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Leptospira seropositivity as a risk factor for Mesoamerican Nephropathy

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ABSTRACT

Background: Leptospirosis is postulated as a possible cause of Mesoamerican Nephropathy (MeN) in Central American workers.

Objectives: Investigate job-specific *Leptospira* seroprevalence and its association with kidney disease biomarkers.

Methods: In 282 sugarcane workers, 47 sugarcane applicants and 160 workers in other industries, we measured anti-leptospiral antibodies, serum creatinine, and urinary injury biomarkers, including neutrophil gelatinase-associated lipocalin (NGAL), interleukin-18 (IL-18), and N-acetyl-D-glucosaminidase (NAG).

Results: *Leptospira* seroprevalence differed among job categories and was highest among sugarcane cutters (59%). Seropositive sugarcane workers had higher NGAL concentrations (relative mean: 1.28; 95% CI: 0.94–1.75) compared to those who were seronegative, with similar findings among field and non-field workers.

Conclusions: *Leptospira* seroprevalence varied by job category. There was some indication that seropositivity was associated with elevated biomarker levels, but results were inconsistent. Additional studies may help establish whether *Leptospira* infection plays any role in MeN among Central American workers.

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Introduction

In several regions of Central America, particularly the Pacific coast lowlands, there is an epidemic of chronic kidney disease (CKD) termed Mesoamerican Nephropathy (MeN) that mainly affects young male agricultural workers [1–7]. The etiology of the disease is unknown and it is not associated with common causes of CKD in the developed world such as diabetes, hypertension and obesity [3,5,7–15]. Although most attention has been focused on the potential relationship to heat stress and recurrent dehydration because of its occurrence in workers performing strenuous manual labor under hot environmental conditions, infectious diseases, particularly leptospirosis, were identified as one possible etiology requiring further study at the First International Workshop on MeN in 2012 [1,5,6,9,16,17].

Leptospirosis is a zoonotic disease caused by pathogenic spirochetes of the *Leptospira spp.* genus that is known to cause acute kidney injury (AKI); notably, both the geography and demographic characteristics of the population at risk of leptospirosis are similar to those of MeN [16,18–26]. Human infection usually occurs after contact with water or soil contaminated by the urine of animal reservoirs [20,21]. Leptospirosis has a wide spectrum of clinical manifestations [20,21,27–38] and, although known to cause AKI, there are only a few studies of its association with CKD [29,31,39–48]. The studies that have evaluated kidney recovery after leptospirosis-induced AKI show that normalization of serum creatinine occurs in the vast majority of patients, albeit with tubular dysfunction that can persist for several months [31,40]. Given the growing body of evidence showing an increased risk of developing CKD after an episode of AKI despite early normalization of serum creatinine [49,50] and a recent study showing an association between chronic human exposure to leptospires and CKD [51], investigations are warranted to assess whether clinically recognized leptospirosis is associated with CKD, as well as whether asymptomatic or mild

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leptospirosis, which is more likely to go unrecognized and is more frequent than the severe cases associated with overt AKI, can cause subclinical kidney injury that subsequently predisposes to CKD.

To explore these questions, we evaluated the prevalence of *Leptospira* seropositivity among workers employed in a region where MeN is common; estimated incident cases of leptospirosis among sugarcane workers within one harvest season; and determined whether *Leptospira* seropositivity was associated with biomarkers of kidney function and injury.

Methods

Study population

Data for this study was collected as part of a prospective study that evaluated biomarkers of kidney function and injury among a population of sugarcane workers in Nicaragua [14,52], including applicants for jobs as field workers who were found to have an elevated serum creatinine during employment screening, as well as a population of miners, construction workers, and port workers who had never worked in the sugarcane industry (Figure 1). All individuals were required to be at least 18 years of age to be eligible for participation. Study protocols were approved by the Institutional Review Boards at the Boston University Medical Center and the Nicaraguan Ministry of Health. All study participants provided written informed consent prior to enrollment in research activities.

Sugarcane workers (n = 282) worked during the harvest at one company in northwestern Nicaragua in one of the following job categories: seed cutter, seeder, agrichemical applicator, irrigator, cane cutter, driver, or factory worker. Participants were enrolled at the pre-harvest screening during a mandatory medical exam of all potential employees (October to December 2010); during this time, questionnaires were administered and biospecimens collected. Workers were re-sampled approximately 4 to 6 months later, near the end of the harvest season (March to May 2011). The final study population was selected from the workers who had questionnaires and biospecimens collected at both time points (refer to Laws et al. 2015 for a detailed description of each job category and sampling methodology) [14].

Sugarcane applicants (n = 47) included individuals who had an elevated serum creatinine (≥1.4 mg/dl) at the pre-harvest mandatory testing conducted by the company. This creatinine threshold was used by the company to exclude individuals from being hired. At the time of enrollment, 59 individuals were identified as having an elevated serum creatinine, of these, we included all individuals with a creatinine of 1.6 mg/dl or above, and a random sample of those with creatinine levels of 1.4–1.5 mg/dl. Questionnaire and biospecimen

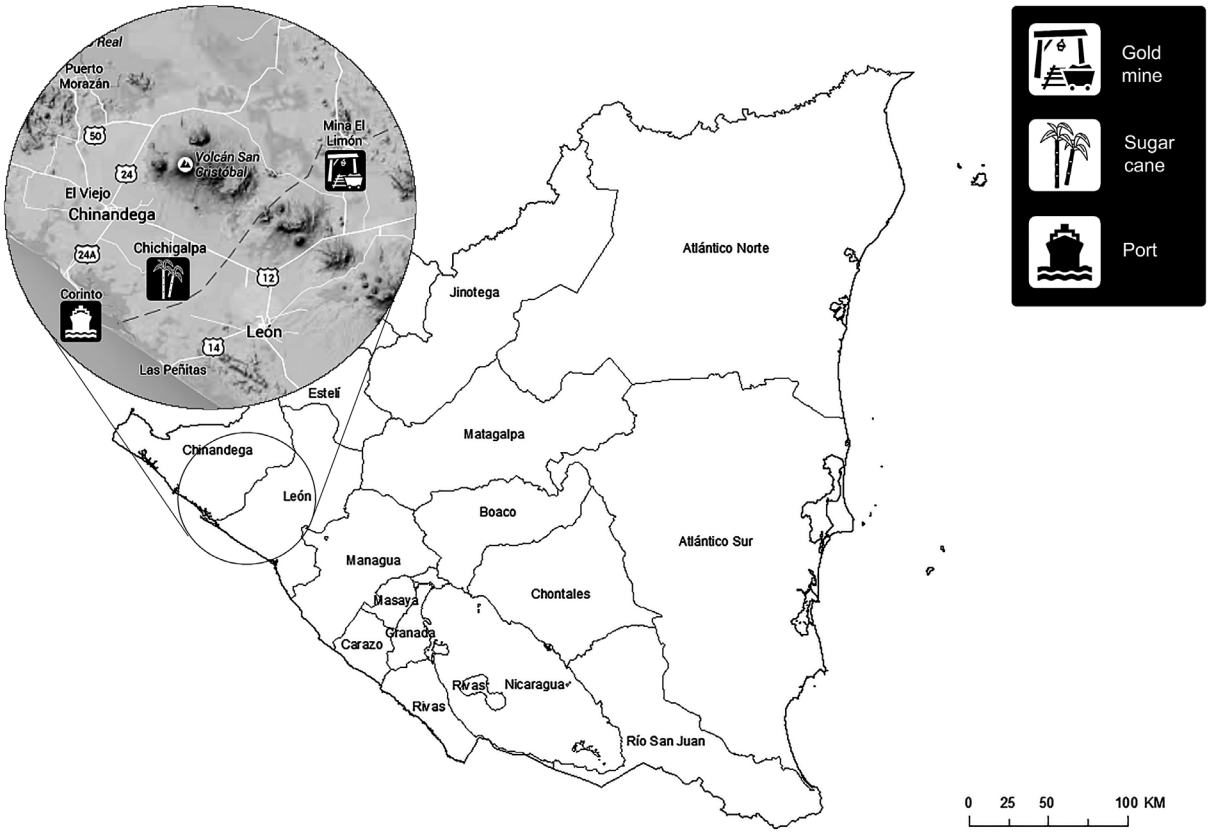

Figure 1. Location of industries in Nicaragua from which study population was recruited.
Notes: Area enlarged shows locations of sugarcane company, gold mine, and port from which study population of workers was recruited. Workers in the construction sector include workers from the entire enlarged area, involved in building of traditional houses and commercial buildings.

collection for these individuals occurred at the time of the pre-harvest screening (October to December 2010).

Workers in other industries ($n = 160$) included miners, construction workers and port workers from northwestern Nicaragua who had never worked in the sugarcane industry. These workers were recruited because they were thought to have an increased prevalence of CKD based on a prior study [13] or anecdotal reports. Miners were recruited from those who worked in the largest gold mine in the region. Construction workers were recruited from laborers actively involved in various aspects of building traditional houses or commercial buildings. Port workers were recruited from those who were affiliated with unions and performed heavy manual labor in the port of Corinto, Nicaragua. Questionnaire and biospecimen collection was completed at their respective work locations at approximately the same time as the late-harvest sampling in sugarcane workers (March to May 2011).

For all workers, biospecimens consisted of blood and urine samples. Additionally, questionnaires were administered that asked about demographics, occupational history, and work practices. Sugarcane workers were sampled twice, before and near the end of the harvest, while the other two categories were sampled once.

Laboratory testing

All blood and urine samples were collected by trained personnel. On the day of collection, samples were processed at the local health center and stored at −20 °C. Within one week, they were transported to the ISO-certified Centro Nacional de Diagnóstico y Referencia (CNDR) in Managua, a division within the Ministry of Health, and stored at −80 °C.

Leptospira serology

Serum and urine samples were shipped to the Bacterial Special Pathogens Branch at the Centers for Disease Control and Prevention (CDC) (Atlanta, Georgia, USA). Serum samples were tested for the presence of anti-leptospiral antibodies using a microscopic agglutination test (MAT) following standard procedures. The CDC's MAT panel was used, which includes 20 reference strains belonging to 17 serogroups [53]. The antigens were 5–7 day-old cultures grown in liquid Elinghausen Mc-Cullough Johnson Harris (EMJH) medium adjusted to the concentration of a 0.5 McFarland standard. MAT was done at doubling dilutions starting from 1:100. Positive samples were titrated up to end titers. The endpoint titer was reported as the highest dilution that agglutinated at least 50% of the cells for each strain tested.

Sugarcane workers were considered seronegative if specimens from both the pre- and late-harvest had titers of <100. Seroconversion or a fourfold rise in titers was considered evidence of recent or current leptospirosis. All titers ≥100 that did not represent either seroconversion or a fourfold rise in titers between paired samples, were considered evidence of past *Leptospira* infection. Some individuals had a negative titer at pre-harvest and a late-harvest titer of 100 or 200. It was unclear whether these represented true seroconversions given the low titers. Since there was a period of several months between samples collected at the pre- and late-harvest, peak titers could in theory be missed. To avoid misclassifying individuals with recent or current infection as past infection with *Leptospira*, anti-leptospiral IgM antibodies in serum were determined on all individuals that had low titer seroconversion or less than a fourfold rise in titers on the MAT using a dipstick ELISA kit (ImmunoDOT, GenBio, San Diego, CA). An ELISA IgM result of 2.0–2.5 dots was considered borderline positive; while 3.0–4.0 dots was considered positive. Since IgM antibodies can remain detectable for prolonged periods of time, only those individuals that had a negative IgM ELISA on the first sample (pre-harvest) and a positive IgM ELISA on the second sample (late-harvest) were considered to have recent or current infection; all others were categorized as past *Leptospira* infection.

Only a single serum sample was collected from sugarcane applicants and workers in other industries. These individuals were considered seronegative if the MAT titer was <100; a titer of ≥800 was considered evidence of recent or current leptospirosis [54]; all other titers were considered evidence of past *Leptospira* infection.

Leptospira urine PCR

Urine was also tested by polymerase chain reaction (PCR) to detect leptospiral DNA. For sugarcane workers, urine from the late-harvest sample was used. Frozen urine was thawed and centrifuged at 3000 ×*g* for 15 min at room temperature. Supernatants were decanted and 10 mL of PBS was added to wash the pelleted urine and centrifuged again. Washing was performed twice; upon decanting the final wash supernatant, pellets were resuspended in 500 µL PBS. DNA from the pellets was extracted with the QIAamp DNA Mini Kit using an automated QIAcube extraction instrument (Qiagen, Valencia, CA) following manufacturer's directions. All urine samples were screened for the presence of *Leptospira* using a published TaqMan PCR assay targeting the gene for the leptospiral outer membrane protein LipL32 [55]; this was performed using an ABI 7500 Real Time System instrument (Applied Biosystems, Inc., San Francisco, CA).

Kidney injury biomarkers

Serum samples were analyzed for creatinine using the kinetic-rate Jaffe method at the Centro Nacional de Diagnóstico y Referencia (CNDR) (Managua, Nicaragua). Based on manufacturer specifications,

0.2 mg/dL was subtracted from serum creatinine results to calibrate to an isotope mass dilution spectroscopy standard prior to use of the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation (with race considered "non-black") to estimate glomerular filtration rate (eGFR) [56].

Urine samples were shipped to the Division of Nephrology and Hypertension at the Cincinnati Children's Hospital Medical Center (Cincinnati, Ohio, USA) for analysis of creatinine, albumin, interleukin-18 (IL-18), neutrophil gelatinase-associated lipocalin (NGAL) and N-acetyl-D-glucosaminidase (NAG). IL-18, NGAL, and NAG are considered biomarkers of kidney tubular injury, and may provide earlier detection of kidney injury and better prognostication in CKD than can be achieved by measuring serum creatinine alone [57–69]. Urine creatinine and albumin were measured by immunoturbidimetry and a colorimetric modification of the Jaffe reaction, respectively. All reported values of urine creatinine (g/L) were above the limit of detection (LOD). The LOD for urine albumin was 1.3 mg/L. NGAL (Bioporto, Gentofte, Denmark) and IL-18 (MBL, Intl., Woburn, MA) were measured by ELISA. All reported values of NGAL (ng/ml) were above the LOD. The LOD for IL-18 was 4 pg/ml. NAG activity was measured using a colorimetric assay (Roche Diagnostics, USA) with a LOD of 0.003 U/L.

Data analysis

The distribution of each biomarker of kidney injury was examined using histograms, graphical displays, and summary statistics. For values below the LOD, the LOD/$\sqrt{2}$ was substituted. To account for urine dilution, urine biomarkers were normalized to urine creatinine (g/L) and expressed as follows: albumin to creatinine ratio (ACR) (mg/g creatinine), NGAL (µg/g creatinine), IL-18 (ng/g creatinine), and NAG (U/g creatinine).

The first set of analyses used logistic regression to assess job category as a predictor of *Leptospira* seropositivity. The second set of analyses used linear regression to assess *Leptospira* seropositivity as a predictor of eGFR, NGAL, IL-18, and NAG. In sugarcane workers, this association was examined at both pre-harvest and late-harvest. There were too few cases classified as recent or current infection to conduct separate analyses restricted to this category. Age, sex, and years employed at the company were included as covariates in all models to control for confounding; job category was included in models that were not already stratified by type of job.

Urine IL-18, NGAL, and NAG normalized to urine creatinine were natural log-transformed to satisfy normality assumptions, and the β estimates for IL-18, NGAL, and NAG were exponentiated (e^{β}) to show differences in means on the multiplicative scale or "relative means."

Results

Study population

Among 489 individuals included in the study (93% men, mean age 35 years (SD = 10.6)), 282 were sugarcane workers (19 seed cutters, 35 seeders, 29 agrichemical applicators, 49 irrigators, 51 cane cutters, 41 drivers, and 58 factory workers), 47 were sugarcane applicants with elevated serum creatinine at pre-harvest, and 160 were workers in other industries (51 miners, 56 construction, and 53 port workers) (Table 1). The average number of years worked among sugarcane worker's categories ranged from 3.7 to 14.4 years, seed cutters and cane cutters had worked for the fewest number of years, while drivers and factory workers had worked the longest.

Leptospira serology and urine PCR

The proportion of seropositive individuals (MAT titer ≥ 100 at any time point), reflecting any prior infection with *Leptospira*, was 29% (n = 142) (Table 2). The highest seroprevalence was observed among cane cutters (59%), and the lowest among port workers (8%) and sugarcane factory workers (9%). Among sugarcane workers, field workers (seed cutters, seeders, agrichemical

Table 1. Characteristics of study population, by job category.

Job Category	*n*	Age Mean (SD)	Male *n* (%)	Years worked Mean (SD)
All workers	489	35 (10.6)	454 (93%)	_
Sugarcane workers	282	34 (10.4)	249 (88%)	9.6 (9.1)
Field Workers	183	31 (9.4)	152 (83%)	7.0 (7.5)
Seed cutters	19	30 (9.2)	9 (47%)	3.7 (4.4)
Seeders	35	30 (8.8)	14 (40%)	5.0 (4.9)
Agrichemical applicators	29	35 (8.2)	29 (100%)	12.0 (8.0)
Irrigators	49	30 (8.8)	49 (100%)	9.7 (8.0)
Cane cutters	51	31 (10.6)	51 (100%)	4.1 (6.8)
Non-Field Workers	99	38 (10.6)	97 (98%)	14.3 (9.9)
Drivers	41	41 (11.2)	41 (100%)	14.4 (8.9)
Factory workers	58	37 (9.8)	56 (97%)	14.3 (10.6)
Sugarcane applicants with elevated serum creatinine	47	33 (8.6)	47 (100%)	4.9 (3.5)
Miners	51	38 (7.9)	48 (94%)	*
Construction workers	56	35 (12.3)	56 (100%)	*
Port workers	53	40 (11.6)	53 (100%)	*

*No data available.

Table 2. *Leptospira* seropositivity by job category.

Job category	Seropositive *n* (%)[a]	Unadjusted OR (95% CI)	Adjusted OR[b] (95% CI)
All workers	142 (29%)	–	–
Sugarcane workers	95 (34%)	–	–
Seed cutters	3 (16%)	2.3 (0.5–11.4)	3.2 (0.6–17.6)
Seeders	13 (37%)	7.2 (2.1–24.7)	10.6 (2.6–43.2)
Agrichemical applicators	10 (35%)	6.5 (1.8–23.1)	6.9 (1.9–24.8)
Irrigators	18 (37%)	7.1 (2.2–23.0)	8.0 (2.4–26.3)
Cane cutters	30 (59%)	17.5 (5.5–55.9)	19.6 (6.0–64.2)
Drivers	16 (39%)	7.8 (2.4–26.0)	7.8 (2.4–25.8)
Factory workers	5 (9%)	1.2 (0.3–4.6)	1.2 (0.3–4.8)
Sugarcane applicants with elevated serum creatinine	20 (43%)	9.1 (2.8–29.3)	9.8 (3.0–32.2)
Miners	16 (31%)	5.6 (1.7–18.2)	5.9 (1.8–19.3)
Construction workers	7 (13%)	1.75 (0.5–6.4)	1.8 (0.5–6.7)
Port workers	4 (8%)	Ref	Ref

[a]"n" represents number of persons in category who are seropositive; "(%)" represents percentage of total number of people in category who are seropositive.
[b]Adjusted for sex, age, and years worked.

Table 3. *Leptospira* serovar distribution among seropositive individuals.[a]

Serovar	*n* (%)
Alexi	4 (2.8%)
Australis	1 (0.7%)
Bataviae	5 (3.5%)
Borincana	1 (0.7%)
Bratislava	58 (40.8%)
Canicola	20 (14.1%)
Cynopteri	2 (1.4%)
Djasiman	8 (5.6%)
Georgia	3 (2.1%)
Grippotyphosa	2 (1.4%)
Icterohaemorrhagiae	11 (7.7%)
Pomona	5 (3.5%)
Pyrogenes	3 (2.1%)
Tarassovi	2 (1.4%)
Wolffi	3 (2.1%)
More than one serovar with equally elevated titers	14 (9.9%)

[a]Serovar with the highest titer by MAT is presented.

applicators, irrigators, and cane cutters) had a higher seroprevalence (40%) than non-field workers (drivers and factory workers) (21%), with an adjusted odds ratio of 3.2 (95% confidence interval (CI): 1.7–6.1). Among specific job categories, the odds of being seropositive for *Leptospira* was highest among cane cutters, seeders, irrigators, drivers, agrichemical applicators, sugarcane applicants, and miners, compared to port workers. Individuals in three job categories had evidence of recent or current leptospirosis: cane cutters ($n = 2$), sugarcane applicants ($n = 3$), and miners ($n = 3$).

The most frequently observed serological reactivity (highest titer by MAT) was to serovar Bratislava ($n = 58$, 40.8%), Canicola ($n = 20$, 14.1%), and Icterohaemorrhagiae ($n = 11$, 7.7%). More than one serovar with equally elevated titers was also frequently found ($n = 14$, 9.9%) (Table 3). All subjects tested had negative urine PCR results, suggesting the absence of chronic colonization of the kidney by leptospires.

Leptospira seropositivity and kidney injury biomarkers

Leptospira seropositivity was not associated with eGFR except among sugarcane applicants with elevated creatinine. Those that were seropositive had lower mean eGFR (*mean difference*: –10.08; 95% CI: –24.12–3.96) compared to those who were seronegative.

At pre-harvest, there was no association between *Leptospira* seropositivity and concentrations of biomarkers of kidney injury among sugarcane workers (Table 4); however, IL-18 was somewhat elevated among seropositive field workers (*relative mean (RM):* 1.30; 95% CI: 0.89–1.88) compared to those that were seronegative. When stratified by job category, seropositive cane cutters had higher IL-18 (*RM*: 1.95; 95% CI: 0.98–3.87) and NGAL concentrations (*RM*: 1.30; 95% CI: 0.79–2.16) compared to those who were seronegative. Seropositive sugarcane applicants had higher NGAL (*RM:* 1.59; 95% CI: 0.81–3.11) and NAG (*RM:* 1.44; 95% CI: 0.85–2.43).

At late harvest, NGAL, IL-18, and NAG were significantly higher among seropositive sugarcane workers as compared to seronegative workers when adjusting only for sex, age, and years worked (results not shown). However, additional adjustment for job category resulted in a decline of 15–22% in the relative means (Table 4). Including job category in the model, seropositive sugarcane workers had higher NGAL concentrations (*RM*: 1.28; 95% CI: 0.94–1.75) compared to those who were seronegative. When stratifying by fieldworker status, seropositive field workers had higher IL-18 (*RM:* 1.31 95% CI: 0.90–1.92) and NGAL (*RM:* 1.31; 95% CI: 0.91–1.89) concentrations compared to those who were seronegative. A similar result was evident among non-field workers (IL-18 *RM:* 1.56; 95% CI: 0.84–2.88; NGAL *RM:* 1.66; 95% CI: 0.92–2.97). When stratifying by individual job categories, higher NGAL concentrations were seen among seropositive seed cutters (*RM:* 2.09; 95% CI: 0.73–5.97), seeders (*RM:* 1.99; 95% CI: 0.74–5.32), drivers (*RM:* 1.52; 95% CI: 0.90–2.57), factory workers (*RM:* 2.84; 95% CI: 0.81–10.03), and higher IL-18 concentrations were observed among seropositive seeders (*RM:* 2.48; 95% CI: 1.14–5.38), as compared to seronegative workers in these jobs. Among workers in other industries, seropositive miners had higher IL-18 (*RM:* 1.48; 95% CI 0.74–2.95) and NAG (*RM:* 1.36; 95%

Table 4. *Leptospira* seropositivity[a] as a predictor of kidney injury biomarkers, by job category in the sugarcane industry.

	eGFR[b]		IL-18[c]		NGAL[c]		NAG[c]	
	Mean difference	Mean difference	Relative mean	Relative mean	Relative mean	Relative mean	Relative mean	Relative mean
Job Category	Pre-harvest (95% CI)	Late-harvest (95% CI)	Pre-harvest (95% CI)	Late-harvest (95% CI)	Pre-harvest (95% CI)	Late-harvest (95% CI)	Pre-harvest (95% CI)	Late-harvest (95% CI)
Sugarcane workers	1.75	1.89	1.21	1.19	1.01	1.28	1.04	1.00
n = 282	(−1.95–5.44)	(−2.52–6.30)	(0.88–1.66)	(0.86–1.65)	(0.76–1.33)	(0.94–1.75)	(0.81–1.35)	(0.79–1.27)
Field workers[a]	0.83	0.34	1.30	1.31	1.05	1.31	1.01	1.13
n = 183	(−3.60–5.26)	(-5.46–6.14)	(0.89–1.88)	(0.90–1.92)	(0.76–1.45)	(0.91–1.89)	(0.77–1.32)	(0.86–1.48)
Seed cutter	4.33	11.73	0.65	1.75	1.60	2.09	0.76	1.40
n = 19	(−13.74–22.40)	(-13.07–36.53)	(0.10–4.23)	(0.29–10.48)	(0.51–5.00)	(0.73–5.97)	(0.34–1.69)	(0.29–6.75)
Seeder	2.97	6.27	1.54	2.48	0.97	1.99	1.16	0.98
n = 35	(−9.73–15.65)	(−7.05–19.59)	(0.51–4.68)	(1.14–5.38)	(0.43–2.21)	(0.74–5.32)	(0.59–2.27)	(0.50–1.90)
Agrichemical applicator	1.96	2.09	0.79	1.82	1.12	0.63	0.72	0.74
n = 29	(−5.96–9.89)	(−6.27–10.44)	(0.25–2.47)	(0.66–5.01)	(0.58–2.15)	(0.24–1.66)	(0.24–2.12)	(0.37–1.45)
Irrigator	1.34	0.54	0.93	0.87	0.67	1.26	1.13	1.39
n = 49	(−6.71–9.40)	(−8.47–9.55)	(0.49–1.76)	(0.42–1.81)	(0.28–1.59)	(0.59–2.67)	(0.60–2.10)	(0.78–2.49)
Cane cutter	1.91	−0.87	1.95	0.69	1.30	0.84	1.03	0.80
n = 51	(−7.25–11.07)	(−15.93–14.19)	(0.98–3.87)	(0.32–1.50)	(0.79–2.16)	(0.45–1.58)	(0.67–1.58)	(0.52–1.22)
Non-field workers[b]	−2.00	1.61	1.28	1.56	0.83	1.66	1.08	1.08
n = 99	(−8.75–4.76)	(−3.87–7.09)	(0.72–2.28)	(0.84–2.88)	(0.50–1.40)	(0.92–2.97)	(0.63–1.86)	(0.68–1.72)
Driver	2.21	1.80	0.94	1.20	0.84	1.52	0.96	0.84
n = 41	(-6.27 - 10.69)	(−5.10–8.70)	(0.42–2.06)	(0.56–2.60)	(0.48–1.46)	(0.90–2.57)	(0.44–2.12)	(0.49–1.44)
Factory	−6.90	2.30	1.12	1.53	0.79	2.84	1.37	1.60
n = 58	(−20.10–6.30)	(−8.65–13.25)	(0.42–2.95)	(0.46–5.10)	(0.27–2.37)	(0.81–10.03)	(0.55–3.45)	(0.64–4.03)
Sugarcane applicant with elevated serum creatinine	−10.08	–	0.84	–	1.59	–	1.44	–
n = 47	(−24.12–3.96)		(0.42–1.70)		(0.81–3.11)		(0.85–2.43)	

eGFR = estimated glomerular filtration rate (ml/min/1.73 m^2), IL-18 = Interleukin 18 (pg/ml), NGAL = Neutrophil gelatinase-associated lipocalin (ng/ml), NAG = N-acetyl-D-glucosaminidase (U/L).

[a]Seronegative individuals in each job category are the reference group.

[b]Results represent linear regression arithmetic differences in mean eGFR between individuals with exposure to *Leptospira* and seronegative individuals.

[c]Results represent linear regression multiplicative differences (relative means) in mean IL-18, NGAL, and NAG between individuals with exposure to *Leptospira* and seronegative individuals.

Notes: Model adjusted for age, years worked (except for sugarcane applicants), and also adjusted for sex in categories with females (seed cutters, seeders, and factory); Sugarcane Workers also adjusted for job category.

Table 5. *Leptospira* seropositivity as a predictor of kidney injury biomarkers, by job category among workers in other industries.[a]

	eGFR[b]	IL-18[c]	NGAL[c]	NAG[c]
Job category	Mean difference (95% CI)	Relative mean (95% CI)	Relative mean (95% CI)	Relative mean (95% CI)
Miner $n = 51$	1.91 (−9.11–12.93)	1.48 (0.74–2.95)	1.05 (0.65–1.69)	1.36 (0.91–2.03)
Construction $n = 56$	10.70 (−7.01–28.40)	1.50 (0.49–4.59)	0.87 (0.30–2.51)	0.53 (0.26–1.04)
Port worker $n = 53$	13.97 (−12.97–40.91)	0.87 (0.28–2.66)	0.50 (0.14–1.76)	0.63 (0.22–1.82)

Notes: Model adjusted for age, and for sex in categories with females (miners). eGFR = estimated glomerular filtration rate (ml/min/1.73 m^2), IL-18 = Interleukin 18 (pg/ml), NGAL = Neutrophil gelatinase-associated lipocalin (ng/ml), NAG = N-acetyl-D-glucosaminidase (U/L).

[a]Seronegative individuals in each job category are the reference group.

[b]Results represent linear regression arithmetic differences in mean eGFR between individuals with exposure to *Leptospira* and seronegative individuals.

[c]Results represent linear regression multiplicative differences (relative means) in mean IL-18, NGAL, and NAG between individuals with exposure to *Leptospira* and seronegative individuals.

CI 0.91–2.03) compared to those who were seronegative (Table 5).

The median urine ACR among sugarcane workers was 2.4 mg/g at pre-harvest and 2.1 mg/g at late harvest (results not shown). At both time points fewer than 5% of workers had an ACR ≥ 30 mg/g (the clinical threshold for albuminuria). Sugarcane applicants had a median ACR of 3.2 mg/g, with 14.9% of them having an ACR ≥ 30 mg/g. Miners, construction workers, and port workers had a median ACR of 3.9, 3.3, and 1.3 mg/g, respectively. The percentage of workers with ACR ≥ 30 mg/g was 21.6% for miners, 10.9% for construction workers, and 7.5% for port workers. There was no association between urine ACR and *Leptospira* exposure (results not shown).

Discussion

Seropositivity to *Leptospira* was high among our study population, with significant differences by job category. We found some evidence that *Leptospira* seropositivity may be associated with elevated levels of biomarkers of kidney injury.

Despite infectious diseases being one of the hypothesized causes of the unexplained epidemic of MeN, there have been no prior studies evaluating them as a possible factor. Leptospirosis is a plausible candidate due to its widespread prevalence in Central America, high infection rates among the type of workers who have been identified as having elevated rates of MeN, and its established association with AKI.

Among sugarcane workers, field workers had higher seroprevalence than non-field workers, which is expected given the higher likelihood of occupational exposure to soil contaminated with *Leptospira*. Cane cutters had the highest seroprevalence at 59%. Among workers in other industries, miners were disproportionately affected. Drivers also had high seroprevalence, which was unexpected given that they have less occupational exposure to soil compared to field workers. However, workers were classified by their present occupation, and many drivers may have been field workers in the past or could be exposed non-occupationally.

There was evidence of recent or current leptospirosis in cane cutters, sugarcane applicants, and miners. Since cane cutters had samples from both pre- and late-harvest, recent or current leptospirosis represent incident cases ($n = 2$, 4%) during the harvest season. Given that *Leptospira* is endemic in regions of Central America, recent or current leptospirosis cases likely approximate the baseline incidence rate in this population, since samples were taken during a period when there was no outbreak. Sugarcane applicants and miners were sampled at only one time point, limiting the options for comparison with sugarcane cutters.

Some leptospiral serovars are commonly associated with particular animal reservoirs [20,21]. In this population, the serovars with the greatest number of seropositive individuals included Bratislava, typically associated with horses; Canicola, typically associated with dogs; and Icterohaemorrhagiae, typically associated with rats. All three animals are present in large numbers in the main geographic areas in which study participants live, but only rats are common in the specific occupational setting in which this study was conducted. However, highest MAT titer to a particular serovar does not necessarily represent the infecting serovar given the occurrence of paradoxical reactions and cross-reactions [70], so these data give only a broad idea of serovars present in this population of workers. Sources of exposure to *Leptospira* likely include contact with soil contaminated by rodent urine in the sugarcane fields and mines, in addition to non-occupational exposures, which are common in rural tropical environments [20,21].

Chronic colonization of the kidney by leptospires has been previously documented and seems to occur in a small percentage of individuals infected by *Leptospira* [71]. One recent study suggests that chronic colonization of kidney tubules by leptospires has a deleterious effect on kidney function [51]. Research on chronic colonization of renal tubules by leptospires and its role in the development of CKD is in its early stages, and the role of this pathophysiological mechanism in the development of MeN is still unknown. The negative urine PCR results in this study suggest the absence of chronic colonization of renal tubules by leptospires in our population, although low levels of leptospires in urine could have gone undetected. Given the apparent infrequent occurrence of chronic colonization among individuals infected by *Leptospira*, and the large scale of the CKD epidemic in Central America, we believe that it is important to consider alternative mechanisms

of renal injury by which infection with *Leptospira* could lead to the development of CKD.

In acute leptospirosis urine PCR is expected to be positive since leptospires are actively shed in the urine. The eight individuals with evidence of recent or current leptospirosis had negative urine PCR results. Since individuals were tested at their respective work locations and did not show evidence of an acute illness, the precise timing of infection is uncertain. Two of the eight individuals with evidence of recent or current leptospirosis were sugarcane workers that had paired samples taken. Even though acute infection occurred within the six-month harvest season, since urine PCR is only positive for a few weeks after acute infection, it is possible that enough time had elapsed for leptospires to be undetectable in urine by the time of the late-harvest sample, explaining the negative urine PCR results.

Leptospira seropositivity was not associated with eGFR except among sugarcane applicants with elevated creatinine, who represent a group of individuals with some degree of kidney dysfunction at the time of testing. It is possible that no association between *Leptospira* seropositivity and eGFR was found among other job categories because the yearly pre-employment screening conducted by the company has created a selection process skewed toward healthier workers, excluding those individuals with elevated serum creatinine.

Biomarkers of kidney injury (IL-18, NGAL, and NAG) were significantly elevated among seropositive sugarcane workers at late-harvest when adjusting only for sex, age, and years worked. This association was attenuated by 15–22% when the model additionally included job category as a covariate, suggesting that job category is acting as a proxy for an unmeasured exposure that is causing the elevation in biomarkers of kidney injury. However, *Leptospira* infection may be acting as a kidney disease susceptibility factor, given the higher concentrations of biomarkers among seropositive workers. If leptospirosis, including the vast majority of cases that do not develop overt AKI, can cause some degree of underlying subclinical kidney injury, individuals that are seropositive to *Leptospira* could be more susceptible to additional kidney insults.

There are some limitations to this study. First, job category was determined based on present occupation, which could have been different at the time of infection with *Leptospira* if the worker held a different job category in the past. This is not a concern for recent or current leptospirosis among sugarcane cutters since infection occurred during the present harvest season. Second, a portion of sugarcane workers tested at pre-harvest were not tested at late-harvest (refer to Laws et al. 2015 for a detailed description of loss to follow-up (LTF) among sugarcane workers) [14]. If sugarcane workers with recent or current leptospirosis left their job due to symptoms and were LTF, the incidence of infection during the harvest season may have been greater than the one observed in this study. Finally, while the overall size of the study was not small, the need to conduct analyses among participants stratified into several job categories limited the precision of the results.

Future areas of research include conducting studies to confirm or disprove the association between *Leptospira* seropositivity and biomarkers of kidney injury seen in this study. In particular, it is important to obtain a better understanding of whether asymptomatic or mild leptospirosis can cause subclinical kidney injury that increases the risk of developing CKD if exposed to other kidney insults. Whether the timing of infection with *Leptospira*, particular serovar, or the occurrence of repeated infections with different serovars plays a role, is currently unknown. The possibility of an interaction between *Leptospira* infection and other hypothesized causes of MeN, such as heat exposure and strenuous work, requires further study.

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