Chapter 4

RESULTS AND DISCUSSION

4.1 AN INTERVENTION STRATEGY TO IMPROVE THE DRINKING WATER QUALITY IN RURAL HOUSEHOLDS

Home based interventions aimed at improving the quality of drinking water at the point-of-use are becoming a feasible and effective way of immediately providing potable water to people who are dependant on untreated water (Sobsey, 2002). During the pilot study (section 2.7) it was seen that the microbiological quality of household water deteriorates during storage at the point-of-use. It was therefore decided that this study will assess the efficiency of the CDC safe water system (chlorine based water treatment combined with safe storage and education) at improving the microbiological quality of stored drinking water at the point-of-use in rural households in South Africa.

4.1.1 Baseline characteristics of households in two rural villages before intervention study

The household demographics of the two villages are indicated in Table 4.1. There were few differences between the 2 study groups with regards to the total number of adult males, adult females and children under the age of 5 years. A total of 524 people lived in the 120 interviewed households and the average number of people per household varied between 4.3 and 4.5.

The majority of households in both villages had between 2 and 5 rooms (Fig 4.1 and 4.2). Approximately 3% of the female heads of households in village 1 and 5% of village 2 households had no formal education. However, 82% of the female heads of households in village 1 and 84% of village 2 households had at least secondary education (Table 4.1). In addition, 68% of the households in village 1 had children (male and female) younger than 5 years of age compared to 73% of the households in village 2 (Table 4.1).

Figure 4.1: **Traditional households in two study villages in the Vhembe region of the Limpopo Province , South Africa**

Figure 4.2: **More western type households in two study villages in the Vhembe region of the Limpopo Province , South Africa**

Table 4.1: Summary of the household demographics indicating the number of people in each household and the level of education of the female head of the household in each of two rural villages in the Vhembe region of the Limpopo Province, South Africa

Demographics	Village 1 households using tap water (n=60 households)	Village 2 households using river water (n=60 households)
People in household		
Adult females	60 (100%)	60 (100%)
Adult males	51 (85%)	51 (85%)
Female children <5 years	22 (37%)	27 (45%)
Male children <5 years	19 (32%)	17 (28%)
Educational level of female head of household		
None	2 (3%)	3 (5%)
Only pre-primary	3 (5%)	0 (0%)
Only primary	2 (3%)	3 (5%)
Only secondary	49 (82%)	50 (84%)
Diploma	3 (2%)	2 (3%)
Degree	1 (1%)	2 (3%)

The households were selected based on the water source type they were using (Fig 3.2 and 3.3). Both villages did not have a committee and none of the households paid for water. Households were asked during the survey to indicate the location of their water collection point. The distance of the water source from the household was calculated for each household by measuring the distance in steps from the household to the specific water collection point of each household. The South African government target for reasonable access to a water source is 0 m to 200 m from the place of dwelling (Republic of South Africa, 1994). In these two villages, many of the people had to walk long distances to obtain water from the source. Approximately 53% of the households in village 1 and 37% of the households in village 2 had their water source located within 100 m from the household, while 47% of the households in village 1 and 63% of the households in village 2 had a water source located within 100 m to 500 m from the household (Table 4.2).

Table 4.2: Summary of the water sources used by the study households in each of two rural villages in the Vhembe region of the Limpopo Province, South Africa

Data	Village 1 households using tap water (n=60 households)	Village 2 households using river water (n=60 households)
Source distance from household:		
< 100 m	32 (53%)	22 (37%)
> 100 m	28 (47%)	38 (63%)
Is water readily available from source?:		
Yes	28 (47%)	60 (100%)
No	32 (53%)	0 (0%)
Alternative water source:		
None	0 (0%)	59 (98%)
Rainwater	1 (2%)	1 (2%)
River	59 (98%)	0 (0%)
Busiest time at primary water source:		
Morning	49 (82%)	40 (67%)
Afternoon	2 (3%)	2 (3%)
No busy time	9 (15%)	18 (30%)
Who fetches the water for the household?		
Only children	8 (13%)	1 (2%)
Only adults	21 (35%)	19 (31%)
Both	31 (52%)	40 (67%)
Number of water collections per day:		
Once	10 (17%)	11 (18%)
Twice	16 (27%)	14 (23%)
Thrice	34 (57%)	32 (53%)
Four times	0 (0%)	3 (4%)
Source water is considered clean	23 (38%)	14 (23%)
Source water is considered clear	57 (93%)	44 (73%)
Source water don't have a smell	5 (8%)	12 (20%)
Source water don't have a taste	12 (20%)	14 (23%)
Use of the source water:		
Drinking	60 (100%)	60 (100%)
Cooking	59 (98%)	59 (98%)
Bathing	42 (70%)	43 (72%)
Treatment of water from primary water source:		
None	56 (93%)	60 (100%)
Sodium hypochlorite	0 (0%)	0 (0%)
Boiling	4 (7%)	0 (0%)

Most of the households in village 1 (82%) and village 2 (67%) reported that mornings can be busy times at the respective sources (Table 4.2). Approximately 53% of the households using tap water in village 1 complained that water was not readily available at the primary water source. Therefore, almost 98% of the households in village 1 resorted to the river in their region as an alternative water source in times when water was not readily available from the communal taps (Table 4.2).

Approximately 34 (57%) households in village 1 and 32 (53%) households in village 2 reported to collect water 3 times per day, 16 (27%) households in village 1 and 14 (23%) households in village 2 collected water twice a day and 10 (17%) households in village 1 and 11 (18%) households in village 2 collected water once a day (Table 4.2). Adults (35% in village 1; 31% in village 2) or both adults and children (52% in village 1; 67% in village 2) were responsible for collection of water for their households (Table 4.2).

All the households in both villages used the primary water source for cooking (98%) and drinking (100%) purposes (Table 4.2). In village 1, 38% of the households considered the tap water as clean; 8% of the households reported that the tap water did not smell and 20% of the households reported that the tap water did not have a taste (Table 4.2). In village 2, 23% of the households considered the water to be clean; 20% of the households reported the water did not smell and 23% of the households reported that they had no problem with the taste of the river water (Table 4.2).

Inadequate or no treatment of stored drinking water remains a problem in low socio-economic households. The majority of households in village 1 (93%) and village 2 (100%), did not use any treatment before consuming the water, while 7% of the households in village 1 indicated that they used boiling as treatment of their drinking water (Table 4.2). This indicated a lack of knowledge and education by the households on the health risks associated with waterborne diseases.

Table 4.3: Summary of the water storage practices in study households in each of two rural villages in the Vhembe region of the Limpopo Province, South Africa

Data	Village 1 households using tap water (n=60 households)	Village 2 households using river water (n=60 households)
Do you store water in your household:		
Yes	60 (100%)	60 (100%)
No	0 (0%)	0 (0%)
Container size in which water is stored inside household:		
20-50 litre	33 (55%)	25 (42%)
50-100 litre	3 (5%)	8 (13%)
100-200 litre	7 (12%)	9 (15%)
>200 litre	17 (28%)	18 (30%)
Container storage conditions:		
Closed indoors	18 (30%)	34 (57%)
Closed outdoors	1 (2%)	2 (3%)
Open indoors	34 (57%)	22 (37%)
Closed indoors	6 (10%)	1 (2%)
Open/closed indoors	1 (2%)	0 (0%)
Open/closed outdoors	0 (0%)	1 (2%)
Number of times storage container is emptied:		
Daily	8 (13%)	17 (28%)
Weekly	27 (45%)	28 (47%)
Monthly	16 (27%)	10 (17%)
Rarely	9 (15%)	5 (8%)
Cleaning of storage containers:		
Daily	7 (12%)	15 (25%)
Weekly	28 (47%)	32 (53%)
Monthly	15 (25%)	12 (20%)
Rarely	10 (17%)	1 (2%)

All the households in both study groups stored their water after collection (Table 4.3). Different size containers were used for this purpose (Fig 2.10 and Fig 2.11), ranging from 20 to 50 litres (55% village 1 households; 42% village 2 households), 50 to 100 litres (5% village 1 households; 13% village 2 households), 100 to 200 litres (12% village 1 households; 15% village 2 households), to > 200 litres (28% village 1 households; 30% village 2 households) (Table 4.3). Several studies have reported that inadequate storage conditions could result in an increase in numbers of some microorganisms such as heterotrophic bacteria and total coliform bacteria over time

(VanDerSlice and Briscoe, 1993; Reiff *et al*., 1996). According to the survey it was evident that 30% of village 1 households and 56.7% of village 2 households stored their water containers indoors with a closed lid, while 57% of village 1 households and 37% of village 2 households stored their drinking water containers indoors in open containers (Table 4.3). Further observations indicated that 15% of village 1 households and 13% of Village 2 households had loose covers on their storage containers. Approximately 58% of village 1 households and 32% of village 2 households had no cover on the storage containers. Earlier studies by Dunker (2001) and Nala and co-workers (2003) have showed that open containers were more at risk of being contaminated by human and animals than containers which were covered. Many of the households in this study kept their water storage containers on the floor which was smeared with fresh cow dung (Fig 4.3). When the cow dung becomes dry, it forms a dust layer which could contain microorganisms. The cow dung also attracted flies which could be potential vehicles of disease and can contaminate water and food supplies in these rural households (Benenson, 1995).

Figure 4.3: A female member of the study community in the Vhembe region of the Limpopo Province, South Africa busy smearing the floors of the dwelling with cattle dung using her bear hands

Most of the households (45% households in village 1; 47% households in village 2) reported to clean the storage container after 7 days (Table 4.3). Consequently, biofilm formation inside household storage containers (Fig 2.14) due to improper cleaning practices could aid in the survival and growth of potential pathogenic disease causing microorganisms (Bunn *et al*., 2002; Momba and Kaleni, 2002; Jagals *et al*., 2003). Jagals and co-workers (2003) showed that biofilm growth in storage containers can be removed or limited with effective cleaning. Bunn and co-workers (2002) and Momba and Kaleni (2002) have showed in two separate studies (South Africa and Gambia) that indicator organisms (total coliforms, faecal coliforms, *E. coli*, *C. perfringens*, somatic and male specific F-RNA bacteriophages) and pathogens (*Salmonella* spp and *Helicobacter pylori*) could survive longer than 48 h in biofilms inside household drinking water storage containers.

Poor sanitation could conditions increase the risk of diseases in a household (WHO, 2002a). A study by Alam and Zurek (2004) has showed that houseflies carry virulent *E. coli* O157:H7 in areas where cattle are kept and this may play an important role in the transmission of this pathogen between cattle and to the household environment. Consequently, observations made by the interviewers included the following: 35% of the households in village 1 and 8% of the households in village 2 had a dirty yard of which 43% households in village 1 and 8% households in village 2 had flies present in the yard. In village 1, 35% households had dirty kitchens and 40% of the village 1 households had flies present in the kitchen. In village 2, 10% of the households had dirty kitchens and 5% of the households had flies present in the kitchen. Garbage containers were absent in 100% of the households in village 1 and 98% of the households in village 2. Approximately 52% of households in village 1 and 37% of households in village 2 had flies in the toilet. Approximately 63% households in village 1 and 37% households in village 2 had a pit latrine. However, 35% of the households in Village 1 and 62% of the households in village 2 had no toilet facilities and had to use the bush near their household to relieve themselves (Table 4.4).

The method used to obtain water from the storage container could contribute to contamination and the spreading of potential disease causing microorganisms between members of the same household (Jagals *et al*., 1999). Approximately 90% of village 1

households and 97% of village 2 households used a mug to collect water from the storage container (Fig 4.4).

Table 4.4: Summary of hygiene and sanitation conditions/practices in study households in each of two rural villages in the Vhembe region of the Limpopo Province, South Africa

Data	Village 1 households using tap water (n=60 households)	Village 2 households using river water (n=60 households)
Toilet facilities:		
Use bush	21 (33%)	37 (62%)
Use neighbour's toilet facilities	1 (2%)	0 (0%)
Have pit latrine	38 (63%)	23 (38%)
Hand wash facility close to toilet	1 (2%)	0 (0%)
Toilet paper available for use when going to toilet	28 (42%)	51 (85%)
Soap present in household	2 (3%)	1 (2%)
Hand washing practices:		
Before eating	58 (97%)	57 (95%)
Before food preparation	3 (5%)	7 (12%)
After being to toilet	29 (48%)	17 (28%)
After cleaning baby's butt	4 (7%)	9 (15%)
Waste storage in households:		
Daily	24 (40%)	42 (70%)
Weekly	19 (32%)	11 (19%)
Monthly	2 (3%)	3 (5%)
Rarely	15 (25%)	4 (7%)
Waste disposal by households:		
Inside or outside yard	2 (3%)	0 (0%)
Only inside yard	2 (3%)	0 (0%)
Only outside yard	56 (94%)	60 (100%)
Animals in or close to household:		
Cats	2 (3%)	8 (13%)
Dogs	10 (17%)	29 (48%)
Poultry	31 (52%)	40 (67%)
Pigs	2 (3%)	2 (3%)
Goats	21 (35%)	10 (17%)
Cattle	7 (12%)	3 (5%)
Donkeys	4 (7%)	0 (0%)

Jagals and co-workers (1999) and Sobsey (2002) indicated that faecally contaminated hands of household members who do not apply personal hygiene practices can contribute to water contamination (Fig 4.4). Observations made during the baseline survey showed that the mug was not washed every time it was used and was left next to the storage container where animals and small children had access to it.

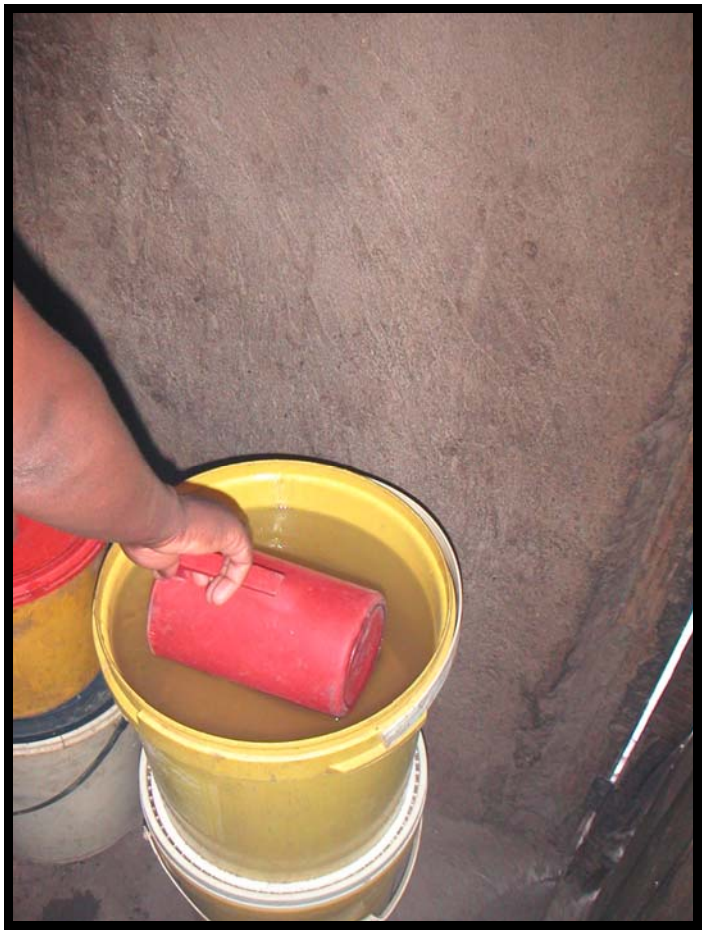

Figure 4.4: One of the study households in the two rural villages in the Vhembe region of the Limpopo Province, South Africa using a mug to collect water from a water storage container

In this study, only 2% households in village 1 and no households in village 2 had a place near the toilet to wash hands. The survey further indicated that approximately 48% households in village 1 and 28% households in village 2 washed hands after going to the toilet. Furthermore, observations during the survey indicated that only 3% of the village 1 households and 2% of the village 2 households had toilet paper available in the toilet. Generally, the toilets were not in good conditions (Fig 4.5 and Fig 4.6).

Figure 4.5: **A typical pit toilet used in both study villages in the Vhembe region of the Limpopo Province, South Africa: no toilet paper available and people used old magazines and newspapers**

Figure 4.6: **A VIP toilet used in both study villages in the Vhembe region of the Limpopo Province, South Africa**

It was also noted that between 95% and 97% of the study population in both villages, reported to wash their hands before eating, while only 5% of the households in village 1 and 12% of the households in village 2 reported to wash hands before they prepared food. This practice was considered a potential risk of faecal contamination of food and water supplies in these households. In addition, only 7% of the mothers in households from village 1 and 15% of mothers in households from village 2 reported to wash their hands after cleaning their baby's buttocks (Table 4.4). This practice was considered another risk of potential contamination of domestic drinking water supplies because studies have indicated that *E. coli* spp, *Klebsiella* spp, *Shigella sonnei* and faecal enterococci can survive between 10 min and 3.5 h on unwashed hands (Knittle, 1975; Casewell and Phillips, 1977; Pinfold, 1990).

Furthermore, 40% of the households in village 1 and 70% of the households in village 2 stored solid wastes on a daily basis (Table 4.4). Approximately 32% of village 1 households and 19% of village 2 households stored solid wastes for 7 days (Table 4.4). In general, only 25% of the study households in village 1 and 7% of study households in village 2 reported to rarely or never store solid wastes. This could be a potential breeding place for flies and pose a health risk to the communities (Table 4.4).

A close living association between the people and animals such as cattle, poultry, donkeys, pigs, goats, dogs and cats was observed in both study villages during this survey (Table 4.4). The majority of households (52% households in village 1; 67% of households in village 2) kept poultry, 35% households in village 1 and 17% households in village 2 kept goats, while 12% of the householdshouseholds in village 1 and 5% of the households in village 2 kept cattle close to the dwelling (Table 4.4). These animals generally walk free in the vicinity of the households (Fig 4.7) and the water sources which increases the risk of waterborne transmission of zoonotic pathogens (Meslin, 1997; Franzen and Muller, 1999; Slifko *et al*., 2000; Enriquez *et al*., 2001; Hoar *et al*., 2001; Leclerc *et al*., 2002; Hackett and Lappin, 2003).

Figure 4.7: Animals like goats moves freely around at one of the study households in the Vhembe region of the Limpopo Province, South Africa

Ignorance and a lack of education concerning waterborne diseases could play an important role in the general health of a household. Results from the baseline survey indicated that only 58% of the households in village 1 and 46% of the households in village 2 reported to have knowledge of waterborne diseases (Table 4.5). This is in spite of the Department of Health and Primary Health Care Clinics in the Vhembe district giving regular education sessions on waterborne diseases to the village communities. However, the clinic staff did mention they have problems reaching all the households due to transport problems and shortage of staff (personal communication with staff members at the various clinics).

During the baseline survey, it was found that 28% of the households in village 1 and 18% of the households in village 2 had a child under the age of 5 years who had suffered from diarrhoea in the last 6 months prior to the survey (Table 4.5). However, the majority of respondents (33% from village 1 households; 32% from village 2 households) had no idea what the cause of the child's diarrhoea were; 50% of the households in village 1 and 47% of the households in village 2 gave contaminated water

as reason and 12% of the households in village 1 and 10% of the households in village 2 mentioned food as a possible cause (Table 4.5).

Table 4.5: Knowledge of waterborne diseases by study households in each of two rural villages in the Vhembe region of the Limpopo Province, South Africa

Data	Village 1 households using tap water (n=60 households)	Village 2 households using river water (n=60 households)
Number of households with knowledge on waterborne diseases	30 (50%)	28 (46%)
Households with children <5 years with diarrhoea in last 6 months	17 (28%)	11 (18%)
What do head of household think caused the child's diarrhoea?		
Dirty water	30 (50%)	28 (47%)
Food	7 (12%)	6 (10%)
No idea	20 (33%)	19 (32%)
Poor hygiene	0 (0%)	2 (3%)
Seasonal change	3 (5%)	0 (0%)
Teething	0 (0%)	5 (8%)
How can diarrhoea in children be prevented?		
Clean water	25 (42%)	21 (35%)
Clean food	1 (2%)	0 (0%)
Medicine	3 (5%)	9 (15%)
No idea	31 (52%)	30 (50%)

It was alarming that 0% of the households in village 1 and 3% of the households in village 2 thought that poor hygiene could be responsible for the child's diarrhoea (Table 4.5). Similarly 52% of households in village 1 and 50% of the households in village 2 had no idea how to prevent the child from getting diarrhoea (Table 4.5). However, 42% of households in village 1 and 35% of households in village 2 did mention that clean (safe) water could prevent diarrhoea in children (Table 4.5).

4.1.2 The effectiveness of a home chlorination intervention study

The intervention households using the 1% and the 3.5% sodium hypochlorite solutions had zero counts for heterotrophic bacteria, total coliforms, faecal coliforms, *E. coli*, faecal enterococci, *C. perfringens*, somatic and male specific F-RNA bacteriophages in the water samples taken from both container types during the formal intervention trial. This indicated that both the 1% and 3.5% sodium hypochlorite solutions were effective home based treatments. Therefore, all the results discussed in this section on household water samples will be on counts obtained for the traditional and CDC safe storage containers in households receiving the placebo solution.

4.1.2.1 The physical quality of the primary water sources and the container stored water used by the two rural villages

The pH values for tap water ranged between 7.0 and 7.1 and for river water varied between 6.8 and 7.7 (Table 4.6). The pH values of both types of containers fell within the South African water quality pH guideline range for domestic use of 6.0 to 9.0 (Table 4.6) (DWAF, 1996). Several studies have indicated that pH could play a role in the survival of microorganisms during disinfection (Engelbrecht *et al.*, 1980; Schaper *et al*., 2002b). A study by Vaughn and co-workers (1986) has showed that viruses are more readily inactivated by chlorine when the water had a pH level of 6 compared to the water samples which had a pH level of 8. In this study no statistical differences (P=0.783) between the tap and river water sources with regards to the pH was found (Tables 4.7 and 4.8). In village 1 no statistical difference were found between the pH values from the communal tap water source and the traditional storage containers (P=0.354) and between the tap water sources and the CDC safe storage containers (P=0.388). In addition, no significant difference were seen in the pH measurements between the two types of water storage containers (P=0.483) (Table 4.6). Likewise, in village 2, no statistical difference were found between the pH values from the river water source and the traditional storage containers (P=0.423) and between the river water source and the CDC safe storage containers (P=0.438) (Table 4.6). In addition, no significant difference were seen in the pH measurements between the two types of water storage containers in Village 2 (P=0.350) (Table 4.6).

Table 4.6: Geometric mean values (95% confidence intervals) of the physical parameters of the water sources and the traditional and the CDC safe storage containers of two rural villages using the placebo solution in the Vhembe region of the Limpopo Province, South Africa

Physical parameters	Village 1 using tap water			Village 2 using river water		
	Communal tap water sources	Traditional water storage container	CDC safe water storage container	River water source	Traditional water storage container	Improved CDC water storage container
pH	7.0 (7.0; 7.1)	7.3 (7.0; 7.8)	7.3 (7.0; 7.8)	7.2 (6.8; 7.7)	7.0 (6.9; 7.2)	7.4 (6.7; 7.6)
Temperature (ºC)	19.4 (18.6; 20.2)	20.2 (19.2; 21.3)	19.4 (18.6; 20.2)	19.3 (15.6; 22.9)	19.3 (18.6; 19.9)	19.7 (18.7; 19.9)
Turbidity (NTU)	0.6 (0.3; 1.0)	0.6 (0.1; 1.1)	0.9 (0.2; 1.5)	5.9 (4.1; 7.7)	4.2 (3.0; 5.3)	3.5 (2.4; 4.6)

The South African recommended guideline values for temperature of domestic water ranged between 18ºC to 24ºC (DWAF, 1996). In this study the temperatures for all water source samples as well as water samples obtained from the traditional and CDC safe storage containers in both villages fell well within this range. This indicated that disinfection of the microorganisms in these water sources might be successful (Table 4.6). Several studies have shown that temperature plays an important role in the survival of microorganisms and the effectiveness of a disinfectant. Atkin and co-workers (1971) and Sattar (1981) have showed that viruses have a tendency to survive longer in groundwater sources than in surface water at similar temperatures due to the effect of temperature and ultra violet sunrays. Carlsson (2003) stated that an increase in water temperatures can result in higher rates of inactivation of microorganisms in water samples.

In this study no statistical differences (P=0.867) between communal tap and river water samples with regards to the temperature readings were seen (Tables 4.7 and 4.8). In village 1 no statistical difference were found between the temperature values from the communal tap water source, the traditional storage containers (P=0.03) and the CDC safe storage containers (P=0.281). In addition, no significant difference were seen in the temperature measurements between the traditional and CDC safe storage containers (P=0.193) (Table 4.6). In village 2, no statistical difference were found between the temperature values from the river water source and the traditional storage containers (P=0.359) and between the river water source and the CDC safe storage containers (P=0.154) (Table 4.6). In addition, no statistical difference between the traditional and CDC safe storage containers in village 2 with regards to temperature could be seen (P=0.462) (Table 4.6).

Turbidity measurements give a general indication of the concentration of suspended clay, silt, organic matter, inorganic matter, plankton and other microscopic organisms in a water source (DWAF, 1996). In this study the river water source samples had turbidity values which exceeded the recommended South African guideline value of 0.1 NTU (Table 4.6) (SABS, 2001). High turbidity values are associated with the survival of microorganisms due to association of the microorganisms with particulate matter in the water (DWAF, 1996). Tap water sources had turbidity values between 0.3 NTU and 1.0 NTU and river water sources had turbidity values ranging from 4.1 NTU to 7.7 NTU

(Table 4.6). Therefore, a significant difference ($P<0.001$) was observed in the turbidity values between the two types of water sources. This suggested that the river water had more nutrients and particulate matter, which could have assisted in the survival and transmission of waterborne diseases due to the association between microorganisms and particulate particles (DWAF, 1996). The turbidity of the water inside the traditional and improved CDC safe storage containers in households using the tap and the river water sources were higher than the South African guideline values of 0.1 NTU (SABS, 2001) (Table 4.6). This could have reduced the effectivity of the disinfectant used in this study and assisted in the survival of microorganisms due to association of the microorganisms with particulate matter in the water (DWAF, 1996).

In village 1, no statistical differences in the turbidity values between the communal tap water source and the traditional storage containers ($P=0.934$) and between the tap water and the CDC safe storage containers ($P=0.439$) were seen. In addition, in village 1, no statistical significant difference were seen between the turbidity measurements of the traditional and the CDC safe storage containers ($P=0.243$) (Table 4.6). While in village 2, a significant statistical difference between the turbidity values from the river water source and the traditional storage containers ($P=0.008$) and between the river water source and the CDC safe storage containers ($P=0.001$) were observed (Table 4.6). The lower turbidity measurements in the storage containers could be due to settlement of particular matter in the containers during storage. However, no statistical significance between the turbidity values from the two types of storage containers were observed ($P=0.814$) in village 2 (Table 4.6).

4.1.2.2 The microbiological quality of the primary water sources and the container stored water in the two rural villages

Microbiological quality of the primary water sources used in both villages was assessed using indicator microorganisms which included heterotrophic plate counts, total coliforms, *E. coli*, faecal coliforms, faecal enterococci, somatic and male specific F-RNA bacteriophages (DWAF, 1996). The presence of these indicator microorganisms in a water sample, were a general guideline to indicate the presence of potential pathogenic bacteria, viruses and parasites and to determine the health risk to consumers (DWAF, 1996).

Heterotrophic plate counts indicated the general microbiological quality of the water samples and mostly included microorganisms such as *Aeromona* spp, *Klebsiella pneumoniae, Enterococcus, Flavobacterium* spp, *Bacillus* spp and *Enterobacter* spp that required organic carbon for growth (DWAF, 1996; WHO, 2002b). Generally heterotrophic microorganisms are considered to be harmless. However, various studies have indicated that some heterotrophic microorganisms might be opportunistic pathogens (Payment *et al*., 1991; WHO, 2002b; Bartram *et al*., 2003). These opportunistic pathogens have been associated with diseases in immunocompromised individuals, infants and the elderly during exposure to or consumption of contaminated water (Payment *et al*., 1991; Bartram *et al*., 2003).

In this study, the heterotrophic bacterial counts for the communal taps and river water as well as the traditional and CDC safe storage container water samples in both villages exceeded the South African recommended guideline value of 100 cfu.ml^{-1} (Tables 4.7 and 4.8) (SABS, 2001). Heterotrophic microorganisms are found as natural inhabitants of water and soil environments and might have been present in the communal tap water and river water sources or due to biofilms inside the reservoir and pipe distribution systems or due to various animal- and human activities inside the river catchment (Bartram *et al*., 2003).

Table 4.7: Geometric mean values (95% confidence intervals) for microbiological indicators of water samples collected over a 4 month period from communal tap water sources and the stored household water in traditional and CDC safe storage containers used by households together with the placebo solution from village 1 in the Vhembe region of the Limpopo Province, South Africa

Water source and container type	Heterotrophic bacteria (cfu.1 ml^{-1})	Total coliforms (cfu.100 ml^{-1})	Faecal coliforms (cfu.100 ml^{-1})	*Escherichia coli* (cfu.100 ml^{-1})	Faecal enterococci (cfu.100 ml^{-1})	*Clostridium perfringens* (cfu.100 ml^{-1})
Communal tap source*	1.6×10^6 (6.6×10^5; 4.2×10^6)	360 (247; 525)	180 (116; 277)	84 (54; 124)	37 (18; 72)	34 (14; 83)
Traditional containers**	3.0×10^7 (7.7×10^6; 1.2×10^8)	783 (435; 1 411)	414 (221; 775)	115 (77; 170)	100 (51; 197)	98 (69; 140)
CDC safe storage containers**	1.7×10^7 (5.0×10^6; 5.4×10^7)	944 (638; 1 390)	578 (409; 816)	120 (74; 196)	105 (47; 233)	90 (40; 199)

*** n= 16 taps**

**** n = 10 households**

Table 4.8: Geometric mean values (95% confidence intervals) for microbiological indicators of water samples collected over a 4 month period from communal tap water sources and the stored household water in traditional and CDC safe storage containers used by households together with the placebo solution from village 2 in the Vhembe region of the Limpopo Province, South Africa

Water source and container type	Heterotrophic bacteria (cfu.1 ml^{-1})	Total coliforms (cfu.100 ml^{-1})	Faecal coliforms (cfu.100 ml^{-1})	*Escherichia coli* (cfu.100 ml^{-1})	Faecal enterococci (cfu.100 ml^{-1})	*Clostridium perfringens* (cfu.100 ml^{-1})
River water source *	2.1×10^6 (1.1×10^5; 3.9×10^7)	844 (691; 1 032)	538 (328; 883)	166 (90; 306)	154 (42; 582)	132 (21; 807)
Traditional containers**	5.3×10^6 (5.5×10^5; 5.1×10^7)	1 345 (1 100; 1 643)	1 025 (784; 1 341)	413 (279; 610)	139 (80; 241)	170 (106; 274)
CDC safe storage containers**	1.0×10^7 (2.2×10^6; 4.8×10^7)	1 380 (1 157; 1 646)	1 090 (855; 1 389)	343 (215; 548)	94 (62; 142)	125 (95; 165)

*** n= 4 sites on river**

**** n = 10 households**

The results further indicated that the counts detected in the household storage containers (traditional and CDC safe storage containers) were higher than the primary water source counts (Tables 4.7 and 4.8). The increase in heterotrophic plate counts in both the traditional and the CDC safe storage containers could be ascribed to: (1) secondary contamination of the stored water, (2) re-growth of some heterotrophic microorganisms, or (3) unhygienic water-handling practices (Nala *et al*., 2003). The higher heterotrophic plate counts in the storage containers indicated an increased risk to people consuming the water for infections by opportunistic pathogenic microorganisms such as *Aeromona* spp and *Pseudomonas* spp, which have been associated with diseases such as diarrhoea, skin, eye and respiratory infections (DWAF, 1996; Bartram *et al*., 2003).

The statistical analysis of the heterotrophic bacterial counts indicated the following:

- No statistical difference (P=0.272) could be seen between the heterotrophic bacterial counts of the river and tap water sources (Tables 4.7 and 4.8).
- In village 1 no statistical differences was found between the tap water source and the traditional storage containers (P=0.359) or between the tap water source and the CDC safe storage containers (P=0.968) (Table 4.7).
- In village 2 no statistical differences was found between the river water source and the traditional storage containers (P=0.196) or between the river water source and the CDC safe storage containers (P=0.303) (Table 4.8).
- In village 1 no statistical differences was found between the traditional storage containers and the CDC safe storage containers (P=0.459) (Table 4.7 and Fig 4.8).
- In village 2 no statistical differences was found between the traditional storage containers and the CDC safe storage containers (P=0.597) (Table 4.8 and Fig 4.8).
- In general no statistical difference with regards to heterotrophic bacteria could be seen between the traditional and CDC safe storage containers using the placebo solution (P=0.974). This showed that the CDC safe storage container alone did not make a difference in improving water at the point-of-use.

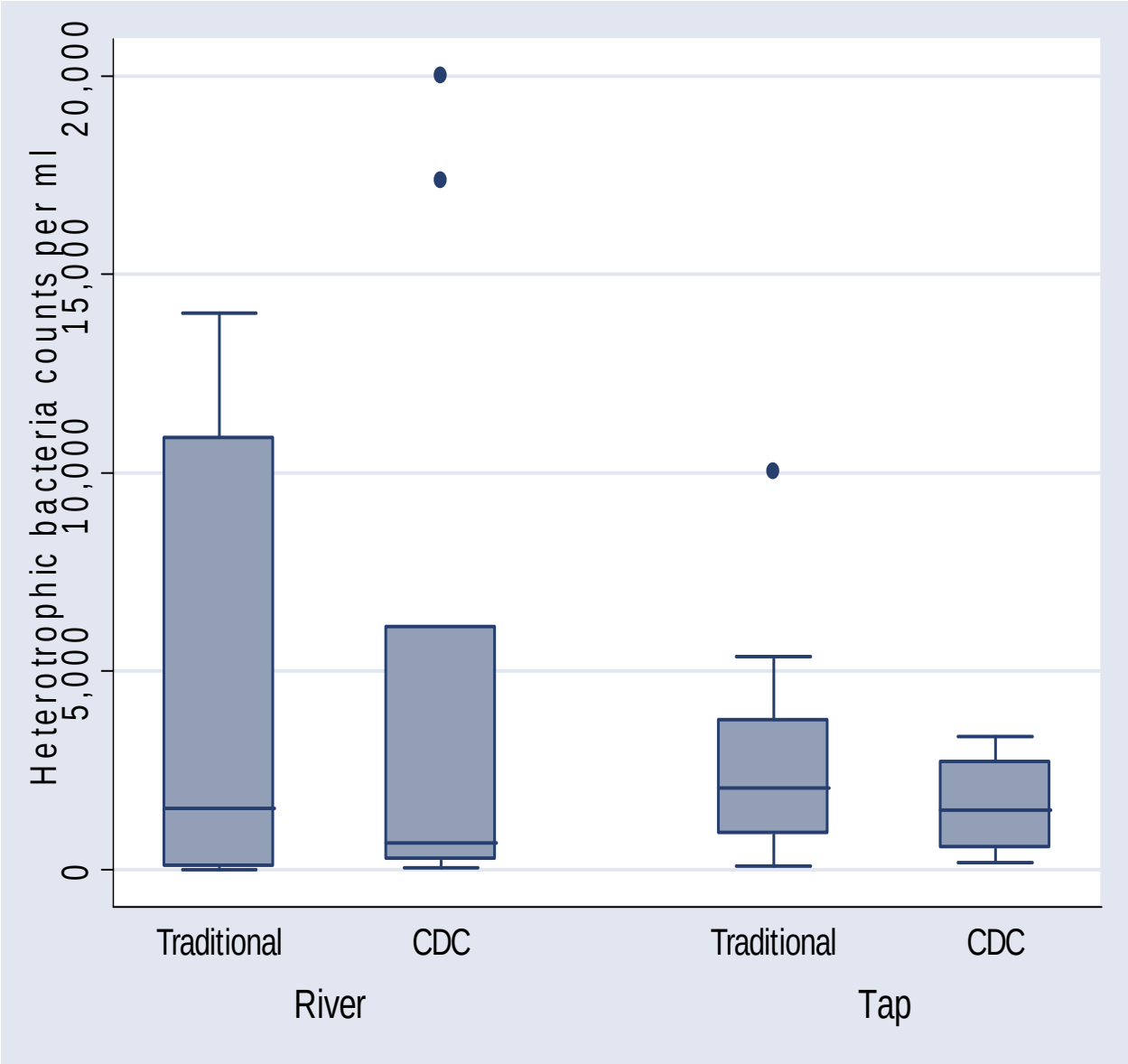

Figure 4.8: **Heterotrophic bacteria distributed by primary water sources and stored water in traditional and CDC safe storage containers from two villages in the Vhembe region of the Limpopo Province, South Africa**

Total coliforms included bacteria of known faecal origin such as *E. coli*, as well as bacteria such as *Citrobacter* spp and *Enterobacter* spp which may be found in faeces and the environment and bacteria such as *Serratia* spp which may replicate in water environments (WHO, 1996). The total coliform bacterial count for tap and river water sampling points as well as the total coliform counts for the stored water in the traditional and CDC safe storage containers in village 1 and village 2 exceeded the South African guideline value of 10 cfu.100ml^{-1} for total coliforms presence in water intended for domestic purposes (Tables 4.7 and 4.8) (SABS, 2001).

The high total coliform counts in the water sources and especially in the storage containers increased the health risk associated with waterborne diseases such as gastroenteritis, dysentery, cholera, typhoid fever and salmonellosis which are caused by pathogenic organisms such as *Salmonella* spp, *Shigella* spp, *Vibrio cholerae*, *Campylobacter jejuni*, *Campylobacter coli*, *Yersinia enterocolitica* and pathogenic *E. coli* (DWAF, 1996). In addition, the increase in the total coliform counts in the traditional and the CDC safe storage containers during storage at the point-of-use in both villages indicated secondary contamination due to unhygienic handling practices and storage conditions (Tables 4.7 and 4.8) (Jagals *et al.*, 1999; Nath *et al*., 2006).

The statistical analysis of the total coliform bacterial counts indicated the following:

- A statistical difference (P=0.004) could be seen between the total coliform bacterial counts of the river and tap water sources (Tables 4.7 and 4.8).
- In village 1 statistical differences was found between the tap water source and the traditional storage containers (P=0.02) and between the tap water source and the CDC safe storage containers (P=0.003) (Table 4.7).
- In village 2 statistical differences was found between the river water source and the traditional storage containers (P=0.0005) and between the river water source and the CDC safe storage containers (P=0.0001) (Table 4.8).
- In village 1 no statistical differences was found between the traditional storage containers and the CDC safe storage containers (P=0.557) (Table 4.7 and Fig 4.9).
- In village 2 no statistical differences was found between the traditional storage containers and the CDC safe storage containers (P=0.829) (Table 4.8 and Fig 4.9).
- In general no statistical difference with regards to total coliform bacteria could be

seen between the traditional and CDC safe storage containers using the placebo solution (P=0.557). This showed that the CDC safe storage container alone did not make a difference in improving water at the point-of-use.

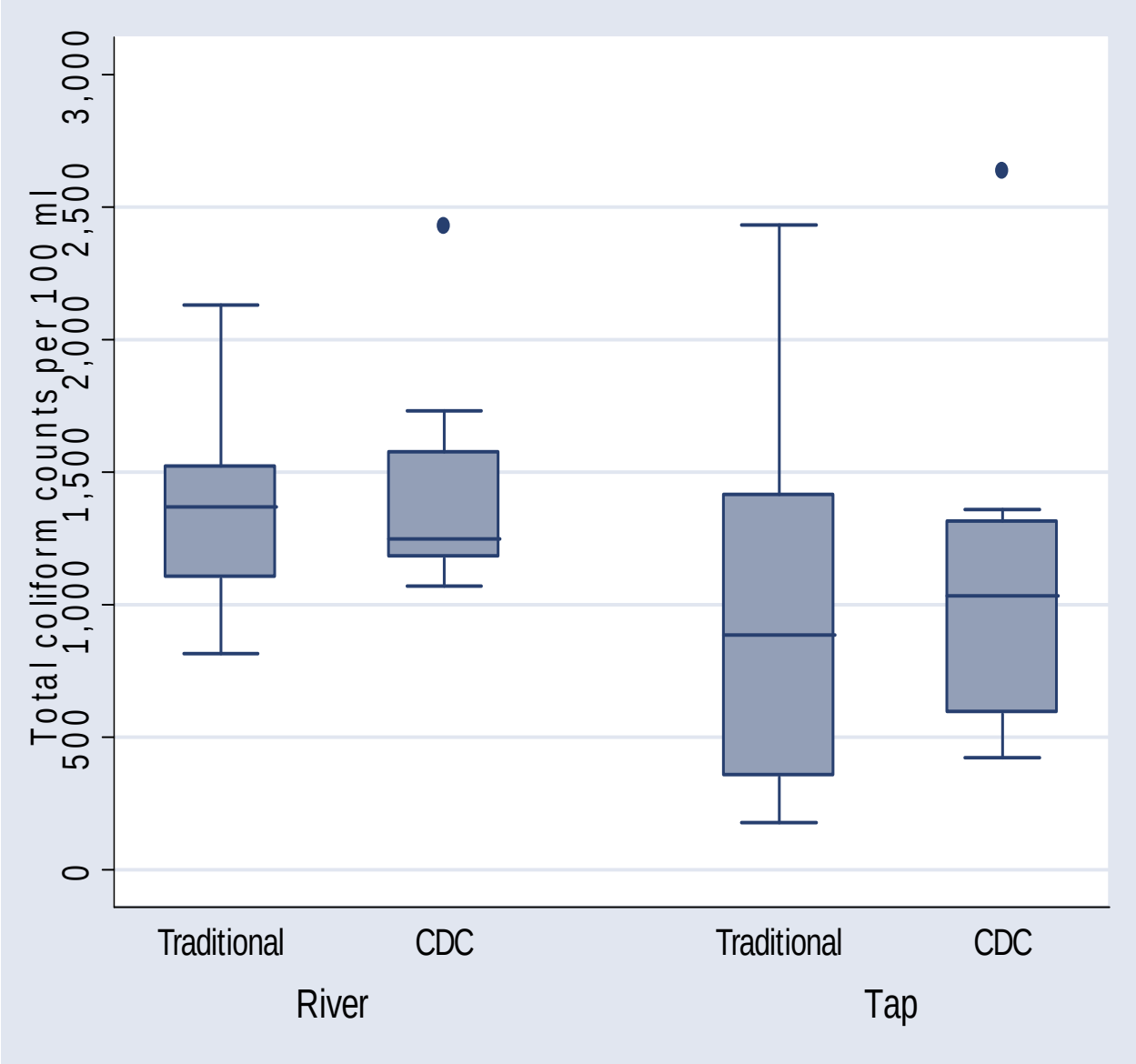

Figure 4.9: **Total coliform bacteria distributed by primary water sources and stored water in traditional and CDC safe storage containers from two villages in the Vhembe region of the Limpopo Province, South Africa**

Faecal coliform bacteria were used in this study to indicate the presence of potential pathogenic microorganisms that is transmitted through the faecal-oral route (DWAF, 1996). The faecal coliform counts in the water sources and the traditional and CDC safe storage containers in village 1 and village 2 households exceeded the South African recommended guideline value of 0 cfu.100 ml^{-1} (Tables 4.7 and 4.8) (SABS, 2001). The high faecal coliform counts in the river water samples indicated that the river has been contaminated due to direct faecal contamination from warm-blooded animals/humans or sewage run-off during rainy periods (WHO, 2002a).

In addition, the increase in faecal coliform counts in the traditional and the CDC safe storage containers in both village households during storage at the point-of-use in both villages (Tables 4.7 and 4.8) were in agreement with results from previous studies indicating the microbiological decrease in water quality after collection (Sobsey, 2002; Fewtrell *et al*., 2005). This increase in faecal coliform counts in the storage containers in both village households, indicated secondary contamination either due to human or animal faecal matter or because of unhygienic storage and handling practices at the point-of-use (DWAF, 1996).

The statistical analysis of the faecal coliform bacterial counts indicated the following:

- A statistical difference (P=0.004) could be seen between the faecal coliform bacterial counts of the river and tap water sources (Tables 4.7 and 4.8).
- In village 1 statistical differences was found between the tap water source and the traditional storage containers (P=0.012) and between the tap water source and the CDC safe storage containers (P=0.0001) (Table 4.7).
- In village 2 statistical differences was found between the river water source and the traditional storage containers (P=0.0004) and between the river water source and the CDC safe storage containers (P=0.0001) (Table 4.8).
- In village 1 no statistical differences was found between the traditional storage containers and the CDC safe storage containers (P=0.306) (Table 4.7 and Fig 4.10).
- In village 2 no statistical differences was found between the traditional storage containers and the CDC safe storage containers (P=0.708) (Table 4.8 and Fig 4.10).
- In general no statistical difference with regards to faecal coliform bacteria could be seen between the traditional and CDC safe storage containers using the placebo

solution (P=0.364). This showed that the CDC safe storage container alone did not make a difference in improving water at the point-of-use.

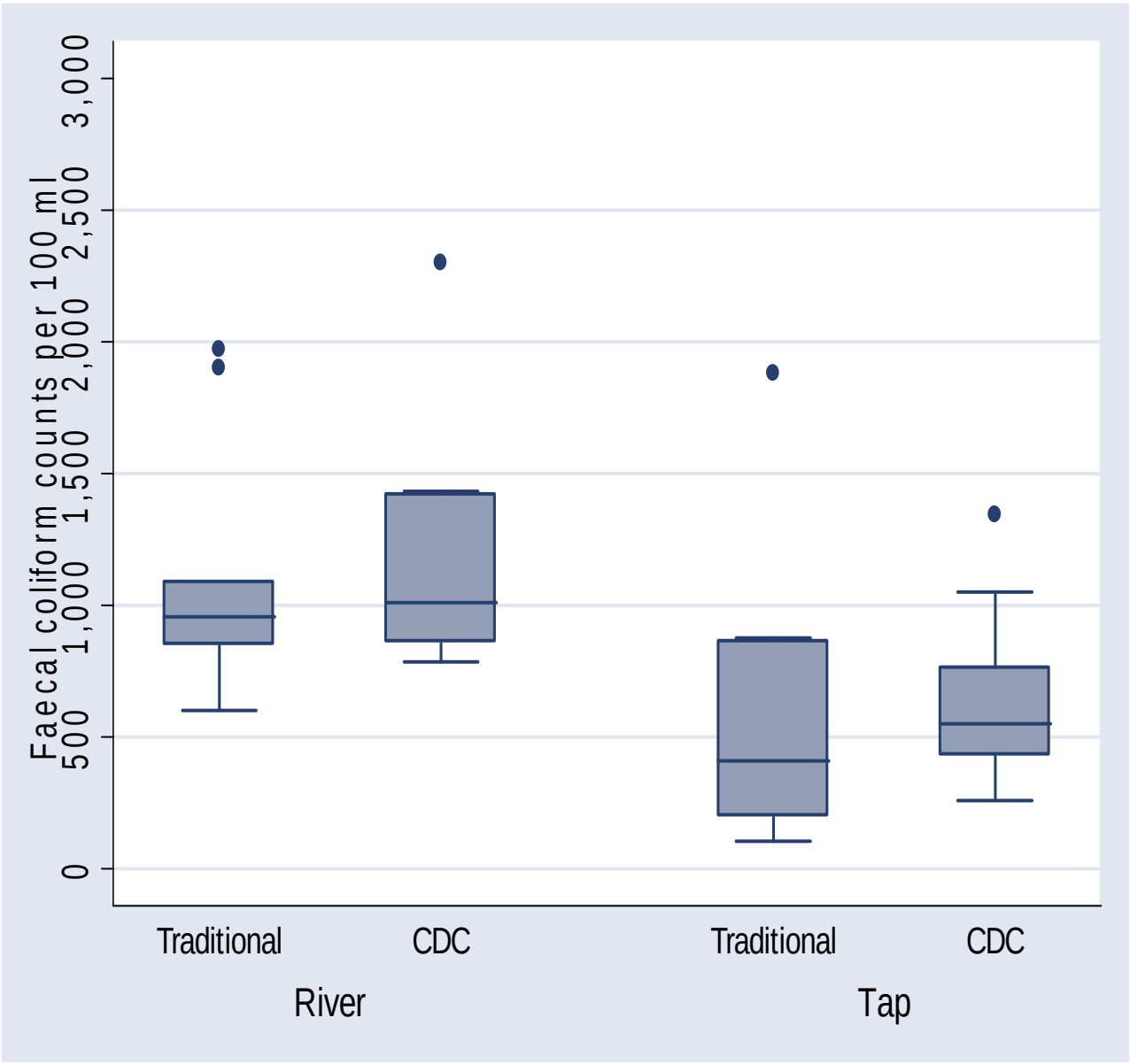

Figure 4.10: Faecal coliform bacteria distributed by primary water sources and stored water in traditional and CDC safe storage containers from two villages in the Vhembe region of the Limpopo Province, South Africa

Although *E. coli* bacteria are found in the faeces of humans and animals, pathogenic *E. coli* strains have virulence factors, which could be responsible for the cause of diseases and therefore implicate a potential risk to the consumers (Kuhnert *et al*., 2000). The detection of *E. coli* in the water samples indicated the presence of faecal pollution from warm blooded animals and humans (Kuhnert *et al*., 2000). During this study the *E. coli* counts exceeded the recommended guideline value of 0 cfu.100 ml^{-1} for both the water sources and the two types of storage containers in both villages (Tables 4.7 and 4.8) (Edberg *et al*., 2000; SABS, 2001). The results of this study showed *E. coli* counts increased after collection and indicated secondary contamination of the stored household water at the point-of-use (Tables 4.7 to 4.8).

The statistical analysis of the *E. coli* bacterial counts indicated the following:

- A statistical difference (P=0.010) could be seen between the *E. coli* bacterial counts of the river and tap water sources (Tables 4.7 and 4.8). This indicated that unimproved sources (River water) were more prone to faecal contamination than improved sources (communal taps) due to human and animal activities in the vicinity of the source.
- In village 1 no statistical differences was found between the tap water source and the traditional storage containers (P=0.109) and between the tap water source and the CDC safe storage containers (P=0.131) (Table 4.7).
- In village 2 statistical differences was found between the river water source and the traditional storage containers (P=0.0005) and between the river water source and the CDC safe storage containers (P=0.007) (Table 4.8).
- In village 1 no statistical differences was found between the traditional storage containers and the CDC safe storage containers (P=0.861) (Table 4.7and Fig 4.11).
- In village 2 no statistical differences was found between the traditional storage containers and the CDC safe storage containers (P=0.501) (Table 4.8 and Fig 4.11).
- In general no statistical difference with regards to *E. coli* bacteria could be seen between the traditional and CDC safe storage containers using the placebo solution (P=0.802). This showed that the CDC safe storage container alone did not make a difference in improving water at the point-of-use.

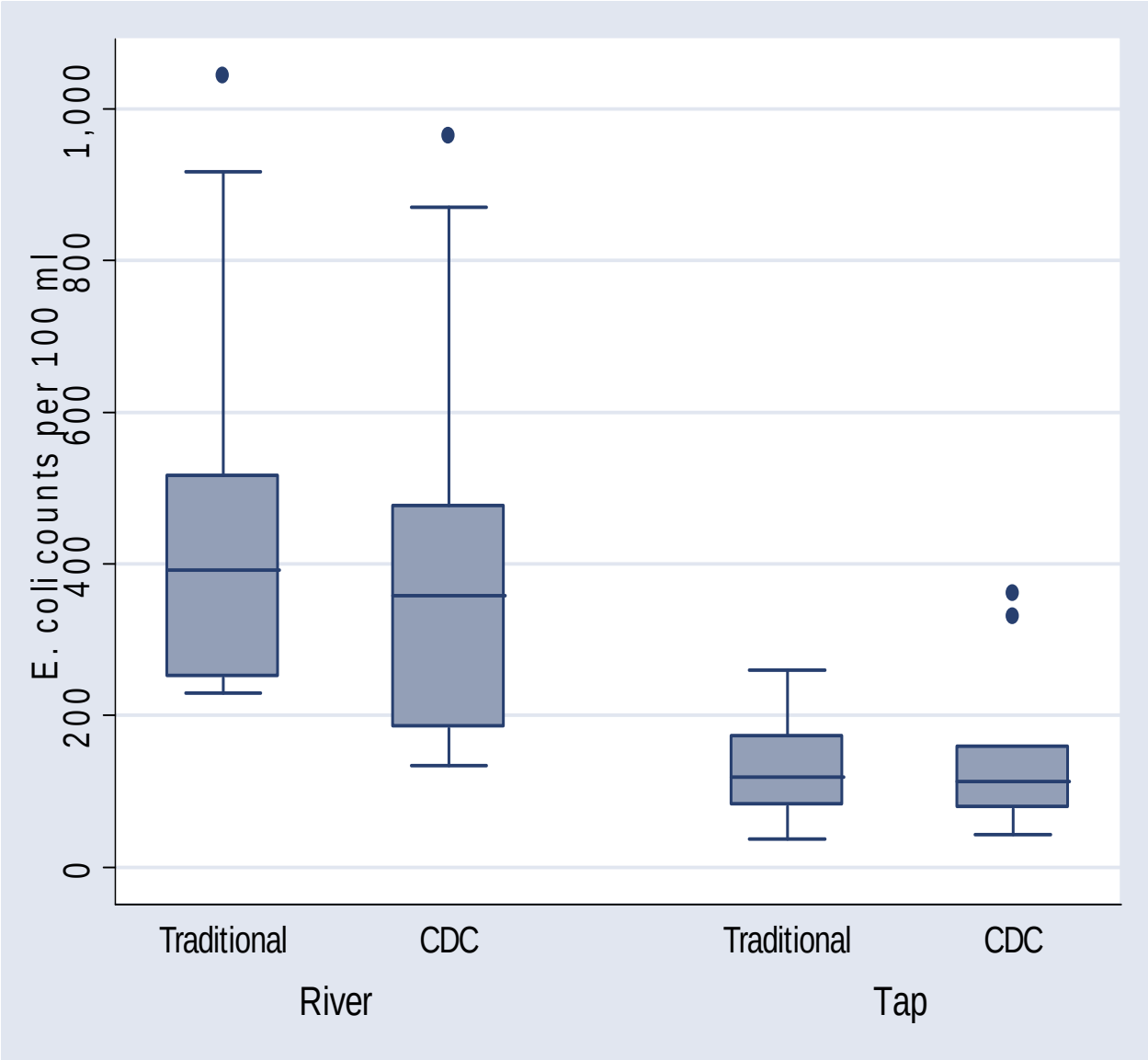

Figure 4.11: ***Escherichia coli*** **bacteria distributed by primary water sources and stored water in traditional and CDC safe storage containers from two villages in the Vhembe region of the Limpopo Province, South Africa**

Faecal enterococci counts in this study were used to indicate the presence of human faecal contamination in the water samples (SABS, 2001). The South African water quality guideline value for faecal enterococci in water intended for domestic use is 0 cfu.100 ml^{-1} (SABS, 2001). However, the faecal enterococci counts for both the water sources exceeded the South African guideline value for safe drinking water (Tables 4.7 and 4.8). In addition it was seen that faecal enterococci counts increased in the traditional and CDC safe storage containers in village 1 in households using communal tap water indicating secondary contamination through unhygienic practices during collection and storage at the point-of-use (Table 4.7). In village 2 households, the faecal coliform counts were similar to that of the water source and even decreased in the CDC safe storage containers which indicated that the collected water was already contaminated or the reduced counts were due to the natural die-off of the bacterial cells in the containers (Table 4.8) (Moyo *et al*., 2004).

The statistical analysis of the faecal enterococci bacterial counts indicated the following:

- A statistical difference (P=0.001) could be seen between the faecal enterococci bacterial counts of the river and tap water sources (Tables 4.7 and 4.8).
- In village 1 statistical differences was found between the tap water source and the traditional storage containers (P<0.001) and between the tap water source and the CDC safe storage containers (P<0.001) (Table 4.7).
- In village 2 no statistical differences was found between the river water source and the traditional storage containers (P=0.597) while for the CDC safe storage containers there was a significant reduction in the faecal enterococci counts (P=0.0001) (Table 4.8).
- In village 1 no statistical differences was found between the traditional storage containers and the CDC safe storage containers (P=0.917) (Table 4.7 and Fig 4.12).
- In village 2 no statistical differences was found between the traditional storage containers and the CDC safe storage containers (P=0.216) (Table 4.8 and Fig 4.12).
- In general no statistical difference with regards to faecal enterococci bacteria could be seen between the traditional and CDC safe storage containers using the placebo solution (P=0.532). This showed that the CDC safe storage container alone did not make a difference in improving water at the point-of-use.

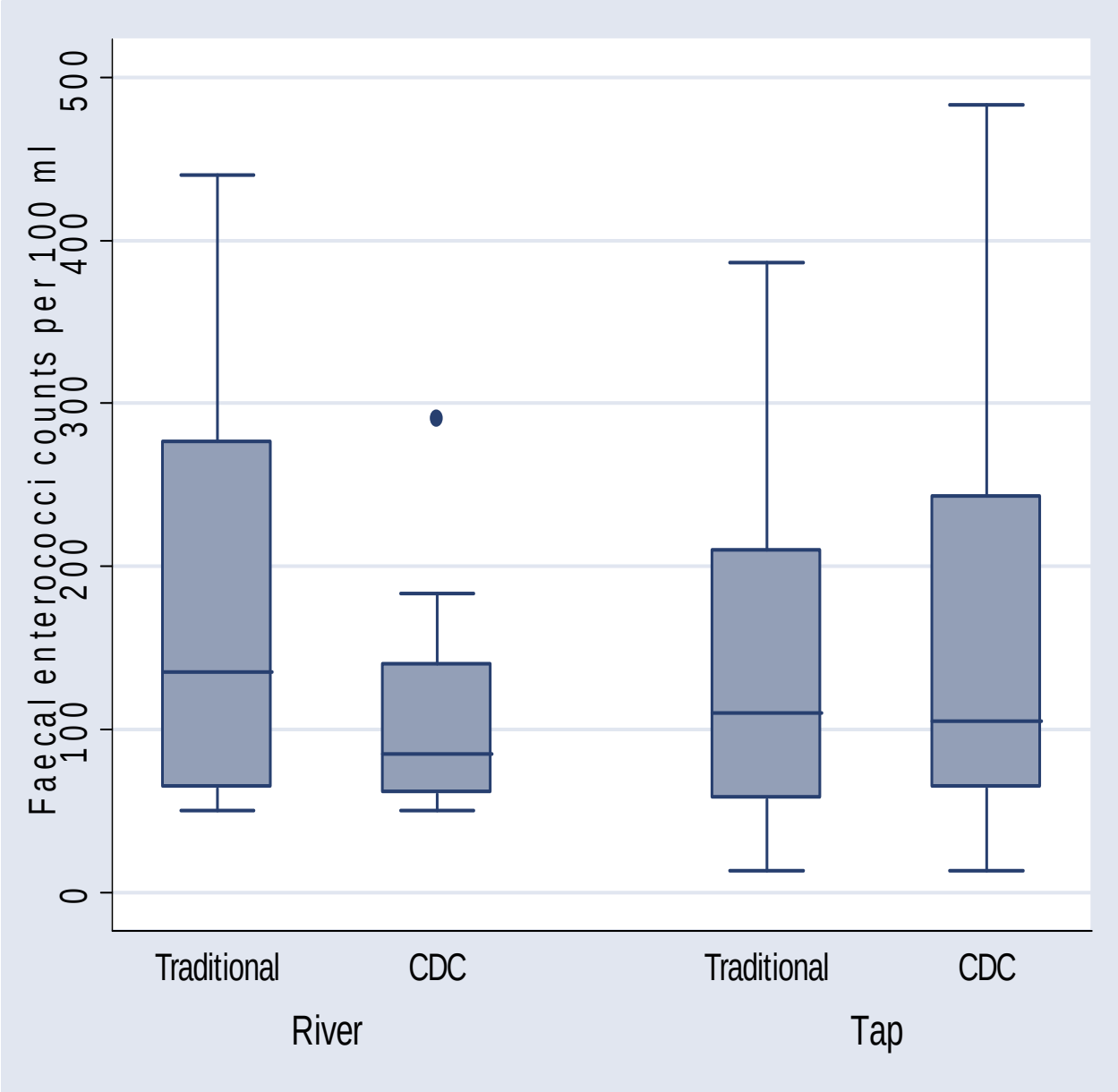

Figure 4.12: **Faecal enterococci bacteria distributed by primary water sources and stored water in traditional and CDC safe storage containers from two villages in the Vhembe region of the Limpopo Province, South Africa**

The direct detection of viruses in water samples would be preferred. However, viral isolation and detection methods are expensive, labour intensive and require skilled personnel. Therefore, indicator organisms such as *C. perfringens*, somatic and male specific F-RNA bacteriophages were used in this study to indicate the potential presence of pathogenic enteric viruses (Grabow *et al*., 1993; Leclerc *et al*., 2000). *Clostridium perfringens* is associated with soil as well as with animal and human faeces and the spores could survive for long periods in the environment such as sediments (Listle *et al*., 2004). Therefore, the presence of *C. perfringens* in the water sources and the traditional and CDC safe storage containers indicated that potential pathogenic viruses (eg. Enteroviruses and Hepatitis A virus) and parasites (eg. *Giardia* and *Cryptosporidium*) could have been present in the water. These pathogens could cause diseases such as hepatitis, meningitis and gastroenteritis (Payment and Franco, 1993).

The statistical analysis of the *C. perfringens* bacterial counts indicated the following:

- A significant statistical difference ($P<0.001$) could be seen between the *C. perfringens* bacterial counts of the river and tap water sources (Tables 4.7 and 4.8).
- In village 1 statistical differences was found between the tap water source and the traditional storage containers ($P=0.0001$) and between the tap water source and the CDC safe storage containers ($P=0.022$) (Table 4.7).
- In village 2 no statistical differences was found between the river water source and the traditional storage containers ($P=0.247$) and between the river water source and the CDC safe storage containers ($P=0.684$) (Table 4.8).
- In village 1 no statistical differences was found between the traditional storage containers and the CDC safe storage containers ($P=0.829$) (Table 4.7 and Fig 4.13).
- In village 2 no statistical differences was found between the traditional storage containers and the CDC safe storage containers ($P=0.216$) (Table 4.8 and Fig 4.13).
- In general no statistical difference with regards to *C. perfringens* bacteria could be seen between the traditional and CDC safe storage containers using the placebo solution ($P=0.401$). This showed that the CDC safe storage container alone did not make a difference in improving water at the point-of-use.

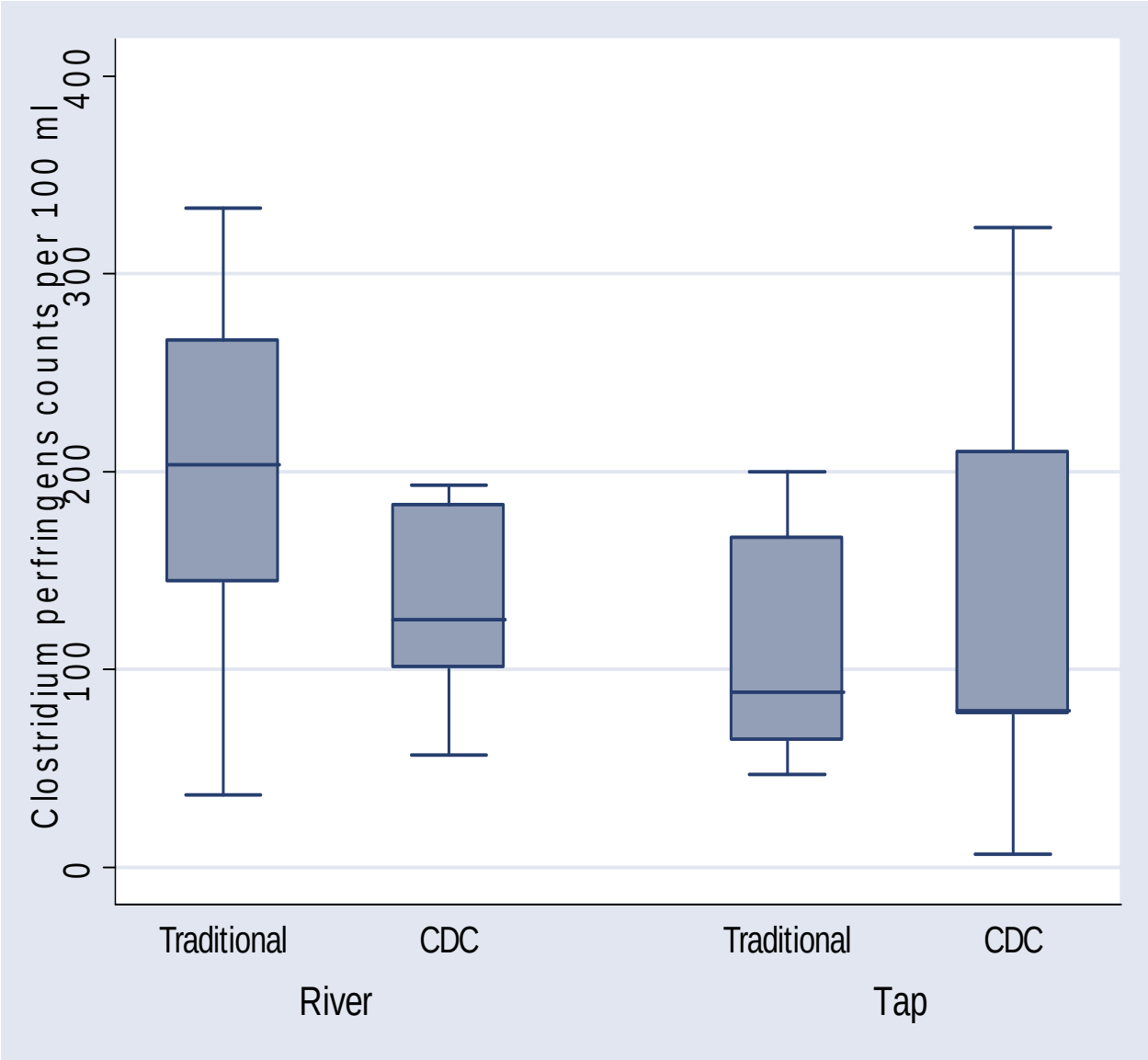

Figure 4.13: ***Clostridium perfringens*** **bacteria distributed by primary water sources and stored water in traditional and CDC safe storage containers from two villages in the Vhembe region of the Limpopo Province, South Africa**

According to the South African guidelines, no somatic bacteriophage counts should be detected in water intended for drinking purposes (SABS, 2001). Table 4.9 showed the presence of somatic and male specific F-RNA bacteriophages in the primary water sources which indicated the potential risk of the presence of human viruses such as Adenoviruses, Astroviruses, Caliciviruses, Enteroviruses, Hepatitis A virus and Rotaviruses which could have caused diseases such as hepatitis, myocarditis and gastroenteritis to consumers (Grabow *et al*., 1993b). The increase in somatic and male specific F-RNA bacteriophage prevalence in household storage containers of the households using communal tap water indicated secondary contamination after collection and during storage at the point-of-use due to unhygienic practices (DWAF, 1996).

Table 4.9: Presence-Absence analyses of source water (communal tap and river water) and stored water (traditional and CDC safe storage containers), from households using the placebo solution in two rural villages in the Vhembe region of the Limpopo Province, South Africa.

Bacteriophages	Village 1			Village 2		
	Communal tap sources (n = 16 taps)	Traditional storage containers (n = 10 HH)	CDC safe storage containers (n = 10 HH)	River water source (n = 4 sites)	Traditional storage container (n = 10 HH)	CDC safe storage container (n = 10 HH)
Somatic	1/16 (6.3%)	9/10 (90%)	10/10 (100%)	4/4 (100%)	10/10 (100%)	10/10 (100%)
Male specific F-RNA	1/16 (6.3%)	8/10 (80%)	9/10 (90%)	4/4 (100%)	10/10 (100%)	10/10 (100%)

In general, the results discussed in this section indicated that the 1% and 3.5% sodium hypochlorite solutions were effective water treatment interventions. Both the traditional and CDC safe water storage containers showed similar results with regards to treatment effectivity in households using either the 1% or 3.5% sodium hypochlorite solutions. Furthermore, the microbial counts obtained from the traditional and CDC safe storage containers in households using the placebo solution, indicated that the container without a sodium hypochlorite solution treatment, do not improve the stored drinking water. Therefore, more intensive marketing of sodium hypochlorite as a water treatment

intervention should be pursued especially in communities where point-of-use water treatments could make a difference in the microbiological quality of drinking water.

4.1.2.3 Association between household demographics and hygiene practices and water quality in the study population

The association (link) between household demographic and hygiene practices and water quality, measured in terms of *E. coli* counts, were assessed using Poisson regression which adequately deals with counts and zeros. All factors included in the baseline household questionnaire (Appendix C) were considered. The factors that were included into the final regression were the following: (1) practice of hand washing before food preparation, (2) container type in use and (3) a compounded variable of source and the distance the household is away from the source. The results are shown in Table 4.10.

Table 4.10 Poisson regression analysis with *E. coli* average counts in households using the placebo solution as measure for water quality

***E. coli* average counts**	**IRR (Incidence Rate Ratio)**	**P-value**	**95% confidence interval for IRR**
Hand washing vs no hand washing	0.58	0.031	(0.349 ; 0.950)
CDC storage containers vs traditional storage container	0.98	0.941	(0.646 ; 1.499)
Living far (>100 m) vs living close (<100 m) to the river source	0.85	0.623	(0.453 ; 1.607)
Living close (<100 m) to a tap source vs living close (<100 m) to the river source	0.26	0.000	(0.132 ; 0.493)
Living far (>100 m) from a tap source vs living close (<100 m) to the river source	0.29	0.005	(0.121 ; 0.681)

Based on the incidence rate ratios obtained in the analysis in Table 4.10, the following could be concluded:

- If hands were washed before food preparation *E. coli* counts were reduced to 58% (P=0.031) of the *E. coli* counts when hands were not washed. Hand washing after defecation and before food preparation is fundamental to food hygiene and several studies have showed that hands could play an important role in the transmission of *E. coli* species (Boyer *et al*., 1975; Harris *et al*., 1985). In addition, Lin and co-workers (2003) have showed that *E. coli* bacteria are harboured under the fingernails and proper washing with soap could decrease the incidence. This was confirmed by studies showing that hand washing decrease diarrhoeal prevalence by 89% (Han *et al*., 1989).
- When living further (> 100 m) away from the river, the *E. coli* counts were 85% (P=0.623) of that when living close (within 100 m) to the river, i.e. relative to living close, however, a 15% reduction in *E. coli* counts were observed in households further than 100 m of the river source. This was contrary to the expectation that it should have been higher. One of the reasons could be that households living far from the primary water source tend to collect more water and store water for longer periods. The storage containers these households are using are larger than 25 litres. The results from this analysis could be explained due to the possible settling of the microorganisms at the bottom of these larger containers. A second explanation could be due to natural die-off of *E. coli* bacteria during the long periods of storage inside these larger containers (Moyo *et al*., 2004).
- When living close (within 100 m) to a tap source, *E. coli* counts was only 26% (P<0.000) of that of *E. coli* counts when living close (within 100 m) to the river. This implied that people using an improved source such as the communal taps, will have less *E. coli* bacteria compared to people using an unimproved water source such as a river.
- When living further (> 100 m) away from a tap source, *E. coli* counts was only 29% (P=0.005) of that of *E. coli* counts when living close (within 100 m) to the river. This implied that people using an improved source such as the communal taps, will have less *E. coli* bacteria compared to people using an unimproved water source such as a river.

- In the CDC container, *E. coli* counts were 98% that of traditional container (P=0.941). The latter is evident from Fig 4.11 and Tables 4.7 and 4.8.

4.1.3 Compliance of study households in the two villages with the intervention

During the intervention study, the presence of a free chlorine residual in both the traditional and the CDC safe containers in the households which used the 1% and 3.5% sodium hypochlorite solutions were measured to determine if these households complied with a point-of-use treatment such as the use of the sodium hypochlorite solutions. In general, the levels of compliance in households for both villages were in agreement with other studies (Table 4.11) (Quick *et al*., 1999; Quick *et al*., 2002; Reller *et al*., 2003; Crump *et al*., 2005). Generally the households in village 1 complied between 60% and 100% (Table 4.11). Households in village 1 not always using the sodium hypochlorite solutions gave two reasons for the low levels of compliance. The first reason was because the people believed tap water was microbiologically cleaner than river water (which they have been using before the introduction of communal taps) and therefore it was not necessary to treat the water (indicated in Table 4.12). The second reason was that households using the 3.5% sodium hypochlorite solution did not like the taste of the sodium hypochlorite in the water, which could be due to the high free chlorine residual of the 3.5% sodium hypochlorite water samples that ranged between 3.8 and 4.5 $mg.l^{-1}$ after 60 min (indicated in Table 4.12). This free chlorine residual is higher than the recommended free chlorine residual level of 0.8 $mg.l^{-1}$ as suggested by the WHO (2004). Unfortunately it was found during this study that the stipulated free chlorine residual level was only achieved after 24 h and not 2 h as implicated by the DOH and DWAF. These high concentrations of chlorine in drinking water can lead to the formation of trihalomethanes (THMs) which have been associated with various types of cancers (Freese and Nozaic, 2004). However, the intervention study indicted that the households in village 2 complied between 90% and 100% and that these households had no complaints about the taste of the sodium hypochlorite in the treated stored water during the intervention study. In households where free chlorine residuals were not detected on the unannounced visits of the research teams to the households, it was due to the households having collected water the previous day and in which the free chlorine residual levels have already dropped to undetected levels.

Table 4.11: Compliance by intervention households who used either a 1% or a 3.5% sodium hypochlorite solution as an intervention strategy together with their traditional or CDC safe water storage containers

Study Population	Container Type	Round 1 water collection		Round 2 water collection		Round 3 water collection	
		1% sodium hypochlorite solution	3.5% sodium hypochlorite solution	1% sodium hypochlorite solution	3.5% sodium hypochlorite solution	1% sodium hypochlorite solution	3.5% sodium hypochlorite solution
Village 1 households using communal taps as primary water source	Traditional	80% (n = 10 households)	70% (n = 10 households)	70% (n = 10 households)	70% (n = 10 households)	70% (n = 10 households)	90% (n = 10 households)
	CDC	70% (n = 10 households)	70% (n = 10 households)	60% (n = 10 households)	100% (n = 10 households)	80% (n = 10 households)	100% (n = 10 households)
Village 2 households using the Sambandou River as primary water source	Traditional	100% (n = 10 households)	100% (n = 10 households)	90% (n = 10 households)	90% (n = 10 households)	100% (n = 10 households)	100% (n = 10 households)
	CDC	90% (n = 10 households)	90% (n = 10 households)	90% (n = 10 households)	100% (n = 10 households)	100% (n = 10 households)	100% (n = 10 households)

A total of 103 (86%) households from village 1 (n = 54 households) and village 2 (n = 49 households) completed the qualitative survey at the end of the intervention study. The survey consisted of observations made by the interviewers and a short questionnaire regarding the use of the intervention and degree of satisfaction or dissatisfaction with the intervention. The results are shown in Table 4.12.

Table 4.12: Summary of the qualitative survey at the end of the formal intervention study by households in each of two rural villages in the Vhembe region of the Limpopo Province, South Africa

Data	Village 1 households using tap water (n=54 households)	Village 2 households using river water (n=49 households)
Use the same container to collect and store water	49 (91%)	43 (88%)
Number of water collections per day:		
Once	16 (30%)	14 (29%)
Twice	4 (7%)	8 (16%)
Thrice	25 (46%)	25 (51%)
Four times	5 (9%)	2 (4%)
Water have a taste after treatment	5 (9%)	33 (67%)
Water have a smell after treatment	1 (2%)	16 (33%)
Like the taste of the water after treatment	48 (88%)	36 (73%)
Will buy sodium hypochlorite for treatment of water in containers	4 (7%)	3 (6%)
Reasons for not buying sodium hypochlorite solution:		
Government must provide	0 (0%)	5 (10%)
Insufficient funds	31 (57%)	7 (14%)
No reason	6 (11%)	35 (71%)
Believe water is already clean	17 (31%)	0 (0%)
Don't want to use it/ don't need it	0 (0%)	2 (4%)
If CDC safe storage container is available at shops – I will replace my traditional containers:	51 (94%)	26 (53%)
Overall satisfaction with CDC safe storage container	54 (100%)	45 (92%)
Problems encountered with CDC safe storage container:		
Broken tap/spigot	0 (0%)	4 (6%)

In general, no problems were reported by the study population concerning the use of the CDC safe water protocol (chlorine based water treatment combined with safe storage). The overall consensus of households in village 1 (100%) and households in village 2 (92%) was that they were satisfied with the CDC safe storage container (Table 4.12). However, 6% of households in village 2 complained about broken taps (spigot) (Table 4.12).

At baseline characteristics of the households, it was seen that the households from both villages were not used to treat their domestic water (Table 4.2). Although this intervention showed a high level of compliance with the sodium hypochlorite solution during the intervention trial (Table 4.11), the survey showed that only 7% of the households in village 1 and 6% households in village 2 are willing to buy the sodium hypochlorite solution to continue treating their drinking water. This indicated that more intensive education interventions are needed to help people understand why they need to change their behaviour (Wilson and Chandler, 1993). It will be necessary to incorporate cultural believes around hygiene behaviours and diarrhoea which is caused by improper hygiene and sanitation practices and faecal contaminated water (Kaltenhaler and Drasar, 1996). It was found that people in rural Vhembe region of South Africa do not consider diarrhoea as a health problem. These communities see diarrhoea as something that is natural and even induce it to “clean” their gastrointestinal systems (both adults and children). Another reason for not continuing in the use of the sodium hypochlorite solution was that 31% of the households from village 1 believed that the water from the communal taps are clean/safe and not in need of treatment (Table 4.12). This could be seen in the addition of the sodium hypochlorite solutions during the intervention trial (Table 4.11). Although 67% of the households in village 2 reported that the water had a different taste after treatment with the 1% and 3.5% sodium hypochlorite solutions, 73% of the households reported to like the taste of the treated water irrespective of the concentration of the sodium hypochlorite solution (Table 4.12). In comparison, only 9% of the households from village 1 reported that water had a taste after treatment with the 1% and 3.5% sodium hypochlorite solutions, while 88% of the households reported to like the taste of the treated water irrespective of the concentration of the sodium hypochlorite solution (Table 4.12).

4.1.4 Sustainability of intervention strategy in two rural villages

The sustainability of the intervention introduced to the study households in each of the two rural villages was assessed twice after the formal intervention trial of 16 weeks. The first visit to the households was unannounced and was carried out 6 months and the second visit was carried out unannounced 12 months after the intervention trial. During both visits to all the households, water samples were collected from the traditional and CDC safe storage containers (depending which containers were given to the specific household) and free chlorine residuals were tested as described in section 3.3.2.1.

The results from the water samples collected from all study households in village 1 for the first and second visits are shown in Tables 4.13 and Table 4.14. The households in village 1 complied with the use of the sodium hypochlorite and this was reflected in the free chlorine residual results and microbiological counts as shown in Tables 4.13 and 4.14. The results from the households using the placebo solution were similar to results seen during the formal intervention trial. Counts for heterotrophic bacteria, total coliforms, faecal coliforms, *E. coli*, faecal enterococci and *C. perfringens* bacteria still exceeded the recommended guideline values for water used for domestic purposes (DWAF, 1996; SABS, 2001) as specified in Table 2.2. The counts for total coliform, faecal coliform and *E. coli* bacteria did increase in the CDC safe storage containers compared to the traditional containers in the households from village 1 using the placebo solution after 6 months (Table 4.13).

However, the results from the 12 month follow up visit (Table 4.14) indicated that the counts for these microorganisms were higher in the traditional containers compared to the CDC safe storage containers. This increase could have been due to biofilm formation inside the containers or natural die-off of the various microorganisms (Momba and Notshe, 2003; Moyo *et al*., 2004). No microbial counts for any of the indicator microorganisms could be detected in households using the 1% and 3.5% sodium hypochlorite solutions in both villages indicating compliance and susceptibility of the intervention protocol (Tables 4.13 and 4.14).

Table 4.13: Geometric mean values (95% confidence intervals) for microbiological indicators of tap water samples collected 6 month after the formal intervention study in traditional and CDC safe water storage containers used by households from village 1 in the Vhembe region of the Limpopo Province, South Africa

Sodium hypochlorite solution	Container type	Heterotrophic bacteria (cfu.1 ml^{-1})	Total coliforms (cfu.100 ml^{-1})	Faecal coliforms (cfu.100 ml^{-1})	*Escherichia coli* (cfu.100 ml^{-1})	Faecal enterococci (cfu.100 ml^{-1})	*Clostridium perfringens* (cfu.100 ml^{-1})
Placebo	**Traditional containers (n = 10 households)**	2.3×10^6 (6.1×10^5; 8.8×10^6)	844 (691; 1 032)	538 (328; 883)	166 (90; 306)	154 (42; 582)	132 (21; 807)
	CDC containers (n = 10 households)	2.2×10^5 (5.2×10^4; 9.5×10^5)	1 345 (1 100; 1 643)	1 025 (784; 1 341)	413 (279; 610)	139 (80; 241)	170 (106; 274)
1%	**Traditional containers (n = 10 households)**	Computation of geometric means and 95% confidence intervals was not feasible due to large number of households with 0 counts					
	CDC containers (n = 10 households)						
3.5%	**Traditional containers (n = 10 households)**						
	CDC containers (n = 10 households)						

Table 4.14: Geometric mean values (95% confidence intervals) for microbiological indicators of tap water samples collected 12 months after the formal intervention study in traditional and CDC safe storage containers used by households from village 1 in the Vhembe region of the Limpopo Province, South Africa

Sodium hypochlorite solution	Container type	Heterotrophic bacteria (cfu.1 ml^{-1})	Total coliforms (cfu.100 ml^{-1})	Faecal coliforms (cfu.100 ml^{-1})	*Escherichia coli* (cfu.100 ml^{-1})	Faecal enterococci (cfu.100 ml^{-1})	*Clostridium perfringens* (cfu.100 ml^{-1})
Placebo	**Traditional containers (n = 10 households)**	1.2×10^6 (4.0×10^5; 4.0×10^6)	606 (304; 1 206)	354 (152; 830)	62 (25; 153)	148 (45; 489)	74 (41; 135)
	CDC containers (n = 8 households)	4.6×10^4 (3.8×10^3; 5.6×10^5)	376 (160; 888)	133 (46; 382)	65 (17; 122)	82 (11; 608)	50 (15; 169)
1%	**Traditional containers (n = 6 households)**	Computation of geometric means and 95% confidence intervals was not feasible due to large number of households with 0 counts					
	CDC containers (n = 8 households)						
3.5%	**Traditional containers (n = 10 households)**						
	CDC containers (n = 6 households)						

The results from the water samples collected from all study households in village 2 for the first and second visits are shown in Tables 4.15 and Table 4.16. Although the formal intervention trial clearly showed effectivity of the intervention strategy and compliance by the households in these villages in the use of the sodium hypochlorite solutions, the results from the two follow up visits indicated a different scenario (Tables 4.15 and 4.16). The results from both visits showed that no household was using the sodium hypochlorite solution after the intervention trial. No free chlorine residual levels were detected in any of the water samples tested during both visits. The microbiological counts for all indicator bacteria in all 60 households exceeded the recommended guideline values for water used for domestic purposes (DWAF, 1996; SABS, 2001) as specified in Table 2.2. The microbiological counts of the water stored at these households indicated a potential risk for waterborne diseases (WHO, 2004).

Several studies have reported on the success of point-of-use devices in communities all over the world (Chapter 2). However, there is still a large gap in the literature on studies which have tested the sustainability of point-of-use interventions. These type of studies are important in order to determine if communities have change their behaviour and adopted the point-of-use intervention as a way of life. Consequently, this is the first study in South Africa to test the sustainability of a point-of-use intervention in a rural setting.

Although it was assumed before the study commenced, that the use of sodium hypochlorite by the rural communities will not be a problem because during diarrhoeal outbreaks the DOH and DWAF provided 3.5% sodium hypochlorite solution to all households in affected communities. Several awareness campaigns and pamphlets are available in all 11 official languages in the Primary Health Care clinics in the rural regions (Appendix B). However, the results of this study have clearly indicated that more should be done to have people change their usual habits which could be harmful for the members inside a close relationship, such as a household.

Table 4.15: Geometric mean values (95% confidence intervals) for microbiological indicators of river water samples collected 6 month after the formal intervention study from traditional and CDC safe storage containers used by households from village 2 in the Vhembe region of the Limpopo Province, South Africa

Sodium hypochlorite solution	Container type	Heterotrophic bacteria (cfu.1 ml^{-1})	Total coliforms (cfu.100 ml^{-1})	Faecal coliforms (cfu.100 ml^{-1})	*Escherichia coli* (cfu.100 ml^{-1})	Faecal enterococci (cfu.100 ml^{-1})	*Clostridium perfringens* (cfu.100 ml^{-1})
Placebo	**Traditional containers (n = 10 households)**	3.4×10^6 (2.9×10^5; 3.7×10^7)	1 196 (973; 1 467)	697 (510; 951)	151 (106; 217)	137 (59; 313)	133 (77; 225)
	CDC containers (n = 10 households)	8.8×10^6 (1.0×10^6; 7.4×10^7)	534 (433; 661)	233 (153; 354)	113 (65; 198)	176 (113; 275)	108 (66; 178)
1%	**Traditional containers (n = 10 households)**	1.6×10^6 (2.7×10^5; 9.1×10^6)	1 392 (630; 3 075)	587 (319; 1 081)	183 (109; 306)	149 (63; 349)	106 (63; 178)
	CDC containers (n = 10 households)	4.9×10^6 (6.1×10^5; 3.7×10^7)	485 (371; 636)	246 (169; 359)	92 (62; 136)	74 (39; 139)	109 (50; 238)
3.5%	**Traditional containers (n = 10 households)**	1.2×10^6 (1.3×10^5; 1.1×10^7)	903 (548; 1 489)	518 (278; 965)	96 (56; 165)	113 (66; 196)	102 (50; 210)
	CDC containers (n = 10 households)	4.5×10^7 (4.3×10^5; 4.6×10^7)	551 (415; 733)	252 (201; 317)	132 (86; 202)	123 (60; 250)	161 (86; 304)

Table 4.16: Geometric mean values (95% confidence intervals) for microbiological indicators of river water samples collected 12 months after the formal intervention study in traditional and CDC safe storage containers used by households from village 2 in the Vhembe region of the Limpopo Province, South Africa

Sodium hypochlorite solution	Container type	Heterotrophic bacteria (cfu.1 ml^{-1})	Total coliforms (cfu.100 ml^{-1})	Faecal coliforms (cfu.100 ml^{-1})	*Escherichia coli* (cfu.100 ml^{-1})	Faecal enterococci (cfu.100 ml^{-1})	*Clostridium perfringens* (cfu.100 ml^{-1})
Placebo	**Traditional containers (n = 9 households)**	2.5×10^6 (1.4×10^5; 4.4×10^7)	1 303 (892; 1 904)	1 086 (733; 1 608)	473 (275; 812)	386 (30; 250)	106 (48; 230)
	CDC containers (n = 7 households)	2.6×10^6 (9.1×10^4; 7.0×10^7)	1 410 (957; 2 077)	1 082 (588; 1 994)	396 (147; 1 066)	99 (49; 202)	113 (64; 199)
1%	**Traditional containers (n = 3 households)**	8.1×10^4 (1.2×10^3; 6.0×10^6)	669 (25; 17 777)	391 (10; 14 693)	86 (8; 892)	193 (69; 538)	113 (40; 320)
	CDC containers (n = 4 households)	1.3×10^5 (1.5×10^3; 1.2×10^7)	638 (138; 2 952)	330 (36; 3 098)	143 (19; 1 068)	155 (23; 1 075)	154 (32; 750)
3.5%	**Traditional containers (n = 6 households)**	2.6×10^5 (8.0×10^3; 8.5×10^6)	1 092 (506; 2 358)	564 (239; 1 334)	203 (104; 400)	188 (111; 312)	93 (64; 136)
	CDC containers (n = 6 households)	1.7×10^5 (2.0×10^4; 1.4×10^6)	617 (74; 2 190)	392 (108; 1 430)	92 (39; 218)	173 (79; 377)	91 (54; 153)

Several reasons could be listed why the intervention was not sustainable in village 2 and was not continued in village 1. Firstly, it will be the cost of the sodium hypochlorite solution. Poor households would rather buy bread and maize meal before spending money on something such as sodium hypochlorite. In addition, these communities are conditioned to the effect that "if" or "when" their water source is found to be contaminated like in the case of a cholera outbreak, the government will provide sodium hypochlorite for them and they don't have to buy it themselves. Secondly, the people in these rural communities are used to the water they consume and don't get ill possibly due to a higher immunity. However, they are not considering the health implications it has on immunocompromised individuals, young children and the elderly. South Africa has a high prevalence of HIV/AIDS infected individuals which could seriously be affected by poor water quality, poor and inadequate sanitation infrastructures and unhygienic practices at the point-of-use. It was found that the study households could not understand why the water should be treated if it does not affect their health. This implied that more vigorous educational programmes should be launched in these rural communities in South Africa. Lastly, in village 2 where the intervention was not sustainable after the initial intervention trial, it did not seem that the community leaders (who were all men) had any interest in water quality issues. In comparison, in village 1, the chief was involved in all community research activities and the results indicated the intervention was sustainable as long as households had a supply of sodium hypochlorite. This clearly showed that the environment must be supportive to make an intervention sustainable in the long run. The results from this study have clearly showed that people need to be educated and behaviour change interventions must be incorporated into point-of-use intervention trials.

4.1.5 Summary of the efficiency of the CDC protocol (CDC safe storage container with a sodium hypochlorite solution) at improving the microbiological quality of stored drinking water in rural households in South Africa

The microbiological quality of the water sources used for domestic purposes by the two study populations were unacceptable and posed a potential health risk to the consumers. The counts for all indicator microorganisms exceeded the SABS (2001) stipulated water quality guideline values and indicated that the water might be harbouring potential opportunistic and pathogenic microorganisms.

This was the first study carried out in South Africa to evaluate the impact of the CDC safe storage container with or without the addition of a 1% or a 3.5% sodium hypochlorite solution on water supplies stored in rural households in the Vhembe region of the Limpopo Province. The results indicated that both the 1% and 3.5% sodium hypochlorite solution interventions was effective and reduced the potential risk of waterborne diseases by improving the microbiological quality (based on indicator microbial counts) of stored household drinking water in the CDC safe storage containers to undetectable counts. These results are in agreement with other studies conducted in developing countries where the CDC safe storage container together with a sodium hypochlorite solution was assessed as a combined intervention strategy (Macy and Quick, 1998; Semenza *et al*., 1998; CDC, 2001; Sobsey, 2002; Sobsey *et al*., 2003).

It was seen that even in the traditional household water storage containers, the numbers of indicator organisms of stored drinking water were reduced to undetectable counts with the use of the 1% and the 3.5% sodium hypochlorite solutions. This is in agreement with earlier studies suggesting that when the traditional household storage container is handled correctly and covered properly, the microbiological quality of the stored drinking water can be protected and the traditional storage container can be used effectively by households which cannot afford the CDC safe storage container (Hammad and Dirar, 1982; Deb *et al*., 1986; Pinfold, 1990).

The increase in the indicator microorganism counts in the traditional and CDC safe storage containers in the households using the placebo solution indicated secondary faecal contamination at the point-of-use due to unhygienic water-handling practices and unsanitary use of utensils and contaminated hands touching the water. In addition, no statistical differences were seen in the prevalence of indicator microorganisms between the traditional and the CDC safe storage containers using the placebo solution in both the study populations. This indicated that the CDC safe container as a single intervention without a sodium hypochlorite solution was not effective in the prevention of secondary contamination and did not significantly improved the microbiological quality of the stored drinking water. This is in agreement with an earlier study conducted by Quick and co-workers (1996) who indicated that the CDC safe storage container without the sodium hypochlorite intervention is not effective in reducing the risk associated with waterborne diseases.

Although this study included an education intervention on the use and cleaning of the CDC safe storage container and the correct addition of sodium hypochlorite solutions to the stored water, the survey indicated an urgent need for behavioural changes in these communities. It seemed that appropriate hygiene practices were not practiced due to cultural believes and financial burdens on the family and the lack of proper sanitation and water infrastructures. In addition, several studies have shown that the addition of sodium hypochlorite to stored drinking water reduced diarrhoea between 44% and 48% (Quick *et al*., 1999; Quick *et al*., 2002). It is however, essential that interventions at the household level should be implemented and promoted by goverment on a larger scale in rural communities to prevent the outbreak of waterborne diseases.

It was evident from this study that the intervention was effective and households complied with the use of sodium hypochlorite as long as they knew that their water will be tested by the research team. However, the results showed that the intervention was not sustainable after 12 months, especially in village 2 where households used the river as a primary water source. The households in village 1 using the tap water continued using the sodium hypochlorite solutions until the bottles were finished but did not purchase new stock to treat the water. The sustainability of the intervention in village 1 could also be biased because of various research activities carried out in the Vhembe region during the past few years which could have alarmed the households that the research team might pitched up at their homes to take a water sample. Consequently, the results suggested that without behaviour change and people taking ownership of the intervention, point-of-use intervention might not be sustainable (Nath *et al*., 2006).

4.2 DETERMINATION OF FAECAL SOURCE ORIGIN IN STORED DRINKING WATER FROM RURAL HOUSEHOLDS IN SOUTH AFRICA USING MALE SPECIFIC F-RNA BACTERIOPHAGE SUBGROUP TYPING

The use of male specific F-RNA bacteriophages genotyping assisted in differentiating between faecal contamination of human and animals, which was used in the determination of intervention strategies, aimed at improving household stored drinking water supplies. This study assessed the prevalence (using the Presence-Absence spot test) and the origin (using oligonucleotide subgroup typing) of male specific F-RNA bacteriophages in water sources and household storage containers in rural communities of the Vhembe region of the Limpopo Province, RSA.

4.2.1 Prevalence of male specific F-RNA bacteriophages in the primary water sources and the household water storage containers in rural households

The prevalence of male specific F-RNA bacteriophages in the primary water sources and in the stored water collected from the traditional household storage containers in the two study villages were assessed using methods describe in section 3.4. All 4 (100%) of the river water and all 7 (100%) of the tap water samples collected during the first and second trips tested positive for the presence of male specific F-RNA bacteriophages (Fig 4.14). During the first water collection trip, only 26 (65%) of the traditional storage containers in the 40 households that used tap water as a primary water source were positive for the presence of male specific F-RNA bacteriophages. In comparison, 36 (90%) of the traditional storage containers in the 40 households that used river water as a primary water source were positive for the presence of male specific F-RNA bacteriophages (Fig 4.14). During the second water collection trip, 12 (30%) of the traditional storage containers in the 40 households using tap water contained male specific F-RNA bacteriophages (Fig 4.14). In comparison 34 (85%) of the traditional storage containers in the 40 households using river water contained male specific F-RNA bacteriophages during the second water collection trip (Fig 4.14).

Generally more of the traditional household water storage containers filled with river water tested positive for the prevalence of male specific F-RNA bacteriophages compared to the traditional household water storage containers filled with tap water (Fig 4.14). This could be due to animals frequently using the river catchment for drinking and then defecating near or in the river water. In village 2 many of the women also use the river for bathing and washing clothes. Consequently the animal and human activities in or near the river in village 2 could have contributed to the presence of male specific F-RNA bacteriophages in the river water samples.

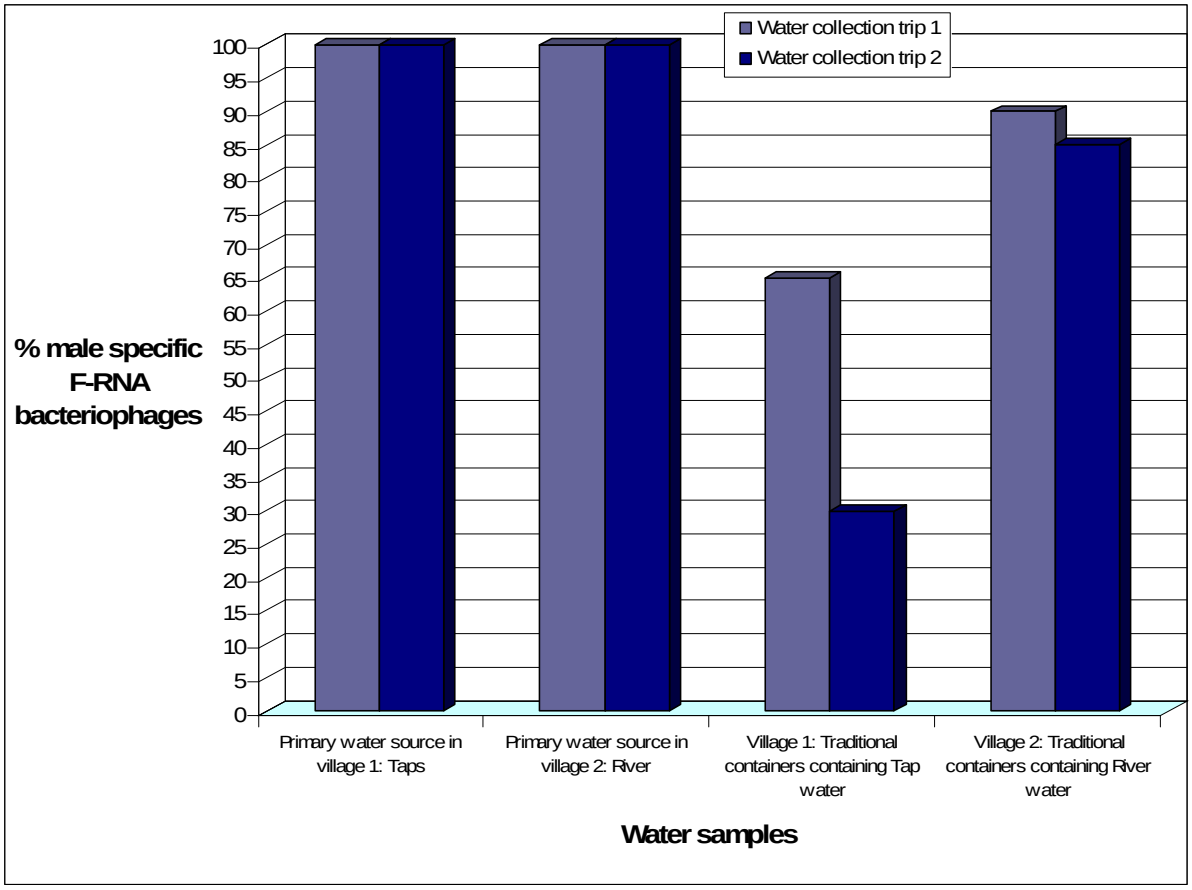

Figure 4.14: Prevalence of male specific F-RNA bacteriophages in primary water sources and stored water in traditional household water storage containers from two villages using different primary water sources

In order to determine the impact of an improved storage container on the origin of faecal pollution the presence of male specific F-RNA bacteriophages in the traditional and CDC safe storage containers were determined (Fig 4.15) during the second water collection trip (section 3.4). In the households which used the tap water as their primary

water source, 6 (30%) of the 20 households contained male specific F-RNA bacteriophages in their traditional storage containers, compared to only 4 (20%) of the 20 households which were provided with the CDC safe storage containers (Fig 4.15).

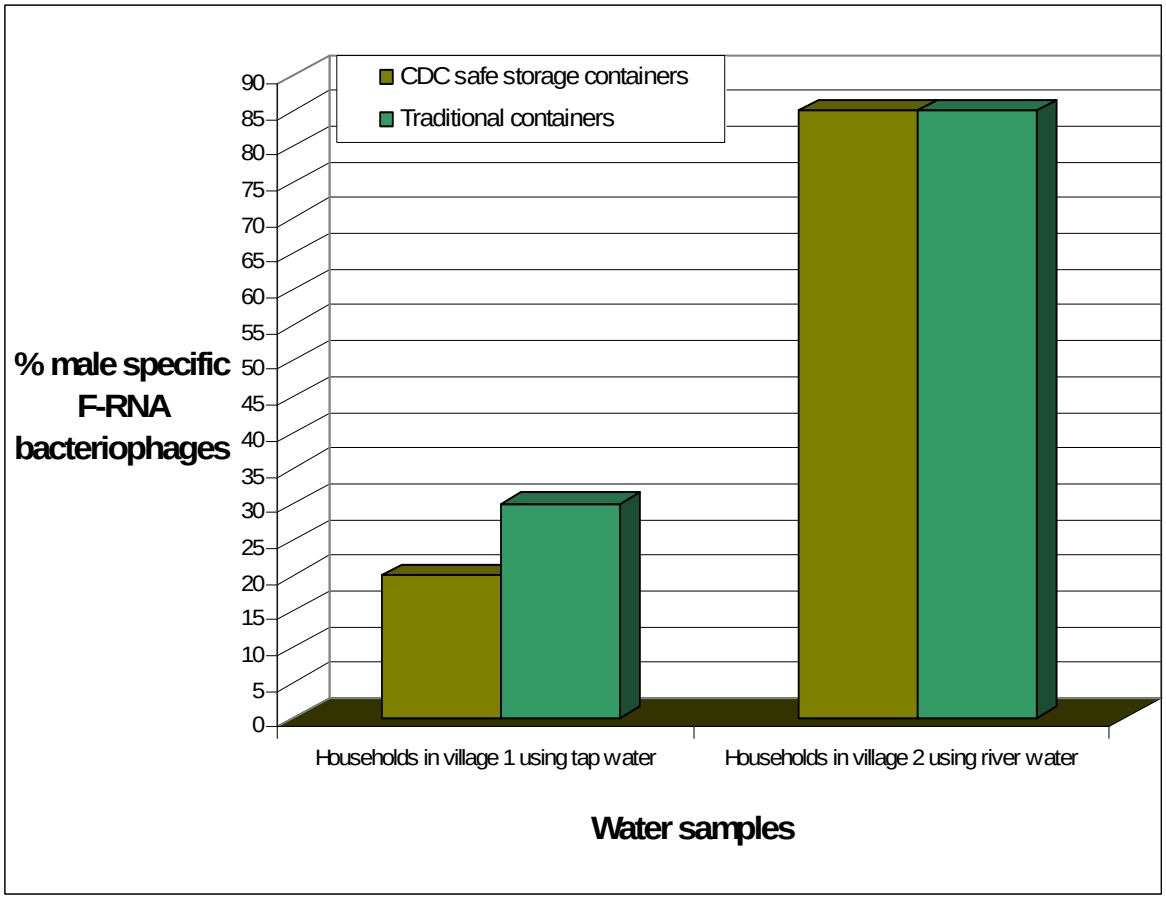

Figure 4.15: Presence of male specific F-RNA bacteriophages in the traditional and CDC safe storage containers in rural households from two villages using different water sources

In the households which used the river water as their primary water source, male specific F-RNA bacteriophages were prevalent in 17 (85%) of the 20 households respectively using the traditional storage containers and the households which were provided with the CDC safe storage containers (Fig 4.15). This indicated that the containers with water from an unimproved source (eg. River water) used in village 2 was more contaminated with male specific F-RNA bacteriophages compared to containers with water from an improved source (eg. tap water) used in village 1 (Fig 4.15).

4.2.2 Origin of male specific F-RNA bacteriophage subgroups in the primary water sources

Genotyping of F-RNA isolates from the communal tap and river water sources for both the villages identified subgroup I male specific F-RNA bacteriophages as the predominant bacteriophage subgroup present (Table 4.17). Subgroup I male specific F-RNA bacteriophages are indicative of animal faecal pollutions, specifically cattle, sheep and pig faeces which are in agreement with earlier studies conducted by Hsu and co-workers (1995), Beekwilder and co-workers (1996) and Uys, (1999). In village 1 using communal tap water sources it was observed that faeces of animals such as pigs, goats and cattle were lying next to the taps. The water reservoir in village 1 was also exposed to small animals, bird droppings and dust particles which might have contained faeces from animals grazing in the vicinity of the reservoir (Fig 4.16).

Figure 4.16: Animals near groundwater reservoir pumping water to communal taps used by study households in village 1 in the Vhembe region of the Limpopo Province, South Africa

The Sambandou River used by households in village 2 was frequently used by domestic animals and cattle for drinking purposes and it was common to find animal faeces (Fig 4.17) in the vicinity of the drinking water sources or close to the areas where people collect their drinking water or even in the water source (Fig 4.18) (Table 4.17). All these animal activities in the vicinity of the water sources contributed to the presence of subgroup I male specific F-RNA bacteriophage contamination that was identified in the water sources. The National Research Council (NRC, 2004) has reported that subgroup I male specific F-RNA bacteriophages are found in both human and animals faeces and sewage. Therefore, it could be possible that the predominance of subgroup I male specific F-RNA bacteriophages in both the water sources and especially in high concentrations in the river source could be due to both animal and human activities in and near the river source (Table 4.17).

Figure 4.17: Animal dung seen in the river water source used by study households in village 2 in the Vhembe region of the Limpopo Province, South Africa

Figure 4.18: Animals drinking and defecating in the river water source used by study households in village 2 in the Vhembe region of the Limpopo Province, South Africa

No male specific F-RNA bacteriophages belonging to subgroups II and III (associated mainly with human faecal pollution) and subgroup IV (associated mainly with animal faecal pollution) have been isolated from the communal tap water source samples. These bacteriophage groups may have had a fast die-off curve or were just not present at all. A study carried out by Schaper and co-workers (2002b) have shown that subgroup I male specific F-RNA isolates were more resistant than subgroup II F-RNA isolates followed by subgroup III male specific F-RNA isolates and lastly subgroup IV male specific F-RNA isolates to chlorine, temperature, pH and salt concentrations in water samples (Schaper *et al*., 2002b). The absence of subgroups II, III and IV in the tap water sources could therefore give a false indication that the subgroup I isolates were primarily of animal faecal origin and not from human origin (Hsu *et al*., 1995; Beekwilder *et al*., 1996; Uys, 1999).

Table 4.17: **Prevalence of male specific F-RNA bacteriophages in river and communal tap water sources in two rural villages in the Vhembe region of the Limpopo Province, South Africa**

Village 1 using communal tap water					**Village 2 using Sambandou River water**				
Male specific F-RNA bacteriophages genotype isolated (percentage %)					**Male specific F-RNA bacteriophages genotype isolated (percentage %)**				
Number of water samples tested	Subgroup I (MS2)	Subgroup II (GA)	Subgroup III (QB)	Subgroup IV (F1)	**Number of water samples tested**	Subgroup I (MS2)	Subgroup II (GA)	Subgroup III (QB)	Subgroup IV (F1)
14*	14 (100%)	0 (0%)	0 (0%)	0 (0%)	8*	8 (100%)	4 (50%)	0 (0%)	0 (0%)

* Water samples collected for round and round 2

However, male specific F-RNA bacteriophages belonging to subgroup II were found in the river water samples (50%) which could indicate possible human pollution of the source water (Fig 4.19) (Hsu *et al*., 1995; Beekwilder *et al*., 1996; Uys, 1999; Brion *et al*., 2002). Subgroup IV bacteriophages have been shown to be associated with bird faeces (Brion *et al*., 2002; Schaper *et al*., 2002a) and even though no subgroup IV male specific F-RNA bacteriophages were identified during this study, both the river and communal tap reservoirs were exposed to faecal contamination from small animals and birds (Table 4.17).

Figure 4.19: People washing clothes in the river water source used by study households in village 2 in the Vhembe region of the Limpopo Province, South Africa

4.2.3 Origin of male specific F-RNA bacteriophage subgroups in the stored household water at the point-of-use in the traditional and CDC safe water storage containers in rural households

A total of 4 (7%) male specific F-RNA bacteriophages belonging to subgroup I male specific F-RNA bacteriophages (associated with animal faecal pollution), was identified in the traditional storage containers in the study households using the tap water source (Table 4.18). Similarly only 1 (5%) of the CDC safe storage containers in the households using tap water sources tested positive for the presence of subgroup I male specific F-RNA bacteriophages (associated with animal faecal pollution) (Table 4.18).

In the study households using the river water source, a total of 37 (62%) of the traditional storage containers contained subgroup I male specific F-RNA bacteriophages (associated with animal faecal pollution) (Table 4.18). Similarly, 9 (45%) of the CDC safe storage containers tested positive for the presence of subgroup I male specific F-RNA bacteriophages (associated with animal faecal pollution) (Table 4.18). Since animals were observed during this study to lick the communal taps in village 1 (Fig 4.16) and defecate in the vicinity of the taps and river water area where people collect their domestic water from, the presence of subgroup I male specific F-RNA bacteriophages (associated with animal faecal pollution) was similar to the results obtained for the two water sources analysed (Table 4.17). This is in agreement with similar studies, which found that the presence of subgroup I male specific F-RNA bacteriophages in water samples primarily indicated animal faecal pollution (Hsu *et al*., 1995; Beekwilder *et al*., 1996; Uys, 1999).

Since it was observed that the storage containers were left out in the yard or stored inside a traditional hut (Table 4.3), in many instances without a cover, the exposure to dust and faecal contamination from domestic animals, insects and poultry could have introduced subgroup I male specific F-RNA bacteriophages to the containers (Rosas *et al*., 2006). Many of these households also used fresh cow dung to smear the floors of their huts (Fig 4.3). The dust that originates from the dried cow dung could have contributed to the contamination of the open water storage containers (Benenson, 1995; Rosas *et al*., 2006).

No subgroup II male specific F-RNA bacteriophages (associated with human faecal pollution) were isolated from the tap water sources (Table 4.17), or in any of the storage containers in village 1 households (Table 4.18). However, subgroup II male specific F-RNA bacteriophages (associated with human faecal pollution) were isolated in both the traditional and the CDC safe storage containers in households from village 2 (Table 4.18). Nine (15%) of the sixty households (40 households from round 1 water collection and 20 households from round 2 water collections) using the traditional water storage containers and nine (45%) of the twenty households (from second water collection trip) using the CDC safe storage containers contained subgroup II male specific F-RNA bacteriophages associated with human pollution (Table 4.18).

Brion and co-workers (2002) have stated that the presence of subgroup II male specific F-RNA bacteriophages was an indication of distant or sporadic faecal pollution of human origin. Studies conducted by Hsu and co-workers (1995), Beekwilder and co-workers (1996) and Uys (1999), have confirmed that subgroup II male specific F-RNA bacteriophages are predominantly found in human faeces and sewage. Consequently, contamination of the stored water in this study by humans might have occurred when members of the households used dirty utensils to transfer the stored water from these large open storage containers to a smaller storage container or directly through faecally contaminated hands – especially by small children touching the storage containers (Fig 2.13) and utensils (Jagals *et al*., 1999). In addition, a study conducted in South Africa and Spain (Schaper *et al*., 2002a), analysed various sewage and faecal samples and showed that faeces from poultry, cattle and pigs could also contribute to the presence of subgroup II male specific F-RNA bacteriophages.

Table 4.18: **Prevalence of male specific F-RNA bacteriophages in stored drinking water containers from rural households in two villages in the Vhembe region of the Limpopo Province, South Africa**

Household storage container	Village 1 using communal tap water Male specific F-RNA genotypes isolated (percentage %)				Village 2 using Sambandou River water Male specific F-RNA genotypes isolated (percentage %)			
	Subgroup I (MS2)	Subgroup II (GA)	Subgroup III (QB)	Subgroup IV (F1)	Subgroup I (MS2)	Subgroup II (GA)	Subgroup III (QB)	Subgroup IV (F1)
Traditional storage containers (n = 60)*	4 (7%)	0 (0%)	0 (0%)	0 (0%)	37 (62%)	9 (15%)	0 (0%)	0 (0%)
CDC safe storage containers (n = 20)**	1 (5%)	0 (0%)	0 (0%)	0 (0%)	9 (45%)	9 (45%)	0 (0%)	0 (0%)

*40 households selected in each village (first water collection round) using traditional storage containers + 20 households (second water collection round) used as control group in each village using traditional storage containers (Household as described in section 3.4.1)

**20 households selected in each village (second water collection round) using CDC safe storage containers (Household as described in section 3.4.1)

A close human to animal association were observed in these rural communities and domestic animals and poultry were frequently seen walking into the household area where the water containers were stored (Fig 4.7). Consequently, the presence of the subgroup II male specific F-RNA bacteriophages in the traditional and especially in the CDC safe storage containers suggested that faecal contamination could also have originated from these domestic animals and cattle at the households as well as from the primary water sources (Jagals *et al*., 1999; Schaper *et al*., 2002a). No subgroup III (associated mainly with human faecal pollution) or subgroup IV (associated mainly with animal faecal pollution such as poultry and pig faeces) (Schaper *et al*., 2002a) were detected in any of the traditional or CDC safe storage containers during the study period (Table 4.18). These results were similar to the results obtained for the primary water sources (Table 4.18). However, according to a 2004 review on *Indicators for Waterborne Pathogens* by the National Research Council of the National Academies of Science (NRC, 2004), subgroup I was found in both human and animals faeces and sewage. Therefore, the absence of subgroup III and IV from the water samples tested during this study, might mean that isolates belonging to these two subgroups might not persist in water as long as subgroups I and II (Schaper *et al*., 2002b). Consequently, subgroups I and II isolates present in these water samples might have been introduced into the water due to both human and animal contamination (NRC, 2004).

4.2.4 Summary of the use of male specific F-RNA bacteriophages subgroup typing to determine the faecal source origin in primary water sources and drinking water stored in traditional and CDC safe storage containers in rural households

This is the first study to use male specific F-RNA bacteriophages to determine the origin of faecal pollution in household storage containers in rural households without adequate water and sanitation infrastructures. The results demonstrated that water from the water sources and the household storage containers were primarily contaminated by animal faecal matter because mainly subgroup I F-RNA bacteriophages (associated with animal faecal pollution) were isolated. In addition, households using an unprotected water source also had subgroup II male specific F-RNA bacteriophages present in the household stored water which could have been either due to poor sanitation and hygienic conditions during storage and handling or due to contamination by animal

faeces (Rosas *et al*., 2006). It was difficult to determine the reason for the human faecal contamination because this study did not focussed on household hygiene practices. It was however, observed that people removed the taps and the caps of the containers because they were afraid the children would break them. This happened in spite of the educational intervention on the proper use of the CDC safe storage container. In addition a recent study suggests that subgroup II male specific F-RNA bacteriophages could have been from faecal samples of poultry and cattle (Schaper *et al*., 2002a). Consequently, it could be speculated that both subgroups I and II isolates could have been introduced to the stored drinking water from both human and animal origin (NRC, 2004). However, it is important to note that Schaper and co-workers (2002a) concluded that the association between the specific subgroups can not be used for absolute distinction between human and animal faecal pollution. Genotyping, therefore, seems not to be such an accurate tool to determine the origin of faecal pollution due to the potential for cross-reactions between some human and animal subgroups (NRC, 2004). This indicated the need for more intensive studies to confirm the specificity of the four subgroups of male specific F-RNA bacteriophages.

The absence of subgroups III and IV male specific F-RNA bacteriophage isolates in both the sources and storage containers indicated (1) no human contamination of the household stored water, (2) isolates from these subgroups does not survive for long periods in the environment and (3) temperature, pH and turbidity of the water could affect the survival of this specific subgroup isolates (Schaper *et al*., 2002b). More studies are therefore needed to investigate the prevalence of male specific F-RNA subgroups in human and animal faeces especially in rural communities where a close living relationship exists between humans and animals.

Although the CDC safe storage container was specifically designed to reduce external microbial pollution of stored drinking water, it was observed that the households did not at all times put the caps and/or the taps/spigot on the CDC safe storage containers exposing the water in these containers to potential faecal pollution. One of the reasons was that the parents were scared that the children would break the tap or through away the cap because children loved to play with the tap which could have increased the risk of faecal contamination of the water. Although this study reported on a small study group, the results clearly illustrated the need to provide these households with proper

water and sanitation infrastructures to reduce the storage period of household drinking water and in the process try to prevent the possible faecal contamination of the stored water. In general this study has found that the use of male specific F-RNA bacteriophage genotyping could be used to some extend to distinguish between human and animal faecal pollution. However, this is an expensive technique which requires skilled personnel and more studies in rural settings are needed. This was however the first study according to the literature to describe the origin of faecal pollution in household stored drinking water in a rural setting.

4.3 SURVIVAL OF INDICATOR AND PATHOGENIC MICROORGANISMS IN DRINKING WATER STORED IN AN IMPROVED HOUSEHOLD STORAGE CONTAINER WITH OR WITHOUT THE ADDITION OF A SODIUM HYPOCHLORITE SOLUTION

Very little information on the survival of pathogenic microorganisms in the CDC safe storage container is currently available. Therefore this study investigated the survival of naturally occurring indicator and selected seeded pathogenic microorganisms in the CDC safe storage container before and after the use of specific concentrations of a sodium hypochlorite solution.

4.3.1 Physical quality of improved and unimproved water sources inside the CDC safe storage container over a period of 5 days

Turbidity, pH and temperature of a water source play an important role in the complete removal of microorganisms during the chemical treatment of the water with sodium hypoclorite (Allwood *et al.*, 2003; Skraber *et al.*, 2004). Additionally, factors such as virus aggregation, viral attachment to surfaces or suspended matter, the initial free chlorine dose and free chlorine residual after disinfection also influence the survival of microorganisms during disinfection (Floyd and Sharp, 1977; Carlsson, 2003).

Studies have showed that viruses tend to survive longer in groundwater than in surface water at similar temperatures (Atkin *et al.*, 1971; Sattar, 1981). A study by Carlsson (2003) indicated that increased temperatures produced higher rates of bacterial and viral inactivation in water. Lund and Ormerod (1995), LeChevallier and co-workers (1996) and Power and Nagy (1999) have showed that temperatures above 5ºC could attribute to the formation of biofilms in drinking water systems which could aid in the survival of microorganisms. In addition, several studies have reported that attachment of organisms to surfaces makes them more resistant to starvation and disinfection due to biofilm formation (Kjellberg *et al.*, 1983; Baker, 1984; LeChevallier *et al.*, 1984; Herson *et al.*, 1987; John and Rose, 2005). In this study, the temperature for both borehole and river water samples ranged between 19 ºC and 24°C and fell within the South African recommended guideline values of 18ºC to 24ºC (DWAF, 1996).

In two separate studies, Engelbrecht and co-workers (1980) and Schaper and co-workers (2002b) have showed that bacteriophages and viruses were affected differently in their susceptibility to chlorine disinfection due to changes in the temperatures and pH parameters of water sources. Grabow and co-workers (1993b) have showed that the higher the pH of the solution, the more resistant microorganisms become to chlorine disinfection. This was confirmed by Vaughn and co-workers (1986) whom have showed that viruses are more readily inactivated by chlorine in pH levels of 6 compared to pH levels of 8. In this study the pH values for borehole water samples ranged between 7.0 and 7.1 and for river water samples varied between 6.8 and 7.7 which fell within the South African water quality pH guideline range for domestic use of 6.0 to 9.0 (DWAF, 1996).

Turbidity in water could be caused by the presence of suspended matter such as clay, silt, organic matter, inorganic matter, plankton and other microscopic organisms (LeChevallier *et al*., 1981; DWAF, 1996). The recommended South African guideline value for turbidity in water to be used for domestic purposes is 0.1 NTU (DWAF, 1996). During this study, the turbidity values for borehole water varied between 0.74 and 1.75 NTU and for river water between 7.04 and 8.30 NTU, which exceeded the South African guideline values. These high turbidity values suggested that microorganisms present in the water source could possibly be associated with particulate matter in the water, which can protect and assist in their survival and reduce the effect of the sodium hypochlorite disinfectant (DWAF, 1996).

4.3.2 Free chlorine residuals in the improved CDC safe storage containers after addition of 1% or 3.5% sodium hypochlorite solutions

Throughout this study, the free chlorine residual of the containers receiving the 1% and 3.5% sodium hypochlorite solutions after sixty minutes were in the order of 0.8 $mg.l^{-1}$ for containers which received the 1% sodium hypochlorite solution and 3.8 $mg.l^{-1}$ for containers which received the 3.5% sodium hypochlorite solution. After 24 h the free chlorine residual levels had dropped to 0 $mg.l^{-1}$ and 0.8 $mg.l^{-1}$ respectively for the 1% and 3.5% sodium hypochlorite solutions. On day 2 no more free chlorine residual were detected in any of the containers. The 3.5% sodium hypochlorite solution had a higher free chlorine residual compared to the 1% sodium hypochlorite solution. Consequently,

the 3.5% sodium hypochlorite solution was more effective for longer periods as would be expected compared to the 1% sodium hypochlorite solution in both the borehole and the river water containers. The free chlorine residuals in the containers receiving the 1% sodium hypochlorite solution indicated that the water was no longer protected after 24 h against secondary contamination, which could be introduced by unhygienic handling and storage practices, dust and animals at the point-of-use (Sobsey, 2002).

4.3.3 Survival of naturally occurring indicator and pathogenic microorganisms in the CDC safe storage container before and after the addition of a sodium hypochlorite solution

The microbiological analyses of the borehole water and the river water samples indicated that ground water was microbiologically of a better quality and less contaminated than surface water when looking at the prevalence of naturally occurring indicator microorganisms in both the water sources (Tables 4.19 to 4.23). Borehole water contained initial counts of heterotrophic bacteria and total coliforms, while river water only contained initial counts of several indicator bacteria which included heterotrophic bacteria, total coliforms, faecal coliforms, faecal enterococci and *C. perfringens* (Tables 4.19 to 4.23). This was in agreement with similar studies conducted by Lehloesa and Muyima (2000) on ground water and communal tap water sources used by rural communities in the Eastern Cape, South Africa. No Enteroviruses were detected in any of the water samples after amplification in BGM cell cultures and molecular detection methods (section 3.5.5), although Enteroviruses have been shown to be sporadically present in untreated water sources (WHO, 1996). In addition, no *Salmonella* spp were detected in any of the original water samples after selective enrichment and enumeration steps (section 3.5.4).

The presence of heterotrophic microorganisms in both water sources indicated the general microbiological quality of water samples (DWAF, 1996; WHO, 2002b). Although heterotrophic bacteria is generally not considered harmful, various studies have indicated that some heterotrophic bacteria such as *Aeromona* spp, *Klebsiella pneumoniae*, *Enterococcus*, *Bacillus* spp and *Enterobacter* spp might be opportunistic pathogens and have been associated with diseases of the respiratory tract and wound

infections (Payment *et al*., 1991; WHO, 1996; WHO, 2002b; Bartram *et al*., 2003; Ehlers *et al*., 2003).

The recommended South African guideline value for heterotrophic bacteria in domestic water is less than 100 cfu.1 ml^{-1} or less than 2 log_{10} (SABS, 2001). The initial heterotrophic plate counts of 9 log_{10} present in both the water sources indicated that the water was unacceptable for human consumption because of the possible presence of opportunistic and pathogenic microorganisms which could cause diseases (Table 4.19) (DWAF, 1996; SABS, 2001; Ehlers *et al*., 2003). Over the 5 day period, the heterotrophic microorganisms declined respectively to 8 log_{10} in borehole water and 7 log_{10} in river water in the containers receiving the placebo solution (Table 4.19).

In the containers filled with borehole water, the 1% sodium hypochlorite solution reduced the numbers of heterotrophic organisms within 60 min to undetectable levels (Table 4.19). However, in the containers filled with river water, the heterotrophic microorganisms were not inactivated within 60 min and were even detected for 5 days during which the heterotrophic microorganism counts decreased from 9 log_{10} to 5 log_{10} (Table 4.19).

The results from this laboratory study for borehole water were in agreement with results from section 4.1 on the effectiveness of the 1% and 3.5% sodium hypochlorite solutions in the CDC safe storage containers for heterotrophic bacteria. However, the turbidity of river water used in the laboratory studies were higher (7.14 NTU to 8.3 NTU) than the turbidity of the river water samples during the field studies (2.4 NTU to 4.4 NTU). This indicated that the higher turbidity of the water used in the laboratory studies could have reduced the effectivity of the 1% sodium hypochlorite solution in killing the heterotrophic bacteria (Table 4.19) (WHO, 1996; Tree *et al*., 2003). It was possible that some of the heterotrophic microorganisms used the nutrients in the turbid water to survive (WHO, 1996). However, in the containers receiving the 3.5% sodium hypochlorite solution, no heterotrophic bacteria survived in the river or borehole water samples after 60 min (Table 4.19). This was in agreement with the results obtained during the field intervention trial studies for heterotrophic bacterial counts in water samples assessed in the CDC safe storage containers (section 4.1).

Table 4.19: The survival of naturally occurring heterotrophic bacteria over a 5 day period detected in the borehole and river water samples before and after the addition of a placebo, 1% or 3.5% sodium hypochlorite solution

Sodium hypochlorite solution	Water source									
	Borehole water					River water				
	Day 0*	Day 0**	Day 1	Day 2	Day 5	Day 0*	Day 0**	Day 1	Day 2	Day 5
Placebo***	9.5	-	9.4	9.0	8.5	9.3	-	8.6	8.5	7.5
1%	9.3	n.d	n.d	n.d	n.d	9.3	6.9	6.2	5.5	5.4
3.5%	9.3	n.d	n.d	n.d	n.d	9.3	n.d	n.d	n.d	n.d

* time = 0 minutes before the addition of the sodium hypochlorite solution
** time = 60 minutes after the addition of the sodium hypochlorite solution
*** placebo = distilled water
n.d = not detected
- = not tested

The presence of total coliforms in both water sources indicated the presence of bacteria which can originate from faecal contamination or from environmental sources such as sewage run offs (Pinfold, 1990). The South African recommended guideline value for total coliforms in drinking water is less than 10 cfu.100 ml^{-1} or 1 $\log_{10}$ (SABS, 2001). In this study the levels of naturally occurring total coliform bacteria determined in borehole (1 $\log_{10}$) and river (4 $\log_{10}$) water samples indicated the likelihood that the water was faecally contaminated by human and animal faeces (Table 4.20) (DWAF, 1996).

Total coliform bacteria decreased in both water sources in the containers receiving the placebo solution over a 5 day period with a higher decline rate in the river water containers (decrease from 4 $\log_{10}$ to 2 $\log_{10}$) (Table 4.20). The higher decline rate could have been due to the decrease in nutrient levels because of competition between microorganisms (LeChevallier and McFeters, 1985; Momba and Notshe, 2003). In the containers receiving the 1% and the 3.5% sodium hypochlorite solutions, no total coliform bacteria in the water samples survived after 60 min (Table 4.20). These results were in agreement with the results obtained during the field intervention trial studies for total coliform counts in water samples assessed in the CDC safe storage containers receiving the 1% and 3.5% sodium hypochlorite solutions (section 4.1).

Table 4.20: The survival of naturally occurring total coliform bacteria over a 5 day period detected in the borehole and river water samples before and after the addition of a placebo, 1% or 3.5% sodium hypochlorite solution

Sodium hypochlorite solution	Water source									
	Borehole water					River water				
	Day 0*	Day 0**	Day 1	Day 2	Day 5	Day 0*	Day 0**	Day 1	Day 2	Day 5
Placebo***	1.9	-	1.7	1.6	1.4	4.1	-	3.9	3.6	2.2
1%	1.9	n.d	n.d	n.d	n.d	4.1	n.d	n.d	n.d	n.d
3.5%	1.9	n.d	n.d	n.d	n.d	4.1	n.d	n.d	n.d	n.d

* time = 0 minutes before the addition of the sodium hypochlorite solution
** time = 60 minutes after the addition of the sodium hypochlorite solution
*** placebo = distilled water
n.d = not detected
- = not tested

The South African guideline value for the prevalence of faecal coliform bacteria in water used for domestic purposes is 0 cfu.100 ml^{-1} or not detected (SABS, 2001). Faecal coliform bacteria were detected only in the river water (3 $\log_{10}$) and not in any of the borehole water samples (Table 4.21). The presence of faecal coliform bacteria in the river water samples in the containers receiving the placebo solution indicated the presence of potential pathogenic microorganisms such as *Salmonella* spp, *Shigella* spp, pathogenic *E. coli* and *V. cholerae* which are associated with waterborne diseases such as salmonellosis, dysentery, gastroenteritis and cholera (DWAF, 1996; SABS, 2001).

The faecal coliform bacteria were still detected after 5 days in the containers receiving the placebo solution with a 1 $\log_{10}$ decrease from day 1 (Table 4.21). In the containers receiving the 1% and the 3.5% sodium hypochlorite solutions, no faecal coliform bacteria in the river water samples survived after 60 min (Table 4.21). These results were in agreement with the results obtained during the field intervention trial studies for faecal coliform counts in water samples assessed in the CDC safe storage containers receiving the 1% and 3.5% sodium hypochlorite solutions (section 4.1). In addition, several previous studies indicating that coliform bacteria are more sensitive to chlorine disinfection than male specific F-RNA bacteriophages and Enteroviruses (Sobsey, 1989; Morris, 1993; Tree *et al.*, 1997).

Table 4.21: The survival of naturally occurring faecal coliform bacteria over a 5 day period detected in the borehole and river water samples before and after the addition of a placebo, 1% or 3.5% sodium hypochlorite solution

Sodium hypochlorite solution	Water source									
	Borehole water					River water				
	Day 0*	Day 0**	Day 1	Day 2	Day 5	Day 0*	Day 0**	Day 1	Day 2	Day 5
Placebo***	n.d	-	n.d	n.d	n.d	3.3	-	3.2	2.9	2.4
1%	n.d	n.d	n.d	n.d	n.d	3.4	n.d	n.d	n.d	n.d
3.5%	n.d	n.d	n.d	n.d	n.d	3.5	n.d	n.d	n.d	n.d

* time = 0 minutes before the addition of the sodium hypochlorite solution
** time = 60 minutes after the addition of the sodium hypochlorite solution
*** placebo = distilled water
n.d = not detected
- = not tested

Naturally occurring faecal enterococci were only detected in river water samples (2 $\log_{10}$) during this study (Table 4.22). The presence of faecal enterococci in water indicates the presence of human faecal contamination in the water samples as well as the potential risk of waterborne diseases (DWAF, 1996). The South African guideline value for faecal enterococci in water to be used for domestic purposes is 0 cfu.100 ml^{-1} or not detected (SABS, 2001). The counts (2 $\log_{10}$) of faecal enterococci in the river water containers receiving the placebo solution exceeded the recommended South African guideline values (0 cfu.100 ml^{-1}) for faecal enterococci counts in water to be used for domestic purposes and indicated the potential risk of transmission of waterborne pathogens which may include viruses and parasites that can survive for longer periods of time in water (DWAF, 1996).

Faecal enterococci bacteria could still be detected in the river water samples receiving the placebo solution after 5 days, although a 1 $\log_{10}$ decrease in the survival could be detected between day 1 and day 5 (Table 4.22). In the containers receiving the 1% and the 3.5% sodium hypochlorite solutions, no faecal enterococci bacteria in the river water samples survived after 60 min (Table 4.22). These results were in agreement with the results obtained during the field intervention trial studies for faecal enterococci counts in water samples assessed in the CDC safe storage containers receiving the 1% and 3.5% sodium hypochlorite solutions (section 4.1). A study by Tree and co-workers (2003) indicated that enterococci bacteria are more resistant than *E. coli* to chlorine

disinfection. However, this was not seen in this study (Table 4.26), which could have been due to the initial differences in the naturally occurring bacterial counts of enterococci and the higher seeded counts for *E. coli* in the CDC safe storage containers.

Table 4.22: The survival of naturally occurring faecal enterococci bacteria over a 5 day period detected in the borehole and river water samples before and after the addition of a placebo, 1% or 3.5% sodium hypochlorite solution

Sodium hypochlorite solution	Water source									
	Borehole water					River water				
	Day 0*	Day 0**	Day 1	Day 2	Day 5	Day 0*	Day 0**	Day 1	Day 2	Day 5
Placebo***	n.d	-	n.d	n.d	n.d	2.8	-	2.5	2.2	1.4
1%	n.d	n.d	n.d	n.d	n.d	2.8	n.d	n.d	n.d	n.d
3.5%	n.d	n.d	n.d	n.d	n.d	2.7	n.d	n.d	n.d	n.d

* time = 0 minutes before the addition of the sodium hypochlorite solution
** time = 60 minutes after the addition of the sodium hypochlorite solution
*** placebo = distilled water
n.d = not detected
- = not tested

Clostridium perfringens is normally present in human and animal faeces, survives longer than indicator microorganisms and serves as an indicator for the presence of resistant microorganisms such as viruses, protozoan cysts and oocysts (Payment and Franco, 1993; WHO, 1996). No *C. perfringens* spores or vegetative cells were detected in the borehole water receiving the placebo solution (Table 4.23). However, containers with river water receiving the placebo solution did have *C. perfringens* vegetative cells and spores present (2 $\log_{10}$) over the 5 day period (Table 4.23).

In the containers receiving the 1% sodium hypochlorite solution *C. perfringens* were not inactivated in 60 min (Tables 4.23). The resistance of the *C. perfringens* bacteria spores and vegetative cells in the storage containers might have been due to survival ability of the spores or the high turbidity values of the river water which influenced the effectiveness of the 1% sodium hypochlorite solution (WHO, 1996; Tree *et al*., 2003). The extended survival of *C. perfringens* in the river water samples indicated the possible presence of more resistant microorganisms such as enteric Adenoviruses, Caliciviruses, Enteroviruses, Hepatitis A virus and Rotaviruses, as well as protozoan

parasites such as *Cryptosporidium*, *Entamoeba* and *Giardia* (WHO, 1996; Carlsson, 2003).

The results from this laboratory study for borehole water indicates that higher turbidity affects the efficiency of a disinfectant such as the 1% sodium hypochlorite solution. The higher turbidity (7.14 NTU to 8.3 NTU) of the water used in the laboratory studies could have assisted the *C. perfringens* bacterial spores to survive (Table 4.23).

Table 4.23: The survival of naturally occurring *Clostridium perfringens* bacteria over a 5 day period detected in the borehole and river water samples before and after the addition of a placebo, 1% or 3.5% sodium hypochlorite solution

Sodium hypochlorite solution	Water source									
	Borehole water					River water				
	Day 0*	Day 0**	Day 1	Day 2	Day 5	Day 0*	Day 0**	Day 1	Day 2	Day 5
Placebo***	n.d	-	n.d	n.d	n.d	2.7	-	2.5	2.3	1.5
1%	n.d	n.d	n.d	n.d	n.d	2.6	1.6	n.d	n.d	n.d
3.5%	n.d	n.d	n.d	n.d	n.d	2.6	n.d	n.d	n.d	n.d

* time = 0 minutes before the addition of the sodium hypochlorite solution
** time = 60 minutes after the addition of the sodium hypochlorite solution
*** placebo = distilled water
n.d = not detected
- = not tested

In the containers receiving the 3.5% sodium hypochlorite solution, no *C. perfringens* vegetative cells or spores in the water samples survived after 60 min (Table 4.23). This indicated that the 3.5% sodium hypochlorite solution was more effective than 1% sodium hypochlorite solution against spore forming microorganisms and could be used successfully for the disinfection of resistant microorganisms in water with high turbidity (Payment and Franco, 1993; WHO, 1996). This was in agreement with the results obtained during the field intervention trial studies for *C. perfringens* bacteria in water samples assessed in the CDC safe storage containers using the 3.5% sodium hypochlorite solution (section 4.1).

4.3.4 Survival of seeded indicator and pathogenic microorganisms in the CDC safe storage container before and after the addition of a sodium hypochlorite solution

To date the only information available on the effect of disinfection procedures on microorganisms in the CDC safe storage container is based on *E. coli* and faecal coliforms (Sobsey, 2002; Sobsey *et al*., 2003). Ashbolt (2004) has shown that the survival of many enteric pathogens is different to the survival of indicator microorganisms. Therefore, the survival of seeded indicator microorganisms (somatic and male specific F-RNA bacteriophages) and pathogenic microorganisms (*S. typhimurium*, *E. coli* and Coxsackie B1 virus) before and after the addition of a placebo, 1% or 3.5% sodium hypochlorite solution in the CDC safe storage container was assessed (Tables 4.24 to 4.28).

Somatic and male specific F-RNA bacteriophages were used in this study as indicators of enteric viruses (Grabow, 2001). These bacteriophages closely resembled human enteroviruses with regard to size, morphology, nucleic acid structure and failure to replicate in water environments (Grabow, 2001). The survival of both somatic and male specific F-RNA bacteriophages during the 5 day period implicated that when pathogenic enteric viruses were present, they could survive in these storage containers for periods longer than 24 h in temperatures of 25ºC (Tables 4.24 and 4.25) (Duran *et al*., 2003).

The somatic bacteriophages decreased from 9 $\log_{10}$ to 5 $\log_{10}$ in the borehole water and from 9 $\log_{10}$ to 6 $\log_{10}$ in the river water samples in the containers receiving the placebo solutions (Table 4.24). The South African water quality guidelines state that somatic bacteriophages must be present in the water sample in concentrations not exceeding 1 cfu.10 ml^{-1} (SABS, 2001). The results in this study have showed that somatic bacteriophages were sensitive to both the 1% and the 3.5% sodium hypochlorite solutions and did not survive longer than 60 min after addition of the solutions (Table 4.24). These results were in agreement with the results obtained during the field intervention trial studies for somatic bacteriophages in water samples assessed in the CDC safe storage containers receiving the 1% and 3.5% sodium hypochlorite solutions (section 4.1).

Table 4.24: The survival of seeded somatic bacteriophages over a 5 day period detected in the borehole and river water samples before and after the addition of a placebo, 1% or 3.5% sodium hypochlorite solution

Sodium hypochlorite solution	Water source									
	Borehole water					River water				
	Day 0*	Day 0**	Day 1	Day 2	Day 5	Day 0*	Day 0**	Day 1	Day 2	Day 5
Placebo***	9.1	-	8.1	6.0	5.3	9.2	-	9.2	8.0	6.2
1%	9.1	n.d	n.d	n.d	n.d	9.2	n.d	n.d	n.d	n.d
3.5%	9.1	n.d	n.d	n.d	n.d	9.1	n.d	n.d	n.d	n.d

* time = 0 minutes before the addition of the sodium hypochlorite solution
** time = 60 minutes after the addition of the sodium hypochlorite solution
*** placebo = distilled water
n.d = not detected
- = not tested

Schaper and co-workers (2002b) have showed that temperature and pH play an important role in the survival of the different genotype groups of male specific F-RNA bacteriophages. In the containers receiving the placebo solution, the male specific F-RNA bacteriophages decreased from 9 $\log_{10}$ to 7 $\log_{10}$ in the borehole water and from 9 $\log_{10}$ to 8 $\log_{10}$ in the river water containers respectively (Table 4.25). In general male specific F-RNA bacteriophages counts were higher over the 5 days in the storage containers receiving the placebo solution in both borehole and the river water samples, compared to somatic bacteriophages (Tables 4.24 and 4.25).

The results indicated that male specific F-RNA bacteriophages were more resistant to environmental conditions than somatic bacteriophages. This is in agreement with earlier laboratory studies carried out during 2003 by two different groups: (1) Allwood and co-workers (2003) have shown that F-RNA bacteriophages are a good indictor for the survival of Noroviruses in water free from disinfectants because it survived longer than Noroviruses during laboratory studies; and (2) Duran and co-workers (2003) have shown that somatic bacteriophages were inactivated significantly easier than male specific F-RNA bacteriophages and *Bacteroides fragilis* bacteriophages in ground water samples.

In the borehole water containers, the 1% sodium hypochlorite solution effectively reduced the male specific F-RNA bacteriophages after 60 min to undetectable levels

(Table 4.25). These results were in agreement with the results obtained during the field intervention trial studies for male specific F-RNA bacteriophages in water samples assessed in the CDC safe storage containers using the 1% sodium hypochlorite solution (section 4.1). However, in river water containers, F-RNA bacteriophages were not inactivated by the 1% sodium hypochlorite solution within 60 min of exposure and could be detected for all 5 days with a decrease in the survival from 9 $\log_{10}$ to 1 $\log_{10}$ (Table 4.25). However, the higher turbidity (7.14 NTU to 8.3 NTU) of the water used in the laboratory studies could have reduced the effectivity of the 1% sodium hypochlorite solution in killing the male specific F-RNA bacteriophages (Table 4.25).

This study showed that male specific F-RNA bacteriophages survived longer compared to Coxsackie B1 viruses (Table 4.28) with the addition of the 1% sodium hypochlorite solution. This was in agreement with a study by Tree and co-workers (2003) which indicated that Poliovirus was more susceptible to chlorine than male specific F-RNA bacteriophages and more resistant to chlorine than bacterial indicators. Consequently the survival of both the male specific F-RNA bacteriophages and Coxsackie B1 viruses during chlorination with 1% sodium hypochlorite solution indicated the suitability of the male specific F-RNA bacteriophages as indicators for the presence of potentially pathogenic enteric viruses in drinking water sources (Grabow, 2001; Allwood *et al*., 2003; Duran *et al*., 2003; Tree *et al*., 2003).

The results further indicated that the 3.5% sodium hypochlorite solution were the most effective sodium hypochlorite solution because no male specific F-RNA bacteriophages survived longer than 60 min after addition of the solution in both water sources (Table 4.25). This was in agreement with the results obtained during the field intervention trial studies for male specific F-RNA bacteriophages in water samples assessed in the CDC safe storage containers after the addition of the 3.5% sodium hypochlorite solution (section 4.1).

Table 4.25: The survival of seeded male specific F-RNA bacteriophages over a 5 day period detected in the borehole and river water samples before and after the addition of a placebo, 1% or 3.5% sodium hypochlorite solution

Sodium hypochlorite solution	Water source									
	Borehole water					River water				
	Day 0*	Day 0**	Day 1	Day 2	Day 5	Day 0*	Day 0**	Day 1	Day 2	Day 5
Placebo***	9.2	-	8.3	8.2	7.3	9.9	-	9.0	9.1	8.0
1%	9.1	n.d	n.d	n.d	n.d	9.1	6.8	5.4	4.5	1.5
3.5%	9.2	n.d	n.d	n.d	n.d	9.2	n.d	n.d	n.d	n.d

* time = 0 minutes before the addition of the sodium hypochlorite solution
** time = 60 minutes after the addition of the sodium hypochlorite solution
*** placebo = distilled water
n.d = not detected
- = not tested

Escherichia coli (ATCC 13706) bacteria were used to indicate the survival of pathogenic microorganisms that can multiply in the gastrointestinal tracts of warm blooded humans and animals (DWAF, 1996). *Salmonella typhimurium* (NCTC 12484) bacteria were used in the study as a typical waterborne pathogen to give information on the possible survival of waterborne pathogens in household water storage containers (Theron and Cloete, 2002). The seeded studies on *E. coli* (Table 4.26) and *S. typhimurium* (Table 4.27) bacteria indicated that these bacteria could survive in the environment because counts for both bacteria were detected during the 5 days in the river and borehole water containers without any sodium hypochlorite solutions. Generally, these two bacteria had a faster die-off than male specific F-RNA bacteriophages (Table 4.25) and Coxsackie B1 viruses (Table 4.28). This natural die-off curve is in agreement with studies carried out by Nasser and Oman (1999), Allwood and co-workers (2003) and Skraber and co-workers (2004) which have showed with laboratory studies that *E. coli* cells decreased faster than male specific F-RNA bacteriophages, Hepatitis A virus or Polio virus type 1.

The survival of *E. coli* bacterial cells in containers containing borehole and river water samples is indicated in Table 4.26. In the river and borehole water containers receiving the placebo solution, *E. coli* bacterial cells were able to survive for 5 days with a decrease in survival from 7 $\log_{10}$ to 3 $\log_{10}$ (Table 4.26). In the borehole water

containers, the 1% sodium hypochlorite solution effectively reduced the *E. coli* bacteria after 60 min to undetectable levels (Table 4.26). However, in river water containers, *E. coli* bacteria were not inactivated by the 1% sodium hypochlorite solution within 60 min of exposure and the bacterial cells survived for 24 h in the river water containers (Table 4.26). The laboratory studies indicated that the higher turbidity of the river water samples could have reduced the effectivity of the 1% sodium hypochlorite solution. However, the containers receiving the 3.5% sodium hypochlorite solution showed complete inactivation of all *E. coli* bacterial cells within 60 min (Table 4.26). The results seen in this study for *E. coli* bacteria is in agreement with results reported by Duran and co-workers (2003) whom have showed that chlorination inactivated bacteria more efficiently than bacteriophages and Enteroviruses. In addition, the temperature could also have played a major role in the survival of the bacteria. During this study the temperatures in the containers ranged between 19°C and 24°C. Flint (1987) has showed that *E. coli* cells survived better at 4ºC compared to 15ºC, 25ºC or 37ºC. Lim and Flint (1989) have shown that *E. coli* can survive up to 12 days without loss of viability dependant on the water temperatures which ranged between 15°C to 37ºC. Both these two studies have showed that *E. coli* bacteria survive better at lower temperatures (Flint, 1987; Lim and Flint, 1989). In addition, this was in agreement with the results obtained during the field intervention trial studies for *E. coli* bacteria in water samples assessed in the CDC safe storage containers after the addition of the 1% and 3.5% sodium hypochlorite solution (section 4.1).

Table 4.26: The survival of seeded *Escherichia coli* bacteria over a 5 day period detected in the borehole and river water samples before and after the addition of a placebo, 1% or 3.5% sodium hypochlorite solution

Sodium hypochlorite solution	Water source									
	Borehole water					River water				
	Day 0*	Day 0**	Day 1	Day 2	Day 5	Day 0*	Day 0**	Day 1	Day 2	Day 5
Placebo***	7.0	-	5.0	4.8	3.2	7.0	-	6.9	4.6	3.9
1%	7.1	n.d	n.d	n.d	n.d	7.0	3.8	n.d	n.d	n.d
3.5%	6.9	n.d	n.d	n.d	n.d	7.1	n.d	n.d	n.d	n.d

* time = 0 minutes before the addition of the sodium hypochlorite solution
** time = 60 minutes after the addition of the sodium hypochlorite solution
*** placebo = distilled water
n.d = not detected
- = not tested

The survival of *S. typhimurium* bacterial cells in containers containing borehole and river water samples is indicated in Table 4.27. In both the river and borehole water containers receiving the placebo solution, *S. typhimurium* bacterial cells were able to survive for 5 days with a decrease in survival from 6 $\log_{10}$ to 3 $\log_{10}$ (Table 4.27). The high turbidity of the river water in this study could have assisted in the survival of the bacteria and protected them from the effect of the sodium hypochlorite solution. The survival of *S. typhimurium* as a typical waterborne microorganism indicated that other waterborne microorganisms such as *Shigella* spp, *V. cholera*, *Yersinia enterocolitica* and *Campylobacter jejuni* could also survive in household storage containers without treatment (WHO, 1996). In the borehole water containers, the 1% sodium hypochlorite solution effectively reduced the *S. typhimurium* bacteria after 60 min (Table 4.27). However, in river water containers, *S. typhimurium* bacteria were not inactivated by the 1% sodium hypochlorite solution within 60 min of exposure and the bacterial cells survived for 24 h in the river water containers (Table 4.27). The containers receiving the 3.5% sodium hypochlorite solution showed complete inactivation of all *S. typhimurium* bacterial cells within 60 min in both types of water samples (Table 4.27). Generally, results from this study is in agreement with a study by Mitchell and Starzyk (1975) which have showed that *S. typhimurium* and *E. coli* cells in river water samples have similar survival patterns.

Table 4.27: The survival of seeded *Salmonella typhimurium* bacteria over a 5 day period detected in the borehole and river water samples before and after the addition of a placebo, 1% or 3.5% sodium hypochlorite solution

Sodium hypochlorite solution	Water source									
	Borehole water					River water				
	Day 0*	Day 0**	Day 1	Day 2	Day 5	Day 0*	Day 0**	Day 1	Day 2	Day 5
Placebo***	6.9	-	4.8	3.5	3.3	6.9	-	5.8	4.5	3.3
1%	6.9	n.d	n.d	n.d	n.d	6.9	2.3	n.d	n.d	n.d
3.5%	6.8	n.d	n.d	n.d	n.d	6.9	n.d	n.d	n.d	n.d

* time = 0 minutes before the addition of the sodium hypochlorite solution
** time = 60 minutes after the addition of the sodium hypochlorite solution
*** placebo = distilled water
n.d = not detected
- = not tested

Although a vaccine strain of Poliovirus type 1 was included in the original protocol, studies on Poliovirus type 1 during this research study were excluded due to the global Poliovirus-containment. Therefore, Coxsackie B1 virus was the only virus used in this study as representative of human Enteroviruses. The Enteroviruses are Picornaviruses containing a single stranded RNA and particles containing 60 molecules each of 4 distinct proteins designated VP1 through VP4 (Rueckert, 1985). The Picornaviruses group contains the Polioviruses, Coxsackie viruses (A and B), Echoviruses and several Enteroviruses (WHO, 1996). Coxsackie B1 virus was used as a representative indicator virus to indicate the survival of human Enteroviruses in stored water containers. Several studies have indicated that human enteric viruses not only survived longer than bacterial indicators, but can also be present when indicator microorganisms are absent (Bosch *et al.*, 1991; Bosch, 1998). Therefore, it was deemed necessary to include a representative viral indicator in this study to assess the survival of viruses in the CDC safe storage container with or without the treatment of a sodium hypochlorite solution.

The results of this study have indicated that Coxsackie B1 virus particles were more persistent and have been detected through-out the 5 day period in the containers receiving the placebo solution (Table 4.28). This is in agreement with a study by Skraber and co-workers (2004) whom have showed that Enteroviruses such as Poliovirus type 1 were more persistent and survived longer than thermotolerant coliforms at various temperatures and pH values. It is however important to highlight that Shuval and co-workers (1971) have shown that Enteroviruses have different stabilities in water. Shuval and co-workers (1971) have found that Polio type 3 and Coxsackie A13 viruses were more readily inactivated than Polio type 1 or Coxsackie B1 virus at different water temperatures. The study of Shuval and co-workers (1971) have showed that Coxsackie B1 virus survived longer than Poliovirus type 1 at temperatures ranging between 23°C to 27 ºC.

The results from this study indicated that in the containers containing the borehole water with much lower turbidity values (between 0.74 and 1.75 NTU) than river water containers (between 7.04 and 8.30 NTU), the 1% and the 3.5% sodium hypochlorite solutions effectively reduced the Coxsackie B1 virus particles after 60 min to undetectable levels (Table 4.28). However, in river water containers, Coxsackie B1 virus particles were not inactivated by the 1% sodium hypochlorite solution within 60

min of exposure, but survived for 2 days in the containers (Tables 4.28). These results were in agreement with several earlier laboratory-seeding studies (Duran *et al*., 2003; Tree *et al*., 2003). Duran and co-workers (2003) have showed that Enteroviruses and bacteriophages were more resistant to chlorination inactivation compared to bacterial cells. Additionally, studies carried out by Kelly and Sanderson (1958) and Shaffer and co-workers (1980) have showed that different strains of Poliovirus type 1 have different rates of chlorine inactivation which enables them to survive chlorine treatment. This indicated the need to conduct more intensive studies on a range of viruses that could potentially affect these rural communities in order to assess the survival of viruses in point-of-use intervention systems.

Table 4.28: The survival of seeded Coxsackie B1 viruses over a 5 day period detected in the borehole and river water samples before and after the addition of a placebo, 1% or 3.5% sodium hypochlorite solution

Sodium hypochlorite solution	Water source									
	Borehole water					River water				
	Day 0*	Day 0**	Day 1	Day 2	Day 5	Day 0*	Day 0**	Day 1	Day 2	Day 5
Placebo***	6.9	-	6.7	6.5	6.0	6.9	-	6.8	6.5	6.2
1%	5.8	n.d	n.d	n.d	n.d	5.8	5.3	2.7	n.d	n.d
3.5%	5.8	n.d	n.d	n.d	n.d	5.8	n.d	n.d	n.d	n.d

* time = 0 minutes before the addition of the sodium hypochlorite solution
** time = 60 minutes after the addition of the sodium hypochlorite solution
*** placebo = distilled water
n.d = not detected
- = not tested

Several other factors could also have influenced the survival of Coxsackie B1 viruses in the river water. One of these factors could be the adhesion of virus particles to the walls of storage containers which was showed to happen when the pH of the water is at 7 or lower (Taylor *et al*., 1981). Ward and Winston (1985) have showed that Poliovirus type 1 adheres to the walls of containers filled with ground water. Bixby and O'Brien (1979) as well as Chattopadhyay and co-workers (2002) have found that virus particles are in competition with organic matter for adsorption sites on the walls of polypropylene storage containers. A study by John and Rose (2005) has showed that the effect of attachment of viruses to solid surfaces is virus dependant. They have however showed

that the survival of Poliovirus and Hepatitis A virus increased when attached to solid surfaces (John and Rose, 2005).

Another factor which could have aided in the survival of Coxsackie B1 virus particles in the river water was the high turbidity values (7.03 NTU to 8.3 NTU). Suspended matter in the water could act as adsorption sites for virus particles and protect them from the effect of disinfectants. Floyd and Sharp (1977) and Young and Sharp (1977) have showed that Enteroviruses in their normal state in fresh water clump together to form aggregates which are capable of protecting viable particles from disinfection and increase their survival. The high turbidity values of the river water used in this study did indicate the presence of particulate matter, which might have influenced the effectiveness of the 1% sodium hypochlorite solution. The survival of Coxsackie B1 virus particles in the river water containers during the 1% sodium hypochlorite solution treatment could therefore be ascribed to either aggregation, high turbidity of the water or due to chlorine resistance (Jensen *et al*., 1980; Hejkal *et al*., 1981). The 3.5% sodium hypochlorite was more effective in killing all viable viruses in both the river and borehole water containers after the addition of the solution (Table 4.28). It is however important to mention the study of Tree and co-workers (2003) whom have showed that indigenous Enteroviruses are more resistant to chlorination than laboratory adapted strains. The Coxsackie B1 virus strain used in this study was a laboratory adapted strain. Therefore, laboratory studies may overestimate the level of human enteric virus inactivation in the field and should only be used as a guideline to assess the efficiency of a disinfection process.

4.3.5 Summary of the survival of selected indicator and pathogenic microorganisms in drinking water stored in an improved household storage container with or without the addition of a sodium hypochlorite solution

In general, the CDC safe storage container proved to be convenient to handle, store the water and protect it from external contamination during storage. The reduction in the numbers of total coliforms, faecal coliforms, *C. perfringens*, somatic bacteriophages, *E. coli* and *S. typhimurium* in the control CDC safe storage containers not treated with a sodium hypochlorite solution (containers receiving the placebo solution) reflected the natural die-off curve of microorganisms under the prevailing storage conditions.

Consequently, this study indicated that even without the addition of a disinfectant, the counts of indicator and pathogenic microorganisms in water stored in the CDC safe storage containers decreased with time if the containers were not exposed to secondary contamination factors such as flies, insects, dust and faecally polluted hands and utensils (Jagals *et al*., 1999; Rose *et al*., 2006). However, microorganisms have been shown to survive in biofilms, which forms inside household storage containers (Fig 2.14) (Momba and Kaleni, 2002). These biofilms might harbour potentially pathogenic microorganisms, which can pose a health risk to consumers (Bunn *et al*., 2002; Jensen *et al*., 2002; Momba and Kaleni, 2002).

The 1% sodium hypochlorite solution was effective in reducing the counts of indicator and the seeded pathogenic microorganisms in the borehole water containers within 60 min to undetectable levels. However, in the river water samples, the 1% sodium hypochlorite dosage did not reduce the numbers of heterotrophic bacteria, *C. perfringens*, *E. coli*, *S. typhimurium*, male specific F-RNA bacteriophages and Coxsackie B1 viruses within 60 min. More resistant microorganisms such as heterotrophic bacteria, male specific F-RNA bacteriophages and Coxsackie B1 viruses were still present after 1 day and male specific F-RNA bacteriophages were detected up to 5 days after treatment with 1% sodium hypochlorite solution. It was evident that the high turbidity levels (7.04 to 8.30 NTU) in the river water did influence the effectivity of the 1% sodium hypochlorite solution. The river water could have contained particulate matter to which microorganisms could have attached for protection (WHO, 1996; Carlsson, 2003). Turbid water could also contain nutrients, which support microbial growth (LeChevallier *et al*., 1981). LeChevallier and co-workers (1981) have showed that water with turbidity between 1 and 10 NTU can result in an eight-fold decrease in efficiency of disinfection and were eight times more likely to carry pathogenic microorganisms.

The results obtained in this study confirmed that the 3.5% sodium hypochlorite dosage successfully reduced the number of a spectrum of microorganisms to undetectable levels within 60 min in the CDC safe storage container. This is the first evidence of successful disinfection by the sodium hypochlorite solution and dosage recommended by the South African Department of Health in the improved CDC safe storage containers.

Although seeding experiments provided valuable information on the inactivation of organisms, the seeded microorganisms used during this study may not always be representative of naturally occurring microorganisms in ground and surface water samples (Tree *et al*., 2003; Schaper *et al*., 2002b). The chlorine resistant parasitic protozoa such as the oocysts of *Cryptosporidium parvum* and various enteric viruses (Hambidge, 2001; Li *et al*., 2002) are of particular concern. Future studies should therefore, investigate the survival of parasites such as *Giardia* and *Cryptosporidium*.

The findings of this study confirmed that the CDC protocol (chlorine based water treatment combined with safe storage and education) offers a user-friendly and relatively inexpensive intervention strategy to control the transmission of enteric waterborne pathogens. The results from this study clearly indicated that the 3.5% sodium hypochlorite concentration was more effective against resistant pathogenic microorganisms compared to the 1% sodium hypochlorite solution used by the CDC. The 3.5% sodium hypochlorite solution, which is prescribed by the South African DOH and DWAF, provided a relatively high free chlorine residual of 3.8 $mg.l^{-1}$ after 60 min which is effective in reducing the health risk associated with waterborne pathogens in households with limited or no existing water and sanitation infrastructures. However, the water is not considered safe to drink before a free chlorine residual level of 0.8 $mg.l^{-1}$ is detected which in this study was the case only after 24 h for the water sources used in this study. Therefore, during this study all households were told to add their sodium hypochlorite solution, shake the container, closed it and let it stand for 24 h before the water was used. The main concern was that water with high concentrations of chlorine can lead to the formation of trihalomethanes (THMs) which have been closely linked with increased incidences of bladder, rectal and colon cancers in older individuals of the world population (Mills *et al*., 1999; Edstrom Industries, 2003; Freese and Nozaic, 2004). However, in these rural communities, the risk of death due to waterborne diseases is far greater than the relatively small risk of people dying from a small risk of getting cancer in their old days (WHO, 2004).

With proper education and follow-up studies the use of the 3.5% sodium hypochlorite solution together with the CDC safe storage containers could benefit the rural communities in South Africa. The CDC safe storage container is currently produced by a company in South Africa for the CDC and their intervention projects in other African

countries. Subsequently with governmental and non-govermental organisation (NGO) sponsorships, it could be available to rural communities in South Africa for less than R10 a container which is an affordable price for the low socio-econnomic communities in desperate need for point-of-use treatment. In addition, the 3.5% sodium hypochlorite solution is already available in all supermarkets in South Africa and most of the rural population have the knowledge on how to use it because of informative pamphlets distributed by the DOH and DWAF during environmental disasters and waterborne disease outbreaks (Appendix B). The combination of an affordable container and sodium hypochlorite solution could improve point-of-use water quality in rural communities in South Africa where problems such as inadequate water and sanitation infrastructures are present.