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Bovine leptospirosis in abattoirs in Uganda: Molecular detection and risk of exposure among workers

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Abstract

Leptospirosis is a zoonotic bacterial disease reported worldwide. In Uganda, seropositivity has been reported in both humans and domesticated animals, including cattle. However, it remains unknown whether cattle are shedding leptospires and thus acting as potential source for human leptospirosis. We conducted this cross-sectional study in two cattle abattoirs in Kampala, Uganda between June and July 2017. Kidney and urine samples from 500 cattle sourced from across the country were analysed by real-time PCR to establish the prevalence of *Leptospira*-positive cattle and risk of exposure to abattoir workers. The species of infecting *Leptospira* was determined by amplification of *secY* gene and compared to reference sequences published in GenBank. Of 500 cattle tested, 36 (7.2%) had *Leptospira* DNA in their kidneys (carriers), 29 (5.8%) in their urine (shedders); with an overall prevalence (kidney and/or urine) of 8.8%. *Leptospira borgpetersenii* was confirmed as the infecting species in three cattle and *Leptospira kirschneri* in one animal. Male versus female cattle (OR = 3, *p*-value 0.003), exotic versus local breeds (OR = 21.3, *p*-value 0.002) or cattle from Western Uganda (OR = 4.4, *p*-value 0.001) and from regions across the border (OR = 3.3, *p*-value 0.032) versus from the central region were more likely to be *Leptospira*-positive. The daily risk of exposure of abattoir workers to ≥1 (kidney and/or urine) positive carcass ranged from 27% (95% credibility interval 18.6–52.3) to 100% (95% CI 91.0–100.0), with halal butchers and pluck inspectors being at highest risk. In conclusion, cattle slaughtered at abattoirs in Uganda carry and shed pathogenic *Leptospira* species; and this may pose occupation-related risk of exposure among workers in these abattoirs, with workers who handle larger numbers of animals being at higher risk.

KEYWORDS
abattoir, *Leptospira*, leptospirosis, real-time PCR, risk of exposure

1 | INTRODUCTION

Leptospirosis is a zoonotic disease reported worldwide, with the highest incidences occurring in subtropical and tropical countries (Bharti et al., 2003). The aetiological agents of the disease are spirochetes from the genus *Leptospira*, comprising 20 species and approximately 250 serovars belonging to 24 serogroups (Cerqueira & Picardeau, 2009). Certain serovars may be regionally endemic and adapted to specific animal hosts, which may remain asymptomatic, but capable of urinary shedding of the bacteria. In contrast,

incidental hosts (most mammalian species) may develop acute to severe disease (Ellis, 2015; Faine, Adler, Bolin, & Perolat, 1999). Humans, domestic animals and wildlife get infected through direct contact of mucosae or damaged skin with *Leptospira* contaminated urine, abortive tissues or indirectly through contaminated water and soil (Faine et al., 1999; Haake & Levett, 2015).

Cattle are known to carry *Leptospira borgpetersenii* serovar Hardjo and *L. interrogans* Pomona. Infections with *L. interrogans* serovars Canicola and Icterohaemorrhagiae as well as other serovars in the Pyrogenes, Icterohaemorrhagiae, Hebdomadis and Tarassovi serogroups have been reported (Faine et al., 1999). Infected cattle may present with non-specific clinical symptoms including high fever, haemolytic anaemia, hemoglobinuria, jaundice, pulmonary congestion and even death (Ellis, 2015; Faine et al., 1999). Laboratory diagnosis of leptospirosis is commonly based on serological testing, with the microscopic agglutination test being the gold standard (Goris & Hartskeerl, 2014). In recent years, molecular diagnostic techniques including real-time PCR have been described (Rojas et al., 2010; Villumsen et al., 2012).

Generally, zoonotic diseases including leptospirosis are underdiagnosed, thus under-reported or even neglected in Africa and elsewhere (Maudlin, Eisler, & Welburn, 2009; Molyneux et al., 2011). The higher awareness of malaria may have contributed to the misdiagnosis and under-reporting of other febrile illnesses (Crump et al., 2013; Leslie et al., 2012), among other factors. In a study to establish the aetiology of fever in a cohort of 870 hospitalized paediatric and adult febrile patients in Northern Tanzania, a clinical diagnosis of malaria was made in 60.7% of the patients, yet only 1.6% actually were confirmed to have malaria. Acute bacterial zoonoses were identified in over 26% of these febrile admissions, of which 14% had brucellosis, 34% leptospirosis, 20% had Q fever and 31% had spotted fever group rickettsioses (Crump et al., 2013). With incidences of malaria declining due to efficient and large-scale control measures, zoonotic diseases such as leptospirosis become increasingly important. In a systematic review by Costa et al., (2015), the global annual incidence of leptospirosis was estimated at 1.03 million cases (95%CI, 305,000–1,750,000) and resultant deaths at 58,900 (95%CI, 23,800–95,800). For East Africa, an annual incidence of 25.6 cases (95%CI 9.3–43.3) per 100,000 population was reported (Costa et al., 2015). In Uganda, seropositivity has been reported in buffaloes (Atherstone, Picozzi, & Kalema-Zikusoka, 2014) and in dogs (Millán et al., 2013), with the first case of clinical canine leptospirosis recently reported (Alinaitwe, Kakooza, Eneku, Dreyfus, & Campos, 2017). Seroprevalence of 19% was reported in cattle from two districts in Uganda (Dreyfus et al., 2017). Additionally, Dreyfus et al., (2016) demonstrated 35% prevalence of anti-*Leptospira* antibodies in health centre patients in Hoima, Uganda; and that skinning of animals was significantly associated with the observed seropositivity. Therefore, a cattle–human transmission pathway was hypothesized. In the present study, we established the prevalence of *Leptospira* infection in slaughtered cattle and estimated the risk of exposure among abattoir workers.

Impacts

- This study will create public health awareness, trigger further research and prompt approaches for prevention and control of leptospirosis in Uganda.
- Particularly, data on circulating *Leptospira* species could inform choice of *Leptospira* vaccine profiles for cattle in Uganda.
- We demonstrated successful adoption of a real-time PCR assay targeting the *lipL32* gene for detection of pathogenic *Leptospira* in a local veterinary laboratory setting. This approach could also be adopted by human clinical laboratories to confirm leptospirosis among cases of acute undifferentiated fever in Uganda.

2 | MATERIALS AND METHODS

2.1 | Research design and study area

This cross-sectional study was conducted in two purposively selected cattle abattoirs in Kampala, Central Uganda: that is, Nsooba slaughter house, Kalerwe (AK) and City abattoir (LC). The selected abattoirs are currently the largest in Kampala (in terms of daily slaughters), and source their slaughter animals from various regions of Uganda and abroad. Kampala is the second most populated district in Uganda, covering about 73 square miles of land, with 1.5 million occupants (Uganda Bureau of Statistics, 2016).

2.2 | Study population and sample size

The study population comprised all cattle received for slaughter at selected abattoirs on the pre-selected days of sampling between June and July 2017. The sample size given an estimated prevalence of 20% (based on a serological survey by Dreyfus et al., 2017), confidence level of 0.95 and precision of 0.05 was 462 samples (Sergeant, 2009).

2.3 | Sampling strategy

Abattoirs were visited on alternating weekdays (twelve at AK and nine days at LC) to prevent introduction of systematic bias. Samples were systematically collected from four randomly selected slaughter lines at abattoir LC. At the second abattoir (AK), there were no slaughter lines; we assumed two virtual spaces along its width and opportunistically sampled animals slaughtered through one virtual space on alternative visits. Demographic data and sources of the slaughtered cattle were captured during collections, while information on: worker positions, number of personnel at each worker position, responsibilities of personnel of each worker position and daily workload of personnel of various worker positions (number of carcasses handled per day) was obtained from abattoir records, through personal observations and consultation with forepersons of worker positions.

2.4 | Sample collection and transportation

From each selected animal, a piece of kidney ≥0.5–1.0 cm thick and extending from the renal cortex to the medulla was collected aseptically into a sterile plastic bag. At least 4 ml of urine retained in the bladder was collected by cystocentesis. The quantities of samples picked were based on estimates of starting material required for the DNA extraction methods used in this study. All samples were kept on ice before delivery to Central Diagnostic Laboratory at College of Veterinary Medicine, Animal Resources and Biosecurity (COVAB), Makerere University, for further processing and storage at −20°C. The samples were kept for not more than 7 weeks before DNA was extracted.

2.5 | DNA extraction from kidney homogenates

The kidney tissue (1.5–2 g) was homogenized in 3 ml of sterile phosphate-buffered saline (PBS), pH 7.4 (Rankem–RFCL), and DNA extracted from the homogenates using the guanidium thiocyanate (GES) method as described by Pitcher, Saunders, and Owen (1989).

Briefly, 250 µl of homogenate was lysed in 500 µl of GES followed by centrifuging and transferring 600 µl of supernatant into a new micro-centrifuge tube. Then, 250 µl of 7.5 M ammonium acetate was added followed by addition of 500 µl of phenol:chloroform:isoamyl alcohol mixture in the ratio of 49.5:49.5:1 (Sigma-Aldrich Chemie) and centrifuging at 15,600 x *g*, 4°C for 15 min. DNA was precipitated using absolute isopropanol and the resultant pellet washed in cold 80% ethanol. The pellet was dried for 15 min, reconstituted in 50 µl of pyrogen-free water and stored at −20°C.

2.6 | DNA extraction from urine

Four ml of urine was centrifuged at 15,600 x *g*, 4°C for 15 min and the resultant pellet reconstituted in 200 µl of PBS. DNA was then extracted using the QIAamp® DNA Mini kit (Qiagen) according to manufacturer´s guidelines for purification from blood or body fluids. Briefly, samples were lysed in a mixture of protease and buffer AL for 30 min at 56°C on a thermoblock (QBT4 Grant instruments). Subsequently, 200 µl of absolute ethanol was added followed by mixing and transferring 500 µl of the mixture into a spin column. The column contents were then spun at 6,000 x *g* for 1 min, before washing through the column with 500 µl each of the ethanol-based buffers AW1 and AW2. DNA was finally eluted in 50 µl of buffer AE (10 mM Tris·Cl; 0.5 mM EDTA; pH 9.0) and stored at −20°C.

2.7 | Real-time polymerase chain reaction

A TaqMan real-time polymerase chain reaction (qPCR) assay previously reported as successful for the detection of pathogenic *Leptospira* (Alinaitwe et al., 2017; Villumsen et al., 2012) was used to determine the infection status of cattle kidney and urine samples in this study. The qPCR targeted the gene *lipL32* which encodes for a major outer membrane protein, only present in pathogenic *Leptospira* species (Haake et al., 2000). Primers and probe were according to Villumsen et al. (2012). The qPCR conditions were validated using dilution series of *Leptospira interrogans* serovar Icterohaemorrhagiae strain RGA and yielded an efficiency of 100.0% and 101.6% on the 7500 Fast and Step One Plus® PCR Systems (Applied Biosystems), respectively. The detection limit was found to be 10 genome equivalents per reaction, the ideal threshold determined at 0.06 and a cut-off set at 40 cycles. No false-positive reactions were observed during the validation process. All reactions were carried out in duplicate on Step One Plus® and 7500 Fast PCR Systems, with recommended default cycling settings (Holding at 50°C for 2 min, 95°C for 10 min and 40 cycles of 95°C for 15 s, 60°C for 1 min). The total reaction volume of 25 µl contained these final concentrations: 1× TaqMan® Universal PCR Master Mix, No AmpErase UNG®, 0.5× TaqMan® Exogenous Internal Positive Control mix (IPC), 0.5× IPC template (Applied Biosystems), 1 µM of each primer, 80 nM of the probe and 2.5 µl of template. For each run, DNA from *L. interrogans* serovar Hardjo strain Hardjoprajtino was included as positive control and pyrogen-free water as a negative control. The IPC made it possible to control for inhibition and thus prevent false-negative results. Samples were considered positive when showing an exponential amplification curve in both replicates at cycle times <40, with the threshold set at 0.06.

2.8 | Identification of infecting *Leptospira* species

In qPCR positive samples with a high *Leptospira* load (C_t Value < 30), amplification and sequencing of the 470 bp fragment of *secY* gene was performed as previously described (Allan et al., 2018; Dietrich et al., 2014). Selection of C_t value cut-off was based on previous experience working with sequences from non-culture clinical samples in similar studies (Allan et al., 2018). Infecting *Leptospira* species were determined by sequence comparison with published reference sequences of the *secY* gene in GenBank (Benson et al., 2012; Victoria et al., 2008).

2.9 | Data analysis

Data were recorded using Microsoft Excel 2010 (Microsoft Corp.) and analysed with Excel, Stata 15 (Stata Corp.) and R software (R version 3.4.3; R Development Core Team, Vienna, Austria, 2015).

2.9.1 | Case definitions

A *Leptospira*-positive animal was kidney and/or urine qPCR positive. A carrier was defined as an animal whose kidney sample was qPCR positive and a shedder as one with a positive urine qPCR result.

2.9.2 | *Leptospira* prevalence and study population characteristics

The overall prevalence of positive animals was calculated (number of positives/study population), and descriptive analysis of population

demographics of the slaughtered animals performed. The agreement between urine and kidney qPCR results was assessed using Cohen's kappa statistic (Thrusfield, 2005).

Difference in *Leptospira* prevalence by region, sex, breed, age and abattoir were described and analysed by Chi-square test (univariable analysis). The outcome of interest in the multivariable logistic regression model was *Leptospira* prevalence in slaughter cattle.

A manual forward and backward selection method was applied to assess the association between exposure variables (abattoir, sex, age, breed and region) with the outcome and to control for confounding variables. Exposure variables were entered in the model if the univariable *p*-value was ≤0.2 or if their presence changed an exposure variable by more than 15% to account for bias and were kept in the model if the Likelihood ratio test was statistically significant ($p \leq 0.05$). In addition, the following interaction terms were tested: "sex*age" and "breed*region." The Hosmer and Lemeshow's statistic was used to test the goodness-of-fit of the model.

2.9.3 | Assessment of risk of exposure to *Leptospira*-positive carcasses

For each abattoir, the risk of exposure to *Leptospira* among workers was estimated firstly deterministically and secondly in a stochastic model, assuming random variation.

Firstly, the number of daily carriers or shedders (*Npos*) a worker at each position is exposed to was estimated using the number of processed animals at the particular worker position and the prevalence of positive kidney/urine samples. A beta distribution was used to account for sampling uncertainty: $\beta(\alpha, \beta)$, where α = number of positive carriers and shedders (pos) +1 and $\beta = N$ (sample size) − pos + 1. The daily risk of a worker at each abattoir being exposed to at least one kidney and/or urine positive carcass was calculated with the formula $1 - (1 - P)^n$, (where *P* is the prevalence and *n* is the number of cattle slaughtered per day), assuming that it only varied randomly and not by any specific bias (season, day of week etc.). This daily risk of exposure and 95% credibility intervals for different worker positions was modelled stochastically using a binomial distribution. The input data were the number of sampled *Leptospira*-positive carcasses, the total number of sampled animals and the number of carcasses handled per year (300 days, taking average absences into account). R codes for the stochastic model are shown in the Appendix 1.

2.10 | Ethical considerations

Approval of procedures was sought from the institutional review board of the College of Veterinary Medicine, Animal Resources and Biosecurity (COVAB), Makerere University (SBLS/REC/17/003) and from the Uganda National Council for Science and Technology (A565). Consent from abattoir representatives was obtained ahead of the study and also at the time of sampling.

3 | RESULTS

3.1 | Study population characteristics

The abattoirs commonly slaughtered indigenous/local breeds of cattle (80.2%), with a slightly higher turnover of female animals (54.4%). The majority of animals were sourced from central and western regions of Uganda. A detailed description of the population characteristics of the sampled animals is shown in Table 1.

3.2 | Prevalence of *Leptospira*

Of 500 cattle, 36 (7.2%) carried *Leptospira* DNA in their kidneys (carriers), and 29 (5.8%) in their urine (shedders); with an overall prevalence

TABLE 1 Composition of the sampled animals (*N* = 500) by sex, breed, age, region and abattoir

Category of variable	Level	Numbers	Proportion (%)
Sex	Female	272	54.4
	Male	228	45.6
Age	Juvenile (<1.5 years)	32	6.4
	Adult	468	93.6
Breed	Local	401	80.2
	Cross	94	18.8
	Exotic	05	1.0
Region animal was sourced	Northern	39	7.8
	Eastern	55	11.0
	Central	200	40.0
	Western	69	19.0
	Across border (mainly Tanzania)	42	8.4
	Undetermined	95	13.8
Sample composition by abattoir	Abattoir LC	313	62.6
	Abattoir AK	187	37.4

(kidney and/or urine) of 8.8%. Of 36 carriers, 21 (58.3%) were also found to be shedding. Eight of the 29 (27.6%) shedders had their kidneys test negative. Up to 99.6% (498/500) replication of the qPCR reactions was obtained in urine samples and in 99.2% (496/500) of the tested kidney samples, with C_t values ranging from 22 to 37 for all positive samples. The six non-replicating signals were very weak (amplification occurring from the 38th to as late as 40th cycle), even after repeated testing. Overall, there was a good agreement between urine and kidney qPCR results (Cohen's kappa statistic 0.622; $p \leq 0.001$). Prevalence of carriers and shedders by abattoir is summarized in Table 2. Furthermore, the comparison of qPCR results between kidney and urine samples is shown in Table 3.

3.3 | *Leptospira* species

Of the 44 *Leptospira*-positive cattle, samples from eight cattle (ten qPCR positive samples) had a C_t value < 30 and were selected for *secY* sequencing and *Leptospira* species identification. Of the ten qPCR positives, *secY* amplification and sequencing was successful from five samples (three kidney and two urine) obtained from four cattle. *Leptospira borgpetersenii* infection was confirmed in three cattle. Sequences from two of these three cattle were 100% identical to *L. borgpetersenii* serovar Hardjo (Hardjo-bovis) from GenBank searches and showed 98%–99% degree of identity to a range of serovars including Hardjo in the other animal. In one animal, *L. kirschneri* infection was confirmed, with 100% sequence identity to several *L. kirschneri* serovars including Cynopteri, Kamituga, Kunming and Mwogolo.

3.4 | Risk factors for *Leptospira* prevalence

While there was no statistically significant association between cross breeds and *Leptospira* prevalence, exotic breeds were 21 times at higher odds of being kidney and/or urine positive than local breeds, once controlled for the effect of sex and region (p-value 0.002; CI 3.12–145.39). Cattle sourced from across the border were three times as likely (p-value 0.032; CI 1.11–10.03) and those from the western region four times as likely (p-value 0.001; CI 1.80–10.98) to be kidney and/or urine positive than cattle from the central region, once adjusted for the effect of sex and breed. Adding the variables "abattoir" or "age" into the model did not improve the fit and thus was removed (Table 4). The Hosmer and Lemeshow's goodness-of-fit test indicated a good fit of the data (p-value 0.4). None of the tested interactions were significant.

3.5 | Cattle slaughter process and worker positions

Formal worker positions/groups in the abattoirs included: offloaders, animal traders, dealers (in plucks, offal, limbs, head, urogenital parts, kidneys and foetuses), restrainers, halal butchers, skinners, eviscerators, carcass upholsters, offal processors, pluck inspectors (veterinarians), veterinary assistants, meat loaders and janitors. The offloaders were charged with taking the animals off the delivery lorries and leading them to the kraal where the animal traders would haggle and buy them. Once bought, the traders would tag their animals and lead them to a holding area with help of specific restrainers. The restrainers were also responsible for delivering the animals to the final slaughter slab and offer a hand to halal butchers who were solely responsible for bleeding and partial decapitation. Skinners and eviscerators would then pick up from the butchers and continue to remove the skin, viscera and other parts that were sold separately from the carcass, including head, limbs and genitalia. These were handed over to respective dealers. With help of veterinary assistants, the dealers of kidneys and plucks (liver, heart and lungs) would deliver these parts for inspection by assigned veterinarians before gathering and processing (majorly washing) these parts for sale. Upholsters would then deliver and hang ready carcasses at sales'

TABLE 2 Prevalence of *Leptospira*-positive carcasses by abattoir

Abattoir	*N* tested	Average daily slaughter	Carriers[a]	Shedders[a]	Overall prevalence
			N pos; % (CI)	*N* pos; % (CI)	*N* pos; % (CI)
AK	187	162	7; 3.74 (1.65–7.87)	7; 3.74 (1.65–7.87)	10; 5.35 (2.74–9.89)
LC	313	221	29; 9.27 (6.39–13.17)	22; 7.03 (4.56–10.60)	34; 10.86 (7.74–14.98)

Abbreviations: CI, Confidence Interval; *N*, number; pos, positive.
[a]A carrier is an animal with a positive kidney qPCR result and a shedder is one with a positive urine qPCR result.

TABLE 3 Comparison of *Leptospira* qPCR results between urine and kidney samples of 500 cattle with absolute numbers (*n*) and row percentages (%)

	Urine negative *n* (%)	Urine positive *n* (%)	Total *n* (%)
Kidney negative *n* (%)	456 (98)	8 (2)	464 (100)
Kidney positive *n* (%)	15 (42)	21 (58)[a]	36 (100)
Total *n* (%)	471 (94.2)	29 (5.8)	500 (100)

[a]There was a good agreement between urine and kidney qPCR results (Cohen's kappa statistic 0.622; $p \leq 0.001$).

TABLE 4 Association between population characteristics of sampled cattle (*N* = 500) and *Leptospira* prevalence in a multivariable logistic regression model

Exposure	Category	Odds ratio (OR)	95% CI	*p*-Value
Sex	Females	Ref[a]		
	Males	3.00	1.47–6.16	0.003
Breed	Local	Ref[a]		
	Cross	1.31	0.62–2.77	0.482
	Exotic	21.29	3.12–145.39	0.002
Region	Central	Ref[a]		
	Western	4.44	1.80–10.98	0.001
	Northern	1.07	0.22–5.21	0.936
	Eastern	1.26	0.39–4.04	0.702
	Across border	3.34	1.11–10.03	0.032
	Undetermined	1.36	0.49–3.80	0.558

Abbreviation: CI, Confidence Interval.
[a]Ref is the parameter in a given category to which the other parameters in the same category were compared.

points, where veterinarians would further inspect and stamp them as a sign of pass for sale. Once sold, a special worker group, the meat loaders would cut, weigh and load the bought meat.

3.6 | Risk of exposure to *Leptospira*-positive carcasses among abattoir workers

The average number of carcasses handled daily per individual worker was similar for most worker positions across the two abattoirs, except for halal butchers, carcass upholsters and pluck inspectors. The daily risk of exposure to ≥1 (kidney and/or urine) positive carcass ranged from 27% (95% credibility interval 18.6–52.3) to 100% (CI 91.0–100.0), with halal butchers and pluck inspectors being highly exposed (Tables 5 and 6).

4 | DISCUSSION

Prevalence of infection with pathogenic *Leptospira* species in up to 8.8% of slaughtered cattle in this study reveals a potential role cattle may play in maintenance and transmission of leptospirosis in Uganda. *Leptospira* may persist in kidneys of infected animals for periods of weeks to years, with possibility of shedding the bacteria in urine and contaminating soil and water sources (Ellis, 2015; Faine et al., 1999). The risk of human and animal infection from such contaminated sources will increase in presence of predisposing seasonal factors including rainfall and flooding (Faine et al., 1999; Haake & Levett, 2015). The rainy seasons in many parts of Uganda usually take place from March–June (first season) and from August–November (second season); with the highest peak being around May and November (Kansiime, Wambugu, & Shisanya, 2013). The sampling time of our study (June and July) took place at the end of the first rainy season, setting favourable conditions for *Leptospira* transmission. Therefore, the prevalence and derived exposure risk may have been a high estimate. However, the slaughter animals were sourced from a wide geographical range with varying climatic conditions/seasons at the time of sampling, thus minimizing this sampling bias. The prevalence of pathogenic *Leptospira* was comparatively higher in kidneys (7.2%) than in urine (5.8%), probably as a result of intermittent urinary

TABLE 5 The daily risk of a worker being exposed to ≥1 (kidney and/or urine) positive carcass (%) for different worker positions at abattoir AK

Worker position	*N* carcasses handled per day	*N* carcasses handled per year (300 days)	Predicted prevalence (%)	*N* pos carcasses handled per year	% Daily risk of exposure (CI)
Pluck dealer, offal handler	5	1,500	7.88	80	34.34 (13.3–40.7)
Restrainer, skinner, urogenital dealer, eviscerator	7	2,100	5.61	112	27.67 (18.6–52.3)
Kidney dealer	10	3,000	7.20	160	47.67 (25.7–65.3)
Offloader	12	3,600	7.45	193	60.00 (30.0 –71.0)
Carcass upholster	30	9,000	5.90	481	86.34 (58.7–95.7)
Pluck inspector	32	9,600	3.71	513	72.00 (61.7–96.7)
Halal butcher	81	24,300	8.36	1,299	100 (91.0–100.0)

Abbreviations: CI, Credibility Interval; *N*, number; pos, positive.

TABLE 6 The daily risk of a worker being exposed to ≥1 (kidney and/or urine) positive carcass (%) for different worker positions at abattoir LC

Worker position	*N* carcasses handled per day	*N* carcasses handled per year (300 days)	Predicted prevalence (%)	*N* pos carcasses handled per year	% Daily risk of exposure (CI)
Skinner, offal handler, carcass upholster	3	900	10.69	98	29.00 (20.3–39.7)
Offloader, urogenital dealer, eviscerator	5	1,500	9.85	163	40.00 (32.7–56.7)
Kidney dealer	7	2,100	14.03	228	53.00 (32.3–56.7)
Pluck dealer, restrainer, halal butcher	8	2,400	10.52	261	58.33 (48.2–72.6)
Pluck inspector	60	18,000	10.88	1955	100 (99.0–100.0)

Abbreviations: CI, Credibility Interval; *N*, number; pos, positive.

shedding or other factors such as low bacterial load and inhibition from high levels of urea that limit PCR detection of leptospires in urine samples (Schrader, Schielke, Ellerbroek, & Johne, 2012). To minimize influence of inhibition factors, all urine samples were collected on ice and immediately frozen at −20°C on arrival to the laboratory. Efficiency of this freezing method was assessed through an experiment in which non-frozen aliquots of urine samples turned out negative with the *Leptospira* specific qPCR assay used in this study, while their corresponding frozen counterparts were qPCR positive. Similar abattoir based studies conducted in New Zealand (Fang et al., 2014) and Tanzania (Allan, 2016) reported a higher prevalence in urine than in kidneys. In both studies, the amount of kidney tissue extracted was much smaller than what we used in our study, which may have led to missing of more localized infections that are common in cases of chronic *Leptospira* infections in cattle. However, it may be more practical to collect and use urine as opposed to kidneys for detection of *Leptospira* infections in cattle.

Male animals in this study were found more likely to carry or shed leptospires than females. A similar trend in male dogs was attributed to roaming (Ward, Guptill, Prahl, Ching Wu, 2004) and in sea lions, to the more migratory activity of males (Norman, DiGiacomo, Gulland, Meschke, & Lowry, 2008). Either way, chances of males getting into contact with *Leptospira* contaminated sources increase consequently. In the current study, the observed association could be more related to the natural mating behaviour of male cattle, such as flehmening on urine of females. Local/native breeds of cattle had less risk of carrying or shedding leptospires as compared to imported/exotic breeds. The majority of local breeds in Uganda are managed under agro-pastoral production systems (Kugonza, Nabasirye, Mpairwe, Hanotte, & Okeyo, 2011; Wurzinger et al., 2006), with access to natural pastures in grazing areas and fallow land. As such these cattle may be constantly exposed to sub-infectious levels of endemic *Leptospira* strains associated with the grazing environment and thus develop immunity against *Leptospira* infection. On the other hand, exotic animals are usually under confinement on commercial ranches, zero grazing farms, and fed improved pastures and supplementary feeds. Given the low number of exotic animals (only 1%) tested in this study, this result should be interpreted with caution. Association of cattle from Western Uganda with a higher *Leptospira* prevalence may indicate that bovine leptospirosis is widespread among cattle in Western Uganda. Moreover, Dreyfus et al., 2016 reported skinning of animals as a risk factor for *Leptospira* seropositivity in humans in Hoima, Western Uganda. However, animals sold for slaughter may not truly depict prevalence of particular diseases in the general population as a result of potential for selection bias (McKenna et al., 2004). In endemic setups, herd owners may cull animals on the basis of particular disease-associated characteristics such as old age or poor reproductive performance. Nevertheless, this does not rule out the usefulness of insights on bovine leptospirosis in Uganda that may be derived from this current study. The definite origin of up to 14% of the animals could not be established mainly because of lack of access to accompanying documentation from their source markets. In future, it may be necessary to design field studies that estimate prevalence of diseases across home grazing areas in regions where slaughter cattle are sourced. The lack of association between age of the animals and *Leptospira* carriage and/or shedding as observed in this study could be because slaughter animals are selected for an ideal slaughter weight and as such, the study population mainly comprised of adult aged cattle. Therefore, the rather small sample size of young animals made it difficult to detect a statistically significant difference.

Sequences obtained from three cattle showed a high degree of similarity to *L. borgpetersenii* serovar Hardjo. Hardjo is commonly associated with cattle around the world and has recently been confirmed in Tanzanian cattle (Allan et al., 2018). Additional detection of *L. kirschneri* infection indicates that multiple *Leptospira* species may be present in cattle in Uganda. The high degree of similarity to multiple reference sequences in this case may require further work to confirm the infecting serovar. *SecY* sequence analysis was performed on a total of ten qPCR positive samples and high-quality sequence was obtained in five (50.0%). This success rate is consistent with other studies that have used this approach (Allan et al., 2018; Dietrich et al., 2014). Failure to obtain *secY* sequence from some of the qPCR positive samples with C_t values < 30 could be attributed to the difference in PCR amplicon length between the

lipL32 qPCR assay (87 bp) and the *secY* typing assay (470 bp). In this regard, any DNA fragmentation resulting from the extraction methods or DNA degradation during sample storage could reduce the chance of getting long amplicons, thus significantly lowering the sensitivity of the conventional PCR assay used during *secY* sequence typing. Another probable reason is mismatch between primers and bacterial sequence. The primers used in this study were based on a primer set from a published MLST scheme (Ahmed et al., 2011) that was adapted for use in the East African region (Dietrich et al., 2014). However, as relatively little is known about the genetic diversity of *Leptospira* in East Africa, primer mismatch may remain a feasible explanation for failure to sequence product from some of the qPCR positive samples. Despite this limitation, our study represents the first information regarding *Leptospira* genotypes circulating in cattle in Uganda and also supports existing serological data that suggest a wide diversity of *Leptospira* species infecting Ugandan cattle (Dreyfus et al., 2017).

Confirmation of renal prevalence and urinary shedding of pathogenic *Leptospira* species among cattle slaughtered at abattoirs in Uganda implies potential occupational risk to abattoir workers and to those involved in obstetrics, milking and animal transportation. Workers who handle many carcasses or are exposed to urine splashes and infectious tissues may be at higher risk of exposure. Pluck inspectors in both abattoirs were at high risk of exposure to *Leptospira*-infected carcasses. This is because inspectors were charged with examining all plucks and carcasses of cattle slaughtered daily, yet they were a small worker group (four at AK and five at LC). Additionally, halal butchers and carcass upholsters of abattoir AK were relatively at a high risk of exposure; with the butchers being at 100% risk. A much lower risk of exposure for halal butchers in abattoir LC was related to the larger worker to cattle ratio: a typical slaughter line handling 10–25 animals was allocated to one or two butchers. At abattoir AK, two butchers were responsible for decapitation of an average of 162 animals daily. Unlike upholsters in abattoir LC who used pulleys to hang and drag carcasses to the sale points, upholsters of abattoir AK had to carry all carcasses on their shoulders, making the activity labour intensive and also increasing the number of carcasses each upholster is in contact with.

While this study estimated the risk of exposure, which was derived from the prevalence and number of carcasses handled, it would further be important to establish the actual infection risk. Estimation of infection risk would depend on additional factors including but not limited to probability of exposure to urine splashes or contaminated tissues, number of hours worked per day, worker position in the slaughter process and whether personal protective equipment is available and appropriately worn (Dorjee et al., 2011; Dreyfus et al., 2014). Therefore, the risk of exposure as estimated in this current study may only give insight on likelihood of an unforeseen risk of *Leptospira* infection among abattoir workers in Uganda. In future, it may be necessary to design epidemiological studies to measure the incidence of leptospirosis among these abattoir workers. Elsewhere, seroprevalence of *Leptospira* among abattoir workers has already been demonstrated: in Tanzania (Schoonman & Swai, 2009), Nigeria (Ezeh, Ellis, Addo, & Adesiyun, 1988), New Zealand (Dreyfus et al., 2014), India (Sharma, Vijayachari, Sugunan, Natarajaseenivasan, & Sehgal, 2006), Colombia (Nájera, Alvis, Babilonia, Alvarez, & Máttar, 2005) and Brazil (Gonçalves et al., 2006). The highest risk of being seropositive has been reported among workers at the slaughter board (Bacic, Zorica, Adamov, & Branka, 1994; Dreyfus et al., 2014) and meat inspection (Blackmore & Schollum, 1982), compared to workers of other areas of the slaughter plants. In all these studies, leptospirosis was regarded as an occupational hazard that warrants institution of appropriate control measures.

Measures directed at limiting the number of *Leptospira*-positive cattle making it for slaughter would theoretically help reduce risk of exposure among workers in the studied abattoirs. This would entail control of *Leptospira* infection at the animal source points (farms), so as to reduce the disease burden in cattle populations. Vaccination of animals is one of such strategies (Bolin & Alt, 2001; Hartskeerl, Collares-Pereira, & Ellis, 2011). Vaccines are serovar-specific, yet data on predominant *Leptospira* serovars in Ugandan cattle is still lacking. Treatment of infected animal herds has also been shown to lessen both urinary shedding and the impact of infection (Alt, Zuerner, & Bolin, 2001; Hartskeerl et al., 2011). Additional indirect measures may be taken at the abattoirs, including sensitization of workers, use of personal protective equipment, revision of workflow and slaughter methods, redistribution of workforce, and reduction of workload and working hours in risky positions.

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CONFLICT OF INTEREST

All authors have declared that no competing interests exist.

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APPENDIX 1

R CODES FOR THE STOCHASTIC MODELS

Work position and input parameters	R code

AK pluck dealer or offal handler, 1,500 animals in 300 days, 5 animals per day, infection probability is 0.053475936
alpha = 11, Beta = 176
	riskkidney2<−c()
	for (i in 1:10,000) {
	prev<−rbeta(1,11, 176)
	kidney1<−matrix(rbinom(1,500,1,prev),ncol = 5)
	kidney2<−as.data.frame(kidney1)
	kidney2$means<−rowMeans(kidney2)
	kidney2$risk<−ifelse(kidney2$means > 0,1,0)
	risk1<−mean(kidney2$risk)
	riskkidney2<−rbind(risk1, riskkidney2)
	}
	quantile(riskkidney2, c(0.025,0.5,0.975))

AK restrainer, skinner, eviscerator, 2,100 animals in 300 days, 7 animals per day, infection probability is 0.053475936
alpha = 11, Beta = 176
	riskkidney2<−c()
	for (i in 1:10,000) {
	prev<−rbeta(1,11, 176)
	kidney1<−matrix(rbinom(2,100,1,prev),ncol = 7)
	kidney2<−as.data.frame(kidney1)
	kidney2$means<−rowMeans(kidney2)
	kidney2$risk<−ifelse(kidney2$means > 0,1,0)
	risk1<−mean(kidney2$risk)
	riskkidney2<−rbind(risk1, riskkidney2)
	}
	quantile(riskkidney2, c(0.025,0.5,0.975))

AK kidney dealer, 3,000 animals in 300 days, 10 animals per day, infection probability is 0.053475936
alpha = 11, Beta = 176
	riskkidney2<−c()
	for (i in 1:10,000) {
	prev<−rbeta(1,11, 176)
	kidney1<−matrix(rbinom(3,000,1,prev),ncol = 10)
	kidney2<−as.data.frame(kidney1)
	kidney2$means<−rowMeans(kidney2)
	kidney2$risk<−ifelse(kidney2$means > 0,1,0)
	risk1<−mean(kidney2$risk)
	riskkidney2<−rbind(risk1, riskkidney2)
	}
	quantile(riskkidney2, c(0.025,0.5,0.975))

(Continues)

APPENDIX 1 (Continued)

Work position and input parameters	R code

AK offloader, 3,600 animals in 300 days, 12 animals per day, infection probability is 0.053475936
alpha = 11,
Beta = 176
	riskkidney2<−c()
	for (i in 1:10,000) {
	prev<−rbeta(1,11, 176)
	kidney1<−matrix(rbinom(3,600,1,prev),ncol = 12)
	kidney2<−as.data.frame(kidney1)
	kidney2$means<−rowMeans(kidney2)
	kidney2$risk<−ifelse(kidney2$means > 0,1,0)
	risk1<−mean(kidney2$risk)
	riskkidney2<−rbind(risk1, riskkidney2)
	}
	quantile(riskkidney2, c(0.025,0.5,0.975))

AK carcass upholster, 9,000 animals in 300 days, 30 animals per day, infection probability is 0.053475936
alpha = 11,
Beta = 176
	riskkidney2<−c()
	for (i in 1:10,000) {
	prev<−rbeta(1,11, 176)
	kidney1<−matrix(rbinom(9,000,1,prev),ncol = 30)
	kidney2<−as.data.frame(kidney1)
	kidney2$means<−rowMeans(kidney2)
	kidney2$risk<−ifelse(kidney2$means > 0,1,0)
	risk1<−mean(kidney2$risk)
	riskkidney2<−rbind(risk1, riskkidney2)
	}
	quantile(riskkidney2, c(0.025,0.5,0.975))

(Continues)

APPENDIX 1 (Continued)

Work position and input parameters	R code

AK inspector, 9,600 animals in 300 days, 32 animals per day, infection probability is 0.053475936
alpha = 11,
Beta = 176
	riskkidney2<−c()
	for (i in 1:10,000) {
	prev<−rbeta(1,11, 176)
	kidney1<−matrix(rbinom(9,600,1,prev),ncol = 32)
	kidney2<−as.data.frame(kidney1)
	kidney2$means<−rowMeans(kidney2)
	kidney2$risk<−ifelse(kidney2$means > 0,1,0)
	risk1<−mean(kidney2$risk)
	riskkidney2<−rbind(risk1, riskkidney2)
	}
	quantile(riskkidney2, c(0.025,0.5,0.975))

AK Halal butcher, 24,300 animals in 300 days, 81 animals per day, infection probability is 0.053475936
alpha = 11,
Beta = 176
	riskkidney2<−c()
	for (i in 1:10,000) {
	prev<−rbeta(1,11, 176)
	kidney1<−matrix(rbinom(24,300,1,prev),ncol = 81)
	kidney2<−as.data.frame(kidney1)
	kidney2$means<−rowMeans(kidney2)
	kidney2$risk<−ifelse(kidney2$means > 0,1,0)
	risk1<−mean(kidney2$risk)
	riskkidney2<−rbind(risk1, riskkidney2)
	}
	quantile(riskkidney2, c(0.025,0.5,0.975))