Short Report: Seroprevalence of *Leptospira* Hardjo in Cattle and African Buffalos in Southwestern Uganda

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Abstract. Leptospirosis, caused by the spirochete bacterium *Leptospira* spp. is a zoonosis, distributed worldwide and classified as an emerging infectious disease. Fatal outcomes to leptospiral infection do occur and the disease can cause abortion and other reproductive problems in cattle, goats, and pigs. In humans the symptoms range from subclinical infection to acute febrile illness, pulmonary hemorrhage and renal failure. Leptospirosis has never been officially reported to the World Health Organization (WHO) or the World Animal Health Organization in animals or humans in Uganda. However, favorable ecological conditions and suitable animal hosts can be found within the country. A commercially available enzyme-linked immunosorbent (ELISA) kit was used to screen sera samples from domesticated cattle and African buffalo (*Syncerus caffer*) at two locations in southwestern Uganda, collected over a 4-year period. Positive samples were found in both cattle and African buffalo samples, from both locations and across the sampling period. Overall seroprevalence was 42.39% in African buffalo and 29.35% in cattle.

Leptospirosis, a zoonotic bacterial infection caused by the spirochete *Leptospira* spp., is distributed worldwide.¹ Leptospire have been found in most mammalian species examined, including marine animals, and on every continent except Antarctica. Leptospirosis, in addition to being the most widespread bacterial zoonosis globally,² is also classified as an emerging infectious disease.³ Leptospirosis is also referred to as Weil's disease, Weil's syndrome, mud fever, or swamp fever.⁴ Often associated with flooding and high seasonal rainfall,² leptospirosis occurs in rural and urban areas in developed and less developed countries, with exposure among the general population a result of daily living activities.⁵

Annually, there are 1 million recorded cases of severe leptospirosis worldwide.⁶ The World Health Organization (WHO) currently estimates an incidence of 10–100 cases per 100,000 people annually in the humid tropics, with case fatality rates ranging from < 5% to 30%.⁷ Human leptospiral infection is usually obtained either indirectly from an animal source through exposure to soil or water that has been contaminated with the urine of infected rats, rodents, domestic pets and livestock,⁸ or directly from contact with the urine and other bodily fluids of infected animals, typically as a result of occupational activities. Human-to-human transmission is rare⁴; despite its widespread prevalence, leptospirosis is rarely considered in the differential diagnosis of febrile illness, particularly in developing countries.⁹

All mammals are capable of being infected with at least one serovar of *Leptospira* spp., with clinical signs just as variable in animals as in humans. Animals are either reservoir hosts, chronically maintaining leptospire in their renal tubes with little or no detectable clinical damage, or accidental hosts.³ Epidemiological information to date identifies rodents as the primary reservoir hosts for most *Leptospira* serovars,⁴ responsible for transferring infection to accidental hosts such as domestic livestock, dogs, and humans.³

Although Uganda has never filed reports of leptospirosis to the World Animal Health Organization or the WHO, studies from surrounding countries have confirmed the presence of the disease in Kenya,¹⁰ Tanzania,¹¹ Sudan,¹² and Democratic Republic of Congo.¹³ Additionally, climatic conditions and the presence of suitable reservoir hosts favor both leptospiral infection and leptospiral survival in the environment.

Sera samples used in this study were originally collected as part of on-going annual disease investigations (tuberculosis, brucellosis, foot-and-mouth disease, Rift Valley Fever, and Rinderpest) in Queen Elizabeth National Park and Bwindi Impenetrable National Park in southwest Uganda by veterinary staff of Conservation through Public Health, from 2007 to 2011. Serum from 92 African buffalo (*Syncerus caffer*) and 92 cattle was selected at random from the collection held by the National Animal Disease Diagnostics and Epidemiology Center (NADDEC) and approved for use in this study by Conservation through Public Health, Uganda Wildlife Authority and the Ministry of Agriculture, Animal Industries and Fisheries.

The commercially available Linnodee Lepto kit (Linnodee Animal Care, Balleycare, UK), a double antibody sandwich enzyme-linked immunosorbent assay (ELISA) kit, has been successfully used to screen cattle sera samples for *L. hardjo* immunoglobulin M (IgM) and IgG antibodies within Africa.¹⁴ Personal communication with Linnodee Animal Care technical staff confirmed the kit had previously been used to screen African buffalo sera samples as well. The test results were expressed as a ratio of the test sample and a mean positive control sera. A sample was recorded as positive if the ratio was > 0.12, indicating exposure to serovar Hardjo.

Of the 184 serum samples screened (see Table 1) with the Linnodee Lepto kit: 66 were positive with an optical density ratio of > 0.12 (35.9%: 95% confidence interval [CI] 29.3–43.0%) and 103 were negative with an optical density ratio of ≤ 0.05 (56.0%: 95% CI 48.8–63.0%). The remainder of the samples was taken to be inconclusive as they fell within the range of > 0.05 ≤ 0.12 (8.3%: 95% CI 5.0–13.0%). Positive samples were found in each of the years that samples were collected (see Table 2).

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TABLE 1
Sera sample details

Species	Location	Year	No. of samples
Cattle	QENP	2008	29
		2011	32
Buffalo	BINP	2011	31
		2007	36
	QENP	2008	22
		2011	34

The seroprevalence results were recalculated to determine the true seroprevalence,¹⁵ adjusting for imperfections in this diagnostic kit based on the stated sensitivity and specificity of the diagnostic test, 94.1% and 94.8%, respectively. With adjustment for true prevalence, overall seroprevalence was 35.1% for all sera samples; the detection in African buffalo was higher than cattle, at 41.8% and 27.2%, respectively (see Table 3). However, despite this difference there was no association between species and seroprevalence at $P = 0.05$. This lack of association between exposure and species may be caused by the low number of sera samples analyzed. The small sample size may also reflect the absence of association between seroprevalence and location as indicated by the χ^2 value of 0.8680 ($P = .05$).

This is the first study to show exposure to serovar Hardjo in domestic livestock, and wildlife in Uganda; indicating the distribution of this bacterium across two animal species from 2007, when the first of these samples were collected. Previously using an agglutination test, Ball¹⁶ confirmed exposure to leptospires in cattle and hippopotamus within Uganda. Recently, Millán and others¹⁷ confirmed exposure to *Leptospira interrogans* using microscopic agglutination in dogs from the same region of Uganda as investigated herein, namely the Queen Elizabeth National Park and Bwindi National Park. The high seroprevalence in cattle is consistent with other published work. For example within-herd leptospiral seroprevalence in cattle was 35% in Cameroon.¹⁴

The findings in this pilot investigation suggest that this zoonotic bacterial infection may be more widespread than previously recognized, with the potential that at least two popular tourism areas are endemic for *L. hardjo*. The initial clinical symptom of leptospirosis, fever, is a common complaint that drives patients to seek health care in Uganda. Current national guidelines state that patients presenting with acute fever, absent of any other signs of clinical disease, be presumptively treated for malaria,¹⁸ despite evidence of pervasive malaria over-diagnosis.¹⁹ Perhaps from these affected regions leptospirosis should now be included within the differential diagnosis and a wider assessment of the risk to human health should now be considered. In addition, although the International Association for Medical Assistance to Travelers, does highlight tourists

TABLE 2
Seroprevalence results by location and year

	No. of samples	Seroprevalence (%)	True prevalence (%)	95% Confidence interval for true prevalence (%)
QENP	153	34.64	33.12	32.43–33.80
BINP	31	41.94	41.32	37.81–44.83
2007	36	47.22	47.27	44.21–50.33
2008	51	23.53	20.62	18.78–22.45
2011	97	38.14	37.06	35.95–38.16

TABLE 3
Overall seroprevalence results

	Number of samples	Number of positive samples	Antibody prevalence (%)	True prevalence (%)	95% Confidence interval for true prevalence (%)
Overall	184	66	35.87	34.50	33.92–35.07
African Buffalo	92	39	42.39	41.83	40.65–43.02
Cattle	92	27	29.35	27.16	26.07–28.25

to Uganda as “at risk” from leptospirosis, the wider dissemination of this message may now be timely.

Furthermore, in Uganda many farmers rely on cattle and their products for their livelihoods and culturally a high priority is placed on cattle production. The impact of serovar Hardjo infection may be even more severe on cattle with co-infection, poor husbandry and feeding practices, and inadequate water and trace mineral intake, conditions all too common in cattle production in Uganda. The economic impact of leptospirosis on livestock production needs to be assessed to determine its priority in comparison to more recognized livestock diseases in Uganda. Leptospirosis may be seen as a “silent” condition, as yet unrecognized, whose presence will increasingly impact both sustainable food production and farmers’ livelihoods in endemic areas; additional research to determine the impact of leptospirosis on livestock production, wildlife conservation, and public health is warranted.

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Disclaimer: Leptospirosis has never been reported to the World Health Organization or the World Animal Health Organization from Uganda. However, favorable environmental and ecological conditions, such as frequent human-livestock and livestock-wildlife contact and high populations of rodents and feral dogs in urban areas, create favorable dynamics for *Leptospira* spp. transmission. This pilot study assessed whether domestic livestock and wildlife had been exposed to *Leptospira* spp. in Uganda and the seroprevalence of *Leptospira hardjo* in domestic cattle and African buffalo. The content of this article has not and will not be submitted for publication elsewhere as long as it is under consideration by the *American Journal of Tropical Medicine and Hygiene*. We have no conflicts of interest. All the authors have participated in the study, are in agreement with the submission of this article and subsequent revisions submitted by the corresponding author.

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