The Microbiological Survey of Potential Water Borne Pathogens in Fresh Water Springs of the Selected Community Located in the Upolu Island, Samoa

F. Latū, P. Amosa, T. Imo, and V. Taufao

Abstract—**The objective of this study was to determine the sanitary quality of three freshwater springs by estimating the concentration of total coliform (TC) and faecal coliform (FC) bacteria as pollution indicators. Water samples were collected twice a month during the period August 2008 to January 2009; from springs on the island of Upolu, Samoa. For bacteriological analysis, the membrane filtration method was used for the two groups of bacteria. All samples from the three sites were found contaminated with total coliform and faecal coliform bacteria and the counts were higher than the maximum microbial contaminant level established by World Health Organization (WHO). The results imply that the springs were heavily polluted by bacteria of faecal origin suggesting that, these springs are potential sources of health hazards which is important from a public health perspective.**

Index Terms—**Total coliform, faecal coliform indicators, Samoa.**

I. Introduction

The current priority strategic areas of development for Samoa have been identified in the Samoa Strategy for the Development of Samoa (SDS), with the vision of an "Improved Quality of Life for All" [1]. Improved life implies access to good quality drinking water for the well being of all citizens. In recent years, there have been reported outbreaks of certain waterborne diseases such as cholera, dysentery and typhoid with the latter occurring in high rates in certain parts of Samoa [2]. In May and June of 2005, there were respectively ten and nineteen confirmed cases of typhoid in Samoa. The severity of the typhoid problem in Samoa has been reiterated by a representative of the World Health Organisation (WHO) to the local media [3].

As there are local communities which use freshwater springs either to supplement their reticulated water supply or as their sole supply for drinking and other domestic purposes, it is important to identify the safety levels of these waters, especially in consumption issues. The Samoa Water Authority (SWA) conducts some microbiological analysis of our national water resources but does not analyse for some of the micro-organisms addressed in this research. Thus, for a community that relies on water springs to supplement or fully provide its water supply, it is important to know whether these water resources are completely safe for consumption. Thus, the main objectives of this study were to: 1) Measure the levels of faecal coliforms and total coliforms in the Vinifou, Tufuiopa and Malie freshwater springs during the sampling period 2) To compare the spatial and temporal distribution of bacterial concentrations in the Vinifou, Tufuiopa and Malie freshwater springs and 3) To compare collected microbiological data with World Health Organization standards to determine the safety of these waters for drinking.

Manuscript received January 18, 2012; revised March 4, 2012. This study was funded by a grant from the Centre of Samoan Studies of the National University of Samoa.

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II. Methodology

A. Site Selection

The three freshwater springs investigated are at Vinifou (latitude 13°84′ S and longitude 171°75′ W), Tufuiopa (latitude 13°84′ S and longitude 171°76′ W) and Malie (latitude 13°48′ S and longitude 171°50′ W) on Upolu Island (13°55′ S, 171°45′ W), Samoa (Fig.1). The two former springs (Vinifou and Tufuiopa) are located in the urban areas while the latter spring (Malie) is located in a rural part of the island. The Tufuiopa fresh water spring is fed by the underground and the spring itself is bordered by a public cemetery to the south and private residences to the east and west directions. The water flows under the village women's committee building in its outflow direction towards the sea to the north. The spring itself is divided into two distinct areas as drinking pool and bathing pool. The depth from the water surface to ground level is approximately 2 m, as it flows directly from underneath the adjacent public cemetery in the south. The drinking well is partially fenced off and receives water from the aquifer before it flows into the bathing pool area and northwards towards the sea. Users need to gain access through a locked gate and descend a series of concrete steps before reaching the drinking water well**.** For the Vinifou fresh water spring, it is located 5 m from the roadside on a sloping gradient and about 5 feet from ground level. The underground water source is fed by a watershed area higher up in the south which is the source for several other fresh water springs in the area. The third fresh water spring sampling site is surrounded by families (3 households, and possibly more) which use this water source during periods when the public water supply is shut off. The concrete well mouth is slightly raised above ground level and the water level is about 120 cm from ground level during dry spells and about 500 cm from ground level during the rainy season. From the well mouth, the west main coast road runs 20 m to the south with surrounding families approximately 15 m to the east, west and northerly (seaward) directions. Access is not restricted to both humans and animals alike by a fence or other means.

Runoff from surrounding areas also contributes to the water in the well during rainy periods.

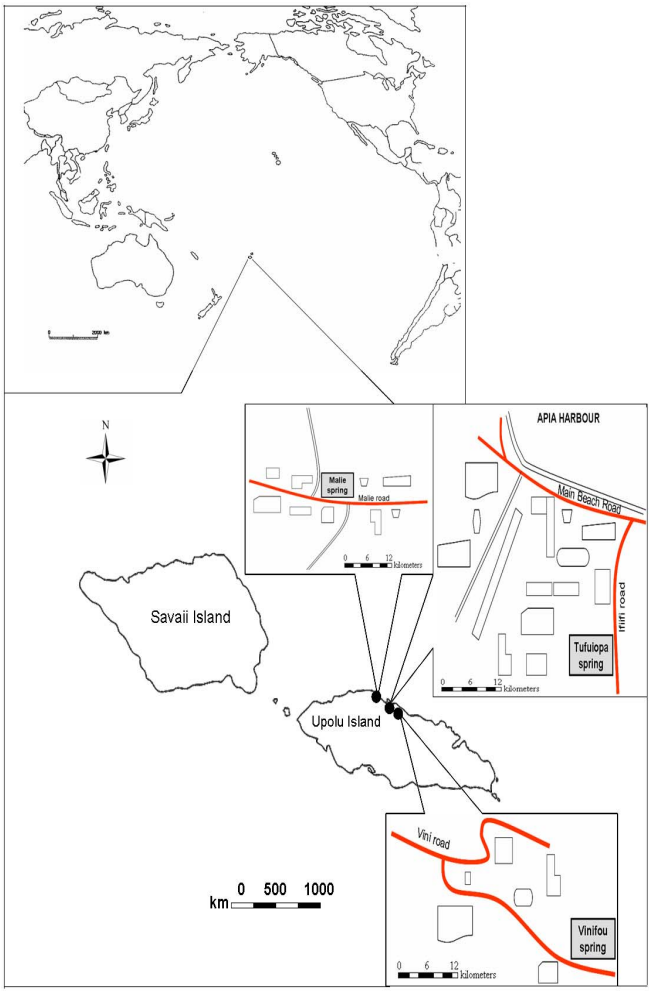

Fig.1. Map of Sampling location

B. Sample Location

Water samples were collected from each of the three springs on the same day at fortnightly intervals from August 2008 to January 2009. This period covered three months of the cool, dry season (Season 1 - period of relatively lower humidity, from August to October) and three months of the hot, wet season (Season 2 - period of relatively higher humidity, from November to January). Triplicate 500 ml samples were collected in week 1 and week 3 of each month giving a total of 36 samples to be analysed from each site. The samples were collected in autoclave sterilized 500 ml glass bottles (Schott Duran GL 45) from a depth of 8 to 10 cm below the surface of the water [4] to avoid contamination from debris and bacteria deposited by wind on the surface water. In each case, the volume exceeded 100 ml required to carry out all tests [5]. The samples were immediately taken to the National University of Samoa Biology laboratory and processed within 5 h of sampling, as compared to 6 h delay time in a comparable study [6] and within 30 h. From each 500 ml sample bottle, 30 ml was drawn and serially diluted ten fold and treated as described subsequently.

C. Culture Media and Bacterial Colony Identification

The use of mFC agar (DIFCO) for the detection of faecal coliform bacteria and the enumeration of total coliforms on mEndo LES agar (DIFCO) has been cited in several studies [5], [7]-[9]. The mFC agar contains selective and differential agents. These include: 1. Rosolic acid, a selective agent added to the agar media during preparation to inhibit bacterial growth except for faecal coliforms. 2. Bile salts which also inhibit non-enteric (bacteria not normally found in intestines of warm blooded animals) bacteria. 3. Aniline blue which indicates the ability of faecal coliforms to ferment lactose acid that causes a pH change in the medium. Lactose utilization (blue color) is the basis for the identification of faecal coliforms as previously stated [5]. The mEndo agar LES contains: 1. Yeast extract that supplies B-complex vitamins and minerals which stimulate bacterial growth. 2. Sodium desoxycholate and sodium lauryl sulfate which act as inhibitors. 3. Basic fuchsin which is a pH indicator. Lactose-fermenting bacteria produce aldehyde that reacts with sodium sulfite and fuchsin to produce red colonies. 4. The development of a metallic sheen occurs when the bacteria produce aldehydes with the rapid fermentation of lactose [10]. These growth media were prepared in accordance with instructions by manufacturer (DIFCO laboratories), poured into 60 x 14 mm disposable petri dishes then placed in the refrigerator at 4°C [5] until use. All growth media were prepared at least 24 h before use.

D. Sample Processing and Culture

For the detection and enumeration of total and faecal coliforms, several methods have been suggested. Membrane filter procedure which is as effective as the multiple tube fermentation procedure shows discrete bacterial colonies that may be further identified, although highly turbid water and non-coliform bacteria can interfere with the test. Although highly effective, the membrane filter procedure requires processing of several sample dilutions in order to obtain filter plates with an appropriate range of colonies to validate enumeration [5]. All 108 water samples collected from the three sites over the study period were prepared and processed in an identical manner. Prior to the beginning of this study, samples were collected and vacuum filtered to establish a baseline count of raw samples. These pre-study trials confirmed that, a ten-fold serial dilution of the raw sample was necessary to obtain a meaningful viable count due to the exceptionally high bacterial content of the original samples. From each 500 ml raw sample, a 30 ml aliquot was added to 270 ml of sterilized water to obtain a 1/10 dilution of the sample. From this dilution, 30 ml was transferred to 270 ml of sterilized water to obtain a 1/100 dilution. This was repeated until a 10^{-5} dilution of the original sample was obtained. In vacuum filtration, 100 ml from the highest dilution (10^{-5}) was vacuum filtered through a 0.45 µm nitrocellulose millipore membrane (Millipore) according to the USEPA membrane filtration method 8074[1]. The membrane was then placed on mEndo agar. Another 100 ml was membrane filtered then placed on mFC agar. The holding funnel was then rinsed with 70% ethanol [11] to remove any residual bacteria. This procedure was repeated for the next highest dilution (e.g. 10^{-4}) to reduce any cross contamination through carry over. This process

[1] Adapted from Standard Methods for Examination of Water and Wastewater, 9222 B and 9221 B

was repeated for all triplicate samples from each site. For detection of faecal coliforms and total coliforms, culture media plates were incubated inverted for 24 h at 44.5°C and 37°C respectively [12].

E. Data Analysis

The validity of the data and potential relationships between the measured parameters (site and monthly variations) were identified by conducting comparative statistical analyses of power transformed results using the R-package statistics software.

III. Results

A. Distribution of Total and Faecal Coliform Bacteria

Fig.2 compares the overall mean of total and faecal coliform bacterial concentrations for the whole six months study period at each site. As shown in Fig.2, the highest mean concentrations for both groups of bacteria were obtained from Malie while Vinifou spring gave the lowest mean counts

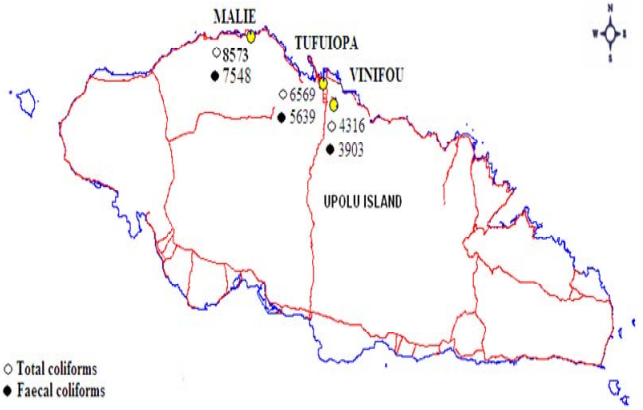

Fig. 2. Mean total and faecal coliforms (cfu/100 ml water) across the three sites over the six month period.

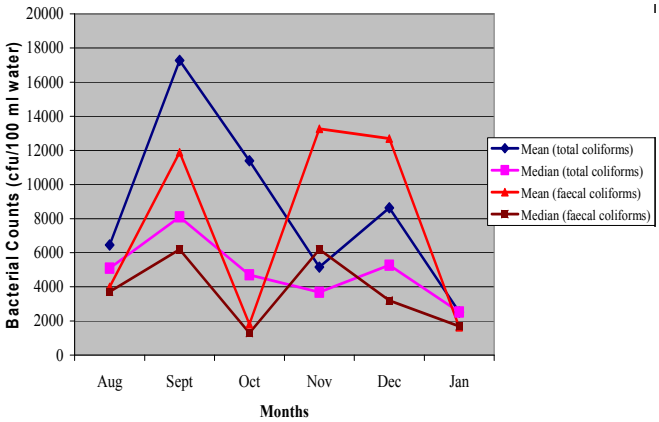

Fig. 3. Monthly means and medians of total and faecal coliforms in the Malie spring.

Fig 3 displays the mean and median of both total and faecal coliform bacteria at Malie spring over the six months from August 2008 to January 2009. For the total coliform experiment and taking the median as the best measure, September gave the highest count. August and October also gave high counts and together they gave high counts for season 1. For season 2, the December count was quite high. For the faecal coliforms, there was a huge drop in October compared to the other months except January (although it is also a bit lower). Again, September gave the highest count with November.

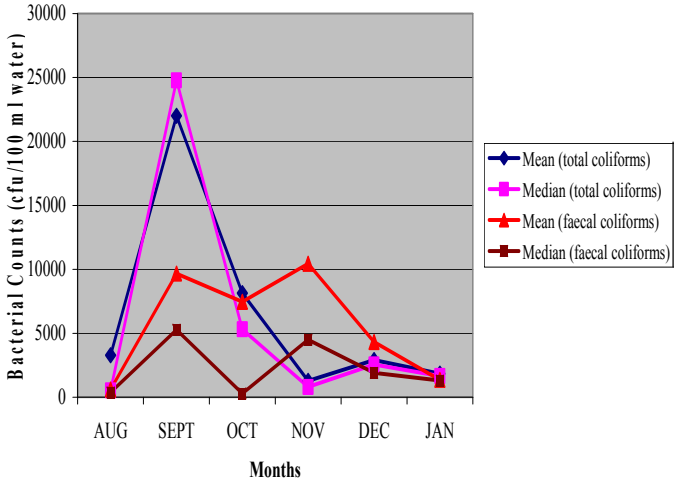

Fig. 4. Monthly means and medians of total and faecal coliforms in the Tufuiopa spring.

At the Tufuiopa spring (Fig.4), September gave the highest counts for total coliforms and the lowest counts in November. Faecal coliforms also fluctuated with the highest count in September followed by a decrease in October. Counts in December and January for both coliforms were quite similar. The ANOVA test and regression fit results shows that there were significant differences in total coliform counts between August and November, August and October and August and September. While counts in November and September were definitely greater than August, the counts in October were less.

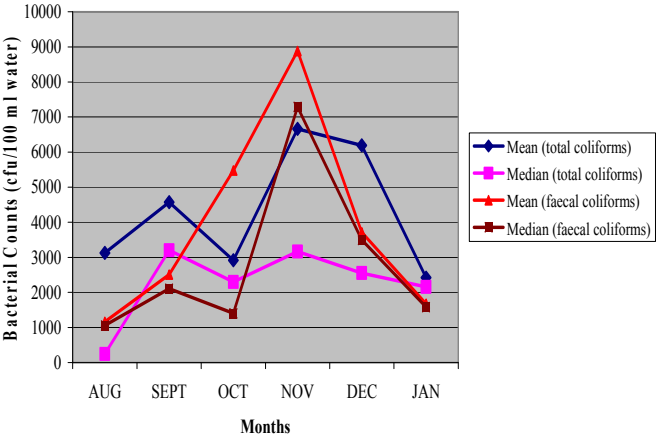

Fig. 5. Monthly means and medians of total and faecal coliforms in the Vinifou spring.

Fig.5 shows changes in the mean and median concentrations of total and faecal coliforms in water samples collected from the Vinifou spring during the six months of this project. The median faecal coliform counts at Vinifou spring were generally higher than the total coliform counts compared to the first two sites. There were increases in faecal coliforms from previous months during September and November with the latter giving the highest monthly number of faecal coliform colonies. Counts were similar for October and November with August giving the lowest count. For total coliforms and taking the median as the best

measure, the highest counts were measured in September and November. Like faecal coliforms, the lowest counts were measured in August. Total coliform counts did not flunctuate as much as faecal coliforms counts.

IV. Discussion

The presence of pathogens and indicator organisms in groundwater and surface water sources depends on various factors such as the physical and chemical features of the catchments area, the extent and array of human activities and animal sources that release pathogens to the environment. In surface waters, potential pathogen sources include point sources like stormwater overflows, as well as non-point sources, such as contaminated runoff from agricultural areas and areas with sanitation through onsite septic systems and latrines, a common feature here in Samoa. Other sources are wildlife and direct access of livestock to surface water bodies [13].

A. Spatial Comparisons of Springs

The coliform composition of water collected from the Malie spring was compared to the coliform composition of water collected from the other two sample collection sites. This inter-site comparison was conducted to determine if there was any variation in the total and faecal composition of water collected from the different sites in the six month period due to topography and conditions of the immediate surrounding of the springs. Fig. 6 shows peak levels of total coliform in September and December in all springs. Malie and Tufuiopa had the lowest bacterial concentrations in November while the lowest levels for Vinifou were measured in January. These trends are confirmed by the regression fit analysis.

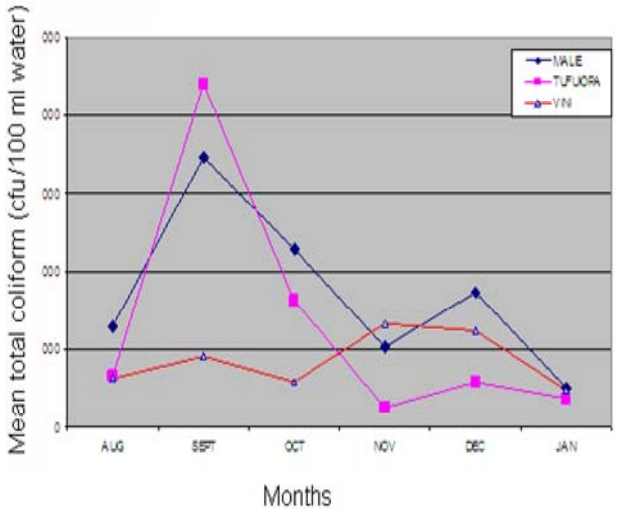

Fig. 6. Display of monthly means of total coliforms in the three springs.

The faecal total coliform levels at the three sites were quite variable with Malie, showing very high levels of bacteria September, November and December (Fig.7). Tufuiopa spring followed a similar pattern in September and November. The lowest levels of faecal coliforms were measured in August and January in all three sites. Malie also showed a huge decline in faecal coliform in October. The trends from statistical analysis confirmed that both Tufuiopa and Vinifou springs had lower levels of faecal coliform bacteria than Malie in all months except October.

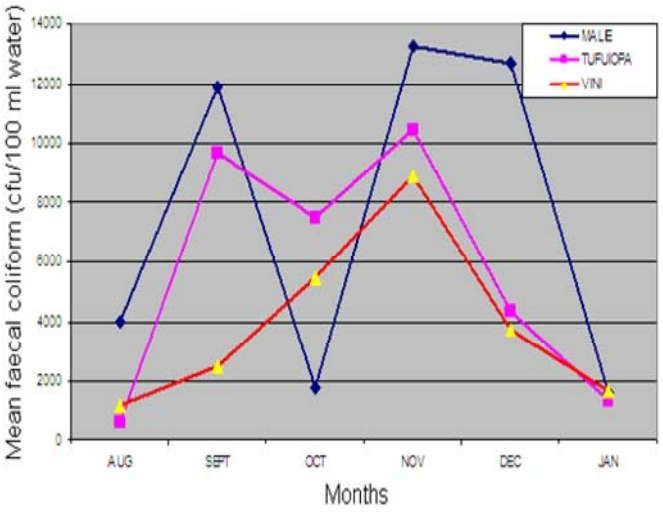

Fig. 7. Monthly means of faecal coliform in water from three spring

B. Spatial and Seasonal Bacterial Variations

This study investigated the levels of indicator bacteria – total coliform and faecal coliform – at three water sources during three months of the wet season and three months of the dry season. The findings have shown that all three sites were highly contaminated. Generally, Malie water was more contaminated with very high concentrations of both total and faecal coliform bacteria than Tufuiopa and Vinifou. Malie also showed significantly higher levels of total coliform during the dry season (August to October) compared to the other two sites. All three water sources have structural protections but whereas the latter two springs have cement protections which are about 1 to 2 m above water surface, Malie is only protected by piled rocks to a height that is levelled with ground surface. While protected water sources are generally less polluted than unprotected sources [14] other studies [15] have found that it is quite possible for protected water sources to be more contaminated than unprotected sources and during dry periods. One possible source of contamination is the greater exposure of the water source to faecal contamination [15]. For the three study sites, the water source is located close to family homes where possibly, children and domestic animals defecate openly. Vinifou spring lies right next to a pig sty and domestic animals frequently roam the water banks. Likewise, domestic animals like dogs and pigs frequent the other two sites. It has also been suggested that a structural difference probably tends to make the unprotected water sources less polluted during the dry period [15]. Of the three study sites, Malie has the least structural protection. The contamination of the Tufuiopa spring may also be related to the high population of local users and its proximity to town as previous studies on the association between water contamination and proximity to towns discovered [16].

V. Conclusion

This research discovered high levels of bacterial

contamination at the three springs which far exceeded WHO standards for safe drinking water in developing countries. The quality of these water sources need to be monitored more frequently to ensure complete safety for community use. A health survey of the three sites should be conducted to identify any frequent water users with possible infections from contaminated water.

ACKNOWLEDGMENT

We are grateful to the Centre for Samoa Studies (CSS) at the National University of Samoa, for the financial assistance which enabled this research to be done. We would also like to express our gratitude to the Mapping Division of the Ministry of Natural Resources and Environment (MNRE) for preparing the topographic maps and providing GPS coordinates for our research sites.

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