

# Bacterial Contamination and Nutrient Concentration of Infant Milk in South Africa:

A Sub-study of the National PMTCT Cohort Study





UPPSALA  
UNIVERSITET

# **Bacterial Contamination and Nutrient Concentration of Infant Milk in South Africa:**

**A Sub-study of the National PMTCT Cohort Study**

Erika Bergström

June 2003

This research was conducted in fulfillment of the requirements of the masters degree in international health, department of women's and children's health, Uppsala University, Sweden.

This study was made possible with funding from SIDA/SAREC, NRF, HST and UNICEF.

The information contained in the publication may be freely distributed and reproduced, as long as the source is acknowledged, and it is used for non-commercial purposes.

# Contents

<b><i>List of Figures</i></b> _____	<b>5</b>
<b><i>List of Tables</i></b> _____	<b>6</b>
<b><i>Acknowledgments</i></b> _____	<b>7</b>
<b><i>Abstract</i></b> _____	<b>8</b>
<b><i>Introduction</i></b> _____	<b>10</b>
Background to the study _____	10
Global health and nutrition among infants and young children _____	10
Breastfeeding & its impact on infants and children’s health _____	11
Replacement feeding _____	12
Sanitary quality of food _____	15
The safety of foods from primary production to final consumption _____	15
HIV and Infant feeding implications _____	16
Study area _____	18
<b><i>Aim of the study</i></b> _____	<b>19</b>
Objectives _____	19
<b><i>Material and Methods</i></b> _____	<b>20</b>
Study population _____	20
Data collection and sampling _____	20
Sample size _____	21
Analysis of milk feeds: Bacterial contamination _____	21
Analysis of milk feeds: Protein concentration _____	22
Data entry & Statistical analysis _____	22
<b><i>Results</i></b> _____	<b>23</b>
General characteristics of the study population _____	23
Infant feeding counselling _____	25
Infant feeding factors and behaviours _____	26
Microbiological results _____	32
Factors associated to contamination of the milk feeds _____	33
Protein results _____	34
Factors associated to over-dilution of the feeds _____	35
Home visits with observations and sample collection _____	36
<b><i>Discussion</i></b> _____	<b>40</b>
Methodological issues _____	40
Heavily contaminated milk feeds _____	41
Over-dilution of milk feeds _____	43
Socioeconomic factors _____	43

Conclusion	44
Suggestions for further studies	44
<b>References</b>	<b>45</b>

## List of Figures

Figure 1.	Distribution of protein results according to age of infant	34
Figure 2.	Protein concentration of available samples obtained during home visits, by age of child.	38
Figure 3.	Protein concentration of demonstration samples prepared during home visits, by age of child	38

## List of Tables

Table 1.	Characteristics of the infants	22
Table 2.	Characteristics of the mothers	23
Table 3.	Living conditions in the households	24
Table 4.	Hand hygiene, infant feeding utensils and water used for feeding	26
Table 5.	Preparations of milk feeds	27
Table 6.	Storage of prepared milk feeds and handling of left over milk	27
Table 7.	Time taken for infant to finish a prepared milk feed after it is poured into the feeding utensil	28
Table 8.	Cleaning of infant feeding utensil	29
Table 9.	Type of commercial milk feeds obtained at the PMTCT-clinic	30
Table 10.	Milk samples collected at the PMTCT-clinic	31
Table 11.	Counts of E-coli and Enterococci in sample milk feeds	31
Table 12.	Protein results compared to recommended levels of protein	33
Table 13.	Available already prepared milk samples collected during home visits	35
Table 14.	Contamination degree of E-coli in milk samples received during home visits	37
Table 15.	Contamination degree of Enterococci in milk samples received during home visits	37

## Acknowledgments

This study was made possible by the SAREC-financed research collaboration between Uppsala University (UU) Sweden and the University of the Western Cape (UWC) South Africa. Additional funding was provided by the Health Systems Trust and UNICEF(South Africa). The success of this research is due to the co-operation and interest of many unique people. My warmest gratitude and thanks go to:

Advisor Dr Ted Greiner, Section for International Maternal and Child Health (IMCH), UU, Sweden.

Co-advisor Nigel Rollins, Department of Paediatrics and Child Health, Nelson Mandela School of Medicine, University of Natal, Durban.

Professor Wim Sturm at Department of Medical Microbiology, University of Natal Durban (UND).

Dr Nceba Gqaleni and Chief Technician Arthur Pietersen at the Division of Human Biochemistry, UND.

Dr Mickey Chopra and Dr Debra Jackson of the School of Public Health, University of the Western Cape for supervision and support.

Ms Tanya Doherty (Health Systems Trust) and Dr Mark Colvin (Medical Research Council) for assisting with planning for field work.

King Edward VIII Hospital Durban through Hospital Manager Dr Z. N Karva and Co-ordinator for Research Programs Mr A.J Seekola, Provincial co-ordinator for the PMTCT-program. Professor R.W Green-Thompson and local Project Manager for the PMTCT-program Dr D. Moodley, KwaZulu-Natal.

Lulama Mdutayana, Infant feeding counsellor at the PMTCT-clinic at King Edward VIII Hospital.

Nozipo Conana at the Department of Medical Microbiology UND and Sue Von Maten at the Agricultural Faculty Department of Animal and Poultry, University of Natal Pietermaritzburg, Kindra Gurpreet, Sarena Zolwa, Logan Pylay and Hlengiwe Mbongwa.

Kristine Ekelund, IMCH and Mosotho Gabriel.

Dr Juana Willumsen.

Recepta AB.

# Abstract

## Background

South Africa provides HIV+ mothers with free commercial infant milk for six months of the infant's life in 18 pilot sites for the prevention of mother-to-child-transmission (PMTCT). Many mothers in the PMTCT-programme choose to accept this formula, but few studies of actual feeding patterns have been conducted. Studies in some PMTCT pilot sites have shown that mothers have difficulties in formula feeding their children. The risks of replacement feeding are well known in general, but very few detailed studies of the issues involved have been in any African setting.

## Aim and Objectives

The aim of this study was to assess how mothers in an urban/peri-urban PMTCT area of South Africa prepare and feed commercial infant milk to their infants and to assess the safety of these feeds. The objectives were to describe the methods of preparation of commercial infant milk and to measure bacterial contamination and protein concentration in these feeds.

## Material and Methods

This cross sectional study was carried out in Nov-Dec 2002. Data collection included structured interviews and collection of commercial infant milk samples among 94 mothers at a PMTCT-clinic. A sub-sample of 22 of these mothers was also visited in their homes. Samples were taken from 21 demonstration feeds made by mothers while the researcher observed. Questions included general characteristics of infant/mother, socio-economic status, ways of feeding, utensils used, methods of cleaning, preparing, storing, and any foods and fluids added in addition to the milk. Feeds were analysed for bacteria indicative of faecal pollution, and for protein concentration as an indicator for the extent of dilution of the feeds. Data were analysed using SPSS. Associations between contamination, concentration and procedures that could contribute to microbiological contamination and incorrect concentrations were explored by using odds ratios and Fisher's Exact Test.

## Results

This was a relatively well-off sample for African conditions. The majority of the participants had 12 or more years of education and 72% possessed refrigerators. Most mothers made mistakes in the cleaning process despite seemingly good quality counselling in formula feeding. *Escherichia coli* were isolated from 64% of the milk feeds and *Enterococci* from 26%; 67% had at least one of these. No contamination of *Shigella* or *Salmonella* was found. A significantly lower risk of contamination was seen among: mothers who boiled water to use directly before preparation instead of boiling and storing it before use; mothers who used feeding cups/containers instead of bottles; ownership of several bottles instead of a few; preparation of several milk feeds per night compared with just one or two; mothers who received free formula milk from the clinic. Sterilising methods like boiling utensils, and use of sterilising solution or bleach water protected against contamination. *E-coli* were isolated from 63% of the available feeds at home and from 33% of the demonstration feeds, *Enterococci* from 56% and 14% respectively.

Over-dilution was found in a total of 28% of the milk samples collected at the clinic and 47% of the samples collected in homes. That only 14% of demonstration feeds were over-diluted suggests that the majority nevertheless knew how to prepare milk feeds correctly. The risk for over-diluted feeds was greater for: older infants; larger feeds compared with smaller amounts prepared at one time; and when there was no access to running water inside the house.

**Conclusion**

Substantial levels of contamination and over-dilution of commercial milk feeds were found in mothers who were relatively well-educated and advantaged for the African setting. This study provides several examples where health worker messages to mothers could be improved to reduce these risks. However, many of the problems identified were not due to lack of knowledge on the part of the mothers and thus may be difficult to avoid.

## **Introduction**

### **Background to the study**

The risks with breast milk substitutes/commercial infant milk feeds is well known through different published reports showing the many difficulties with safe practice of replacement feeding and potential risks of health hazards for the baby, especially in developing countries.

South Africa provides HIV+ mothers with free commercial infant formula/milk for the first 6 months of life as a part of a pilot programme for the prevention of mother-to-child-transmission (PMTCT) in 18 centres in the country. Many mothers in the PMTCT-program choose to accept this, but few studies of actual feeding patterns have been conducted. Studies in some PMTCT pilot sites have shown that mothers have some difficulties in formula feeding the children (Chopra M et al. 2000), and further research on this has been requested by the South Africa National HIV/AIDS programme.

This study is a sub-study of the larger “Good Start Infant Feeding Study”, a National Prevention of Mother-to-Child-Transmission Cohort in South Africa conducted by University of Western Cape, Health System Trust and Medical Research Council about infant feeding behaviours and factors influencing on mothers choices during the first year of life.

This study has focused on how mothers within the PMTCT-program in South Africa formula feed their children with the data collection conducted during November and December 2002. The study took place in Durban, South Africa, with interviews conducted at a PMTCT-clinic.

### **Global health and nutrition among infants and young children**

Each year more than 10 million infants and young children in developing countries die before reaching age five (WHO/WHA April 2002). Of these 10 million, 70 % of the children die of infections like acute respiratory infections, diarrhoea, measles and malaria or from malnutrition (WHO/UNICEF 2001). Often the infectious disease is associated with malnutrition (WHO/UNICEF 2001; Motarjemi et al. 1993).

Of 10.9 million deaths annually among children under five years of age, 60% of the deaths are directly or indirectly due to malnutrition, (WHO/UNICEF 2001).

Among infants and young children in developing countries inappropriate feeding practices are major contributing factors to the high morbidity and mortality followed by unsustainable socioeconomic development and ongoing poverty (WHO/WHA April 2002).

Each year 55% of infant deaths caused by diarrhoeal diseases and acute respiratory infections may be related to inappropriate feeding practices (WHO/WHA May 2002).

## **Breastfeeding & its impact on infants and children's health**

Breastfeeding is a major contributor to child health by protecting the infant and child against infections and death. Providing the mother is given the right information and support from the family, community and from the health care system, exclusive breastfeeding from birth is possible for most mothers except in a few medical conditions (WHO/WHA May 2002).

### **Protective factors & flora**

The protective factors in breast milk vary depending on the pathogens the infant is exposed to and on the chronological age and maturity of the infant (Lewis-Jones et al 1985).

In a breastfed infant, *Lactobacillus bifidus* is the normal flora of the intestinal tract while the number of other flora like gram-negative bacteria is small (Yoshioka, Iseki, Fujita 1983). A higher growth of *Lactobacillus bifidus* keeps the growth of other micro-organisms low (Lawrence 1994).

Pathogenic micro-organisms in the flora do exist even in small numbers. It has been shown that while the gut flora consist mainly of bifid bacteria, other micro-organisms like streptococci, bacterioides, clostridia, micrococci, enterococci and E-coli are also found in the infant's gut. Larger amounts of solid food added the diet of older infants also showed increased growth of the E-coli in the flora (Yoshioka, Iseki, Fujita 1983).

### **Less infectious diseases & death**

Differences in infant mortality rate between developing and industrialized countries are related to higher rates of infectious diseases in the developing countries. Infant mortality reflects the differences in sanitation, nutrition, housing and socio-economic status (Janine M et al. 1984).

In a pooled analysis of data from Brazil, Pakistan and the Philippines done by WHO's Collaborative Study Team, the effect of not breastfeeding on the risk of death due to infectious diseases was assessed. The results showed that protection provided by breast milk steadily declined with age. (WHO 2000).

In a population-based case-control study of infant mortality in Brazil, results showed (Victoria et al. 1987) that completely weaned (no breast milk) infants had 14.2 times a greater risk of death from diarrhoeal diseases than infants not receiving any supplementation with replacement milk.

A Malaysian study found a relative risk of 5 deaths after 1 week of age when not breast fed compared with breast fed infants in households without piped water or a toilet (Habicht, DaVanzo, Butz 1988).

In a longitudinal study of feeding practices and morbidity from infectious diseases among infants in Peru, the incidence and prevalence rate of diarrhoea among infants aged less than 6 months given nothing else than breast milk, were less than among infants given replacement milk and other fluids in addition to breast milk. (Brown et al. 1989).

Another longitudinal study in the Philippines examined the relationship between breastfeeding and diarrhoeal morbidity and age-specific effects of infant feeding patterns. Supplements of water, teas and other fluids not containing nutrients in addition to breast milk increased the risk of diarrhoea two or three times compared to exclusive breastfeeding. (Popkin et al. 1990).

### **Nutrients and energy**

Due to its unique composition including nutrients, non-nutrient growth factors, immune factors, hormones and other bioactive components, breast milk is well known to be the ideal food in fulfilling the needs of the infants during the first six months of life (Breastfeeding 2001).

It can provide the total needs for energy and nutrients for the first six months life, and often continues to provide half of all requirements during the following six months and one-third during the child's second year (WHO May 2001).

### **Replacement feeding**

Only under exceptional circumstances and in a few medical conditions may breastfeeding not be the recommended feeding method. When that is the case, a replacement feed with a suitable breast-milk substitute is recommended. The replacement feed can be the mother's own breast milk expressed before giving to the child, breast milk from a healthy wet-nurse, breast milk from a human milk bank, or a breast milk substitute, i.e. commercial milk feeds.

To prevent health hazards due to inappropriate preparation of replacement feeding, adequate instructions for appropriate preparation must be provided by health workers with close follow up of the infants.(WHO April 2002)

### **Contaminated infant formula, a result of lack of hygiene and sanitation**

A review of research concerning risk factors for diarrhoea, found that food contamination is a major contributor among infants and young children. Together with breastfeeding, safe water supplies and sanitation, food safety must also be given high priority to prevent diarrhoeal diseases (Motarjemi 1993).

Introduction of complementary foods and the use of artificial baby milk have been associated with a high degree of bacterial contamination. Isolates from most of the major bacterial pathogens have been reported, especially from milk. A predominant cause of diarrhoea in developing countries is *Escherichia coli* (E-coli) (Morais, Gomes, Sigulem 1997).

A community-based study carried out in an urban slum in India examined the bacterial contamination of supplementary milk feeds to infants and children up to 2 years of age. Results showed 53.7% contamination of milk feeds directly taken from the feeding utensils. Sixteen per cent of the bacteria analysed were enteropathogenic; *E.coli* presented 13.4% of the pathogens, *Klebsiella* 5.4%, *Enterobacter* 5.4%, *Pseudomonas aeruginosa* 4.7% and *Shigella* spp. 2.7%. Higher degree of contamination could significantly be associated with

lower income, lower caste, illiteracy among the mothers, unclean utensils, and lack of hand hygiene in the mother (Ray, Nath, Reddy 2000).

In Nigeria mothers working in an urban market with children up to 2 years of age were randomly asked for samples of food and water intended for the children. The samples were collected at home or at the market. At home the child was taken care of by older siblings or house-helpers. The samples were analysed for coliform content. Storage time was significantly associated with a higher degree of bacterial contamination and the amount of bacteria was higher in foods for the children left at home. Reheated food compared with freshly-cooked food was also more contaminated. Poorer hygiene was significantly associated with handling of the food for children left at home by someone else than the mother (Iroegbu et al. 2000).

In a longitudinal study of lactation and infant growth in rural Thailand, bacterial contamination of complementary food was examined. Significantly related to increased bacterial contamination were bottle feeding and pre-mastication/mashing of the food. Lower degree of contamination was related to using boiled water to prepare the foods or boiling the foods to make soups. Preparing the food in and serving the food from a banana leaf reduced the degree of contamination. Prolonged storage time was associated with increased contamination (Imong et al. 1995).

In a study carried out in an outpatient clinic for infants in urban Ethiopia, samples from feeding bottles prepared for infants were collected. Among other bacteria analysed, Salmonella could be identified in three of the total 100 samples. The bottles contained cow's milk and gruel from a cereal blend. Salmonella, normally killed at cooking temperature, are discussed as post-cooking contamination, an important factor that influences the hygienic quality of the complementary food (Erku, Ashenafi 1998).

### **Sterilisation – the recommended method**

Using a sterile method during preparation of individual feeds and cleaning of utensils has been generally accepted since the beginning of the first half of 1900. In the 1950s, the possibility of using a clean method instead of a sterile method was raised and questioned. The Committee of Fetus and Newborn of the American Academy of Pediatrics continues to recommend a sterilizing method for formula feeding. The "aseptic/clean method" may be acceptable in certain situations but increases risks of health problems due to other surrounding contaminating sources (The Committee on Fetus and Newborn 1961; Gerber et al. 1983).

In a US survey of mothers of relatively low socioeconomic status, neither terminal sterilisation nor the aseptic method was followed by the majority. Enteric organisms were found in half the feeding bottles and more than half the formula stored in bulk. Only in homes rated as "excellent in housekeeping" was there no bacterial growth. More education of mothers was recommended. (Kendall, Vaughan, Kusakeioglou 1971).

The introduction of chemical disinfection by using a solution of sodium hypochlorite (the Milton method) for bottles and teats among mothers in Sweden, showed a significant decrease in bacterial contamination compared to other methods like rinsing the feeding utensils with hot water or boiling the utensils. The recommendation of rubbing the teats with salt before sterilising was followed by most, 74%, of the mothers regularly and by 11% occasionally. Fifteen percent of the mothers did not rub the teats at all. (Söderhjelm 1972).

## **Risk and health hazards with incorrect dilutions of replacement feeds**

Incorrect dilution of commercial infant formula milk is commonly followed by serious health risks and in some cases death of the infant. Research from the seventies up to today confirms the difficulties with safe replacement feeding. In a hospital in England, among infants with dehydration, those with hypernatremia had been fed with milk feeds of greater sodium concentration and osmolality than the infants with normal sodium level in the blood. Most feeds were too concentrated (Chambers, Steel 1975).

A prospective study in Jordan measured the effect of feeding practices on children up to 1.5 years of age. The children were admitted to the hospital for hypernatraemic dehydration (serum sodium level  $\geq 150$  mmol/L) caused by gastroenteritis. Thirty-six per cent of the children with hypernatraemic dehydration had been given powder milk compared with 15% of children in the control group. In the hypernatraemic group, 7.5% of the children were breast fed compared with 60% of the infants in the control group. Six children with hypernatraemia suffered from convulsions and two died (Abu-Ekteish, Zahraa 2002).

A similar case study from the USA describes the health complications in a seven week old girl fed with undiluted evaporated milk for 5 days by the grandmother who believed the baby “needed richer milk”. The infant suffered from severe hypertonic dehydration with hyperglycaemia, intravascular coagulation, renal failure, gangrene of the legs and coma (Cyril et al 1975).

## **Difficulties in correctly mixing infant formula milk**

The reasons for improperly mixed infant formula are many and complex. As the above case study from USA describes, the grandmother aimed to give “richer milk” by not diluting the liquid formula, resulting in traumatic health problems for the baby (Cyril 1975).

A similar case study also from USA describe inadequate “directions for use” of the formula powder as the probable cause of hyperosmolar formula fed to a newborn followed by hypovolemic shock, intestinal ischemia with full-thickness gangrene of the colon (Wilcox, Fiorello, Glick 1993).

A study performed in England, report large inaccuracies in measuring formula powder among mothers. Mothers attending an infant welfare clinic were asked to measure milk powder from a standard packet. The correct scoop was provided and also a knife for levelling if they normally used that. They were asked to scoop in the same way as they did at home, twice in the normal way and once as if they were in a hurry. Results showed a wide range in the weight of the powder, the heaviest scoop (5.6g) being double the weight of the lowest scoop weight (2.8g). All mothers reported following the instructions from the manufacturer. Some mothers reported adding an extra scoop to “satisfy” the baby. In general the scoop was heaped if they didn’t use a knife to level it off (Jeffs 1989).

A study conducted at a Baby Health Clinic in Australia reported that 30 % of milk samples collected from infant bottles were not correctly prepared when compared with the manufacturer’s instructions. In 19% of cases, potentially serious errors had been made. (Lilburne et al. 1988).

A case report from USA describes the complications resulting when a young mother was sent home after delivery with a “starter pack” of concentrated liquid formula. Instruction in how to prepare this formula was given and when this starter pack was consumed she changed over to a powdered formula of same brand. The mother mixed this powder in the same way with equal parts of water as for the liquid formula. She used the same diluting can from the starter pack resulting in four times the normal concentration. These feeds were given for approximately two weeks until the baby’s death at three weeks of age (Coodin, Gabrielson 1971).

In urban and rural areas in Indonesia, mothers attending mother-child-clinics/centres were asked to provide milk samples from feeding bottles prepared for their infants. The nutritional content of the feeds were examined, here the protein content, to determine the prevalence of over or under diluted milk. Of total 52 samples, one third were less than 50% of proper strength and half the samples were within +/- 20% of the manufacturer’s recommended protein concentration. Overall one in four feeds had a proper concentration. The mothers had adequate level of education and better than the average economic situation. Written instructions on the formula tins were available (Surjono et al. 1980).

## **Sanitary quality of food**

The use of indicator organisms, coliforms and enterococci, are widely accepted to indicate contamination of food (Jay 1986).

### **E-coli as an indicator of food sanitation**

The coliform bacteria include *Esherichia coli* (E-coli), *Enterobacter aerogenes*, *Citrobacter* and *Klebsiella*. E-coli, a faecal indicator, can be found in the intestinal tract of humans and animals, while *E. aerogenes* is associated with non faecal materials. Approximately 90% of faecal coliforms consist of E-coli (Jay 1986).

### **Enterococci as an indicator of food sanitation**

The enterococci are members of the genus *Streptococcus* of which *S. faecalis* and *S. faecium* constitute the enterococcus group. Enterococci have been employed as indicators of faecal pollution when found in food. However, some of the strains can be found on plants without any sanitary significance when later found in foods (Jay 1986).

## **The safety of foods from primary production to final consumption**

The Hazard Analysis Critical Control Points system (HACCP) (<http://www.fao.org/DOCREP/005/Y1579E/y1579e04.htm>) is a preventive strategy used to identify microbiological hazards associated with food preparation and handling at different stages and to assess the risks. The system also identifies critical control points in the food chain where controls can be applied to prevent, eliminate or reduce hazards (Ehiri et al. 2001).

## **Standards of pathogenic micro-organisms in infant formula and raw breast milk**

According to the United Nations Food and Agriculture Organization (FAO)'s standards of infant formula and follow-up formula, the products should not contain any pathogenic micro-organisms or any substances originating from micro-organisms in amounts that constitute a health hazard for the baby (Codex standard for infant formula 1997).

Similarly, no pathogenic micro-organisms should be present in raw human milk fed to infants. The recommendation from the US Food and Drug Administration and the Centers for Disease Control is to heat treat donor milk before use (Arnold, Tully 1992).

Carrol et al. (1979) recommend microbiological screening of all raw donated milk before use in neonatal units. No general accepted microbiological criteria are set concerning the degree of bacterial contamination considered to be safe when feeding it raw, except that no potential pathogens (like *Staphylococcus aureus*, enterobacteria and group-B streptococci) should be present.

## **Standards of protein content in infant formula**

To afford a satisfactory margin of safety based on theoretical calculations and epidemiological data regarding the potential renal solute load of infant formulas, the recommendation was to decrease the upper limit for protein content, down to 3.2 g per 100 kcal. (Ziegler, Fomon 1989).

According the Codex Alimentarius Commission's Standards for infant formula (for newborn children up to 12 months of age) the protein content in infant formula should not be less than 1.8 gram (g) per 100 available calories (=kilocalorie/kcal) and not more than 4 g per 100 kcal. For follow-up formula (for infants from the 6 months on and for young children 1-3 years of age), the protein content should not be less than 3.3 g per 100 kcal and not more than 5.5 g per 100 kcal (Codex standard for infant formula 1997).

## **HIV and Infant feeding implications**

The HIV virus passes into breast milk and may infect the breastfed child. For mothers facing both HIV and poverty the choice of infant feeding method is a difficult dilemma. The risk of illness, malnutrition and death due to unsafe replacement feeding might in some cases be higher than the risk of HIV transmission to the child through breastfeeding.

## **Recommendations by WHO**

WHO recommends in their "Global Strategy for Infant and Young Child Feeding" breastfeeding the infant exclusively (nothing other than breast milk) for the first six months of life (WHO 2001), and continuing to breastfeed while introducing nutritionally safe and adequate amounts of complementary food to meet nutritional requirements up to the age of two years or beyond (WHO 2002).

Mothers infected with HIV should be counselled on feeding options and if the chosen replacement feeding fulfils the criteria of being safe, acceptable, feasible, affordable and sustainable, the mothers should be advised to avoid breastfeeding their infants. Under other circumstances, WHO's recommendation is to breastfeed exclusively for the first months followed by cessation as soon as this is feasible (WHO 2000).

## Study area

South Africa is divided into nine provinces with a total population of approximately 41 million people; 77% Africans, 11% white, 9% coloured (mulatto), and 2-3% Indian (Statistics South Africa 1996).

In 1999, the under-5 mortality rate for South Africa was 69 per 1000 live births, down from 130 in 1960, and the infant mortality rate was 54 per 1000 live births. Approximately 10% of the children 0-3 months old in 1995-2000 were exclusively breastfed the day before their mother was interviewed. Nine per cent of under-fives were moderately or severely underweight and 23% suffered from stunting. In 1999, 92% of the urban population used improved drinking water sources and 99% had adequate sanitary facilities, for the rural population these figures were 80% and 73% respectively. During 1995-1999, the literacy rates among males and females over 15 years of age were 67% and 66% respectively. Life expectancy in 1999 was 52 years (Unicef 2001).

Facing an HIV/AIDS epidemic, the government of South Africa decided in 2000 to start a pilot programme for the prevention of mother-to-child transmission (PMTCT) of HIV. As part of the programme, the country provides HIV+ mothers with free commercial infant formula/milk for the first six months of life. A change in the programme has been made and now mothers who chose to start with breastfeeding are provided with free formula during the six months after breastfeeding stops.

Eighteen pilot sites were set up in health facilities in the nine provinces. These sites have specially educated infant feeding counsellors who give pre- and post- delivery counselling to the mothers about HIV testing and feeding options for the infants based on WHO's guidelines (WHO/UNICEF 1993). Positive and negative factors related to breastfeeding and replacement feeding are discussed with the mothers. The aim is that the mother should take an informed choice in how she wants to feed her child. When the mother knows she is HIV positive and has taken her decision, education is given concerning how to make the feeding option safer for her baby. A mother who does not know her HIV status or has tested negative is recommended to breastfeed. Counsellors follow up on HIV positive mothers after delivery at the PMTCT clinics.

This study was performed in Durban, located on the east coast, KwaZulu-Natal province (KZN), South Africa. KZN has a population of approximately 8.5 million, mainly African people (Statistics South Africa 1996). The climate in the province is tropical.

## **Aim of the study**

The aim of this study was to assess how mothers in an urban/peri-urban PMTCT area of KZN, South Africa prepare and feed commercial infant milk to their infants and to assess the safety of these feeds.

## **Objectives**

- ? To describe the method of preparation of commercial infant milk, used by selected mothers in one area included in the South Africa National PMTCT programme.
- ? To measure bacterial contamination in commercial infant milk feeds prepared by these mothers.
- ? To measure the protein concentration of these feeds (as an indicator of extent of dilution of the milk powder).

## **Material and Methods**

### **Study population**

Mothers who attended the PMTCT clinic and exclusively or partly fed their infants with formula at the time of the interview, were included in the study. These mothers were attending the clinic on set dates for follow up consultation by the doctor or to obtain free supplies of infant formula.

Written and verbal information about the study were given out to the participants and signed informed consent forms were obtained.

### **Data collection and sampling**

The questionnaires were pre-tested during a field visit in 2002 on eight mothers attending a baby clinic in a rural area of KZN. Later the same year, a revised questionnaire was pre-tested on 22 mothers attending the chosen PMTCT clinic just before the start of the data collection. Samples of bottle contents were also obtained each time and handling and analytical methods were refined.

The data were collected by using structured interviews. The interview consisted of questions about the general characteristics of the infant and mother, socio-economic and educational conditions, different ways of feeding the infant commercial infant milk, feeding utensils used and their methods of cleaning, preparation and storage of milk feeds, and foods and fluids added in addition to milk. Each interview took approximately 10-15 minutes.

At the time of the interview, samples were taken from:

- ? Any available infant formula milk already prepared and ready for the child to drink or
- ? Milk that was being consumed by the child, or had been drunk from within the past half hour

The samples were analysed for bacterial contamination at the Department of Medical Microbiology, University of Natal Durban, and for nutrient concentration at the Agricultural Faculty, Department of Animal and Poultry, University of Natal Pietermaritzburg.

Each questionnaire and sample was identified only with an ID-number. No personal details of the woman were recorded as so to ensure confidentiality.

## Sample size

It was estimated that the prevalence of contaminated infant milk feeds among mothers with low socio-economic status was going to be around 80%. To show a difference in prevalence of 30% between mothers with higher socio-economic status at 95% significance level and with 80% power, the study population required a total sample size of 90 mother-and-infant pairs. It was expected that this sample size also would allow for analysis of differences in the concentration of the milk feeds.

## Sub-sample

In addition, a sub-sample of mothers at the clinic was asked for permission to visit them in their homes in order to take samples of already made up feeds and to ask them to make demonstration feeds. The mothers were aware that questions about formula milk would be asked but not that samples would be collected during the home visit. The mothers were provided with infant formula powder for the demonstration feeds of the same kind they received for free at the clinic.

The sub-samples functioned as a control group in order to evaluate if any differences of bacterial contamination and nutrient concentration could be seen between samples taken during clinic attendance and samples taken during home visits.

## Analysis of milk feeds: Bacterial contamination

### The following analyses were conducted:

- ? Colony count of E-coli
- ? Colony count of Enterococcus
- ? Colony count of Shigella
- ? Count of Salmonella

Bacteria	Agars used for plating	Amount of milk plated on respectively Agar plate
E-coli	MacConkey Agar	1, 10 and 100 micro litre
Enterococcus	Hoyle's Agar	1, 10 and 100 micro litre
Shigella	XLD	100 micro litre
Salmonella	XLD	100 micro litre

### Milk samples taken during clinic attendance

Samples of available milk feeds taken at the clinic were directly put on ice in sterile containers in a cool box maintained at 4-8° C and transported to the laboratory within 2 hours. At the lab the samples were stored in a refrigerator at 4-5° C to be handled the same day. The milk samples were plated out on Agar plates by using Finn pipette Digital instruments with sterile pipettes. One, 10 and 100 micro litre of milk were plated out according to the above table. After culturing, the Agar most convenient to read the colony-forming units (CFU) from for respectively bacteria was used. The Agars were cultured at 37° C for +/- 24 hours in an Oxygen incubator before analysis.

## **Milk samples from home visits**

The sub-samples taken during home visits from available milk feeds were divided into two aliquots:

1. Plated directly on specific Agars (named above) on site by using sterile plastic pipettes plating 10 micro litre of milk on each Agar. These were directly put on ice in a cool-box and transported to the lab as soon as possible to be cultured according the same procedure as described for the samples received at the clinic.
2. Transported as soon as possible to the lab in sterile containers not put on ice, to be plated, cultured and analysed the same day according the procedure as described for the samples received at the clinic.

Comparing these two results helps to identify if bacterial growth increased during storage/transportation of the milk from the mothers' home to the clinic/lab.

## **Analysis of milk feeds: Protein concentration**

Samples of available milk feeds taken both at clinic and during home visits were directly put on ice in clean containers in a cool box and transported to a freezer the same day to be kept frozen (-20° C) until analysis. The samples were analysed in a LECO FP2000 Nitrogen Analyser using the Dumas Combustion Method. Each sample was analysed with duplicates with the mean value as final result.

The results from the lab were given in percentages of protein (gram protein/100 millilitres prepared formula). According to the manufacturer's lable, the amount of kilocalories per 100 ml in the formula milk was 67. The following equation was used in this study to calculate the amount of protein/100 kcal in each sample:

$$X = (Y / 67) \times 100.$$

X is total gram protein/100 kcal. Y is grams of protein measured /100 ml of formula milk.

## **Data entry & Statistical analysis**

Data were entered and analysed using EPIINFO6 and SPSS (Statistical Program for Social Science). Associations between contamination, concentration and procedures that could contribute to microbiological contamination and incorrect concentrations were explored by using mainly Odd Ratios. Fisher's Exact Test was also used, based on chi-square tests for significances, when the number of mothers in some groups was less than five.

A group of variables that hypothetically measure socioeconomic status (SES) was chosen and correlated with each other by using Pearson correlation. The variables that were best correlated with each other were added together forming a summated SES index. The variables chosen were those having the higher  $r_{kk}$  coefficient, calculated according to the following equation (Nunnally J.C (1967) as cited by Greiner T (1977)):

$$r_{kk} = k \bar{r}_{ij} / 1 + (k-1) \bar{r}_{ij}$$

The letter k is the number of variables used and  $\bar{r}_{ij}$  the average correlation of the variables. The reliability coefficient  $r_{kk}$  can vary from -1 to 1. A larger value indicates a more reliable index. SES was composed of the variables: electricity + stove + fridge + toilet + TV + radio + water source + phone.

## Results

### General characteristics of the study population

A total of 94 mother and infant pairs were interviewed at the PMTCT-clinic, King Edward VIII Hospital in Durban. The mothers lived in central Durban, in surroundings areas and townships far away on farms.

People attending the free public hospitals in the area can in general not afford their own medical insurance or medical aid and have no employer who pays it for them. A common problem for many of the mothers living outside of Durban city, was to afford transportation costs for mini taxis to attend the clinic for the follow up consultations or to collect the formula tins.

The mean age of the mothers was 28 (16-40) years and the mean age of the infants was 5.7 (0.3-16) months. The mean birth weight was 2.9 (0.9-4.2) kg.

The general characteristics of the infants and the mothers are listed in Table 1 and 2, and the living conditions in the households in Table 3, below.

Most of the families had access to piped drinking water, flush toilet, electricity and fridge, radio, TV and stove. Piped water in the area was known to be clean and safe for drinking, with no treatment needed for adults. Around 38% of the mothers normally stored the water in a container inside the house. Most of them did not have access to running water inside the house.

**Table 1. Characteristics of the infants (N=94)**

		N	%
Gender	Boys	48	51
	Girls	45	48
	Missing data	1	
Age in months	<6	59	63
	6-12	26	28
	>12	9	9
Birth weight in grams	<2500	22	23
	2500-2999	21	22
	3000-3499	28	30
	3500-3999	21	22
	>4000	1	1
	Missing data	1	

**Table 2. Characteristics of the mothers (N=94)**

	N	%
Maternal age in years		
<25	24	26
25-29	38	40
30-34	20	21
>34	12	13
Marital status		
Married	8	8
Partner	59	63
Single	26	28
Missing data	1	
Highest level of education achieved/last standard passed		
Diploma/college/university	9	10
Completed high school*	41	44
Started but not completed high school**	36	38
Higher primary school or less***	6	6
No school	1	1
Missing data	1	
Main activity during the day		
At home	67	71
Unqualified job	19	20
Qualified job****	4	4
School	1	1
Mix of being at home and at work	2	2
Missing	1	
Main provider of income in the child's house		
Mother herself	21	22
Father of child/husband/partner	20	21
Mother's own parent	32	34
Mother's grandparent/child grant/relative or A mixed income sources	18	19
Missing data	3	

\* 12 years of public school

\*\* 8-11 years of public school

\*\*\* 1-7 years of public school (includes lower and higher primary school)

\*\*\*\* Higher education than high school needed

**Table 3. Living conditions in the households**

		N	%
Water source for drinking	Piped - inside house	62	66
	Piped - in the yard	16	17
	Piped - public tap	12	13
	Borehole/well	2	2
	River	2	2
Toilet	Flush toilet	66	70
	Latrine	28	30
Fuel for cooking	Electricity	70	75
	Paraffin	22	23
	Wood/open fire	2	2
Electric stove	Yes	74	79
	No	20	21
Fridge	Yes	68	72
	No	26	28
Radio	Yes	81	86
	No	13	14
TV	Yes	70	74
	No	24	26
Phone at home or cell phone	Yes	52	55
	No	42	45
Car	Yes	7	7
	No	87	93

## Infant feeding counselling

The infant feeding counsellor at the PMTCT-clinic was a key informant during the data collection. According to her, she gave the mothers counselling on feeding options first at the prenatal clinic and later postnatal at the PMTCT-clinic. She tried to see all mothers individually at the PMTCT-clinic to follow up feeding counselling given earlier, but also in groups to discuss different infant feeding topics. A major topic discussed during the counselling was, as specified in the guidelines, the importance of food hygiene:

a) always wash hands after toilet visits and changing nappies, and before food preparations and feeding the child, b) keep utensils and feeding areas clean, c) safe boiled water, and d) safe storage of food.

In order to guide the mothers in their choices of feeding method, the counsellor discussed the cost of teats, bottles, fuel and sterilising methods required in addition to the formula the mothers would receive for free. She also discussed the cost involved once they purchased their own formula after the free six months.

In relation to commercial formula feeding and its risks, the general recommendation given is to cup feed the infant and not bottle feed. Just enough formula milk should be prepared for the infant for each feed unless the mother has access to a fridge. The formula milk is not considered to be safe after one hour at room temperature. The milk can be stored for as long as one day if it is kept in a fridge. Left over milk after the child has finished drinking should not be stored to be given to the infant later, rather thrown away or given to an older sibling or used for cooking. Boiled water can be stored in a flask.

The recommended cleaning methods after each feed were first to scrub the bottle with a bottle-brush in hot water with soap, rinse with clean warm water and finally to use a sterilising method such as boiling the utensils for more than five minutes or using a sterilising solution or bleach water. Delaying the cleaning procedure leads to dried milk in the bottle, which is difficult to remove and increases the risk for bacterial growth. The teat should be rubbed with salt before the sterilising. Feeding cups can be washed in hot water with soap and rinsed well afterwards. No sterilising method is needed for feeding cups.

Recommendations on how to mix the formula milk were also included in the counselling. Formula milk should be prepared directly in the feeding utensil by adding the correct amount of milk powder to the water.

All counselling seemed to be given theoretically with no practical exercise in cleaning or mixing done together with the mothers. Although mothers often had to wait for many hours to see the doctor, no special area for formula preparation or cleaning of the utensils was provided in the waiting area at the PMTCT-clinic. There was an area for changing the nappies near the toilet. They could however ask the staff for boiled water to use in making feeds.

The feeding counsellor said she felt she had a good relationship with the mothers and that they were interested in the information they received from her. However, sometimes it was difficult to get their attention and interest during the group counselling sessions or they did not feel comfortable to openly ask question. She expressed a desire to have more time for individual counselling with the mothers.

## **Infant feeding factors and behaviours**

Data on how well mothers said they implemented the recommendations described above are presented in Tables 4-10.

It did not seem to be routine to wash hands with soap before preparing the milk feeds; 32% of the mothers stated that they did it now and then. Cup feeding was not common. Around 41% of the mothers owned feeding cups but 97% of the entire sample stated that they normally used infant feeding bottles. Most of the bottles brought to the clinic by the mothers seemed to be made of plastic, although it was not observed if they were made of hard or soft plastic. None of the mothers used glass bottles.

**Table 4. Hand hygiene, infant feeding utensils and water used for feeding. (N=94)**

		N	%	
Hand washing before preparing the feed	Always	45	48	
	Often	19	20	
	Sometimes	26	28	
	Seldom	1	1	
	Never	3	3	
Infant feeding utensil normally used	Infant feeding cup	3	3	
	Infant feeding bottle	91	97	
Infant feeding utensils in possession	Feeding bottles	1	7	
		2-3	46	
		3-4	26	
		5-8	24	
	Teats	1	3	
		2-3	44	
		4-5	31	
		6-12	14	
	Feeding cups	1	35	
		2	3	
		3	1	
	Normal cup	1	3	
	Other items	Yes	36	38
		No	58	62
Water source used for the milk feeds	Piped water - inside house	66	70	
	Piped water – yard	13	14	
	Piped water – public tap	11	12	
	Borehole/well	2	2	
	River	2	2	

All mothers boiled the water before preparing the milk feeds (Table 5). Either they boiled the water directly before each feed or boiled and stored it to use later.

Around 10% of the mothers prepared enough milk each time for more than one feed. The remaining milk was stored for later use. As shown in Table 6, during the day, 46% of the mothers normally prepared and gave the feeds directly when the child wanted to feed while the remaining 54% of mothers stored the milk feeds in different ways until the baby wanted to feed. At night, the number of prepared milk feeds not stored but given directly after preparation decreased to 37%. Mothers prepared up to five feeds at night. Most commonly the mothers prepared two feeds and also fed the baby two times at night.

The majority of mothers kept left-over feeds to use later (Table 6). In addition, many mothers leave the bottle with the baby for such long periods of time that bacterial growth is inevitable (Table 7).

**Table 5. Preparation of milk feeds (N=94)**

		N	%
Person normally preparing the milk feed	Mother herself	82	87
	Her own mother	6	6
	Friend/nanny	5	5
	Father of child	1	1
Preparation of water for the feeds	Boil the water before use	94	100
	-before each feed	39	41
	-boil and store hot in flask	45	48
	-boil and store covered	9	10
	-missing data	1	
Amt formula normally prepared at one time	75-100 ml	12	13
	125-150 ml	29	31
	175-200 ml	16	17
	250-375 ml	34	36
	500-1000 ml	3	3
Amt formula normally given at each feed	75-100 ml	14	15
	125-150 ml	32	34
	175-200 ml	17	18
	250 ml	31	33
Preparation frequency for night feeds	Prepare all at once before night	49	52
	Prepare when time for feeding	44	47
	Do not feed at night	1	1

**Table 6. Storage of prepared milk feeds and handling of left over feeds (N=94)**

		N	%
Storage of day feeds	Do not store, give directly	43	46
	Covered, at ambient temperature	36	38
	Fridge	9	10
	Bottle warmer	4	4
	Flask	2	2
Storage of night feeds	Do not store, give directly	35	37
	Covered, at ambient temperature	45	48
	Fridge	4	4
	Bottle warmer	7	7
	Flask	2	2
Handling of left over feeds	Store the feed to use later	57	61
	Throw away	32	34
	Always finish the feed	5	5

**Table 7. Time taken for the infant to finish a prepared milk feed after it is poured into the feeding utensil (N=94)**

		N	%
Time taken to finish the milk feed	<1 hour	49	52
	1-2 hours	25	26
	3-4 hours	12	13
	Half a day	5	5
	All day	4	4

Many different cleaning methods for the feeding utensils were described by the mothers (Table 8). Most of them, 89%, used the described cleaning method after/before each feed before using the same utensil again for a new feed.

The first step, scrubbing the utensils in water with soap using a bottle-brush, was followed by most of the mothers. Hot water, as recommended, was used by only 34% and cold or warm water was used by 89% of the mothers. 12% of mothers mentioned that they did not boil the bottles, just placed them into boiled water and washed them.

The recommendation to use a sterilising method was followed by 76%. Some mothers used more than one of the recommended steps. Not always all of them ended the cleaning procedure with a sterilising step, 13% first sterilised the bottles by boiling them or putting them in sterilising solution or bleach water, and then washed/rinsed them with water and soap.

**Table 8. Cleaning of infant feeding utensils**

		N	%
Person normally cleaning the feeding utensils	Mother herself	86	92
	Her own mother	6	6
	Father of child/Nanny	2	2
Cleaning times	Before/after each feed	84	89
	A few times per day	7	7
	Once a day	2	2
	Missing data	1	
Cleaning equipment	Bottle-brush	91	97
	Cloth	5	5
	Soap/detergent	68	72
	Salt	8	9
	Nothing available	1	1
Use of a recommended sterilising method	Yes	71	76
	No	23	24
Type of sterilising method(s) normally used	Boiling utensils	35	37
	- >5 minutes	26	74
	- <5 minutes	1	3
	- boiling time unknown	8	23
	- together with soap, salt and/or bleaching water	8	23
	Sterilising solution	24	26
	Bleach water	18	19

### Other cleaning methods also used

A question was asked if they used the same cleaning method for the teat as for the bottle. The majority, 78%, said they did so. Other mothers reported that they:

***“use salt to rub the teat with”, “use a teat brush instead of the bottle brush”, “boil the teat but not the bottle” or “sterilize the bottle but not the teat”.***

To find out if they sometimes used any other methods, they were asked “What happens if there is dried milk in the bottom of the bottle (or cup if that was used)”? Other methods reported by 17% of the mothers included:

***“use bleach water and soak the bottle for a while”, “use a different brush”, “use detergent and brush”, “use hot water with soap and shake”, “use hot water and shake”, “use hot water and bottle brush”, “soak with hot / boiled water”, “use hot water with salt and shake / soak / rinse / wash”, “use salt and brush”, “use a cloth and a brush” or “use some sort of sterilising method”.***

At night, 74% of the mothers used another previously cleaned bottle if they had to make a new feed. . Among the mothers that used the same feeding utensil, 7% did not clean it before they reused it. Of the 18% that did clean the utensils at night, all except one mother mentioned other cleaning methods than the ones they had used during the day. Reported methods from the interviews included:

*“use hot water, soap and shake”, “use hot water, soap and brush”, “rinse in cold water”, “hot water and shake”, “rinse in dish water”, “cold water and brush”, rinse in hot water” or “shake with boiled water and change the teat”.*

## **Type of feeds**

Of the 65 mothers receiving free formula milk from the clinic (Table 9), 17% mentioned spontaneously that the formula was not enough and that they had to buy more. Of the 69% of the mothers who stated that they received the free formula (Pelargon) from the clinic, 7% used a different formula brand at the time of the interview because they had run out of the Pelargon.

It did not seem very common to add anything to the milk feed . At the time of the interview, 15% had added some type of cereal (Nestum or Cerelac) or homemade porridge into the feeds.

**Table 9. Type of commercial milk feeds obtained at the clinic (N=94)**

		N	%
Received free formula milk from the clinic	Yes	65	70
	No	29	31
Type of formula brand for milk sample	Nan Pelargon (free from clinic)	59	63
	Nan	21	22
	Lactogen	5	5
	Nespray	4	4
	Infacare	3	3
	SMA	1	1
	Fresh cow milk	1	1

## **Milk samples collected at the clinic**

One milk sample was collected from each mother at the PMTCT-clinic, in total 94 samples. More than 70% of the mothers had prepared feeds considered too old to be safe for feeding (more than one hour since preparation). It was observed that some mothers brought boiled water stored in a clean feeding bottle with formula powder separately in a box. When it was time for feeding they mixed the feed by putting the powder into the bottle. It is unclear why more mothers did not follow this method instead of preparing the feed at home with risk of too long storage.

All mothers (except from one who didn't know) stated that they had boiled the water before preparing the feeds. Mothers preparing the feeds at the clinic had access to boiled water through the staff. One mother had had brought a prepared unused feed from the night before

to the clinic. Since the baby did not drink it, she saved it to use it the following day. See Table 10.

**Table 10. Milk samples collected at the PMTCT-clinic. (N=94)**

		N	%
Time passed since the milk feed was prepared	<1 hour ago	11	12
	1-2 hours ago	17	18
	3-4 hours ago	51	54
	>4 hours ago	15	16
Site where mother prepared the milk feed	At home	81	86
	At clinic	13	14
Type of stove used for boiling water	Electric stove	75	80
	Paraffin cooker	18	19
	Don't know	1	1
Had child drunk from the feed before sample was taken?	Yes	68	72
	No	22	23
	Missing data	4	

## Microbiological results

Many of the milk feeds collected were contaminated with the measured pathogens (Table 11). In 64% of the milk samples, E-coli colonies were isolated and in 26% of the samples Enterococci could be found.

Of the samples containing E-coli, 44% were contaminated with >1000 colony-forming units (cfu)/ml and in samples containing Enterococci, 17% were contaminated with >1000 cfu/ml. None of the 94 milk samples were contaminated with Shigella or Salmonella.

**Table 11: Counts of E-coli and Enterococci in sample milk feeds**

No. of cfu/ml	E-coli		Enterococci	
	N	%	N	%
0	33	35	64	68
1-10	2	2	1	1
11-100	4	4	3	3
101-1000	13	14	4	4
1001-10 000	12	13	12	13
10 001-100 000	19	20	3	3
100 001- 200 000	4	4	-	
> 200 000	6	6	1	1
Missing	1		6	
<b>Total contaminated</b>	<b>60</b>	<b>64</b>	<b>24</b>	<b>26</b>

## **Factors associated to contamination of the milk feeds**

### **Boiling water for the feeds**

Mothers who boiled the water directly before preparation of each milk feed, were significantly protected against E-coli contamination in their feeds when compared to the cases where mothers boiled and stored water in a flask or a covered container before preparation (Odds Ratio/OR=0.4 (Confidence interval/CI=0.157-0.907)).

### **Type of feeding utensil**

Infant feeding bottles had a significantly higher risk of contamination with E-coli than feeding cups/containers ( $p=0.014$ , Fisher's Exact Test). No feeds from cups/container out of 4 were contaminated while 60 out of 89 bottle feeds were contaminated.

### **Number of bottles**

Mothers with several bottles (4-8) were significantly protected against E-coli contamination compared with those who owned fewer (OR=0.361 (CI=0.148-0.882)).

### **Number of feeds prepared at night**

Among mothers who prepared milk feeds at night for their infants, the number of times they prepared the feeds significantly influenced on the risk of contamination. Mothers preparing feeds between 3-5 times were protected against Enterococci contamination compared with mothers preparing 1-2 feeds at night (OR=0.533 (CI=0.383-0.745)).

### **Cleaning methods**

Use of a sterilising method (boiling/sterilising solution/bleach water) at the end of the cleaning process had a protective effect against E-coli contamination of the milk feeds (OR=0.311 (CI=0.211-0.865)). Using a sterilising method in general, whether it was before or after rinse/wash, also had a protective effect against E-coli contamination ( $p=0.045$ , Fisher's Exact Test).

Use of sterilising solution had a significantly protective effect against Enterococci contamination (OR=0.186 (0.040-0.867)).

A few mothers (5) used a cloth to clean the bottles which significantly increased the risk of feeds being contaminated with Enterococci ( $p=0.018$ , Fisher's Exact Test).

### **Free milk from the clinic**

Mothers receiving free formula had significantly less risk of feeds being contaminated with E-coli or Enterococci compared with mothers buying formula (E-coli; OR=0.207 (0.064-0.664) and Enterococci; OR=0.3 (CI=0.114-0.822)).

## Living location

Twenty two of the total 94 mothers in the study were visited by the researcher in their homes. More mothers living outside Durban had feeds contaminated with E-coli than mothers living in central Durban ( $p=0.056$ , Fisher's Exact Test).

## Factors not significantly associated to contamination

No significant associations were found between contamination and factors like storage of the milk feeds during the day, left over milk stored to be used later, foods/fluids added into the feeds in addition to milk powder, formula brand and to socio-economic and educational conditions.

## Protein results

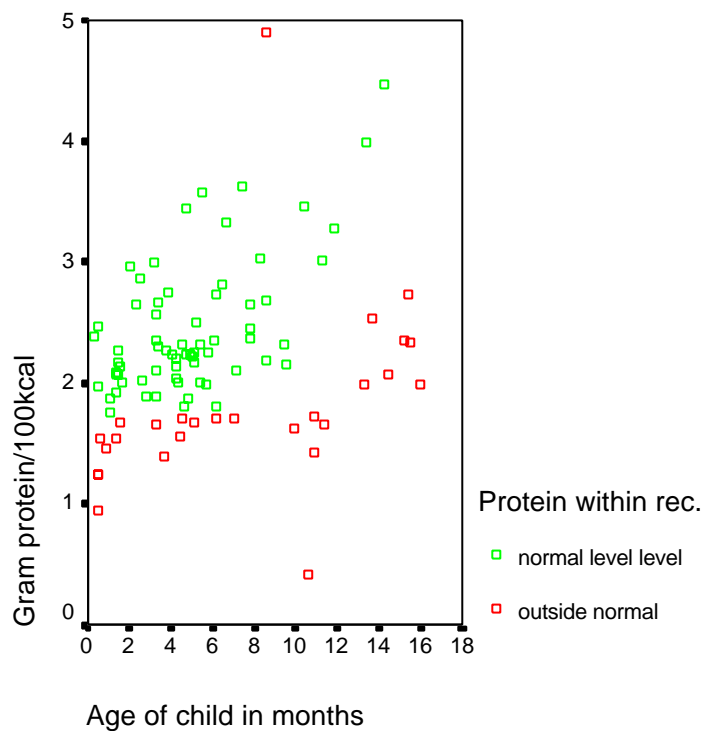
Over-concentration of milk feeds did not seem to be a problem among the mothers compared with over-dilution. More than 70% of the mothers prepared feeds with acceptable levels of protein. In total, 28% of the infants at the clinic received milk feeds of too low a concentration of protein (and thus formula powder). Over-dilution was seen in 22% of infants 0-12 months of age and in 78% of the samples of the few older infants. All samples, except for two, obtained from mothers with babies over 12 months had over-diluted feeds, even when feeds were prepared with follow-up formula or milk aimed for older children (Nespray). See Table 12.

**Table 12. Protein results compared to recommended levels of protein (N=94)**

	N	%
Milk samples within reference value	67	71
Milk samples outside reference value	27	29
Level of protein for infants 0-12 months of age: < 1.8g / 100kcal	19	22
1.8-4.0g / 100kcal (ref)	65	77
> 4.0g / 100kcal	1	1
Level of protein for infants >12 months of age < 3.0g / 100kcal	7	78
3.0-5.5g / 100kcal (ref)	2	22
> 5.5g / 100kcal	0	0

Figure 1 shows the distribution of the protein results by age of the infants. The results are marked with different colours depending on if the results correspond with Codex Alimentarius recommended levels of protein or not.

**Figure 1. Distribution of protein results according to age.**



## **Factors associated to over-dilution of the feeds**

### **Age of infant**

Results overwhelmingly show that infants under six months of age were at lower risk of being given over-diluted milk feeds than infants older than six months (OR=0.365 (CI=0.144-0.926)) and even more so for children younger than 12 months of age compared with those older than this (OR=0.084 (CI=0.016-0.436)).

To the question what the mothers gave the infants to eat the day before the interview (“yesterday”), all but two of the mothers with infants above six months of age reported that food in addition to the formula milk was also given.

### **Amount of formula prepared**

Mothers preparing smaller amounts of formula at one time (75-150ml) were significantly at less risk of preparing over-diluted milk feeds than mothers preparing larger amounts of milk (175-1000ml) (OR=0.358 (0.133-0.962)).

### **Main source of water for the milk feeds**

Mothers with no running water inside the house to use for the infants milk feeds were at greater risk of preparing over-diluted milk feeds compared with mothers who had running water inside the house (OR=2.971 (CI=1.136-7.772)).

## Free milk from the clinic

Free formula was more likely to be over-diluted, though this did not quite reach statistical significance (OR=2.521 (0.978-6.501), Pearson Chi-square p=0.052)

## Factors not significantly associated to over-dilution

No significant associations were found between over-dilution and factors like foods/fluids added into the feeds in addition to the formula powder, formula brand and to socio-economic and educational conditions.

## Home visits with observations and sample collection

A total of 22 mothers interviewed at the PMTCT clinic were visited in their homes. The mothers knew the date for the home visit and that a few more questions about formula feeding would be asked. They were not informed that new milk samples would be asked for.

Seventeen milk samples from already prepared and available feeds were obtained. One sample was not analysed for enterococci at the lab due to technical problems. In addition, 21 feeds were prepared and demonstrated by the mothers. Pelargon was provided for this purpose to all except two of the mothers who wished to use their own formula. Most of the already available feeds, 71%, had been prepared using the free formula/Nan Pelargon.

Table 13 shows when the available milk feeds had been prepared. Around 70% of these mothers kept feeds older than one hour, most of them at ambient temperature.

**Table 13. Available already prepared milk samples collected during home visits (N=17)**

		N	%
Person who had prepared the milk feed	Mother herself	14	82
	Her own mother	2	12
	Baby sitter, 11 years old	1	6
Time passed since the milk feed was prepared	<1 hour ago	5	29
	1-2 hours ago	4	24
	3-4 hours ago	3	18
	>4 hours ago	5	29
Storage of the available milk feeds	Covered, at ambient temperature	13	76
	Fridge	2	12
	Bottle warmer	1	6
	Prepared just before arrival	1	6

All demonstration samples were made in infant feeding bottles except in three cases where the mother used a cup, a cup with drinking spout or a plastic milk container. Usually nothing was added to the milk feeds except in a few cases where cereals or some type of homemade porridge was added.

## **Observed cleaning methods used in the homes**

Different cleaning methods and equipment were observed in the homes similar to what has already been described. Three of the 22 mothers did not use a sterilising method as mentioned at the clinic. Six mothers had changed to another sterilising method than mentioned earlier and a few used salt because no sterilising solution was available. Some of the mothers did not have any detergent or bottlebrush available and rinsed/washed by hand and in some cases boiled the utensils.

Some mothers had invested in a sterilising solution. One mother boiled the bottle in the electric water kettle used also to boil the water for the feeds. One mother stored empty sterilised bottles in the fridge until use to protect against contamination.

For the mothers who had access to detergent, soap, bottle brush, pots, buckets and kitchen space, the cleaning procedure could be performed very effectively. In cases where there was no kitchen or cleaning area available, or lack of detergent or a bottlebrush, the mothers seemed to struggle a bit with the cleaning procedure but no feeding utensil observed during the home visits was visibly dirty.

Some 62% of mothers used a sterilising method (boiled the utensils, used sterilizing solution or bleach water). I observed that, after the sterilizing procedure, mothers used the bottlebrush, hands and/or towel to rinse/wash and/or dry the bottle before use, which could lead to contamination. Many of the mothers rinsed the bottle to clean off spilled powder or squeezed the teat with their fingers while shaking it to mix to milk powder. Some mothers used a fitted plastic lid to preventing the milk from dripping out from the teat while shaking the bottle. One mother mentioned that she did not change the sterilising solution daily as recommended, but did so about every other day. We did not ask routinely about this and thus do not know how common it was to change the sterilization solution daily.

Some mothers tried to keep a clean area for the infant feeding utensils and for milk preparations but in several homes the kitchen space was very limited if it existed at all, for example in rented rooms. In these cases, it did not always seem sufficiently hygienic.

## **Observations of milk feed preparations**

The water was boiled before each preparation during the demonstrations. In 90% of the cases, water was boiled using electricity. Many of the mothers used electric water kettles instead of boiling water in a pot on an electric stove. Ten percent of the mothers used paraffin cookers.

In general the mothers seemed to be well informed about how to prepare the feeds, how to measure the powder with the scoops, and the number of scoops to add to a specific amount of water. The most commonly observed potential source of (relatively small) error was to first measuring the powder into the bottle and then pouring the water into the bottle afterwards.

## Microbiological results of milk samples obtained during home visits

The same degree of E-coli contamination was found in available samples plated directly in the homes and samples obtained at the clinic (63% versus 64%). The degree of Enterococci contamination for the home samples was higher compared to the clinic samples (56% versus 26%).

Table 14 shows a contamination degree of E-coli of 63% of the available milk samples plated directly at home and a contamination degree of 81% when the same milk samples had been stored and transported without refrigeration. This suggests that bacterial growth would appear to be larger with prolonged storage time of the feeds. However, no clear difference of Enterococci contamination could be seen between samples plated directly in the homes and samples plated at lab after being stored without refrigeration.

The demonstration samples, taken directly from the feeding utensils after preparation, were in general less contaminated than the available samples, both for E-coli and for Enterococci. .

**Table 14: Contamination degree of E-coli in milk samples obtained during home visits**

	Available samples		Demonstration samples	
	Plated at site	Plated at lab	Plated at site	Plated at lab
% of samples contaminated	63% (10/N)	81% (13/N)	33% (7/N)	33% (7/N)

**Table 15: Contamination degree of Enterococci in milk samples obtained during home visits**

	Available samples		Demonstration samples	
	Plated at site	Plated at lab	Plated at site	Plated at lab
% of samples contaminated	56% (9/N)	44% (7/N)	14% (3/N)	10% (2/N)

## Protein results for milk samples obtained during home visits

The protein results for these already prepared milk feeds corresponded with those from the milk samples collected at the PMTCT clinic. Figure 2 shows that 44% of the infants up to 12 months of age were given feeds considered having too low a level of protein.

Figure 3 shows that 14% of the milk feeds prepared at the time of the home visit were over-diluted among mothers with infants up to 12 months of age. The only mother with a child above 12 months of age made an over-diluted feed.

Figure 2. Protein concentration of available samples obtained during home visits, by age of child.

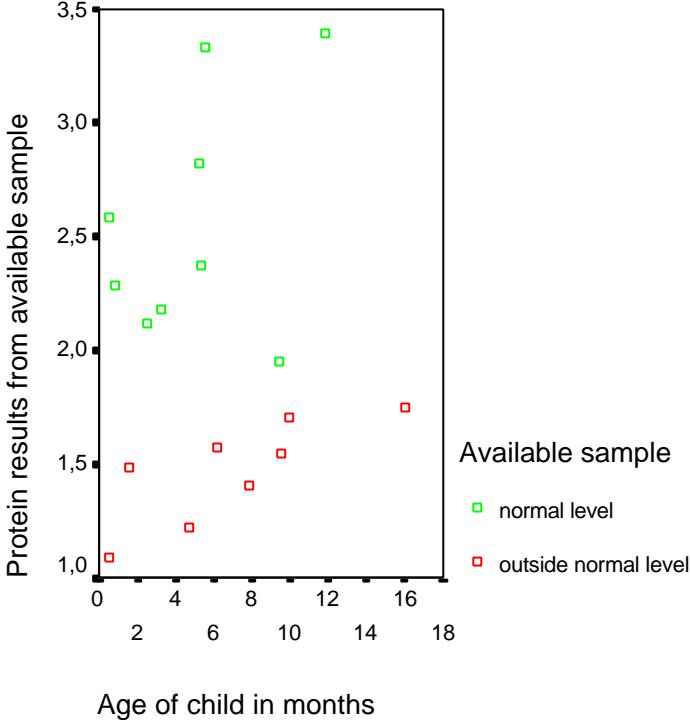
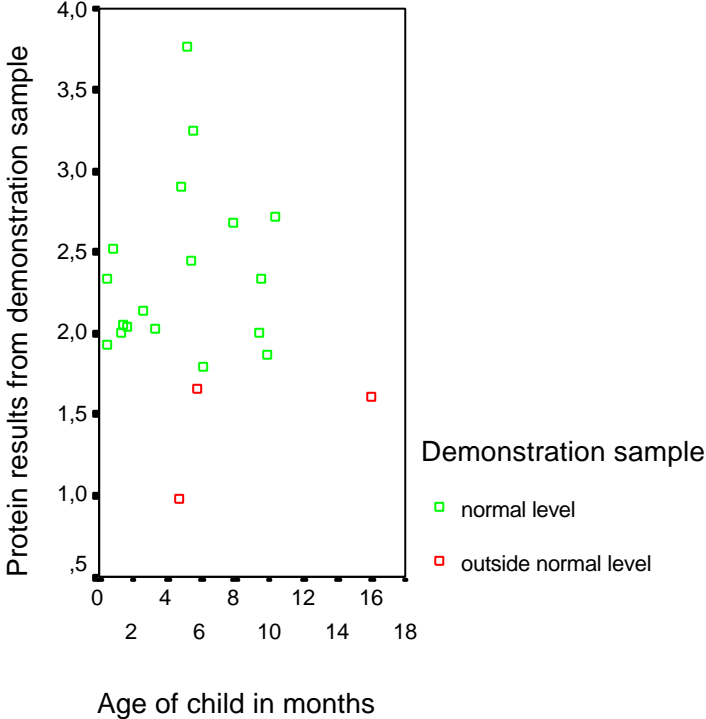


Figure 3. Protein concentration of demonstration samples prepared during home visits, by age of child



## **Discussion**

### **Methodological issues**

The PMTCT-clinic at King Edward VIII hospital in Durban was selected purposively. Its location next door to the Department of Medical Microbiology and Human Division of Biochemistry allowed milk samples to be transported to the lab within a short time for bacterial analysis or frozen storage for later analysis of protein content.

The clinic was open Monday to Friday (except for Tuesdays) for consultations and mothers could collect their free formula on any weekdays. The high number of mothers attending the clinic each week facilitated attainment of the required sample size within the available time.

The study participants were selected at the clinic and almost all mothers who were asked to participate in the study at the clinic gave their permission; three or four mothers refused. More difficult was the recruiting of mothers for home visits. Nonverbally, we sensed that many mothers may have feared disclosure of their HIV status to family members or neighbours and did not agree to any visit. Some mothers lived far away on farms and could not give sufficient information about their address or directions for later home visits, which prevented us from having access to mothers from a wider variety of different living conditions. Thus the home visit sample was not only small, but rather highly selected.

The purpose of the home visits was to collect available milk samples from feeding utensils and from demonstration feeds, and if possible to observe infant feeding procedures like cleaning methods and preparation. All home visits were made to so-called formal settlements (houses or flats/rooms) except for one mother who lived in a shed in an informal area outside Durban. However, at the time of the visit, she could not be found.

The mothers interviewed at the clinic may represent a relative cross section of the population within the PMTCT-programme in a formal settlement in an urban/peri-urban area of Durban, South Africa. Similar studies in other PMTCT-clinics sited in rural areas would probably provide complementary information on other living conditions that would be of value to the PMTCT-programme. In general the mothers in the present study seemed to have a relatively good living standard.

The co-worker who facilitated the study through interviews and translation when necessary worked at the clinic as an infant feeding counsellor. She was selected by the doctor at the clinic who worked with the mothers and who had earlier positive experiences in research with her. Of great value was that the counsellor's efforts were appreciated by the mothers, and contributed to their comfort. The mothers got support from staff and from each other concerning infant feeding choices, but also about their own health situation. For many, the clinic was the only place to share their HIV status and concerns.

On the other hand, the infant feeding counsellor might have influenced the validity and reliability of the responses. Some of the interviews took place in private and some were carried out by the field co-worker alone. Some mothers preferred to sit among the other mothers and did not choose privacy. Interviewing is a skill always with a risk of getting

“expected” answers. Even when a “good feeling” is achieved during the interview, circumstances can affect the information provided during data collection with a result of incorrect significant associations found, or not found.

## **Heavily contaminated milk feeds**

Internationally accepted criteria do not seem to exist concerning standards for pathogenic micro-organisms in infant formulas. This study refers to the Codex Alimentarius Commission’s Standards, which has established the criteria that the product should not contain any pathogenic micro-organisms (Codex standard for infant formula 1997). With respect to E- coli, 64% of the samples failed to meet the Codex standard and with respect to Enterococci, 26% failed. It is perhaps questionable whether this criterion is possible to meet in developing countries with a lack of resources. A common attitude among many health workers seems to be that these infants “need to face some bacteria to get used it,” seemingly overlooking the risk to the health of this vulnerable group of infants that already are faced with lack of the protective factors in breast milk and in some cases are infected with HIV.

If we nevertheless use the bacteriological criteria set in Finland for raw breast milk given to all newborns, milk feeds with <1000 cfu/ml potential pathogens, 44% of the feeds were excessively infected with E-coli and 17% with Enterococci. The E-coli bacteria are known to be a predominant cause of diarrhoea in developing countries (Morais, 1997). This degree of contamination is unacceptable in other parts of the world and should also be for these infants within the PMTCT program.

The organisms E-coli and Enterococci indicate faecal pollution and lack of sanitary quality (Jay 1986). Overall, more milk feeds were contaminated with E-coli than with Enterococci and none were contaminated with Salmonella or Shigella.

The present study suggests that a lack of sanitary quality was related to the mothers’ handling of the formula feeds and the feeding utensils. The risk for contamination decreased if the water used for the feeds was used directly after boiling instead of boiled and stored before preparation, if feeding cups/containers were used instead of feeding bottles, if the mother had several bottles available and if she prepared a few feeds per night instead of just one or two. Mothers using a sterilising method like boiling the utensils, using a sterilising solution or bleach water were also significantly protected for having contaminated milk feeds. These results regarding risk factors and risk behaviours correspond with the counselling guidelines of preparation and handling of the milk feeds.

Despite their following the recommended methods of preparation and cleaning, many mothers still had contaminated feeds. Most feeds had been prepared long before the infant had completed drinking them. Some infants drank milk prepared half a day or more before consumption. That this did not relate statistically to contamination levels could have been due to inaccurate or incomplete reporting in this regard. On the other hand, the protective effect against contamination when more than two feeds per night were prepared may have indicated the importance of avoidance of prolonged storage of the feeds. Another possible explanation could be that mothers gave an adjusted answer, afraid that an answer not corresponding with recommended storage guidelines might affect them negatively in relation to the formula distribution.

Simple achievements, likely to reduce contamination of feeding utensils and milk feeds to some extent, should be recommended and repeated to the mothers by the health workers. Health workers must give the mothers clear instructions on the period of time that can elapse between when non-refrigerated milk is prepared and when consumption must be completed. Even though most mothers had access to a fridge, most stored their milk feeds at room temperature during the day and even more did so at night. Health workers should realize that low-income mothers are likely not to throw away left over milk. They should give clear instructions on how to use leftovers and to remind mothers to store the leftovers in the fridge if storage is likely. They should also be aware of the possibility that mothers prepare larger amounts of formula than needed for each feed to save time during a day. Health workers probably have not adequately instructed mothers that sterilization procedures must be the final step before preparing milk in bottles and that the teats must not be touched after this. Since shaking the bottle is the easiest way to mix the milk powder, an alternative must be taught, if handling of teats is to be avoided. The plastic lid, enclosed with many bottles in the shops, prevents against some contamination if it is handled in a hygienic way. The mothers should be advised not to use cloths in the cleaning procedure, rather a functional bottle-brush. Even more importantly, health workers should continue to recommend the use of feeding cups instead of infant feeding bottles.

Infant feeding involves many critical steps during handling, storage, preparation and cleaning, with great demands upon good hygienic practice. These data confirm the difficulties mothers face in attempting to practice safe replacement feeding even when the methods taught to them were largely followed.

Mothers who received free formula from the clinic were significantly protected against bacterial contamination. Breastfeeding mothers who wanted to stop were also permitted to start with free formula for six months. Some of them had started to buy their own formula and now came to the clinic to get the permission for free formula. It is quite possible that mothers receiving free formula were currently receiving more information about formula feeding and hygiene than other mothers.

Pelargon claims that its low pH is protective against bacterial growth, but although all free formula was Pelargon, we found no correlation between the presence of contamination with faecal bacteria and the type of formula used. It is unclear whether the lack of correlation was due to a higher number of mothers receiving free formula at the time of the interview (65) compared with the number of Pelargon milk samples (59) collected.

## **Over-dilution of milk feeds**

In contrast to findings from developed countries (Hall 1976; Abu-Ektes, Zahraa 2002), over-concentration of milk feeds prepared by the mothers within the PMTCT-programme did not seem to be a problem. Only one sample above recommended level of protein was found among the samples received from the PMTCT-clinic. Over-dilution, when compared to Codex Alimentarius Commission's Standards (Codex standards for infant formula 1997), was however a problem. In 28% of the clinic milk samples and 47% of the home visit samples the amount of protein was too low.

Although no broad conclusions can be drawn as to whether the measured low levels of protein in this study constitute a risk of malnutrition among the formula fed infants in this PMTCT-program, it is clear that it occurs in a substantial proportion of infants already constituting a vulnerable group.

The proportion of milk samples with inadequate protein concentrations taken at the clinic was significantly lower among younger infants and among mothers who prepared smaller amounts of formula at one time. In theory, the amount and concentration of formula milk needed increases with the age of the child. Why improperly prepared formula milk occurred more frequently among the older infants is not clear but may be related to complementary feeding of other foods or a desire to make the formula to last longer. Over-dilution among older infants could be compensated through complementary foods with a high nutrient density, but this is highly unlikely. Most studies show that complementary foods used by low-income families tend to be too low in nutrient density. Thus maintaining a proper concentration of formula is probably equally important at all ages. The fact that over-dilution was a problem even among younger infants in milk sampled at home visits is also of concern.

The demonstration feeds showed that the majority of visited mothers could prepare milk feeds with correct concentrations. Thus improving education to mothers about how to make up feeds is unlikely to overcome this problem.

No significant association was found between contaminated milk feeds and over-diluted milk feeds. Thus these two problems are independent and it would be incorrect to say that a mother making one type of mistake was more likely to make the other.

## **Socioeconomic factors**

When examining simple relationships, as this study has done, there is always a risk that confounders interfere with the findings. Analysis was done according to infant age to control for its effect. In theory, socio-economic status (SES) could be a major factor explaining much of the findings. However in the present study this is unlikely, since none of the SES indices that we created had any significant correlation with bacterial contamination or incorrect dilution of the milk feeds. This may have been due to our sample being relatively homogenous with too few differences in the socioeconomic indicators measured. Other socio-economic indicators that were not measured, such as type of housing, might have worked better. If mothers did not give accurate answers concerning their socio-economic status the relationship with bacterial contamination might not be correct.

If more or better questions concerning socioeconomic status had been asked or a sample with a more varied SES status had been chosen, it might have been possible to develop a better SES indicator. This in turn could be used to create a simple, sensitive and specific algorithm to identify mothers, likely to be able to formula feed safely versus those unlikely to do so, a first step towards an evidence-based approach to risk assessment that is, advising mothers on which feeding approach is likely to lead to the best outcome for their children.

## **Conclusion**

We found substantial contamination and over-dilution of commercial infant milk feeds prepared by mothers within the National PMTCT-programme in an urban/peri-urban area of Durban, South Africa. Our findings suggest that, while most mothers seem to understand and follow the instructions they are given on cleanliness and hygiene when they have adequate facilities, health workers need to give better advice and education to mothers, especially about terminal sterilisation, avoidance of mixing by holding the nipple and shaking the bottle, of storage and refrigeration of prepared feeds, rapidity with which they should be consumed, and the handling of leftovers. Most mothers appear to understand how to prepare feeds with correct concentrations, yet often over-dilute feeds. The provision of free formula appears to worsen this problem.

These results also provide a further warning: even in urban South Africa, there are serious risks involved when mothers who bottle feed do not have facilities for hygienic preparation of feeds. Since, within this sample there was no association between SES and over-dilution, and only 14% of demonstration feeds were over-diluted, we conclude that in a group at this level of SES status, education is unlikely to greatly reduce this risk. Either this level of risk must be accepted or another alternative such as exclusive breastfeeding advised.

## **Suggestions for further studies**

One difficulty today in the counselling of the mothers is to identify the mothers not likely to formula feed safely even with good counselling. To be able to identify these mothers a simple tool or a simple, sensitive and specific algorithm must be developed in order to be able to guide health workers in advising mothers in their choice of infant feeding method. The findings of the present study did not provide this information and thus further research of this type is required.

The PMTCT-clinic in this study did not yet have any statistics on morbidity or mortality for infants, let alone a system for linking them to feeding status. Differences in health status were therefore difficult to analyse but are of obvious concern. Staff felt that the breastfed infants were healthier during the first year than the formula fed infants. Particularly in the African setting, studies are needed to find out the relationship between feeding methods and the health and survival of the infants.

## References

- Arnold L DW, Tully M R (1992). Guidelines for the establishment and operation of a human milk bank. West Hartford, Conn. *Human Milk Bank Association of North America*.
- Abu-Ekteish F, Zahraa J (2002). Hypernatraemic dehydration and acute gastro-enteritis in children. *Annals of Tropical Paediatrics*, 22: 245-249.
- ACC/SCN (2000). Fourth Report on the World Nutrition Situation. Geneva: ACC/SCN in collaboration with IFPRI.
- Breastfeeding (2001). Part 1: The evidence for breastfeeding. *Pediatric Clinics of North America*. 48(1) February 2001.
- Brown K H et al (1989). Infant-feeding practices and their relationship with diarrheal and other diseases in Huscar (Lima), Peru. *Pediatrics*, 83(1):31-40.
- Carroll L et al. (1979). Bacteriological criteria for feeding raw breast-milk to babies on neonatal units. *Lancet*, 2: 732-733.
- Chambers T L, Steel A E (1975). Concentrated milk feeds and their relationship to hypernatraemic dehydration in infants. *Archives of Disease in Childhood*, 50:610-615.
- Chopra M et al. (2000). HIV and infant feeding. Summary of the findings and recommendations from a formative research study with the Khayelitsha MTCT Programme, South Africa. University of Western Cape.
- Codex standards for infant formula (1997). [ftp://ftp.fao.org/codex/standard/en/CXS\\_072e.pdg](ftp://ftp.fao.org/codex/standard/en/CXS_072e.pdg) & [ftp://ftp.fao.org/codex/standard/en/CXS\\_156e.pdf](ftp://ftp.fao.org/codex/standard/en/CXS_156e.pdf)
- Coodin F J, Gabrielson I W (1971). Formula fatality. *Pediatrics*, 47: 438-443.
- Cyril A L et al. (1975). Hazards of overconcentrated milk formula. *JAMA*, 232(11): 1136-1140.
- Ehiri J E et al. (2001). Critical control points of complementary food preparation and handling in eastern Nigeria. *Bulletin of the World Health Organization*, 79(5): 423-435.
- Erku W A, Ashenafi M (1998). Prevalence of food-borne pathogens and growth potential of salmonella in weaning foods from Addis Ababa, Ethiopia. *East African Medical Journal*, 75(4): 215-218.
- Gerber M A et al. (1983). Sterilization of infant formula. *Clinical Pediatrics*, 22(5): 344-349.
- Greiner T (1977) The influence of infant food advertising on infant feeding practices in St. Vincent. Thesis for Masters of Science in Nutrition *Ithaca NY: Cornell University*,
- Habicht J-P, DaVanzo J, Butz W P (1988). Mother's milk and sewage: Their interactive effects on infant mortality. *Pediatrics*. 81(3):456-460.

- Hall D M B et al. (1976). Artificial feeding of black infants. *South African Medical Journal*, 8(50(20)): 261-263.
- Imong S M et al. (1995). Maternal behaviour and socio-economic influences on the bacterial content of weaning foods in rural northern Thailand. *Journal of Tropical Pediatrics*, 41: 234-240.
- Iroegbu C U et al. (2000). Bacteriological quality of weaning food and drinking water given to children of market women in Nigeria: implications for control of diarrhoea. *Journal of health, population, and nutrition*, 18(3): 157-162.
- Janine M et al. (1984). Mortality and infectious diseases associated with infant-feeding practices in developing countries. *Pediatrics*, 74 (suppl):702-727.
- Jay J M (1986). Modern food and microbiology. 3<sup>rd</sup> edition. 407-436. New York: Van Nostrand Reinhold Company.
- Jeffs S G (1989). Hazards of scoop measurements in infant feeding. *Journal of the Royal College of General Practitioners*, 39: 113.
- Kendall N, Vaughan V C, Kusakeioglu A (1971). A study of preparation of infant formulas. *American Journal of diseases of Children*, 122: 215-219.
- Lewis-Jones D I et al (1985). The influence of parity, age, and maturity of pregnancy on antimicrobial proteins in human milk. *Acta Paediatrica Scandinavica* 74:655.
- Lilburne A M et al (1988). Infant feeding in Sydney: a survey of mothers who bottle feed. *Australian Paediatric Journal*, 24: 49-54.
- Morais T B, Gomes T A T, Sigulem D M (1997). Enterocoagulative Escherichia coli in infant feeding bottles. *The Lancet*, 349: 1448.
- Motarjemi Y et al. (1993). Contaminated weaning food: a major risk factor for diarrhoea and associated malnutrition. *Bulletin of the World Health Organization*. 71(1):79-92.
- Nunnally. J C (1967). *Psychometric Theory*. New York: McGraw Hill.
- Popkin B M et al. (1990). Breast-feeding and diarrheal morbidity. *Pediatrics*, 86(6): 874-882.
- Principles for the establishment and application of microbiological criteria for foods (1997). <http://www.fao.org/DOCREP/005/Y1579E/y1579e04.htm>
- Ray G, Nath G, Reddy D C S (2000). Extents of contamination of top milk and their determinants in an urban slum of Varanasi, India. *Indian Journal of Public Health*, 44(4): 111-117.
- Statistics South Africa (1996). The people of South Africa Population Census. Census in brief. Report No 03-01-11 (1996). *Statistics South Africa. Pretoria*.
- Surjono D (1980). Bacterial contamination and dilution of milk in infant feeding bottles. *Journal of Tropical Pediatrics*, 58-61.

Söderhjelm L (1972). Infant feeding hygiene in Sweden: a survey of bottle and teat hygiene. *Acta Paediatrica Scandinavica*, 61:565-570.

The Committee on Fetus and Newborn (1961). Report of Committee on Fetus and Newborn. Sterilization of milk-mixtures for infants. *Pediatrics*, 28:674-675.

UNICEF (2001). The State of the Worlds Children 2001.

Victoria C G et al. Evidence for protection by breast-feeding against infant deaths from infectious diseases in Brazil. *The Lancet*. 1987; August 8.

WHO (2001). Global strategy for infant and young child feeding: the optimal duration of exclusive breastfeeding. Fifty-fourth World Health Assembly May 2001. A54/INF.DOC./4.

WHO (2002). Infant and young child nutrition. Fifty-fifth World Health Assembly. *WHA 55/25 May*.

WHO (2002). Infant and young child nutrition. Global strategy and young child nutrition. Fifty-fifth World Health Assembly. *WHA 55/15 April*.

WHO (2000). New data on the Prevention of Mother-to-child Transmission of HIV and their Policy Implications. Conclusions and recommendations. WHO Technical Consultation on Behalf of the UNFPA/UNICEF/WHO/UNAIDS Inter-Agency Task Team on Mother-to-Child Transmission of HIV. Geneva. October.

WHO/UNICEF (1993). HIV/AIDS counsellor training course. Participants manual. WHO/CDR/93.5 and UNICEF/NUT/93.3. Geneva: WHO.

WHO/UNICEF (2001). Model chapter for textbooks. IMCI. Integrated Management of Childhood Illness. *WHO/FCH/CAH/01.01*.

WHO/University of California, Davis (1998). Complementary feeding of young children in developing countries: A review of current scientific knowledge. Geneva: WHO.

WHO (2000). WHO Collaborative Study Team on the role of breastfeeding on the prevention on infant mortality. Effect of breastfeeding on infant and child mortality due to infectious diseases in less developed countries: a pooled analysis. *The Lancet*. 355(5) 451-455.

Wilcox D T, Fiorello A B, Glick P L (1993). Hypervolemic shock and intestinal ischemia: A preventable complication of incomplete formula labelling. *The Journal of Pediatrics*, 122(1): 103-104.

Yoshioka H, Iseki K I, Fujita K (1983). Development and differences of intestinal flora in the neonatal period in breast-fed and bottle-fed infants. *Pediatrics* 72:317.

Ziegler E E, Fomon S J (1989). Potential solute load of infant formulas. *Journal of Nutrition*, 119: 1785-1788.