

**PARTICIPATORY EPIDEMIOLOGY OF COMMON DISEASES WITH SPECIAL
REFERENCE TO CRYPTOSPORIDIOSIS IN DAIRY CALVES AND CHILDREN
ATTENDING HEALTH FACILITIES IN MALINDI DISTRICT, COAST PROVINCE.
KENYA**

**A THESIS SUBMITTED IN PARTIAL FULFILMENT FOR THE DEGREE OF MASTERS
OF SCIENCE IN VETERINARY EPIDEMIOLOGY AND ECONOMICS**

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FACULTY OF VETERINARY MEDICINE

UNIVERSITY OF NAIROBI

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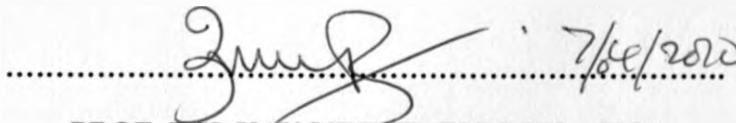
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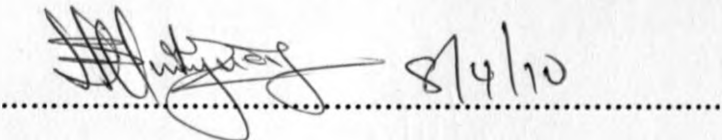
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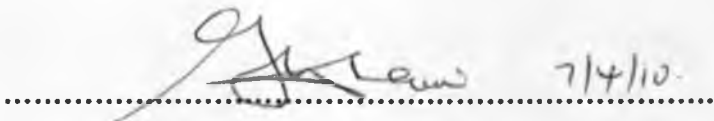


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DEDICATION

This is for you my dear parents, Mum, Maryam and Father, Ali for your love and tender care all the way through the sweet and sour experiences of life. You were my first teachers and I thank you for all the sacrifices you made for me and for bringing me this far. May Allah bless you all.

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LIST OF ABBREVIATIONS

Dr.....	Doctor.
PCR.....	Polymerase chain reaction.
DNA	Deoxyribosenucleic acid.
rRNA	Ribosomal ribosenucleic acid.
RFLP	Restriction fragment length polymorphism.
RAPD	Rapid amplification of polymorphic DNA.
AIDS	Acquired immune deficiency syndrome.
IFN- γ	Gamma Interferon.
IgG	Immunoglobulin G.
HIV	Human immunodeficiency virus.
H&E	Haematoxylin and Eosin.
ITS	Internal transcribed spacer.
rDNA	Ribosomal DNA.
IgA	Immunoglobulin A.
RNA	Ribosenucleic acid.
HAART	Highly active antiretroviral therapy.
NTZ	Nitazoxanide.

ABSTRACT

Cryptosporidium is an important parasite that causes a potentially life-threatening disease in immunocompromised individuals worldwide. The disease also contributes significantly to morbidity among calves and children in developing countries. Diagnosis of infection with this parasite was previously based on identification of acid-fast oocysts in stool; however, of late immunoassay techniques and PCR-based assays have been used to increase the sensitivity of detection. The organism is a major public health concern as its oocysts are resistant not only to common antiseptics. There are currently no drugs that cure cryptosporidiosis unlike other coccidian and enteric parasites. Prevention remains the most effective method of control.

Reports on cryptosporidiosis in Kenya remain scanty. Few studies have been done to assess the prevalence of the disease. The status of cryptosporidiosis in animals and man in Malindi District, Coast province of Kenya is not known. This study was designed to determine the prevalence of cryptosporidiosis in dairy calves and children in Malindi district. The objectives of the study were to provide baseline data on cryptosporidiosis, to determine the important risk factors and community perception associated with the disease.

The research was carried out in Malindi and Magarini administrative divisions of Malindi district in Coast province of Kenya. The participatory methodologies and acid fast staining of the samples collected were used to collect and analyze data. Semi structured interviews were used to come up with a list of eight diseases and or conditions in each division. In Malindi, helminthiasis, ectoparasites, coccidiosis, eye problems, pneumonia, East Coast Fever, Foot and Mouth disease and diarrhea were the diseases listed while in Magarini, helminthiasis,

pneumonia, East Coast Fever, anaplasmosis, coccidiosis, foot and mouth disease, navel ill and mucoid diarrhea were listed. In both divisions, cryptosporidiosis was not listed by the participants but it was added to the list as it was the disease of interest. Seasonal calendar was used to get the seasonality of the diseases.

By using pair wise ranking, the relative importance of the listed diseases as perceived by the farmers was established. In Malindi, helminthiasis was the most important followed by ectoparasites and diarrhea while in Magarini; East Coast Fever was the most important followed by pneumonia and bloody diarrhea. Proportional piling was used to establish the perception of the farmers on morbidity and mortality of the listed diseases. In Malindi division, ectoparasites East Coast Fever, pneumonia had the highest morbidity and mortality. In Magarini, East Coast Fever and pneumonia had the highest morbidity and mortality with morbidity. Matrix scoring assessed the farmers' ability to identify the listed diseases by using disease-sign matrix. In Malindi division, participants demonstrated good agreement for five out of the twelve disease signs and moderate agreement in six out of the twelve of the disease signs. There was poor agreement in one disease sign. In Magarini division, analysis of the disease-sign matrix scores demonstrated good agreement between the participants for 5 out of the 11 disease signs and moderate agreement between another 5 out of the 11 disease signs. There was poor agreement in one disease sign.

On methods of disease prevention and control, matrix scoring established that the participants strongly associated deworming with prevention and control of helminthiasis having a Kendall's coefficient of 0.982 in Malindi and 0.964 in Magarini. Vaccination was strongly associated by

the participants with foot and mouth disease having a Kendall's coefficient of 0.964 and 0.946 in Malindi and Magarini respectively. Proportional piling established the perception of the farmers on the effectiveness, financial implications, ease of use and action needed whether group or individual action of some disease prevention methods against the listed diseases. Participants in both division demonstrated that hygiene was the most efficient method in controlling cryptosporidiosis. Economic importance of the listed diseases was established by the use of proportional piling. Participants in both divisions demonstrated that decreased growth rate and decreased income were the most important economic importance indicators associated with cryptosporidiosis.

Thirty-two calves tested positive for cryptosporidiosis giving a prevalence rate of 10.6 % (32/300) in dairy calves under the age of six months while 9 children tested positive for cryptosporidiosis giving a prevalence rate of 6.4 % (9/140) in children under the age of five years in dairy production zone areas of Malindi district. All the positive cases in children were referred to the health institutions for management. The results of the study identified the risk factors involved with the disease in Malindi. In calves, risk factors therefore identified were general hygiene of the calf pens, housing, method of feeding, colostrums intake, use of feed supplements and the water used for drinking. In children, risk factors were unsafe drinking water, contact with pets and contact with farm animals and chicken and hygiene and sanitation.

Cryptosporidiosis is a diarrheal condition common in calves under the age of six months and children under the age of five years. Practising good hygiene and drinking of safe water will help a lot in reducing cases of the disease. Washing hands thoroughly with soap and water after using the toilet or before handling or eating food is highly recommended. Drink water which is safe.

All foods eaten raw should be properly washed with the water which is safe before eaten and avoid direct exposure to pets and farm animals. If exposure cannot be avoided, wash hands well immediately after contact. Routine testing of purified and unpurified water to check for the presence of *Cryptosporidium parvum* oocysts is recommended.

1 INTRODUCTION

Cryptosporidium is one of the several protozoan genera in the phylum *Apicomplexa*. The organism is an unobtrusive coccidian parasite of worldwide distribution capable of infecting many species of mammals including man, birds, reptiles and even fish. *Cryptosporidium* exists in the environment as a 5- μ m-diameter oocyst, which contains four sporozoites.

The discovery of *Cryptosporidium* is usually associated with E.E Tyzzer, who in 1907 described a cell-associated organism in the gastric mucosa of mice (Keusch *et al.*, 1995). *Cryptosporidium* has gained much attention in the last twenty years as clinically important human pathogen. Interest in *Cryptosporidium* heightened in 1971 when *Cryptosporidium* was found to be associated with diarrhea in cows (Panciera *et al.*, 1971). The first two cases of human cryptosporidiosis were reported in 1976 (Nime *et al.*, 1976). Infections in immune competent and immune suppressed people are worldwide. In the early 1980s the strong association between cases of cryptosporidiosis and immunodeficient individuals such as those with acquired immune deficiency syndrome (AIDS) brought *Cryptosporidium* to the forefront as a ubiquitous human pathogen (Payne *et al.*, 1984).

Presently the increasing population of immunocompromised persons and the various outbreaks of cryptosporidiosis through waterborne infection have placed an even greater emphasis on this pathogen (Juraneck, 1995). The protozoan parasite *Cryptosporidium parvum* is a leading cause of infectious diarrhea in humans and livestock with fecal-oral transmission. (Guerrant, 1997; Fayer *et al.*, 2000).

In mammals the organism usually infects the gastrointestinal tract particularly the distal small intestine (Fayer and Ungar 1986). In birds it favours the respiratory tract (Slavin, 1955), in reptiles it favours the gastric mucosa (Dillehay *et al.*, 1986) and in fish, *Cryptosporidium* species have been found as developmental stages in intestinal villi and in intestinal contents (Pavalsek, 1983).

After ingestion of the free living stages, the oocysts, endogenous stages become established just within the luminal margin of the gut linings cells where each develops, inside a vacuole bounded by membranes derived from both host and parasite cells (Fayer, and Ungar, 1986). The life cycle resembles that of other coccidia and when completed tiny oocysts are shed in the faeces. Unlike many other coccidian, *Cryptosporidium* can sporulate while still attached to the gut epithelium and thus can infect a new host as soon as they are shed. *Cryptosporidium* can infect several different hosts; survive most environments for long periods of time due to its "hardy cyst" (Keusch *et al.*, 1995). It inhabits all climates and localities (Keusch *et al.*, 1995). The length of the life cycle is probably influenced to some extent by the species, age and immune status of the host, and in particular strains of the parasite involved. However, experimental transmissions in young ruminants and other species have shown that the cycle can be completed in 3 to 4 days, (Tzipori, 1983).

Infection is generally self-limiting, followed by variable protective immunity involving humoral and cell-mediated responses, except in the immune-suppressed, where infection may be prolonged and fatal (Guerrant, 1997; and Kosek *et al.*, 2001). The spectrum of illness caused by *Cryptosporidium* is linked closely with the level of host immunity.

Clearance of this intestinal pathogen is related directly to the ability to mount an effective cellular immune response at the site of infection in the intestinal mucosa (Flanigan *et al.*, 1992). Little is known about the pathogenesis of the parasite and no safe and effective treatment has been successfully developed to combat cryptosporidiosis (Juranek, 1995).

Reports of cryptosporidiosis in Kenya remain scanty. Few studies have been done to assess the prevalence or the possible zoonotic importance of the disease. One such study was carried out in Kiambu district in Central province, Kenya by Simwa and others. The study was conducted to assess the prevalence of the infection among children less than five years of age in a rural community (Simwa *et al.*, 1989).

A total of 1420 diarrhoeic stool specimens from children less than five years were processed for bacteriology and parasitology. They were also examined for *Cryptosporidium* oocysts, using the modified Ziehl-Neelsen (ZN) acid-fast stain. Three point eight percent (3.8%) of all the diarrhoea samples were positive for oocysts. Three hundred and twenty (320) non-diarrhoeic stools from children in the same age group were also examined and were all negative for *Cryptosporidium* oocysts. The results of this study would suggest that infection with *Cryptosporidium* is associated with acute childhood diarrhoea in Kenya (Simwa *et al.*, 1989).

One of the reasons why reports in Kenya remain scanty is probably due to under reporting and misdiagnosis, as it is not routinely investigated in clinical laboratories. Cryptosporidiosis is not among the diseases that are routinely reported by the medical

services hence its role in enteric disease is largely unreported. The status of cryptosporidiosis in animals and man in Malindi District, Coast province of Kenya is not known.

1.1 Objectives of the study

The broad objective of this study was to determine the prevalence of cryptosporidiosis and the risk factors associated with the disease in Malindi district.

The specific objectives were:

- i. To determine the community perception of cryptosporidiosis in Malindi district, Coast province, Kenya.
- ii. To provide baseline data on the status of cryptosporidiosis in Malindi district, Coast province in Kenya.
- iii. To determine important risk factors associated with cryptosporidiosis in both humans and calves.

2 LITERATURE REVIEW

2.1 Epidemiology of Cryptosporidiosis

2.1.1 Historical background

In 1907 Ernest Edward Tyzzer clearly described a protozoan parasite he frequently found in the gastric glands of laboratory mice. He recognized asexual and sexual modes of reproduction followed by spore (oocyst) formation, noting the presence of an attachment organelle at all stages. In 1910 Tyzzer described the same organism in much greater detail proposing *Cryptosporidium* as a new genus and *C. muris* as a new species. In 1912 Tyzzer identified and named a new species, *Cryptosporidium parvum*, providing great morphological detail. He distinguished the new species from *C. muris* by experimentally infecting mice and showing that *C. parvum* was smaller and developed only in the small intestine.

Tyzzer was the first to report on avian cryptosporidiosis in 1929. He found all the developmental stages in the chicken cecal epithelium. For nearly half a century after Tyzzer's work, *Cryptosporidium* was not regarded as economically or medically important and was therefore of interest primarily to the taxonomists. Rush *et al.*, (1987) presented the first report linking cryptosporidiosis with morbidity and mortality and also named a new species, *Cryptosporidium meleagridis*. Diarrhea and a low death rate were found in 10 to 14-day old turkey poults. However, it was not until 1971 when *Cryptosporidium* was found to be associated with bovine diarrhea by Panciera *et al.* and its veterinary interest was stimulated. In 1976 Nime and colleagues as well as Meisel *et al.*, independently reported the first cases of human cryptosporidiosis. In 1982 physicians in Boston, Los Angeles, New

York, Philadelphia, and San Francisco reported to the Centre for Disease Control that 21 males had severe protracted diarrhea caused by *Cryptosporidium* in association with Acquired Immune Deficiency Syndrome (AIDS) (Anonymous., 1982). Medical and veterinary interest in the epidemiology, diagnosis, treatment, and prevention increased substantially thereafter throughout the world.

2.1.2 Aetiology

Cryptosporidium is one of the several protozoan genera in the phylum *Apicomplexa* (Levine, 1980). All are referred to as coccidia. *Cryptosporidium* is an oval shaped protozoan parasite found in man, mammals, birds, fish and reptiles. The parasite replicates intracellularly in the brush border of the small intestine. Infective oocysts are shed into the lumen and passed in the faeces (Flanigan and Soave, 1993).

Several species of *Cryptosporidium* were named after the host in which they were found. There are eight valid named species of *Cryptosporidium*. These species are *Cryptosporidium muris* (Tyzzer, 1910), *Cryptosporidium parvum* (Tyzzer, 1912), *Cryptosporidium meleagridis* (Slavin, 1955), *Cryptosporidium wrairi* (Netterling *et al.*, 1971), *Cryptosporidium felis* (Iseki, 1979), *Cryptosporidium serpentis* (Levine, 1980), *Cryptosporidium nasorum* (Hoover *et al.*, 1981), and *Cryptosporidium baileyi* (Current *et al.*, 1986). *Cryptosporidium* appears to be infectious for 79 species of mammals including man (Fayer *et al.*, 1990).

2.1.3 Life cycle

Cryptosporidium is taxonomically classified as a Sporozoa, since its oocyst releases 4 sporozoites (its motile infectious agents), upon excystation (Flanigan and Soave, 1993). The life cycle is complex. There are both sexual and asexual cycles and there are 6 distinct developmental stages (Keusch *et al.*, 1995). When oocysts in food, water, or the general environment are ingested or inhaled by a suitable host, sporozoites excyst and parasitize epithelial cells of the gastrointestinal or respiratory tract.

The life cycle of *Cryptosporidium* is illustrated in figure 1.

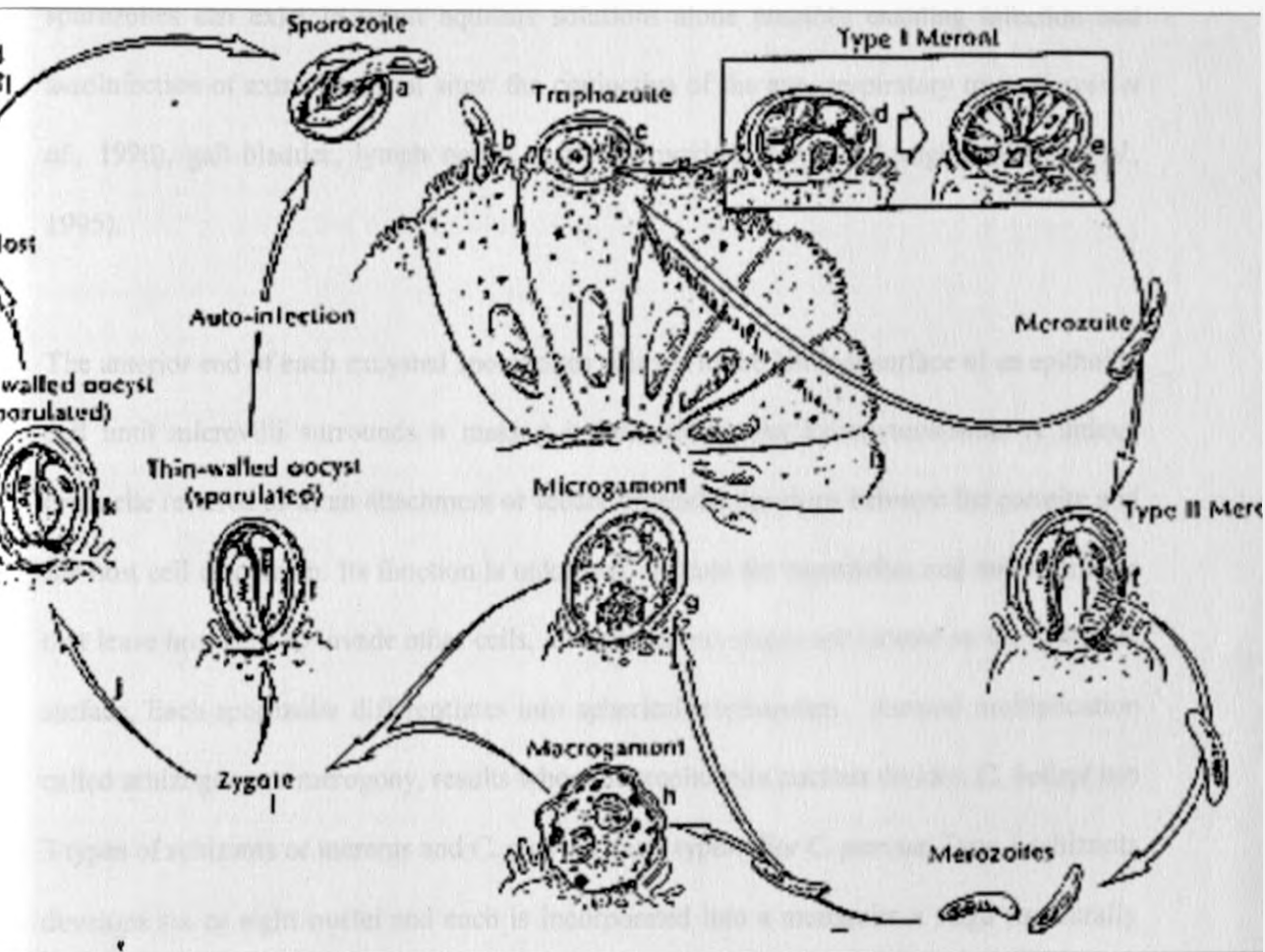


Figure 1: Life cycle of *Cryptosporidium* (Current and Blagburn, 1990)

The sporulated oocyst is the only exogenous stage consisting of 4 sporozoites within a tough two-layered wall. It is excreted from the body of an infected host in the faeces. The endogenous phase begins after a suitable host ingests the oocyst. Sporozoites excyst from the oocyst and parasitize epithelial cells of the gastrointestinal and respiratory tract. Figure 2 shows a sporozoite release from *Cryptosporidium* oocyst. For most coccidia, excystation of sporozoites require exposure to reducing conditions followed by exposure to pancreatic enzymes and /or bile salts; for *Cryptosporidium* such exposure may enhance excystation but sporozoites can exist in warm aqueous solutions alone possibly enabling infection and autoinfection of extra intestinal sites: the conjunctiva of the eye, respiratory tract, (Fayer *et al.*, 1990), gall bladder, lymph nodes, testicles, ovaries, uterus and vagina (Fletz *et al.*, 1995).

The anterior end of each excysted sporozoites adheres to the luminal surface of an epithelial cell until microvilli surrounds it making it intracellular but extracytoplasmic. A unique organelle referred to as an attachment or feeder organelle develops between the parasite and the host cell cytoplasm. Its function is unknown. Except for merozoites and microgametes that leave host cells to invade other cells, all endogenous stages are located on the epithelial surface. Each sporozoite differentiates into spherical trophozoites. Asexual multiplication called schizogony or merogony, results when the trophozoite nucleus divides. *C. baileyi* has 3 types of schizonts or meronts and *C. parvum* has 2 types. For *C. parvum* Type 1 schizonts develops six or eight nuclei and each is incorporated into a merozoite a stage structurally similar to the sporozoite (Fayer *et al.*, 1990).

Each mature merozoite leaves schizonts to infect another host cell and develop into another Type 1 or Type 2 schizonts that produce 4 merozoites. It is thought that only merozoites from type 2 schizonts initiate sexual multiplication (gametogony) upon infecting new host cells by differentiating into either microgamont (male) or macrogamont (female) stage. Each microgamont becomes multinucleate and each nucleus is incorporated into microgamete, a sperm cell equivalent. Macrogamont remain uninucleate, an ovum equivalent. It is assumed that only fertilised macrogamonts develop into oocyst that sporulate in situ and contain 4 sporozoites. Oocysts in the intestinal tract are excreted with feces whereas those in the respiratory tract exit the body with the respiratory or nasal secretions (Fayer *et al.*, 1990). Some reports suggest that oocysts with thin walls release sporozoites that autoinfect the host whereas those with thicker walls leave the body to infect other hosts. (Current, 1985, Current 1988).

The prepatent period is the shortest time after ingestion of infective oocyst for the parasite to complete the endogenous life cycle and excrete newly developed oocyst. This time varies with the host and species of *Cryptosporidium*. Experimentally determined prepatent periods for *C. parvum* ranges from two to seven days for calves, two to fourteen days for dogs, three to six days for pigs, two to five days for lambs (Fayer *et al.*, 1990) and four to twenty two days for humans. (Dupont *et al*; 1995). The patent period, is the duration of oocyst excretion experimentally determined for *C. parvum* range from 1 to 12 days for calves, 3 to 33 days for dogs, 5 to 14 days for pigs (Fayer *et al.*, 1990) and 1 to 20 days for human (Dupont *et al.*, 1995).

2.1.4 Occurrence and distribution

Cryptosporidium is an important and widely distributed enteric pathogen of young livestock and humans and its common in other hosts in which it is often asymptomatic. *Cryptosporidium* causes acute self-limiting gastroenteritis in immunocompetent individuals and persistent and potentially fatal infection in the immunocompromised worldwide (Fayer and Ungar, 1986).

The organism has a wide host range and isolates from mammals have been successfully transmitted to both homologous and heterologous host species. Because the parasite can cross host species barriers, infections in other agricultural animals, rodents, wildlife species and companion animals must be regarded as possible reservoirs of infection both for livestock and humans. The parasite has been recorded in about 79 mammalian species worldwide (Addy and Atkins-Bekoe, 1986).

Most data on the incidence of *C. parvum* infection in domestic animals which have been recorded world wide have been reported in cattle (Fayer and Ungar, 1986; O'Donoghue, 1995). Much survey data from the U.S. indicates an enormous variation in incidence (Garber *et al.*, 1994). In New Zealand 37% of 550 samples from scouring calves were positive for oocysts (Townsend and Lance 1987). In Trinidad and Tobago 8.7% of calves less than 24 weeks were infected (Kaminjolo *et al.*, 1993). In U.K. and the Netherlands five surveys of naturally infected housed calves were sampled on alternate days for 3 to 4 weeks and fecal smears were examined for oocysts had prevalence rates that varied from 60% to 100% (Blewett, 1989).

While there is less information on infected sheep than cattle, distribution also appears to be worldwide. Naturally reared lambs on a farm in Idaho, the earliest lambs appeared healthy, but scouring commenced midway through lambing and of 60 animals infected 12 died (Angus *et al.*, 1982). Of 196 neonatal deaths on 5 sheep farms in Chile, oocysts were found in 7.7% (Valenzuela *et al.*, 1991). Some evidence of seasonality in prevalence was observed in lambs on farms in Northern Spain, where prevalence was greater in spring (90% of farms) than in autumn (40% of farms) (Matos-Fernandez *et al.*, 1994).

In Denmark, an outbreak of cryptosporidiosis in kids from a dairy goat flock of 250 reached a morbidity rate approaching 100% and mortalities of 20 to 40% inspite of good husbandry (Bennett *et al.*, 1985). In Northern Spain up to 70% of kids sampled were infected and all the farms visited had cryptosporidiosis in their flocks (Matos-Fernandez *et al.*, 1994). Cryptosporidiosis has been recognized as a common gastrointestinal problem in calves in the United Kingdom (Current and Garcia, 1991). The U.K. Veterinary Investigation Service diagnosed 2,177 episodes of infection in calves in 1994 versus 216 in 1984. While this is not the total number of animals affected it reflects samples from sites where one or more calves were infected at a specific time.

Infection in pigs has been reported sporadically worldwide but the prevalence remains unclear, as most studies have involved only small numbers of animals. In a California auction yard, over a 3-month period it was found that 5% of 200 pigs were infected with *Cryptosporidium* (Tacal *et al.*, 1987). In Trinidad and Tobago 54 of 275 piglets sampled were infected with *Cryptosporidium* (Kaminjolo *et al.*, 1993).

Infection in horses was first described in immunocompromised Arabian foals (Synder *et al.*, 1978). Infection rates in non-diarrheic animals of 2.4 to 60% have been recorded (Lengronne *et al.*, 1985) and it appears that the infection which may be widespread may occasionally produce clinical symptoms. Studies in France reported a symptomatic infection with oocyst shedding in yearlings and infection rates in mares as high as 80% with possible transmission from mares to foals (Chermette *et al.*, 1987).

Prevalence data for infection in domestic dogs are sparse. Individual cases in young dogs have been reported, often with concurrent infection such as canine distemper virus (Fukushima and Helman, 1984). Faecal analysis of 57 adult dogs in Finland, (Pohjola, 1984) 200 in Federal Republic of Germany (Augustin-Bichl *et al.*, 1984) and 101 in Edinburgh area of Scotland (Simpson *et al.*, 1988) failed to detect oocyst, while in California, only 4 of 200 impounded stray dogs were infected (El-Ahraf *et al.*, 1991). In more surveys 0.01% of canine stools from public parks in Scotland contained oocysts (Grimason *et al.*, 1993), while 19.6% of faecal samples taken from public parks in Australia proved to be positive (Johnston and Gasser, 1993).

In Kenya, reports on cryptosporidiosis remain scanty and few studies have been done to assess the prevalence or zoonotic importance of the disease. Such a study was conducted to assess the prevalence of the infection among children less than five years of age in a rural community in Kiambu District, Kenya by Simwa *et al.*, (1989).

2.1.5 Pathophysiology

In general, diarrhea develops when intestinal absorption is impaired or intestinal secretion is enhanced. Both of these processes are regulated by the intestinal epithelial cells, which are infected by *Cryptosporidium* (Clark and Sears, 1996). Several investigators have identified impaired glucose-stimulated sodium and water absorption and/or increased chlorine secretion in experimental models of cryptosporidiosis (Argenzio *et al.*, 1993). In addition to these transport defects, abnormalities in the barrier properties of the intestinal epithelium, mediated in part by intercellular junctional complexes, contribute to *Cryptosporidium* diarrhea. Griffiths *et al.*, (1994) and Adams *et al.*, (1994) found evidence of permeability defects and decreased resistance across *C. parvum*-infected intestinal cell lines. In addition, these workers found that *C. parvum* infection of these monolayers resulted in the release of cytoplasmic lactate dehydrogenase, consistent with cellular injury, which ultimately resulted in cell death.

Another group has suggested that *Cryptosporidium* induces apoptosis in biliary epithelial cells, but this mechanism of cell death has not been confirmed in vivo (Chen *et al.*, 1998). Malabsorption and abnormal intestinal permeability (decreased vitamin B₁₂ absorption, decreased D-xylose absorption, and abnormal lactose/mannitol permeability test) have been confirmed in people with AIDS and cryptosporidiosis (Goodgame *et al.*, 1995). One mechanism for the induction of intestinal secretion by *Cryptosporidium* may involve the stimulation of prostaglandin production by intestinal epithelial cells (Laurent *et al.*, 1998).

2.1.6 Biochemistry

Like other protozoan parasites, *Cryptosporidium* appears incapable of de novo purine synthesis and relies on salvage pathways for hypoxanthine, guanine, and adenine. Studies with radiolabeled purine precursors (formate and glycine) indicate that these compounds are incorporated into host cells but not intracellular *C. parvum*. Enzymatic activity necessary for purine salvage (hypoxanthine, guanine, and xanthinephosphoribosyl-transferase) was identified in *C. parvum* sporozoites and may localize to a single enzyme. Such an enzyme may serve as an antiparasitic drug target (Doyle *et al.*, 1998). Keithly *et al.*, (1997) identified a polyamine biosynthesis pathway in *C. parvum* that is found chiefly in plants and some bacteria but not mammalian cells. The lead enzyme of this pathway, arginine decarboxylase, is sensitive to a specific, irreversible arginine decarboxylase inhibitor, which reduces the intracellular growth of *C. parvum*.

Another potential drug target is the shikimate pathway, in which (in plants) chorismate is converted to *p*-aminobenzoic acid, folate, and other aromatic compounds (Roberts *et al.*, 1998). *Cryptosporidium* and other Apicomplexan parasites were found to be sensitive to glyphosate, an inhibitor of the shikimate pathway. This inhibition also provides circumstantial evidence for the existence of a plastid-like organelle in *Cryptosporidium*, similar to that described for *Plasmodium* and *Toxoplasma* (Kohler *et al.*, 1997).

2.1.7 Molecular Genetics

Karyotypic analysis suggests that *C. parvum* contains eight chromosomes, ranging in size from 0.945 to 2.2 Mb, giving a total haploid genome size of approximately 10.4 Mb. Blunt *et al.*, (1997) also identified a low-molecular-weight molecule that may correspond to the

35-kb circular, extrachromosomal DNAs (plastids) found in other Apicomplexan parasites. Subsequently significant progress was made toward understanding the *C. parvum* genome through expressed sequence tag (EST) and genome sequence survey (GSS), DNA-sequencing projects, as well as a genome-mapping project. To date, the EST project has isolated and partially sequenced 567 ESTs, with 37% of the unique clones demonstrating significant homology to GenBank sequences. A summary of this data can be found on the World Wide Web (Davis *et al.*, 1999).

Of the two projects to sequence random fragments of *Cryptosporidium* genomic DNA, the first determined the sequence of 1,507 fragments, totaling more than 888,000 bp of new sequence. 27% of these sequences demonstrated homology to GenBank sequences. The second GSS project sequenced 654 fragments, totaling more than 324,700 bp, with 16% of the unique sequences demonstrating homology to GenBank sequences. In addition to these sequencing projects, a complete map of the eight *C. parvum* chromosomes was completed (Piper *et al.*, 1998). These projects greatly facilitated future studies of this organism.

rRNA gene structure is central to the phylogenetic classification and genotyping of microbial organisms; therefore, the characterization of the *C. parvum* rRNA gene organization by Le Blancq *et al.*, (1997) was an important milestone in *Cryptosporidium* research. These investigators found that the small- and large-subunit rRNAs were 1.7 and 3.6 kb, respectively; a 151-bp putative 5.8S rRNA was also identified. Like other eukaryotes, the rDNA unit is arranged as a 5' small-subunit rRNA-internal transcribed spacer 1 (ITS1)-5.8S rRNA-ITS2-large-subunit rRNA 3' complex. There appeared to be five copies of the rDNA per haploid genome, which were not organized in the conventional head-to-tail arrangement

but, rather, were dispersed throughout the genome to at least three different locations. Interestingly, there were two distinct types of rDNA units (four copies of type A and one copy of type B) which contained marked differences in the ITS regions.

Knowledge of this intra-organismal heterogeneity is crucial when interpreting the results of genotyping of *C. parvum* isolates based on rRNA heterogeneity. Similar rDNA organization is found in the Apicomplexan protozoa *Plasmodium*, *Babesia*, and *Toxoplasma*. In *Plasmodium*, the two classes of rRNAs are differentially expressed during the life cycle of the parasite. Such information on developmental expression is not yet available for *C. parvum*. Curiously, another Apicomplexan parasite, *T. gondii*, has an entirely different rDNA organization, containing multiple copies of tandemly arrayed rDNA units. The phylogenetic and biologic consequences of these differences have not been resolved.

Khramstov *et al.*, (1997) found *C. parvum* to contain two small extrachromosomal, cytoplasmic, virus-like double-stranded RNAs. These RNAs (1,786 and 1,371 bp) each contain a single open reading frame that encodes a putative RNA-dependent RNA polymerase and a protein with limited homology to mammalian mitogen-activated protein kinases, respectively. Virus-like particles were not observed in *C. parvum* sporozoites by electron microscopy, but other data suggested that the RNAs were unencapsidated. Although there are several examples of protozoan viruses including *Giardia*, *Trichomonas*, and *Leishmania*, these viruses do not resemble the *C. parvum* like RNAs. To date, these RNAs have been identified in many laboratory and commercial samples of *C. parvum* but have not been found in five non-laboratory members of the genus (Khramstov *et al.*, 1997).

2.1.8 Genetic diversity

Paralleling the clinical diversity of cryptosporidiosis is increasing evidence of molecular heterogeneity among *C. parvum* isolates. Western blot analysis of *C. parvum* oocyst antigens with a *Cryptosporidium* specific monoclonal antibody revealed heterogeneity among several human, calf, and lamb isolates (Nichols *et al.*, 1991). A second study of *C. parvum* antigens also found heterogeneity among human isolates and between human and animal isolates when using polyclonal and monoclonal antibodies against *C. parvum* antigens in Western blots (Nina *et al.*, 1992).

Two-dimensional gel electrophoresis of *C. parvum* sporozoite proteins from five different isolates has revealed a 106-kDa peptide which differed in its isoelectric point in several of the isolates and a 40-kDa protein in one isolate which was not present in the others (Mead *et al.*, 1990). Finally, isoenzyme typing of *C. parvum* isolates from different geographical locations revealed two isoenzymes of phosphoglucomutase and hexokinase that segregated according to human or animal origin (Awad-El-Kariem *et al.*, 1995).

Genetic studies of *Cryptosporidium* undertaken by Ortega *et al.*, (1991) supported the evidence of isolate diversity obtained by analysis of sporozoite proteins and suggest that there are at least two subtypes of human isolates. Restriction fragment length polymorphism (RFLP) analysis of *C. parvum* genomic DNA from three bovine and three human isolates revealed polymorphism between the human and bovine isolates and among the human isolates.

Heterogeneity among *C. parvum* isolates was also demonstrated using the random amplification of polymorphic DNA (RAPD) (Welsh and McClelland, 1993). According to one study, RAPD analysis of 25 *C. parvum* isolates revealed two genotypes; one genotype was unique to human isolates, while the other was found predominantly in isolates from calves and lambs (Morgan *et al.*, 1995). In similar studies Carraway *et al.*, (1996) examined five *C. parvum* isolates by RAPD and also found genetic heterogeneity among human and animal isolates.

In addition an RFLP analysis of a repetitive DNA sequence in 23 human and calf *C. parvum* isolates revealed the same profile in all calf isolates but two patterns among the human isolates, one of which was identical to the profiles in the calf isolates (Bonnin *et al.*, 1996). Sequence analysis of three *C. parvum* genes (encoding dihydrofolate reductase-thymidylate synthase, α -tubulin, and β -tubulin) from a calf isolate and a human isolate has also identified an unexpectedly high level of polymorphism (Vasquez *et al.*, 1996).

Several investigators have since used RFLP and DNA sequencing of polymorphic genes to determine the genotype of larger numbers of *C. parvum* isolates from animals, humans infected in outbreaks, and people with AIDS. These studies continued to support the concept that humans can be infected by two genetically distinct types of *Cryptosporidium*, designated genotype 1 (human type) and genotype 2 (bovine type). These studies also suggested that cattle are exclusively infected by genotype 2 isolates and that most human infections are caused by genotype 1- parasites. Several of the human infections caused by genotype 2 isolates appeared to be zoonotic, with an identifiable bovine source. Unfortunately, division of *C. parvum* isolates into two discrete groups is probably an

oversimplification. First, several investigators have found evidence of isolates containing a mixture of genotype 1 and genotype 2 alleles (Carraway *et al.*, 1997). The subdivision of *C. parvum* into two species, *C. hominis* and *C. parvum*, has been proposed to replace the type 1 and 2 subspecies designations, respectively (Morgan-Ryan *et al.*, 2002). *C. hominis* was considered as not naturally infective for animals with few exceptions and experimentally, some *C. hominis* isolates were infective at high doses for calves, lambs, and piglets but not for cats, dogs, and rodents, with some exceptions (Ebeid *et al.*, 2003).

A study whose aim was to investigate the infectivity of *C. hominis* in an alternative laboratory rodent, the Mongolian gerbil provided findings that represented the first report of reproducible and stable experimental *C. hominis* infection in the Mongolian rodent gerbil (Xiao and Morgan-Ryan, 2004). In humans, the most commonly detected genotypes are *Cryptosporidium hominis* (*C. parvum* genotype 1 recognized as a distinct species) and the bovine *C. parvum* genotype 2 (Morgan-Ryan *et al.*, 2002). *C. hominis* naturally infects humans almost exclusively, and experimentally, *C. hominis* infections were obtained in calves, lambs, and pigs with at least some isolates but not in rodents commonly used for propagating *C. parvum* genotype 2, with some exceptions (Morgan-Ryan *et al.*, 2002).

Second, multilocus genotype determination failed to identify recombinant genotypes, suggesting that most genotype 1 and genotype 2 isolates were reproductively isolated populations (Spano *et al.*, 1998). Spano *et al.* (1998) and Widmer *et al.* (1998) however identified polymorphism in the β -tubulin gene intron which might have arisen from a recombination event. Although these studies contributed significantly to the understanding

of genetic diversity among *Cryptosporidium* isolates, most were small studies with geographically diverse isolates from sporadic outbreaks. Larger studies focused on specific populations, such as people with AIDS or children in developing countries are necessary before we can have a more complete understanding of the molecular epidemiology of cryptosporidiosis.

Several investigators speculated that *Cryptosporidium* isolates may also vary in virulence, in part explaining the clinical diversity observed in cryptosporidiosis. One such study suggested that genotype 1 isolates were less virulent than genotype 2 isolates in in vitro assays measuring the disruption of intestinal cell monolayers (monolayer resistance and cytoplasmic lactate dehydrogenase release) (Widmer *et al.*, 1998). The experimental data indicated that the host ranges for genotype 1 and 2 isolates were different, with genotype 1 isolates being infectious mainly to humans and primates and genotype 2 isolates being infectious to most mammals (Widmer *et al.*, 1998).

2.1.9 Immunology

Much of what is known about the immune response to *Cryptosporidium* has been learned from experimental murine models. While such models are useful, they have several limitations. First, immunocompetent adult mice are not susceptible to *C. parvum* infection. For unclear reasons, only immunocompetent mouse pups (younger than 26 days) were susceptible to infection (Novak and Sterling, 1991). In addition few murine models completely mimicked human infection, since infected mice did not typically develop diarrhea. According to Griffiths *et al.* (1998) exceptions to this were the gamma interferon (IFN- γ) knockout mice, which could be infected by relatively few oocysts and would

experience weight loss and wasting before eventually dying. Mice with severe combined immunodeficiency (SCID mice) were also susceptible to *C. parvum* infection, and were widely used to study the immunology of cryptosporidiosis (McDonald *et al.*, 1992).

The two key immune components necessary for prevention and/or resolution of cryptosporidiosis as shown by these studies are CD4⁺ lymphocytes and IFN- γ (Chen *et al.*, 1993). Depletion of IFN- γ by intraperitoneal injection of anti-IFN- γ antibodies resulted in a shortened prepatent period, increased oocyst excretion, and early disease and death (McDonald and Bancroft, 1994). Also, a human case of protracted cryptosporidiosis in a patient with IFN- γ deficiency has been reported (Gomez Morales *et al.*, 1996). Selective immune cell reconstitution experiments have defined an important role for CD4⁺ lymphocytes in the prevention or resolution of cryptosporidiosis (Chen *et al.*, 1993) as well as a possible role for CD8⁺ lymphocytes (McDonald and Bancroft, 1994). Similarly, mice which lack functional CD4⁺ lymphocytes (major histocompatibility complex class II-deficient mice) were more susceptible to infection than were control mice and were unable to clear the infection; CD8-deficient mice (major histocompatibility complex class I-deficient mice) resolved the infection (Aguirre *et al.*, 1994).

Also, mice deficient in T-cell receptor α (present on most CD4⁺ lymphocytes) were more susceptible to infection than were controls. Gamma/delta-T-cell-deficient neonatal mice were more susceptible than control mice but were able to clear the infection (Waters and Harp, 1996). Since the cytokine interleukin-12 can induce IFN- γ production, it is not surprising that treatment of mice with interleukin-12 prevented or greatly reduced the severity of infection (Urban *et al.*, 1996). In experimental murine infections, neither tumor

necrosis factor nor natural killer cells were important in resolving infection (Chen *et al.*, 1993). The mechanisms by which *Cryptosporidium*-infected intestinal epithelial cells initiate immune responses were not entirely clear but one apparent mechanism in human cells involved the production of tumor necrosis factor alpha, interleukin-8, and C-X-C chemokines by infected mucosa (Laurent *et al.*, 1997).

2.1.10 Host range

Cryptosporidium has been reported in fresh water and marine fish (Hoover *et al.*, 1981), in an amphibian (Crawshaw and Mehren, 1987), in 57 species of reptiles (O'Donoghue, 1985) and in over 30 species of birds (O'Donoghue, 1985) including domesticated chicken, turkeys and a wide variety of wild and captive birds. *Cryptosporidium* has been reported also in 79 mammalian species (O'Donoghue, 1985) and in humans with the first case reported in 1976 (Nime *et al.*, 1976). *Cryptosporidium* has also been isolated in non-human primate for example, rhesus monkey (Kaup *et al.*, 1994).

2.1.11 Method of transmission

The method of transmission is considered to be by oocysts that are fully sporulated and infective when they are passed in the faeces (Angus, 1983). Large numbers of organisms are passed in the faeces during the patent period allowing environmental contamination to build up rapidly. With the auto infective cycle of the parasite minimal numbers of ingested oocysts can establish a clinical infection. Transmission can occur directly, e.g. from calf to calf or indirectly via fomite or human transmission; from the contamination in the environment or faecal contamination of the feed and water supply. *Cryptosporidium* are

non-host specific parasites where oocysts from the faeces of wild mice could infect calves which may develop non-fatal clinical cryptosporidiosis (Klesius *et al.*, 1986).

Cryptosporidial infection can be transmitted from faecally contaminated food and water, from animal-person contact and via person-person contact (Fayer *et al.*, 1990). The probability of transmission from just a small amount of contamination is fairly high as determined by a study by Dupont *et al.*, (1995) who showed that the 50% infective dose of *C. parvum* is only 132 oocysts for healthy persons with no previous serological immunity to cryptosporidiosis.

There have been six major outbreaks of cryptosporidiosis in the United States as result of contamination of surface (rivers and lakes) drinking water (Juraneck, 1995). One of the major outbreaks occurred in Milwaukee in 1993 and affected over 400,000 persons. Food can also be a source of transmission where the offending foods are contaminated by infected or asymptomatic human carriers. The first documentation of this type of infection occurred at a county fair in Maine, where children who drank apple cider contaminated by animal faeces developed cryptosporidiosis (Juraneck, 1995). The oocysts don't survive cooking but food contamination can occur in beverages, salads or other foods not heated or cooked after handling. Transmission of *C. parvum* from household pets to humans is extremely rare, but there is a definite correlation between infection in calves and humans (Juraneck, 1995).

Person to person transmission is also common. *Cryptosporidium* transmission occurs at high frequency in day-care centres, where infants or younger children are clustered within

classrooms, share toilets and common play areas. Day care employees can become easily infected with *C parvum* through careless diaper changing or through washing the laundry of infected children.

Nosocomial settings are also a major forum for cryptosporidial transmission. There have been several reports of both transmission from patients to health care staff and patient-to-patient transmission. An outbreak in a bone-marrow transplant unit occurred where five patients developed cryptosporidiosis after a sixth, infected patient was admitted to the unit (Casemore *et al.*, 1994). Five cases of cryptosporidiosis were confirmed among nursing staff, and infection most likely occurred from major environmental contamination due to the patients' intractable diarrhea and vomiting (Casemore *et al.*, 1994). Various routes of transmission such as aerosol infection is fairly likely since *Cryptosporidium* oocysts are shed in large numbers during acute infection and are immediately infective to other people (Casemore *et al.*, 1994).

2.1.12 Resistance to environment

The thick walled oocysts are resistant to most disinfectants and can survive for several months in cool and moist conditions. The infectivity of the oocysts can be destroyed by ammonia, formalin, freeze-drying and exposure to temperatures below 0°C and above 65°C. (Tzipori, 1983), ammonium hydroxide, hydrogen peroxide, chlorine dioxide, 10% formol saline and 5% ammonia are effective in destroying the infectivity of oocysts (Campbell and Current, 1983). The infectivity of the oocysts in calf faeces is reduced after 1-4 days of drying (Anderson, 1986).

2.1.13 Risk Factors

The factors that make animals susceptible to infection and that predispose infected animals to develop clinical disease are not well understood. In calves concurrent infection with other enteropathogens especially rotavirus and corona virus are common and epidemiological studies suggest that diarrhea is more severe with mixed infections (Robert *et al.*, 1991). In general, mixed infections are most common but cryptosporidial infections can be significant in its own right and one study found *C. parvum* as the only pathogen identifiable in 51% of 277 diarrheic calves. (Moore and Zeman, 1991). In two other studies involving diarrheic calves submitted to diagnostic laboratories cryptosporidia were the only pathogens isolated in 51% and 55% of the cases while in 25% and 39% of the cases the protozoan agent was found in combination with rotavirus and corona virus (Krogh and Henriksen 1985).

Immunologically compromised animals are more susceptible to clinical disease than immunocompetent animals but the relationship between disease and failure of passive transfer of colostral immunoglobulins is not clear. The disease can be reproduced in both colostrum deprived and colostrums fed calves. In the field clinical disease can occur in calves that have serum IgG concentration in excess of 10 mg /ml, however, the shedding of the organism has been observed to be high in calves with low absorptive efficiency of IgG from colostrum and low serum IgG concentrations (Lopez *et al.*, 1988). Case fatality rates in cryptosporidiosis are generally low unless there are other complicating factors.

In humans, exposure factors, such as farming and social and behavioural patterns have increased exposure to infection. These risk factors are as described by Fayer and Ungar, (1986). Deficient immunity (Acquired Immune Deficiency Syndrome (AIDS) and other

acquired or congenital immunodepression, immunosuppression or malnutrition is one of the main risk factors. Others include zoonotic contact through leisure activity e.g. camping and farm visits or even occupational exposure like veterinary, agricultural, nursing, medical laboratory or child daycare.

Poor hygienic and sanitary conditions including drinking water and food, deficient sewerage or waste disposal and poor fly control are the other important risk factors. Exposure to untreated surface or recreational water or inadequately treated water supply can also put one at risk of being infected with *Cryptosporidium* parasites. Consumption of raw foods such unpasteurized milk or contact with a case of diarrhoea including daycare attendance; household contact or parenting and travelling from urban to rural areas can also put one at risk of contracting cryptosporidiosis.

2.1.14 Pathogenesis

Following ingestion of the oocyst there is excystation (release of infective sporozoites), merogony (asexual multiplication), gametogony (gamete formation), fertilization, oocyst wall formation and sporogony (sporozoite formation) (Current, 1985). Thus the oocyst of *Cryptosporidium* species can sporulate within the host cell, in contrast to the oocyst of *Eimeria* and *Isospora* species that do not sporulate until they are passed from the host to the environment, and they are infective when passed in the faeces (Current, 1985). Sporulated oocyst can exist in the intestine before being excreted in the faeces and the infection can persist until the immune response of the animal eliminates the parasite.

In naturally occurring cases in calves the cryptosporidia are most numerous in the lower part of the small intestine and occasionally in the cecum and colon (Pearson *et al.*, 1982). The middle and the lower portion of the jejunum and ileum have the largest number of organisms following experimental infection of the newborn calves. In the initial stages of the experimental disease the infection predominates in the small intestine but later the large intestine is also affected (Tzipori *et al.*, 1983).

The intracellular stages of the organism are within a parasitophorus vacuole that is confined to the microvillus region of the host cell (Current, 1985). The cryptosporidia appear free in the lumen of the intestine and attach to the microvilli of the villus epithelial cells. The parasitophorus envelope of the trophozoites and schizonts are derived from the microvilli, and the intracellular location of the organism is confined to fusion of the organism with the apical cytoplasm of the epithelial cells and their enclosure by host membranes (Pearson and Logan, 1983). Thus the organism is intracellular but extra-cytoplasmic

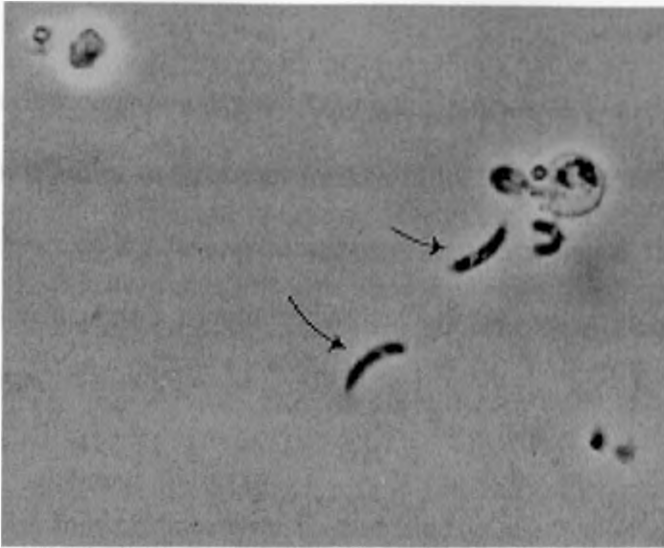


Figure 2: Phase contrast photograph of sporozoite release from *Cryptosporidium oocyst* (Flanigan and Soave 1993).

A sporozoite specific lectin adherence factor has been identified as the agent of attachment to the intestinal surface (Keusch *et al.*, 1995).

2.1.15 Clinical signs

2.1.15.1 Clinical signs in calves

Persistent discharge of yellow, watery faeces containing mucus is suggestive of cryptosporidiosis (Sanford and Josephson, 1982). The most prominent signs of cryptosporidiosis are seen in preweaned calves and include diarrhea accompanied by lethargy, inappetance, fever, dehydration and/or poor conditions, (Current, 1985).

Varying degrees of apathy, reduced feed intake and dehydration maybe present. Only rarely do severe dehydration, weakness and collapse occur as in other causes of acute diarrhea in

neonatal calves. The persistent nature of the diarrhea leads to a marked energy deficit and the calves die of inanition at three-four weeks of life. In the experimental disease in calves, depression and anorexia are the earliest and most consistent clinical findings. Feed intake is reduced and combined with persistent diarrhea over several days may cause emaciation (Fayer *et al.*, 1990).

However, recovery may occur between six and ten days after the onset of diarrhea. Both the incubation period and the clinical course of the diarrhea in calves affected with cryptosporidiosis tend to be a few days longer than diarrhea caused by rotavirus, corona virus or enterotoxigenic *E. coli* (Fayer *et al.*, 1990).

2.1.15.2 Clinical signs in children

Cryptosporidium infection was first recognized in humans because of its association with severe diarrhea. The various symptoms differ greatly between immunocompetent and immunocompromised individuals. Diarrhea is the most noteworthy symptom.

Characteristically, it is voluminous and watery, and often called cholera-like. As many as 71 times stool has been voided by patients and 12 to 17 per day have been reported. However less fulminant diarrhea also occurs, even in HIV-infected persons (Andreani *et al.*, 1983).

Mucous may be associated with diarrhea, but blood or leucocytes are rarely reported. As much as 25-Kg weight loss have been reported (Goodstein *et al.*, 1989). Crampy abdominal pain sometimes quite severe may occur. Less frequent symptoms accompanying diarrhea include low grade fever (<39°C) perhaps actually caused by other concomitant infections

particularly in immunologically deficient hosts; general malaise, weakness, or fatigue; a loss of appetite, nausea and vomiting. Toxic megacolon has been associated with cryptosporidiosis in one HIV-infected patient with severe diarrhea (Connolly and Gazzera 1987). Other clinical symptoms besides diarrhea associated with cryptosporidiosis include cholecystitis, hepatitis, pancreatitis, reactive arthritis, and a variety of respiratory symptoms.

Gallbladder disease primarily acalculous cholecystitis, but sometimes sclerosing cholangitis has been increasingly reported especially in HIV infected patients in whom chronic gallbladder carriage may be responsible for the inability to eradicate intestinal cryptosporidiosis (Davis *et al.*, 1996). Usually symptoms include fever, right upper quadrant non-radiating pain, nausea and vomiting, jaundice may also occur. *Cryptosporidium* infection of the respiratory tract particularly in immunologically impaired patients has become the subject of a growing number of case reports (Forgacs *et al.*, 1983).

While *Cryptosporidium baileyi* infecting poultry produces respiratory symptoms in poultry it has been shown to cause infection in humans. Respiratory symptoms associated with *Cryptosporidium baileyi* infection have been non specific: shortness of breath, hoarseness, wheezing, croup and, most often cough (Forgacs *et al.*, 1983).

2.1.15.3 Clinical signs in other species

In goats acute diarrhea and dehydration are the main clinical signs (Tzipori *et al.*, 1983). The persistent diarrhea results in marked loss of body weight and an appearance of emaciation in some cases. In experimental disease in lambs, depression, diarrhea and reduced feed intake are common and recovery occurs within a few days. More severe

clinical manifestation have been observed in the field in lambs subjected to environmental cold stress and those that are energy deficient due to an inadequate intake of colostrum.

Avian cryptosporidiosis can be respiratory, enteric or renal. Generally, clinical signs include coughing, sneezing, dyspnea and rales (Lindsay and Blagburn, 1990). The persistence of diarrhea usually suggests the presence of cryptosporidiosis.

2.1.16 Necropsy findings

Varying degrees of dehydration, emaciation and serous atrophy are present in animals which have had persistent diarrhea for several days. On biopsy or autopsy of immunocompromised patients with diarrhea, organisms have been located throughout the gastrointestinal tract from the esophagus and stomach to rectum and even appendix (Benkov *et al.*, 1985). In the intestine there is villus atrophy in the ileum, cecum, and colon. *Cryptosporidium parvum* is most often in the distal small intestine associated with villous atrophy, villous fusion, and metaplasia of the surface of the epithelium to low columnar or cuboidal cells, degeneration or sloughing of individual enterocytes and shortening of the microvilli (Baxby and Blundell, 1983).

Histologically the Cryptosporidia are associated with the microvilli of the villus epithelial cells. Large numbers of the parasites are embedded in the microvilli of jejunal and ileal absorptive enterocytes (Sanford and Josephson 1982). Mononuclear cells and neutrophils infiltrate the lamina propria (Belchev *et al.*, 1987). Cecum, colon and duodenum can also be

infected (Baxby and Blundell, 1983). Crypts in all sites become dilated and contain necrotic debris or dead leucocytes.

In the intestines of calves with cryptosporidiosis, the villi are shorter than normal and there is crypt hyperplasia and infiltration with a mixture of inflammatory cells (Heine *et al.*, 1984). In figure 4 blunting or loss of villi, elongation of crypts, and infiltration of the lamina propria are described.



Figure 3: Duodenal biopsy showing histologic changes which are not characteristic (H and E stain) (Chichino *et al.*, 1991).

2.2 Diagnosis

Diagnosis of cryptosporidiosis in humans and animals has progressed from histological staining of gut or other tissue biopsy specimens to simple and more sensitive assays that are designed to detect oocysts or other antigens in stools samples.

Preservation and storage of specimens containing oocysts is done as follows:

a) Preservation of specimen

For the diagnosis of cryptosporidiosis, stool specimens are submitted as fresh material or in 10% formalin or sodium acetate-acetic acid-formalin (SAF) preservatives. If parasites are not going to be used for subsequent tests, fixed specimens are recommended because of biohazard considerations. Fresh or preserved stool specimens can be examined as wet mounts or can be concentrated and stained to aid in the visualization of *Cryptosporidium* oocysts (Current, 1986).

b) Storage of specimens

Potassium dichromate solution (2 to 2.5 % w/v in water) is used routinely as a storage medium to preserve viable oocysts. When stored at 4° C in potassium dichromate solution, *Cryptosporidiu* oocysts remain viable for at least three months, and some may retain infectivity for up to 12 months (Current, 1986). Since the percentage of viable oocysts begins to decrease after three months of cold storage, it is advisable to generate fresh oocysts every three to four months (Current, 1986).

Some researchers, (Dubey *et al.*, 1989), preferred to store oocysts of *Cryptosporidium* species in balanced salt solution supplemented with antibiotics because chromium is an environmental contaminant requiring special disposal and handling procedures. Hank's balanced solution (HBSS) containing 10,000U penicillin, 10 mg streptomycin; 0.05 mg fungizone; and 500 U/ml mycostatin, is recommended for the storage of *Cryptosporidium* species oocysts and also for the storage of sporocysts of *Sarcocystis* species.

2.2.1 Staining methods for microscopical oocyst detection

Oocysts can be demonstrated in faeces by non invasive Giemsa staining techniques (Pohlenz *et al.*, 1978). In 1981 the Ziehl-Nielsen acid fast staining technique finally provided clinical and research laboratories with a simple and effective method for identifying oocysts in stool samples: bright red oocysts against a background of blue green faecal debris and yeasts (Henriksen and Pohlenz, 1981).

The acid fast staining technique has been modified and improved upon in the intervening years with the description of hot (Garcia *et al.*, 1983) and cold modified acid fast stains (Ma and Soave, 1983). Further modifications included the incorporation of dimethylsulfoxide (DMSO) into the acid fast stain (Bronsdon, 1984) and the incorporation of the detergent tergitol into the modified cold kinyoun acid fast method (Ma, 1986). An advantage of the acid fast staining methods is that other parasites like *Isospora* and *Cyclospora* can be detected in the faecal smears that would go unidentified if specific immunofluorescence or enzyme immunoassays were used. The modified acid-fast stain is traditionally used to detect the presence of cryptosporidial oocysts as shown on figure 4.

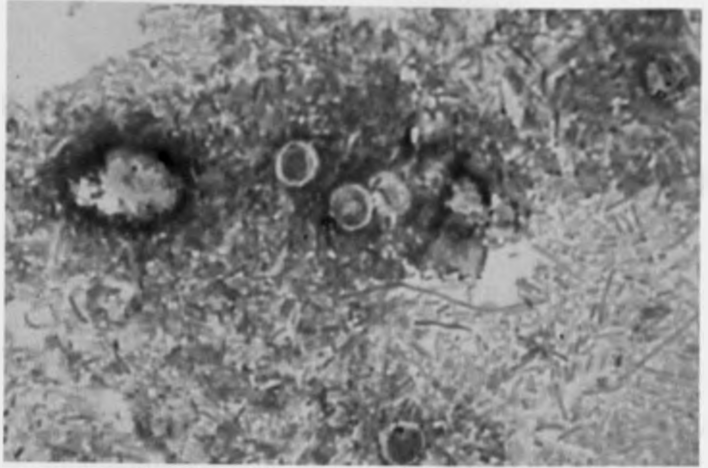


Figure 4: *Cryptosporidium parvum* oocysts: Oocysts appearing as bright organisms containing some dark granules and usually have a central structure (Modified acid fast stain) (Chichino *et al.*, 1991).

Alternatives to the bright field microscopic acid fast methods include neofuchsin (Chichino *et al.*, 1991), the hot safranin-methylene blue stain (Baxby *et al.*, 1988), Kohn's stain (Asahi *et al.*, 1988), modified Koster stain (Kageruka *et al.*, 1984), carbol-methyl violet and tartrazine (Mitacek and Vitorec, 1985). Fluorescent stains include auramine O, (Payne *et al.*, 1983), auramine-carbol-fuschin (Casemore *et al.*, 1985), and acridine orange (Ma and Soave, 1983).

2.2.2 Immunofluorescence microscopy for oocyst detection

Immunologic technique for the detection of cryptosporidia in stool specimens has been described (Casemore *et al.*, 1985; Stibbs and Ongerth, 1986). Indirect immunofluorescence assays were described for the detection of oocysts employing convalescent human serum (Casemore *et al.*, 1985) and oocyst-immunized rabbit antiserum (Stibbs and Ongerth, 1986). Immunofluorescent assays employing oocyst-reactive monoclonal antibodies

introduced (Sterling and Arrowwood, 1986). Immunofluorescence assays demonstrating cryptosporidial life cycle stages like oocysts in infected tissues or biopsy specimens have been reported (Bonnin *et al.*, 1990).

2.2.3 Enzyme immunoassays for Cryptosporidial antigen detection

Faecal enzyme immunoassays have been reported utilizing oocyst reactive monoclonal antibodies (Chapman *et al.*, 1990). The monoclonal antibodies were adapted for antigen detection in an antigen-capture enzyme immunoassay. Enzyme immunoassay is less sensitive than the modified Ziehl-Nielsen stain or immunofluorescence assay especially when oocyst numbers were small. An indirect double antibody enzyme immunoassay using polyclonal antisera has also been developed (Ungar, B.L.P., 1990), but the test was not as sensitive as the immunofluorescent procedure.

2.2.4 Other immunoassays for Cryptosporidial antigen detection

Oocyst detection in stool samples employing latex beads coated with antisera from oocyst-immunized rabbits has been reported (Pohjola *et al.*, 1986). This agglutination assay was applied to homogenized stools and gut content samples from *C. parvum* infected mice. The assay was rapid and simple to perform but lacked specificity. A flow cytometry-based assay was reported for quantitation of oocysts in faeces of experimentally infected mice (Arrowwood *et al.*, 1995). The assay used an oocyst-specific monoclonal antibody (IgG, 0W50) previously employed to detect oocysts in specimens by immunofluorescence microscopy (Garcia *et al.*, 1992).

2.2.5 Genetic methods for detecting oocysts

Genetic methods for detecting oocysts have been developed that identify and amplify *Cryptosporidium* nucleic acids using the Polymerase Chain Reaction (PCR) (Johnson *et al.*, 1983). Oocysts were detected by PCR, but direct use of PCR did not distinguish between live and dead oocysts since oocyst DNA is apparently preserved at least a week after cell death. Several *Cryptosporidium* DNA and RNA regions have been sequenced and reported to be valuable as targets for parasite detection and determination of viability.

Studies on the genetics of *Cryptosporidium* species are ongoing. DNA and isoenzyme characterization studies have shown significant genotypic differences between isolates. Pulsed-field electrophoretic studies have resolved 5-6 chromosomes for *C. parvum* isolates, although additional ones may be present as well. Chromosome size has not been well-characterized; sizes ranging from 900 to 3300 kb (depending on the technique employed) have been obtained.

No difference was found in the chromosomal banding patterns of *C. parvum* isolates from humans, calves or horses. Significant differences were observed between *C. parvum* and *C. baileyi* isolates (Laxer *et al.*, 1992). Most studies have detected significant molecular variation between *Cryptosporidium* species, as well as in different isolates of the same species.

The genetic basis of this variation needs to be characterized, with respect to virulence characteristics, immunogenicity and drug susceptibility. Several studies have identified recombinant or partial fusion antigens (Current and Garcia, 1991). These include a

sporozoite protein of 140 000 mol. wt., a large sporozoite and merozoite glycoprotein (300 000 mol. wt), another large sporozoite glycoprotein (900 000 mol. wt) encoded by a single copy gene on the largest chromosome, an oocyst wall protein (190 000 mol. wt) and an epitope shared by two recombinant proteins (15 000 and 60 000 mol. wt) (Current and Garcia, 1991). Other genes identified include a potential ATPase, a structural protein, a DNA-binding protein, and various cytoskeletal proteins (Current and Garcia, 1991).

After a *C. parvum* genomic DNA sequence was randomly selected for analysis, a 400-base sequence was specifically amplified using appropriate oligonucleotide primers and yielded a unique pattern upon restriction endonuclease digestion (Laxer *et al.*, 1991). The assay was reportedly sensitive and the authors predicted it would detect "small" numbers of oocysts in clinical or environmental samples. This DNA sequence (probe) was the basis of a PCR-based viability assay reported to function by detecting DNA in sporozoites released during excystation, relying on the digestion of free DNA before excystation and assuming that sporozoite DNA detected following excystation treatment was evidence of viability (release of sporozoites) (Mosier *et al.*, 1992).

A more PCR-based viability assay used a truncated DNA sequence derived from a *C. parvum* oocyst protein sequence to detect free sporozoites following excystation (Wagner-Wiening and Kimmig, 1995). A PCR-based assay targeting a small (18S) subunit rRNA sequence was reported to be as sensitive as immunofluorescence for detecting oocysts in water including waste water (Johnson *et al.*, 1983). The assay detected as few as 1 to 10 purified oocysts but was up to 1000-fold less sensitive in the presence of environmental

contaminants. Flow cytometry purification of environmental samples largely restored the sensitivity.

Mouse monoclonal antibodies have also been used to detect oocysts in fecal and water samples by immunofluorescence (Sterling and Arrowood, 1986). Most infections are diagnosed in the laboratory on the basis of acid-fast or immunofluorescence (IFA) staining of oocysts in fecal smears (Guerrant, 1997).

PCR methods are being used as well for *C. parvum* DNA detection in fixed tissue (Laxer *et al.*, 1992; Guerrant, 1997). This method has the advantage of accurately distinguishing *C. parvum* from non-*C. parvum* species.

2.2.6 Differential diagnosis of cryptosporidiosis

These are diseases and conditions which have clinical signs or show clinical picture which is similar to cryptosporidiosis. Important differential diagnosis of cryptosporidiosis includes:

- 1) Other protozoa: *Giardia*, *Isospora*, *Microsporidia*, *Cyclospora*, *Eimeria*, *Toxoplasma*.
- 2) Enteric bacteria: *Clostridium difficile*, *Salmonella* species, *Shigella* species, *Campylobacter* species, *Mycobacterium avium* complex.
- 3) Viruses: *Cytomegalovirus*, *Adenovirus*.
- 4) Adverse reactions to drugs: didanosine, clarithromycin, zidovudine.
- 5) HIV enteropathy.

2.3 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 spp.*, and *Plasmodium spp.*), 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 *et al.*, 1998).

In animals

Numerous compounds have undergone efficacy evaluation against *Cryptosporidium* species in non human hosts (Mead *et al.*, 1995). Most studies evaluating potential anti-cryptosporidial agents were conducted in laboratory rodents (Rasmussen *et al.*, 1995). Many compounds, maduramicin, alborixin, lasalocid, several aromatic amidines, salimomycin, dehydroepiandrosterone, paromomycin, L-arginine, glucanthine, clarithromycin, azithromycin, erythromycin, oleandomycin, spiramycin, halofuginone, metronidazole, sulphadimethoxine, sulfamerazine, sulfamethazine, sulfaquinoxaline, mepacrine, norfloxacin and mefloquine show promise in laboratory rodents (Rasmussen *et al.*, 1995).

Potential anti-cryptosporidial agents in ruminants include paromomycin, lasalocid, halofuginone and sulfaquinoxaline which possessed demonstrable or partial activity against *C. parvum* infections in ruminant species (Fayer and Ellis, 1993). Though little

information is available in animals other than rodents and ruminants, some success was achieved in treating cryptosporidiosis in felids and elapid snakes (Cranfield and Graezyk, 1994).

Animals suffering from cryptosporidiosis may require oral or parenteral rehydration with fluids and electrolytes in addition to anti-diarrheals and attempted chemotherapy with putative anti-cryptosporidial drugs. Rehydration is particularly important in young animals, immunocompromised animals or those suffering from intercurrent disease. The latter should receive appropriate antibiotic therapy if bacterial co-pathogens are involved.

In bovine cryptosporidiosis no specific anti-cryptosporidial drug has been identified. A few anti-coccidial or anti-protozoal drugs have demonstrable action upon the parasite. Claims for the efficacy of halofuginone lactate in calf cryptosporidiosis have been made by French scientists (Yvore and Naciri, 1989). In uncontrolled field trials, 67 diarrheic dairy calves under 4 weeks old, which were excreting *Cryptosporidium species* but no other detectable enteropathogen, were treated on 3 consecutive days with 0.5 mg / kg halofuginone lactate in aqueous solution. At 5 days after the start of the treatment, 97 % had stopped excreting oocysts, though only 68 % had ceased to scour, while altogether 22% of the treated calves died despite additional rehydration and other non specific supportive treatment (Fitzgerald, 1988).

Decoquinatate is a non antibiotic synthetic molecule, active on certain protozoa: coccidia, toxoplasma, cryptosporidia, neospora. This drug has also been tried in cryptosporidiosis therapy (Redman and Fox, 1994).

Several trials have been conducted for the evaluation of decoquinatone in treating cryptosporidiosis in ruminants. One such trial is the evaluation of decoquinatone to treat experimental cryptosporidiosis in kids, (Mancassola *et al.*, 1997). The purpose of this trial was to evaluate the effects of decoquinatone at 2.5 mg / kg / day for 21 days to prevent an experimental cryptosporidiosis in kids. Decoquinatone is well-tolerated by animals and may be recommended in the prevention of ruminant cryptosporidiosis, a disease which has very limited treatment options (Mancassola *et al.*, 1997).

Sulfaquinoxaline has been used either alone or combined with other sulfonamides or vitamins, (Contrepois and Vallet, 1984). The first priority which should usually be undertaken before the pathogen status is established, is to employ oral or parenteral rehydration measures based on glycine / electrolyte solution, perhaps coupled with the use of kaolin based absorbent and demulcent suspensions and / or antispasmodic drugs and to institute sensible isolation and hygiene measures to combat the spread of infection. Passive protection by normal or hyperimmune colostrum (Fayer *et al.*, 1989) is of very dubious value and probably does little to influence the scale or course of naturally occurring outbreak of cryptosporidiosis in calves (Current, 1985/a). Thus consideration of the housing and management arrangements in any given situation is important.

In humans

In immunocompetent individuals, general supportive care is the only treatment for the illness (Flanigan and Soave, 1993). Oral or intravenous rehydration and replacement of electrolytes may be necessary for particularly voluminous, watery diarrhea. Oral rehydration

include: gatorade, bonillon or oral rehydration solution containing glucose, sodium bicarbonate and potassium.

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; however, as in any diarrheal illness, hydration must be carefully monitored. In individuals with persistent diarrhea, an underlying immunodeficiency (HIV infection and congenital immunodeficiency) should be considered. In developing countries, children with cryptosporidiosis often have associated (or subsequent) malnutrition, which should be addressed.

In immunocompromised hosts, particularly AIDS patients with CD4 cell counts below $200/\text{mm}^3$, cryptosporidiosis can be life-threatening and must be treated aggressively. Initially, the nutritional, hydration, and electrolyte status of the patient should be assessed and corrected with intravenous hydration, if necessary. Antimotility agents, such as opiates and somatostatin analogues, may also be used.

In people with AIDS, the ideal treatment involves partial restoration of immune function with highly active antiretroviral therapy (HAART). Several case reports have demonstrated the resolution of cryptosporidial diarrhea coincident with a rise in CD4 cell count upon combination antiretroviral therapy (Carr *et al.*, 1998). If HAART therapy is not possible, several antibiotics that have some limited efficacy against *Cryptosporidium* (paromomycin, nitazoxanide, azithromycin) (Rasmussen *et al.*, 1995) should be considered.

For immunocompromised patients with cryptosporidiosis, several antimicrobial agents have been tested as possible treatments for the illness. Antibiotics such as spiramycin and dicalzuril sodium have produced partial responses in patients (a partial decrease in diarrhea or partial decrease in stool oocyst number), but have not yielded reliable reproducible results (Flanigan and Soave, 1993). One particular antimicrobial agent, paromomycin, has been shown to decrease the intensity of infection and improve intestinal function and morphology (Goodgame, 1996).

Nitazoxanide (NTZ) is the latest drug to be widely tested against human cryptosporidiosis. Nitazoxanide is a nitrothiazole benzamide with broad antimicrobial activity. An open-label study of NTZ in 15 Mexican AIDS patients with cryptosporidiosis found parasite clearance in nearly 100% of patients, triggering larger studies in the United States (Feregruno *et al.*, 1996). Another small, uncontrolled African study of NTZ also demonstrated that it has some efficacy (Dumbo *et al.*, 1997). Unfortunately, one larger clinical trial and in vivo animal studies have been less encouraging (Davis *et al.*, 1996).

Although antibodies do not provide protection from cryptosporidial infection, several studies have been done to show that antibodies in the intestinal lumen may help clear or even prevent infection. The feeding of bovine colostral immunoglobulins to patients has been shown to ameliorate symptoms of *Cryptosporidium* infection in humans (Heyworth, 1992). It has also been shown that the release of intestinal IgA accompanies this clearance of infection. In addition, anti-sporozoite antibodies have blocked the infectivity of *C. parvum* sporozoites in mice by inhibiting their ability to attach to the surface of the intestinal mucosa.

A study by Doyle *et al.*, (1993) reproduced the inhibition of *C. parvum* infection by hyperimmune bovine colostrum in vitro. A combination of pharmacologic and immunologic therapy may be the most effective scenario for combating *C. parvum* infection in immunodeficient persons. Effective treatment of humans suffering from cryptosporidiosis may require oral or parenteral rehydration with fluids and electrolytes, in addition to antidiarrheals and attempted chemotherapy with putative anti-cryptosporidial drugs. Rehydration is particularly important in children and immunocompromised individuals or those suffering from intercurrent disease.

2.4 Prevention and Control

2.4.1 Animals

Prevention of cryptosporidiosis in animals is best achieved by eliminating contact with viable oocysts. This is difficult, particularly on farms and in zoos, given the resistance of oocysts to disinfectants. Prevention is largely based on knowledge of the biology, life cycle and modes of transmission of *Cryptosporidium* species. A combination of hygienic practices, effective chemotherapy and supportive measures should result in effective control of most outbreaks of animal cryptosporidiosis.

The main target for any control regime should be the oocysts. The primary aim should be to kill them; failing this, effort should be concentrated upon delaying exposure of the vulnerable animal to any infected area for as long as possible. Stringent disinfection is the usual means adopted to free premises from pathogens. However with *Cryptosporidium* species, this is by no means straightforward, as the oocyst is remarkably impervious to

chemical agents. The only rational methods available are steam heat, 10% formalin or various concentrations of household ammonia (Angus, 1983). A spray disinfectant that generates release of ammonia on the working surfaces has been developed commercially (OO-CIDE, Antec International Ltd., Chilton Industrial Estate, Sudbury, Suffolk, England) and destroys coccidial and cryptosporidial oocysts (Blewett, 1989).

In vitro experiments have shown that high concentrations of hydrogen peroxide also can kill cryptosporidial oocysts (Blewett, 1989). Elimination of infection from calf pens and houses is an important primary strategy in controlling clinical cryptosporidiosis.

Available evidence suggests that it is very difficult, if not impossible, to keep calving areas free of cryptosporidial oocysts. Excretion of oocysts by the dams and contamination of the calf during the birth process cannot be ruled out; thus, control of this stage might be difficult. Where calves are to suck their dams, the best that can be achieved is to clean out the rearing area daily and to limit the amount of potentially infected faeces in the vicinity. However, in dairy units, calves are removed from their dams often after only one feed of colostrum.

There is evidence from reports of cryptosporidiosis in very well-managed rearing units that oocysts excretion is delayed when calves are individually penned for a period after they are born (Blewett, 1989). In two instances calves did not develop clinical disease until they were moved to communal housing (Blewett, 1989). If calves can be kept clear of infection for the first 2 to 3 weeks of their lives, the clinical effects of *C. parvum* infections are likely to be innocuous and transient. Furthermore, the structure of calf pens can have a bearing on the

availability of infective oocysts. An epidemiological study in Brazil found that the use of raised slatted floors reduced *C. parvum* infection significantly when compared with floors made of concrete or bare earth.

Where disinfection is not feasible, other measures have to be adopted like efforts being made to reduce exposure to infection by redistributing the animals. For example, where an explosive outbreak is under way an outdoor calving paddock, uncalved cows and cows with recovered, nondiarrheic calves should be moved away to another field. While acutely diarrheic calves and dams should be housed if possible. Later, the indoor premises could be completely disinfected.

Measures designed for controlling outbreak of cryptosporidiosis on the farm are equally applicable to other enteric infections, (Fayer *et al.*, 1990). The following principles should be adopted:

1. Calf rearing should be done on an “all-in, all-out” basis.
2. Individual pens should be stringently disinfected between batches of calves.
3. Calves should be born and raised in a clean, dry environment.
4. Ideally, newborn calves should be penned individually for 2 to 3 weeks.
5. Sick calves should be removed immediately from the company of healthy calves.
6. Healthy calves should have different attendants than those of sick calves.
7. Attendants should- keep boots, protective clothing, among others as free of feces as possible.
8. Utensils should be heat sterilized, if possible, daily.
9. Vermin, farm dogs, and cats should be controlled.

10. Colostrum management and nutrition should be satisfactory.

11. Appropriate prophylactic measures against other agents, such as rotavirus or Enterotoxigenic E.Coli K99+ vaccine, should be employed.

2.4.2 Humans

There is no medication that prevents cryptosporidiosis. To prevent cryptosporidiosis one needs first to practice good hygiene. Wash hands thoroughly with soap and water after using the toilet or before handling or eating food especially for persons with diarrhea. Wash hands after every diaper change, especially if one works with diaper-aged children. It is important to protect others by not swimming especially if experiencing diarrhea (Fayer *et al.*, 1990)

Avoiding water that might be contaminated by avoiding swallowing recreational water and drinking untreated water from shallow wells, lakes, rivers, springs, ponds and streams is important. If unable to avoid drinking or using water that might be contaminated, then treat the water by heating to a rolling boil for at least 1 minute or using a filter that has an absolute pore size of at least 1 micron. Chemical disinfection of *Cryptosporidium* cannot be relied on because it is highly resistant to inactivation by chlorine or iodine (Jakubowski, 1995).

It is also important to avoid food that might be contaminated. Wash and /or peel all raw vegetables and fruits before eating. Use uncontaminated water to wash all food that is to be eaten raw and avoid eating uncooked foods when travelling in countries with minimal water treatment and sanitation systems.

2.5 Participatory appraisal

Participatory appraisal is a method of involving the community in reviewing their situation, ranking opportunities and risks and in some cases plans and implementing them. It is an extension of rapid appraisal methods of social research, when the researcher visits the surveyed community and brief interview with available community members is able to review specific issues and obtain a quick snapshot of key indicators. Various participatory appraisal methods are available which are used to investigate animal health issues. These methods include a variety of interviewing methods, scoring and ranking tools and visualization tools such as seasonal calendars, maps, venn diagrams and flow diagrams. Collated descriptions of these methods were produced by Cornwall (Andrea Cornwall, 1992).

2.5.1 Review of participatory methodologies

2.5.1.1 Interviewing methods

Interviewing methods have been widely used during surveys. Not only are they useful methods in their own right but they were often considered to be an important component of other methods (Pretty *et al.*, 1995). Most if not all participatory appraisal methods involve interviewing skills and it was often the follow-up questions asked after the completion of a diagram, map or scoring tool which provided the most insightful information (Pretty *et al.*, 1995). In relation to veterinary medicine the use of semi-structured interviews in participatory appraisal has some overlap with history taking and diagnosis in veterinary practice.

2.5.1.2 Visualization method

a) Seasonal calendars

Seasonal calendars are used to illustrate temporal variation in disease incidence and parasite populations. In addition to depicting livestock disease incidence, seasonal calendars also could show livestock movements, human labour patterns, key animal management practices and rainfall (Catley and Ahmed, 1996).

2.5.1.3 Ranking and scoring methods

Ranking and scoring methods require the informants to assess the relative importance of different items. Ranking usually involve placing items in order of importance (first, second, third among others) whereas scoring methods use counters such as seeds, stones or beans to attribute to a specific score to each item. The methods enable informants identify and compare their own criteria of describing livestock diseases. They provide important data on morbidity, mortality and other important aspects of livestock diseases which are important in the particular study (Catley and Mohammed, 1996).

An example of ranking tool is pair-wise ranking and this requires informants to compare items or problems in pairs and decide which was most important. The results are usually presented in a simple matrix and a total rank for each item calculated.

Scoring methods can use various scoring procedures but, in general a pile of counters is used to measure an indicator. For example informants could be asked to score livestock diseases out of 10 for mortality and morbidity. In this example morbidity and mortality are

the important aspects of livestock disease which could be used to compare one disease with other. Other types of scoring methods can enable informants to identify and compare their own criteria for describing items such as livestock species or livestock diseases.

Scoring methods could be made more visual by using at least two methodological variations. In matrix scoring, a matrix is drawn on the ground with items along one axis and indicators along the other axis. Counters such as stones could be used to score the relationship between the items and the indicators. When the matrix is completed, the researcher is able to use the visual display as an aid to further questioning about different types of health problems which the participants had identified. A series of short semi-structured interviews could evolve and form the matrix in order to follow-up interesting results and cross-check information. A matrix drawn on the ground is also useful because it could easily be expanded in order to illustrate other groups of indicators or items which arose from the follow-on questioning (Rees *et al.*, 1998).

The other visually orientated scoring method is proportional piling. In this method a large pile of counters is used to represent a sum total of different items which shared similar features but varied in quantity and relative importance. The counters are then divided by the participants to show the relative sizes or importance of different items. Typically about 100 counters are used in the original pile (Grandin and Young, 1996).

3 MATERIALS AND METHODS

3.1 Description of the study area

The study area in Malindi district Coast province of Kenya is shown shaded in Figure 5. The district is one of the seven districts in Coast province. It borders Kilifi District to the south, Tana River District to the north and North West and Indian Ocean to the east. It lies between latitude 2° 20' and 4° south and longitude 39° and 40° 14' east and is 0-418 metres above sea level .The district covers an area of 7,605 km² and is divided into three administrative divisions namely, Malindi, Marafa and Magarini as shown in Figure 8.

The district has a monsoon type of climate with hot and humid conditions all the year round. It is hot and humid from January to April while June to August is the coolest period. Average annual temperatures range from 22.3°C to 26.6°C in lowlands while the hinterlands temperatures range from 30°C to 34°C. The area receives bimodal rainfall with long rains in mid-April to June and short rains in August to October. The mean annual rainfall is 1200mm (District development office, 2001).



Source: (www.justkenya.org)

Figure 5: Map of Kenya showing study area.

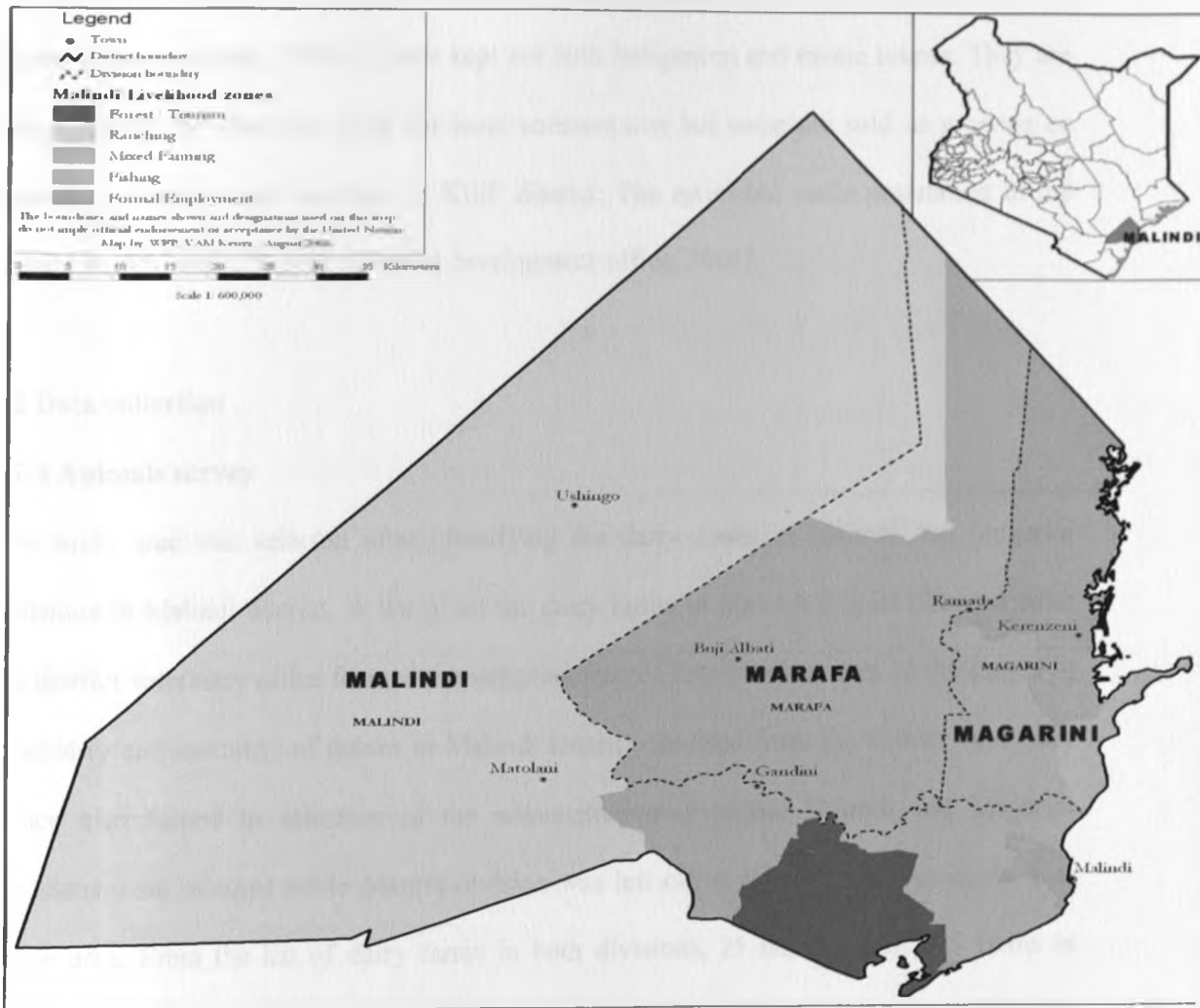


Figure 6: Administrative boundaries of Malindi district (Source: WFP / VAM KENYA)

The district population is 305,143 with an average population density of 36 persons per square kilometre (GoK, 1999). Cattle kept are both indigenous and exotic breeds. They are reared mainly for meat and milk for local consumption but some are sold as growing or fattening stock to cattle ranchers in Kilifi district. The estimated cattle population in the district in 2001 was 136,600, (District development office, 2001).

3.2 Data collection

3.2.1 Animals survey

The study area was selected after identifying the dairy zones in Malindi and Magarini divisions in Malindi district. A list of all the dairy farms in Malindi district obtained from the district veterinary office formed the sampling frame. Veterinary records on the causes of morbidity and mortality of calves in Malindi District obtained from the district veterinary office also helped in selection of the administrative divisions. Malindi and Magarini divisions were selected while Marafa division was left out as it was a predominantly beef cattle area. From the list of dairy farms in both divisions, 25 farms out of 185 farms in Malindi division were selected and 25 farms out of another 185 farms in Magarini division were selected using a simple random sampling procedure (random numbers table).

The sampling unit of interest and analysis was the average dairy farm, which was defined as a farm with ≥ 5 adult cows. The fifty farms formed the clusters and random sampling of the clusters was done to actually select the calves from which faecal samples were obtained. Sampling of the calves was done proportional to the size of the total herd in the respective farms. The owners of the selected farms were invited to participate in the study through a

letter that included a brief description of the study objectives and what was expected of the farmers. During the first visit, the farmers were requested to reaffirm their willingness to participate in the programme which they did. The sample size needed was calculated by the following formula:

$$n = (Z^2pq) / L^2 \text{ (Martin } et al., 1988).$$

$$n = (4*0.25*0.75) / 0.05^2$$

$$n = 300$$

Where n = number of calves needed for the study (sample size).

Z² = approximate square of Z (standard normal distribution unit) which provides a 95% confidence level.

p = estimated prevalence rate as obtained from work done by Fayer and others (1990).

q = 1 - estimated prevalence rate.

L = allowable error or required precision.

Confidence intervals were calculated to indicate the population prevalence using the method below.

To determine if we were to use normal distribution:

$n * p = 300 * p$; and $n * (1-p) = 300 * (1-p)$, where p = estimated prevalence rate as obtained from work done by Fayer and others (1990).

We were to use normal distribution when n is greater than 5.

Variance of p = $[p(1-p)]/n$ and standard error = $\sqrt{[p(1-p)]/n}$

Confidence interval = $p \pm Z_{\alpha/2} * SE$.

Three hundred calves were selected from the fifty farms and sampling was done proportional to the size of the farm. The farm data and calf management practices were

gathered through personal interviews with the farmers and the animal handlers conducted by the investigator personally to ensure consistency using a semi-structured questionnaire (Appendix 8.1) constructed in English language, but were translated in Kiswahili language in 43 of the 50 farms visited. The selected farms were visited three times every month and information on the socio-demography of the farmer, the farmer's experience and awareness of diarrhoea diseases and contact with other domestic animals collected using a questionnaire (Appendix 8.1). Primary data involved collection of faecal samples from calves of up to 6 months of age. The faecal samples were placed in clean polypots and preserved with 70% alcohol. A criterion was used to assess the hygiene and housing of the calves. This criterion is as shown in Table 1.

Table 1: Criterion used to assess the hygiene and housing of the calves

Rating	Characteristics
Good	Separate calf pens which are dry, spacious, no feces, cleaned daily, calves were clean, concrete floor, with hay or sand as bedding
Moderate	Separate calf pens which had concrete floor, not washed daily, feces present in pen, and on calves, hay as bedding
Poor	Floor not concrete, not dry, feces around, calves dirty, hay as bedding

3.2.2 Children survey

The survey began with a desk study at the Malindi District Hospital where the investigator went through the hospital records provided by the District Medical Officer of Health. The officer also provided the investigator with a list of the hospitals and all other health institutions in the selected study area from which 30 health institutions were randomly selected using random numbers table. The management of the health institutions was invited to participate in the study through letters that included a brief description of the study objectives and the expected roles of the institutions. The institutions agreed to collaborate in the programme. The study sample was obtained by selecting every fifth child less than five years of age attending the clinic or health centre on that particular visit. This age was selected because diarrhoea is third among the list of the leading causes of death in children under 5 years in Malindi district and cryptosporidiosis has a diarrhoeal syndrome.

The sample size needed was calculated by the following formula as described by Martin *et al.*, (1988).

$$n = (Z^2pq) / L^2$$

$$n = (4*0.12*0.88) / 0.05^2$$

$$n = 132.$$

Where n = number of children needed in the study (sample size).

Z^2 = approximate square of Z which provides a 95% confidence level.

p = estimated prevalence rate as obtained from work done by Fayer and others (1990).

q = 1- estimated prevalence rate.

L = allowable error or required precision.

Confidence intervals were calculated to indicate the population prevalence using the method below.

To determine if normal distribution was to be used:

$n \cdot p = 140 \cdot p$; and $n \cdot (1-p) = 140 \cdot (1-p)$, where p = estimated prevalence rate as obtained from work done by Fayer and others (1990).

We were to use normal distribution when n is greater than 5.

Variance of $p = [p(1-p)]/n$ and standard error = $\sqrt{[p(1-p)]/n}$

Confidence interval = $p \pm Z_{\alpha/2} \cdot SE$

One hundred and forty children were selected, seventy children from each division. During the visits to the health institutions, primary data was collected through collection of fecal samples from the children of up to 5 years of age. The parents or guardians were requested to give consent before their children could participate in the study by signing a consent form (Appendix 8.4) which was drafted in Kiswahili for ease of understanding by the participants. The parents were also requested to fill patient particulars form (Appendix 8.5). This was necessary so as to be able to trace the patient for further treatment and advice in case the child tested positive for cryptosporidiosis. The faecal samples were placed in clean polypots and preserved in 70% alcohol. Additional data were collected using a questionnaire (Appendix 8.2) administered via personal interviews with the children's parents or guardians. The questionnaires were constructed in English, but they were administered in Kiswahili to improve clarity on various issues. A third questionnaire (Appendix 8.3) was administered to the health service providers to obtain additional data especially to ascertain the estimated prevalence and risk factors of the disease according to the clinicians.

A criterion was used to assess storage of water among the participants. This criterion is as shown in table 2.

Table 2: Criterion used to assess storage of water among the participants

Rating	Characteristics
Good	Clean plastic water tanks installed or use of clean plastic drums to store the water.
Moderate	Use of clean plastic jerrycans or metal drums
Poor	Non specific containers used which are not clean.

3.2.3 Participatory Appraisal

This part of the study was conducted through holding of separate participatory workshops in the two divisions. The selected farmers whose farms were randomly identified for animal data survey were requested to attend the workshops for the participatory work. In this study the methods used were semi structured interviews, seasonal calendar, pair wise ranking, matrix scoring, and proportional piling to gather data on the following:

- 1) The most important calf diseases in the divisions.
- 2) Seasonality of the calf diseases.
- 3) Perception of the farmers on morbidity and mortality of the listed calf diseases.
- 4) Farmers' ability to identify or recognize the listed diseases.
- 5) Farmers' perception of the prevention and control methods against the listed diseases.

6) Economic importance of the calf diseases listed.

3.2.3.1 Relative importance of the diseases

The workshop participants listed the common calf diseases. Local names for the diseases were used. The clinical manifestations of these were solicited in order to find their English corresponding names. Pairwise ranking process was explained to the participants before the ranking process to find the most important calf diseases.

3.2.3.2 Seasonality of the diseases

The participants were asked to describe the seasons found in Malindi. They used their local names and this was translated to the Gregorian yearly calendar by trying to identify the season at the time of interview. The participants were then asked to show in which seasons the listed and ranked diseases in section 3.2.3.1 occurred in their herds.

3.2.3.3 Perception of the farmers' on morbidity and mortality of the diseases listed

The perception of the farmers' on morbidity and mortality of the listed diseases was assessed using proportional piling. Proportional piling method was explained to the participants before the scoring. Local names for the diseases were employed and the terms morbidity and mortality were explained to the participants. The participants were given 100 stones to distribute in a pile to illustrate their perception on the morbidity and mortality of the listed diseases. The participants first divided a pile of 100 stones on the proportion of healthy or sick calves in their respective herds during the last year and further score from the sick group against each of the listed calf diseases. Then from the sick group, the farmers

were requested to again score each disease from the list against 3 parameters which were dead, recovered and sold.

3.2.3.4 Farmers' ability to identify the listed calf diseases

Matrix scoring method was developed according to the method for livestock-disease scoring described by Catley and Mohammed (1996) to get the farmers' ability to identify the listed diseases. The participants developed a list of indicators for the diseases. Twenty six indicators were listed. These indicators were categorised by the participants into 12 'disease-signs', 9 prevention and control methods and 5 parameters to assess the economic importance of the listed diseases. In Magarini division the same exercise was conducted and the participants came up with a list of 24 indicators. These indicators were then categorised by the participants 11 'disease-signs', 8 prevention and control methods and 5 parameters to assess the economic importance of the listed diseases.

The key used in analysis of the scores for the scoring methods in the study was based on the results from the Kendall's coefficient of concordance. A coefficient of between 0.1 to 0.3 was considered poor or low agreement, while those between 0.4 and 0.6 were being considered moderate agreement. Coefficients of 0.7 to 1 were considered to be high or good agreement. This key was used in analyzing the level of agreement in all the exercises involving scoring or ranking methods in both divisions.

3.2.3.4.1 'Disease sign' matrix for the listed diseases

To construct the 'disease-sign', reference to a traditional game called 'kigogo' was made. This game uses two parallel rows of 12 shallow holes in the ground or a wooden plank and involves the movement of seeds, cowrie shells or stones between the holes by the players. Local familiarity with this arrangement was adapted to form the disease-signs matrix by making 12 columns and 8 rows of shallow holes in the ground, in Malindi division and 11 columns 8 rows of shallow holes in the ground, in Magarini division. The matrix had diseases along one axis and the signs on the other axis.

For each disease-sign, informants were asked to score the listed diseases by dividing a pile of 20 stones. The more important a particular disease-sign for that disease, the greater the quantity of stones assigned to it. After each disease-sign had been scored, the informants were asked to check their scores and confirm that as a group. They agreed that the scores were correct. When all the disease-signs had been scored, the results were recorded and additional questions were asked by the researcher to cross-check and probe the outcome.

3.2.3.5 Farmers' perception of the prevention and control methods

After the list of indicators was obtained (section 3.2.3.4) in each division, the next activity was to develop another matrix with diseases on one axis and the prevention and control methods on the other. For each disease-method, the informants were asked to score the listed diseases by dividing a pile of 20 stones. The more appropriate a particular prevention/control method for that disease, the greater the quantity of stones assigned to it. After each disease-method had been scored, the informants were asked to check their scores

and confirm their scoring as a group. When all the disease-methods had been scored, the results were recorded and additional questions were asked by the researcher to cross-check and probe and verify the scores.

3.2.3.6 Farmers' perception of the prevention and control methods

Proportional piling was then conducted to assess the participants' perception on the prevention and control methods for each of the diseases against the parameters identified. For each disease two methods which received the highest scores in the previous exercise of 'disease-prevention and control matrix' were picked to be used in the assessment. Five parameters were listed for the assessment of the methods against the diseases. For each disease, the participants were asked to score out of 5 stones each of the two methods selected against the disease. The more important the method of disease prevention or control with references to the parameter in question, the higher the points it gets.

3.2.3.7 Economic importance of the listed diseases

From the list of economic indicators listed in each of the two divisions, the participants were asked to score the listed diseases by dividing a pile of 20 stones. The more important economic parameter is for that disease, the greater the quantity of stones assigned to it. After each disease-parameter had been scored, the informants were asked to check their scores and confirm that as a group. They agreed that the scores were correct. When all the disease-parameter had been scored, the results were recorded.

3.3 Sample processing / analysis

The fecal samples collected were placed in clean polypots and stored in 70% alcohol. The fecal samples were taken to the laboratory where they were stained using acid-fast staining.

3.3.1 Acid-fast staining

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 15minutes, 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 centrifuged at 3000g for 15minutes. The aqueous layer was decanted and the pellet stored at +4°C prior to use, Modified Ziehl- Neelsen (MZN) method, (Pohjola *et al*, 1984).

Using the concentrated pellets obtained from the procedures above, moderately thick fecal smears dried in air were 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 and air dried and examined under a x100 magnification.

A positive slide showed oocysts presenting as round object, usually 4-5 μ m in diameter, with some degree of red staining of the mass filling the oocysts whereas most fecal debris and yeast cells take up the colour of the counterstain as shown in figure 7.

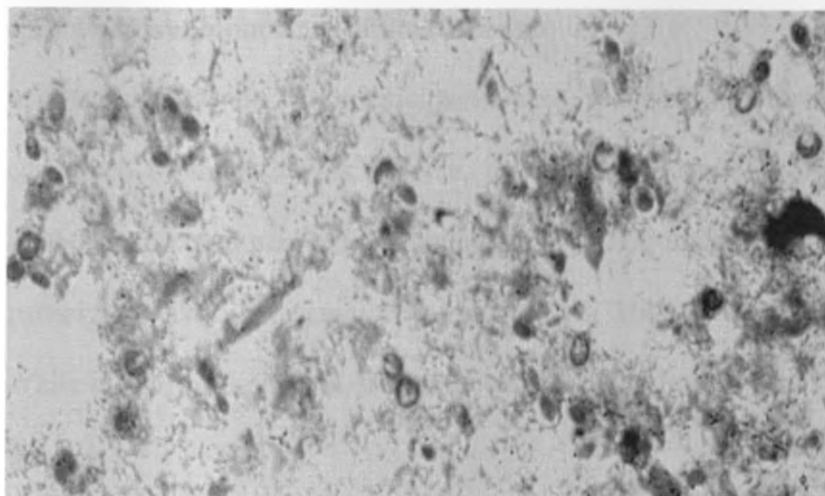


Figure 7: Acid-fast staining of *Cryptosporidium* oocysts (Magnification 825x).

(Source: www.soton.ac.uk)

3.4 Data analysis

Data was entered in Excel 2000 and was analyzed using Genstat. Descriptive statistics was computed using Genstat. Association between the risk factors and disease was assessed using chi-square and the strength of the association measured by odds ratio.

Analysis of the two matrices was done and assessment of agreement between the participants was also determined by calculating Kendall's Coefficient of Concordance. Any disagreement between the participants in scoring the disease signs or disease causes could be seen at this stage. This was to assess the local ability of the community to distinguish between diseases with similar clinical signs and also their ability to identify cryptosporidiosis.

4 RESULTS

4.1 Participatory Appraisal

4.1.1 Relative importance of the diseases

In Malindi division, the participants were grouped into three groups. The groups comprised of three classes, women, older men and younger men. Each group had a minimum of seven people and maximum of ten. Through semi-structured interviews, the participants listed 6 diseases and 8 conditions. The results were as shown in Table 3. While in Magarini division, the participants listed 8 diseases and 1 condition as shown in Table 4.

The farmers listed some diseases and conditions in their local names. Coccidiosis was locally referred to as “Kuharisha damu”, Foot and Mouth disease was locally referred to as “Aweb”. Pneumonia was locally called “Monia”. “Macho mekundu” locally referred to eye problems while “Mba” was a local name for dermatomycosis. Ectoparasites were locally called “Wadudu wa ngozi” while accidents were referred locally to as “Ajali”. Others were:

- a) “Minyoo” referring to helminthiasis.
- b) “Ngai” referring to East Coast Fever.
- c) “Kuharisha” referring to diarrhea of unknown etiology to the farmers.
- d) “Choo Kavuu” referring to hard feces e.g. in constipation.
- e) “Kutapika” referring to vomiting.
- f) “Nyongo” referring to Anaplasmosis.
- g) “Kuharisha kamasi” referring to mucoid diarrhea.

h) "Maumbile" referring to physical abnormalities.

From the pair wise ranking, the relative importance of each of the diseases listed by the farmers in Malindi division was established as shown on Table 3. Helminthiasis locally called 'minyoo' was the most important, being number one on the list.

Further discussion with the participants in Malindi division revealed that among the list of diseases and conditions listed, 6 diseases and two conditions are the ones most important to them.

These were the following:

1. Helminthiasis ("Minyoo).
2. Ectoparasites ("Wadudu wa ngozi").
3. Coccidiosis ("Kuharisha damu").
4. Eye problems ("Macho mekundu").
5. Pneumonia ("Monia").
6. East Coast Fever ("Ngai").
7. Foot and Mouth disease ("Aweb").
8. Diarrhea ("Kuharisha").

In Magarini division, the results of the pairwise ranking were as shown on Table 4. The list of most important diseases in Magarini division is as shown below:

- 1) "Minyoo" is the local name for helminths.
- 2) Pneumonia was locally referred to as "Monia".
- 3) "Ngai" is the local name for East Coast Fever.

- 4) "Nyongo" is the local name for anaplasmosis.
- 5) The locals referred coccidiosis as "Kuharisha damu".
- 6) "Aweb" is the local name for Foot and Mouth disease.
- 7) Navei ill was locally referred to as "Kufura kitovu".
- 8) "Kuharisha kamasi" referring to mucoid diarrhea.
- 9) "Maumbile" referring to physical abnormalities.

In the lists in both the divisions, cryptosporidiosis was not included by the farmers. The disease was added to the lists by the investigator as it was the disease of interest. The investigator described the clinical signs of cryptosporidiosis to the farmers to see if they have observed the disease in their calves. The farmers agreed they had seen the disease. The locals described a syndrome of profuse watery or mucoid diarrhea which does not respond to treatment and mostly affecting very young calves, those less than one month old. The farmers agreed they had seen the disease. The farmers described the syndrome in Kiswahili. To quote the farmers' description: "*Ndama wetu zinaharisha sana, choo chao ni majimaji sana au mara nyengine makamasi na haisikii dawa. Ndama wachanga ndio wanalemewa zaidi na ugonjwa*". This translated to literally mean: "*Our calves diarrhea profusely and the diarrhea is watery or sometimes mucoid and it does not respond to treatment. The very young calves are the ones which are more severely affected by the disease.*" From this cryptosporidiosis was then substituted for "Kuharisha" in the farmers list of most important diseases.

On further discussion with the farmers in Magarini they all agreed to remove abnormalities from the list which ranked last and so the first eight were taken as the most important calf diseases. From here the ranks of the diseases were established East Coast Fever locally called 'ngai being number one. There was a slight variations from the final eight diseases listed in Magarini from those listed in Malindi division. In Magarini division, Navel ill and Anaplasmosis were listed in the final list of eight diseases. These diseases were not listed in Malindi; instead ectoparasites and eye problems were among the final eight.

Table 3 Pairwise ranking of the most important calf diseases in Malindi division

	Min yoo	Ngai	Kuha-Risha	Choo KavU	Kuta-Pika	Nyongo	CCC	FMD	Pn	EP	Hernia	DMC	Hair Balls	Ectop.	Acc	Rank
Min-Yoo		Min-yoo	Min-Yoo	Min-Yoo	Min-Yoo	Min-Yoo	Min-yoo	Min-Yoo	Min-yoo	Min-Yoo	Min-yoo	Min-Yoo	Min-yoo	Min-yoo	Min-yoo	1
Ngai			Kuha-Risha	Choo KavU	Ngai	Nyongo	CCC	Ngai	Pn	Ngai	Ngai	Ngai	Ngai	Ectop.	Ngai	8
Kuha-Risha				Kuha-Risha	Kuha-risha	Kuha-risha	Kuha-risha	Kuha-risha	Kuha-risha	Kuha-risha	Kuha-risha	Kuha-risha	Kuha-risha	Ectop	Kuha-risha	3
Choo KavU					Choo KavU	Nyongo	CCC	Choo kavu	Pn	EP	Choo kavu	Choo kavu	Choo kavu	Ectop	Choo kavu	8
Kuta-Pika						Nyongo	CCC	Kuta-pika	Pn	EP	Kuta-pika	DMC	Kuta-pika	Ectop	Acc	12
Nyongo							CCC	Nyongo	Pn	EP	Nyongo	Nyongo	Nyongo	Ectop	Nyongo	6
CCC								CCC	CCC	CCC	CCC	CCC	CCC	Ectop	CCC	4
FMD									Pn	EP	FMD	DMC	FMD	Ectop	Acc	13
Pn										EP	Pn	Pn	Pn	Ectop	Acc	6
EP											EP	EP	EP	Ectop	EP	5
Hernia												DMC	Hair Balls	Ectop	Acc	15
DMC													DMC	Ectop	Acc	11
Hair Balls														Ectop	Acc	14
Ectop															Ectop	2
Acc																8

Key: CCC = Coccidiosis. FMD = Foot and Mouth disease. Pn = Pneumonia. EP = Eye problems. DMC = Dermatomycosis. Acc = Accidents

Ectop = Ectoparasites. Nyongo = Anaplasmosis. Kutapika = Vomiting. Kuharisha = Diarrhea. Choo kavu = Hard feces. Minyoo = Helminths.

Ngai = East Coast Fever.

Table 4 Pairwise ranking of the most important calf diseases in Magarini division

	Aweb	Monia	Kuharisha damu	Minyoo	Kufura Kitovu	Ngai	Nyongo	Kuharisha Kamasi	Maumbile	Rank
Aweb		Monia	Kuharisha damu	Aweb	Kufura Kitovu	Ngai	Aweb	Kuharisha Kamasi	Aweb	6
Monia			Monia	Monia	Monia	Ngai	Monia	Monia	Monia	2
Kuharisha damu				Kuharisha damu	Kuharisha damu	Ngai	Kuharisha damu	Kuharisha damu	Kuharisha damu	3
Minyoo					Kufura Kitovu	Ngai	Nyongo	Kuharisha kamasi	Minyoo	8
Kufura Kitovu						Ngai	Nyongo	Kufura Kitovu	Kufura Kitovu	4
Ngai							Ngai	Ngai	Ngai	1
Nyongo								Nyongo	Nyongo	4
Kuharisha Kamasi									Kuharisha kamasi	6
Maumbile										9

Key: Aweb = Foot and Mouth disease. Monia = Pneumonia Kuharisha damu = Coccidiosis. Minyoo = Helminths. Kufura kitovu = Navel ill.

Ngai = East Coast Fever. Nyongo = Anaplasmosis. Kuharisha kamasi = mucoid diarrhea. Maumbile = Physical abnormalities.

4.1.1.2 Seasonality of the diseases

In both Malindi and Magarini divisions, after the pair wise ranking, seasonality of the most important diseases listed was established. Seasonal calendar was the participatory appraisal tool used. Through semi-structured interviews with the participants, it was agreed among all the participants that both division has four weather seasons. The participants related the weather pattern with the flow of tourist in the district. They reported that the tourists' numbers decrease at the beginning of April and mid October due to the rains starting. The tourist season is at its peak beginning September and at the start of February. The participants first named the seasons as shown in Table 5 below:

Table 5: Seasons in Malindi district as perceived by the farmers.

Season in Swahili	Period of the year	Predominant weather
Mwaka	April to end of August	Long rains
Kaskazi ya Vuli	September to mid October	Dry period
Vuli	Mid October to end of January	Short rains
Kaskazi ya Mwaka	February to March	Dry period

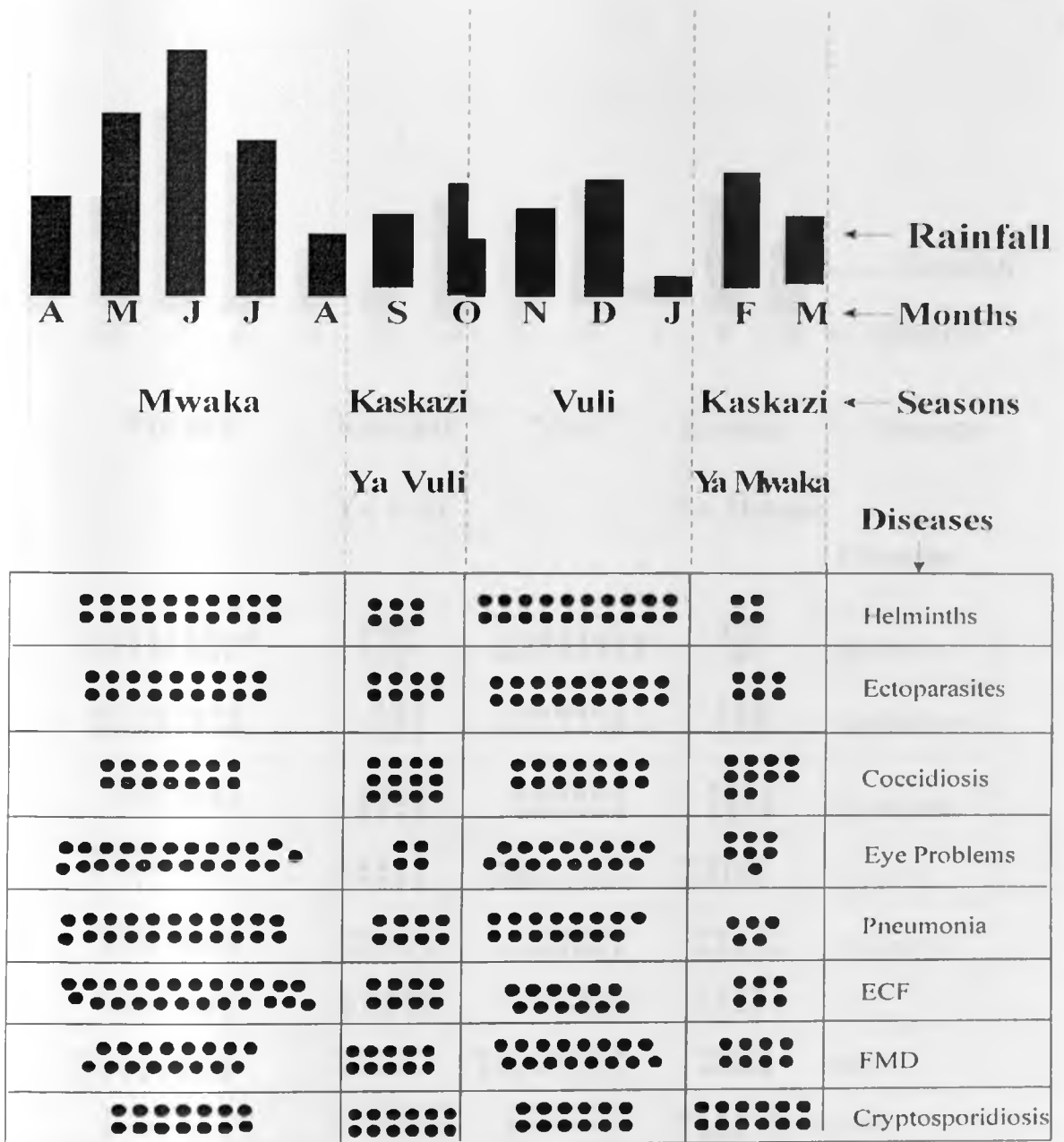
They marked the beginning of the year by the beginning of the long rains. The long rains begin in April to the end of August referred to locally as "Mwaka" while the short rains begin mid October locally referred to as "Vuli". The shorter dry season begins in September referred to as locally "Kaskazi ya Vuli" and the longer dry period begins in February referred to as locally "Kaskazi ya Mwaka". The seasonality of the most important diseases in the division was then discussed among the participants and the results were recorded.

In both divisions, helminthiasis locally called "*Minyoo*" was listed to be a condition occurring throughout the year but cases increase during the rainy seasons. In Malindi division, ectoparasites or "*Wadudu wa ngozi*" had cases increasing during the rainy seasons but cases were said to be seen throughout the year. Coccidiosis or "*Kuharisha damu*" occurs throughout the year in both divisions and that it has no specific season. Navel ill or "*Kufura Kitovu*" was said to occur throughout the year in Magarini division, but cases tend to drop during the dry periods. Cryptosporidiosis was listed as a disease occurring throughout the year with no specific season in both divisions.

Eye problems or "*Macho mekundu*" especially pink eye was one the main problem listed by farmers in Malindi division although eye conditions can be caused by various other organisms or even physical trauma. The condition was reported to be more prevalent during the rainy periods and the cases reduce in number during the dry periods. In both divisions, pneumonia, "*Monia*" had cases highest during the "*Mwaka*" season and during the "*Vuli*". East Coast Fever or "*ngai*" is most common during the rainy seasons. In Magarini division, Anaplasmosis or "*Nyongo*" was reported to be most common during the rainy seasons

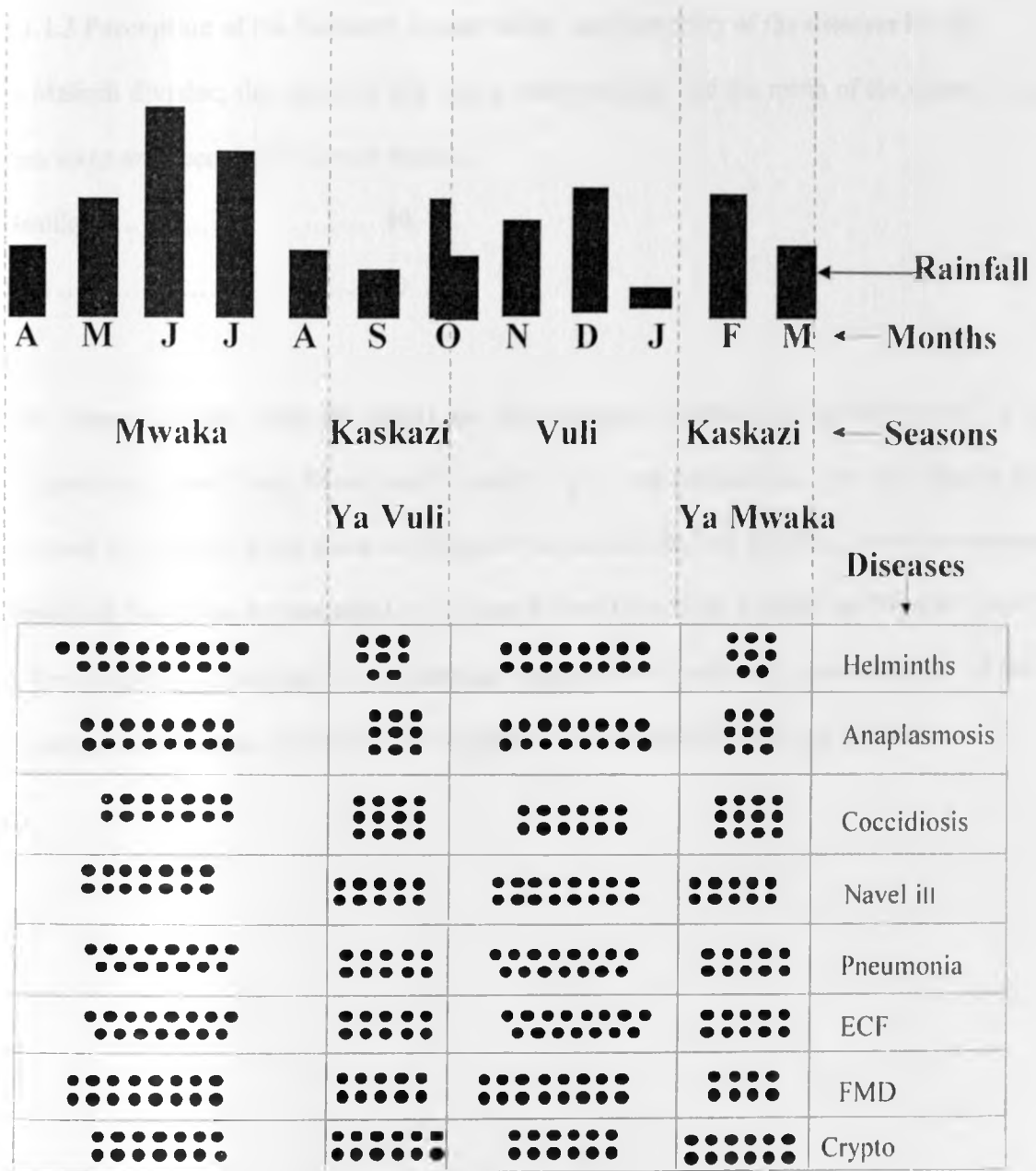
In Malindi and Magarini divisions, Foot and Mouth Disease or "*Aweb*", is the most irregular disease as far as seasonality is concerned because outbreaks are usually associated with movement of infected animals from neighbouring district, Tana River district.

A seasonal calendar depicting both the rainy and dry seasons together with the diseases and their seasons was then drawn as shown in figure 8 and figure 9.



Key: ECF: East Coast Fever; FMD: Foot and Mouth Disease.

Figure 8: Disease - season calendar of the most important calf diseases in Malindi division (n = 50).



Key: ECF: East Coast fever; FMD: Foot and Mouth Disease; Crypto: Cryptosporidiosis

Figure 9: Disease - season calendar of the most important calf diseases in Magarini division (n = 50).

4.1.1.3 Perception of the farmers' on morbidity and mortality of the diseases listed

In Malindi division, the results of the scores were recorded and the mean of the scores calculated then taken and recorded as shown below:

Healthy 40
Sick 60

The farmers scored relatively highly on four diseases, helminthiasis locally called 'minyoo', ectoparasites, East Coast Fever locally called 'ngai' and pneumonia. Foot and Mouth Disease received a relatively good score as compared to coccidiosis, eye infection and cryptosporidiosis. Results of the scores are presented in a Figure 10 and table 6 for Malindi and Figure 11 and Table 6 for Magarini. A summarized percentage comparison of morbidity and mortality of the most important calf diseases in Malindi and Magarini was recorded as shown on Table 6.

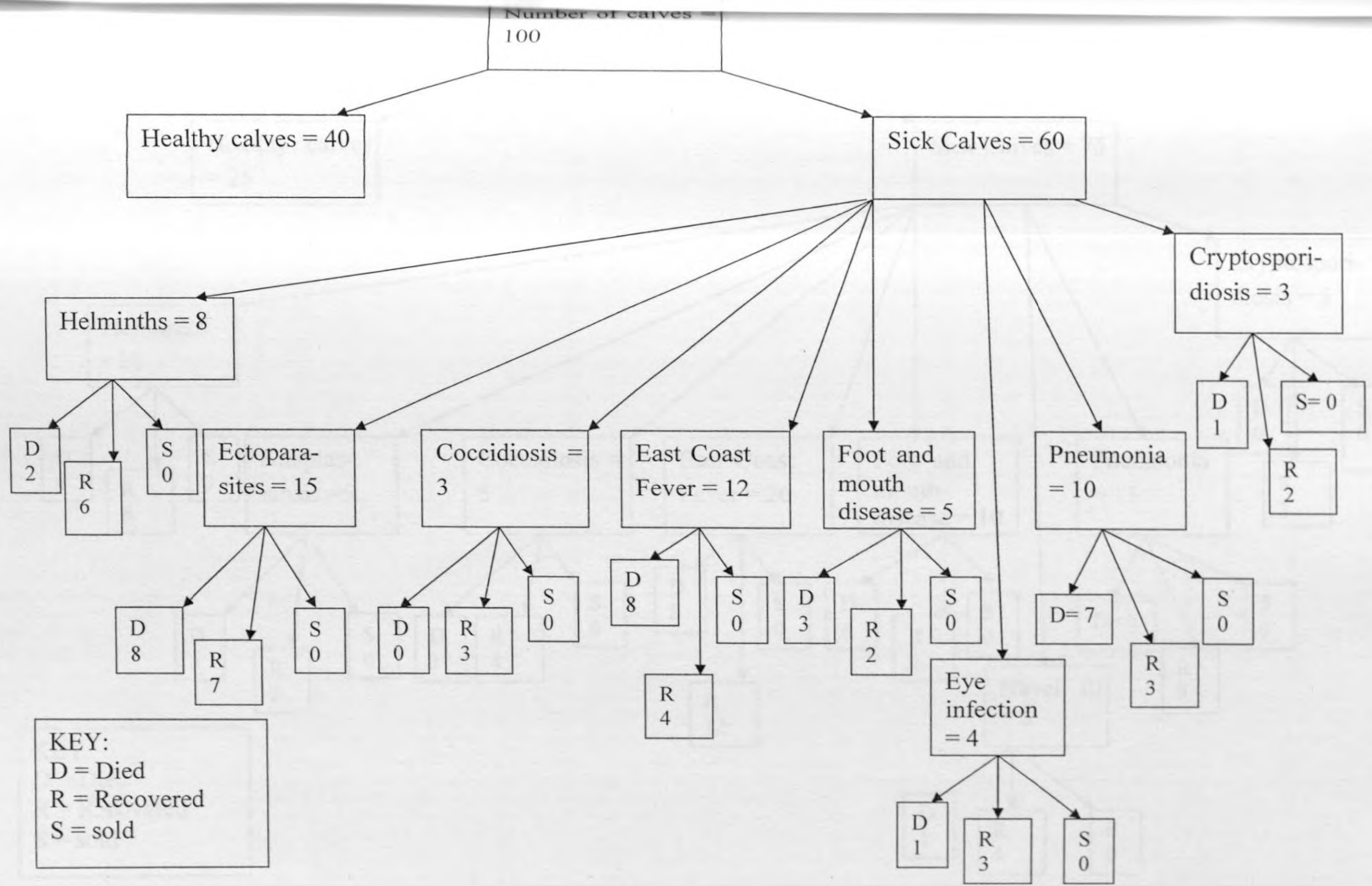


Figure 10.....Perception of morbidity and mortality of the most important calf diseases in Malindi division

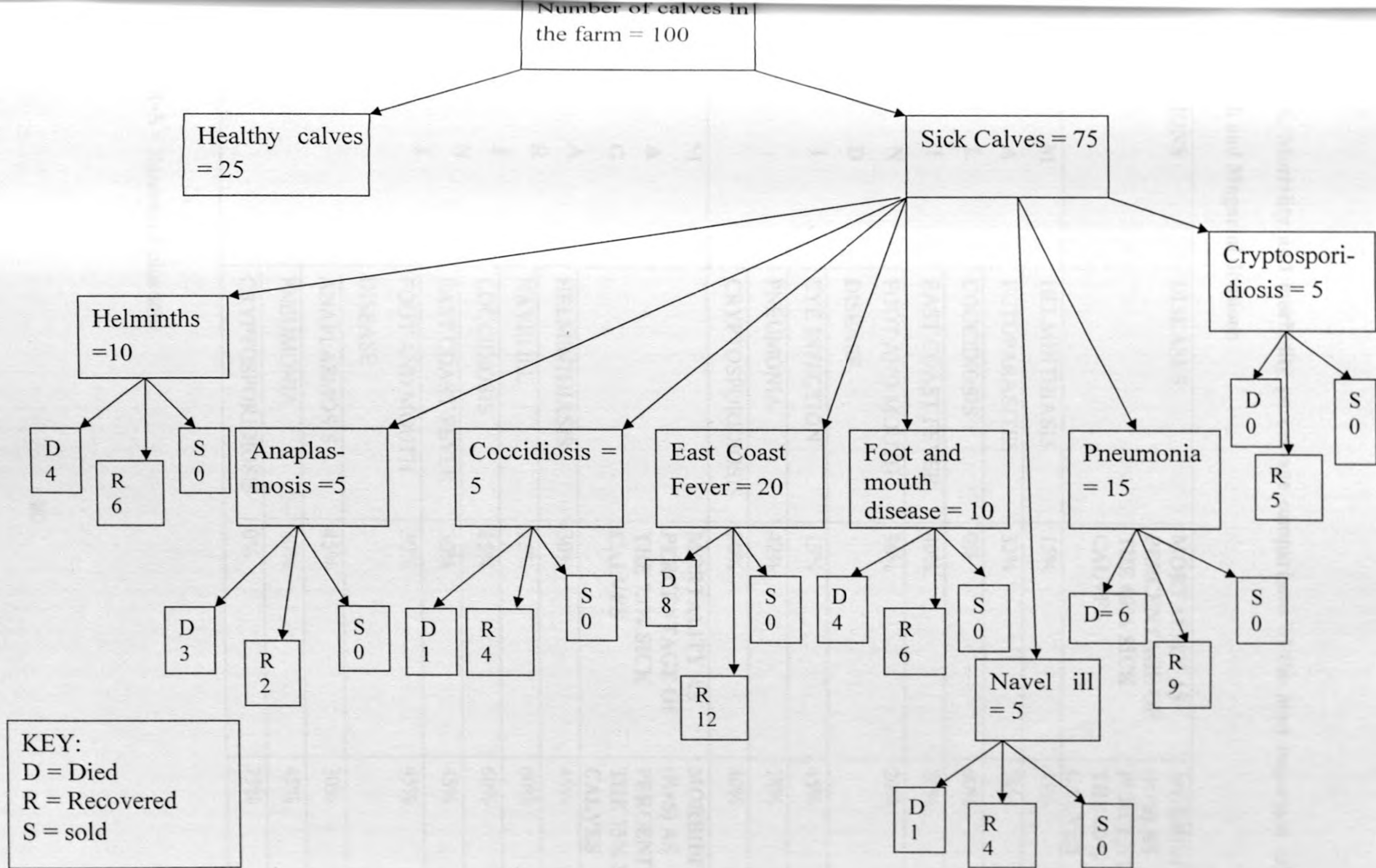


Figure 11.....Perception of morbidity and mortality of the most important calf diseases in Magarini division

Table 6: Mortality and morbidity percentage comparisons of the most important calf diseases in Malindi and Magarini divisions

DIVISIONS	DISEASES	MORTALITY AS PERCENTAGE OF THE 60% SICK CALVES	MORBIDITY (R+S) AS PERCENTAGE OF THE 60% SICK CALVES
M A L I N D I	HELMINTHIASIS	15%	45%
	ECTOPARASITES	32%	28%
	COCCIDIOSIS	0%	60%
	EAST COAST FEVER	40%	20%
	FOOT AND MOUTH DISEASE	36%	24%
	EYE INFECTION	15%	45%
	PNEUMONIA	42%	18%
	CRYPTOSPORIDIOSIS	20%	40%
M A G A R I N I		MORTALITY AS PERCENTAGE OF THE 75% SICK CALVES	MORBIDITY (R+S) AS PERCENTAGE OF THE 75% SICK CALVES
	HELMINTHIASIS	30%	45%
	NAVEI ILL	15%	60%
	COCCIDIOSIS	15%	60%
	EAST COAST FEVER	30%	45%
	FOOT AND MOUTH DISEASE	30%	45%
	ANAPLASMOSIS	45%	30%
	PNEUMONIA	30%	45%
	CRYPTOSPORIDIOSIS	0%	75%

Key: R+S = Recovered plus sold.

4.1.1.4 Farmers' ability to identify the listed diseases

The disease signs listed in Malindi division were watery diarrhea, pot belly, and bloody diarrhea, seeing parasites, red eyes, swollen lymph nodes, dyspnea, cough, sudden death, lameness, mucoid diarrhea and lacrimation while those listed in Magarini division were the same apart from lacrimation, red eyes and seeing parasites which were replaced with emaciation and mouth ulcers. The results were recorded and respective means of the scores calculated and recorded as shown in Table 7 and Table 8. The level of agreement tested by calculating Kendall's coefficient of concordance. The black dots represent the means of the scores (number of stones). P – Values for the respective coefficients were determined using the chi – square tables and recorded in the respective tables.

Table 7: Matrix scoring results for the disease-sign matrix for diseases in Malindi division (n = 50; W = Kendall's Coefficient)

Diseases → <u>Signs</u> ↓	Minyoo (Helmi- ths)	Ectoparasites	Cocci- Diosis	Eye Problems	Pneumonia	Ngai (East Coast fever)	Aweb (Foot and mouth disease)	Crypto- sporidiosis	P - values for W for disease- signs
Watery diarrhea W = 0.218	●●●● ●●●		●●● ●●					●●●● ●●●●	P < 0.25
Pot belly W = 0.329	●●●●● ●●●●	●	●●				●●	●●● ●●●	P < 0.05*
Bloody diarrhea W = 0.329	●●●		●●●●● ●●●●●			●●●		●●●●	P < 0.05*
Seeing parasites W = 0.96		●●●●●●●● ●●●●●●●● ●●							P < 0.01*
Red eyes W = 0.964				●●●●●●●● ●●●●●●●●					P < 0.01*
Swollen lymph nodes W = 0.67		●●●● ●●●●				●●●● ●●●● ●●			P < 0.05*
Dyspnea W = 0.285	●●●				●●●● ●●●●	●●●● ●●●			P < 0.25
Cough W = 0.215	●●● ●				●●●● ●●●●	●●● ●●●			P < 0.25
Sudden death W = 0.148		●	●		●●● ●	●●●● ●●●	●●● ●●●		P < 0.25
Lameness W = 0.98							●●●●●●●● ●●●●●●●●		P < 0.01*
Mucoid diarrhea W = 0.314	●●● ●●●							●●●●●● ●●●●●●	P < 0.05*
Lacrimation W = 0.982				●●●●●●●● ●●●●●●●●					P < 0.01*

*= Significant p-value ≤ 0.05

Table 8: Matrix scoring results for the disease-sign matrix for diseases of calves in Magarini division (n = 50; W = Kendall's Coefficient)

Diseases → <u>Signs</u> ↓	Minyoo (Helminths)	Navel ill	Coccidiosis	Nyongo (Anaplasmosis)	Pneumonia	Ngai (East Coast Fever)	Aweb (Foot and mouth disease)	Crypto- sporidiosis	P – values for W for disease-signs
Watery diarrhea W = 0.254	•••• •••		•••• ••					•••• •••	P < 0.25
Pot belly W = 0.634	•••••• ••••••							•••• ••••	P < 0.05*
Bloody diarrhea W = 0.54			•••••• ••••••			•••••• ••••••			P < 0.05*
Emaciation W = 0.212	•••••• •••••			•••• ••••		•••• ••			P < 0.25
Mouth ulcers W = 0.946							•••••••••• ••••••••••		P < 0.01*
Swollen lymph nodes W = 0.467		•• ••		•• ••		•••••• ••••••			P < 0.05*
Dyspnea W = 0.326				•• •	•••• ••	•••• ••••	•• ••		P < 0.05*
Cough W = 0.293				•• ••	•••••• ••••••	•••• ••••			P < 0.05*
Sudden death W = 0.119			•• •	•• ••	•• •	•••• ••	•• ••	•	P < 0.25
Lameness W = 0.467		•• ••		••		••	•••••• ••••••		P < 0.05*
Mucoid diarrhea W = 0.246		•••••• ••••	•• ••					•••••• ••••	P < 0.25

*= Significant p-value ≤ 0.05

Analysis of the disease-signs matrix scores in Malindi division, demonstrated good agreement between the participants' groups for five out of the twelve disease signs and moderate agreement in six out of the twelve disease sign. There was poor agreement in one disease sign. The participants did not agree in the disease causing 'sudden death'. Red eyes and lacrimation were strongly associated with eye problems with a coefficient close to one. Lameness was strongly associated with Foot and Mouth Disease while the participants depended on largely seeing the ectoparasites for them to identify the problem. Swollen lymph node locally called '*tezi* or *kanjir*' was moderately associated with East Coast Fever and ectoparasite.

In Magarini division, analysis of the disease-signs matrix scores demonstrated good agreement between the participants' groups for 5 out of the 11 disease signs and moderate agreement between another 5 out of the 11 disease signs. There was low agreement in one of the disease sign which was disease causing sudden death. The participants highly associated mouth ulcers with Foot and Mouth Disease and lameness. Pot belly was also seen to be closely related with helminthiasis. According to the participants, watery diarrhea was related to helminthiasis, coccidiosis and cryptosporidiosis.

Swollen lymph nodes locally called '*tezi* or *Kanjir*' were closely related with East Coast Fever, as was the case with mucoid diarrhea and cryptosporidiosis. Loss of body condition or emaciation was related to helminthiasis, Anaplasmosis locally called '*nyongo*' and East Coast Fever. According to the participants bloody diarrhea is associated with coccidiosis and East Coast Fever. Dyspnea and cough was associated with pneumonia and East Coast Fever.

4.1.1.5 Farmers' prevention and control methods

In Malindi division, these were deworming, dip / spray, injectables, clear bushes, isolation, hygiene, housing, vaccination and rotational grazing. In Magarini division, the indicators were the same as in Malindi division except for injectables which was not mentioned. In both divisions, the prevention and control measures the farmers are using in their farms was assessed using matrix scoring. The results in Malindi and Magarini divisions were recorded as shown in Table 9 and Table 10.

Table 9: Matrix scoring results for disease-control methods matrix for diseases of calves in Malindi division (n = 50).

Diseases → <u>Methods</u> ↓	Minyoo	Ectoparasites	Cocci- diosis	Eye Problems	Pneumonia	Ngai	Aweb	Crypto	P – value for W for disease- methods
Deworming W = 0.982	●●●●●●●● ●●●●●●●●								P < 0.01*
Dip / Spray W = 0.221	●	●●●●● ●●●●		●●	●	●●●● ●●●			P < 0.25
Injectables W = 0.232	●●●	●●● ●●			●●● ●	●●●● ●●●●			P < 0.25
Clear bushes W = 0.215	●●● ●	●●●● ●●●●			●●	●●● ●●●			P < 0.25
Isolation W = 0.344			●●● ●●	●● ●	●● ●		●● ●●	●●● ●●	P < 0.05*
Hygiene W = 0.282			●●●● ●●●●	●● ●●	●●			●●● ●●●	P < 0.25
Housing W = 0.229			●●● ●●●	●●● ●●	●●● ●●●			●●●	P < 0.25
Vaccination W = 0.964							●●●●●●●● ●●●●●●●●		P < 0.01*
Rotational grazing W = 0.367	●●●	●●●● ●●		●● ●●		●●●● ●●●			P < 0.05*

Key: “Minyoo”: Helminthiasis; “Ngai”: East Coast Fever; “Aweb”: Foot and Mouth Disease; “Crypto”: Cryptosporidiosis.

* = Significant p-value ≤ 0.05

Table 10: Matrix scoring results for disease-control methods matrix for diseases of calves in Magarini division (n = 50).

Diseases → Methods ↓	Minyoo	Navel ill	Coccidiosis	Nyongo	Pneumonia	Ngai	Aweb	Crypto	P – value for W for disease- methods
Deworming W = 0.964	●●●●●●●● ●●●●●●●●								P < 0.01*
Dip / Spray W = 0.455				●●●●● ●●		●●●●● ●●●●●			P < 0.05*
Clear bushes W = 0.1537	●●● ●●			●●● ●●●	●● ●●	●●● ●●			P < 0.25
Isolation W = 0.226		●● ●●	●●● ●●●				●●●● ●●●●	●●	P < 0.25
Hygiene W = 0.234	●●●● ●●●●	●●●● ●●●						●●● ●●	P < 0.25
Housing W = 0.946					●●●●●●●● ●●●●●●●●				P < 0.01*
Vaccination W = 0.946							●●●●●●●● ●●●●●●●●		P < 0.01*
Rotational grazing W = 0.621	●●●●● ●●●●●			●● ●●		●●● ●●●			P < 0.05*

Key: “Minyoo”: Helminthiasis; “Ngai”: East Coast Fever; “Aweb”: Foot and Mouth Disease; “Crypto”: Cryptosporidiosis;
 “Nyongo”: Anaplasmosis.

* = Significant p-value ≤ 0.05

In Malindi division, analysis of the matrix scores demonstrated good agreement between the participants' groups in two out of the nine prevention and control measures. There was moderate agreement in the rest seven methods. The farmers strongly associate deworming with helminthiasis with a coefficient of 0.982. Vaccination was the absolute best method in controlling and preventing Foot and Mouth Disease. Dipping or spraying with acaricides was the best way to prevent or control East Coast Fever and ectoparasites.

In Magarini division, analysis of the matrix scores demonstrated good agreement between the participants' groups for four out of the eight methods. There was moderate agreement in three other methods. But the participants did not agree in one method, as there was poor agreement on the 'clear bushes' method with a coefficient of 0.1537. The participants strongly associated deworming with prevention and control of helminthiasis. Dipping or spraying with acaricides was the best methods in preventing East Coast Fever and Anaplasmosis that are tick - borne diseases.

Housing was closely associated with pneumonia as was vaccination in the case of Foot and Mouth Disease. The participants moderately agreed that rotational grazing can help in controlling helminthiasis, Anaplasmosis and East Coast Fever. The participants moderately agreed that hygiene can help in the control of navel ill and cryptosporidiosis which are diarrheal syndromes and helminthiasis. There was moderate agreement in the method that isolation of the sick calf could help in controlling Foot and Mouth Disease and the diarrheal syndromes including navel ill, coccidiosis and cryptosporidiosis.

4.1.1.6 Farmers' perception of the prevention and control methods

The parameters that the participants came up with were effectiveness referring to how effective is the method in prevention and or control of the diseases. Others were financial cost or the amount of money involved in using the method, user friendly referring to ease of use of the method and group action was the other indicator which referred to if the method required action by a group of farmers for the desired results to be achieved. The fifth indicator was individual action referring to if the method only required action by only one farmer for desired results to be achieved. In both divisions, the means of the scores were calculated and recorded. In Malindi division, the results were as shown in Table 11.

Table 11: Proportional piling results for the farmers' perception on the prevention and control methods for the calf diseases in Malindi division (n= 50).

Diseases ↓	Methods ↓	Parameters				
		Effectiveness	Financial cost	User friendly	Group action (Effort by group of farmers)	Individual action (Effort by one farmer)
Minyoo (Helminths)	Deworming	●●● ●●	●● ●	●●● ●●		●●● ●●
	Rotational grazing	●● ●	●	●● ●●		●●● ●●
Eye problems	Hygiene	●● ●●	●● ●	●● ●		●●● ●●
	Housing	●● ●	●●	●		●● ●●
Ectoparasites	Dip / spray	●●● ●●	●●● ●●	●● ●●	●	●●● ●●
	Housing	●● ●	●● ●	●●		●●
Pneumonia	Housing	●●● ●●	●●● ●●	●●● ●●		●● ●●
	Hygiene	●● ●	●● ●●			●●● ●●
Coccidiosis	Housing	●●● ●●	●●● ●●	●● ●	●	●●● ●●
	Hygiene	●● ●●	●● ●●	●● ●		●●● ●●
Ngai (East Coast Fever)	Dip / spray	●●● ●●	●●● ●●	●●● ●●	●●● ●●	●●● ●●
	Rotational grazing	●● ●	●●● ●●	●● ●●	●●● ●●	●●● ●●
Foot and Mouth Disease	Vaccination	●●● ●●	●●● ●●	●● ●●	●●● ●●	●●● ●●
	Isolation	●●● ●●				●●● ●●
Crypto-sporidiosis	Hygiene	●●● ●●	●● ●●	●● ●		●●● ●●
	Housing / Isolation	●● ●	●●● ●●	●● ●		●●● ●●

In Magarini division the same exercise was repeated and the results were then recorded as shown in Table 12 below.

Table 12: Proportional piling results for the evaluation of prevention and control methods for the calf diseases in Magarini division (n = 50).

Diseases ↓	Methods ↓	Parameters				
		Effectiveness	Financial cost	User friendly	Group action (Effort by group of farmers)	Individual action (Effort by one farmer)
Minyoo (Helminths)	Deworming	●●● ●●	●●● ●●	●●● ●●		●●● ●●
	Rotational grazing	●●	●●	●●		●●● ●●
Navel ill	Hygiene	●●	●●	●●● ●●		●● ●●
	Housing	●	●● ●●	●		●●
Nyongo (Anaplas- mosis)	Dip / spray	●●● ●●	●●● ●●	●●● ●●	●	●●● ●●
	Housing	●	●●	●●		●●
Pneumonia	Housing	●●● ●●	●●● ●●	●●● ●●		●●● ●●
	Hygiene	●	●	●		●●● ●●
Coccidiosis	Housing	●●	●●	●●		●
	Hygiene	●● ●	●● ●	●● ●		●● ●
Ngai (East Coast Fever)	Dip / spray	●●● ●●	●●● ●●	●●● ●●	●●	●●● ●●
	Rotational grazing	●●	●●	●●		●●
Foot and mouth disease	Vaccination	●●● ●●	●●● ●●	●● ●	●●● ●●	●●● ●●
	Isolation	●● ●	●●			●●● ●●
Crypto- sporidiosis	Hygiene	●●● ●●	●● ●	●● ●●		●●● ●●
	Isolation	●●● ●●	●● ●	●●		●●● ●●

In Malindi division (Table 11), the participants demonstrated that deworming was the most efficient method in controlling helminthiasis and they classified it as an individual effort and the financial cost being moderate as per the farmers' perception and on reference to the relative prices of dewormers. According to the farmers, hygiene of the calf pens seemed to be more efficient as per the high scores it obtained against other parameters used. Dipping or spraying with acaricides received high scores against ectoparasites indicating its efficiency and the method was user friendly and individual effort was required.

Proper housing of the calves was perceived by the participants to be largely associated with prevention and control of pneumonia as it received high scores for all the parameters. Proper housing received high scores in all the parameters in the control and prevention of coccidiosis but closely followed by hygiene of the calf pens. Dipping or spraying with acaricides was perceived to be very efficient in prevention and control of East Coast Fever as it received high scores in all the parameters. Vaccination according to the scores by the participants was seen to be very efficient in the prevention and control of Foot and Mouth Disease. The participants' perception on hygiene and housing or isolation of the sick calves was seen to be moderately associated with prevention and control of cryptosporidiosis.

In Magarini division (Table 12), the participants perceived that deworming was very efficient in controlling helminthiasis according to their scores. According to the participants, hygiene and housing was seen to be moderately efficient in controlling navel ill or colibacillosis because of the moderate scores it received.

According to the participants, dipping or spraying with acaricides was very efficient in controlling Anaplasmosis and East Coast Fever as the methods received high scores. The method was perceived to be effective although the financial cost was high due to high prices of drugs but it was user friendly.

Housing was perceived to be very efficient in the control of pneumonia according to the participants as it received high scores. The method was perceived to be very effective, user friendly and requires individual action although the financial cost was high according to the farmers due to the expenses involved in construction of the houses. Hygiene was perceived to be only moderately efficient in the control of coccidiosis.

Vaccination of calves with Foot and Mouth Disease was perceived to be very efficient in the prevention of the disease as it got high scores for all the parameters as shown in Table 12. The method was perceived to be highly efficient, user friendly and it requires both individual and group effort but the financial cost was high. Hygiene was perceived to be moderately efficient in controlling cryptosporidiosis.

4.1.1.7 Economic importance of the listed diseases

The economic importance parameters listed were increased cost of rearing, increased cost of treatment, decreased growth rate, decreased income and increased mortality. The means of the scores were calculated and recorded as shown in the Tables 13 and Table 14.

Table 13: Proportional piling results for the economic importance of the calf diseases in Malindi division (n = 50).

Indicators ↓	Diseases ↓							
	Minyoo (Helminths)	Ectoparasites	Coccidiosis	Eye problems	Pneumonia	Ngai (East Coast Fever)	Aweb (Foot and Mouth Disease)	Crypto-sporidiosis
Increased cost of rearing	•• •	••• ••	•• •	•• ••	•• ••	•• •	••• ••	•• •
Increased cost of treatment	•• ••	•••• ••••	•••• •••	•••• •••	••• ••	••• ••	••	•• ••
Decreased growth rate	•••• ••••	•• •	•• ••	•	••	•	•• •	••• ••
Decreased income	•• ••	••	•• •	•••• •••	•• ••	••• ••	•• ••	••• ••
Increased mortality	•	••	•• •	•	••• ••	•••• ••	••• •••	•• •

Table 14: Proportional piling results for the economic importance of the calf diseases in Magarini division (n = 50).

<u>Indicators</u> ↓	Diseases							
	Minyoo (Helimiths)	Navel ill	Coccidiosis	Nyongo (Anaplas- mosis)	Pneumonia	Ngai East Coast fever)	Aweb (Foot and mouth disease)	Crypto- sporidiosis
Increased cost of rearing	•• ••	••	•••• ••	•	•• •	•••• ••	••	•• ••
Increased cost of treatment	•• •	•••••• ••••••	•• ••	•••••• ••••	•••• ••••	••••	••••	•• ••
Decreased growth rate	••••• ••••	••	•• •	••	•	••	•••• ••••	•••• ••••
Decreased income	•••• ••	••	••••• ••••	••	•••• ••	•••• ••	•• •	•••• ••
Increased mortality	•	•• •	•	•••• ••	•••• ••	•••• ••	•••• ••••	•

In Malindi division (Table 13) helminthiasis was highly associated with decreased growth rate. According to the farmers, the cost of treatment was greatly increased in the cases of ectoparasites. Coccidiosis was seen to increase cost of treatment in cases of the disease based on the scores. Eye problems seem to have an effect of increasing the cost of treatment due to the high prices of drugs used in treatment of the eye related conditions and hence decreases income from the animals. This is as seen by the scores the parameters received in relation to eye problems.

Pneumonia had a moderate effect on almost all the parameters. Pneumonia moderately increased the cost of rearing that is relative increase in expenditure of rearing the calves, increased cost of treatment in relation to relative increase of expenditure in purchasing of drugs for treatment, increased mortality and decrease income obtained according to the farmers' perception. East Coast Fever was highly associated with increased mortality, increased cost of treatment and decreased income according to the farmers' perception. The farmers reported that the drugs to treat east coast fever are expensive and hence this decreases the income they would have expected from the animals.

Increased cost of rearing due to vaccines being expensive and increased mortality received high scores in relation to Foot and Mouth Disease. This means that according to the participants, Foot and Mouth Disease increases cost of rearing and mortality rate. Cryptosporidiosis decreases growth rate and income as these received moderate scores.

In Magarini division, helminthiasis was seen to be related closely with decreased growth rate as the parameter got high scores. Helminthiasis also causes decrease in income as it was seen from the scores. Navel ill or colibacillosis has a high cost of treatment that is relative increase in expenditure of purchasing drugs for treatment, as the parameter received a high score. Coccidiosis was seen to be largely decreasing income and increasing the cost of rearing. Anaplasmosis was observed to be associated closely with increase in cost of treatment in relation to relative increase of expenditure in purchasing of drugs for treatment.

Pneumonia increases cost of treatment, increases mortality rate and decreases income to be obtained from the animals. According to the participants East Coast Fever increases cost of rearing and mortality rate and decreases income. It also moderately increases the cost of treatment in relation to relative increase of expenditure in purchasing of drugs for treating the disease.

Foot and Mouth Disease was seen to moderately increase mortality and decrease growth rate. According to the participants, cryptosporidiosis moderately increased cost of rearing, mortality rate and treatment and decreased growth rate.

4.2 Sample Analysis

4.2.1 Calf fecal samples

Of the three hundred fecal samples collected from farms within the two divisions, 32 samples (10.6%) stained positive for *Cryptosporidium* parasite as seen in figure 12 while 268 samples (89.33%) stained negative for the parasite.

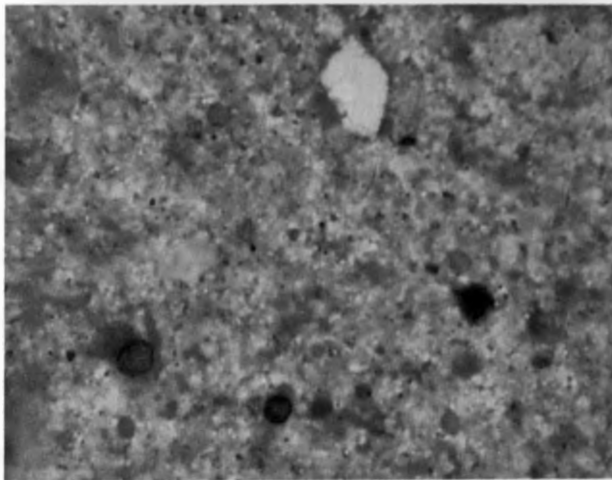


Figure 12: Oocysts of *Cryptosporidium species* staining red on a green background (Magnification 1000x).

In total, 32 out of 300 calves tested positive for cryptosporidiosis, giving a prevalence rate of 10.6 % in dairy calves under the age of six months in Malindi district.

Confidence intervals were then calculated to indicate the population prevalence.

$$p = 32 / 300 = 0.106.$$

Upper confidence limit = 0.14106; Lower confidence limit = 0.0711.

4.2.2 Children fecal samples

Of the 140 fecal samples collected from children from hospitals and health centres in the two divisions, nine samples (6.4%) stained positive for *Cryptosporidium* parasite as seen in figure 13 while 131 samples (93.6%) stained negative for the parasite.

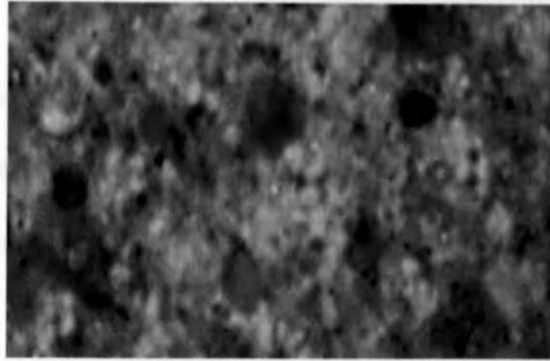


Figure 13: Oocysts of *Cryptosporidium* species staining red on a green background. (Magnification 1000x)

In total, 9 out of 140 children tested positive for cryptosporidiosis which gives a prevalence rate of 6.4 % in children under the age of five years in dairy animal zone areas of Malindi district.

Confidence intervals were then calculated to indicate the population prevalence.

$$p = 9 / 140 = 0.064.$$

Upper confidence limit = 0.1052; Lower confidence limit = 0.023.

4.3 Analysis of secondary data

4.3.1 Calves

Table 15: Socio-demographic characteristics of respondents participating in the participatory epidemiology of common diseases with special reference to cryptosporidiosis in dairy calves in Malindi District, Coast Province, Kenya.

	Malindi division n = 25	Magarini division n = 25
Respondent is the owner of the farm	10	18
Respondent not owner of the farm	15	7
Sex of the respondent		
a) Male	17	19
b) Female	8	6
Relationship to the owner		
a) Spouse	2	-
b) Child	6	3
c) Parent	-	-
d) Other relative	5	3
e) Not related	2	1
Period spent on the farm		
a) Less than 5 years	4	8
b) Between 5 - 10 years	13	11
c) More than 10 years	8	6
Age of the respondent		
a) Less than 18 years	-	-
b) Adult (more than 18 years)	25	25

Table 16: Information on farmers' experience and awareness of respondents participating in the participatory epidemiology of common diseases with special reference to cryptosporidiosis in dairy calves in Malindi District, Coast Province, Kenya.

	Malindi division n = 25	Magarini division n = 25
Period spent as a livestock farmer in the region		
a) Less than 5 years	4	8
d) Between 5 - 10 years	10	9
b) More than 10 years	11	8
Mean number of cattle owned		
a) Cows	21	17
b) Heifers	13	9
c) Bulls	3	5
d) Castrates	2	5
e) Female calves	15	10
f) Male calves	9	14
Mean number of other type of livestock kept		
a) Pigs	-	-
b) Goats	9	15
c) Sheep	3	7
d) Rabbits	-	-
e) Chicken	175	85
f) Others	-	-

4.3.1.1 Calf housing

In Malindi division, hygiene, spacing and house design was quite good in 14 farms (28%), notably the individual calf pen housing seen in Barani farm. Calf housing in 36 of the (72%) farms was poor in terms of hygiene, spacing and the house design. In Magarini division, 17 farms (34%) house their calves in calf pens. Of these only 9 farms (52.9%) had calf pens which were quite good in terms of hygiene, spacing and the house design. The rest 47.1%, the conditions were quite poor. Thirty three participants (66%) do not house their calves separately. They were left together with the older animals in the same herd.

4.3.1.2 Calf feeding

Eighteen farmers (36%) in Malindi division used mainly bucket feeding method, but the calf were left to suckle colostrum in the first 24 hours of life. Notably in Makitosha farm, the owner usually set aside three to four animals to nurse the weak calves although not born by them, that is the farmer practices fostering. Seven participants (14%) allow the calves to suckle throughout the night that is they are left with the dams and the cows are milked in the evening only. During the rainy season when production increases and the market for the milk shrinks some well established farms such as Barani and Makitosha do skimming of the milk and feed the skimmed milk to the calves. In Makitosha farm the owner may increase the number of animals which will nurse the weak calves during the times of excess supply of milk.

In Magarini division, 14 farmers (28%) used mainly bucket feeding, but the calf were left to suckle colostrum in the first 24 hours of life. Eleven of the other participants (22%) allowed the calves to suckle through out the night i.e. they were left with the

dams and the cows were milked in the evening. One farmer, Mjanaheri farm, has been doing skimming of the milk and feed the skimmed milk to the calves during the rainy season in times of excess supply of milk. In 18 farms (36%) in Malindi division, the owners were very keen to feed colostrum, but in the rest 7 farms (14%), the participants seem not to be very keen in colostrum feeding. In Magarini division, 14 participants (28%) were keen about colostrum intake but the rest 11 participants (22%) were not. In all the farms, no farmer used milk replacers. This could be due to abundance of milk as marketing of milk was one of the key problems affecting the dairy industry in Malindi district.

Twelve participants (24%) in Malindi used maize germ meal and wheat bran as feed supplements to their calves. Two farmers (4%), Makitasha and Barani farms prepared their own feed formulations to feed to their calves. The other 11 participants (22%) did not use any feed supplements for their calves. In Magarini, only five participants (10%) used maize germ meal and wheat bran as feed supplements to their calves. The rest of the participants did not use any feed supplements to their calves.

4.3.1.3 Type of water

In Malindi division, only four participants (8%) were using tap water in their farms. The rest of the participants in Malindi division, used water from the well in their farms. It was this same water which was also made available to the calves. No farm had ever analyzed the water they provided to the calves or treated the water with any kind of disinfectant. In Magarini division 22 participants (44%) used water from the well in their farms and it was the same water provided to the calves. The rest of the participants (6%) in the division used water from the dams, river, streams, rain water

collecting by the roadside or any other available water source. The same water was then provided to the calves. As for Malindi, no farm had ever analyzed the water they provided to the calves or treated the water with any kind of disinfectant.

4.3.1.4 Risk factors

The risk factors therefore identified were poor hygiene of the calf pens, poor housing of the calves, method of feeding, poor colostrums intake, use of feed supplements and unsafe water used for drinking.

4.3.2 Children

4.3.2.1 Sanitary facilities

In Malindi division, 48 participants (34.2%) interviewed had proper sanitary facilities where 23 (16.4%) came from households where the facilities were shared only by the members of the same family. The rest 22 participants (15.7%) in Malindi division had poor sanitary facilities. In Magarini division, 36 participants (25.7%) interviewed had proper sanitary facilities and here 16 (11.4%) came from households where the facilities were shared only by the members of the same family. The rest 34 participants (24.3%) had poor sanitary facilities.

4.3.2.2 Type of water

In Malindi division, the source of water used for domestic purposes by the families was from the local municipal council for 54 families (38.5%) and from the wells and boreholes for 16 families (11.4%). In Magarini division, 27 participants (19.3%) used water from the local municipal council for domestic purposes and the rest of the

participants (30.7%) interviewed relied for water from the wells, boreholes and dams available in the area for domestic use.

4.3.2.3 Storage of water

Storage of the water was good with all the participants in both divisions. In Malindi division, 9 families (6.42%) among the ones getting water from local municipal council also boiled the water used for drinking but the rest among the ones in the division did not. In Magarini division, none of the participants boiled water for drinking or used any kind of disinfectants to treat the water.

4.3.2.4 Contact with pets, chicken or farm animals

All the participants interviewed in both the divisions did keep either pets (cats or dogs) or farm animals (cattle, sheep, goats or chicken) or both. In Malindi division, only 8 families (5.71%) take their children to the farms, and the children come into contact with the pets and farm animals but the rest (44.3%) do not take their children to the farms and the children hence do not come into contact with the animals especially calves and pets at the farm. In Magarini division, children from 39 families (27.8%) often come into contact with the animals or pets and their parents frequently took them to the farms when they visited, there was no contact with pets or farm animals in the rest of the participants. Contact with the animals especially calves and pets occurred while playing with the animals at the farm.

4.3.2.5 Risk factors

The risk factors therefore identified were unsafe drinking water, contact with pets and contact with farm animals and chicken, hygiene and sanitation.

4.3.3 Health service providers

From questionnaire in appendix 8.3 the disease situation as perceived by health service providers was assessed. Information obtained from the administration of this questionnaire is as below.

4.3.3.1 Occurrence of cryptosporidiosis

Diarrhea cases are very common in the area especially in children under the age of five years. The cases of diarrhea tended to increase during the rainy seasons and decreased during the dry periods.

4.3.3.2 Relationship of cryptosporidiosis with nutrition, hygiene and sanitation

The health service providers perceived that there was a relationship between malnutrition and cryptosporidiosis. They reported that from their experience at their respective health centres, the immune system of undernourished children was usually weak hence the chance of them being infected with the disease was higher. They also perceived that there was a relationship between hygiene and sanitation and increase in cases of the disease.

4.3.3.3 Method of diagnosis and treatment of cryptosporidiosis

Method of diagnosis of the disease by the health centres if the disease was to be suspected and if it was to be investigated was acid-fast staining and treatment is mainly antibiotics and supportive treatment.

4.4 Test for association

4.4.1 Calves survey

4.4.1.1 Chi – square test

Chi – square test for association was used to determine if there is any association between the risk factors of cryptosporidiosis and the disease.

The risk factors that had been identified from questionnaires administered and from literature were the poor hygiene of calf pens, poor mode of housing of the calves, method of feeding, poor colostrums intake, no feed supplements and unsafe water used for drinking.

The strength of the association was then calculated using odds ratio.

The hypothesis, assumptions and decision rule below applied to all the other risk factors, that is they were the same.

i) Hypotheses

H_0 : there is no association between the risk factors and disease.

H_a : there is association between the risk factors and disease.

ii) Assumptions

- Independent observations.
- The expected value of any cell is at least 5.

iii) Decision rule: Reject H_0 with 95% confidence if $\chi^2 > \chi^2_{(1, \alpha = 0.05)} = 3.84$

So we reject the null hypothesis and accept the alternate hypothesis with the conclusion that there is association between the risk factors and the disease.

Table 17: Risk factors for cryptosporidiosis in dairy calves under the age of six months and their respective Chi-square statistics.

Risk factor ↓	Factor present and disease present	Factor present and disease absent	Factor absent and disease present	Factor absent and disease absent	Test statistic
Poor hygiene	25	90	7	178	23.69
Poor calf Housing	23	103	9	165	12.82
Method of feeding					
a) Bucket feeding	24	113	8	155	12.33
b) Nursed by dams	27	97	5	171	27.12
Poor colostrum intake	22	125	10	143	5.595
No feed supplements	21	120	11	148	4.96
Unsafe water	27	137	5	131	12.88

4.4.1.2 Strength of association

Strength of the association was calculated using odds ratio which is the cross product ratio. Results are as shown in the Table 18.

Table 18: Odds ratios for risk factors associated with cryptosporidiosis in calves less than six months of age in Malindi District

	Risk factor	Odds Ratio
1.	Poor hygiene of the calf pens	7
2.	Poor housing of the calves	4
3.	Method of feeding of the calves: a)Bucket feeding	4
	b)Nursing by dams	9.5
4.	Poor colostrum intake	2.5
5	Use of feed supplements	2.35
6.	Unsafe water provided to the calves	12.88

From the strength of association above, it is evident that some of the risk factors are more important than others. The farmers cannot ignore unsafe water provided to the calves, general hygiene of the calf pens, nursing by dams and bucket feeding as methods of feeding the calves and mode of housing. The risk factors have a higher chance of putting the calves in danger of getting cryptosporidiosis.

4.4.2 Children survey

The risk factors that were identified were unsafe drinking water, contact with pets, contact with farm animals and chicken and poor hygiene and sanitation.

4.4.2.1 Chi – square test

i) Hypotheses

H_0 : there is no association between the risk factors and disease.

H_a : there is association between the risk factors and disease.

ii) Assumptions

- Independent observations.

(iii) Decision rule: Reject H_0 with 95% confidence if $\chi^2 > \chi^2_{(1, \alpha = 0.05)}$; $\chi^2_{(1, \alpha = 0.05)} = 3.84$

So we reject the null hypothesis and accept the alternate hypothesis with the conclusion that there is association between the risk factors and the disease.

The hypothesis, assumptions and decision rule above were applied for all the other risk factors, that is, they were the same.

Table 19: Risk factors for cryptosporidiosis in children under the age of five years and their respective Chi-square statistics.

Risk factor ↓	Factor present and disease present	Factor present and disease absent	Factor absent and disease present	Factor absent and disease absent	Test statistic
Unsafe water	7	52	2	79	4.9
Contact with pets	7	47	2	84	5.97
Contact with farm animals and chicken	7	47	2	84	5.97
Poor hygiene and sanitation	7	49	2	82	5.4

Fisher's Exact test was also carried out to determine the significance of the results and they were seen to be significant hence the risk factors were significant.

4.4.2.2 Strength of association

Strength of the association was calculated using odds ratio, results were recorded as shown in Table 20.

Table 20: Odds ratios for risk factors associated with Cryptosporidiosis in children less than five years of age in Malindi District.

	Risk factor	Odds Ratio
1.	Unsafe drinking water	5.3
2.	Contact with pets	6.25
3.	Contact with farm animals and chicken	6.25
4.	Poor hygiene and sanitation	5.8

From the strength of association above, it is evident the parents or guardians cannot ignore the risk factors as they have a higher chance of putting the children in danger of getting cryptosporidiosis.

5 DISCUSSION

5.1 Calves survey

5.1.1 Participatory appraisal

The validity of the results produced by the participatory appraisal tools and the use of the other diseases showed that the participants' descriptions of the diseases were in line with modern veterinary knowledge.

In the two divisions, it appeared that diarrhea was common although Foot and Mouth Disease, pneumonia, and East Coast Fever are given more emphasis and were named 'killer diseases' by the farmers due to their high mortality rate among calves especially those under the age of six months.

In Malindi and Magarini divisions cryptosporidiosis was strongly associated with diarrhea. In Malindi division the participants had moderate agreement on hygiene, isolation of the sick calf and housing as the methods to control cryptosporidiosis. In Magarini division the participants also had moderate agreement on the methods of controlling cryptosporidiosis, and had moderate scores for hygiene and isolation of the sick calf. This shows that in both divisions, the participants had moderately agreed that hygiene and isolation of the sick calf can help control cryptosporidiosis; this is the case scientifically as hygiene and isolation of the sick calf can help control cryptosporidiosis (Quigley, 1997).

In Malindi division, the participants scored hygiene highly as the most effective method in controlling cryptosporidiosis that requires individual action and its user friendly with a moderate financial cost according to the farmers' perception of the relative prices of drugs. Housing as a method of controlling cryptosporidiosis (Lassen

et al., 2009), was seen to be moderately effective may be due to the high financial cost associated with the construction as seen by the proportional piling scores. In Magarini division, the participants had moderate scores for hygiene and isolation of the sick calf as methods used in controlling cryptosporidiosis which requires individual action. The methods are also seen to be having moderate financial cost and user friendly. This is evident from the proportional piling scores.

Cryptosporidium species is considered to be an important agent in the aetiology of the neonatal diarrhoea syndrome of calves, lambs and goat kids, causing considerable direct and indirect economic losses, (de Graaf *et al.*, 1999). In Malindi division, cryptosporidiosis was seen to be increasing the cost of treatment, decreases growth rate and thus decreases income. It appears that these economic parameters received high scores. The disease is also believed to increase the cost of rearing and having a moderate mortality rate. In Magarini division, the disease is seen to increase the cost of rearing, increase cost of treatment, decreases growth rate with a high mortality rate. But according to the participants, the disease has only a moderate decrease in income from the livestock.

Cryptosporidiosis is a diarrheic syndrome which affects mainly the very young calves especially those which are less than one month old, (de la Fuente *et al.*, 1999) The livestock keepers in Malindi and Magarini divisions characterized cryptosporidiosis in calves using a local criterion which has similarities with the one used by veterinarians. The farmers described the syndrome in Kiswahili. To quote the farmers' description: "*Ndama wetu zinaharisha sana, choo chao ni majimaji sana au mara nyengine makamasi na haisikii dawa. Ndama wachanga ndio wanalemewa zaidi na ugonjwa*".

This translated to literally mean: “*Our calves diarrhea profusely and the diarrhea is watery or sometimes mucoid and it does not respond to treatment. The very young calves are the ones which are more severely affected by the disease.*” This showed that although the farmers did not have the scientific name for the disease, they could describe its syndrome. Although they were not conversant with the actual clinical signs of cryptosporidiosis which are non-specific the participants reported that that if feed intake is reduced and combined with persistent diarrhea over several days which may cause emaciation this is most probably cryptosporidiosis although they did not have a specific name for it.

Regarding helminthiasis, in Malindi division ‘minyoo’ was strongly associated with watery diarrhea, pot belly and was ranked first in the list of the most important calf diseases in the area. From the matrix scores on the disease-sign matrix, it appeared that the participants are able to identify the condition. This is evident even from the scores on the disease-sign matrix. The farmers are able to identify the condition and they are also using the right methods in prevention and control of the condition. One important clinical sign which can be visibly identified and was left out by the participants is ‘rough coat’ of the calves which is most often associated with helminthiasis (Adebisi, 2008).

Helminths are also responsible for significant economic losses in calves, (Siddiki *et al.*, 2010). The participants also associate decreased growth rate with helminthiasis indicating that helminthiasis causes decreased growth rate and hence decreases income to be obtained from the animals. This was as seen from the scores of proportional piling on the economic importance of the most important calf diseases in the area. This

situation was similar in Magarini division. Participants in Magarini division also related helminthiasis and decreased growth rate which has a direct effect in reducing the income level to be obtained from the calves.

Coccidiosis is transmitted by ingestion of sporulated oocysts. Infection is acquired from contaminated feed, water, and soiled pastures, or by licking a contaminated hair coat (Georgi., 1985). Signs of the disease include anorexia, loss of weight, and hemorrhagic and mucoid diarrhea (Georgi., 1985). In severe cases, feces are liquid, bloody and may contain strands of intestinal mucosa (Ernst and Benz, 1986). In Malindi division, the participants strongly associated bloody diarrhea with coccidiosis by giving it high scores on the matrix scoring although the agreement was moderate.

Proper sanitation and good animal husbandry practices are important in preventing coccidiosis. Water and feed troughs should be constructed and located to reduce fecal contamination. Newborn dairy calves should be provided with clean, dry quarters when removed from the dam to prevent coccidiosis (Ernst and Benz, 1986). There was moderate agreement on the prevention and control methods of coccidiosis with hygiene, isolation of the sick calf and housing receiving moderate to high scores. In Magarini division, there was high agreement on bloody diarrhea which was strongly associated with coccidiosis. Isolation of the sick calf was seen to be the best method on preventing coccidiosis as seen on the matrix scores.

Bovine cryptosporidiosis in neonatal calves can cause a profuse yellowish to yellow-grey watery to mucoid diarrhea (Ridley and Olsen, 1991). Mucoid diarrhea was associated with cryptosporidiosis in Malindi division while in Magarini division it was

closely associated with cryptosporidiosis and navel ill or colibacillosis. This was evident in the matrix scores of the disease-sign matrices. Navel ill or colibacillosis which is a diarrheal syndrome whose occurrence can be influenced by several husbandry factors (Wray and Thomlinson, 1975), was listed among the most important calf diseases in Magarini division. The participants perceived that isolation of the sick calf and hygiene are the best methods in prevention and control of the disease. This was as seen in the scores of the 'disease method' matrix.

The presence of mixed infections or diarrhea caused by other organisms such as rota and corona viruses posed a big challenge to the livestock keepers when they were asked about them. This was because they did not have either the most-appropriate way to diagnose, prevent or control the problem or enough qualified trained personnel to address the problem. Therefore this was one of the problems faced by the livestock keepers in both the two divisions.

5.1.2 Secondary data

From the questionnaires, although in the participatory appraisal seminars the farmers mentioned hygiene, spacing and house design as being important in the control of cryptosporidiosis, housing of calves in Malindi, in 14 farms (28%) was quite good in terms of hygiene; spacing and the house design notably the individual calf pen housing seen in Barani farm. Calf housing in 36 of the (72%) farms was so poor in terms of hygiene, spacing and the house design. In Magarini division, 17 farms (34%) house their calves in calf pens. Of these only 9 farms (52.9%) had calf pens which were quite good in terms of hygiene, spacing and the house design. This was seen to contribute significantly to the diarrhea problem in their farms. This can be linked to

cryptosporidiosis; as cryptosporidiosis is a diarrheal syndrome and hygiene and proper calf housing or isolation of the sick calf can help control cryptosporidiosis (Quigley, 1997).

Unsafe water, (José *et al.*, 2006) provided to the calves and poor colostrum intake, (Goyena *et al.*, 1997) contributed to the calves being infected with *Cryptosporidium* parasite. Calves exposed to unsafe water or had poor colostrum intake had higher chances of getting cryptosporidiosis than those which had not. Method of feeding the calves and use of feed supplements were also some of the other risk factors identified. Calves left to be nursed by their dams were seen to have high chances of getting cryptosporidiosis. Use of feed supplements to calves assists in controlling calf scours (Maas, 2002). Calves provided with feed supplements were seen to have less chances of getting cryptosporidiosis than those which were not.

From the chi-square test of association, it was seen that there was an association between the risk factors and the disease although the strength of the association was stronger in some risk factors than the others. This showed that exposure to the risk factors would most probably lead to the calves coming down with cryptosporidiosis.

5.2 Children survey

5.2.1 Secondary data

5.2.1.1 Children

Cryptosporidiosis is common in children under the age of five years (Wangechi *et al.*, 2006). This is according to the information obtained from the questionnaires (Appendix 8.3) administered to the health service providers. Cases of the disease are thought to increase during the rainy season. Also from the questionnaires (Appendix 8.2.) it was seen that the use of water which is suspected to be contaminated contribute to prevalence of diarrhea cases, which cryptosporidiosis is one of them. In Malindi division, the source of water used for domestic purposes by the families was from the local municipal council for 54 families (38.5%) and from the wells and boreholes for 16 families (11.4%). In Magarini division, 27 participants (19.3%) used water from the local municipal council for domestic purposes and the rest of the participants (30.7%) interviewed relied for water from the wells, boreholes and dams available in the area for domestic use.

In Malindi division, 9 families (6.42%) among the ones getting water from local municipal council also boiled the water used for drinking but the rest among the ones in the division did not. In Magarini division, none of the participants boiled water for drinking or used any kind of disinfectants to treat the water. This hence exposed their children to unsafe water for drinking hence putting them at risk of getting cryptosporidiosis and other water-borne diseases.

Children coming into contact with pets, chicken and farm animals had higher chances of getting cryptosporidiosis than those which had no contact. This could be due to the

fact that the animals or chicken could already be infected or incubating the disease and could easily infect the children who come into contact with the animals or the chicken. Transmission is fecal-oral route hence children coming into contact with the animals's or the chicken feces have higher chance of getting cryptosporidiosis (Fayer *et al.*, 1990). In both the divisions, all the participants were the ones who fed their children, and the only recreation facility visited was the beach which was also not frequent.

5.2.1.2 Health service providers

The health service providers also perceive that there was a relationship between hygiene and sanitation and increase in cases of the disease in children under the age of five years. This was because the disease usually was associated with poor hygiene as the main mode of transmission was fecal oral route. These beliefs were not confirmed as more research was needed to establish the relationship.

Diarrhea cases are very common in the area especially in children under the age of five years. The cases of diarrhea tended to increase during the rainy seasons and decreased during the dry periods. Method of diagnosis of the disease by the health centres if the diseases were to be suspected and if it was to be investigated was acid-fast staining and treatment is mainly antibiotics and supportive treatment. The prognosis of the disease was usually fair if the disease was treated and guarded if it's not treated. The health centres in Malindi district were not well equipped to deal with diagnosis of cryptosporidiosis with the exception of two private health centres. It was also noted that *Cryptosporidium* organism is not routinely investigated in the laboratories and this could be accounting to a big percentage of the cases going undetected. The health service providers also perceived that the disease could be prevented or controlled

through better hygiene standards and especially the use of safe water used for domestic purposes.

It was also seen that the health service providers associate malnourishment, poor sanitation and hygiene with increase in diarrhea cases, which cryptosporidiosis being one of them. This was also not confirmed as further research was needed to establish the relationship.

From the chi-square test of association, it was seen that there was an association between the risk factors and the disease although the strength of the association was stronger in some risk factors than the others. This showed that exposure to the risk factors would most probably lead to the calves coming down with cryptosporidiosis.

6 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusion

Cryptosporidiosis is a diarrhea condition common in calves under the age of six months and children under the age of five years. This is due to their immune system as it is not fully developed. As it was seen from the analysis of the samples the prevalence of cryptosporidiosis in calves is 10.6 % and in children its 6.4 %. These prevalence rates could be reduced if the following recommendations could be carried out. Several risk factors were associated with cryptosporidiosis in calves (Starkey *et al.*, 2006) which included poor hygiene of the calf pens, poor housing of the calves, method of feeding, poor colostrums intake, no use of feed supplements and unsafe water used for drinking. In children, the risk factors included (Wangeeci *et al.*, 2006; Wasif *et al.*, 2004) unsafe drinking water, contact with pets, contact with farm animals and chicken and poor hygiene and sanitation.

Practising good personal hygiene will reduce the chances of infection. Supervise children to make sure they wash their hands well when they come from the toilet if they are old enough to use it by themselves or if younger children come into contact with their feces, their hands should be thoroughly washed with soap and warm water.

Parents should also try and avoid direct exposure to pets, chicken, cattle and other farm animals. If exposure cannot be avoided and if children come into contact with pets, chicken or farm animals, their hands should also be washed thoroughly. Children should also be taught and trained that before eating food, they should wash their hands well.

Water used for drinking must be safe. The parents or guardians should know the source of water they use for domestic purposes. Water given to the children for drinking

should be boiled for it to be safe for drinking or where possible to be filtered to remove *Cryptosporidium* parasites. Children should be taught to avoid swallowing water when swimming in rivers, the ocean, and swimming pools.

Food provided to the children should be safe to eat. Parents and guardian need to prepare food carefully and well to avoid chances of infection by *Cryptosporidium* parasites. Wash all vegetables or fruits that are given to the children to be eaten raw, even those that are peeled before eating. Water used to wash them should be clean and safe. Only pasteurized milk, dairy products, juices, and ciders to be provided to the children to avoid chances of infection. Parents and guardians should comply with any water advisories issued by local authorities and professional advice from health service providers on the control of water borne diseases.

As there are currently no reliable curative therapies for cryptosporidiosis, the best hope lies in prevention of the disease. Good hygiene of the calf pens and equipment used and good calf housing will reduce the risk of calves getting infected. Water provided to the calves need to be safe to avoid infecting the calves by using contaminated water and also the use of feed supplements and ensuring calves take enough colostrums reduces the risk of infection. The parents or guardians need to practice good hygiene and have proper sanitation and only use safe water to reduce risk of infection of their children by *Cryptosporidium* species. Contact with pets, animals and chicken should be avoided whenever possible and where contact occurs, hands should be washed thoroughly with soap and water to avoid infection just in case the animals or chicken are shedding oocysts.

All the positive cases in children were referred to the nearby hospital or health centre for further treatment and advice after the children were traced by using appendix 8.5 which had details of the postal and physical addresses to enable ease of locating the children.

6.2 Recommendations

Since *Cryptosporidium* has the potential to infect many people from a point-source outbreak, much research still needs to be done.

Recommendations from the results

- Calculate the relative risks of infection from drinking water, contact with farm animals, pets and chicken to see where focus on preventative strategies should be placed.
- Organise for farmers trainings to educate farmers about the disease and the risk factors associated with the disease.
- Liase with health facilities in the study area to organise for an awareness campaign on the disease and its risk factors.
- Farmers and parents to be informed on the risks involved in drinking unsafe water or providing contaminated water to the calves.
- Improve communication, diagnoses and reporting cases in order to identify outbreaks.

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8 APPENDICES

8.1 Appendix 1

Questionnaire on the participatory epidemiology of common diseases with special reference to cryptosporidiosis in dairy calves in Malindi District, Coast Province, Kenya.

Enumerator's name:..... Date:.....

Name of respondent:.....

1 DESCRIPTION OF THE AREA:

Village:.....

Sub location:.....

Location:.....

Division:.....

2 SOCIO-DEMOGRAPHIC INFORMATION

2.1 Is the respondent the owner of the farm?

(Tick where appropriate)

Yes:..... No:.....

2.2 What is the sex of the respondent

(Tick where appropriate)

Male.....

Female

2.3 If no, what is your relationship with the owner?

(Tick where appropriate)

Spouse:.....

Child:.....

Parent:.....

Other relative:.....

Not related:.....

2.4 What is your main occupation?

(Tick where appropriate)

Commercial farmer:.....

Subsistence farmer:.....

Pastoralist:.....

Public sector worker:.....

Private sector worker:.....

Others (specify):.....

2.5 For how long have you lived in this farm?.....(years)

2.6 What is your age in completed years?.....

3 FARMERS EXPERIENCE AND AWARENESS.

3.1 For how long have you been a livestock farmer in the
area?..... (Years)

3.2 Indicate below the number and class of cattle owned

Class	Number
Cows	
Heifers	
Bulls	
Castrates	
Female calves	
Male calves	
Total	

3.2 What other type of livestock do you keep?

pigs:.....

goats:.....

sheep :.....

chicken:.....

rabbits :.....

others (specify)

3.3 What is the total number of calves on your farm?

3.4 How do you house your calves?

(Tick where appropriate)

Calf pens:.....

Free range:.....

Housed with the other animals:

Others (specify) :.....

3.5 How often do you clean the calf pen or where you keep the calves:

.....

3.6 How do you clean your calf pen.....

3.7 Do you have a cattle dip?

(Tick where appropriate)

Yes:.....

No:.....

3.8 How do you feed your calves?.....

3.9 How soon after birth did the calves start suckling colostrum?

(Tick where appropriate)

< 6 hours after birth.....

> 6 hours after birth.....

> 12 hours after birth.....

> 24 hours after birth

Did not suckle at all.....

3.10 For approximately how many hours did the calf suckle the colostrums after birth?

.....

3.11 If the calf did not suckle the colostrum, did you feed milk replacers?

.....

3.12 What feed supplements do you give the calves?

.....

3.13 Have the calves suffered from any diarrheal disease?

(Tick where appropriate)

Yes:.....

No:.....

3.14 What ages and sexes of the calves are affected by the diarrheal diseases?

.....

.....

3.15 Is there seasonality or other timing to the appearance of the diseases?

.....

.....

3.16 Do the diseases usually affect one animal or a group of animals at the same time?

and do the diseases spread from animal to animal?

.....

3.17 What causes the diseases: natural / physical causes, supernatural / non-physical causes or both? Describe.

.....
.....
.....

3.18 Are there ways to prevent / avoid these diseases? If so what are they?

.....
.....

3.19 Describe the main symptoms of each of the diarrheal diseases named, if possible in order of progression and timing. What is the first symptom seen and when in each of the diseases? Also, what is the symptom if any which makes you decide it is a particular disease?

.....
.....
.....
.....
.....
.....
.....

3.20 Are traditional treatments available for each of the diseases? Basically what are they? Where and how are they obtained? What happens when they are used? (Please be as specific as possible).

.....
.....

3.21 Are modern treatments available for each of the diseases? What are they? Where and how are they obtained? What happens when they are used? (Please be as specific as possible).

.....
.....
.....

3.22 What usually happens if the animal is not treated?

.....
.....

3.23 When did you last have, or hear of, an animal with a particular diarrheal disease? What did you do and what happened to the animal?

.....
.....
.....
.....

3.24 What treatment was given to the calves under age of 6 months which had diarrhea?

3.25 Did the sick calves recover fully from diarrhea?

(Tick where appropriate)

Yes:..... No:.....

3.26 How many of the sick calves died?.....

4 CONTACT WITH OTHER DOMESTIC ANIMAL

4.1 What other species of domestic animals do you keep?

.....

4.2 What other breeds of the animals do you keep?

.....

4.3 Do they use the same water supply as the calves?

.....

4.4 Are the calves kept separate from the other domestic animals?

.....

4.5 How are the calves attended to in terms of feeding and water?

.....

.....

.....

5 WATER SOURCES AND TREATMENT

5.1 What is the main source of water?

(Tick where appropriate)

Tap water:.....

Well/bore-hole water.....

River/stream/lake.....

Rain water.....

5.2 Is the water given to the calves treated?.....

5.3 If yes, what chemicals are used to treat the water?

.....

5.4 For how long have you used this treatment method?.....

8.2 Appendix 2

Questionnaire on the participatory epidemiology of Cryptosporidiosis in children attending health facilities in Malindi District, Coast province, Kenya.

Enumerator name: Date

Name of the respondent :

1 DESCRIPTION OF AREA

Village :

Sub-location :

Location: Division:

2 SOCIO-DEMOGRAPHIC INFORMATION

2.1 What is your age (years)

2.2 Sex of respondent

(Tick where appropriate)

Male.....

Female.....

2.3 Are you a parent or guardian?.....

2.4 If parent, how many children do you have?

(Tick where appropriate)

1-3

4-6

7-10

11 and above

2.5 How many children are below the age of 5 years?.....

2.6 What is the occupation of

Father.....

Mother.....

Guardian.....

2.7 Do you share sanitation facilities with other occupants in the house you are living in?

(Tick where appropriate)

yes.....

no.....

3 WATER AND FOOD HYGIENE

3.1 Where do you obtain water for domestic use?

(Tick where appropriate)

Tap.....

Well / bore hole.....

River / lake / swamp.....

Other (specify).....

3.2 Do you experience water shortage / scarcity in your area?

(Tick where appropriate)

Yes.....

no.....

3.3 How is the availability of the water?

(Tick where appropriate)

Regularly available.....

Availability irregular.....

Not available

3.4 Where do you store water for domestic use?

(Tick where appropriate)

Water tanks.....

Jerricans.....

Buckets / other containers.....

3.5 Do you boil drinking water before use?

(Tick where appropriate)

Yes.....

No.....

3.6 Who feeds your child?

(Tick where appropriate)

Self.....

Maid.....

Older sibling.....

neighbour.....

4 HEALTH AND RECREATION

4.1 Where is the most popular water recreation facility in your area?

(Tick where appropriate)

Swimming pools.....

Public beaches.....

Water slides.....

4.2 How often do you take your child to swim at the beaches?

(Tick where appropriate)

Very frequently.....

Occasionally.....

Rarely.....

Never.....

4.3 Who treats the child when sick?

(Tick where appropriate)

The local medical doctor.....

Herbalist.....

Self.....

5 PETS AND OTHER FARM ANIMALS

5.1 Do you keep any pets or farm animals in / near the house?

(tick where appropriate)

yes no.....

5.2 How often do the children (below 5 years) come into contact with the domestic / farm animals ?

(Tick where appropriate)

Frequently.....

Occasionally.....

Never.....

5.3 Do you take the children with you when working in the garden?

(Tick where appropriate)

yes..... no.....

8.3 Appendix 3

Questionnaire on the participatory epidemiology of Cryptosporidiosis in children attending health facilities to be administered to health service providers in Malindi District, Coast Province, Kenya.

1 IDENTIFICATION INFORMATION:

Name of health officer:.....

Age :.....

Sex :.....

Designation / Title:.....

Name of institution :.....

Province :.....

District :.....

Division:.....

Date:.....

2 PREVALENCE PROFILE:

2.1 How long have you been working in this institution?

2.2 What is the estimated prevalence of diarrhea in this institution?

.....

2.3 How do you isolate the causative organism in the diarrhea cases?

.....

2.4 If you do isolate for the causative agent, have you encountered cases of cryptosporidiosis in your hospital?

2.5 How do you rate the disease?

Rare..... common

2.6 What is the estimated prevalence of the disease?.....

2.7 What is the age of children mostly affected by the disease?

.....

2.8 Are there ways to prevent / avoid this disease? If so what are they?

.....

.....

.....

.....

2.9 Describe the main symptoms, if possible in order of progression and timing. What is the first symptom seen and when? Also, what is the symptom if any which makes you decide it is this specific disease?

.....

.....

.....

.....

2.10 What is the frequency of the cases of Cryptosporidiosis?

.....

2.11 In what season of the year is the disease mostly frequent? (tick as appropriate)

a) Rainy season:.....

b) Dry season:.....

c) Throughout the year:.....

2.1 Do you have any cases at the institution at the moment?

.....

2.13 If yes in 10 above, state how many patients?

3 CAUSES, ISOLATION OF THE ORGANISM AND TREATMENT:

3.1 What is the main source of drinking water in your division? (tick as appropriate)

- a) Tap:.....
- b) Well:.....
- c) Borehole:.....
- d) Stream:.....
- e) Others (specify)

3.2 Would you say there is a relationship between sanitation and the prevalence of the disease in the area?.....

3.3 What symptoms do children suffering from the disease show?

.....
.....
.....

3.4 Do the children also suffer symptoms of malnutrition?

3.5 How do people in your area seek health service?

.....
.....

3.6 What methods do you use in diagnosis of the disease?

.....
.....
.....

3.7 What treatment do you administer to the patients suffering from Cryptosporidiosis?

.....
.....

3.8 What is the prognosis of the disease?

a) If treated?

b) If not treated?

4 PREVENTION AND CONTROL:

4.1 Does the health institution have the medical facilities and equipment to deal with cases of this disease?

4.2 If your answer above is no, please suggest what should be done to address the problem ;

.....
.....
.....

4.3 What advice should be provided to parents and guardians in order to minimize cases of cryptosporidiosis in children?

.....
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.....

4.4 Is it possible to eliminate the disease in the area?

4.5 If no, please explain why is it not possible to eliminate the disease :

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.....

8.4. CONSENT FORM

TAARIFA YA IDHINI YA MAKUBALIANO

KICHWA CHA UCHUNGUZI: Ugonjwa wa kuharisha kwa ndama na watoto katika wilaya ya Malindi.

VYUO VYA UTAFITI: 1. University of Nairobi.
2. Department of Veterinary, Malindi.
3. Kenya Medical Research Institute, Nairobi

WATAFITI WAKUU: 1. Dr. Hassan Ali Mohamed (BVM)
2. Prof. Erastus K. Kang'ethe.

TAARIFA YA IDHINI

Mwenye kujitolea kwa uchunguzi huu ni lazima apewe nakala ya taarifa hii kabla aandikishwe kwenye uchunguzi. Umeombwa uhusike katika uchunguzi huu. Azma ya taarifa hii ni kukueleza utaratibu wa utafiti na kupata idhini yako ya kuhusika katika uchunguzi huu.

DHAMIRA NA MANUFAA YA UCHUNGUZI

Wewe unaishi katika eneo ambalo umohatarini kuambukizwa ugonjwa wa kuharisha. Lengo la uchunguzi huu ni kukuwezesha kuuelewa zaidi ugonjwa huu. Manufaa ya kujihusisha na utafiti wetu kwako ni kwamba utaweza kufanyiwa uchunguzi bure bila malipo ili kudhihirisha chanzo cha ugonjwa huu na namna bora zaidi ya kuutibu. Zaidi ni kuwa kuelewa magonjwa ya kuharisha kutasaidia kuboresha tiba na njia za kuyazuia au kuyakinga.

UCHUNGUZI NI WA NINI

Huu ni utafiti wa utabibu wa kudhihirisha chanzo cha ugonjwa wakuharisha katika eneo hili na kukusanya sampuli kwa ajili ya upelelezi katika vyumba vya uvumbuzi ili tuweze kuuelewa vizuri ugonjwa huu.

KANUNI ZA UCHUNGUZI

Wakati wa uchunguzi, utaombwa utupatie sampuli moja au zaidi za kinyesi. Sampuli hizo zitatumiwa kuchunguwa sababu ya kuharisha kwa mtoto na pia wepesi wa nguvu za dawa kupigana na maradhi na pia jinsi au asili ya vidudu au viini. Pia kuna uwezekano kuwa sampuli ambazo utatupatia kwa utafiti huu zaweza kutumiwa katika utafiti mwengine. Hutopatiwa ilani ikiwa jambo hili litafanywa mbeleni.

JUKUMU / UTAKALIFU

Uchunguzi huu hautakuwa na madhara yoyote ama taklifu yoyote kwako. Kushiriki kwako katika uchunguzi huu hakutachelewesha huduma ya afya ambayo hupewa kwa kawaida.

UDHIBITI WA SIRI

Taarifa yote itakayokusanywa kukuhusu itahifadhiwa kwa siri. Kinyesi kitatumiwa tu kwa uchunguzi na yakini ya ugonjwa. Jina lako halitachapishwa kwenye jarida au nakala yoyote kwenye uchunguzi huu au mwengine. Jina lako halitawekwa kwenye tarakilishi za utafiti wa kompyuta. Kila sampuli ya kinyesi itapewa alama ya nambari ya utambulishi.

HAKI ZAKO

Ikiwa katika wakati wa utafiti una maswali kuhusu katiba na namna ya utafiti, au unaamini kuwa umepata madhara yoyote kutokana na utafiti huu tafadhali wasiliana na:

1. Dr. Hassan Ali Mohamed- University of Nairobi.
2. Prof. E. Kang'ethe- University of Nairobi, Kabete Campus PHPT Department.
3. Dr. C. K. Malenga- Deputy District Veterinary Officer, Malindi.

KUSHIRIKI KWA KUJITOLEA

Uamuzi wako wa kushiriki katika utafiti huu ni wa hiari na kukataa kushiriki hakutakuletea hatia au kukosa kwa huduma ambazo ungekuwa umepatiwa. Pia, waweza kujiondoa katika utafiti wakati wowote bila ya hatia au kunyimwa huduma za afya. Ukiamua kujiondoa kwenye utafiti, ni lazima urudi kwa daktari wa utafiti na umjulishe hivyo.

Daktari ana haki ya kukutoa katika utafiti wakati wowote ikiwa amelazimika kufanya hivyo. Sababu zaweza kuwa ukosefu wa kutoa kiwango na sampuli ya kinyesi kinachotakiwa, kukosa kukamilisha taarifa au nakala ya afya, makosa kwenye taarifa ya hospitali na pia makosa katika kuzinakili nambari au tarakimu za utafiti.

IDHINI YA KUJIANDIKISHA

Nimesoma kwa makini taarifa niliyopewa na kuielwa vizuri. Maswali yote niliyouliza yamejibiwa kwa kuridhisha. Nimepewa nakili ya taarifa hii ya makubaliano. Naelewa kuwa niko huru kujiondoa katika utafiti wakati wowote bila ya kupata hasara au madhara yoyote mbeleni.

Mimi nimekubali kushiriki katika utafiti:

SAHIHI YA MWENYE KUJITOLEA:

JINA:

TAREHE :

ANWANI YA MWENYE KUJITOLEA:

SAHIHI YA AFISA WA AFYA:

JINA : TAREHE.....

35. PATIENT'S PARTICULARS FORM

PART I

SUR NAME:

OTHER NAMES

AGE :

GENDER :

PARENTS/GUARDIAN'S NAME :

POSTAL ADDRESS :

TEL. NO. :

PHYSICAL ADDRESS :

STREET :

VILLAGE :

LANDMARK :

DISTRICT :

DIVISION :

LOCATION :

SUB-LOCATION :

SUBCHIEF'S NAME:

PART II

NAME OF HEALTH INSTITUTION :

ENUMERATOR'S NAME :

DATE OF VISIT :

SAMPLE BOTTLE NO.: DATE TAKEN:

ENUMERATOR'S SIGNATURE :