

**SUMMARY REPORT:
Boston University investigation of chronic kidney disease in Western Nicaragua, 2009-2012**

August 2012

Prepared by:
Daniel Brooks
Michael McClean

**On behalf of the Boston University Team
(in alphabetical order):**

Juan José Amador
Kate Applebaum
Ann Aschengrau
Bruce Cohen
James Kaufman
Rebecca Laws
Damaris Lopez
Eloesa McSorley
Alejandro Riefkohl
Oriana Ramirez Rubio
Madeleine Kangsen Scammell
Daniel Weiner

Boston University School of Public Health
715 Albany St - Boston, MA 02118

INTRODUCTION

Since 2009, our research team from the Boston University School of Public Health (BU) has been investigating an epidemic of chronic kidney disease (CKD) of unknown cause in Nicaragua. The work was requested by members of a Dialogue Table consisting of a local community group, the Chichigalpa Association for Life (ASOCHIVIDA), and the management of Nicaragua Sugar Estates Limited (NSEL), a major sugar producer in western Nicaragua that operates the Ingenio San Antonio (ISA) sugar mill. ASOCHIVIDA currently represents approximately 2,000 former NSEL workers and community members who are affected by the disease.

In March 2008, ASOCHIVIDA filed a complaint with the Compliance Advisor Ombudsman (CAO). The CAO is an independent office that handles complaints from communities who feel they have been adversely affected by the investments of the World Bank Group's private sector arm (IFC), which had provided funding to NSEL to support its operations. With parties' agreement, CAO convened a Dialogue Table through which the members mutually determined their priorities, and facilitated an open, competitive selection process with the dialogue participants to select a group of scientists to conduct research on the causes of the CKD epidemic. In April 2009, ASOCHIVIDA and NSEL selected BU by consensus from a pool of nine applicants.

The parties at the Dialogue Table gave BU the mandate to answer the following questions:

1. What are the causes of CKD in the Western Zone of Nicaragua – an area that includes the Ingenio San Antonio and its sugarcane plantations?
2. Is there any relationship between the practices of the Ingenio San Antonio and the causes of CKD?

The BU team first conducted a Scoping Study from June to December 2009, which summarized the available information on CKD in the region, identified data gaps, and recommended research activities to address those gaps. We then conducted the following six research activities (in order of the date when reports have been made public):

- An **Industrial Hygiene/Occupational Health Assessment** (August 2010), which evaluated the potential hazards associated with chemicals and work practices at ISA;
- An **Investigation of Water Quality** (August 2010), which included the analysis of a large number of contaminants in water samples collected from locations selected by ASOCHIVIDA;
- A **Qualitative Analysis of Interviews with Physicians and Pharmacists** (February 2012), which summarized data from semi-structured interviews with health professionals in Nicaragua to assess their perceptions regarding renal disease in the region;
- A **Pilot Cohort Study** (February 2012), which assessed the feasibility of conducting a complete retrospective cohort study to evaluate the relationship between work practices at ISA and CKD;
- An **Investigation of Biomarkers in Workers** (April 2012), which evaluated biological markers of kidney injury and CKD in ISA workers, miners, construction workers, and port

workers; and

- An **Investigation of Urinary Biomarkers in Adolescents** (June 2012), which evaluated evidence of subclinical kidney damage among adolescents in different areas of Nicaragua.

We have produced separate detailed reports that describe the methods, results, and conclusions for the Scoping Study as well as for each of the six research activities. These seven reports are all posted and publicly available on the CAO website:

- http://www.cao-ombudsman.org/cases/case_detail.aspx?id=82; and
- <http://www.cao-ombudsman.org/cases/document-links/links-82.aspx>.

The purpose of this report is to summarize the work conducted by BU to date, describe our current perspective on the possible causes of the CKD epidemic, and suggest some areas for future research.

Throughout this report, we use the terms ‘evidence’ and ‘lack of evidence.’ A lack of evidence could mean one of two things: (1) a hypothesized association has been studied and no link has been found, or (2) a hypothesized association has not been studied (or only minimally studied) and so little is known. For most of the conclusions presented in this report, the latter reason was the basis for citing a lack of evidence.

THE SCOPING STUDY (DECEMBER 2009)

In February 2009, NSEL and ASOCHIVIDA agreed to support a scoping study to summarize and evaluate the available information on CKD in the region, identify data gaps, and recommend research activities to address those gaps. The Scoping Study assessed whether further research into the causes of CKD was necessary (i.e., whether there was already sufficient evidence to reach a conclusion), feasible, and likely to be successful in answering the two questions that had been posed by the dialogue participants.

Following an evaluation of all available data, our overall conclusion was that the causes of CKD in the Western Zone of Nicaragua were unknown and that the relationship between the disease and ISA work practices was also unknown. Additionally, we concluded that the epidemic of CKD did not appear to be caused by diabetes or hypertension, which are the main causes in most high income as well as many low or medium income countries, and that the clinical characteristics of the disease, including apparent mild to moderate proteinuria, small kidneys on ultrasound, and the relatively slow rate of progression, suggest that the form of CKD is tubulointerstitial disease. We also concluded that the occurrence of CKD was higher in the departments of León and Chinandega than in other areas of Nicaragua, higher among men than among women, and more common in young age groups than one would expect when comparing to disease patterns in other populations. We also noted that the prevalence of CKD appears to be highest in certain occupations (such as agriculture and mining), but that this association does not necessarily mean that occupational exposures are the cause of CKD; however, the association does suggest that an occupational etiology, either singly or contributory, is a plausible hypothesis that needs to be addressed.

Accordingly, we identified the following hypotheses that merited consideration: exposure to agrichemicals; volume depletion and muscle damage; systemic infections such as leptospirosis, hantavirus and malaria; exposure to heavy metals; exposure to aristolochic acid; medications;

alcohol consumption; kidney stones and structural kidney disease; diabetes; hypertension; glomerulonephritis; urinary tract infection; and genetics.

We also recommended the following research activities to investigate the above hypotheses and address the questions posed by dialogue participants: 1) an industrial hygiene assessment of current work practices at NSEL; 2) environmental sampling; 3) key informant interviews; 4) a pilot retrospective cohort study of past and present sugar cane workers and an associated medical records review; 5) worker biological testing; 6) adolescent urinalysis; 7) post-mortem kidney biopsies; and 8) worker observation studies.

We presented the scoping study to Dialogue Table participants in December 2009 and discussed a list of research tasks we felt were important to implement. The dialogue participants agreed to continue supporting research activities and in January 2010, CAO prepared a Terms of Reference (TOR) for further work based on the research tasks presented by BU. The TOR was then discussed and agreed to by Dialogue Table participants.

The current status of research activities proposed in the scoping study is as follows:

Research Activity	Status	Date made public
Industrial Hygiene/Occupational Health Assessment	Completed August 2010	August 2010
Environmental Sampling	Completed August 2010	August 2010
Key Informant Interviews	Completed September 2011	February 2012
Pilot Retrospective Cohort Study and Medical Records Review	Completed February 2012	February 2012*
Worker Biological Testing	Completed March 2012	April 2012**
Adolescent Biological Testing	Completed May 2012	June 2012
Kidney Biopsies	Not initiated	Not applicable
Worker Observation Studies	Proposed for future research	Not applicable

* Expanded medical record review in progress

** Additional leptospirosis analysis in progress

INDUSTRIAL HYGIENE/OCCUPATIONAL HEALTH ASSESSMENT

From September 2009 to August 2010 the BU team conducted an industrial hygiene (IH) assessment to evaluate the current work practices at ISA during the 2009-2010 zafra (harvest), as well as the chemicals used at ISA both currently and in the past. In particular, we were asked to address the following questions posed by dialogue participants:

1. Is there evidence that current work practices or chemicals used by ISA currently or in the past **cause** CKD?
2. Is there evidence that current work practices or exposure to chemicals used by ISA currently or in the past are **associated** with:
 - a. CKD (defined by high creatinine/reduced kidney function)?
 - b. Acute kidney damage in humans or animals?

There were three main components of the IH Assessment designed to address these questions: (1) a

background appraisal of current ISA activities (prior to the site visit), (2) a site assessment at ISA, and (3) a toxicological review of chemicals used currently and in the past at ISA. The background appraisal included a desk review of current best practice in agricultural industries (with particular emphasis on sugar cane operations) and the collection and review of information about sugar cane operations and work practices at ISA. The site assessment at ISA (April 2010) included a walk-through of the facility, interviews, records review, and worker observations. Representatives from both NSEL and ASOCHIVIDA, as well as retired workers from ISA, participated in the site assessment. The toxicological review of chemicals used currently and in the past at ISA included a determination of a list of chemicals for review, a review of information from government agencies and published scientific literature for these chemicals, and ultimately a determination regarding whether any of these chemicals have a known association with kidney damage or more specifically chronic kidney disease.

After conducting these three components of the IH Assessment, we responded to the questions posed by the dialogue participants based on the current scientific information available. Our specific responses consider both the likelihood of exposure to the agents evaluated as well as the likelihood of causing CKD and/or acute kidney damage:

1. We found no evidence that the current work practices or the chemicals used by ISA currently or in the past are generally accepted causes of CKD.
- 2a. We found very limited evidence that current work practices or exposure to chemicals used by ISA currently or in the past might be associated with CKD. This association is plausible but not established.
- 2b. We found evidence that agents evaluated at ISA might be associated with acute kidney damage, but do not have the information that would allow us to determine if exposure levels are sufficient to result in acute kidney damage. In theory, even repeated subclinical acute kidney damage could lead to CKD, but this mechanism has not been proven.

More specifically, we identified and evaluated five agents hypothesized to be related to CKD development and/or acute kidney damage and found the following:

- We found no evidence in our review of the medical literature that any of the 36 agrichemicals evaluated in this report are generally accepted causes of CKD and found no evidence that any of these 36 agrichemicals are associated with CKD¹.
- We found no evidence in our review of the medical literature that heat stress (volume depletion and muscle damage) and systemic infections (leptospirosis and hantavirus) are generally accepted causes of CKD, and we found only very limited evidence that exposure to these agents is associated with CKD.
- We found evidence in our review of the medical literature that heavy metals and silica are generally accepted causes of CKD. However, we did not know the extent of worker exposure to these agents at ISA.

We noted that all five agents could cause acute kidney damage in humans or animals under certain exposure scenarios. However, we did not have evidence that exposures at ISA have caused acute

¹ Some of the chemicals evaluated may cause kidney damage with short-term or prolonged over-exposure, but the link between kidney damage and developing CKD is not known.

kidney damage or whether acute kidney damage might have led to CKD. In theory, repeated subclinical acute kidney damage could lead to CKD, but this potential mechanism has not been proven.

The dialogue participants asked us to assess whether there is evidence that current work practices or chemicals used by ISA currently or in the past cause CKD. This question was intended to address whether particular practices or chemicals are “generally accepted” causes of CKD. Based on our review of the information gathered during the IH Assessment and the current scientific information available, we concluded that none of the current work practices or the chemicals used by ISA are generally accepted causes of CKD. This conclusion does not rule out the possibility that one or more of these agents might in fact cause CKD, but new scientific research and insights will be necessary to establish whether a link actually exists.

The worker biological testing section of this report takes the industrial hygiene assessment work a further step and describes a study we designed and implemented to look at indicators of subclinical kidney damage in workers in different industries, including sugar cane production, mining, construction and port work. The final section of this report outlines future research activities we think would be useful to help identify if repeated subclinical kidney injury that may result from work in certain occupations could lead to the development of CKD. These activities involve conducting additional research on workers, including collecting samples and analyzing them for indicators of kidney damage, heat stress, volume depletion, muscle damage, agrichemicals and metals.

ENVIRONMENTAL SAMPLING

Between April and August 2010, BU evaluated water samples to better understand environmental conditions at ISA. BU worked with ASOCHIVIDA members to identify locations on the ISA property where they were concerned about water quality, e.g. locations that receive runoff from field and plant activities or where there is high potential for exposure among workers or community members. Six locations on ISA property were selected as sampling locations, three from field and plant wastewater lagoons, and three from wells, including one small, hand-dug well still used by workers for drinking, a deep well with a dedicated water pump that the population uses for swimming and bathing, and a deep well with a dedicated pump located in a parking area that is currently used to fill trucks. A water sample was collected at each location in April 2010 and analyzed at an independent laboratory (ALS Environmental Lab in Houston, Texas, USA) for the following 183 chemicals:

- 14 *Metals*, including antimony, arsenic, beryllium, **cadmium**, chromium, copper, **lead**, nickel, selenium, silver, thallium, **uranium**, zinc and mercury;
- 48 *Volatile Organic Compounds* (VOCs), including common industrial solvents and chemicals;
- 65 *Semi-Volatile Organic Compounds* (SVOCs), including Atrazine and common industrial solvents and chemicals;
- 22 *Organochlorine Pesticides*, including DDT, Lindane, Dieldrin, Chlordane, Endosulfan and Toxaphene, as well as others;
- 20 *Organophosphorus Pesticides*, including **Diazinon**, Chlorpyrifos, Parathion and others;
- 13 *Herbicides*, including **2,4-D**, **Paraquat**, and **Glyphosate**;
- One *Nematicide*, **DBCP** (Nemagon).

The chemicals shown in **bold type** in the list are those that were determined to have some evidence of being associated with kidney effects under certain exposure scenarios, as identified in the Industrial Hygiene/Occupational Health Assessment. Glyphosate and 2,4-D are the two agrichemicals that are used most extensively at ISA (and worldwide).

For the six water samples, ALS produced 1,033 individual results for the chemicals listed above. The concentration of each individual chemical was below the analytical detection limit in the vast majority of instances (1,011 of the 1,033 total analyses or 98%). For the 22 analyses with a concentration above the detection limit, concentrations were well below the USEPA water quality criteria (MCL or RBC), indicating that the water meets USEPA limits. In summary, at the time samples were collected at the six locations on ISA property selected by ASOCHIVIDA members, water quality analyses indicated there was no evidence that consumption of the water would be harmful to human health.

This study was smaller in scope than the effort we had originally proposed, which would have included samples taken from both soil and water and from a substantially larger number of sites. However, based on the results of this study, short half-lives of many of the chemicals of interest, and the lack of an established connection between these chemicals and CKD, we concluded that the large amount of funds that such a study would cost could be put to better use, specifically by taking biological samples from workers at ISA and other industries as described later in this report.

KEY INFORMANT INTERVIEWS

Between November 2010 and February 2012 the BU team conducted a qualitative study using key informant interviews to examine health professionals' perceptions regarding etiology and treatment of CKD and the reported high occurrence of urinary tract infections (UTI) among men. In addition, we wanted to learn more about health professionals' understanding of a dysuria syndrome popularly called "chistata", which occurred frequently among residents of the region. The interviews addressed each of these conditions and their treatment approaches, focusing on the use of potentially nephrotoxic medications. In November 2010, BU interviewed ten randomly selected practicing physicians and nine pharmacists from Western Nicaragua.

Interview results indicated that health professionals perceived CKD as a serious and growing problem in the region, primarily affecting young men working as manual laborers. These findings were consistent with the existence of an epidemic of CKD, describing characteristics similar to those noted in prevalence studies conducted in the region. These include CKD being more frequent among men, starting in young adulthood, and associated with mainly agricultural work but also occurring in miners and construction workers. That diabetes and hypertension cannot explain the CKD epidemic in Nicaragua was also highlighted in the interviews.

Exposure to heat, physical work and dehydration/volume depletion emerged as the factors most commonly believed by both physicians and pharmacists to cause CKD. This viewpoint is consistent with regional studies that have concluded that CKD appears more common in people engaged in occupations in which strenuous work is undertaken at high ambient temperature. Exposures to agrichemicals, heavy metals, alcohol consumption and infectious diseases were also mentioned in physician and pharmacist interviews and have been the focus of additional causal hypotheses in the region.

Interviewees provided inconsistent perspectives regarding the role of drinking water in CKD, with half the pharmacists believing dehydration (i.e., not drinking enough water) leads to CKD, and the other half believing that drinking contaminated water may cause CKD.

According to both physicians and pharmacists, urinary tract infection (UTI) is one of the main causes of dysuria (known as chistata in Nicaragua). This belief is of particular interest since UTIs are generally quite rare in males. Physicians acknowledged that the diagnosis of UTI was almost never based on microbial culture.

Interviews indicate that patients with CKD or at high risk for CKD may be receiving nephrotoxic drugs, and a number of physicians suggested that the use of these medications for UTI, pain, and other conditions might be contributing to CKD. In particular, aminoglycoside antibiotics and chronic use of non-steroidal anti-inflammatory drugs (NSAIDs) are associated with acute kidney injury (AKI) in a dose- and duration-dependent manner. This association is even stronger in the setting of volume depletion, which may be further aggravated due to the use of diuretics. Information obtained from these interviews suggests that diuretics, antibiotics and NSAIDs are widely used and sold over the counter for symptoms that may be related to volume depletion; accordingly, we concluded that use of these medications coupled with volume depletion should be further evaluated as causal factors for CKD in this region. Additionally, the true cause of pain and UTI-like symptoms, the potential associations among heat stress, UTI diagnosis and nephrotoxic medication use, and their contribution to the development of CKD warrant further investigation.

PILOT RETROSPECTIVE COHORT STUDY AND MEDICAL RECORDS REVIEW

Between March 2011 and February 2012, BU conducted a retrospective cohort pilot study of individuals who had worked at ISA. This study was identified during the Scoping Study as a key activity to explore potential causes of CKD. Because the proposed study would be costly and was estimated to require 2-3 years to complete, a pilot study was undertaken to:

- Determine whether a retrospective cohort study is actually feasible;
- Assess the strength of evidence regarding any connection between work at ISA and CKD that would likely be gained from such a study;
- Outline the methods by which a retrospective cohort study could be conducted, along with estimated cost and duration; and
- Collect information from medical records that might inform hypotheses regarding the causes of CKD and inform future studies.

We found that the record system at ISA had many strengths and had improved over time. However, there were two main limitations in the ISA record system from the perspective of conducting a retrospective cohort study: 1) lack of detail in job titles during the 1960s-1980s and 2) lack of any employment information for all categories of workers employed by contractors, with the exception of cane cutters for whom the information was still less available and of lower quality than for ISA employees. In addition, although the ISA records contained standard identification information (social security number and "cedula", the national ID number), health centers and hospitals assigned their own identification number to each patient. As a result, we were unable to locate the medical records for ISA workers when we went to search for them at these medical facilities.

Accordingly, we concluded that it would be feasible to conduct a retrospective cohort study with the following restrictions: (1) only temporary employees and cane cutters could be included in the study, and (2) follow-up for CKD occurrence should begin with the 2004-2005 zafra. Furthermore, the conclusion was based on the likelihood that two additional activities would provide key data on possible confounders and the number and identity of individuals who developed CKD: construction of electronic databases at several health centers and extensive community outreach. While it appears that these methods could be successful, they should be tested before committing to a large-scale study. Nevertheless, while a study would be feasible and provide valuable information, the limitations described above also affected the strength of the conclusions that could be generated.

In addition to providing information about feasibility and strength of evidence from a possible future study, medical record abstraction conducted as part of the pilot study also provided an opportunity to gain new knowledge relevant to possible causes of CKD. Data from the medical record abstraction confirmed a low prevalence of diabetes and hypertension, which are common causes of CKD in other settings. The data also added to the evidence from other sources that the underlying cause of CKD represents a tubulointerstitial rather than glomerular process, based on the findings of low-grade proteinuria and elevated white blood cells in the urine. Finally, the medical record abstraction documented evidence of the frequent occurrence in younger men of dysuria (“chistata”) and the diagnosis of UTIs among a large majority of the men, as well as the frequent use of NSAIDs. The evidence led us to conclude that UTIs are most likely over-diagnosed, and that there must be another cause for complaints of dysuria and the frequent presence of white blood cells in the urine.

Overall, the results of the pilot cohort study suggested that while a retrospective cohort study of ISA workers would provide valuable information, it would also take several years, be more expensive than originally estimated because of the additional efforts required to collect data, and would yield results that may have a limited ability to establish a link between occupational exposure and CKD and that therefore would not be of sufficient definitiveness to address the concerns of the Dialogue Table stakeholders.

WORKER BIOLOGICAL TESTING

From October 2010 to April 2012, the BU team conducted an investigation of biological markers of kidney injury and CKD among workers in Western Nicaragua. Workers in the sugarcane industry were recruited from ISA and tested at the beginning and during the latter part of the 2010-2011 zafra, of whom 284 were randomly selected for laboratory analysis within pre-specified job categories. Workers in three other industries (miners, construction workers, and port workers) who had never worked in the sugarcane industry were also tested once between March and June of 2011 (164 workers). The analyses included: serum creatinine, urinary biomarkers of kidney injury, urine dipstick measures, urine cultures, and heavy metals. The findings are summarized below, organized according to each of the six objectives:

Objective 1: Evaluate characteristics of the disease to determine whether kidney damage is tubulointerstitial or glomerular. Our results suggest that the type of kidney damage occurring in ISA workers, as well as among workers in other industries, is primarily tubulointerstitial in nature. At late-zafra, cane cutters had the highest concentration of urinary NGAL and NAG, both of which

are biomarkers of tubulointerstitial kidney injury. Additionally, protein and glucose were only rarely detected in urine, providing little evidence of glomerular disease². This finding is important because the primary causes of CKD globally are diabetes and hypertension, which more often result in glomerular disease manifestations.

Objective 2: Evaluate biomarkers of kidney injury and CKD among ISA workers by investigating changes during the zafra and differences by ISA job. We found that biomarkers of kidney injury and CKD were generally highest among cane cutters and seed cutters and lowest among factory workers. Estimated glomerular filtration rate (eGFR) was lowest (indicating reduced kidney function) among cane cutters and seed cutters at both pre-zafra and late-zafra (as compared to ISA workers in other jobs), though the difference was highest at late-zafra. This was consistent with the finding that cane cutters and seed cutters experienced the largest decreases in eGFR during the zafra, as compared to ISA workers in other jobs. Because the health surveillance program at ISA screens out workers based on creatinine levels, the prevalence observed in our study population is almost certainly lower than among all seed cutters and cane cutters who have ever worked at ISA. But even with the health surveillance program, the prevalence of CKD stage 3 or higher among cane cutters (6%) and seed cutters (12%) between the ages of 20 and 59 years old was much higher than expected in a population of relatively young men³.

For biomarkers of tubulointerstitial kidney injury, late-zafra concentrations of NGAL and NAG were highest among cane cutters, approximately 3 times as high as among factory workers. The increases during the zafra were highest among cane cutters for NGAL and among both seed cutters and cane cutters for NAG. Interestingly, NGAL and NAG among ISA workers were significantly associated with decreased eGFR at late-zafra but not at pre-zafra. Overall, these results suggest that cane cutters and seed cutters experience tubulointerstitial injury during the zafra that may be increasing their risk of CKD.

Objective 3: Determine whether there is evidence of kidney injury or CKD among workers in other industries who have never worked in the sugarcane industry. Our results provide evidence of CKD among workers in other industries who have never worked in the sugarcane industry. Typically, prevalence of CKD stage 3 or higher in a population less than 60 years old would be expected to be quite low (see footnote 2). However, in workers between the ages of 20 and 59, we found that the prevalence of CKD (Stages 3 and 4) was 6% among miners, 3% among construction workers, and 8% among port workers. The prevalence of CKD in these industries was much higher than expected in a population of relatively young men. The percentage of workers with CKD in these industries can not be directly compared to workers at ISA because the workers in these other industries are not subject to the same pre-employment surveillance program. Additionally, NGAL and NAG among workers in other industries were significantly associated with decreased eGFR, suggesting that workers in these other industries experience tubulointerstitial kidney injury that may be increasing their risk of CKD.

Objective 4: Analyze heavy metals in biological samples collected at both pre-zafra and late-zafra to characterize metals exposure in the region and explore relationships with biomarkers of kidney

² The one possible exception was that miners had protein detected in urine with higher frequency (based on dipstick data) and at higher concentrations (based on urinary ACR) than ISA workers, port workers, or construction workers.

³ For example, the prevalence of CKD stage 3 or higher in men in the United States between the ages of 20 and 59 is 1%.

injury and CKD. Biomarkers of metals exposure among ISA workers were generally low, did not differ by job, and did not increase during the zafra. There was no evidence that lead, cadmium, or uranium are associated with biomarkers of kidney injury or CKD, and some evidence that high exposure to arsenic (total) is associated with biomarkers of CKD (i.e., lower eGFR)⁴.

Objective 5: Culture urine samples collected from ISA workers at late-zafra to investigate the frequent clinical diagnosis of urinary tract infections (UTIs) among young men in this region. Physicians and pharmacists report a high occurrence of diagnosed UTIs based on testing positive for leukocyte esterase and/or reporting symptoms, a strange phenomenon given that UTIs among men in the global population are quite rare. We also had documented frequent diagnoses of UTI in the medical records at ISA. To shed more light on this situation, we cultured urine samples from 50 men who were positive for leukocyte esterase and/or reported having symptoms during the past 24 hours, yet none of these men were found to have a positive urine culture. Additionally, nitrites were very rarely detected in urine, further supporting the absence of UTIs. We cannot rule out the possible role of sexually transmitted diseases or certain other infections, since we did not specifically culture for these pathogens.

Testing positive for leukocyte esterase or blood in urine can be a sign of inflammation. The percentage of ISA workers who tested positive was higher at late-zafra than at pre-zafra for both of these markers. We also found that leukocyte esterase positivity was associated with biomarkers of tubulointerstitial injury (NGAL and NAG) among workers at ISA and in other industries, providing further evidence that leukocyte esterase positivity in this study population is predominantly a marker of inflammation. The combined results for the urine cultures, leukocyte esterase, nitrite, and blood in urine are suggestive of inflammation and not infection.

Objective 6: Determine whether hydration practices or alcohol consumption are associated with biomarkers of kidney injury or CKD. Self-reported consumption of water, bolis, and/or alcohol was not associated with biomarkers of kidney injury or kidney function.

ADOLESCENT BIOLOGICAL TESTING

From October 2011 to June 2012, the BU team investigated the same biological markers of kidney injury as in the worker biological testing (NGAL, NAG, IL-18, ACR) among 200 adolescents in Nicaragua for the purpose of assessing whether kidney damage may have already started during adolescence and before entering the workforce. We collected urine samples from children between the ages of 12 and 19 at four schools that were selected to represent a range of characteristics that have been hypothesized to be associated with the occurrence of CKD among adults in Nicaragua. Ranked from lowest to highest hypothesized risk, the schools were in the following locations: (1) Jinotega, located at high altitude, with low mortality from CKD⁵; (2) Masaya, located in the Pacific region at low altitude with mortality from CKD at the median for all departments; (3) north Chichigalpa, located in northwest Nicaragua in an area with the highest mortality from CKD in the country; and (4) south Chichigalpa, located in the same area and in which most of the students are children of current or former sugarcane workers. From each of the four groups, we collected a urine

⁴ Total arsenic in urine includes all species of organic and inorganic arsenic. In general, inorganic arsenic is more toxic than the organic form. Arsenic speciation (inorganic versus organic) was not possible as part of this investigation but should be considered in future studies.

⁵ Based on data from the Nicaraguan Ministry of Health for 2010.

sample from 25 boys and 25 girls (50 from each group). Urine samples were analyzed for urinary biomarkers of kidney injury and urine dipstick measures. The primary objective was to determine whether there is evidence of kidney injury among adolescents, and if so, assess whether the biomarkers of kidney injury are different by sex, age, or geographical location. The findings are summarized below, organized according to each of the two objectives:

Objective 1: Evaluate whether there is evidence of kidney injury among adolescents. When we compared the concentrations of biomarkers of kidney damage among Nicaraguan adolescents to those reported in other studies, which included both individuals with kidney conditions and those who were apparently free of kidney damage (“normal controls”), we found that the median level of IL-18 among Nicaraguan adolescents at all schools was higher than expected. IL-18 is a biomarker of damage to the renal tubules. This suggests that there may in fact be early kidney damage present in adolescents in Nicaragua, and that the damage is occurring in the tubules, which is consistent with the type of CKD that appears to be responsible for the epidemic.

Objective 2: Evaluate whether there are differences in the prevalence of early-stage kidney damage by sex, age, and school consistent with the patterns of the CKD epidemic among adults. Contrary to our expectations based on the higher frequency of CKD among adult males, girls had higher levels than boys for all biomarkers, with particularly large differences for ACR and IL-18. Results according to school were more in accordance with our *a priori* hypothesis. The results for NAG were most consistent in this regard, with the relative concentrations rank ordered by school according to risk profile (Jinotega, Masaya, north Chichigalpa, south Chichigalpa) in both boys and girls. Both schools in Chichigalpa demonstrated some evidence of tubular kidney damage among both boys and girls, but boys at the school in south Chichigalpa exhibited the most consistent evidence across all three tubular biomarkers. The biomarker ACR, which is primarily associated with glomerular damage, did not demonstrate these patterns.

Overall, the results suggest some evidence of early-stage kidney damage in adolescents in Nicaragua that appears to extend to all regions studied, but with higher frequency in Chichigalpa. It is important to note that a study such as this one, in which apparently healthy adolescents are tested in a community setting using novel biomarkers of kidney injury is unique. We are not aware of any other studies of its kind. The results of the study are provocative, yet at the same time puzzling in some ways. Owing to the uniqueness of the study, as well as the relatively small number of participants, we believe it is important to consider these results as preliminary and avoid drawing firm conclusions until we and/or other researchers have had a chance to replicate them and further consider issues that may have arisen in the design, implementation, or analysis of the study.

SUMMARY OF FINDINGS

In the Scoping Study (December 2009), we identified the following hypotheses that merited further investigation: exposure to agrichemicals; volume depletion and muscle damage; systemic infections such as leptospirosis, hantavirus and malaria; exposure to heavy metals; exposure to aristolochic acid; medications; alcohol consumption; kidney stones and structural kidney disease; diabetes; hypertension; glomerulonephritis; urinary tract infection; and genetics.

During the past two years, we conducted six research activities that have allowed us to investigate many (but not all) of these hypotheses. Above we described our results by research activity, but here we summarize our findings by hypothesis.

- *Exposure to agrichemicals.* As part of the IH Assessment, we conducted an extensive literature review of 21 agrichemicals that are currently used at ISA (based on information from ISA) and another 15 agrichemicals that may have been used at ISA in the past (based on information from ISA, former workers, and other sources). Based on our review of the medical literature, we did not find evidence that any of the 36 agrichemicals are generally accepted causes of CKD. However, the results of the literature review indicated that two of the 36 agrichemicals (2,4-D and paraquat dichloride) have ‘strong’ evidence of an association with acute kidney damage in humans or animals under certain exposure scenarios. Four others (captan, cypermethrin, glyphosate and DBCP) were determined to have ‘good’ evidence of an association with acute kidney damage in humans or animals under certain exposure scenarios. The remaining 30 agrichemicals were determined to have limited or no evidence of an association. The results of this literature review do not rule out the possibility that one or more of these agents might in fact cause CKD, but new scientific knowledge and insights will be necessary to establish whether any link actually exists.

As part of the environmental sampling effort, we analyzed water samples for 56 agrichemicals and found that only four were present at concentrations above analytical detection limits (2,4-D; beta-BHC; delta-BHC; and heptachlor). However, at the time samples were collected, none of the 56 agrichemicals were present at concentrations above US EPA drinking water criteria.

As part of the biological testing of ISA workers, we found that biomarkers of kidney injury and CKD were generally highest among cane cutters and seed cutters, while pesticide applicators were consistently found to have concentrations that were among the lowest by job. We also found that other occupations (miners, port workers, and construction workers) with no occupational exposure to agrichemicals also had high biomarkers of kidney injury and CKD. However, these assessments of job do not specifically investigate the potential relationship between agrichemical exposure and the risk of developing CKD.

- *Volume depletion and muscle damage.* In the worker biological testing study, biomarkers of kidney injury and CKD were generally highest among cane cutters and seed cutters, two groups of field workers that were identified in the IH report as having the highest potential for volume depletion and muscle damage. In addition, other occupations (miners, port workers, and construction workers) involving strenuous manual labor also had high biomarkers of kidney injury and CKD. However, our activities to date did not specifically include an assessment of volume depletion and muscle damage.
- *Systemic infections.* We were able to obtain supplementary funding outside of the dialogue process to measure leptospirosis antibodies in the biological samples that were collected as part of the biological testing effort. Once we receive the leptospirosis data from the analytical laboratory at the U.S. Centers for Disease Control, we will evaluate differences in infection rates by job and explore relationships with biomarkers of kidney injury and CKD. Once available, these results will be summarized in a report, shared with the dialogue participants, and eventually made public. We were not able to address other infectious agents such as hantavirus, malaria, or sexually transmitted diseases as part of the research activities conducted to date.
- *Exposure to heavy metals.* As part of the environmental sampling effort, we analyzed water

samples at ISA for 14 metals and found that the metals associated with CKD were not detected and that only two were present at concentrations above analytical detection limits (zinc and copper). In addition, at the time samples were collected, none of the 14 metals analyzed were present at concentrations above US EPA drinking water criteria. Additionally, based on the results of the biological testing effort, there was no evidence that lead, cadmium, or uranium (metals associated with CKD development) were associated with biomarkers of kidney injury or CKD in persons tested. However, there was some evidence that high exposure to arsenic (total) is associated with biomarkers of CKD (i.e., lower eGFR)⁶.

- *Exposure to aristolochic acid.* We were not able to address this hypothesis as part of the research activities conducted to date.
- *Medications.* Interviews with health professionals indicate that patients with CKD or at high risk for CKD may be taking nephrotoxic drugs. In particular, aminoglycoside antibiotics and chronic use of NSAIDs are associated with AKI in a dose- and duration-dependent manner. This association is particularly notable in the setting of volume depletion, which may be further aggravated due to the use of diuretics. Information obtained from these interviews suggests that diuretics, antibiotics and NSAIDs are widely used and sold over the counter for symptoms that may be related to volume depletion; accordingly, acute kidney damage coupled with volume depletion should be further evaluated as causal factors for CKD in this region.
- *Alcohol consumption.* Self-reported alcohol consumption was not associated with biomarkers of kidney injury or kidney function among workers at ISA or in other industries. However, since previous investigations in the region have found that self-reported alcohol consumption is associated with increased risk of CKD, alcohol consumption should continue to be evaluated as a potential risk factor in future investigations.
- *Kidney stones and structural kidney disease.* We were not able to address this hypothesis as part of the research activities conducted to date.
- *Diabetes, hypertension, and glomerulonephritis.* During informational interviews, health professionals in Western Nicaragua shared their belief that diabetes and hypertension cannot explain the CKD epidemic. Data from the medical record abstraction revealed a low prevalence of diabetes and hypertension. In addition, findings of low-grade proteinuria and elevated white blood cells in the urine of those tested in clinics indicate the underlying cause of CKD represents a tubulointerstitial rather than glomerular process. Our biological testing results also suggest that the type of kidney damage occurring in ISA workers, as well as among workers in other industries, is primarily tubulointerstitial in nature. Additionally, protein and glucose were only rarely detected in urine, providing little evidence of glomerular disease. This finding is important because the primary causes of CKD globally are diabetes and hypertension, which more often result in glomerular disease manifestations.
- *Urinary tract infection.* Physicians and pharmacists report a high occurrence of diagnosed UTIs

⁶ Total arsenic in urine includes all species of organic and inorganic arsenic. Arsenic speciation (inorganic versus organic) was not possible as part of this investigation but should be considered in future studies. We have not yet investigated exposure to mercury, which could also be worth considering in future studies.

among men, a strange phenomenon given that UTIs among men of working age in the global population are quite rare. We cultured urine samples from 50 men who were positive for leukocyte esterase and/or reported having symptoms during the past 24 hours, yet none of these men were found to have a positive urine culture. Additionally, nitrites were very rarely detected in urine, further supporting the evidence that UTIs are improperly diagnosed.

- *Genetics.* We were not able to address this hypothesis as part of the research activities conducted to date. However, we have been in communication with potential collaborators who have expertise in this area.

FUTURE RESEARCH

During the course of our research in Nicaragua over the past three years, we have learned that CKD epidemics with strikingly similar characteristics are evident in other Central American countries such as El Salvador, Guatemala, and Costa Rica, as well as in other tropical regions such as Sri Lanka and India. Agricultural workers in tropical developing countries work in conditions that predispose them to a combination of chronic volume depletion, muscle damage, and exposure to agrichemicals. While chronic volume depletion and muscle damage alone are unlikely explanations for the CKD epidemic, such factors may magnify the effect of low-level exposure to nephrotoxic agents that alone would not result in CKD. Such low-level exposures might occur at work but could also be due to non-occupational factors that are ubiquitous in a region and cause repeated subclinical acute kidney injury that only progresses to CKD in subpopulations that also experience chronic volume depletion and/or muscle damage. It remains unclear whether repeated subclinical kidney injury can progress to CKD, but this is an important question that requires attention.

The idea that subclinical kidney damage may be starting before people enter the workforce was strengthened by the results of the adolescent biological sampling study, and suggests that early life environmental, medical, and/or genetic factors are also an important area for future research. In the Scoping Study, we downplayed the value of studying the potential for a genetic role in the epidemic, because we thought there was not enough known about the genetics of CKD to warrant such an effort. However, since that time, there have been important advances in research on genetic risk factors for non-diabetic CKD⁷. As a result, we now think that efforts to determine whether there is a genetic component to the epidemic now rate a higher priority.

Considering all of the findings to date, the BU team is in agreement that the highest priority research activities are to conduct studies to

1. Investigate the potential combined effect of chronic volume depletion and muscle damage in workers on biomarkers of kidney injury and CKD.
2. Further investigate the biomarkers of subclinical kidney damage in adolescents; and
3. Investigate the role of genetics, if any, in development of CKD.

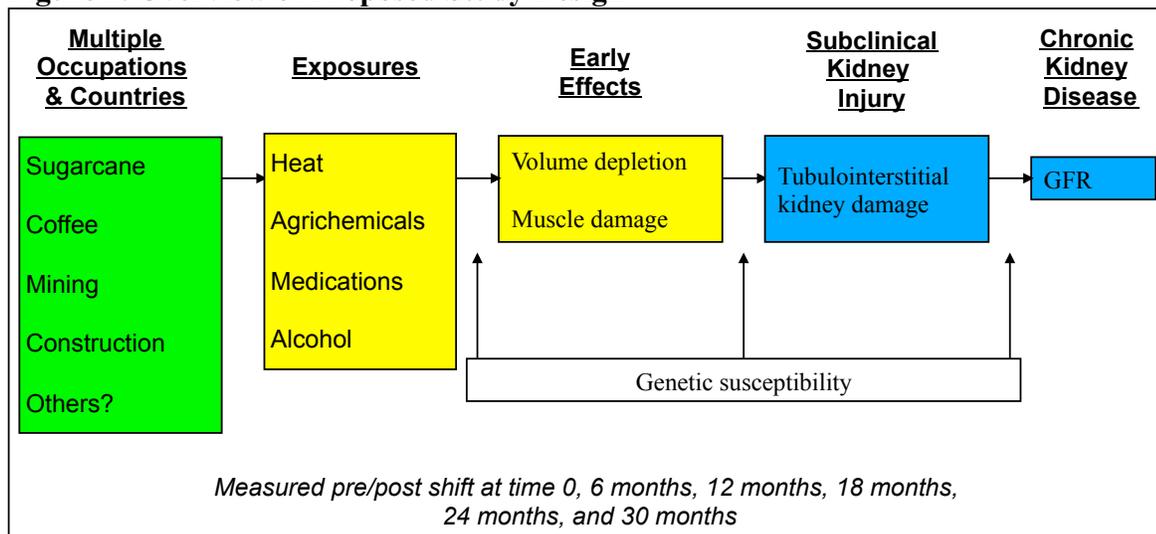
The following sections briefly describe these three studies.

⁷ See Friedman DJ, Pollak MR. Genetics of kidney failure and the evolving story of APOL1. *J Clin Investigation* 2011;121(9):3367-3374.

Study 1: Investigation of kidney injury and CKD development in workers: We are designing the worker investigation so that we can also investigate the potential for repeated subclinical kidney injury to progress to CKD. Given the increasing evidence that the epidemic is not limited to only sugarcane workers or only to Nicaragua, we feel it is important to conduct this effort in multiple industries (including sugarcane) and in multiple Central American countries (including Nicaragua).

Figure 1 presents an overview of the proposed study design. We plan to recruit workers from multiple industries (e.g., sugarcane, coffee, construction) in three different Central American countries (likely Nicaragua, Guatemala, and El Salvador). We will follow a cohort of workers (representing multiple occupations and regions) and obtain repeated measures of exposure to heat and agrichemicals (exposure/dose), volume depletion and muscle damage (early effect), tubular markers (subclinical kidney injury), and eGFR (CKD) at 6-month intervals over a 30-month period (6 rounds of analysis). Biological samples will be collected at pre-shift and post-shift during each round of sampling, allowing us to assess biomarkers of volume depletion, muscle damage, and pesticide exposure.

Figure 1. Overview of Proposed Study Design

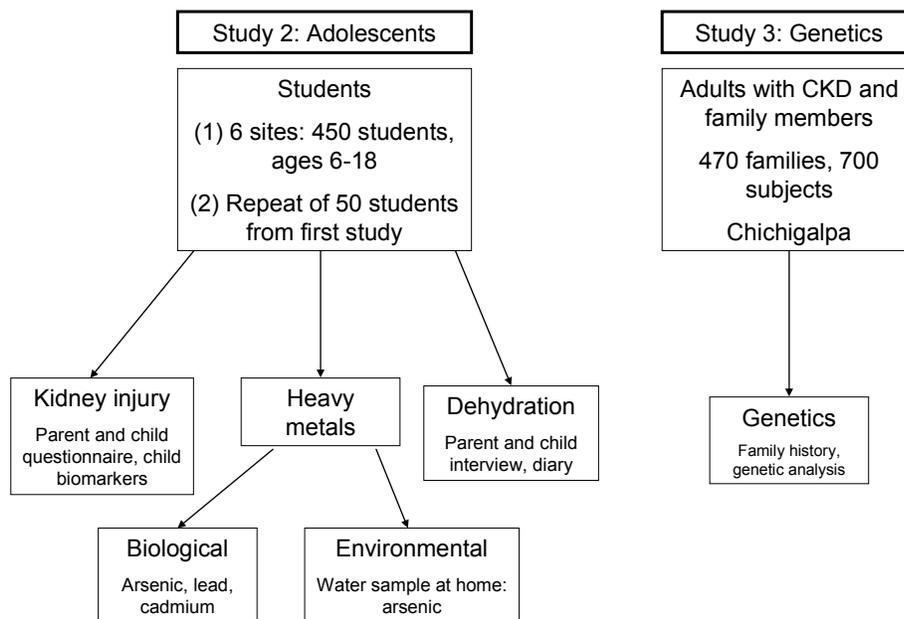


Though the primary specific aims will be to investigate the role of volume depletion and muscle damage, the same study could also be used to address the role of other factors in CKD development such as agrichemicals, genetics, nephrotoxic medications, and/or alcohol consumption.

Study 2: Further investigation of adolescents. As noted before, the adolescent biological testing study found some evidence that kidney injury may be starting during childhood. Because of the uniqueness of the methods used in the study and the fact that there were some counterintuitive findings, we would like to continue to investigate the pattern of early kidney injury in children to determine if our preliminary findings are accurate and to develop a better understanding of kidney injury among Nicaraguan youth. We would like to conduct another study of early kidney damage among adolescents to see if our results can be replicated. In addition, we would like to collect samples from younger children to see if there is evidence of kidney damage at an even earlier age. At the same time, we would like to use the same study population to assess hypotheses regarding factors occurring during childhood, specifically heavy metals and chronic volume depletion. An outline of a possible study is shown in Figure 2.

Study 3: Genetic risk factors. As previously noted, the new knowledge gained by researchers about genetic susceptibility to non-diabetic CKD makes it important to consider genetics as a potential contributor to the excess of CKD in Central America. Therefore, we would like to better understand the patterns of disease in families and conduct genetic analysis. The best study design for this purpose is a population in which CKD is common and in which individuals with the disease are easily identified and family members are accessible. Based on these criteria, we think that Chichigalpa would be an excellent location for this study. An outline of a possible study is also shown in Figure 2.

Figure 2. Overview of studies 2 (adolescents) and 3 (genetics)



In addition to the three studies just described, there are other important areas of research that are worth pursuing, including the following:

1. Prospective monitoring of biological sampling cohort: The biological testing of workers involved collection of samples at baseline and approximately 5-6 months later for ISA workers and at a single time for workers in the other industries. We would like to follow up with these workers and collect additional information to determine how their renal health status may have changed over time. Following these workers would also give us the chance to learn more about the activities of ISA workers during the period of time they are not working at ISA as well as how workers in the other industries may change the type of work they do, which is important for understanding their overall exposure to occupational risk factors.
2. Fructose consumption: There is some evidence in laboratory tests on animals that excessive fructose consumption, combined with volume depletion, may lead to CKD. Some of the samples collected from workers and adolescents during our investigation could be sent for

analysis to the University of Colorado at Denver, where a leading expert in the field would analyze these samples to test this hypothesis.

3. Prevalence study in Rivas: We are providing assistance to a Boston-area researcher planning to conduct a study of the prevalence of CKD in Rivas. This would be the only population-based prevalence study in Nicaragua conducted outside of León and Chinandega except for a study in the city of Managua in 2003, and would help to characterize the extent of the epidemic. The Rivas Comprehensive Health Service System (SILAIS) is sponsoring the study.
4. Hydration practices away from work: One factor potentially affecting volume depletion and susceptibility to CKD is fluid consumption away from work. We would like to gain a better understanding of the amount and type of fluids consumed by community residents.
5. Cause of UTI symptoms: Having established that the urinary symptoms common among young men don't appear to be UTI, their cause and whether it is at all related to kidney damage remains unanswered. We would like to do additional testing and analysis to try to learn more about this condition.
6. CKD progression: A better understanding of the factors that affect the progression of CKD could help improve the medical care and survival of individuals who are diagnosed at a relatively early stage. We would like to better understand the natural history of the disease and determine whether there are any as yet unidentified factors that impact positively or negatively on the rate of progression.
7. Improve surveillance in region: The systems to understand the most basic facts about the CKD epidemic—whether it is increasing, decreasing, or remaining at the same level—are technically possible but not in place. We would like to assist in setting up and evaluating such a system.