Microbial and metal water quality in rain catchments compared with traditional drinking water sources in the East Sepik Province, Papua New Guinea

Helena M. Horak, Joshua S. Chynoweth, Ward P. Myers, Jennifer Davis, Scott Fendorf and Alexandria B. Boehm

ABSTRACT

In Papua New Guinea, a significant portion of morbidity and mortality is attributed to water-borne diseases. To reduce incidence of disease, communities and non-governmental organizations have installed rain catchments to provide drinking water of improved quality. However, little work has been done to determine whether these rain catchments provide drinking water of better quality than traditional drinking water sources, and if morbidity is decreased in villages with rain catchments. The specific aim of this study was to evaluate the quality of water produced by rain catchments in comparison with traditional drinking water sources in rural villages in the East Sepik Province. Fifty-four water sources in 22 villages were evaluated for enterococci and *Escherichia coli* densities as well as 14 health-relevant metals. In addition, we examined how the prevalence of diarrhoeal illness in villages relates to the type of primary drinking water source. The majority of tested metals were below World Health Organization safety limits. Catchment water sources had lower enterococci and *E. coli* than other water sources. Individuals in villages using Sepik River water as their primary water source had significantly higher incidence of diarrhoea than those primarily using other water sources (streams, dug wells and catchments).

Key words | diarrhoeal illness, microbial water quality, Papua New Guinea, rain catchments

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INTRODUCTION

In 2006, the mortality rate for children under 5 years old in Papua New Guinea (PNG) was 73 per 1,000 live births, and PNG was ranked as 52nd in under-five mortality in a 2006 survey of 194 countries (UNICEF 2008). Between 2000 and 2003, diarrhoeal illness accounted for 15% of mortality for children under five in PNG (WHO 2006*a,b*). Common causes of water-borne infectious diarrhoeal diseases of public health importance include rotavirus, *Shigella*, *Cryptosporidium parvum*, *Campylobacter* and enteropathogenic *Escherichia coli* (Howard *et al.* 2000). The spread of these diarrhoeal pathogens is influenced by a lack of proper water supply systems, insufficient volumes of safe water at the household level, poor sanitation and unhygienic conditions (Kingston 2004).

The most widely used definition of 'access to water-supply services' is access to at least 20 litres per person per

day from an 'improved' water source within 1 kilometre of the user's dwelling (WHO/UNICEF 2006). An improved water source has construction which generally protects the water sources from contamination, particularly by faecal matter (WHO/UNICEF 2006). Surveys conducted in 2006 estimate that only 39% of the total population in PNG had improved drinking water coverage: 88% within urban areas and 32% in rural areas (WHO/UNICEF 2008). By comparison, 41% of the sub-Saharan Africa rural population has access to improved drinking water coverage (WHO/UNICEF 2006).

In general, PNG water supply systems face challenges of drought, poor water quality due to irregular maintenance, and land compensation demands (Kingston 2004). In rural areas, the Ministry of Health is responsible for construction, operation and maintenance of the supply system, but there is little to no regular surveillance of these activities (Kingston 2004). While multiple agencies use different standards for drinking water quality, the PNG's published regulation is the *Public Health (Drinking Water) Regulation 1984* which specifies that non-disinfected community or individual community supplies in PNG should have zero *E. coli* per 100 ml, and no more than three coliform organisms per 100 ml (PNG Office of Legislative Counsel 1984). Within the communities included in this study, the water sources have little to no monitoring, and the majority of water system construction, operation and maintenance is carried out by communities and NGOs with little or no involvement by government agencies.

Within the Sepik Province, water is central to village food production. Sago, the primary local carbohydrate/starch source is locally produced by repeatedly filtering river water through shredded sago palm fibres (Attenborough & Alpers 1992). A major concern with sago processing is bacterial contamination—a recent study of sago starch in the lowlands of PNG found frequent isolation of high levels of faecal coliforms and *E. coli*, and less frequent isolation or lower levels of various other diarrhoeal pathogens including *Bacillus cereus, Salmonella* and coagulase-positive staphylococci (Greenhill *et al.* 2007). Sago samples stored for shorter durations had higher levels of *E. coli* and faecal coliforms, and levels decreased with storage time (Greenhill *et al.* 2007). Since longer storage was associated with lower bacterial levels, the authors suggest that faecal coliforms

and other pathogens are environmentally derived, and potentially due to sago processing with contaminated water (Greenhill *et al.* 2007).

Fishing is also an important water-centred activity in PNG and the primary protein source for villagers (Attenborough & Alpers 1992). There are concerns that fish in PNG are becoming contaminated with inorganic pollutants from pollutant-laden surface waters. In PNG, the most prominent example of inorganic pollutant contamination is the Ok Tedi copper mine's industrial waste disposal into the Fly River. Since commencement of mining, fish catches have declined (Swales *et al.* 1998). Elevated levels of metals are found in fish (Swales *et al.* 1998) as well as in human hair samples (Jones *et al.* 1987). Currently, proposed copper and gold mining projects in the Sepik region contribute to concerns about how these projects will affect water quality in traditional ground and surface water sources.

One solution that has been proposed to address the problem of water contamination and dependence on traditional surface and ground water sources in the Sepik region is the construction of rain catchments. Rain catchments are well-established systems for delivering potable water that are particularly relevant in high rainfall areas such as the East Sepik Province, which receives 190–400 cm of rainfall annually (Hanson *et al.* 2001). Within this study area, there are over 200 installed water tanks. Catchments collect rainwater from a zinc-alum-coated corrugated metal roof through a gutter that drains into storage containers. The gutters have screens at the site where the pipe enters the storage tank to minimize outside pollution. Older storage containers are composed of galvanized steel with a bitumen-based coating. More recent installations are 8,000 litre plastic UV stabilized polypropylene tanks manufactured within the country. Villagers are expected to pay a quarter of the cost of the tank, with the remainder subsidized by a local missionary group. The complete installation inclusive of the tank, building, construction and transport of a single tank system costs US$6,000. The approximate cost to the villages is US$2,000–2,500 for transportation and installation; the remaining costs are subsidized.

In general, the quality of rain catchment water varies depending on location, topography, weather (Evans *et al.*

2006; Pathak & Heijnen 2006; Evans et al. 2007b) and characteristics of the rain catchment (Dillaha & Zolan 1985; Chang et al. 2004). Although drinking rainwater has been associated with isolated outbreaks of gastrointestinal illness (Palmer et al. 1983; Merrit et al. 1999; Taylor et al. 2000), well-maintained roof catchments can provide acceptable water for drinking and cooking (Dillaha & Zolan 1985; Evans et al. 2007a). In a study looking at highly credible gastrointestinal illness in 4–6 year olds, the consumption of properly harvested untreated rainwater did not increase the risk of gastrointestinal illness when compared with consumption of chlorinated and filtered public main water (Heyworth 2001).

Within the East Sepik Province, over 200 rain catchments have been installed into local villages over the past 25 years. Generally, villages rely on one primary water source, but may have access to other sources as well. Many local anecdotes suggest that water quality of the rain catchments is superior to that of traditional drinking water sources including the Sepik River, stream and dug wells. However, because of the difficulty in conducting high quality research in extremely resource-constrained settings, testing the water quality in rain catchments has not been conducted before this investigation. The specific aims of this study are: (1) to test the hypothesis that water quality (as measured using faecal indicator bacteria and metals) is superior in rain catchments compared with other traditional drinking water sources; and (2) to test the hypothesis that prevalence of diarrhoeal illness is lower in villages that have rain catchments. This is the first known study to broadly examine the microbiological and chemical quality of rain catchments and other drinking water sources in this region.

VILLAGES

Our field sites consisted of 22 villages distributed around the Sepik River (Figure 1). All samples and data were collected in July 2006, which is the early-to-mid dry season. Water quality data and population data were collected at all 22 villages, but health data were only collected in 16 villages during a scheduled clinic run by a visiting medical team. The visiting medical team was composed of
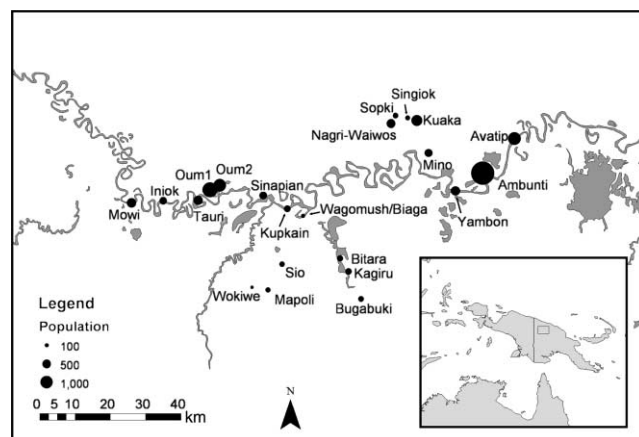

Figure 1 | Map of the Sepik River basin depicting water collection sites and village names. Circle size corresponds to village population based on our informal census. Water boundaries are drawn from the Global Lakes and Wetlands Database (Lehner & Döll 2004) as well as personal unpublished geodata collections.

medical students and undergraduate students, under the supervision of a Stanford emergency room physician who has been visiting the region every two years for the past decade. The clinics provide medical supplies, medical treatment for patients and training for local medics. The villages that did not have access to clinics because of distance and/or time constraints were visited independently of the medical team for water testing.

The median population of the 22 villages is 387, with ranges from 67 to 3,500. Villages considered to be major population centres included in the study were Ambunti (population 3,500), Oum-1 (population 1,500) and Oum-2 (population 1,059).

We categorized drinking water sources in each village into four groups: rain catchment, the Sepik River, stream and dug well. Catchments, described previously, provided water that appeared clear in colour with no sediments. The Sepik River category includes water collected directly from the river, as well as tributaries that feed into the Sepik River. Based on recommendations by the local population regarding locations where drinking water was typically collected, water was sampled from the banks and/or the middle of the river, the latter of which was generally swiftly flowing. The width of these river sources ranges between 20 m and 500 m. The Sepik River water is muddy yellowish-brown in colour, with heavy silt content and occasional debris such as sticks and leaves. The river's

characteristics were described by the locals as typical for the early-to-mid-dry season. The stream category includes natural springs and briskly flowing streams which come from rocky outcroppings. Both springs and streams provide water year-round. Water from this category was clear in colour with little/no sediments, and aquatic life such as fish and tadpoles was observed in some sources. The dug wells are uncovered, manually dug pits in the ground, ranging from 0.25 m to 1.0 m in depth. Dug wells allow access to the surficial aquifer. The dug well water was darker brown in colour than the Sepik River water, with heavy silt content. The dug wells are primarily centred in the Nagri region, which is located in the northern East Sepik Province. This region does not have access to as many Sepik River sources.

MATERIALS AND METHODS

Sample collection

In total, 54 drinking water sources were sampled in 22 villages within East Sepik Province, PNG, during July 2006 (Figure 1). The water samples included 15 catchments, 25 Sepik river water sites, 9 streams and 5 dug wells. All water sources were tested for enterococci and for health-relevant metals and metalloids (silver, aluminium, arsenic, calcium, cadmium, chromium, copper, iron, mercury, magnesium, nickel, phosphorus, selenium and zinc). A subset of water sources were assayed for *E. coli*, since a complete sampling was precluded by a problem with the microbial media (described below). The water sources tested for *E. coli* included 10 rain catchments, 22 Sepik river water sites, 8 streams and 4 dug wells.

Water samples (500 ml) were taken in polyethylene collection bottles that were rinsed with boiled water and vigorously rinsed at least three times in the sample water prior to sample collection. Occasionally, rinsing with boiled water between samples was not possible due to the remoteness of the sampling sites. For these samples ($n \sim 6$), bottles were rinsed vigorously in sampling water at least three times, and the research team sampled waters in order of presumed cleanest to dirtiest. Each sample was divided for bacterial quantification and metal analysis. Care

was taken not to dip the collector's hands into the water source, nor to disturb sediment in the water source. During sample collection, the date and time of collection, name of the village and water source location by GPS were noted.

Microbiological analysis

A 50 ml aliquot of each water sample was filtered through 0.45 µm pore size sterile membrane filters (HA type, Millipore, Molsheim, France) which were subsequently placed on mEI agar (Difco Laboratories, Detroit) and MI agar (Difco Laboratories, Detroit) for enterococci and *E. coli* enumeration, respectively. After filtration, the filter funnel and base were rinsed in boiled water. The mEI and MI plates were incubated aerobically at $35 \pm 0.5°C$ for 24 hours. Although the standard mEI incubation temperature is $41 \pm 0.5°C$, power constraints limited the incubation temperature to $35 \pm 0.5°C$. The impact of this incubation temperature on colony counts is explored in the results section. The total time from sample collection to incubation was no longer than 1 hour. Results were recorded as colony forming units (CFU) per 100 ml. Colonies were individually counted up to 500 CFU, and estimated up to 800 CFU by counting 1/8 of the plate and scaling accordingly. CFUs higher than the maximum were recorded as the maximum limit (assumed to be 800 CFU per plate). Plates with no CFUs were recorded as half the detection limit (1 CFU per plate).

To test the efficacy of using boiled water to clean the filtration funnel and base, controls were included with 50 ml of sterile bottled water. Colonies that grew on control plates were noted, and were assumed to have come from the filtration device, and subtracted from the final bacterial counts of samples filtered at the same analysis time. On average, only 0.07 CFU appeared on the control mEI plates and 0.15 CFU appeared on the control MI plates–indicating the boiled water was sufficient for cleaning the filtration device between samples. If a control plate had greater than 5 CFU, all the samples filtered at that time were discounted ($n = 12$).

All samples were incubated in a modified Minicooler incubator controlled by RancoETC-111000 thermostat (Ranco, Chihuahua, Mexico), powered by a standard 12 V car battery. The temperature maintained within the

incubator was $35 \pm 0.5°C$ for 24 h, with the temperature periodically reconfirmed in field conditions.

We characterized the water quality in the various water sources using the enterococci and *E. coli* as separate indicators for water quality. Using the *E. coli* measurements and WHO Water Quality Guidelines for Rural Drinking Water Supplies, we assigned water with < 10 CFU/100 ml as good indicating low risk, 10–100 CFU/100 ml as questionable indicating intermediate risk, and > 100 CFU/100 ml as poor indicating high risk (WHO 1997). We applied the same categorization for enterococci measurements even though specific enterococci criteria have not been published by the WHO.

Towards the end of the study, a percentage of MI media developed unknown yellow/cream coloured growth on the periphery of the plate, consistent with fungi. These plates were discarded and not included in the study. However, as a consequence, not all samples were tested for *E. coli* on MI media due to lack of supplies.

Comparison of mEI agar performance at two temperatures

Because our study utilized the incubation temperature of $35 \pm 0.5°C$, which is lower than standard mEI incubation temperature of $41 \pm 0.5°C$, we tested the two different incubation temperatures in laboratory conditions. Six surface water samples (1 l) from Lake Lagunita, Stanford, California, were collected in sterile polyethylene collection bottles. Prior to filtration, the water sample was gently agitated, and identical volumes between 1 and 100 ml were filtered on two different 0.45 μm pore size sterile membrane filters. The membrane filters were subsequently place on mEI agar (Difco Laboraories, Detroit). A total of 12 pairs of samples were incubated aerobically at $35 \pm 0.5°C$ or at $41 \pm 0.5°C$ for 24 hours, followed by enumeration and analysis by linear regression.

Metal analysis

The water samples analysed for metal content were those from 15 catchments, 25 Sepik river water sites, 9 streams and 5 dug well sources. In duplicate, the drinking water samples were filtered using a 0.2 μm pore size filter syringe into 15 ml polypropylene bottles, and acidified with two drops (0.2 ml) of concentrated trace-metal grade hydrochloric acid. Two months post-collection, trace elements were measured in the aqueous phase using inductively coupled plasma–optical emission spectrophotometry (ICP-OES) (IRIS model; Thermo Jarrell Ash, Franklin, Massachusetts) with a 10% accuracy range. The final metal measurement of each sample was determined as the mean between the two duplicates. All reaction-ware used in the experiment was rinsed in 0.5 M hydrochloric acid before use.

A multi-element analysis was carried out for each sample for the following elements: aluminium, arsenic, cadmium, calcium, chromium, copper, iron, magnesium, mercury, nickel, phosphate, selenium, silver and zinc. The metals chosen for analysis are designated as inorganic chemical contaminants by the World Health Organization (WHO 2006*a,b*) and/or the United States Environmental Protection Agency (USEPA 2003). The metals denoted in the World Health Organization standards are based on credible evidence of occurrence of the chemical in drinking water, combined with evidence of actual toxicity or significant international concern (WHO 2006*a,b*). Using US EPA standards, the chosen metals are either National Primary Drinking Water Standards, which are legally enforceable standards that apply to public water systems, or *National Secondary Drinking Water Standards*, which are non-enforceable guidelines for contaminants that may cause cosmetic (skin or tooth discoloration) or aesthetic effects (such as taste, odour or colour) (USEPA 2003). Detection limits were defined by three times the standard deviation of seven blanks.

Health data

The diarrhoea health data were collected in 16 selected villages during the same period as the drinking water source testing. The data were retrospectively compiled from the medical notes at the conclusion of clinic sessions with villagers. The data included the numbers of patients seen by the medical team and the number of these patients citing complaints of diarrhoea. The clinic sessions were open to specific health concerns, as well as general wellness visits–patients were self-referred. The total number of patients

seen in the clinics ranged from 2 to 85; the median number of patients was 11. The number of patients under-five years of age seen in the villages ranged from 0 to 22, with a median of 4. The number of patients greater than or equal to 5 years of age ranged from 1 to 58, with a median of 6. The number of patients without a recorded age ranged from 0 to 6, with a median of 1. In addition, the primary water source where the majority of the population obtained drinking water was identified by speaking with the local healthcare workers as well as local villagers.

Statistical analysis

Using SPSS statistical software, data were analysed using non-parametric methods which included Yates' chi-square and Kruskal-Wallis one-way analysis of variance tests. In addition, the microbiological data were log-transformed and analysed by two sample t-tests. The significance level used in all analyses was $p = 0.05$.

RESULTS

Comparison of mEI agar performance at two temperatures

A total of 12 samples were analysed by membrane filtration onto mEI media and incubated at $35 \pm 0.5°C$ and $41 \pm 0.5°C$ for 24 hours. The mEI colonies at $35 \pm 0.5°C$ were lighter blue in colour compared with the mEI colonies at $41 \pm 0.5°C$. Colony counts ranged from 0 to 185 CFU, with no temperature consistently over- or underestimating colony counts. Linear regression indicates that the $R^2 > 0.9$ (Figure 2). In addition, the difference between concentrations measured by incubation at the two temperatures was not significantly different from 0 ($t = -0.953$, df $= 15$, $p = 0.4$, paired t-test).

Microbiological quality of drinking water sources

The enterococci densities for all water sources ranged between below the detection limit (recorded as half the detection limit) and 624 CFU/100 ml (Figure 3). The median enterococci densities measured for catchment, Sepik River, stream and dug well sources were 4, 76, 60,
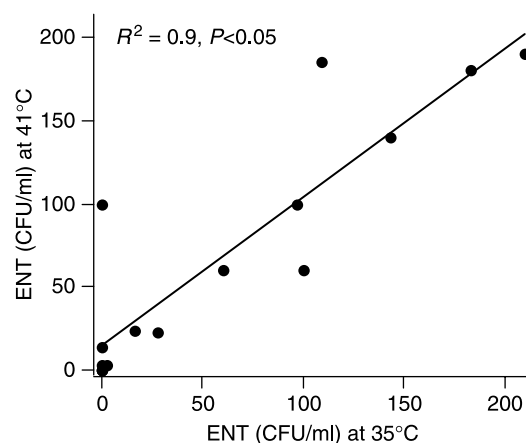
$R^2 = 0.9$, $P<0.05$

Figure 2 | Comparison of two incubation temperatures (35°C and 41°C) for determining concentrations of enterococci in water samples. Identical volumes of the same water sample were filtered, placed on mEI agar, and incubated either at 35°C (x-axis) or 41°C (y-axis). Line shows least-squares regression.

132 CFU/100 ml, respectively. The catchment sources had the lowest measured median enterococci density, as well as the smallest interquartile range (25th percentile of 2 CFU/100 ml and 75th percentile of 14 CFU/100 ml). The dug well sources had the highest measured median enterococci densities; however, one dug well source measured below the lower detection threshold. Using Kruskal Wallis statistical testing, the catchment sources had significantly lower enterococci levels compared with Sepik River ($H = 13.926$, df $= 1$, $p = 0.000$), stream ($H = 9.843$, df $= 1$, $p = 0.002$) and dug well sources ($H = 5.003$, df $= 1$, $p = 0.03$). In addition, the streams had significantly lower enterococci levels compared with Sepik River ($H = 5.727$, df $= 1$, $p = 0.02$). No other comparisons achieved statistical significance.

The *E. coli* concentrations for all water sources ranged from below the lower limit of detection (recorded as 1 CFU/100 ml) to above the upper limit of detection (1,600 CFU/100 ml) (Figure 3). The median *E. coli* levels measured for catchment, Sepik, stream and dug well sources were 35, 371, 117 and 569 CFU/100 ml, respectively. The Sepik River had the widest interquartile range, with 25th and 75th quartiles of 165 CFU/100 ml and 926 CFU/100 ml, respectively. The catchment sources were significantly lower in *E. coli* levels compared with Sepik River and stream sources ($H = 7.766$, df $= 1$, $p = 0.005$). Although they did not achieve statistical significance, comparisons between catchment and dug
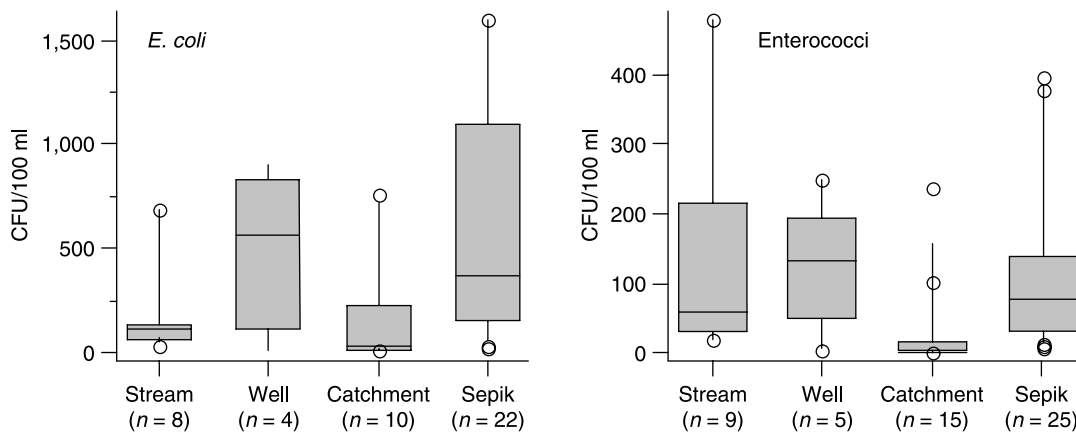
Figure 3 │ Box and whiskers plots illustrate the range of concentrations of *E. coli* (left) and enterococci (right), measured in the different water source categories: stream, (dug) well, catchment and Sepik. For each box, the line within the box represents the median, the top and bottom of the box represent the 75th and 25th percentiles, respectively, and the top and bottom whisker extend to the 90th and 10th percentiles, respectively. Observations beyond the 10th and 90th percentiles are shown as open circles.

well ($H = 1.138$, df $= 1$, $p = 0.3$) and catchment and stream sources ($H = 1.283$, df $= 1$, $p = 0.3$) suggested lower *E. coli* levels in the catchment sources. For the dug wells, the lack of statistical significance is probably a consequence of the small number of dug well sources tested ($n = 4$). For the streams, the lack of statistical significance is likely to be a combination of lower *E. coli* levels in streams as well as a small sample size ($n = 8$).

The characterization of water from each water source into good, questionable and poor categories based on enterococci is summarized in Figure 4 and Table 1. For catchment enterococci measurements, 66.7% (10/15 sources) were in the good category, 20% (3/15 sources)

were in the questionable category, and 13.3% (2/15 sources) were poor. Of the Sepik River sources, 4% (1/25 sources) were in the good category, 64% (16/25 sources) were in the questionable category, and 32% (8/25 sources) were in the poor category. The stream sources had 0% (0/9 sources) in the good category, 55.6% (5/9 sources) in the questionable category, and 44.4% (4/9 sources) in the poor category. The dug wells had 20% (1/5 sources) in the good category, 20% (1/5 sources) in the questionable category, and 60% (3/5 sources) in the poor category.

When applying the above guidelines for water quality to *E. coli* alone (Figure 4 and Table 1), only one dug well water source (25%, 1/4 sources) was in the good category.
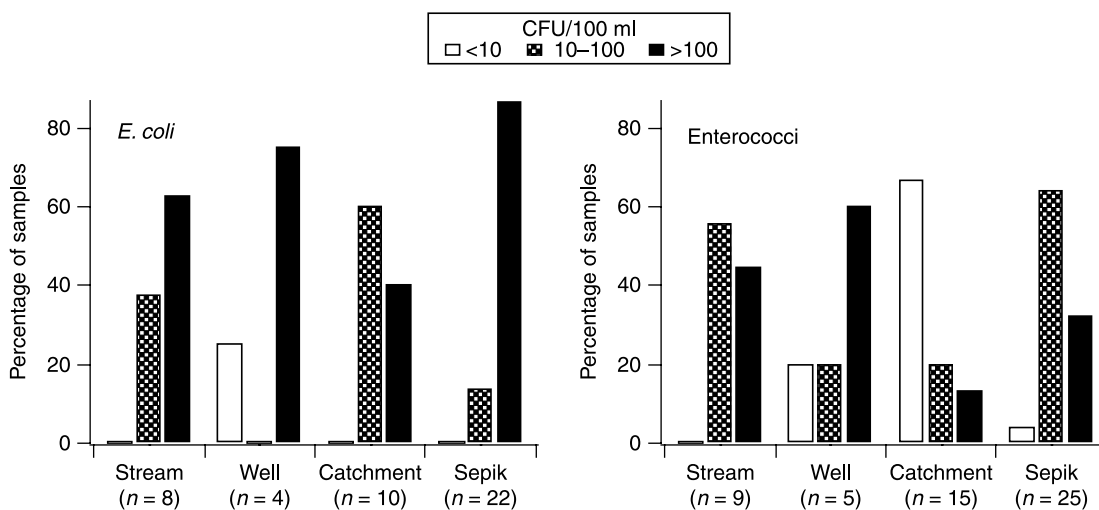

Figure 4 │ Percentage of samples with concentrations of *E. coli* (left) and enterococci (right) of < 10, 10–100 and > 100 CFU/100 ml for each water source. Number of samples in each category is also denoted.

Table 1 | Water sources are classified into good (<10 CFU/100 ml), questionable (10–100 CFU/100 ml) and poor (>100 CFU/100 ml) using enterococci (columns) and *E. coli* (rows) as separate indicators. Water sources are represented by the percentage in each classification. In parenthesis, the ratio of the number of samples in that classification over the total samples in that water source. All samples have enterococci measurements. Samples without *E. coli* measurements are listed as 'No measure'

	Enterococci (CFU/100 ml)		
E. coli (CFU/100 ml)	<10 Good	10–100 Questionable	>100 Poor
<10 Good	–	–	Well 20% (1/5)
10–100 Questionable	Catchment 33% (5/15) Sepik 4% (1/25)	Sepik 4% (1/25) Stream 33% (3/9) Well 20% (1/5)	Catchment 13% (2/15) Sepik 4% (1/25)
>100 Poor	Catchment 13% (2/15)	Catchment 13% (2/15) Sepik 48% (12/25) Stream 11% (1/9)	Sepik 28% (7/25) Stream 44% (4/9) Well 40% (2/5)
No Measure	Catchment 27% (4/15) Well 20% (1/5)	Catchment 7% (1/15) Sepik 12% (3/25) Stream 11% (1/9)	–

The remaining three dug wells (75%, 3/4 sources) were in the poor category. All of the remaining water sources were in the questionable or poor category. The catchments had the highest percentage in the questionable category – 60% (6/10 sources). The Sepik River sources had the lowest percentage in the questionable category – 13.6% (3/22 sources). The streams had 37.5% (3/8 sources) residing in the questionable category.

Using both indicators together allows more confidence in water quality classification (Table 1). Because only one tested water source (dug well) was characterized as 'good' using *E. coli* as a metric, no water source–not even the catchment water–was deemed 'good' using both *E. coli* and enterococci densities as metrics. The samples that were deemed 'poor' by both metrics included none of the catchments, 28% of the Sepik River, 44% of the stream, and 40% of the dug well samples. The classification of the remaining water sources varied depending on the indicator used to make water quality assessments.

Bank and middle of Sepik River measurements

The Sepik River data can be further characterized into bank and middle measurements. While the *E. coli* levels significantly differed between bank and middle measurements, no significant differences or trends were noted in the enterococci data. The median *E. coli* levels of the middle ($n = 8$) and bank ($n = 7$) are 170 and 1,400 CFU/100 ml, respectively. By Kruskal Wallis testing, this difference is statistically significant ($H = 5.769$, df $= 1$, $p = 0.02$) with samples collected at the bank measuring higher *E. coli* levels. For enterococci, this comparison does not show a significant difference ($H = 1.817$, df $= 1$, $p = 0.2$). In addition, at three locations along the river, paired sample measurements were taken at both the bank and middle from the same locations. The median *E. coli* levels for the bank and middle of the Sepik River were 368 and 1,600 CFU/100 ml respectively. The difference is statistically significant ($H = 4.355$, df $= 1$, $p = 0.04$). For enterococci data, this comparison does not show a significant difference ($H = 0.048$, df $= 1$, $p = 0.8$).

Metal analysis

The majority of the metal measurements of water sources were below WHO and/or USEPA thresholds, leaving these water sources to be classified as good in terms of metal content. For two metals the majority of samples were above EPA thresholds: 59% of iron samples (32/54 samples) and 94% of silver samples (51/54 samples) (Table 2).

For iron levels, 100% (25/25 sites) of the Sepik sources and 60% (3/5 sites) of the dug well sources were above

Table 2 | Mean, median and ranges for metal content from all drinking water sources. WHO and EPA standards are listed, as well as percentage of samples which exceed these standards

Metal content of drinking water (mg l^{-1})				Standard (mg l^{-1})		% Above	
Metal	Mean	Median	Range	WHO	USEPA	WHO	USEPA
Silver*	0.2622	0.2730	0.0387–0.4563	N/A	0.1000		94
Aluminium*	0.1329	0.1366	0.0464–0.2078	N/A	0.2000		4
Arsenic	0.0014	0.0010	<0.0000–0.0063	0.0100	0.0100	0	0
Calcium	6.9470	6.2863	0.0569–26.535	N/A	N/A		
Cadmium	0.0010	0.0000	<0.0000–0.0078	0.0030	0.0050	15	6
Chromium	0.0008	0.0005	<0.0000–0.0039	0.0500	0.1000	0	0
Copper	0.0061	0.0023	0.0001–0.1745	2.0000	1.3000	0	0
Iron*	0.1647	0.1013	0.0101–0.6478	N/A	0.0500		59
Mercury	0.0005	0.0001	<0.0000–0.0047	0.0060	0.0020	0	6
Magnesium	3.8957	2.6528	0.0064–19.65	N/A	N/A		
Nickel	0.0034	0.0031	<0.0000–0.0123	0.0700	N/A	0	
Phosphorus	0.0170	0.0100	<0.0000–0.0981	N/A	N/A		
Selenium	0.0017	0.0003	<0.0000–0.0079	0.0100	0.0500	0	0
Zinc*	0.4433	0.1525	0.0231–2.5535	N/A	5.0000		0

*National Secondary Drinking Water Standards, United States Environmental Protection Agency. N/A, no published WHO or EPA standard.

the EPA threshold (0.05 mg/ml). The Sepik sources were three to four times the EPA threshold; the highest level was 12 times the EPA threshold. The dug well sources were two to four times the EPA limit. The majority of the catchment and stream sources were below the EPA limit.

For silver levels, 94% of all sources were above the EPA threshold (0.10 mg/ml). The majority of the sources were approximately two to three times the EPA threshold; the highest level was 4.5 times the EPA level. The level of silver did not vary according to source.

Health data

In each village, the primary water source was identified by speaking with the village leader and/or healthcare worker. Hereafter, each village is described by its primary water source (for example, 'stream village', etc.) (Table 3). In the 16 villages with health data, 38% were catchment villages (6/16 villages), 44% were Sepik River villages (7/16), 13% were stream villages (2/16) and 6% were dug well villages (1/16).

The health data collected at the villages suggests that having a primary water source that was not the Sepik River (including catchment, stream and dug well) was associated with fewer patients with complaints of diarrhoea (Table 3). When considering the four primary water sources and whether the individual patient had complaints of diarrhoea (Table 3), significant differences were found for patients of all ages ($\chi^2 = 12.2$, df = 3, $p = 0.007$) but not for children under five ($\chi^2 = 7.67$, df = 3, $p = 0.05$). In both cases, the percentage of individuals reporting diarrhoea was greater in Sepik villages than other villages. When comparing the Sepik villages with all others (catchment, stream and dug well), there was a significant difference for patients of all ages ($\chi^2 = 10.57$, df = 1, $p = 0.001$) and in children under five ($\chi^2 = 4.99$, df = 1, $p = 0.03$). Comparisons of catchments versus all other water sources (Sepik River, stream and dug well) did not achieve significance in patients of all ages ($\chi^2 = 0.01$, df = 1, $p = 0.9$) nor in children under five ($\chi^2 = 0.05$, df = 1, $p = 0.8$).

DISCUSSION

Within this study, the levels of enterococci were significantly lower in catchments compared with Sepik sources, streams and dug wells. Mean and/or median levels of enterococci were comparable to or lower than those in

Table 3 | Population and primary water source for each village. Patients are grouped into under five years of age, five-or-greater years of age, or unknown/undocumented age. The final column combines patients of all ages. Percentage of patients in each age group that visited the clinic with diarrhoeal complaints. In parenthesis, the number of patients with diarrhoeal complaints is expressed over the total number of patients seen. N/A indicates that no individuals in given category attended the clinic

Village number	Village pop.	Primary water source	% < 5 years old with diarrhoea	% ≥ 5 years old with diarrhoea	% Unknown age with diarrhoea	% All ages with diarrhoea
1	249	Catchment	N/A (0/0)	0 (0/1)	0 (0/1)	0 (0/2)
2	527	Catchment	0 (0/7)	0 (0/26)	N/A (0/0)	0 (0/33)
3	198	Catchment	N/A (0/0)	0 (0/2)	0 (0/2)	0 (0/4)
4	211	Catchment	0 (0/3)	6 (1/18)	0 (0/1)	4.5 (1/22)
5	1,500	Catchment	100 (3/3)	0 (0/11)	0 (0/1)	20 (3/15)
6	280	Catchment	25 (1/4)	50 (2/4)	N/A (0/0)	37.5 (3/8)
7	67	Stream	0 (0/7)	0 (0/8)	0 (0/6)	0 (0/21)
8	182	Stream	18 (4/22)	5 (3/58)	0 (0/5)	8.2 (7/85)
9	406	Dug well	0 (0/6)	7 (1/15)	N/A (0/0)	4.8 (1/21)
10	260	Sepik	0 (0/3)	0 (0/3)	N/A (0/0)	0 (0/6)
11	385	Sepik	20 (1/5)	0 (0/1)	N/A (0/0)	16.7 (1/6)
12	590	Sepik	43 (3/7)	9 (1/11)	0 (0/4)	18.2 (4/22)
13	545	Sepik	100 (2/2)	0 (0/5)	0 (0/3)	20 (2/10)
14	1,059	Sepik	50 (2/4)	14 (1/7)	0 (0/1)	25 (3/12)
15	100	Sepik	43 (3/7)	0 (0/2)	0 (0/1)	30 (3/10)
16	389	Sepik	50 (1/2)	50 (1/2)	0 (0/1)	40 (2/5)

other studies of rain catchments (Simmons *et al.* 2001; Ariyananda 2003; Evans *et al.* 2007a).

Although only one water sample (from a dug well) met the standards of *PNG's Public Health (Drinking Water) Regulation 1984* (PNG Office of Legislative Counsel 1984), *E. coli* levels were significantly lower in catchments compared with Sepik sources. Comparisons between catchments with streams and with dug wells did not achieve statistical significance; however, trends suggested lower levels of *E. coli* in catchments compared with other sources. The lack of statistical significance was probably due to the small number of dug wells and streams sampled. In our study, the mean and/or median level of *E. coli* in rain catchments was comparable to or higher than those in other microbiological studies of rain catchments (Ariyananda 2003; Evans *et al.* 2007a; Sazakli *et al.* 2007).

No catchments in our study had *E. coli* concentrations below our detection limit, which is in disagreement with other studies of rain catchments (Dillaha & Zolan 1985; Pinfold *et al.* 1992; Ariyananda 2003; Evans *et al.* 2007a; Sazakli *et al.* 2007). One possible explanation is that the Sepik Province of PNG is located in the tropics which may

have natural reservoirs of these organisms, confounding the results of using *E. coli* and enterococci to assess water quality (Fujioka *et al.* 1988; Hazen 1988; Rivera *et al.* 1988; Hardina & Fujioka 1991; Fujioka *et al.* 1999). Our use of both *E. coli* and enterococci to assess water quality, rather than just one indicator, provides us with more confidence in our findings of poor and questionable water quality in the Sepik Province in PNG. Using both metrics, catchments were the only water type that did not have samples in the poor category (Table 1).

Our findings were shared with local medics and community leaders. The catchments with the highest densities of enterococci ($n = 1$, 236 CFU/100 ml) or *E. coli* ($n = 2$, 406 CFU/100 ml and 758 CFU/100 ml) were, in all three cases, identified as having sources of contamination. Two of the catchments had broken screens, and the third catchment had children throwing stones, sticks and other debris onto the roof and gutter flowing into the tank. None of the tanks received regular maintenance and cleaning. Our results highlight the importance of rain catchment maintenance to ensure good water quality.

Although collected sample sizes were small, the data support the local belief that collected drinking water is cleaner in the middle of the Sepik; we found lower *E. coli* densities in the middle of the river compared with the bank. Therefore, if water must be collected from the Sepik River, villagers should be advised to collect away from the banks.

Our data on concentrations of 14 metals suggested that the majority of metal indicators are well below EPA thresholds and the water is considered 'safe'. The two metals that were above the EPA threshold, silver and iron, are considered 'secondary' metals, which are relevant to the cosmetic and aesthetics of the drinking water, but not correlated with any significant health effects. Metal concentrations should continue to be monitored in this region, particularly if new industries or factories are created that may produce metal waste.

While inorganic pollutants and chemicals in the rainwater are a concern for heavily industrialized areas, the East Sepik Province in PNG currently has limited industrial activity, which does not appear to contribute significantly to metal levels in rain catchments nor in other traditional water sources. The greatest concern in this region remains bacterial contamination and infectious disease, which accounts for a large percentage of morbidity and mortality.

Our health data suggest that having a catchment, stream or well as a primary water source rather than the Sepik River was associated with fewer diarrhoea complaints. However, there are a number of important limitations to our health data. Individuals attending the clinic were self-referred; hence the health data did not represent a true cross-section of the populations in the villages. In several villages, the clinics were well-publicized with many villagers presenting with health complaints or general health checks. Other villages did not receive this publicity; hence the clinics were less well attended. In addition, the health data represent a snapshot of the health of the population. It is likely that both water quality and diarrhoeal incidence vary by season; our cross-sectional research design does not allow us to investigate whether the associations we documented in health and drinking water source persist throughout the year. Our results suggest that providing drinking water sources that reduce reliance on the Sepik River may lead to less diarrhoeal illness.

In general, the local perspectives on rain catchments were extremely positive—villagers cited better taste and perceived cleaner water, as well as anecdotal evidence of improved village health. Provided that there are no leaks and the catchment is used as a drinking water source only, the rain catchments can supply a village for a period of three weeks. Even during the dry season, rainfall generally occurs within three to four weeks; hence water shortage from the rain catchments is rarely an issue with proper use.

The data provided by our study suggest that the belief that rain catchment water is safer is corroborated by lower levels of bacterial indicators compared with traditional water sources. This study also demonstrates the feasibility of conducting high-quality laboratory measurements of bacterial indicators in the field under the constraints of extremely poor resource settings.

CONCLUSIONS

This study examined the quality of rain catchments and traditional drinking water sources in the East Sepik Province, PNG. We found that rain catchments have lower enterococci and *E. coli* levels compared with other traditional water sources including the Sepik River, streams and dug wells. Only rain catchments had no sources classified as poor using both microbial indicators to assess quality. We also determined that *E. coli* levels were significantly lower in the middle of the Sepik River compared with the bank. No significant differences were noted in enterococci measurements. Of the 14 tested health-relevant metals, only iron and silver measured above the United States Environmental Protection Agency recommended drinking water limits. Iron was elevated three to four-fold in the Sepik River sources and two to four-fold in dug wells, and silver was elevated two to three-fold in all sources. These are secondary metal measures relevant to water taste rather than health. Finally, we found that individuals in villages relying primarily on Sepik River water for drinking water reported significantly higher incidence of diarrhoea than those using primarily other water sources (streams, dug wells and catchments). Our results support the use of rain catchments for providing an improved source of drinking water in this region.

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