POTENTIAL HEALTH RISK POSED BY CALVES IN TRANSMISSION OF ZOOONOTIC CRYPTOSPORIDIUM PARVUM IN URBAN SMALL HOLDER DAIRY PRODUCTION IN DA GORETTI DIVISION OF NAIROBI KENYA

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FACULTY OF VETERINARY MEDICINE

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DECLARATION

This is my original work and has not been presented for a degree in any other university.

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DEDICATION

To my beloved family
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Special thanks go to the Director of Veterinary Services and the Ministry of livestock and Fisheries for allowing me to undertake this course.

The technical members of the Department of Public Health, Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Nairobi cannot escape mention for their valuable assistance.

Last but not least, I whole heartedly express my gratitude to my husband for financial support, encouragement and understanding and my children for their patience.
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ABSTRACT

_Cryptosporidium parvum_, commonly referred to as Crypto, is a protozoan parasite (a one-celled organism) that cause diarrhea in calves (as well as other animals). This extracytoplasmic organism invades enterocytes (cells that line the intestines) in the distal small intestine and large intestine.

The prevalence of _Cryptosporidium_ spp. in healthy asymptomatic calves was investigated in urban small holder dairy farms in Dagoretti Division, Nairobi, Kenya on a population of 117 calves of 7-30 days old purposively selected from a population of 296 dairy farmers between August 2006 and May, 2007. The detection of _cryptosporidium_ spp. was based on sodium chloride floatation technique and modified acid fast stain (Modified Ziehl-Neelsen). Additionally, positive samples on Modified Ziehl-Neelsen (MZN) and 20% of the negative samples were examined by Indirect Fluorescence test (IFA) method as a comparative test. The prevalence based on Modified Ziehl-Neelsen tests was 23.4% (27/117). There was significant agreement (Kappa=0.45) between the MZN and IFA test. Isolates of _Cryptosporidium_ were examined via bidirectional DNA sequencing which revealed that they were _Cryptosporidium rynaee_, which is considered to be non zoonotic.

Using questionnaires, information regarding risk factors for cryptosporidiosis in both calves and human was collected. The risk factors assessed with regard to cryptosporidiosis in calves were management (housing and feeding) system, while with regard to human infection risk factors included close association with calves, protective clothing, washing of hands after handling of manure and level of awareness about cryptosporidiosis were considered.
Ninety five percent (95%) of the respondents did not use any protective clothing, 77.3% did not wash hands after handling animals and manure waste.

In a participatory manner, farmers developed mitigation measures that would minimize risks associated with handling of calves. Personal hygiene was the single most important mitigation measure for reducing the risk of disease transmission. Other critical mitigation measures were hygiene of calf-pens and milking areas.

There is evidence that dairy calves in Dagoretti have high level of cryptosporidiosis infection. Further, cryptosporidiosis is not well known or understood within the dairy and close neighboring community in Dagoretti. Calf-borne Cryptosporidia needs to be typed in order to find if it contains an appreciable proportion of the zoonotic strain C.parvum. Therefore the broad mitigation measures developed should be applied to reduce the human health risks.
CHAPTER 1

1.0 Introduction

1.1 Urban dairy farming.

Urban agriculture is the production, processing, and marketing of food (vegetable, fruits, eggs, meat, mushroom, and others) and other agricultural products (flowers, herbs, ornamental plants, tree seedlings, and others) in the inner city and peri-urban areas. It is classified as crop-livestock farming or on-plot type of agriculture where farming is carried out on the plots around houses (Mougeot, 1999). Given the rapid rates of urban population growth, as stated by the UN-Habitat (2004) predicting that by 2030, 60% of the population will live in cities, agricultural production, primarily in the hands of poor smallholders, require improvement in order to meet the urban demand. Dairy production significantly contributes to productivity and sustainability of crop-based small-holder farming systems. Most Kenyan cities are now practicing smallholder dairy farming in the urban and peri-urban areas generally keeping one to two cows (mostly Holstein Fresian or Arshire) which comprise 50% of the herd and the other half consisting of female calves and heifers (Staal et al., 1997a). Cattle are stall fed mainly by cut napier grass and crop residues especially from maize and bananas, supplemented with forage gathered from common properties around the farms. Following the study done in Dagoretti on “Characterizing the benefits and health risks in urban smallholder dairy production in Dagoretti, Nairobi, Kenya”, IDRC file 102019-004 (Kange’the et al., 2005), it was brought to light that the reason for dairy farming households engaging in urban
dairy production was its potential to increase the food availability and income. On average each household produces about 10 kg of milk daily, of which a quarter is used for home consumption. This contributes directly into the nutritional needs of the families and indirectly, releases approximately Ksh. 46,080 annually per household that could have been used to buy other nutritious foods the families may be lacking and the rest sold locally to consumers through informal markets. The direct sale of milk in the division amounted to Ksh. 48,822,816.00 giving an average earning of Ksh 50,857.00 per lactation in a dairy keeping household. Farmers also captured the value of animal waste nutrients through farm use of animal manure to grow crops enabling them to improve the yields by approximately 66% thus increasing food availability for household consumption and even for sale. A threat to this enterprise especially in less favored regions of the world, is the impact of neonatal diseases, particularly enteric infections. Cryptosporidiosis is only one of several which need to be controlled.

1.2 Health Risks

*Cryptosporidium* is an important parasite that is very prevalent on dairy farms and is capable of causing diarrhea on its own or in combination with other agents. Calves usually are believed to be infected shortly after birth and shed the organism with in 5-7 days through feces contaminating the environment. This protozoan parasite can infect also humans. The adult household members are likely to clean the cattle shed, milk and take care of the animals including calves while children are likely to be infected as they play in standing water contaminated with cattle feces.
Despite the benefits derived from the agricultural activities, the previous study identified a health risk posed to the urban dairy farmers by zoonotic diseases like Cryptosporidiosis. Health risks associated with livestock keeping have not been well documented. Among all animals present on a dairy farm, the highest morbidity and mortality rates generally occur in calves prior to weaning. The hazard identified showed that the knowledge of cryptosporidiosis among dairy farming was scanty as indicated by the low response rate (20%). Zoonoses risks were not highly considered when the health risk factors were discussed until they were brought to the picture by the research team of their existence (Kang’ethe, et al. 2005). Data on the prevalence and risk factors of cryptosporidiosis in calves is lacking. Such data is necessary for the individual households that are engaged in urban dairying, health service providers and also policy makers. The information, if available, can be used by farmers to mitigate the risks of transmission to themselves, employees and consumers of their products.

For health service providers, it offers an opportunity to better diagnose disease and provide appropriate health care. For instance, cryptosporidiosis is not the first enteric diseases in differential diagnosis list causing diarrhea in calves. Others include Escherichia infection and Salmonellosis. Availability of the prevalence rates of such zoonotic diseases is essential in provision of good health care.

1.3 Overall Objective

The role played by calves in the transmission of cryptosporidiosis in human to improve human health and protect livestock based urban livelihoods
1.3.1 Specific Objectives

a) To improve the understanding of risks to human health caused by Cryptosporidium infection of caves in Dagoretti.

b) To determine the prevalence of zoonotic Cryptosporidium infection in calves

c) To characterize isolates of Cryptosporidium from caves using molecular and phylogenetic analysis and compare with human isolates.

d) Based on the evidence, suggest important measures to reduce the spread of zoonotic cryptosporidiosis in the human population

1.4 Justification

Although the medical community increasingly recognizes the zoonotic potential of Cryptosporidium, the public is largely unaware that they could be infected through animal contact, handling of manure, person to person, food borne and water contamination. A previous study (Kang’ethe et al, 2005) indicated that Dagoretti community had negligible knowledge on the existence of the disease with about 7 percent having knowledge that the disease could be transmitted by cattle and one percent through manure. This clearly showed that they were at risk of contracting the disease from the practices associated with urban dairy farming. Young children, pregnant women and elderly persons are more at risk followed by those with weakened immune system and in the latter, the disease maybe serious and life threatening.
Reports on the impact of urban agriculture on household nutrition are encouraging. A study done in the Kenyan coast (Mutiso, 1993) showed that children from cattle owning household had a higher height for age Z-score (HAZ) indicating lower levels of stunting compared to those from non cattle owning households. In Kenya Lee-Smith and Memon, 1994, indicated that urban farmers produced more than 77 percent of their food largely for home consumption. Urban agriculture is therefore assuming great importance in the supply of food and other human essentials from the sale of agricultural products for the increasing urban population. Therefore quality control measures are required to reduce the health risks posed by the intensification of livestock production that creates conducive environment for the multiplication of zoonotic parasitic infections. Following the Da goretti study initiated in 2005, it was shown that adult animals and calves were shedding oocysts at 12.1 and 11% respectively. In order to associate the causative agent found in man and the reservoir host (calves), a detailed health risk analysis of cryptosporidiosis was carried out to elucidate the role of zoonotic bovine *cryptosporidium* infection in human population engaged in urban dairy production. Routes of exposure to the people were assessed and possible measures taken to manage the exposure.

1.5: Research expected output

Health risk analysis of cryptosporidiosis in urban smallholder dairy production, roles played by calves in the transmission, its prevalence and mitigation strategies that when adopted could lead to a reduction of the risk of disease.
1.6 Impacts (short and long term)

By addressing potential health risks, this study would contribute towards securing the support of public and private sector partnership for specifically urban agriculture dairy farmers, in addressing issues on public health policy implication and in the long term, assist in developing policy guidelines that will result in safe urban agriculture and prevent contamination of dairy products consumed in urban and rural areas.
CHAPTER 2

2.0 Literature review

2.1: Introduction and history of Cryptosporidium species

Only two species of *Cryptosporidium* have been identified in ruminants. The intestinal species, *C. parvum*, is the common cause of ruminant cryptosporidiosis (Current, 1988). However, an abomasal species, *C. muris*, has been found in cattle in the U.S. (Anderson, 1987) and in mountain gazelles in the Federal Republic of Germany (Pospischil et al., 1987). The first report of *Cryptosporidium* species infection in cattle appeared in 1971 (Panciera et al., 1971). Endogenous cryptosporidial stages were detected on histological examination of jejunum of an 8-month-old heifer which had chronic diarrhea. Later, other reports followed in which similar infections were found in diarrheic 2-week-old calves (Meuten et al., 1974). In 1976, Canadian workers reported the occurrence of *Cryptosporidium* species infection in calves aged less than 2 weeks with acute diarrhea, from beef and dairy herds (Morin et al., 1976). The parasite was found in histological sections of the lower jejunum and ileum, associated with slight villi stunting. A variety of other enteropathogens, both viral and bacterial, were associated with the presence of the parasite in most instances, but in some cases no other pathogen were detected.

The role of *Cryptosporidium* species as a primary enteropathogen was uncertain until 1980, when evidence from field studies showed that the parasites was capable of causing clinical
diarrhea in calves in the absence of concurrent infection with common viral or bacterial agents normally incriminated in outbreak of neonatal calf diarrhea (Tzipori et al, 1980). Experimentally, these outbreaks were confirmed when infective oocysts were isolated in the distal small intestines and associated them to C. parvum infection (Tzipori et al, 1983). However, an abomasal form of Cryptosporidiosis was found in cattle in the Central U.S. in 1985 (Anderson, 1987), caused by a species of Cryptosporidium apparently indistinguishable from C. muris, the species originally found in a mouse stomach by Tyzzer in 1907.

In dairy animals, C. parvum has been associated with diarrhea affecting primarily calves up to 30 days of age (Fayer and Ungar, 1986; Snodgrass et al, 1986; Harp et al, 1990; Moore and Zeman, 1991; McCluskey et al, 1995; Olson et al, 1997; Wade et al, 1999). It is generally believed that the illness in calves results in high morbidity and low mortality (Kirkpatrick, 1985; Mann et al, 1986; Sobieh et al, 1987; Scott et al, 1995). Other clinical signs reported in calves include dullness, lethargy, anorexia, fever, dehydration, and loss of condition (Angus, 1991). Calves infected with C. parvum are reported to shed peak numbers of oocysts in diarrheic feces ranging from $10^6$ to $10^7$ per gram of feces (Blewett, 1988; Fayer, 1997a). The potential hazard infected calves pose to environmental contamination emphasizes the importance of management practices in controlling infection.

Few studies have been conducted to evaluate the role of management on the risk of infection in dairy cattle (Quigley et al, 1994; Garber et al, 1994). There is need to identify ways by which these perceived risks from domestic animals can be managed. The design of strategic
plans to control the perpetuation of the infection in the target population depends largely on understanding the factors that lead to the introduction, transmission, and spread of infection in animals. Epidemiologic studies that take into consideration the multifactorial nature of infections will no doubt provide valuable effective information to design strategies to prevent the occurrence of infection in animals and ultimately reduce public concern.

2.2: Cryptosporidium

Cryptosporidium is one of several protozoan genera in the phylum Apicomplexa which develop within the gastrointestinal tract of vertebrates throughout their entire life cycles. More than 22 species have been described based upon the host from which they were originally isolated (Fayer et al., 1997a) However, only 13 species are considered valid by many investigators. The valid species list is as in the Table 1.
Illness in human is confined primarily to infections associated with *C. parvum* (O’Donoghue, 1995). Other species as *C. baileyi* (Ditrich, 1991), *C. felis* (Morgan et al, 2000a), *C. meleagris* (Morgan et al, 2000a), *C. muriis* (Katsumala et al, 2000) *C. canis* (Morgan et al, 2000a) from other animals have been found to cause infection in humans, particularly, in the immunocompromised individuals. Molecular studies have enhanced the knowledge of the

\[\text{Table 1:13 valid Cryptosporidium species}\]

<table>
<thead>
<tr>
<th>Cryptosporidium species</th>
<th>Size (µm)</th>
<th>Host</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. andersoni</em></td>
<td>5.5 × 7.4</td>
<td>Bovines</td>
<td>Abomasum</td>
</tr>
<tr>
<td><em>C. baileyi</em></td>
<td>4.6 × 6.2</td>
<td>Birds</td>
<td>Cloaca, bursa, respiratory tract</td>
</tr>
<tr>
<td><em>C. canis</em></td>
<td>5.0 × 4.7</td>
<td>Canids, human</td>
<td>Small intestine</td>
</tr>
<tr>
<td><em>C. felis</em></td>
<td>4.5 × 5.0</td>
<td>Felids, human</td>
<td>Small intestine</td>
</tr>
<tr>
<td><em>C. galli</em></td>
<td>8.0–8.5 × 6.2–6.4</td>
<td>Birds</td>
<td>Proventriculus</td>
</tr>
<tr>
<td><em>C. hominis</em></td>
<td>4.5 × 5.5</td>
<td>Human</td>
<td>Small intestine</td>
</tr>
<tr>
<td><em>C. meleagris</em></td>
<td>4.5–5.0 × 4.6–5.2</td>
<td>Birds, human</td>
<td>Intestine</td>
</tr>
<tr>
<td><em>C. molnari</em></td>
<td>4.7 × 4.5</td>
<td>Fish</td>
<td>Stomach</td>
</tr>
<tr>
<td><em>C. muriis</em></td>
<td>5.6 × 7.4</td>
<td>Rodents, human</td>
<td>Stomach</td>
</tr>
<tr>
<td><em>C. parvum</em></td>
<td>4.5 × 5.5</td>
<td>Ruminants, human</td>
<td>Intestine</td>
</tr>
<tr>
<td><em>C. saurophilum</em></td>
<td>4.2–5.2 × 4.4–5.6</td>
<td>Lizards, snake</td>
<td>Intestinal and cloacal mucosa</td>
</tr>
<tr>
<td><em>C. serpentsis</em></td>
<td>4.8–5.6 × 5.6–6.6</td>
<td>Snakes, lizards</td>
<td>Stomach</td>
</tr>
<tr>
<td><em>C. suis</em></td>
<td>5.1 × 4.4</td>
<td>Pigs, human</td>
<td>Small intestine</td>
</tr>
<tr>
<td><em>C. wrairi</em></td>
<td>4.0–5.0 × 4.8–5.6</td>
<td>Guinea pigs</td>
<td>Small intestine</td>
</tr>
<tr>
<td><em>C. bovis</em></td>
<td>4.2–4.8 × 4.8–5.4</td>
<td>Ruminants</td>
<td>Small intestine</td>
</tr>
<tr>
<td><em>C. scophthalmi</em></td>
<td>3.0–4.7 × 3.7–5.0</td>
<td>Fish</td>
<td>Intestine</td>
</tr>
</tbody>
</table>

(Xiao and Ryan, 2004)
epidemiology of human cryptosporidiosis and have shown that the vast majority of human cases are caused by *Cryptosporidium hominis* (synonymous with *Cryptosporidium parvum* genotype (1) and *C. parvum* (synonymous with *C. parvum* genotype 2) (McLauchlin et al., 2000; Anon, 2002, Morgan-Ryan et al., 2002).

Infected individuals show a wide spectrum of clinical presentations, but the pathogenicity of *Cryptosporidium* varies with the species of parasites involved and the type, age, and immune status of the host. In many animals, *Cryptosporidium* infections are not associated with clinical signs but with an acute, self-limiting illness. In industrialized countries, epidemics of cryptosporidiosis can occur in adults via the food-borne or waterborne route (Mackenzie et al., 1994). Zoonotic infections via direct contact with farm animals have been reported many times but the relative importance of direct zoonotic transmission of cryptosporidiosis is not entirely clear (Miron et al., 1991). One major problem in understanding the transmission of *Cryptosporidium* infection is the lack of morphologic features that clearly differentiate one *Cryptosporidium* species from many others (Fail et al., 2003). Hence, one cannot be sure which *Cryptosporidium* is involved when one examines oocysts in clinical specimens under microscopy. To assess the genetic diversity and evolution of *Cryptosporidium* parasites, molecular and phylogenetic analysis have been used to understand the genetic structure and relationship of *Cryptosporidium* parasites (Xiao et al., 1999a,b).
Transmission is by fecal-oral spread of the oocyst stage with contaminated feed or water or by inhalation. Cross transmission studies previously discussed demonstrate a lack of host specificity for Cryptosporidium parvum. Isolates from human are infective to a variety of mammals and those from one mammalian species are infective to others. From these studies cattle, particularly calves, and other livestock are implicated as a source of human infection. The high prevalence of cryptosporidiosis in neonatal calves, the close contact during their care and the human contact during milking of their dams provide opportunities for transmission to farmers, handlers and veterinarians (Dubey et al, 1990).

The most dangerous source of infection for a calf is its neighbor particularly where animals are communally housed and crowded and calves reared outdoors in permanent paddock where infection is spread rapidly because the cows’ teat and udders become contaminated with infected calf feces in their lying areas (Reynolds et al, 1986).

Animal attendants can spread infection to young calves, their beddings and feeding utensils through contaminated clothing, footwear, and hands. Vermin (mice and rats) carry Cryptosporidium and experimental cross-infection of mice and calves with calf isolate and mouse isolate (Klesius et al, 1986), has led to the conclusion that such isolates are freely interchangeable in the farm environment.

Sub clinical infections have been confirmed in adult cattle (Mann et al, 1986) that calving cows could excrete sufficient numbers of oocysts to infect their offspring at birth.
Animal to person (zoonotic) transmission has been described from farm animals, household pets and laboratory animals. Infected calves, cats or dogs have been likely source (Koch et al, 1983). At least 20 cases of laboratory workers have been reported (Anderson et al, 1982), the majority in contact with infected calves.

Agriculural uses of sewage sludge, manure, pose a health risk. This has led to contamination of drinking water in rivers or wells which frequently have caused several outbreaks of cryptosporidiosis in the industrialized countries (MacKenzie et al, 1994). Crops and vegetables can be contaminated directly by use of contaminated farmyard manure on the field. Calf manure is more often directly disposed to farms as farm manure.

Water for drinking or swimming can serve as a vehicle for the transmission of the oocyst stage. Contaminated surface water has been implicated as the source of human cryptosporidiosis (Anon, 1986). The high prevalence of oocysts in untreated surface water is thought to come from runoff from the fields, pastures, or areas of wildlife or livestock activities.

Transmission by arthropods associated with feces in areas lacking good sanitation might be another source of infection. In Peru, Cryptosporidium parvum oocyst wall material was detected by immunofluorescence microscopy on flies in homes using specific monoclonal antibody (Stering et al, 1987).

Airborne transmission of the oocyst stage has been implicated as a source of respiratory cryptosporidiosis. It was reported that a veterinary scientist who inhaled the air from the end of the inserted stomach tube used to treat an infected calf was found infected (Hojlyng et al, 1984).
A number of studies has shown that person to person transmission occur within day care centers, households, hospital patients, urban environments with high population densities. The spread of infection follow direct, through fecal-oral route or indirect through formites.

Under favorable conditions of high humidity, temperatures <20º C; (Casey et al., 2004) the oocyst of *C. parvum* can survive in the environment for about 6 months, after that infectivity rapidly decreases (Fayer et al., 1998b). Neither moderate freezing nor heating to 50º C completely inactivates oocysts (Fayer, 1994; Olson et al., 1999). Because of their size and slow sedimentation rate oocysts can remain in water bodies for prolonged time periods (Sre´ter and Sze´ll, 1998).

2.4: Morphology and life cycle of *Cryptosporidium*

The small size of the *Cryptosporidium* oocysts makes them indistinguishable at the species level based on morphology by light microscope (Fal et al., 2003). The oocysts are spherical or ovoid in appearance and contain 4 naked parallel sporozoites surrounded by a smooth oocyst wall. At the wall, a faint suture can be seen through which the sporozoites exit during excystation (Morgan-Ryan et Al, 2002). There is some variation from species to species. The length of the oocyst ranges from 4.5 to 7.5 μm and the width from 4.2 to 5.7 μm (Marquardt et al., 2000). At the electron microscope level the sporozoites of *Cryptosporidium* show some of the elements of the apical complex; such as an electron dense collar similar to a conoid, micronemes and electron-dense bodies that may be similar to rhoptries (Marquart et al., 2000). The two apical rings can also be seen at the tip of the zoite. The pellicle is similar to that of other apicomplexans and subpellicular tubules are also present. They lack a true
conoid, perhaps rhoptries, mitochondria, and a cytosome (Marquardt et al. 2000). The life cycle is shown as below;

![Life cycle diagram]

Fig 1 The life cycle of *Cryptosporidium* (Marquardt et al. 2000).

The exogenous stage of the *Cryptosporidium* life cycle is a sporulated, thick walled oocyst which is excreted in the faeces of an infested host. However, the endogenous part of the life cycle begins when the infectious oocysts are ingested either by contaminated water or feed. In the gastrointestinal lumen the oocyst will release sporozoites, which parasitize the villous
enterocytes. The developing stages remain at the luminal surface of the enterocytes. But they are covered by the plasma membrane of the host cell. Thus they are spoken of as being intracellular but extracytoplasmic. The sporozoites differentiate, intracellularly, into trophozoites (uninucleate meronts) that undergo asexual multiplication by nuclear division leaving behind type I and type II meronts. Type I meronts produce six to eight merozoites, which in turn invade epithelium cells and form type II meront. Type I merozoites can go to type II meront or return to form another generation of type I. The type II meronts produces merozoites. They will initiate sexual multiplication as they differentiate into either male micro-gamonts (Rimhanen-Finne, 2006). The fertilized macro-gametes develop into thick walled oocysts, which are excreted from the body or into thin walled oocysts. The latter is responsible for repeated asexual and sexual multiplication (autoinfection) within the same host.

The excreted oocysts are immediately infective to other hosts, and so-called thin-walled oocysts can release their sporozoites while still in the intestines and reinitiate the developmental cycle via autoinfection without an environmental stage, which leads to the production of very high oocyst numbers (Current, 1985). Despite sexual development of the parasite both clonal and panmictic populations have been identified by multilocus genotyping using mini- and microsatellite markers, indicating that both self-fertilization and out-crossing occur (Mallon et al. 2003).

The pre-patent period varies with the host and species of cryptosporidia (Current and Hayness, 1984) i.e,
The Cryptosporidium oocysts are immediately infectious upon excretion and stable in the environment but cannot multiply outside the host. The oocysts resist most disinfectants and water treatment agents. Infection with Cryptosporidium through fecal excretion plays a crucial role in the spread of the disease.

### 2.6: Cryptosporidiosis in calves

Table 2: Infection pattern of cryptosporidiosis in cattle (O’Handley et al. 1999).

<table>
<thead>
<tr>
<th></th>
<th>C. parvum</th>
<th>C. andersoni</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age of oocyst shedding</td>
<td>1-5 weeks</td>
<td>&gt; 7 weeks</td>
</tr>
<tr>
<td>Duration of shedding</td>
<td>1-2 weeks</td>
<td>5 months-years</td>
</tr>
<tr>
<td>Age of peak shedding</td>
<td>1-2 weeks</td>
<td>N/A</td>
</tr>
<tr>
<td>Age of onset diarrhea</td>
<td>1-2 weeks</td>
<td>N/A</td>
</tr>
<tr>
<td>Duration of diarrhea</td>
<td>1-3 weeks</td>
<td>N/A</td>
</tr>
<tr>
<td>Duration of peri-parturient shedding</td>
<td>0-2 weeks</td>
<td>0-13 weeks</td>
</tr>
</tbody>
</table>

This pattern reveals that **C. parvum** is the main cause of cryptosporidiosis in pre-weaned calves and adult cows act as asymptomatic carriers while **C. andersoni** mostly affect post-weaned calves.

The life cycle of **C. parvum** in calves is complete in about 4 days through the fecal-oral route. The infection causes diarrhea associated with profuse shedding of infective oocysts in the order...
of $10^6$ to $10^7$ /g of feces (Current, 1985). At least one large survey of the agents causing diarrhea in neonatal calves (Reynolds et al, 1986) found a highly significant association between Cryptosporidium species infection and diarrhea. Varying of dullness, anorexia, fever and loss of condition can occur. Diarrhea in C. parvum infection is usually mild (Anderson and Bulgin, 1981) though occasionally severe but transient resulting in varying degree of morbidity, but generally low mortality (De.Leeuw et al, 1984).

Infection has been confirmed in calves as young as 4 days (Snodgrass et al, 1980) and as old as 4 weeks (Tzipori et al, 1980). Because the life cycle of C. parvum in calves is only 4 days, clinical infection in a calf as young as 4 days would indicate heavy environmental infection in the calving area which shows the possible relationship between management practices in calf rearing unit. Calves between the ages of 1-2 weeks are highly vulnerable to other viral diseases and usually result to multiple infections.

2.6: Cryptosporidiosis in human

Cryptosporidium parvum has been recognized as a human pathogen since 1976 with the first cases being identified in a previously healthy 3-year old child from a farming community in rural Tennessee (Nime et al, 1976). Later another case was reported in a 39 year old college administrator who lived on cattle farm (Meisel et al, 1976).

During 1976-1982, the disease was reported rarely and occurred predominantly in immunocompromised persons. In 1982, the number of reported cases began to increase as a result of the acquired immunodeficiency syndrome (AIDS) epidemic. Initially, the increase
was limited to immunocompromised persons; however, outbreaks and sporadic infections in immunocompetent persons were identified with the aid of newly developed diagnostic techniques.

Cryptosporidiosis is increasingly recognized as an important agent of diarrhea in normal and immunocompromised humans, in particular, in infants and young children in developing countries in whom it is the major cause of persistent diarrhea (Lima and Guerrant, 1992). In human literature reveals that cryptosporidiosis accounts for up to 20% of the entire childhood diarrhea in the developing countries (Mosier and Oberst, 2000) because most people live in crowded dwellings, each occupied by several households and domestic animals. The majority of families have access to a pit latrine and shallow well and no system existed for the removal of waste. These home settings permit the household members to come in contact with animals, resulting in a number of human-animal contacts each year (Molbak et al, 1992).

Farming families may be especially susceptible to infection as indicated by Rahaman et al, (1984), in Bangladesh that showed 9.1% (7 of 77) of family members and infected farm attendants shed oocysts.

The primary mode of transmission for enteric pathogens is the fecal-oral route because animal fur, skin, and saliva (Keen and Elder, 2002) can become contaminated with fecal organisms. Transmission has also occurred from fecal contamination of food and water (Anon, 2000).
Illness has also been associated with contaminated clothing and shoes (Kiang et al., 2003), and with bedding, flooring, barriers, and other environmental surfaces.

Major outbreaks have been associated with drinking of contaminated water. There have been several human outbreaks of cryptosporidiosis associated with drinking and recreational water, occupational and food contamination (Centers for Disease Control and Prevention, 1995).

In immunocompetent patients, infections lead to invasion and destruction of the intestinal epithelium, causing disturbances of resorption and brush border enzymes with temporal secretory diarrhea lasting 1-30 days, accompanied by abdominal pain, vomiting and weight loss. Asymptomatic excretion of oocysts can occur. Clinical signs are usually transient resulting to total recovery. In immunocompromised individuals however, chronic diarrhea develops frequently leading to considerable fluid and weight loss and even death. Besides the upper intestines, the parasites also infect other organs like liver and bile ducts and even the airways (Farthing, 2000). The 50% infective dose has been reported to be as low as 9 oocysts (Feng et al., 2003).

The finding of C. parvum, C. meleagridis, C. felis, C. canis, C. muris and pig genotype 1 in humans suggests that farm animals, domestic pets, and some wildlife can be potential sources of human cryptosporidiosis (Current et al., 1983). Animals have been attributed to be the main source of C. parvum and other zoonotic cryptosporidium spp found in human and environment (Stantic-Pavlinic et al., 2003).
2.7: Diagnosis of cryptosporidiosis

Several techniques have been used in the identification of *Cryptosporidium* parasites in animal and human samples. These included:

2.7.1: Staining techniques

Most human and animals with cryptosporidiosis pass enough oocysts in their stools so that most of the concentration and/or staining techniques are adequate for detection and diagnosis and have been proved to be very efficient in detecting of oocysts.

A large number of staining techniques are based on the direct examination of wet smears, or smears treated with lugol, phenic fuchsin, methana mine silver, nigrosin, auramine-rhodamine, aridine orange, Giemsa, methylene blue/eosin,safranin-methylene blue and modified periodic acid-Schiff (Garcia et al, 1983). The diagnosis was improved by the discovery of the acid-fast property of *Cryptosporidium* oocysts. There are several staining techniques based on the original Ziehl-Neelsen staining (Pohjola et al, 1985). In this acid-fast staining, the oocysts of *Cryptosporidium* species are revealed as intense pink-reddish forms against a green or blue background depending on the dye used in the counter-staining step. On the other hand, counterstaining using carbol fuchsin, frequently stain for yeasts and the inexperienced may be confused by these yeast cells resembling oocysts in general appearance. The modified acid-fast staining has shown to be effective, reliable and feasible at any laboratory of clinical analysis due to the easy execution and low cost but do not allow distinguishing of species morphologically when oocysts are detected in faecal samples.
2.7.2: Immuno-Fluorescence Assays

These are more sensitive methods that use an antibody specific to Cryptosporidium to detect oocysts in specimens that contain few parasites and large amount of debris. These specimens include fecal samples from asymptomatic carriers or filtrates from surface of drinking water samples. This diagnostic technique is able to detect concentration as low as 1 to 10 oocysts/g feces. Previous evaluations of direct immunofluorescent microscopy with bovine fecal samples have shown that this procedure can detect oocyst concentration as low as 1000 to 6000 oocysts/g (Xiao and Herd, 1993). Given that calf faecal materials has a higher fat content than adult bovine fecal materials, the use of this method improves the chances of detecting the oocysts. Both polyclonal and monoclonal antibodies can be used in direct or indirect immunofluorescence assay (Garcia et al. 1989, Madore et al, 1987).

2.7.3: Enzyme immunoas says (EIA)

Commercial Enzyme Immunoassays (EIAs) have been developed to replace time-consuming microscopic methods. Cryptosporidium specific antigens (CSA) are associated with Cryptosporidium infection and are used as the basis for fluorescent and antigen capture immunoassays. This specific antigen has been found not to cross-react with those of other enteric parasites. CSA is derived from a protein extracted from the oocysts and is stable during transport through the intestinal tract of the host as well as during procedures used to collect and transport clinical specimens. This method performed better than microscopic methods with almost equal sensitivity with immunofluorescence assay.
2.7.4 Enzyme Linked Immuno sorbent Assay (ELISA)

The use of sero-diagnosis techniques for monitoring exposure of human and animals to Cryptosporidium species has been limited. Specific anti-Cryptosporidium IgG and/or IgM can also be detected by an enzyme-Linked immunosorbent assay (Current and Bick, 1989). This can be used in monitoring the infection. Additional evaluation are needed to confirm the utility of this serologic procedures for diagnosing and monitoring infections, to determine the prevalence of prior exposure in selected study population, and to determine if there is any correlation between the presence of Cryptosporidium-specific serum antibodies and resistance to re-infection.

2.7.5: Molecular identification of species/genotype of Cryptosporidium in biosamples

Polymerase chain reaction (PCR)-based assays provide a more useful alternative for detecting and quantifying Cryptosporidium in water, stool, and tissue/organ samples. A study report described PCR method that identified Cryptosporidium at the species level and required no DNA probes (Awad-EI Kariem et al, 1994). The procedure was based on the use of the published 18S rRNA genes of Cryptosporidium parvum and C. muris. The direct sequencing of either the 18S rRNA or Cryptosporidium oocyst wall protein (COWP) gene is the most useful tool for accurate identification of Cryptosporidium species (Abe et al, 2000). This technique has been used to compare Cryptosporidium isolates at the 18S small-subunit (ssu) rRNA gene locus. The assays have been developed to detect different Cryptosporidium species or genotypes and applied for the characterization of isolates of human and animal
origins. The identification of the species and the genotypes is based on sequence analysis or on restriction fragment length polymorphism (RFLP). Several real-time PCR procedures for detection and genotyping of Cryptosporidium parvum have also been developed and evaluated (Higgins et al, 2001). These molecular techniques have been used further to test samples which had been categorized as queried or Eimerian positive by microscopic methods. Molecular methods contribute to the accuracy and timely diagnosis of cryptosporidiosis in livestock with the further benefit of providing epidemiological data for both comparative phylogeny studies and disease control.

2.8: Treatment

One of the most biologically intriguing, and clinically frustrating, features of cryptosporidiosis is its resistance to antimicrobial drugs. Unlike many of its relatives (Toxoplasma gondii, Eimeria, and Plasmodium), there is no curative therapy for cryptosporidiosis, despite in vitro and in vivo testing of hundreds of compounds. One possible explanation for this is that Cryptosporidium establishes a compartment within the host cell, which is morphologically different from the setting used by the related parasites. This unique parasitophorous vacuole may somehow shelter the parasite from antimicrobial drugs (Griffiths, 1998).

Treatment of cryptosporidiosis in animals involves both prophylactic and chemotherapeutic drugs along with other preventive measures since there is no approved effective treatment. However, calves do need intensive supportive care. Sick calves should be housed in clean,
warm, and dry environment. They need fluid therapy to counteract and prevent further dehydration as well as electrolytes to replace those lost due to diarrhea. They also need nutritional support to give them energy to fight disease and repair their bodies.

In human, because the clinical course of cryptosporidiosis depends largely on the immune status of the host, treatment options vary accordingly (Griffiths, 1998). In immunocompetent adults and children, no specific therapy is indicated, since the disease is self-limiting; often associated (or subsequent) with malnutrition, which should be addressed. However, as in any diarrheal illness, dehydration must be carefully monitored. In individuals with persistent diarrhea, an underlying immunodeficiency (HIV infection, congenital immuno deficiency, etc.) should be considered. Nitazoxamide in immunosuppressed individuals have been tried. For individuals with AIDS, anti-retroviral therapy (HAART) has been used to improve immune status.

2.9.1 Control of cryptosporidiosis in calves

As elimination is difficult and relapse can occur, the control of human and animal cryptosporidiosis must focus on prevention targeting mainly the destruction of the oocyst in the environment. The following measures are designed for the controlling of outbreaks of cryptosporidiosis on the farms as applied with other enteric infections:-

Calf rearing should be on an all in all out basis. Individual pens should be stringently disinfected between batches of calves (Blewett, 1988). Calves should be born and raised in a clean, dry environment. Ideally, newborn calves should be penned individually for 2-3 weeks (Blewett, 1989). Sick calves should be removed immediately from the company of health
calves. Healthy calves should have different attendants from those of sick calves. Utensils should be heated and sterilized if possible daily. Attendants should keep boots, protective clothing as free of faces as possible. Vermin, farm dogs and cats should be controlled. Colostrum management and nutrition should be satisfactory. (Fayer, et al 1989). Approximate prophylactic measures against other agents such as rotavirus or ETEC-K99t vaccines should be employed.

2.9.2 Control of cryptosporidiosis in human

Sites that might be endemic need to boil all tap water regardless of whether an outbreak is ongoing or not depending on the source of water supply for an individual and the risk. Patients should be advised to avoid swimming in the water that may be risk of contamination e.g. lake water, or rain water. Education programs are important means of prevention of cryptosporidiosis targeting immunocompromised patients, the dairy farms, the water supply sectors, swimming pool and recreational water operators and general public. The programs should include information on risks of Cryptosporidium infection/contamination from various exposures, advised accordingly on minimizing the risks. There should be initiatives to promote the HACCP techniques in dairy farms.
CHAPTER 3

3.0: Materials and methods

3.1: Study site

The study was conducted in Dagoretti division in Nairobi during the period of August 2006 to December 2007. Dagoretti division is located in the Western part of Nairobi West District of Kenya at an altitude of 1860m above sea level (Fig 2). It consists of five locations (Uthiru, Ruthimitu, Mutuini, Waithaka, Riruta/Kawangware) with an area of 38.7 Km² (AMREF 2002). The division was previously a peri-urban but due to the expansion of Nairobi city, was incorporated in urban centre from the year 1963. The annual rainfall is bimodal, the long rains occur in mid March through July and the short rains in October and November. The mean annual rainfall varies from 750mm – 1800mm. The cattle production systems in the division are urban smallholder dairy production, classified as on plot and off plot. Urban agriculture on plot is carried out on the plots around houses while in off plots farmers utilize the public open space, utility service plots and any agricultural allotments within the city using cut and carry method of fodder supplementation (Lee-smith and Menon 1994). At present, the division has livestock population estimated as; 6948 cattle, 2192 goats, 1960 sheep, 2889 pigs, and 34,170 poultry (Ministry of Livestock Development 2000 Annual Report) with population of 240,509 (1999 population census) up from 41,409 in 1969. The land size has decreased and the livestock production is mainly under zero-grazing
The system is considered more to be intra-urban than peri-urban (Ekuttan, 2006) since it is within the city boundaries.

Figure 2: Map of Dagoretti Division. (Kang’ethe et al, 2005)
3. 2: Study design

A longitudinal study was conducted to establish the prevalence of Cryptosporidium and identify factors associated with the risk of infection of calves with C. parvum from September, 2006 to August, 2007.

3. 3: Calf sampling frame and sampling

The target population consisted of smallholder dairy farmers in Dagoretti Division, Nairobi, Kenya. Using a purposive sampling, pre-weaned calves between the ages of 7-30 days old were sampled from 296 dairy farmers chosen randomly from the sampling frame of 920 dairy households. The sampling scheme was based on the previous study (Kang’ethe et al. 2005) which estimated that there was at least one calf in every other three households. Dams’ gestation period were obtained from farms’ records or estimated when records were not available. Farmers notified the research team when each dam calved for sampling calves between 7-30 days old. Fecal samples were collected from the calf’s rectum using clean, disposable plastic gloves and transported to the laboratory in a cool box. No calf was sampled more than once during the study. A total of 143 fecal samples were collected and analyzed for the presence of Cryptosporidium species using a concentration floatation and centrifugation method.
3. 4: Household survey

A questionnaire was administered to the head of dairy household or any other members of the household during calf sampling (Appendix 1). The questionnaire focused on pre-weaning calf management, knowledge on Cryptosporidium infection and farm practices that may predispose household members to the infection. Information on awareness, transmission and prevention of cryptosporidiosis were collected.

3. 5: Participatory focused group discussion

This phase of the study was done using participatory approach tools. The community groups were selected in the five administrative locations in the study area. The community groups in the location consisted of both men and women and the team facilitators. The tools used consisted of a check list, scoring and ranking. The disease under study was introduced and through group discussions, knowledge gaps regarding the disease were identified. The discussion was designed to elicit information on the risk factors for cryptosporidiosis in both calves and humans. Questions that were asked covered general information on the farms i.e attitude and perception of the people towards the disease, calf management practices, handling of manure, disease and its control measures, nutrition, delivery of animal health services and gender disaggregated data showing the division of farm activities amongst the family members (Appendix 2).
3. 6: Detection of Cryptosporidium oocysts

3. 6.1: Microscopic examination (ME)

A sodium chloride floatation technique was used to concentrate the Cryptosporidium oocysts in calf feces. Ten grams of feces was suspended in distilled water and strained through a layer of cheese cloth to withhold the large debris. After sedimentation through centrifugation at 3000xg for 15 minutes, the supernatant was decanted and the sediment suspended in a sodium chloride solution (1.27 sp gravity). This was centrifuged at 3000xg for 10 minutes resulting in three layers; sediment, sodium chloride and aqueous layers. The upper aqueous layer containing the oocysts was washed with distilled water and centrifuged at 3000g for 15 minutes. The aqueous layer was decanted and the pellet stored at + 4°C prior to use.

3. 6.1.1: Modified Ziehl-Neelsen (MZN) Method (Pohjola et al, 1985)

Using the concentrated pellets obtained from 3.6.1 procedures above, moderately thick fecal smears were made, dried in air and placed in multi-slide carriers for fixation and staining in batches of up to 20 slides as follows; absolute alcohol fixation 1-2 minutes; Kinyoun’s carbol fuschin 8-10 minutes; followed by thorough rinsing; complete decolorization in 3% acid/alcohol; rinsing; counter-stain in 1% malachite green for 3-5min; rinsed air dried and examined under a x100 magnification. A positive control slide was stained with the samples each day as a quality control measure. A positive slide showed oocysts presenting as round
object, usually 4-5µm in diameter, with some degree of red staining of the mass filling the oocysts.

All positive samples and 20% of randomly selected negative samples were further tested using immunoflorescent antibody (IFA) technique.

3.6.1.2: Direct Immunofluorescence Assay (IFA) Test

The quantitative IFA was carried out by using the commercial Merifluor Cryptosporidium/Giardia Kit (Meridian Diagnostics, Inc., Cincinnati, Ohio) by modifying the method of Xiao and Herd (1993). The manufacturer’s recommendations were followed. The modification was by increasing the sample volume 1-5g to 10g of feces and changing sucrose to cesium chloride density gradient centrifugation and from bright field and phase—contrast to immunoflorescence microscopy. Using the concentrated pellets obtained from 3.6.1 procedures above, an aliquot of 10µl was pipetted into a treated IFA-slide well (11mm diameter) of a three well glass microscope slide and spread over the entire well and air dried at room temperature. A drop of detecting reagent and counter stain were added, mixed thoroughly and incubated in the humid chamber for 30 minutes at room temperature. The excess buffer was removed using the blotting paper. A drop of mounting media was added and the well covered with cover slip and examined at 100 x magnification under a fluorescence microscope. The number of Cryptosporidium oocysts was obtained by multiplying the total number of oocysts on the smear by 50. The IFA was used both as a comparison and quantitative assay to estimate
the intensity of the oocyst excretion in the calf samples. Any specimen containing one or more oocysts was considered positive for the presence of \textit{Cryptosporidium}.

\textbf{3. 6. 2: Nested PCR-RFLP for 18S rRNA}

DNA extracted samples using the QIAamp DNA Stool Mini Kit according to the manufacturer’s instructions (QIAGEN, QmbH, Germany) were used to process the targeted 18S rRNA gene in a two-step nested PCR protocol utilizing two oligonucleotide primer sets (Xiao \textit{et al}, 1999a). The primary set was added to the calf DNA extract and PCR amplification carried out to amplify an 830bp fragment of the 18S rRNA using primers \texttt{5'-TTCTAGAGCTAATACATGCG-3'} and \texttt{5'- CCCATTTCTCCGAAACAGGA -3'} for the primary PCR, while primers \texttt{5'-GGAAGGG TTGTATTTATTAGATAAAG-3'} and \texttt{5'AAGGAGT AAGGAACCAACCTCCA -3'} were used for the secondary PCR. Briefly, the PCR mix consisted of 5ml 10X perking Elmer PCR buffer, 8ml dNTP (1.5nM), 1.25\textmu l of each primer, 6.0 \textmu l mgcl\textsubscript{2} (2.5nM), 27.75 \textmu l distilled water and 0.25 \textmu l Taq polymerase in a final volume of 50 \textmu l. PCR was performed as follows: After an initial denaturation step of 3 minutes at 94 \degree C, a set of 35 cycles of runs, each consisting of 45 sec at 94 \degree C, 45 sec at 58 \degree C and 60 sec at 72 \degree C followed by a final extension for 7 minutes at 72 \degree C was performed. Visualization of the PCR product was through gel -electrophoresis.

Samples were sequenced and informative restriction sites were identified on multiple alignments. Aliquots of the nested PCR products were digested with the endonucleases VspI
and ssp1 and the resulting fragment separated by gel-electrophoresis (Matura et al., 2007). RFLP band patterns were compared to those known pattern for Cryptosporidium species.

3. 7: Data management and analysis.

All the data obtained from the field was recorded in a notebook and later entered into a computer using Microsoft Excel for ease of handling. The data was later transferred to Genstat® Discovery Edition 2 and descriptive statistics of continuous variables done.

The two tests, MZN and IFA were compared using Kappa test.

**Table 3: MZN and IFA comparison test**

<table>
<thead>
<tr>
<th>Tests</th>
<th>IFA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Modified Ziehl-Neelsen Test</strong></td>
<td>Samples</td>
</tr>
<tr>
<td>Positive</td>
<td>A</td>
</tr>
<tr>
<td>Negative</td>
<td>C</td>
</tr>
<tr>
<td>Total</td>
<td>a+c</td>
</tr>
</tbody>
</table>

Kappa test statistic was used to assess the level of agreement between the two tests (Martin et al., 1987).
CHAPTER 4

4.0: RESULTS

4.1: Household Characteristics

The majority of the dairy farming households were headed by males (72%; 213/296) while the majority of the respondents were female (72%; 212/296). About 84% (246/296) of the respondents were either household heads or their spouses.

Table 4 summarizes the distribution of dairy households in Dagoretti.

Table 4: Demographic characteristics of dairy households

<table>
<thead>
<tr>
<th>Household characteristic</th>
<th>Gender</th>
<th></th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Female</td>
<td>Male</td>
<td></td>
</tr>
<tr>
<td>Respondents</td>
<td>212 (72)</td>
<td>84 (28)</td>
<td>296</td>
</tr>
<tr>
<td>Household heads</td>
<td>83 (28)</td>
<td>213 (72)</td>
<td>296</td>
</tr>
</tbody>
</table>

Figures in brackets represent percentages

About 53% (157/296) of the total respondents were elderly (>50 years). At six households the respondents were youths 13-19 years of age. There were some age categories in which there appeared to be an uneven split in gender. In dairy households there were significantly fewer men than women in the 40-49 and 50-65 year age groups and more men than women members aged 20-29 years and this is the categories who were at the peak of child bearing age as shown in Table 5.
### Table 5: Household characteristics of the Dairy households in Dagoretti

<table>
<thead>
<tr>
<th>Age</th>
<th>Number of respondent</th>
<th>Sex</th>
<th>Female</th>
<th>P value</th>
<th>C.I</th>
</tr>
</thead>
<tbody>
<tr>
<td>13-19</td>
<td>6 (2.0)</td>
<td>4 (5)</td>
<td>2 (1)</td>
<td>0.036</td>
<td>(-0.086, 0.009)</td>
</tr>
<tr>
<td>20-29</td>
<td>29 (9.8)</td>
<td>13 (15)</td>
<td>16 (8)</td>
<td>0.039</td>
<td>(-0.164, 0.006)</td>
</tr>
<tr>
<td>30-39</td>
<td>50 (16.9)</td>
<td>12 (14)</td>
<td>38 (18)</td>
<td>0.451</td>
<td>(-0.055, 0.127)</td>
</tr>
<tr>
<td>40-49</td>
<td>54 (18.2)</td>
<td>8 (10)</td>
<td>46 (22)</td>
<td>0.014</td>
<td>(0.038, 0.206)</td>
</tr>
<tr>
<td>50-65</td>
<td>99 (33.4)</td>
<td>28 (33)</td>
<td>71 (33)</td>
<td>0.979</td>
<td>(-0.118, 0.121)</td>
</tr>
<tr>
<td>&gt;65</td>
<td>58 (19.6)</td>
<td>19 (23)</td>
<td>39 (18)</td>
<td>0.409</td>
<td>(-0.146, 0.061)</td>
</tr>
<tr>
<td>Total</td>
<td>296</td>
<td>84 (100)</td>
<td>212 (100)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figures in brackets represent percentages

### 4.2: Knowledge on the diseases transmitted through handling cattle dung

Results from the household survey showed that a half (50%, 149/296) of the cattle keepers (respondents) had general knowledge of the transmission of various diseases through handling cow dung. Knowledge of cryptosporidiosis among dairy households was still scanty as indicated by the low percentage of the respondents. Of the 149 who mentioned handling of cow dung as a means of disease transmission, 19.5% (29/149) listed cryptosporidiosis as one of the disease that can be transmitted through that pathway (Table 6). There was no significant difference in the proportion of male and female respondents who believed that disease could be contracted through contact with cattle dung in either the dairy households.
There was no significant difference between male and female who mentioned the diseases listed in Table 6.

4.3: Sources of information on cryptosporidiosis to dairy farming households

Knowledge on cryptosporidiosis was obtained from various sources. A total of 28% (83/149) respondents had gained information from the community meetings and crypto project and 13% (37/149) of the respondents from extension workers. Fifteen percent (46/149) of the respondents had gained information from sources such as family members, neighbors and reading of books or from radio/TV. This implied that the source of information in other dairy farming communities about cryptosporidiosis was totally absent. More women had knowledge from the community/project source than the men. Table 7 summarizes the respondents’ sources of information in the study area.

### Table 6: Knowledge of disease transmission in dairy farming households by gender

<table>
<thead>
<tr>
<th>Characteristic (knowledge of)</th>
<th>N=(no. of respondents)</th>
<th>Total proportion (%)</th>
<th>Proportion of yes counts by gender</th>
<th>P-value</th>
<th>Confidence interval (C.I)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Know of disease transmitted through cattle dung</td>
<td>296</td>
<td>50.3 (149/296)</td>
<td>Male 52% (44/84) female 49.5% (105/212)</td>
<td>0.658</td>
<td>(-0.004-0.022)</td>
</tr>
<tr>
<td>Cryptosporidiosis</td>
<td>149</td>
<td>19.5 (29/149)</td>
<td>Male 16% (7/44) female 21% (22/105)</td>
<td>0.594</td>
<td>(-0.05152,0.09240)</td>
</tr>
<tr>
<td>Worm</td>
<td>149</td>
<td>14.8 (22/149)</td>
<td>Male 16% (7/44) female 4% (15/310)</td>
<td>0.710</td>
<td>(-0.08102,0.05587)</td>
</tr>
<tr>
<td>E.coli</td>
<td>149</td>
<td>6.71 (10/149)</td>
<td>Male 11.4% (5/44) female 4.8% (5/105)</td>
<td>0.123</td>
<td>(-0.09050,0.01863)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>149</td>
<td>29.53 (44/149)</td>
<td>Male 27.3% (12/44) female 28.6% (32/105)</td>
<td>0.860</td>
<td>(-0.08092,0.09709)</td>
</tr>
</tbody>
</table>
Table 7 Sources of information on cryptosporidiosis to dairy farming households by gender.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Proportion of yes count</th>
<th>Gender counts</th>
<th>p value</th>
<th>C.I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extension agents</td>
<td>13%(37/149)</td>
<td>Males 22% (10/44) Family 25.7% (27/105)</td>
<td>0.874</td>
<td>(-0.1038, 0.1222)</td>
</tr>
<tr>
<td>Family members</td>
<td>4%(6/149)</td>
<td>Male 6.8% (3/44) Female 3% (3/105)</td>
<td>0.235</td>
<td>(-0.064, 0.021)</td>
</tr>
<tr>
<td>Neighbors or other community members</td>
<td>13%(20/149)</td>
<td>Male 11.4% (5/44) Family 14.3% (15/105)</td>
<td>0.729</td>
<td>(-0.050, 0.072)</td>
</tr>
<tr>
<td>Read books</td>
<td>11%(16/149)</td>
<td>Male 16% (7/44) Female 8.6% (9/105)</td>
<td>0.845</td>
<td>(-0.074, 0.09083)</td>
</tr>
<tr>
<td>Community meeting/project</td>
<td>28%(83/149)</td>
<td>Male 27% (23/44) Female 28% (60/105)</td>
<td>0.049</td>
<td>(-0.13, 0.010)</td>
</tr>
<tr>
<td>Radio/TV</td>
<td>2%(3/149)</td>
<td>Male 0% (0/44) Family 3% (3/105)</td>
<td>0.273</td>
<td>(-0.0017, 0.030)</td>
</tr>
</tbody>
</table>

4.4 Knowledge of the modes of transmission of cryptosporidiosis

A majority of the household 83.2 % (124/149) respondents said that the disease was transmitted through handling cow dung. Five point four percent (8/149) attributed it to handling of cattle, 12.1% (18/149) through drinking water. Other routes of transmission are as shown in the Table 8. There was no significant difference between males and females respondents on the modes of transmission of cryptosporidiosis.
Table 8: Knowledge on the modes of transmission of cryptosporidiosis.

<table>
<thead>
<tr>
<th>Pathway of disease transmission</th>
<th>Proportion of respondents (yes)</th>
<th>p-value</th>
<th>Confidence Interval (C.I)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total (n=149)</td>
<td>Female (n=105)</td>
<td>Male (n=44)</td>
</tr>
<tr>
<td>Manure</td>
<td>30 (20.1)</td>
<td>30 (28.6)</td>
<td>0</td>
</tr>
<tr>
<td>Handling cattle</td>
<td>8 (5.4)</td>
<td>5 (4.8)</td>
<td>3 (6.8)</td>
</tr>
<tr>
<td>Handling other animals</td>
<td>2 (1.3)</td>
<td>1 (1.0)</td>
<td>1 (2.3)</td>
</tr>
<tr>
<td>Washing water</td>
<td>3 (2.01)</td>
<td>3 (2.9)</td>
<td>0</td>
</tr>
<tr>
<td>Drinking water</td>
<td>18 (12.1)</td>
<td>13 (12.4)</td>
<td>5 (11.4)</td>
</tr>
<tr>
<td>Drinking milk</td>
<td>67 (45)</td>
<td>44 (41.9)</td>
<td>23 (52.3)</td>
</tr>
<tr>
<td>Food</td>
<td>33 (22.1)</td>
<td>22 (21.0)</td>
<td>11 (25)</td>
</tr>
<tr>
<td>Handling dung</td>
<td>124 (83.2)</td>
<td>87 (83)</td>
<td>37 (84.1)</td>
</tr>
<tr>
<td>Don’t Know</td>
<td>6 (4.03)</td>
<td>6 (5.7)</td>
<td>0</td>
</tr>
</tbody>
</table>

Figures in brackets represent percentage

4.5. Knowledge on the methods of protection against cryptosporidiosis

Half of the respondents (51%, 151/296) said that one could protect him/herself from the disease through various methods most of them citing the use of protective clothing (80.8%, 122/151) as the main mode of prevention. Other modes of prevention mentioned were as shown from Table 9. There was no significant difference between the protective methods mentioned by male and female respondents.
Figures in brackets represent percentages.

**Table 9: Knowledge on the methods of protection of Cryptosporidium infection**

<table>
<thead>
<tr>
<th>Preventive measures</th>
<th>Proportion of respondents(n=151)</th>
<th>P value</th>
<th>Confidence interval.(C.I)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total n=151</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Male n=45</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Female n=106</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boil Milk</td>
<td>42(27.8)</td>
<td>0.734</td>
<td>(-0.07114, 0.1017)</td>
</tr>
<tr>
<td>Protective Clothing</td>
<td>122(80.8)</td>
<td>0.251</td>
<td>(-0.1980, 0.05249)</td>
</tr>
<tr>
<td>Wash vegetables/ fruits</td>
<td>3(2.0)</td>
<td>0.273</td>
<td>(-0.001748, 0.03005)</td>
</tr>
<tr>
<td>Protect water source</td>
<td>2(1.0)</td>
<td>0.496</td>
<td>(-0.03215, 0.01777)</td>
</tr>
<tr>
<td>Boil Water</td>
<td>12(7.9)</td>
<td>0.698</td>
<td>(-0.06215, 0.04239)</td>
</tr>
<tr>
<td>Wash Body</td>
<td>10(6.6)</td>
<td>0.550</td>
<td>(-0.02756, 0.05541)</td>
</tr>
<tr>
<td>Wash Hands</td>
<td>32(21.1)</td>
<td>0.973</td>
<td>(-0.07693, 0.07963)</td>
</tr>
<tr>
<td>Don’t know</td>
<td>11(7.3)</td>
<td>0.033</td>
<td>(0.02203, 0.08174)</td>
</tr>
</tbody>
</table>

4.6.1 Knowledge on farm practices

Almost every household (98%, 290/296) grew food crop for home consumption and 96% (284/296) of them used cattle manure either alone or in combination with chemical fertilizer and/or manure from other animal species (Table 10). Ninety seven point six percent (289/296) of the dairy households heaped manure as a mode of treatment. Manure could act as a reservoir of Cryptosporidium oocysts and improper handling could predispose one to contamination and subsequent infection.
Table 10: Use of manure/chemical fertilizer in crops by gender.

<table>
<thead>
<tr>
<th>A type of fertilizer used on crops</th>
<th>Number of respondents</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Female (n=212)</td>
<td>Male (n=84)</td>
<td>Total (n=296)</td>
<td>Percent (%)</td>
</tr>
<tr>
<td>Chemical fertilizer alone</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0.34</td>
</tr>
<tr>
<td>Chicken manure alone</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>0.68</td>
</tr>
<tr>
<td>Chicken manure/chemical</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>0.68</td>
</tr>
<tr>
<td>Cow manure alone</td>
<td>74</td>
<td>25</td>
<td>99</td>
<td>33.45</td>
</tr>
<tr>
<td>Cow manure/chemical</td>
<td>50</td>
<td>32</td>
<td>82</td>
<td>27.7</td>
</tr>
<tr>
<td>Cow and other manure + Chemical</td>
<td>34</td>
<td>13</td>
<td>47</td>
<td>15.88</td>
</tr>
<tr>
<td>Not applicable</td>
<td>6</td>
<td>0</td>
<td>6</td>
<td>2.03</td>
</tr>
<tr>
<td>other manure + cow manure, no Chemical</td>
<td>44</td>
<td>12</td>
<td>56</td>
<td>18.9</td>
</tr>
<tr>
<td>Unknown count</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0.34</td>
</tr>
<tr>
<td>Total</td>
<td>212</td>
<td>84</td>
<td>296</td>
<td>100</td>
</tr>
</tbody>
</table>

4.6.2: Respondents’ attitude and perception of cryptosporidiosis in dairy farming households.

In the focus group discussion, there were 86 participants of which 34 were males and 52 were females. These were representatives selected as contact farmers (active and leading local farmers) from each location in the study area who would pass extension messages to other farmers.
Table 11: Number of participants by location

<table>
<thead>
<tr>
<th>Location</th>
<th>Sex</th>
<th></th>
<th></th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>female</td>
<td>Male</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mutuini</td>
<td>14</td>
<td>8</td>
<td></td>
<td>22</td>
</tr>
<tr>
<td>Riruta/Kawangware</td>
<td>6</td>
<td>3</td>
<td></td>
<td>9</td>
</tr>
<tr>
<td>Ruthimitu</td>
<td>20</td>
<td>11</td>
<td></td>
<td>31</td>
</tr>
<tr>
<td>Waithaka</td>
<td>8</td>
<td>8</td>
<td></td>
<td>16</td>
</tr>
<tr>
<td>Uthiru</td>
<td>4</td>
<td>4</td>
<td></td>
<td>8</td>
</tr>
<tr>
<td>Grand Total</td>
<td>52</td>
<td>34</td>
<td></td>
<td>86</td>
</tr>
</tbody>
</table>

Most participants (58.1%; 50/86) considered themselves to be at risk of cryptosporidiosis of which 68% (34/50) were women. They associated the risk to close contact with cattle, lack of protective clothing and consumption of contaminated water, milk or food. Forty one point nine percent (36/86) did not consider themselves to be at risk of which 55.6% (20/36) said that they were informed while the rest 44.4% (16/36) said that they had not heard about the disease.

About 77.9% (67/86) of the participants said that calves could be a source of cryptosporidiosis since they are prone to diseases just like the adults. Of the participants 5.8% (5/86) also mentioned that they were more in contact with the calves than they were with adult cattle.

4. 6.3: Respondent s’ calf management

Of the participants, 13.9% (12/86) did not separate the calves from the dam immediately, 47.7% (41/86) separated immediately after birth (<12 hours) to prevent the calf from suckling
and to avoid low milk production while 38% (33/86) separated after sometime (>12 hours). Fifty two percent (45/86) housed the calves within the cattle shed and 44.6% (25/86) housed the calves in the kitchen while 10.5% (9/86) kept their calves away (1.5-6.0m) from the cattle shed. Seventy eight percent (66/85) housed their calves singly while the rest housed the calves together.

Sixty four percent (50/79) hand fed the calves with colostrums while 27.9% (24/86) combined hand feeding and letting the calf suckle from the dam. The remaining 6% (5/78) left the calf to run with the dam for days (up to a month).

Eighty six percent (67/78) of the participants fed the calves with colostrum in less than 12 hours after birth while 12% (8/78) fed colostrum later than 12 hours.

Eighty four percent (59/70) of the participants housed their calves in pens with unpaved floor, 12.9% (9/70) did so on raised wooded while 2.9% (2/70) had concrete floor. Sixty seven percent (42/63) used straw/dry grass as beddings, 19% (12/63) sawdust, and 9.5% (6/63) either used wood shavings, sugarcane husks, wastepaper or no beddings.

In treatment of calves, 51% (40/79) separated the sick calves from the healthy ones and treated while 49.4% (39/79) treated without separation. Fifty four percent (43/79) of the participants called a doctor. Six of those who did not separate their sick calves had no knowledge that the sick calves could transmit disease to the healthy ones. Of the participants, 52.7% (41/58) consulted an animal health specialist in case of calf scour while 24.3% (17/58) cut back on milk.
Eight seven percent (68/78) watered their calves introducing in small amounts to avoid water poisoning while the rest did not water the ir calves at all.

**4. 6. 5: Respondent response in management of manure**

Ninety two percent (79/86) of the participants changed the calf beddings. Of these 36.4% (28/77) changed daily while the rest did so when the beddings were soiled (3 days to 2 weeks) and 6.5% (5/77) added a fresh layer on top of the soiled one. Eighty one (63/78) used wheelbarrow, 34.6% (27/78) sacks, and 5% (4/78) buckets to collect calf dung/manure. Forty nine percent (38/78) of the participants also used spades or bare hands although 97.4% (76/78) of the participants used the same equipments to clean the cowshed for the adult cattle without cleaning/disinfecting them in between use. Forty seven percent (36/78) used protective clothing when cleaning the calf-pen, 55.6% (20/36) used old cloths, 44.4% (16/36) gumboot, and 19.4% (7/36) gloves. Others did not use any protection because they either did not have or did not think that dung/manure could predispose them to disease.
4. 6. 6: Farm activities among the household members

Dairy household members were probed on the behavioral changes that related to farming practices. These included education to change cultural tendencies in dairy allocation. This was done to investigate the involvement of gender in balancing practices hence fair distribution of tasks in farming enterprise. Table 12 shows the responses of the focus group discussion participants:

Table 12: Male respondents’ on farm activities among the household members.

<table>
<thead>
<tr>
<th>Activities per household member</th>
<th>Male household</th>
<th>Female household</th>
<th>Male worker</th>
<th>female worker</th>
<th>Boy child</th>
<th>Girl child</th>
<th>Others*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feeding</td>
<td>30.1 **</td>
<td>33.2</td>
<td>18.2</td>
<td>0</td>
<td>12.4</td>
<td>5.6</td>
<td>0.4</td>
</tr>
<tr>
<td>Cleaning of calf-pen</td>
<td>34.2</td>
<td>24.8</td>
<td>21.4</td>
<td>0</td>
<td>17.9</td>
<td>0.5</td>
<td>1.2</td>
</tr>
<tr>
<td>Manure disposal</td>
<td>33.9</td>
<td>17.4</td>
<td>27.3</td>
<td>0</td>
<td>19.0</td>
<td>0.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Watering of calves</td>
<td>23.1</td>
<td>33.9</td>
<td>26.5</td>
<td>0</td>
<td>7.7</td>
<td>2.8</td>
<td>2.4</td>
</tr>
<tr>
<td>Giving treatments</td>
<td>56.5</td>
<td>33.3</td>
<td>4.0</td>
<td>0</td>
<td>5.2</td>
<td>0.6</td>
<td>0.4</td>
</tr>
</tbody>
</table>

*Other members include grand children, and an animal health specialist in treatment of the calves, **Figures represent percentages
Table 13: Female respondents’ on farm activities among the household members.

<table>
<thead>
<tr>
<th>Activities per household member</th>
<th>Male household</th>
<th>Female household</th>
<th>Male worker</th>
<th>Female worker</th>
<th>Boy child</th>
<th>Girl child</th>
<th>others *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feeding</td>
<td>8.6**</td>
<td>69.3</td>
<td>10.1</td>
<td>0</td>
<td>4.2</td>
<td>6.1</td>
<td>0</td>
</tr>
<tr>
<td>Cleaning of calf-pen</td>
<td>9.1</td>
<td>46.8</td>
<td>29.1</td>
<td>0</td>
<td>6.2</td>
<td>8.0</td>
<td>0.3</td>
</tr>
<tr>
<td>Manure disposal</td>
<td>11.7</td>
<td>49.7</td>
<td>27.8</td>
<td>0</td>
<td>7.5</td>
<td>3.3</td>
<td>0</td>
</tr>
<tr>
<td>Watering of calves</td>
<td>7.3</td>
<td>54.9</td>
<td>14.7</td>
<td>0</td>
<td>6.9</td>
<td>11.9</td>
<td>4.3</td>
</tr>
<tr>
<td>Giving treatments</td>
<td>16.2</td>
<td>49.7</td>
<td>8.0</td>
<td>0</td>
<td>3.6</td>
<td>0.8</td>
<td>16.1</td>
</tr>
</tbody>
</table>

- Other members include grand children, and animal health specialist in treatment of the calves.
- **Figures represent percentages.

From the above tables 12 and 13 most calf management activities were carried out by female, male household and male worker and these were likely to be at high risk compared to other members of the family (Appendix 3).

4.7: Household practices mitigating against cryptosporidiosis in smallholder dairy farmers in Dagoretti, Nairobi Kenya

4.7.1: Respondent’s control measures in dairy farming households

Responding to the control measures they would employ against cryptosporidiosis, the following were mentioned and scored as in the tables below:
Table 14: Female respondents’ scores on household practices mitigating against cryptosporidiosis.

<table>
<thead>
<tr>
<th>Preventive measures proposed by female respondents (%)</th>
<th>Females average score of preventive measures per location</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Riruta (n=6)</td>
</tr>
<tr>
<td>--------------------------------------------------------</td>
<td>--------------</td>
</tr>
<tr>
<td>Personal hygiene</td>
<td>0</td>
</tr>
<tr>
<td>Wear protective clothing when handling manure</td>
<td>14.5</td>
</tr>
<tr>
<td>Cleaning cowshed</td>
<td>16.8</td>
</tr>
<tr>
<td>Clean milking equipment s</td>
<td>13.8</td>
</tr>
<tr>
<td>Boil milk</td>
<td>10.8</td>
</tr>
<tr>
<td>Direct run-off away from source of water</td>
<td>11.0</td>
</tr>
<tr>
<td>Wash vegetables/fruits</td>
<td>9.0</td>
</tr>
<tr>
<td>Wash hands</td>
<td>15.8</td>
</tr>
<tr>
<td>Avoid cut grass by roadside</td>
<td>-</td>
</tr>
<tr>
<td>Compost manure</td>
<td>-</td>
</tr>
<tr>
<td>Clean waste and disposal equipments</td>
<td>-</td>
</tr>
<tr>
<td>Boil drinking water</td>
<td>8.3</td>
</tr>
<tr>
<td>Clean milking area</td>
<td>-</td>
</tr>
<tr>
<td>Total score</td>
<td>100</td>
</tr>
</tbody>
</table>

NB-members of Mutuini were time barred for pilling and scoring control measures and the Uthiru participants were combined (male and female).
Table 15: Male respondents’ scores on household practices mitigating against cryptosporidiosis.

<table>
<thead>
<tr>
<th>Preventive measure proposed by male respondents</th>
<th>Males average score of preventive measures per location (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mutuni (n=8)</td>
</tr>
<tr>
<td>Personal hygiene</td>
<td>25.3</td>
</tr>
<tr>
<td>Wear protective clothing</td>
<td>14.8</td>
</tr>
<tr>
<td>Clean shed</td>
<td>27.0</td>
</tr>
<tr>
<td>Clean milking equipment s</td>
<td>9.5</td>
</tr>
<tr>
<td>Boil milk</td>
<td>10.4</td>
</tr>
<tr>
<td>Direct run-off away from source of water</td>
<td>-</td>
</tr>
<tr>
<td>Wash vegetables/fruit</td>
<td>-</td>
</tr>
<tr>
<td>Wash hands</td>
<td>13.0</td>
</tr>
<tr>
<td>Compost manure</td>
<td>-</td>
</tr>
<tr>
<td>Boil drinking water</td>
<td>-</td>
</tr>
<tr>
<td>Clean milking area</td>
<td>-</td>
</tr>
<tr>
<td>Total score</td>
<td>100</td>
</tr>
</tbody>
</table>
Table 16: Summary of average scores on household practices mitigating against cryptosporidiosis by gender

<table>
<thead>
<tr>
<th>Preventive measure proposed by respondents</th>
<th>Average score of preventive measures per gender (%)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males score(n=30)</td>
<td>Females score(n=48)</td>
</tr>
<tr>
<td>Personal hygiene</td>
<td>16.3</td>
<td>12.2</td>
</tr>
<tr>
<td>Clean cow shed</td>
<td>11.3</td>
<td>15.5</td>
</tr>
<tr>
<td>Wear protective clothing</td>
<td>13.6</td>
<td>12.8</td>
</tr>
<tr>
<td>Clean milking equipments</td>
<td>12.2</td>
<td>13.5</td>
</tr>
<tr>
<td>Boil milk</td>
<td>14.2</td>
<td>11.4</td>
</tr>
<tr>
<td>Wash hands</td>
<td>15.9</td>
<td>6.5</td>
</tr>
<tr>
<td>Wash vegetables/fruit</td>
<td>5.8</td>
<td>6.5</td>
</tr>
<tr>
<td>Direct run-off away from source of water</td>
<td>3.3</td>
<td>7.9</td>
</tr>
<tr>
<td>Clean milking area</td>
<td>2.5</td>
<td>10.2</td>
</tr>
<tr>
<td>Boil drinking water</td>
<td>2.1</td>
<td>6.4</td>
</tr>
<tr>
<td>Clean waste and disposal equipments</td>
<td>-</td>
<td>2.9</td>
</tr>
<tr>
<td>Compost manure</td>
<td>2.8</td>
<td>-</td>
</tr>
</tbody>
</table>

4.8.1: Prevalence of cryptosporidiosis in calves

One hundred forty four calves aged between 7 and 30 days were sampled during the sampling season. Of these, 117 fecal samples were examined for the presence of Cryptosporidium oocysts to investigate the prevalence of Cryptosporidium infection. The prevalence value was obtained by taking the number testing positive on Modified Ziehl-Neelson (MZN) test and dividing with the total number of calf samples studied.
The oocysts of \textit{Cryptosporidium} were found in all age categories. The highest prevalence (23.53\%) was found in calves aged between 15-21 day old (Table 17) with the overall prevalence of 23.1\% (27/117).

<table>
<thead>
<tr>
<th>Age of calves (days)</th>
<th>Number of examined samples</th>
<th>Number of calves infected</th>
<th>Infection rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7-14</td>
<td>25</td>
<td>7</td>
<td>8.3</td>
</tr>
<tr>
<td>15-21</td>
<td>34</td>
<td>8</td>
<td>23.53</td>
</tr>
<tr>
<td>22-30</td>
<td>58</td>
<td>12</td>
<td>20.7</td>
</tr>
<tr>
<td>Total</td>
<td>117</td>
<td>27</td>
<td>23.1</td>
</tr>
</tbody>
</table>

\textbf{Table 17: Prevalence of Cryptosporidium species in calves.}

4.8.2: \textbf{Comparison of Modified Ziel-Neelson and Immunofluorescence Assay (IFA) using Kappa statistic.}

To compare the detection efficiency of the method used for examination of fecal specimens, all positive samples (27 calf faecal samples) and 20\% of the negatives (28 faecal samples) randomly selected were subjected to immunofluorescence assay (IFA) test of which 23.6\% (13/55) of the samples tested were positive (Table 18). Sensitivity and specificity of the modified ziehl-Neelsen test was 69.2\% and 57.1\% respectively using IFA test as a Gold standard test. Agreement between the two tests was 0.45. This was a moderate agreement. This finding showed that the two tests were measuring the same thing.
**Table 18: comparison tests results (MZN and IFA)**

<table>
<thead>
<tr>
<th></th>
<th>IFA</th>
<th></th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>MZN</td>
<td>+</td>
<td>-</td>
<td>Total</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>18</td>
<td>27</td>
</tr>
<tr>
<td>-</td>
<td>4</td>
<td>24</td>
<td>28</td>
</tr>
<tr>
<td>Total</td>
<td>13</td>
<td>42</td>
<td>55</td>
</tr>
</tbody>
</table>

MZN: Modified Ziehl-Neelsen and IFA: Immunofluorescence Assay, +: positive, -: negative.

Intensity of oocysts excretion in calves < 1 month from the 13 positive samples on IFA ranged between $2.5 \times 10^6 - 5.0 \times 10^7$ oocysts/gm of feces with an average of $1.83 \times 10^7$ oocysts/gm of feces.

**4.8.3: The speciation of cryptosporidia in calves using PCR.**

Thirteen positive samples on IFA were sent to Cornell University for sequencing and identification of which 15% (2/13) amplified, sequenced and identified as having *Cryptosporidium ryanae* (*Cryptosporidium* deer-like). The two samples were both from male calves, 14 days old. The rest of the samples did not amplify.
CHAPTER 5

5.0 Discussion

Urban agriculture has the potential to help meet the Millennium Development Goals (MDGs) of the United Nations. Urban food production particularly of roots and tuber crops, bananas, fruit trees, vegetables and small scale livestock production contribute to food security and income generation. It provides a safety net for the poor, who do not have access to credit or other form of savings. This helps to address Millennium Development Goals 1 of eradication of poverty and hunger. As other urban centres, Dagoretti study demonstrates that more women than men were involved in urban dairy production and the majority of them were in the middle age to early elderly age. These are vulnerable groups (old and child bearing women) with low body immunity. Other studies have shown gender differences in urban and peri-urban agriculture (Atukunda, et.al. 2003) where women were mainly involved in farming while men engaged in various forms of employment. There is a general notion that more women than men are active in urban agriculture (Wilbers, 2004). In parts of Africa urban farming is predominately a women affair. This attributed to the fact that women continue to bear the main responsibility for household nourishment and well being. Additionally women have low educational status than men thus finding it difficult to secure a formal wage employment (Hovorka, Alice, 2003). Urban agriculture therefore, presents a livelihood strategy which gives good return to limited resource input. However, the study revealed the participants had limited knowledge that unhygienic practices of handling cattle (and their neonates) and their waste (dung) could expose them to health risks.
From the previous study conducted in the same study area by Kang’ethe et al. (2005), recording a herd cryptosporidiosis prevalence of 11% in calves, the community had no or little knowledge (1%) about the existence of cryptosporidiosis. Among the respondents interviewed with regard to the general disease transmission through handling cattle dung, 50.3% were fully aware of the various diseases that could be transmitted and of these, 19.5% (29/149) regarded cryptosporidiosis to be among the diseases transmitted through dung.

Like other control programmes in the world the project realized that education of the local population was the basis for the control of cryptosporidiosis in the dairy households since it is essential to reduce risks associated with animal contact. Experience from outbreaks suggests that people knowledgeable about potential risks were less likely to become ill (CDC, 2004 and 2005). Being one of the emerging zoonotic diseases that cause diarrhea in human and animals, the study emphasized on public education on the risk pathways. Therefore the more people know about hygienic husbandry practices the better they are able to keep themselves and their families well. From this study it was found that the main source of information on the knowledge of cryptosporidiosis in Dagoretti division was through the project team members and extension workers who are Government officers who were collaborators in this project. This implied that few dairy farmers or their neighboring communities are aware of this disease. The research project meetings have raised awareness of the disease and it hoped that further work with the community will continue to educate and promote good hygiene.
The mode of transmission was found to be important factor associated with the occurrence of the disease. In the current study, most of the respondents knew the mode of transmission with half of the respondents saying transmission occurred through handling of manure. When probed further more than 80% of those answering in the affirmative said that the disease was transmitted through handling the dung. However only 5% or less of respondents believed it could be transmitted from handling cattle. Hunter et al, (2003) showed the significance of cattle in the zoonotic transmission of Cryptosporidium in the United Kingdom during the 2001 Foot and Mouth disease outbreak where the animal contact restrictions was put in place during that outbreak. This resulted in a significant reduction in the number of cases of human cryptosporidiosis caused by Cryptosporidium parvum. Water being one of the risk pathways of infection with cryptosporidiosis, only 2% of the respondents interviewed associated the risks of exposure to cryptosporidiosis through contaminated water. However, waterborne outbreaks have been blamed on agricultural sources such as cattle facilities; manure runoff from feedlots, and spraying of liquid manure on field (Casey, 1991) and dairy cattle has been implicated to be the main source with special emphasis on calves (Kirkpatrick, 1995; Xiao and Herd, 1994).

Several outbreaks indicate that failure to understand the mode of disease transmission and properly implement disease prevention recommendations can lead to transmission as indicated in Minnesota outbreak of cryptosporidiosis involving infections of students at farm program where specific recommendation (washing hands with soap after touching a calf and
washing hands before going home) were provided to the teachers but were inadequately implemented (Kiang et al, 2003). The same observation was seen in this study where majority of dairy respondents interviewed, 96% (284/296) used cattle manure to grow food on the homestead and 51% believed that they could protect themselves from these diseases. Most of them cited wearing of protective clothing (86.5% 122/151) and 22.7% (32/151) washing hands as the mode of protection. In practice, it was observed that only 12% (36/296) and 7% (21/296) of households’ members wore protective clothing and washed their hands respectively during cattle fecal sampling. They put more emphasis on boiling of milk compared to other protective measures most probably due to sensitization from first phase of the project (Kang’ethe et al, 2005). It was clear that few dairy farmers were aware of this disease, cryptosporidiosis. Even those farmers who had heard of the disease did not understand the mode of transmission hence the preventative measures. The research project meetings have raised awareness of the disease and it is hoped that further work with the community will continue to educate and promote good hygiene.

Fayer et al 1998, indicated that application of manure on the farm poses a risk to several foodstuffs such as fruits and vegetables which could be contaminated with cryptosporidium oocysts. The association of oocysts contamination of these produce is important from public point of view as these products are consumed raw without thermal processing to inactivate contaminating oocysts due to polluted environment with agricultural run-off.
**Respondents' attitude and Perception of cryptosporidiosis in dairy farming households**

From the outcome of this survey, it was found that both men and women had equal chances of exposing themselves to *Cryptosporidium*. They associated the risks to close proximity of the cow shed to their residential houses, contact with cattle and manure and lack of protective clothing. The observed directional association of pre-weaning practices that were associated with decreased risk of infection is consistent with what is reported in the literature. For example, Quigley et al. (1994) found that there was an increased risk of infection with *C. parvum* if the calves were left to nurse with the cow while hand feeding eliminated the contact between the calf and the cow, and the time the calf spent in the calving pen so that the risk of transmission of infection is reduced.

Cryptosporidiosis is an infectious disease and any process that increases the probability that a susceptible animal will come into contact with the infectious agent will be a risk factor for the disease. In this study some farmers practice group housing of calves which increases the risk of infection with *Cryptosporidium*. This finding is consistent with the recommendation in the literature of not grouping animals together because this increases contact between animals and the risk of contracting infectious diseases (Heath, 1992, Garber, et al, 1994). Furthermore, susceptible calves in contact with other calves were likely to be exposed to *Cryptosporidium* oocysts from an animal that is shedding the organisms.

Examining the constituents of the pre-weaning indices it was found that addition of clean bedding and daily disposal of bedding significantly decrease the risk of infection with *C.
This finding is consistent with general housing recommendations to decrease the risk of exposure to pathogens (Fayer and Ungar, 1986; Heath, 1992). Although it is presumed that improved hygiene will stop the spread of an infectious disease, biosecurity breakdown could directly enhance the spread of the disease in the farm. It has been observed from this study that few farmers had biosecurity program. The process of bedding removal involves walking and using equipment between animal groups and pens. In this process, personnel and equipment becomes fomites for spreading infection as is emphasized from a study by Maldonado- Camargo et al, (1998). Calf to calf contact, crowding, or continuous use of facilities, the distance between the calf-pen and the main cattle shed posed a greater risk in the transmission of the disease. This prolongs the survival rate and increases the number of pathogens in the calf environment.

Also in the calf management, the data indicated that most calves were fed on colostrum during the first 24 hour of life. Its impact on health, as indicated by Quigley et al, (1994) did not reduce the risk of Cryptosporidium infection but the importance of adequate consumption of colostrum during this period is well recognized in improving the immunity in calves (Fayer and Ungar, 1986; Heath, 1992).

In terms of farm daily activities in calf management, there was no gender balance in the farm chores. The mean scores of various activities indicated that so much was left to particular members of the households, the women household members carrying the bigger burden, over-exposing them. Education is required to change cultural tendencies in duty allocation and balanced distribution of tasks in early childhood.
Being a profitable enterprise, urban smallholder dairy farmers need to identify the preventive measures for the protection against associated risks with *Cryptosporidium* infection. From the study control measures suggested by the participant were scored and ranked. If seriously followed, would promote small urban dairy production with reduced chances of disease transmissions.

It was important that the risks to human health from this zoonosis are accurately estimated and put in context with the risks to livelihoods and nutrition from restricting urban farming. In this study, the prevalence of *Cryptosporidia* shedding among un-weaned animals was 23.1%, a level significantly higher than the overall herd level prevalence of 11% from the previous study (Kang’ethe et al, 2005) and in keeping with other reports in the literature that this age group has the highest prevalence of the organism (Sartin et al, 2004). Reports from many studies have shown that infection is common in calves (Mohammed, Wade, and Schaaft, 1999) with highest rate of shedding observed between ages of one to three weeks with peak shedding at 12-16 days of age (Nydam et al, 2001).

As indicated by the Kappa statistic of 0.45, there was a moderate degree of agreement between the modified Ziel-Neelsen (MZN) method and direct Flourecsence Antibody test (FAT) method used in this study. For the purpose of reporting prevalence and investigating risk factors in the present study, the results of MZN were used. The decision was based on the fact that all samples were subjected to this test and thus all had equal chances of becoming
positive. Additionally, this test remains in common use among practitioners and in diagnostic laboratories worldwide.

At least one limitation should be considered in interpreting this result. The investigation did not detect the species of Cryptosporidium oocysts that were isolated by MZN and IFA. Speciation could have given the nature of zoonotic type of Cryptosporidium. However, a few samples were sent to Cornell University whereby 2/13 samples amplified, sequenced and identified as Cryptosporidium rynae. Based on morphological, molecular and biological data, this parasite, recognized as a new species is found only in Bos Taurus calves (Fayer et al, 2008) and is not zoonotic.

For the purpose of understanding the risks associated with Cryptosporidiosis in calves, this study focused on animals that were less than 30 days of age. The rationale for this strategy was that from previous studies (Wade et al, 2000, Sartin et al, 2004, Starkey et al, 2005) among the populations of cattle, the risk of infection is limited to young calves that are typically less than 30 days of age. Given these findings and the potential for increased statistical power, this study opted to restrict the analysis to calves less than 30 days of age.

From this study, dairy calves in Dagoretti have high level of cryptosporidiosis infection. Further, Cryptosporidiosis is not well known or understood within the dairy and close neighboring community in Dagoretti. These cattle borne oocyst present a hazard to immunocompromised people co-existing with the cattle in the division.
For effective foreseen changes in dairy farmers in minimizing health risks associated with *cryptosporidium* infection, relevant information should be passed to the community through the active leading farmers (Training of trainers-T.O.T). The information involves:

- training of production benefits of controlling cryptosporidiosis
- hold awareness campaigns of the risks of zoonosis including cryptosporidiosis with farmers
- Educate relevant groups about hygienic practices.
- Encourage farmers groups that are self-regulatory to practice hygienic dairy production.
- Disseminate educational materials
- Massive awareness campaigns including the media.
CHAPTER 6

6.1: Conclusion

There was evidence that the community knowledge on cryptosporidiosis and other zoonoses is low. Training members to train other members is hoped will in the long term increase their knowledge base and mitigate health hazards associated with urban dairy farming.

Cryptosporidium prevalence in calves was medium. Despite the challenges in determining the genotype of cryptosporidium species present in calves in comparison to human isolates it is evident that the Doagoretti community is at risk of being infected. The mitigation measures developed were broad, targeting high risk age group of calves between 7-30 days old and vulnerable groups all aimed at reducing exposure to cryptosporidiosis and other diseases.

Therefore the outcome challenge for Dagoretti dairy farmers is the adoption and practice of safe hygienic dairy farm methods. This could control the associated diseases including the zoonotic ones like cryptosporidium infection which would lead to acceptance of urban dairy production when evaluating externalities in terms of health risks associated with urban dairying.

6.2: Recommendation

It is therefore recommended that the various benefits of urban dairying, as shown in this study, should be considered, when evaluating the negative externalities in terms of health risks of urban dairy production. Despite the presence of health hazards, urban smallholder dairy production in Dagoretti is a profitable livelihood and there is room for improvement of
husbandry practices that would reduce exposure to health hazards and increase productivity and incomes.
CHAPTER 7

7.0 References


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CHAPTER 8

8.1 Appendix 1

Dairy household questionnaire


Q2) what diseases can people get from handling cow dung?

? Crypt o,

? worms,

? E.Coli,

? diarrhea,

? unsure,

? not ap plicable,

? Others

Q3) how is the disease transmitted?

? Phy sically handle manure,

? Phy sically handling cattle

? Phy sically handling other anima ls

? drink ing dirty water.

? washing in dirty water.

? drinking contaminated mil k.
eating contaminated food.

handling manure.

don’t know.

unsure.

not applicable.

others.

Q4) what can a person do to protect themselves?

Open response write in

(Researcher to categorize before data entry, check all those that apply)

q wear protective clothing (boots, gloves, etc)

q wash hands

q wash body generally

q boil drinking water

q treat drinking water in some other way (filter, chemical)

q boil milk

q wash fruits and vegetables

q protect water sources from manure

q don’t know
Q5) Do you wear any protective clothing when working with calves?

no   yes   decline to answer   problem with question_ __________________________

b) If yes, how often?

Always   Occasionally

Q6) Does this homestead grow food crops?

q   no
q   yes
q   decline to answer

Q7) If yes to Q6, How do you fertilize these crops? (Check 1 or write in response)

q   chemical fertilizers alone
q   cattle manure alone
q   chicken manure alone
q   both cattle manure and chemical fertilizer
Q8) is manure composting practiced?

- no
- yes
- decline to answer
- Problem with question

Q9) Cleanliness of calf pen Closest to picture 1, 2, 3 or 4. Unable to observe why

Q10) Cleanliness of calf Closest to Picture 1, 2, 3, or 4. Unable to observe why

Q11) Did HH member helping wear protective clothing? : No Yes Unable to observe why 

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84
Q12) Did HH member helping wash hands after assisting? : No Yes Unable to observe

Q13) The calf-pen or enclosure has a cemented floor? : No Yes Unable to observe why
Health risk posed by calves in transmission of zoonotic Cryptosporidium parvum in urban smallholder dairy production in Dagoretti division of Nairobi Kenya

Author: Dr Nyongesa C. N. (J5 6/7299/05)

Focus Group Discussion Guide (participatory methodology)

(Segregated into male and female dairy farmers)

Location…………………………

Name of moderator……………………………………

Venue of FGD……………………………………..

Time started………… Time ended…………………….

Number of participants……………………………

Sex…………

Date……………………

Introduction

Many diseases of newborn calves can be controlled by well-designed health management programs that define the care and housing of a calf at birth and the application of proper preventive measures (including sound nutritional programs) for the newborn calf. This maximizes the potential productivity of the overall dairy herd and minimizes simple human
exposure to infectious agents e.g. *Cryptosporidium* parasites as learned from the on going study.

**Attitude and perceptions**

a) Do you consider yourself /family at risk of cryptosporidiosis?

b) If yes, do you consider calves to be the source or casual to cryptosporidiosis?

c) If no, why don’t you consider yourself/family to be at risk?

**Farm Practices**

Probe on whether the following is done when the calf is born (how many do the practice)

a) Whether the new born calf is separated from the dam (not, immediately, after >12hrs)

b) How do they feed the calves on colostrums? (none, left with dam, hand fed or combination)

c) When do you start feeding the calves with colostrums? (<12hrs or > 12hrs)

d) How far is the calf pen from the cattle shed? (ask them to approximate the distance)

e) Whether the calf pen has Concrete /Unpaved floor

f) What type of beddings do they use for calves? (none, straw/hay, sawdust or waste paper others)

g) How do they house their calves? (singly/together)
h) How do they handle the sick calves? (no action taken, separate from the health one and treat or treatment without separating from others)

i) How do you treat calf scour (diarrhea)? (no treatment at all, cut back on milk, electrolytes or antibiotic)

j) What specific practices can lead to one being exposed to the health risks?

Probe on the following practices and who does them. (Score activity involvement by proportionate piling of 50 pebbles).

<table>
<thead>
<tr>
<th></th>
<th>Male hh</th>
<th>Female hh</th>
<th>Male worker</th>
<th>Female worker</th>
<th>Boy hh</th>
<th>Girl hh</th>
<th>Others (specify)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feeding</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cleaning calf pen/s</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disposal of manure</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Watering the calves</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Giving treatment</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Handling of manure

a) Do you clean/change beddings of their sheds? How often? If some don’t, what do they do?

b) Do you use protective clothing when cleaning the shed and disposing off manure?

c) How do you carry the dung to the manure pit? (wheelbarrow, sack, buckets and others)
Do you apply the same methods to adult cattle?

Control measures

What preventive measures (PM) can one take to reduce exposure to cryptosporidiosis from handling manure? List and Rank. Score by proportionate pil ing of 50 pebbles.

<table>
<thead>
<tr>
<th>Participants</th>
<th>PM 1</th>
<th>PM 2</th>
<th>PM 3</th>
<th>PM 4</th>
<th>PM 5</th>
<th>PM 6</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>50</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>50</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>50</td>
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<td>50</td>
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<td>20</td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>
Appendix 3

Figure 3: Average score (%) of activities per household member.