Risk Exposure to Agents Causing Waterborne Diseases in the El Ahogado Basin, Mexico

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ABSTRACT: The risk exposure to agents causing waterborne diseases (WBD), such as bacteria, parasites, and metals (Cr, Cd, Mn, and Pb) in a group of volunteers ($n = 20$) and an external group of three locations of the El Ahogado Basin, Mexico, is reported. A complete blood count (CBC) was made on the volunteers, and factor analysis was used for the CBC. Bacteria and parasitological agents were also monitored at the six sites of the El Ahogado Basin between 2008 and 2010. The measured blood concentrations of Cu, Mn, and Pb were 37.1, 4.6, and 7.1 μg/g, indicating the lowest exposure of volunteers to Mn and Pb and the highest exposure to Cu. The volunteer groups of the El Ahogado Basin are more exposed to agents causing WBD compared to the volunteers of the external group. *Water Environ. Res.*, **85,** 2175 (2013).

KEYWORDS: cross-sectional study, environmental epidemiology, biomarkers, unsafe water, water quality.

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Introduction

In many parts of the world there are people who are in contact with unsafe water. Because of the direct use of water from both rivers and canals in agricultural areas, inadequate treatment systems of surface waters, and poor water distribution in underdeveloped countries, there is significant morbidity caused by waterborne diseases (WBD) (Galea and Vlahov, 2005; World Health Organization and United Nation Children's Fund Joint Monitoring Programme for Water Supply and Sanitation, 2005; Pimentel et al., 2007; Imagawa et al., 2010). Human diseases and intoxications are produced by unsafe water (Arnone and Walling, 2007). Infectious diseases transmitted by water are caused by exposure to enteric pathogens, such as viruses, bacteria, protozoa, and helminthes. The primary sources of these enteric pathogens are the discharges of wastewaters into natural surface water resources (Straub and Chandler, 2003; Craun and Calderon, 2006; Chandran et al., 2011). Unsafe water also contains metals that originate from natural processes, or from industrial or municipal discharges (Chary et al., 2008; Singh et al., 2010). Humans can be exposed to metals through various routes, such as the ingestion of contaminated water, or through the ingestion of small soil particles that can be deposited on unwashed vegetables and fruits (Miller et al., 2006; Castro-González and Méndez-Armenta, 2008). The common symptoms of infections caused by enteric waterborne pathogens are diarrhea, nausea, and cramps; for metal intoxication, an overexposure of Cu, Pb, Cd, and Mn can yield gastrointestinal outcomes.

The assessment of human exposure to environmental pollutants causing diseases, such as metals, can be made by either monitoring these agents in the environment through human wastes, or through human body fluids and tissues. In environmental epidemiology studies, the use of biomarkers (mainly blood and urine) provides meaningful data for the assessment of exposure to metals of individuals, groups, or populations (Christensen, 1995; Wilhelm et al., 2004; Smolders et al., 2010; Cortes et al., 2011).

Most of the observational epidemiologic studies about waterborne risks are relatively recent, and have been focused on groundwater and drinking water consumption (Craun and Calderon, 2006). These works include cohort, case-control, and ecological studies (Fraser and Cooke, 1991; Nchito et al., 1998; Strauss et al., 2001; Robertson et al., 2002; Kapperud et al., 2003; Zmirou-Navier et al., 2006). However, environmental epidemiology studies must consider surface waters that transport water from run-offs, sewages, and other types of wastes. When the latter types of studies are conducted, it is possible to assess whether exposure to environmental pollutants causes morbidity of a given population. Furthermore, these studies can also provide important information to help understand the health risks, and to locate potential sources of pathogens and metals when run-offs and sewages are included. Consequently, it is necessary to conduct additional environmental epidemiology studies for the control of emerging and re-emerging WBD (Chugh, 2008) that include indicators such as pathogens, protozoa, helminths, and metals associated with health disorders by direct or indirect contact with surface waters.

The use of a general indicator for health and nutritional status of an individual for the assessment of possible disorders caused by pathogens, parasites, and metal exposure is the complete blood count (CBC) (González-Madroño et al., 2011; Ramzan et al., 2011). For example, white blood cells significantly increase during a helminth infection (eosinophilia) or bacterial infection (neutrophilia) (Campbell and Smith, 1996), and can be detected by a CBC. Also, the presence of Cd causes eosinophilia (Flora, 2009), and poisoning by Pb and Cd cause anemia (Fonte et al., 2007). The presence of these metals can be associated with the

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levels of hemoglobin and hematocrit (Ahamed et al., 2007) and can be measured by the red blood count of a CBC. Thus, indicators associated with disorders by direct or indirect contact with unsafe water can be measured indirectly by a CBC.

In many underdeveloped countries like Mexico, water bodies are used as captors of wastewaters of which people have easy access, thus establishing contact with unsafe water. One example is Metropolitan Guadalajara (Mexico's second largest city) which spans six municipalities (counties), four of which are geographically located within the El Ahogado Basin. This basin is characterized by unplanned industrialization and urbanization (Córdova et al., 2007) where significant agricultural activities exist; the entire transportation system of wastewater consists of open canals along which human settlements have developed, facilitating human contact with wastewater.

The aim of this study is to report about the presence of indicators comprised of bacterial enteric pathogens (genera *Vibrio, Aeromonas, Salmonella, Shigella, Pseudomonas,* and *Legionella*), parasites (protozoa and helminths), and four metals in three volunteer groups from locations in the El Ahogado Basin, as well as one external group outside the basin (which was not taken as a control group) used to compare the results. Therefore, this work promotes the application of environmental epidemiology studies in areas with urban developments, and where industrial and agricultural activities exist. It is anticipated that in the future, this work can be adapted to other basins in Mexico and Latin America where transportation of wastes through open air canals persists. The assessment was a cross-sectional study that included routine stool tests, CBC, and analyses of whole blood of the biomarkers Pb, Cd, Cu, and Mn (metals associated with municipal and industrial dischargers), which are associated to hematological and gastrointestinal outcomes.

Methods

Study Area and Localities Selected for Sample Collection of Volunteers. The El Ahogado Basin covers an area of approximately 520 km^2 and is located south of Metropolitan Guadalajara (Figure 1). The population density is approximately 100 inhabitants per hectare (Comisión Estatal del Agua y Saneamiento, 2003; Instituto Nacional de Estadística y Geografía, 2005a, 2005b). Approximately 50% of the population lacks sanitation infrastructure and many villages or towns have no drinking water supply. Most of the drinking water (approximately 60%) comes from Lake Chapala (Comisión Estatal del Agua y Saneamiento, 2003). In this basin, there are canals that transport wastewater to the open air, along which are human settlements; these canals are not separated by physical barriers, nor do they have appropriate signalization. Three locations were selected for the study of pathogens, biomarkers, and CBC as shown in Figure 1: Santa Cruz del Valle in the municipality of Tlajomulco, Las Pintas de Arriba in the municipality of Tlaquepaque, and San José del Castillo in the municipality of El Salto.

The Santa Cruz del Valle location is characterized by almost no urban infrastructure. The streets are unpaved, most of the residents have no sewer infrastructure, and drinking water is supplied by special trucks or from wells inside the houses. Santa Cruz del Valle is located between Las Pintas Canal and Arroyo Seco (two surface water bodies of the El Ahogado Basin). In some sections of Santa Cruz del Valle, sugarcane, corn, some

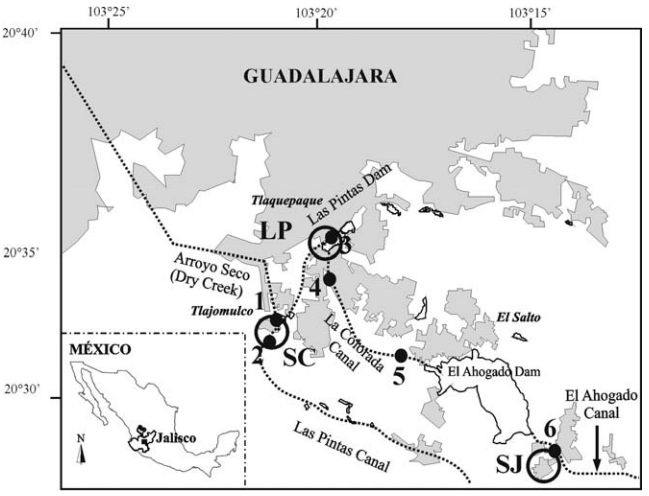

Figure 1—Study area and locations of sites and localities sampled at El Ahogado Basin. Sites of water bodies are numbered, as defined in Table 1. Groups of volunteers defined by circles (SC = Santa Cruz del Valle; LP = Las Pintas de Arriba; SJ = San José del Castillo).

vegetables, and root crops are grown. The Las Pintas de Arriba location is a settlement where a variety of activities exist, such as the buying and selling of used items, brick making, cattle raising, and general trade. Most homes have municipal water and sewer services. In this area there are three water bodies: Las Pintas Dam, and the canals of Las Pintas and La Colorada. Until 1991, water from Las Pintas Dam and Las Pintas Canal was used to satisfy the water demands of Metropolitan Guadalajara. However, given the increasing demand for water, this canal was reopened in April 2011. The unused water of the dam is discharged into the La Colorada Canal, which also captures the wastewater generated by residents and businesses. The San José del Castillo location is south of the primary industrial corridors of Metropolitan Guadalajara. The predominant activity is informal commerce with many street vendors, and with small houses built of inexpensive materials, some with sheet metal or tile roofs. Not all houses have drinking water or electricity. North of San José del Castillo is the El Ahogado Dam that receives water from the La Colorada Canal and discharges from the Guadalajara international airport. The dam also receives treated and untreated wastewater from industrial corridors of the municipality of El Salto. Water from the dam is discharged into the El Ahogado Canal and, finally, into the Santiago River (not shown on Figure 1 map).

Sampling Sites for Water Analyses. Six sites of the El Ahogado Basin were selected to collect water samples for the analysis of generic pathogenic bacteria associated with waterborne diseases as shown in Figure 1: Arroyo Seco (Dry Creek) (site 1), Las Pintas Canal (site 2), Las Pintas Dam (site 3), two sites at the La Colorada canal (sites 4 and 5), and the El Ahogado Canal (site 6). The sample collections were made in August 2008 and April 2010. The water samples for enteric bacteria identification were collected using Moore swabs and sterile glass bottles. For parasite analysis (amoeba, pathogenic intestinal eggs, oocysts, and larval forms), samples were collected in previously treated (HNO_3, 15% v/v) three-liter polyethylene containers.

Selection and Sampling of Volunteers. To assess the exposure of volunteer groups to unsafe water, three groups of inhabitants were selected who lived within 1000 meters of the wastewater captors. A total of 80 volunteers were selected: 20 at each of the three locations, and 20 outside of the El Ahogado Basin. The groups were identified according of the name of the location to which they belonged, as shown in Figure 1: Santa Cruz del Valle, Las Pintas de Arriba, and San José del Castillo. The external group of volunteers was selected as a non-exposure group, and the results of this group were used to compare the exposure indicators of unsafe water to the other groups. An informed consent signature was obtained from all participants. The inclusion criteria used for this study were: (a) the ages of individuals were between 10 and 20 years old, (b) the individuals lived within 500 to 1000 meters from a wastewater captor body, (c) the individuals resided in the area a minimum of 10 years, (d) individuals were not under any medical treatment, (e) all individuals were nonsmokers, and (f) for women, no individual was pregnant or nursing. For underage volunteers, permission was requested and granted by a parent or guardian before participation in the study. The inclusion criteria were based on the assumptions that the volunteers were healthy, but exposed directly or indirectly to unsafe water. The external group volunteers were selected by using the same inclusion criteria, with the exception that they resided in the Atemajac Basin north of the Guadalajara metropolitan area, and were not living near any open wastewater canal. This study was not blind and the volunteers were informed about the purpose of the study. The results of the stool analysis, CBC, and biomarkers were subsequently provided to the volunteers.

Water Samples for Isolation and Identification of Pathogens. For the recovery of *Salmonella, Shigella, Vibrio,* and *Aeromonas* genera, water samples were taken using Moore swabs (Bravo Fariñas et al., 1989; Orta de Velázquez et al., 2002; Fernández et al., 2003). One Moore swab was used for each pathogen with the exception of *Vibrio* and *Aeromonas*, which were investigated using the same swab. For the analysis of *Pseudomonas* and *Legionella*, a one liter sample was collected in a sterile bottle. The *Salmonella* swab was cultured in lactose broth for enrichment, and incubated at 35 °C for 24 hours. For the selective enrichments, broths of selenite, cysteine, and tetrathionate were used, which were incubated at 42.4 °C for 24 hours. A loopful of each enrichment broth was streaked on selective bismuth sulfite agar, *Salmonella-Shigella* agar, and brilliant green agar. For *Shigella,* the swab was enriched in gram negative Hanja broth at 35 °C for 24 hours and isolated in *Salmonella-Shigella* agar, brilliant green agar, and MacConkey agar. Both *Salmonella* and *Shigella* agars were incubated at 35 ± 0.5 °C for 24 hours. For *Vibrio* and *Aeromonas*, the selective enrichment was completed in alkaline-buffered peptone water and incubated at 35 ± 0.5 °C for different lengths of time (7, 18, and 24 hours). For each time period, a loopful of alkaline peptone water was streaked on thiosulfate-citrate-bile-sucrose agar and on starch ampicillin agar (1% v/v). The agar plates were incubated at 35 °C for 24 hours. *Pseudomonas* and *Legionella* were investigated using one milliliter aliquots of the sample collected in a sterile bottle. *Pseudomonas* was cultivated using plates of cetrimide agar (1% glycerol) and MacConkey plates, both incubated at 35 ± 0.5 °C for 24 hours. The *Legionella* aliquot was treated with one milliliter of 0.02 N H_2SO_4 (to remove associated biota), and cultivated in BCYE (buffered charcoal yeast extract) agar supplemented with L-cysteine and GVPC (glycine-vancomycin-polymyxin and cyclohexamide supplement) (Merck KGaA, Darmstadt, Germany), then incubated at 35 ± 0.5 °C in a CO_2 atmosphere for 5 to 10 days. All the procedures for isolation and biochemical identification of pathogens, both in water and stool samples, followed diagnostic tests for pathogens (Forbes et al., 2002; Winn et al., 2006).

For pathogen presumptive biochemical identification, the tests of oxidase, catalase, sulfide-indole motility, Voges Proskauer, citrate, triple-sugar iron agar, and lysine dehydrolase were applied to pathogens isolated from the water samples. For *Aeromonas*, the amylase test was also performed. The cetrimide agar was subjected to ultraviolet light (254 nm) to observe fluorescence, typical of *Pseudomonas* in this medium. The identification of pathogens was confirmed with the API 20E system (BioMérieux, Marcy l'Etoile, France), and the Duopath test (Merck KGaA) was used to confirm the genus *Legionella*.

Processing of Stool Samples for Pathogen Analyses. For the serial stool analysis (ova and parasite examination), three samples were collected from each volunteer every other day. The samples were kept cold in a plastic box with ice packs while being transported to the laboratory. The pathogen investigations in stool were conducted by direct and indirect stool-culture methods (Winn et al., 2006). For the direct method, two grams of stool were suspended in 10 milliliters of physiological saline solution, and two loopfuls were inoculated in liquid broths and agar plates. Green brilliant agar, bismuth sulfite agar, *Salmonella-Shigella* agar, and cetrimide agar were inoculated and incubated at 35 ± 0.5 °C for 24 hours. For the indirect method, the investigation of pathogenic bacteria followed the methodology of the direct method, except that a previous selective enrichment procedure on the stool samples was applied. Presumptive and confirmatory pathogen identification in stool samples was performed using the same procedure as was used for isolation and identification of pathogens in water samples.

Processing of Water and Stool Samples for Ova and Parasite Examination. The water samples were left to settle for 24 hours at 4 °C, then separated by decantation; the supernatant liquid was then discarded. The resulting sludge (approximately 100 milliliters) was used in subsequent analyses. In the case of stool samples, approximately one gram of feces was used to prepare a suspension containing 10 milliliters of saline solution. Three different techniques (direct mount, flotation-sedimentation with $ZnSO_4$ [33.3% v/v], and formol-ether concentration) were applied to both water and stool samples (Navone et al., 2005) for ova and parasite examination. For *Cryptosporidium* identification, the alcohol-acid coloring technique of Ziehl-Nelsen was used (Hycel, Monterrey, México). The microscopic observations were made with a Leica CME microscope at 10 and 40 × for identification of ova and parasitic forms (Leica Microsystems Inc., Buffalo, New York).

Blood Test and Biomarkers. Blood samples for the CBC and biomarkers were collected from the cubital vein into a vacutainer containing K_2 EDTA (ethylenediamine tetra-acetic acid) and heparin as anticoagulants (BD Vacutainer, Franklin Lakes, New Jersey). The blood samples for CBC were processed the same day. The blood samples for metal analysis were stored at –20 °C until subsequent digestion and analysis. Samples from the group of volunteers were collected from January to November 2009. The CBC was made using a five milliliter blood sample in an Ac.T Coulter (Coulter Corp., Miami,

Table 1—Presence of pathogens and parasites at six sites of El Ahogado Basin in surface water during four sampling campaigns (2008 to 2010).

	Site					
	Arroyo Seco #1	Las Pintas Canal #2	Las Pintas Dam #3	La Colorada Canal #4	La Colorada Canal #5	El Ahogado Canal #6
Vibrio spp.	+	+	+	+	+	+
Aeromonas spp.	+	+	+	+	+	+
Salmonella spp.	+	−	−	+	−	+
Shigella spp.	−	−	+	−	−	−
Pseudomonas spp.	+	+	+	+	−	+
Hookworm	+	−	+	+	+	−
Strongyloides stercolaris	−	−	+	−	+	−
Ascaris lumbricoides	−	−	+	−	−	−
Entamoaeba coli	+	−	+	−	−	+

Florida). The variables evaluated were: leukocyte count (white blood count), red blood count, hemoglobin, hematocrit, mean corpuscular volume, corpuscular hemoglobin concentration, mean concentration corpuscular hemoglobin, and platelets. The leukocyte differential count was manually obtained in a Wright smear stain (Hycel) at 100 × objective by a manual cell counter. The cells quantified were lymphocytes, monocytes, neutrophil, eosinophils, basophils, segmented, and bands.

Heparinized blood was used for the metal determinations. The samples were digested following NIOSH method 8005 (National Institute for Occupational Safety and Health, 1994). Briefly, ten milliliters of each whole blood sample, previously tempered and homogenized, were placed in a flask, and a five milliliter mixture of HNO_3, $HClO_4$, and H_2SO_4 at a ratio of 3:1:1 v/v (Sigma-Aldrich, St. Louis, Missouri) was added. Digestion was conducted by placing the samples on a hot plate at 110 °C for two hours, and the heat was then increased to 250 °C. The final product (one to two milliliters) was diluted to 10 milliliters with deionized water and placed in previously treated (HNO_3, 15% v/v) 15-milliliter conical tubes (BD Falcon, Baltimore, Maryland). Detection and quantification of total metal in whole blood was made by an Agilent 7500 series ICP-MS system (Agilent Technologies, Palo Alto, California). The calibration curves were constructed using a blank (HNO_3, 5% v/v) and seven standard concentrations (0.0005 to 0.5 mg/L^{-1}) from a Multi-element Calibration Standard 3, 10 ppm multimetallic solution (PerkinElmer, San Jose, California). For quality control purposes, the Standard Reference Material (SRM) 1577c lyophilized bovine liver (NIST, Gaithersburg, Maryland) was used. The standard was digested the same way as the blood samples from volunteers. Commercially available deionized-water trace select was used (Hycel) for dilution, preparation of standards, and washing of equipment. The recoveries for SRM 1577c were determined.

Data Analysis. The data of water analyses, stool, and blood generated were subjected to different statistical treatments. The presence of pathogens (frequencies expressed as percentages) was calculated for various pathogenic bacteria found in the surface water bodies and in the volunteer groups. The relative risk per location was calculated with the results of parasitological analysis in stool samples using Epi-Info software (Centers for Disease Control and Prevention, Atlanta, Georgia). The relative risk is a measure of causality association, and is used in prevalence outcomes in cross-sectional studies (Schmidt and Kohlmann, 2008). The relative risk compares the frequency of occurrence of the damage among individuals having a risk factor, and those who do not have any risk. According to relative risk, the risk of acquiring parasitosis is higher and a causal relationship exists if the lower limit of the confidence interval is > 1; if the lower limit of the confidence interval is ≤ 1, the causal relationship is not present. An analysis of variance (ANOVA) was performed on the data generated from the CBC and biomarkers using StatGraphics Centurion XV software to conduct the Duncan test (Statpoint Technologies Inc., Warrenton, Virginia) to find statistically significant differences in the four volunteer groups. Additionally, factor analysis with varimax rotation was applied to CBC and biomarkers data to extract the most representative components in all blood variables analyzed.

Results

Microorganisms in Surface Water Samples. The recoveries of pathogenic bacteria were higher during August 2008 and 2009. The results shown in Table 1 indicate the presence of bacteria and parasite pathogens in all sampled sites. However, it can be seen that the presence of pathogens was not homogeneous at all sites; the frequencies of *Vibrio* spp., *Aeromonas* spp., and *Pseudomonas* spp. were 37, 32, and 18%, respectively, whereas the frequencies of *Salmonella* spp. and *Shigella* spp. were lower (11 and 3%, respectively). *Legionella* spp. was not found in any sample. The recoveries of parasites were scarce for both protozoa and ova or larvae. *S. stercoralis* and hookworm were morphologically identified in water samples from sites 1, 3, 4, and 5 in the first two sampling campaigns (August 2008 and April 2009). Hatched eggs of *A. lumbricoides* were found at site 3. *Entamoaeba coli* was found at sites 1, 3, and 6, and were the only protozoa identified in April 2009. *Cryptosporidium* oocyst was not observed in any sample.

Microorganisms in Stool Samples. The frequency of pathogenic bacteria in samples from all volunteer groups was limited. Only one volunteer with *Salmonella* spp. was found at Santa Cruz del Valle. Some commensal bacteria were identified in volunteers, and these included *P. aeruginosa*, *E. coli*, *K. pneumoniae*, *P. rettgeri, P. mirabilis, E. cloacae,* and *M. morganii* (frequency not shown). The frequencies of parasitic pathogens in volunteers at Santa Cruz del Valle were corresponded to *E. histolytica*. One single case of helminthiasis (*E. vermicularis*) was found within this group, with the rest of the volunteers having commensal parasites. Common parasitoses were caused by two

Table 2—Frequency of parasites in stool samples of volunteers at three locations and the external group. Frequencies expressed as percentages.

Parasite	SC[a] ($n = 20$)	LP[b] ($n = 20$)	SJ[c] ($n = 20$)	Total	EG[d] ($n = 20$)
Blastocystis hominis	13	21	29	63	22
Endolimax nana	26	24	26	76	17
Entamoaeba coli	23	18	6	47	17
Entamoaeba hartmanni	26	27	23	76	39
Entamoaeba histolytica	7	6	6	19	4
Enterovius vermicularis	1	0	0	1	0
Giardia lamblia	1	3	3	7	0
Himenolephys nana	0	0	3	3	0
Iodamoeba bütschlii	1	0	6	7	0

[a] SC = Santa Cruz del Valle.
[b] LP = Las Pintas de Arriba.
[c] SJ = San José del Castillo.
[d] EG = external group.

or three commensal parasites in volunteers at Santa Cruz del Valle, a situation that repeated in volunteers from the other groups. There were no worms present in stool samples from volunteers of Las Pintas de Arriba, whereas in the San José del Castillo group, only *H. nana* was identified. *Entamoaeba histolytica* and *G. lamblia* were the most frequent pathogenic protozoa in the three groups of the El Ahogado Basin (Table 2). The external group showed lower frequencies of parasitism compared to volunteers from the other three groups. The relative risk for each parasite found in volunteers was calculated (Table 3). The highest relative risk value corresponded to the commensal parasite *E. nana* (4.25; 3.58 to 166.55, 95% confidence interval) in Santa Cruz del Valle, whereas for the noncommensal species, the relative risk changed to *E. histolytica* (5.00; 0.59 to 314.95, 95% confidence interval) for the same location.

CBC and Biomarker. By comparing the results of the blood count from the three volunteer groups to those of the external group, the ANOVA results indicated that there were no statistically significant differences between the averages ($P = 0.3673$, 95% confidence interval) and the median ($P = 0.506$, 95% confidence interval) of the 15 hematological variables measured (Figure 2). However, individual data show that the maximum count of eosinophils was for Santa Cruz del Valle (22%) and San José del Castillo (24%), and in these same locations the lowest hemoglobin concentrations (7 mg/dL^{-1} and 12.3 mg/dL^{-1}) were measured. The results of factor analysis provided further information about blood variables indicated in the volunteers of each group. In this case, the blood variables were classified into four factors: a) infection factor formed by white blood count, neutrophil, eosinophils, lymphocytes, and segmented; b) inflammation factor formed by basophils, bands, platelets, and monocytes; c) malnutrition factor composed of mean corpuscular volume, corpuscular hemoglobin concentration, and mean concentration corpuscular hemoglobin; and d) anemia factor, formed by red blood count, hemoglobin, and hematocrit. For the four four volunteer groups, five components were extracted, which explained 66% of the variability (Table 4). The first component (18%) was formed only by the variables of the infection factor, the second (15%) by the anemia factor, the third component (13%) included the variables of the infection and inflamation factors, the fourth component (10%) was formed by the malnutrition factor, and the last component (10%) included the infection, inflamation, and malutrition factors.

Table 3—Relative risk in three groups of volunteers at locations in El Ahogado Basin and total relative risk of basin (sum of groups). For relative risk values, confidence interval (CI) ranges given in parentheses.

	Cases of El Ahogado Basin	Cases of external group	Relative risk (95% CI)
Santa Cruz del Valle, commensal:			
E. coli	16	4	4.00 (2.80–100.95)
E. nana	17	4	4.25 (3.58–166.55)
E. hartmanni	17	9	1.89 (1.29–46.28)
Santa Cruz del Valle, noncommensal:			
E. histolytica	5	1	5.00 (0.59–314.95)
I. bütschlii	1	0	
B. hominis	9	5	1.80 (0.54–11.93)
G. lamblia	1	0	
E. vermicularis	1	0	
H. nana	0	0	
Las Pintas de Arriba, commensal:			
E. coli	6	4	1.50 (0.32–9.94)
E. nana	0	0	
E. hartmanni	8	9	0.89 (0.19–3.40)
Las Pintas de Arriba, noncommensal:			
E. histolytica	2	1	2.00 (0.10–130.99)
I. bütschlii	0	0	
B. hominis	7	5	1.40 (0.34–8.09)
G. lamblia	1	0	
E. vermicularis	0	0	
H. nana	0	0	
San José del Castillo, commensal:			
E. coli	2	4	0.50 (0.04–3.66)
E. nana	9	4	2.25 (0.67–17.90)
E. hartmanni	8	9	0.89 (0.19–3.40)
San José del Castillo, nonommensal:			
E. histolytica	2	1	2.00 (0.10–130.99)
I. bütschlii	2	0	
B. hominis	10	5	2.00 (0.66–14.50)
G. lamblia	1	0	
E. vermicularis	0	0	
H. nana	1	0	
Basin complete, commensal:			
E. coli	24	4	2.00 (0.73–12.17)
E. nana	33	4	2.75 (1.34–22.07)
E. hartmanni	33	9	1.22 (0.48–4.72)
Basin complete, noncommensal:			
E. histolytica	9	1	3.00 (0.41–154.58)
I. bütschlii	3	0	
B. hominis	26	5	1.73 (0.67–9.05)
G. lamblia	3	0	
E. vermicularis	1	0	
H. nana	1	0	

The concentration levels of metals in whole blood ranged from 5.1 to 37.1 μg/g of Cu, not detected, to 4.6 μg/g of Mn, not detected, to 7.1 μg/g of Pb; one volunteer from Las Pintas de Arriba presented 0.02 μg/g of Cd (Table 5). Statistically significant differences were found between averages ($P < 0.005$, 95% confidence interval). The group from Santa Cruz del Valle showed the highest dispersion compared to the rest of the groups (Figure 3). From the Duncan test ($P \leq 0.05$, 95%

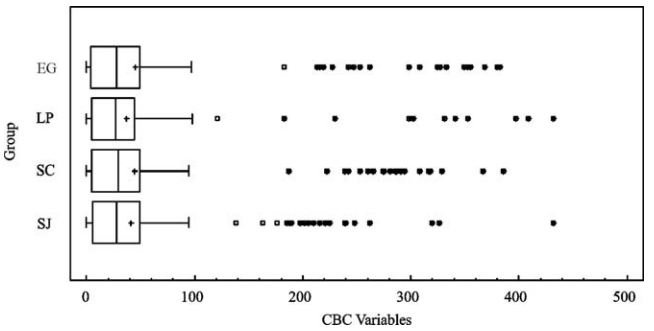

Figure 2—Box plot of complete blood count (CBC) in groups of volunteers of El Ahogado Basin and external group (SC = Santa Cruz del Valle; LP = Las Pintas de Arriba; SJ = San José del Castillo; EG = external group).

confidence interval), two homogeneous groups were found. The first was composed of volunteers from San José del Castillo, Las Pintas de Arriba, and the external group; and the second was composed of volunteers from Santa Cruz del Valle. The distribution of exposure to biomarker was low for Mn and Pb, and remained constant until quartile 3 (Q_3) of the four groups. The Cu concentration increased reaching its highest levels in Q_3 (Figure 4); for this biomarker, more than 15% of volunteers in the four groups were measured with more than 20 μg/g of Cu. From the factor analysis, two components were extracted. The first component represented 52% of the variance and was formed by Pb, Mn, and Cu. The second component included only Cd and represented 25% of the variance. From the first component extracted, loads of Pb (0.93) and Mn (0.87) were the most significant. For quality control, the percent of recovery on a dry matter basis in SRM 1577c was 158% for Cd, 156% for Cu, 213% for Mn, and 125% for Pb.

Table 4—Loading of complete blood count and white blood cell differential at locations and group outside basin, for blood sample data set. Significant loads in bold.

	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
WBC[a]	0.214	−0.037	**0.678**	−0.010	0.155
RBC[b]	−0.073	**0.756**	−0.002	−0.505	−0.086
HGB[c]	0.098	**0.688**	0.085	0.234	0.079
HCT[d]	0.121	**0.823**	−0.070	−0.091	−0.049
MCV[e]	0.188	0.069	0.028	**0.830**	−0.178
MCH[f]	−0.088	−0.059	−0.064	**0.789**	0.062
MCHC[g]	0.001	0.007	−0.013	−0.016	**0.756**
Platelets	0.127	−0.415	0.481	−0.133	0.117
Lymphocytes	**-0.870**	−0.124	0.031	−0.083	−0.134
Monocytes	−0.080	−0.087	−0.778	0.105	−0.062
Neutrophils	**0.921**	0.130	−0.035	0.083	−0.114
Eosinophils	−0.144	−0.086	0.096	0.020	0.743
Basophils	0.054	0.021	0.129	−0.063	**0.650**
Segmented	**0.821**	−0.110	0.266	−0.067	−0.133
Bands	0.338	−0.003	**-0.720**	−0.196	0.023

[a] WBC = white blood count.
[b] RBC = red blood count.
[c] HGB = hemoglobin.
[d] HCT = hematocrit.
[e] MCV = corpuscular volume.
[f] MCH = corpuscular hemoglobin concentration.
[g] MCHC = concentration corpuscular hemoglobin.

Table 5—Statistics of concentrations of biomarkers in four groups of volunteers (concentrations in mg/kg^{-1}).

Biomarker	Group	Mean	Min	Max	SD [a]
Mn	SC[b]	0.8	0.2	4.6	0.9
	LP[c]	0.6	0.3	1.0	0.2
	SJ[d]	0.3	0.0	0.9	0.2
	EG[e]	0.3	0.0	0.5	0.1
Cu	SC	24.5	12.7	37.1	7.3
	LP	15.5	11.7	21.8	3.3
	SJ	11.5	5.1	16.9	3.4
	EG	8.9	5.8	14.9	2.5
Cd	SC	0.0	0.0	0.0	0.0
	LP	0.0	0.0	0.02	0.0
	SJ	0.0	0.0	0.0	0.0
	EG	0.0	0.0	0.0	0.0
Pb	SC	1.7	0.4	7.1	1.5
	LP	1.2	0.5	3.4	0.7
	SJ	0.6	0.0	1.3	0.4
	EG	0.4	0.0	2.6	0.8

[a] SC = Santa Cruz del Valle.
[b] LP = Las Pintas de Arriba.
[c] SJ = San José del Castillo.
[d] EG = external group.
[e] SD = standard deviation.

Discussion

Microorganisms in Water and Stool Samples. The recovery of pathogenic bacteria was higher during the rainy season of 2009 (August) as compared to the other sampling campaigns. The rainy events and WBD were significantly and positively related, and the relationships were much stronger for outbreaks (Arnone and Walling, 2007) caused by an overflow of the surface waters. The presence of pathogens is primarily affected by temperature and ultraviolet light (Brookes et al., 2004) and, therefore, the count of pathogens during the warm and rainy summer was expected. Las Pintas Dam (site 3) showed the highest number of pathogens and parasites. This result can be explained because this water body is a static system, and mixing is low compared to that of canals (sites 1, 2, 4, 5, and 6) even during the rainy season causing higher residence time of

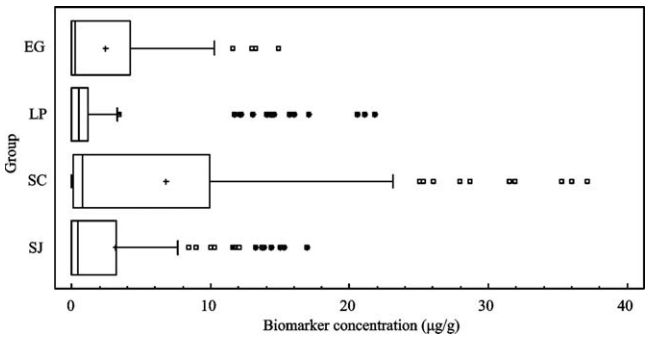

Figure 3—Box plot of biomarkers in volunteer groups of El Ahogado Basin and external group. (SC = Santa Cruz del Valle; LP = Las Pintas de Arriba; SJ = San José del Castillo; EG = external group).

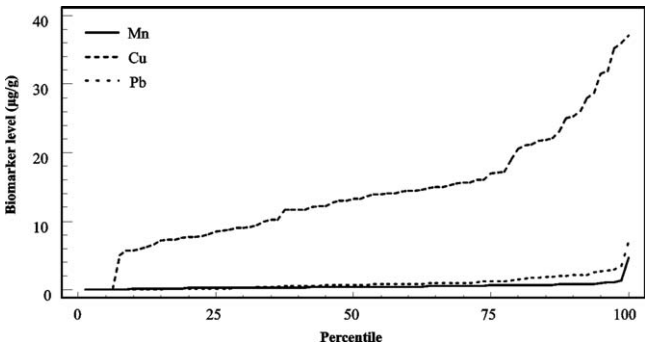

Figure 4—Percentile distributions on biomarkers of total blood samples of volunteer groups of El Ahogado Basin and external group.

substances and microorganisms, thus promoting growth of indigenous flora and pathogens. Another reason for the greater presence of microorganisms at Las Pintas Dam is its geographic location, which is within a zone with different types of activities (commercial, agricultural, industrial), and recreational activities at the dam mainly during summer, causing significant anthropogenic pressure. Recreational activities can contribute to increased pathogen concentrations in reservoirs (Brookes et al., 2004).

Aeromonas spp. and *Vibrio* spp. were recovered from all water samples. The mobile species of *Aeromonas* can be pathogenic to humans, and eleven species of *Vibrio* are pathogenic to humans (Prasanthan et al., 2011). The *Aeromonas* spp. and *Vibrio* spp. are natural inhabitants of the surface water (Zuckerman et al., 2007), and contact with human populations can be considered a health risk. The larval parasites such as *S. stercolaris* (sites 3 and 4), and hookworm (sites 1 through 5) were found, and ova of *A. lumbricoides* were detected at site 3 of the El Ahogado Basin. The presence of these parasites can be attributed to the fact that urban wastewaters are directly discharged to these water bodies. Agricultural activities frequently take place on grounds close to the locations of Las Pintas de Arriba and San José del Castillo, where sites of Las Pintas Dam and La Colorada Canal are included, and whose waters are used for irrigation of seasonal crops. These practices pose potential negative consequences to public health and to agricultural sustainability (Ensink et al., 2010). The presence of pathogenic bacteria in feces of all volunteers was limited, and commensal bacteria and parasites were predominant. The noncomensal parasites *G. lamblia* and *E. histolytica* were identified most frequently in the three groups of the El Ahogado Basin, whereas *E. histolytica* was the only parasite identified in the external group. These parasites have shown the highest amount of outbreaks of protozoan WBD recorded in Europe and the United States, and they have been responsible for several serious outbreaks worldwide over the past ten years. The importance of *E. histolytica* is because of the high probability of re-infection (Bakir et al., 2003). From the relative risk analysis, it was found that there was a significant risk of acquiring a commensal-type parasitism (*E. nana*) in the three volunteer groups studied, whereas the risk was insignificant for parasites such as *G. lamblia*, and *H. nana*. In summary, the only risk of acquiring non-commensal parasitism in the three groups of the El Ahogado Basin is caused by *E. histolytica* and *B. hominis*, both enteric pathogenic protozoa transmitted by the waterborne route associated with gastrointestinal disorders.

Blood Test: CBC and Biomarker. The CBC can be used as a general indicator of health and nutritional status for the assessment of possible illness caused by pathogens, parasites, and metal exposures. Particularly, eosinophils are used as indicators of parasitism (Campbell and Smith, 1996). In this study, the eosinophils count showed a high number in the San José del Castillo and Santa Cruz del Valle groups. The highest frequencies of intestinal parasites, and the identification of some larvae, namely *H. nana* (at San José del Castillo) and *E. vermicularis* (at Santa Cruz del Valle), were found at these two locations. From the ANOVA results on the CBC, no statistically significant differences were observed within the hematologic variables between the groups of volunteers. Typically, the values of the variables hemoglobin and hematocrit were low in all groups studied, including the external group. From the factor analysis results, it was clearly established that both infection and anemia factors were the most important factors of this study, and indicated a non-ideal health status in the volunteers of the different groups, including the control group. In the case of the infection factor, the results obtained can be attributed to both the sanitary conditions at the El Ahogado Basin (such as poor sanitary infrastructure, and the presence of wastewaters in the open air) and to external conditions of the basin that can cause infections in the volunteers. The poor environmental conditions and poor sanitary infrastructure at the El Ahogado Basin locations are typically more notorious than those of the external group, thus making inhabitants of this basin more vulnerable to the exposure of contaminated water and more prone to acquiring diseases associated to contact with unsafe water.

Some metals are required for human life, such as Mn, whereas others are considered ultratrace metals and their concentrations in humans should be maintained at minimum levels, such as Cu (Nielsen, 1996). The levels of Cu and Mn in blood samples of the volunteer groups were lower compared to dietary reference intakes recommended for dietary elements, which are 890 mg/kg^{-1} for Cu, and 1.6 to 2.2 mg/kg^{-1} for Mn per day (United States Department of Agriculture, 2001), except for one volunteer in Santa Cruz del Valle in whom a concentration of 4.6 mg/kg^{-1} of Mn was detected. Lead, a toxic metal without reference in dietary intakes, is not considered part of the ultratrace elements, though an estimate of blood-lead concentration (5.7 µg/dL^{-1}) associated with a tolerable intake has been established at 0.005 mg/kg^{-1} (Food and Agriculture Organization and World Health Organization Joint Committee, 1993). For comparison, only three volunteers from the external group and two volunteers from San José del Castillo were within this recommended limit. Overall, compared to the four groups, the volunteers of San José del Castillo showed the highest mean concentrations of biomarkers; therefore, this group had the greatest exposure to metals associated with industrial wastewaters dischargers.

Conclusions

Cross-sectional measurements used in this environmental epidemiology study proved to be useful in evaluating exposure of agents associated to WBD across the three groups of volunteers at the El Ahogado Basin. The study monitored pathogens in surface water and stools. It was found that the groups of volunteers in the basin are more likely to have a pathogen

causing a health disorder that originated from contact with unsafe water, compared to the volunteers of the external group. The susceptibility of acquiring a health disorder was demonstrated by the fact that the highest frequencies of pathogen and commensal parasites were found in the stool samples of the volunteer groups, whereas these frequencies were the lowest for the external group. In addition to the pathogen parasites, the relative risk of acquiring parsitoses by *B. hominis* (associated with a WBD) was the most significant in the volunteer groups of the El Ahogado Basin. The finding of helminthes parasites (*A. lumbricoides*, *S. stercolaris*, and hookworm) pose a latent risk of acquiring parasitoses when the inhabitants of the El Ahogado Basin come in direct or indirect contact with the water at the sites of this basin. The results of CBC revealed a clear state of non-ideal health in the groups of volunteers of the basin, and this state of health was confirmed by the factor analysis data where the infection factor presented the highest variability. The levels of biomarkers were higher in the volunteer groups of the El Ahogado Basin, particularly in San José del Castillo, where the highest concentrations of Mn, Cu and Pb were detected and were associated with wastewater discharges from an industrial source. It was demonstrated that in El Ahogado Basin, there exists a risk for inhabitants who do not use any type of protection to minimize the possibility of acquiring diseases related to contact with unsafe water, caused by the dischargers of wastewaters. The results of this work can be used to create more stringent sanitary regulations in basins where wastewater dischargers are transported through open air canals such as the El Ahogado Basin. The inclusion of interferences from a bias, and factors such as changes in water sources, infrastructure, and mobility of the community are not within the scope of this study. These factors should be considered in future epidemiological studies designed to reveal causalities, such as cohorts and case-control studies, to this and other basins having conditions similar to the El Ahogado Basin.

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