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1 **Molecular detection and typing of pathogenic *Leptospira* species from livestock and small**

2 **mammals in Uganda**

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21 ***Abstract***

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Leptospira are bacteria that cause leptospirosis in both humans and animals. Human *Leptospira* infections in Uganda are suspected to arise from animal-human interactions. From a nationwide survey to determine *Leptospira* prevalence and circulating sequence types in Uganda, we tested 2030 livestock kidney samples, and 117 small mammals (rodents and shrews) using a real-time PCR targeting the *lipL*32 gene. Pathogenic *Leptospira* species were detected in 45 livestock samples but not in the small mammals. The prevalence was 6.12% in sheep, 4.25% in cattle, 2.08% in goats, and 0.46% in pigs. Sequence typing revealed that *L. borgpetersenii*, *L. kirschneri*, and *L. interrogans* are widespread across Uganda, with 13 novel sequence types identified. These findings enhance the East African MLST database and support the hypothesis that domesticated animals may be a source of human leptospirosis in Uganda, highlighting the need for increased awareness among those in close contact with livestock.

Introduction

Leptospira is a genus of spirochete bacteria which includes pathogenic species that cause leptospirosis in humans and animals. Leptospirosis is spread worldwide, with an estimated one million cases and 58900 deaths annually [1]. The genus *Leptospira* comprises approximately 64 genomospecies and over 250 serovars [2]. Although regional endemicity of certain *Leptospira* serovars and host-adapted types have been reported, small mammals, such as rodents and shrews are regarded as the main reservoirs in many instances [3]. Animal reservoirs do not show symptoms but are capable of shedding leptospires in urine for prolonged periods, consequently contaminating water and soil [4]. Infection in humans and domestic animals occurs through direct contact of mucosae or damaged skin with infected urine or abortive tissues or indirectly through contaminated water and soil [2,3].

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44 In Uganda, there is growing evidence of *Leptospira* infection among febrile patients, and
45 domesticated animals are speculated to be the source [5–7]. In one study, seroprevalence of
46 35% was estimated, with those involved in the skinning of cattle having 12 times higher odds of
47 being seropositive [6]. Follow-up surveys of cattle, goats, sheep, and pigs across the country
48 revealed *Leptospira* seroprevalence rates of 19.3% to 27.8% [8–11]. Although this could mean
49 endemicity and widespread *Leptospira* exposure among domestic animals in Uganda, the public
50 health relevance of such exposures remains unresolved. Only animals with ongoing clinical
51 infection or chronic carriers pose risk of infection to humans and other animals or have the
52 potential to contaminate the environment.

53 In Uganda, *Leptospira* infection based on real-time PCR assays has only been demonstrated in
54 cattle, dogs, and pigs, with limited sequence typing data [14–16]. In the present study, we
55 sampled livestock and small mammals at slaughter facilities across Uganda, to determine the
56 status of *Leptospira* infection and circulating sequence types. Slaughter facilities offered
57 convenient access to kidney specimens for PCR testing, enabling the detection of *Leptospira* in
58 large livestock populations with wide geographical coverage. These facilities can also
59 concentrate zoonotic agents and potentially spread infections to nearby communities through
60 environmental contamination or by attracting disease reservoirs like small mammals [15].

61 **Materials and Methods**

62 *Research design*

63 Between December 2021 and October 2022, we conducted a cross-sectional study in selected
64 livestock slaughter facilities across three of the four geographical regions of Uganda (East,

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North and Central). In each region, the district with the largest number of daily slaughters for all species was selected as the study site, except in the East, where no one district slaughtered the highest number of all the livestock species. Instead, two study sites were recruited. The selected study sites were Lira in the North, Kampala in the Central, and Mbale and Soroti in the East (Figure 1). No site was recruited in the Western region following notification by key informants that a significant proportion of the livestock slaughtered in Kampala (our study site in Central) came from the West, and previous studies in slaughter facilities in Kampala have reported similar findings [15, 16].

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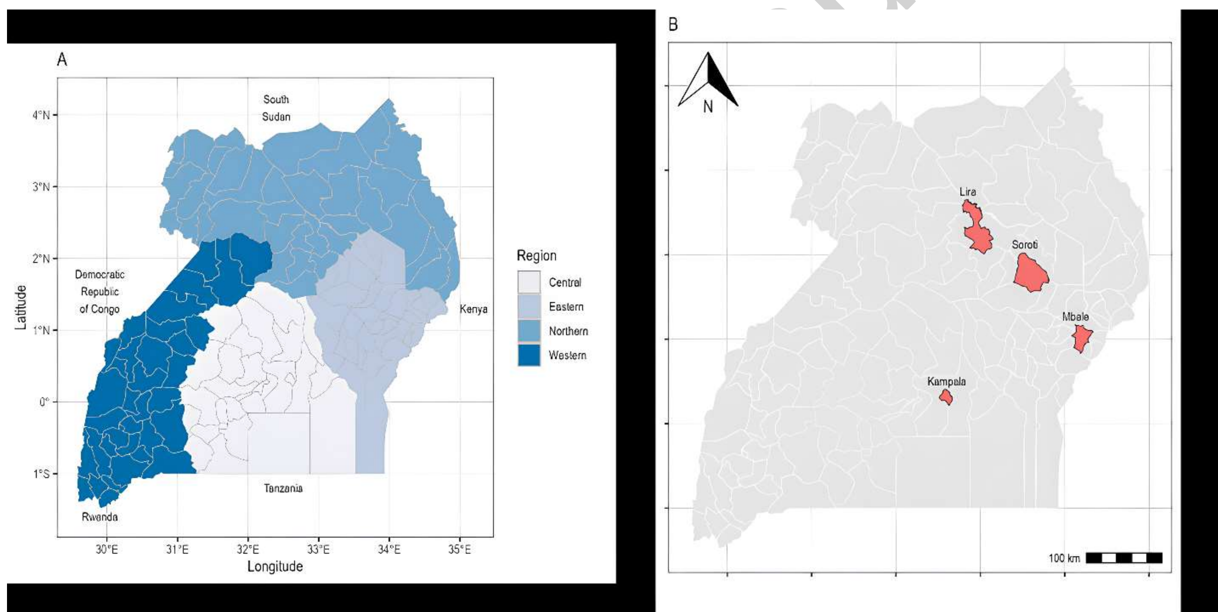

*Figure 1. Map of Uganda showing the regions (**a**) and districts selected as sites for this cross-sectional study (**b**). Source of shapefiles: Uganda - Subnational Administrative Boundaries - Humanitarian Data Exchange (humdata.org) and World Administrative Boundaries - Countries and Territories — Opendatasoft*

Sample size

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79 Sample sizes were calculated in Epitools-epidemiological calculators [16], to estimate the
80 overall true prevalence of *Leptospira* in Uganda without aiming to compare differences
81 between the regions. The minimum sample estimates were 316 cattle (based on 7.2%
82 prevalence in Ugandan slaughter cattle [13]), 53 each for goats and sheep (based on 1.2%
83 prevalence reported in Tanzania [17]), 114 for pigs (assuming a conservative prevalence of 5%),
84 and 99 for small mammals (based on 3.5% prevalence from an unpublished survey conducted
85 by the first author and colleagues at a wildlife-human interface in southwestern Uganda in
86 2016). The estimates considered a *lipL*32 real-time PCR with a sensitivity of 93% and specificity
87 of 98.3% [18] and an error margin of 5%. However, as many samples as could be tested for each
88 species were considered since these samples had already been collected to match the sample
89 sizes for estimating *Leptospira* seroprevalence in the same population. *Leptospira* prevalence
90 data for goats are based on reports from countries neighboring Uganda due to missing local
91 reports at the time the study was designed.

92 *Sampling of livestock*

93 At each site, collection of samples from cattle and small ruminants (goats and sheep) was
94 alternated daily over a 30 day-period to minimize the overrepresentation of animals with the
95 same population characteristics. Pigs were sampled for 16 consecutive days, except in the
96 Eastern region, where sampling was only possible for 10 days due to Easter festivals. An extra
97 pig slaughter facility was enrolled in the East to compensate for this difference in sampling
98 time. Consecutive collection of pig samples was considered because the daily slaughter stock
99 turnover ranged between 80 and 100% in all sites at the time.

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100 On each collection day, slaughtered animals were sampled opportunistically (the next animal
101 was selected when the previous animal was completely sampled). From every animal chosen, a
102 random piece of kidney that included the cortex and medulla and weighed at least five grams
103 was collected aseptically into a sterile screw cap container. The sample volume was as required
104 for the tissue homogenization methods used in this study. Age (young, adult), sex (male,
105 female), and breed (local, exotic, or cross) were noted for each animal, and information on the
106 district of origin was obtained from consultation with the traders or animal movement permits
107 held at the slaughter facilities. Samples were loaded in an ice-cooled box and dispatched daily
108 to the Central Diagnostic Laboratory at the College of Veterinary Medicine, Animal Resources
109 and Biosecurity (COVAB), Makerere University, Uganda. Samples arrived at COVAB on the same
110 day except during collections from the Eastern and Northern regions, where arrival was the
111 next day.

112 *Sampling small mammals*

113 Small mammals were trapped at the same slaughter facilities where livestock were sampled,
114 and in consenting homesteads within a 500-meter radius from the slaughter facilities. The
115 number of homesteads enrolled per region was aimed at a cumulative trap effort of 200 trap
116 nights, except in the central region where the effort was doubled because of the reported
117 scarcity of rodents. For every homestead, two to five small Sherman traps (HB Sherman Traps,
118 Tallahassee, USA) were set in houses, stores, kitchens, poultry houses, or surrounding
119 vegetation. The traps were baited with a combination of ground nuts, peanut butter, sweet
120 bananas, tomatoes, and silver cyprinid, depending on what was commonly reported as gnawed
121 by small mammals in each homestead. Trapping was done overnight, with the baits and

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122 successful traps replaced each morning. Captured animals were euthanized using diethyl ether
123 and transported in ice-cooled boxes to the Central Diagnostic Laboratory, COVAB, Makerere
124 University, where species identification was performed by an experienced zoologist based on
125 phenotypic characterization and measurements of morphometric features [19]. The
126 determination of sex and approximate age were based on external sexual characteristics. This
127 was followed by dissection and extraction of the kidney, spleen, and part of the liver.

128 *Preparation of tissue homogenates and DNA extraction*

129 Three grams of livestock kidney tissue was homogenized and reconstituted in 6 mL of sterile
130 phosphate-buffered saline (pH 7.4; Rankem–RFCL, India). For the small mammals, 50%
131 homogenate was prepared from a pool of both kidneys, the spleen, and part of the liver.
132 Homogenization was achieved by crushing the tissues in stomacher bags (BA6040, Stomacher®
133 80, Seward Ltd., UK) using a ceramic pestle. DNA was extracted from 100 µl of tissue
134 homogenate using the QIAamp DNA Mini Kit for blood or tissue (Qiagen, Hilden, Germany)
135 according to the manufacturer´s guidelines. A dry spin was applied, and the DNA was eluted in
136 buffer AE in two successive steps of 50 µl each and stored at –20 °C. For every extraction run, a
137 *Leptospira*-positive homogenate was included as a positive extraction control, and pyrogen-free
138 water was used as a negative extraction control.

139 *Isolation of Leptospira species*

140 Kidney homogenates from 25% of the livestock samples and from all the small mammals
141 collected each day were cultured to isolate leptospires. Three 10-fold serial dilutions of each
142 homogenate were made in 5 mL of commercial formulations of Ellinghausen-McCullough-

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143 Johnson-Harris (EMJH) medium in which supplements of albumin, polysorbate 80 and
144 additional growth factors have been added (BD Difco™ *Leptospira* Enrichment EMJH, product
145 279510, USA). The primary inoculates (dilution of 1/10) were discarded, and the two
146 subsequent dilutions were incubated at 29.5 °C for 2 days before checking for any signs of
147 turbidity. Subsequent subcultures with visible turbidity were then made in 5 mL of fresh EMJH
148 in which 5'-fluorouracil had been added at a concentration of 200 mg/L and examined every 7-
149 14 days under a dark field microscope for visible leptospires. Cultures in which no visible
150 turbidity or leptospires were observable after 14 weeks were autoclaved and discarded. DNA
151 was isolated from suspected cultures and the presence of pathogenic leptospires tested using a
152 real-time PCR as described below.

153 *Real-time polymerase chain reaction (PCR)*

154 A TaqMan PCR assay targeting the *lipL*32 gene was used to detect pathogenic *Leptospira* in the
155 DNA from livestock and rodent samples. The primers and probes used in this study were
156 described previously by Villumsen et al [20] and synthesized by Eurofins Genomics, France. The
157 presence/absence of the bacteria was determined on a Quantistudio™ 5 PCR System (Applied
158 Biosystems, Foster City, CA, USA) under the following conditions: pre- and post-cycling at 60 °C
159 for 30 seconds, holding at 50 °C for 2 minutes, 95 °C for 10 minutes and 45 cycles of 95 °C for 15
160 seconds and 60 °C for 1 minute. The final concentrations of the mixture in a reaction volume of
161 20 µl were: 1x TaqMan™ Fast Advanced Master Mix, 0.5x TaqMan® Exogenous Internal Positive
162 Control mix (IPC), 0.5x IPC template (Applied Biosystems, Foster City, CA, USA), 1 µM each
163 primer, 80 nM probe and 2.0 µl of DNA template. DNA from *L. interrogans* serovar
164 Icterohaemorrhagiae (strain RGA) and from a positive extraction sample were included as

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165 amplification controls, and 10X Block-Exp IPC® (Applied Biosystems, Foster City, CA, USA) and

166 pyrogen-free water were used as negative amplification controls. A positive sample was one

167 that showed an exponential amplification curve in fewer than 41 cycles, with the fluorescence

168 threshold set at 0.06.

169 *Identification of infecting Leptospira species*

170 *Leptospira*-positive samples with cycle threshold (Ct) ≤36 cycles were typed using nested single-

171 locus sequence typing (SLST) of the *secY* gene as described previously [21], and sequences of

172 245 bp fragments were searched against the BLASTn database for species identification

173 (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Multilocus sequence typing (MLST) was performed on

174 the *secY*-positive samples using Scheme 1, which targets seven housekeeping genes, namely,

175 *glmU*, *pntA*, *sucA*, *tpiA*, *pfkB*, *mreA*, and *caiB* [22]. The sequences were submitted to the

176 PubMLST *Leptospira* database (http://pubmlst.org/leptospira, accessed in November 2023) to

177 determine the allele and allelic profiles for sequence type identification. The sequences were

178 analyzed using Bionumerics software 7.6.3 (Applied Maths, Belgium). *SecY* sequences and

179 concatenated sequences from the MLST were imported to R 4.1.1 [23] using the Biostrings and

180 msa packages, where multiple sequence alignments were generated using the clustal omega

181 method, and distance matrixes were computed. Phylogenetic trees were constructed using the

182 neighbor–joining method.

183 *Data analysis*

184 The data were entered into Microsoft Excel® and analyzed in R version 4.1.1 [23]. Descriptive

185 analysis of population demographics by animal species, breed, age, sex, and region of origin

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186 was performed, and the true *Leptospira* prevalence was calculated using the *epi.prev* function

187 of the *EpiR* package, based on the Rogan-Gladen estimator. The input sensitivity and specificity

188 of the PCR were 86% and 100%, respectively [20], with the method set to "blaker".

189 *Ethical considerations*

190 This study was approved by the Institutional Animal Care and Use Committees of the

191 International Livestock Research Institute (Approval Number ILRI-IACUC2022-17), the School of

192 Biosecurity, Biotechnical and Laboratory Sciences, College of Veterinary Medicine, Animal

193 Resources and Biosecurity (COVAB), Makerere University (Approval number

194 SBLS/HDRC/20/012) and the Uganda National Council for Science and Technology (Approval

195 Number HS1563ES).

196 **Results**

197 *Population characteristics of the sampled livestock and small mammals*

198 Of the 2030 livestock sampled, 820 cattle, 335 goats, 114 sheep, and 761 pigs were included.

199 Up to 78.47% (n=1593) of the animals were adults. There were more female animals sampled,

200 except for cattle, where 57.56% (472/820) were males (Table 1). Cattle, goats, and sheep were

201 predominantly local breeds, while 65.70% (500/761) of the pigs were crossbred. The origin of

202 3.94% (n = 80) of the animals sampled could not be determined due to lack of access to

203 accompanying documentation.

204 With a total of 877 trap nights, 117 small mammals were captured from the three regions,

205 yielding an overall trap success rate of 13.34%. Most of the captures were from the Eastern

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206 (40.17%, n = 47) and Northern regions (37.61%, n = 44). Despite doubling the trapping effort in
207 the Central region, only 26 small mammals were captured (4.81% success with 457 trap nights).
208 There were more male (70.09%, n = 82) and adult (92.31%, n = 108) small mammals captured.
209 The house rat (*Rattus rattus*) was the most common (65.81%, n = 77). The African pygmy mouse
210 (*Mus minutoides;* 18.80%, n = 22), the house mouse (*Mus musculus;* 4.27%, n = 5), the African
211 grass rat (*Arvicanthis niloticus*; 2.56%, n = 3) and the African giant shrew (*Crocidura olivieri;*
212 8.55%, n = 10) were also captured.

213 *Prevalence of Leptospira infection in livestock and small mammals based on the lipL32 PCR*

214 *Leptospira* infection was detected in 45 of 2030 livestock samples by PCR. Most of the infected
215 livestock were adult (91.1%, 41/45), or from the Northern region (57.8%, 26/45) (Table 2). The
216 estimated true prevalence of infection was highest in sheep (6.12%; 95% CI = 2.69–12.89),
217 followed by cattle (4.25%; 95% CI = 2.91–5.98), goats (2.08%; CI = 0.91–4.38) and pigs (0.46%; CI
218 = 0.12–1.31). Further statistical analysis of the association between *Leptospira* infection and
219 age, sex or region of origin was not performed due to the low number of positives observed
220 (Table 2). None of the 117 small mammals were infected (0%; CI = 0.00–3.55). Culturing yielded
221 four presumptive *Leptospira* isolates from two cattle, one goat and one house rat. However,
222 the *lipL*32 PCR analysis of DNA from these isolates was negative, implying that they may have
223 been nonpathogenic *Leptospira* species, and thus were not followed further.

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226 Table 1: Population characteristics of the livestock (n= 2030) sampled during a cross-sectional
227 study in slaughter facilities in Uganda

Category	**Levels**	**number of sampled animals (%)**				
		Cattle	**Goats**	**Sheep**	**Pigs**	**Total**
Sex	Female	348 (42.44)	178 (53.13)	63 (55.26)	444 (58.34)	1033 (50.89)
	Male	472 (57.56)	157 (46.87)	51 (44.74)	317 (41.66)	997 (49.11)
Age	Adult*	737 (89.88)	289 (86.27)	102 (89.47)	465 (61.10)	1593 (78.47)
	Juvenile	83 (10.12)	46 (13.73)	12 (10.53)	296 (38.90)	437 (21.53)
Breed	Cross	130 (15.85)	61 (18.21)	2 (1.75)	500 (65.70)	693 (34.14)
	Exotic	0 (0.00)	0 (0.00)	0 (0.00)	2 (0.26)	2 (0.01)
	Local	690 (84.15)	274 (81.79)	112 (98.25)	259 (34.03)	1335 (65.76)
Region of origin	Central	153 (18.66)	28 (8.35)	7 (6.14)	421 (55.32)	609 (30.00)
	Eastern	107 (13.05)	39 (11.64)	5 (4.38)	119 (15.64)	270 (13.30)
	Northern	454 (55.37)	191 (57.01)	70 (61.40)	210 (27.59)	925 (45.57)
	Western	43 (5.24)	65 (19.40)	25 (21.93)	1 (0.13)	134 (6.60)
	Across Tanzania border	12 (1.46)	0 (0.00)	0 (0.00)	0 (0.00)	12 (0.59)
	Undetermined	51 (6.22)	12 (3.58)	7 (6.14)	10 (1.31)	80 (3.94)
Total		820 (100)	335 (100)	114 (100)	761 (100)	2030 (100)

228 **Adult cattle were defined as ≥1.5 years, a goat as one ≥7 months and a pig ≥6 months*

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230 Table 2: Proportion of *Leptospira*-infected livestock by species, sex, breed, age, and region of
231 origin

Category	Levels	Number of positive samples (%)				
		Cattle	Goats	Sheep	Pigs	Total
Sex	Female	5 (1.44)	6 (3.37)	6 (9.52)	3 (0.66)	20 (1.94)
	Male	25 (5.30)	0 (0.00)	0 (0.00)	0 (0.00)	25 (2.51)
Age	Adult*	27 (3.66)	6 (2.08)	6 (5.88)	2 (0.43)	41 (2.57)
	Juvenile	3 (3.61)	0 (0.00)	0 (10.53)	1 (0.33)	4 (0.92)
Breed	Cross	4 (3.08)	0 (0.00)	0 (0.00)	2 (0.4)	6 (0.87)
	Exotic	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
	Local	26 (3.77)	6 (2.19)	6 (5.36)	1 (0.39)	39 (2.92)
Region of origin	Central	10 (6.54)	0 (0.00)	0 (0.00)	1 (0.24)	11 (1.81)
	Eastern	4 (3.74)	0 (0.00)	0 (0.00)	0 (0.00)	4 (1.48)
	Northern	14 (3.08)	5 (2.11)	5 (7.14)	2 (0.95)	26 (2.81)
	Western	1 (2.34)	1 (1.53)	0 (0.00)	0 (0.13)	2 (1.49)
	Across Tanzania border	1 (8.33)	0 (0.00)	0 (0.00)	0 (0.00)	1 (1.46)
	Undetermined	0 (0.00)	0 (0.00)	1 (14.29)	0 (0.00)	1 (1.25)
Total		30 (3.66)	6 (1.79)	6 (5.26)	3 (0.39)	45 (2.22)

232 **Adult cattle were defined as ≥1.5 years, a goat as one ≥7 months and a pig ≥6 months*

233 *Leptospira species and sequence types*

234 Of the 45 *Leptospira lipL32*-positive samples, 31 had a Ct ≤36 cycles. *SecY* typing was successful
235 in 30 (96.77%) of the samples. *L. borgpetersenii* was the most prevalent *Leptospira* specie (n =
236 16) and was mostly found in cattle (n=13), with goats, pigs, and sheep each having a positive
237 sample. *L. kirschneri* was detected in 5 cattle, 3 goats, and 4 sheep, and *L. interrogans* was
238 detected in 2 pig samples. MLST revealed 16 different sequence types (STs) in 29 of the 30 secY-
239 positive samples, with ST152 and ST360 being the most prevalent and being detected in five
240 animals each. ST 380 was detected in three animals, and ST 369 and ST 24 in two animals each.
241 ST 62, ST 357, ST 359, ST 364, ST 365, ST 368, ST 371, ST 374, ST 377, ST 379, and ST 381 were
242 found in one animal each. Several single-nucleotide polymorphisms were observed in the genes

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243 sequenced via MLST, leading to the identification of new alleles for these housekeeping genes

244 and, consequently, 13 novel STs that were registered in the PubMLST database (Figure 2).

245 These comprise ST 357, ST 359, ST 360, ST 364, ST 365, ST 368, ST 369, ST 371, ST 374, ST 377,

246 ST 379, ST 380, and ST 381.

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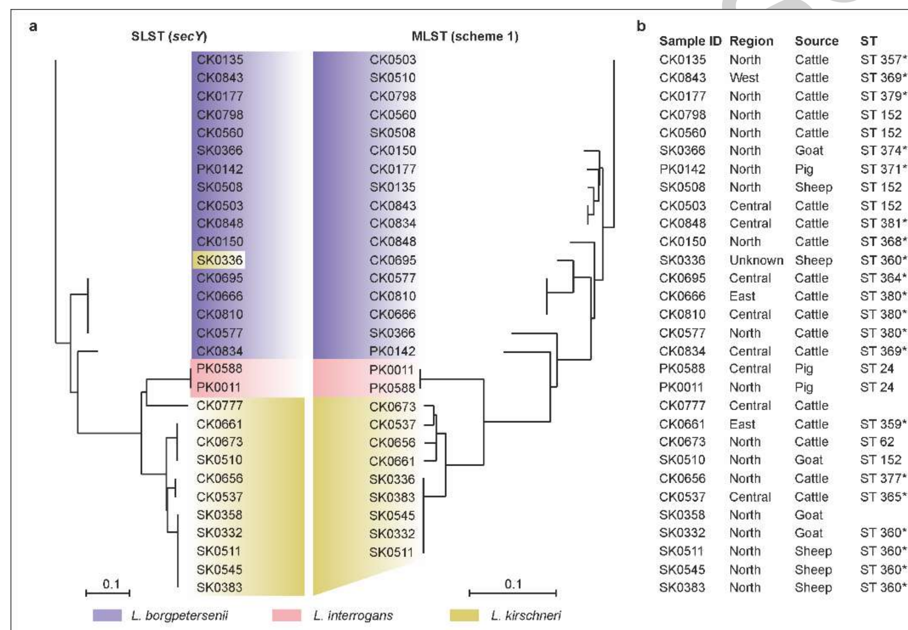

Sample ID	Region	Source	ST
CK0135	North	Cattle	ST 357*
CK0843	West	Cattle	ST 369*
CK0177	North	Cattle	ST 379*
CK0798	North	Cattle	ST 152
CK0560	North	Cattle	ST 152
SK0366	North	Goat	ST 374*
PK0142	North	Pig	ST 371*
SK0508	North	Sheep	ST 152
CK0503	Central	Cattle	ST 152
CK0848	Central	Cattle	ST 381*
CK0150	North	Cattle	ST 368*
SK0336	Unknown	Sheep	ST 360*
CK0695	Central	Cattle	ST 364*
CK0666	East	Cattle	ST 380*
CK0810	Central	Cattle	ST 380*
CK0577	North	Cattle	ST 380*
CK0834	Central	Cattle	ST 369*
PK0588	Central	Pig	ST 24
PK0011	North	Pig	ST 24
CK0777	Central	Cattle	
CK0661	East	Cattle	ST 359*
CK0673	North	Cattle	ST 62
SK0510	North	Goat	ST 152
CK0656	North	Cattle	ST 377*
CK0537	Central	Cattle	ST 365*
SK0358	North	Goat	
SK0332	North	Goat	ST 360*
SK0511	North	Sheep	ST 360*
SK0545	North	Sheep	ST 360*
SK0383	North	Sheep	ST 360*

250 Figure 2. The phylogenetic relationship of leptospires detected in various slaughter animals by

251 (**a**) single locus and multilocus sequence typing, with the region, source of samples, and the

252 sequence types (ST) identified (**b**). MLST alignment utilized concatenated sequences of the

253 seven scheme 1 genes, and novel sequence types are denoted by an asterisk (*). The sequence

254 type could not be identified for SK0358 and CK0777 due to failure in the amplification of the

255 *caiB* and *tpiA* genes, respectively.

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256 **Discussion**

257 We detected infection with pathogenic *Leptospira* of the species *L. borgpetersenii*, *L. kirschneri*
258 and *L. interrogans* among apparently healthy cattle, goats, sheep, and pigs, suggesting their
259 role as *Leptospira* carriers in Uganda. This finding has important implications for public health
260 and animal health, as it highlights the potential risk of transmission through contact with these
261 animals. *Leptospira* infection in livestock results in reproduction and production losses, such as
262 milk yield reduction, stunting, abortions, and deaths. This could have far-reaching economic
263 effects since cattle, goats, sheep, and pigs are the most common livestock kept in Uganda, and
264 are a source of livelihood for up to 70% of households [24]. Infected livestock may also carry
265 and shed leptospires in urine for weeks to years, consequently contaminating soil and water
266 sources, and posing risk of infection for humans [4].

267 From a systematic review of leptospirosis in Africa, livestock particularly cattle appear to be
268 important hosts of several *Leptospira* serogroups, though few data are available to allow
269 comparison of *Leptospira* infection in linked human and animal populations [25]. In East Africa,
270 *Leptospira* exposures have been reported among febrile patients, slaughterhouse workers, and
271 sugarcane plantation workers [26–28]. In Uganda, human *Leptospira* exposures have earlier
272 been speculated to result from animal contact [5–7]. Findings from the current study indirectly
273 build onto this speculation, especially that *Leptospira* sequence types identified in the current
274 study belong to *L. borgpetersenii*, *L. kirschneri* and *L. interrogans,* the same *Leptospira* species
275 previously reported in febrile patients in Uganda [6], and elsewhere in East Africa [6,26,29,30].

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276 The MLST results revealed the circulation of the same *Leptospira* sequence types within
277 livestock species from different regions of Uganda, implying widespread *Leptospira* infection.
278 This could be explained by animal movements and trade across regions within Uganda [31] and
279 the neighboring countries [32]. Twelve cattle sampled in our study were reportedly sourced
280 from across the Tanzanian border, and one was *Leptospira* positive but did not qualify for
281 sequencing (had a Ct of 38). *Leptospira* sequence type (ST) 152, one of the most detected STs in
282 our study, was also detected in isolates from cattle in Tanzania [17]. Furthermore, the sharing
283 of ST 152 between goats and cattle in the current study may imply interspecies transmission or
284 a common source of infection, since cattle and goats are usually kept together in Uganda[33].
285 The identification of several other new STs within *L. borgpetersenii and L. kirschneri* in the
286 current study may mean that the *Leptospira* strains circulating in Uganda are both novel and
287 genetically diverse. While we also intended to characterize the local strains further by next-
288 generation sequencing, we failed at isolating pathogenic leptospires in the present study.
289 Future studies should consider isolation from clinical cases; target multiple sample types,
290 including urine, blood, or kidney tissue; and employ a prescreening test, such as PCR.

291 Comparable levels of *Leptospira* infection as found in livestock in the current study, have been
292 reported elsewhere in East Africa. For example, in a cross-sectional study of livestock sampled
293 from slaughterhouses in Tanzania [17], pathogenic *Leptospira* infection was detected in 7.1%
294 cattle (n = 452), 1.2% goats (n = 167) and 1.1% sheep (n = 89). Earlier studies in Uganda
295 revealed *Leptospira* prevalence of 8.8% (n=500) in slaughter cattle [13], and 10.5% (n= 649) in
296 slaughter pigs [14], compared to 4.3%; and 0.5% respectively reported in the current study. This
297 could be because the other studies employed a more comprehensive sampling approach, which

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298 included kidneys, urine, and reproductive tissue, despite being based in slaughter facilities from
299 only one region of Uganda and studying one livestock species each. In the current study,
300 *Leptospira* prevalence in pigs was still comparably lower than in the other livestock species
301 possibly due to the limited exposure risk associated with the semi-intensive systems under
302 which most pigs in Uganda are kept. Further statistical analysis of the association between
303 *Leptospira* infection and region of origin or age and sex was not performed due to the low
304 number of positive samples detected.

305 The absence of PCR-positive results in the 117 rodents or shrews captured near slaughter
306 facilities in Uganda suggests that small mammals have a limited role in the community spread
307 of *Leptospira*. This could also indicate that slaughter facilities in Uganda do not significantly
308 contribute to *Leptospira* concentration. However, these conclusions may be undermined by the
309 fact that slaughtered livestock originate from various locations and spend minimal time at these
310 facilities. The predominance of the *Rattus rattus* species, known for staying close to human
311 settlements with minimal habitat sharing with other rodents, may also have influenced the
312 findings. The prevalence of *Leptospira* infection among *Rattus rattus* species is generally low
313 even in environments where a high *Leptospira* prevalence is reported [34, 35].

314 Despite reports of *Leptospira* infection in rodents in some parts of Africa [36, 37], their role as
315 *Leptospira* reservoirs in East Africa seems limited. A two-year cross-sectional survey conducted
316 at 12 randomly selected sites in Tanzania revealed no *Leptospira* infection in any of the 384
317 rodents captured [17]. The first author of the present study has earlier participated in two
318 independent captures of small mammals conducted in a rural agricultural environment and at a
319 wildlife-human interface in Uganda and found *Leptospira* infection in 2.6% (n = 234), and 3.5%

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320 (n = 198) respectively (unpublished). Despite this, small mammals or wildlife reservoirs may still
321 contaminate environmental sources such as water, and soil in grazing fields from which
322 domesticated animals are indirectly infected. Given their close interaction with humans and
323 larger urine volumes, livestock are likely the more important carriers and sources of human
324 *Leptospira* infection in Uganda, compared to small mammals.

325 Our study documents the livestock reservoirs of pathogenic leptospires in Uganda and the
326 circulating *Leptospira* species and sequence types among these reservoirs, with the long-term
327 goal of informing prevention and control measures for leptospirosis in Uganda. The *Leptospira*
328 sequence types identified in the present study, including the novel ones, contribute to the
329 MLST database for East Africa and offer a basis for further research to isolate and identify the
330 serogroups and serovars to which these novel sequence types could belong. Our findings also
331 build onto the existing hypothesis that domesticated animals could be a source of human
332 *Leptospira* infection in Uganda, emphasizing the importance of raising awareness among
333 individuals in regular contact with livestock, such as farmers, slaughterhouse workers, and
334 veterinarians.

335 **Data availability**

336 All necessary data have been presented in the manuscript, and further specific requests can be
337 through the corresponding authors.

338 **Author Contributions**

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339 Conceptualization: LA, SD, CK, JJH, AMS, KR, MHR and EAJC; Funding acquisition: MHR, AMS,
340 EAJC and KR; Investigation: LA, JB, VK, CK, CJA, MW, PB, JAH and MHR; Methodology: LA, SD,
341 CK, AL, CJA, AMS, EAJC, MW, JAH, PB and KR; Formal analysis: LA, MW and SD; Supervision: SD,
342 CK, AMS, EAJC and KR; Visualization: LA, MW, JAH and SD; Writing original draft: LA, CK and SD;
343 Review and editing: All authors.

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349 **Competing interest**

350 The authors declare none.

351

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