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The prevalence of *Cryptosporidium parvum* and its relationship to the nutritional status of HIV- positive children suffering from diarrhea in Western Kenya

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Abstract
In this study, prevalence of *Cryptosporidium parvum* was studied in relationship to nutrition amongst HIV positive children with diarrhea as a presenting sign. Watery to loose stool and whole blood specimen were collected from completely randomized inpatient and out patient children. The collected whole blood specimens were screened using ELISA, and Particle Agglutination (Serodia) techniques for HIV (Japan). Stool specimen for those who were confirmed to be HIV positive were processed for microscopy using Kinyoun's technique . The results obtained were analyzed using linear regression for t, P at 95% Confidence Interval for significance levels in Minitab. The findings show that diarrhea in immunocompromised children in Western Kenya was majorly due to *Enterobacterieceae* (92.5%) and partly due to *C. parvum* infection (7.5%). Consequently this adversely affected growth and development in terms of weight and age. Based on prolonged diarrhea duration amongst these children it is clear that there were other co-infecting factors that aggravate the problem. It is therefore concluded that *C. parvum* is more prevalent in HIV negative children than in HIV positive children with diarrhea; and during onset of diarrhea, HIV positive children develop mild malnutrition as diarrhea duration progressed from normal nutrition to malnutrition. However in HIV negative children, nutrition status does not rapidly advance to a malnutrition state as observed in HIV positive children.

Introduction

The genus *Cryptosporidium* (Fayer, 1997) is a member of the phylum *Apicomplexa* along with the related genera of *Toxoplasma, Eimeria* and *Plasmodium*. It is a unicellular coccidian parasite, which infects the epithelial cells lining the gut and the respiratory tract (Ebrahim, 1993). Phylogenetically, *C. parvum* isolates from humans; farm animals; companion animals; and rodents; are morphologically and developmentally similar. However differences in the host specificity, prepatent and patent periods, and pathogenicity have been observed and these have been used previously as the basis for identifying *C.warairi*, and *C. felis* as unique species due to genetic differences with other *Cryptosporidium* species (Lihua, 1999).

Other than genetic differences between human and bovine isolates of *C. parvum* oocyte, inter and intra-species biological difference in *Cryptosporidium* have been infrequently substantiated by molecular studies (Lihua, 1999).

C. parvum is associated with two fold greater hazard of health problems than other AIDS- defining diagnosis (Peng, 1997). It is a major cause of secondary infection and wasting syndrome 'slim disease" in people with HIV/AIDS. It came to light with the spread of HIV infection in Central and Eastern Africa in 1985 (Ebrahim, 1993). In cases of AIDS, infection with *Cryptosporidium* species causes diarrhea, which becomes progressively worse (Ebrahim, 1993). The parasite is transmitted to humans by drinking *C. parvum* contaminated water, contact with infected animals, and contact with infected persons feces (Norman, 1996). Clinical manifestations of Crypto-sporidiosis depend on the immune status of the host (Paget, 1991). *Cryptosporidium parvum* causes a self-limited diarrhea illness in immunocompetent adults, but a protracted severe diarrhea illness in immunocompromised patients (Clark, 1996). In immunocompetent hosts, infection results in passage of four to six watery or mucoid motions a day for up to two weeks, flu-like non-inflammatory gastro-intestinal illness characterized by malaise, anorexia, vomiting, abdominal pain, cramps and sometimes fever (Paget, 1991). Diarrhea may occasionally be much more severe in immunocompromised hosts with defects in either humoral or cell-mediated immunity; where infection is frequently persistent and the resulting micropathology leads to impaired intestinal function as in AIDS patients. This may contribute to early mortality. Statistically, Cryptosporidiosis causes 73% mortality in HIV infected patients with low $CD4^+$ counts especially when the billary tract is involved (Tzipori, 1999).

Patients with $CD4^+$ lymphocyte counts of $< 100 \times 10^6$/liter are at increased risk of contracting clinical Cryptosporidiosis (Tzipori, 1999) and because of the low $CD4^+$ counts the probability of *Cryptosporidum* infection leading to severe diarrhea may be experienced with subsequent early mortality (Una, 2000). It is also evident that HIV infected patients with $CD4^+$ T cells counts of at least 180×10^6 / liter display self-limited disease. However individuals with a $CD4^+$ T cell count $<50 \times 10^6$/ liter have increased incidence of billiary disease, which is usually an indicator of chronic Cryptosporidiosis. Usually such individuals have a

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decreased survival time when compared to those individuals with higher $CD4^+$ cell counts (Tzipori, 1999). Histology of the infected intestine reveal epithelial cell damage, primarily of enterocytes on the villi. Since these villous enterocytes are largely responsible for glucose-stimulated Na^+ and H_2O absorption, the morphological results are consistent with the impaired glucose-stimulated Na^+ and H_2O absorption (Tzipori, 1999). Inflammatory cells recruited to the lamina propria or other sub - epithelial cells in the *C. parvum*, may produce prostaglandin E_2 (PGE_2). PGE_2 stimulate active Cl^- secretion and inhibit electroneutral NaCl absorption possibly via elevation of intracellular cAMP levels. PGE_2 has therefore been associated with diarrhea caused by *Entamoeba histolytica*, and *Salmonella ssp* (Clark, 1996).

The main objective of this study was to determine the prevalence of *Cryptosporidium parvum* infection and its relationship to the nutritional status of HIV- positive children suffering from diarrhea in Western Kenya.

Materials and Methods

The study involved collection of blood and stool samples from both HIV positive and HIV negative children suffering from diarrhea within period of six months. A total of 93 children were involved in the study, where 50 were HIV negative and 43 HIV positive.

Collection of blood samples

Whole blood from children on different medications whose parents/guardian had consented to participate in the project was collected from peripheral vein using a butterfly vein scalp needle into sterile 4 ml, 12 × 75 mm k_2 EDTA: 7.2 mg vacutainer ^{TM} polystyrene tubes and taken to KEMRI – JICA/HIV/AIDS laboratory in Kisumu for HIV analysis within 4-6 h of sample collection.

Collection of stool samples

Stool was collected either in the morning between 8.00 and 11.00 am or at 8.00 -11.00 pm. Anthropometric measurements namely, age, sex, and weight were recorded before collecting the sample. The collected stool samples were stored in a cool box with ice bags at 8 °C and then taken to the health center laboratory from where they were transported to Maseno University Department of Zoology laboratory within 18 h from collection time.

HIV/AIDS testing

The collected whole blood samples in 4 ml, 12 × 75 mm k_2 EDTA: 7.2 mg vacutainer^{TM} polystyrene tubes were centrifuged at 3000 g (Beckman Optima^{TM} TLX Ultracentrifuge) for 3 min for plasma collection in the virology laboratory at KEMRI- Kisumu. The obtained serum was tested for the presence of HIV antibodies using graphite coated antigen from Particle Agglutination test Kit for Agglutination (PA) Serodia (Japan) (procedure as per the protocol). Positive results were achieved if there was a pink agglutination on the surface of the micro well and negative results were declared if there was no agglutination and the graphite pink particle settled at the bottom of the micro well plate. The results obtained were then compared to the direct ELISA Abbott HIV 1 / 2 g Kit Germany for confirmation. The obtained data was then used for correlation purposes with other study parameters.

Detection of *Cryptosporidium* oocysts

Stool samples were analyzed for the presence of *Cryptosporidium parvum* oocyst using Kinyoun's technique; modified Ziehl-Nelsen technique (Baker, 1978).

Anthropometric measurements and determination of nutritional status Nutritional status was determined by comparing the nutritional data, age, weight collected from the cited Health Centers to reference standards. NCHS (USA, National Center for Health Statistics) reference data recommended by WHO (1979) was used as reference standard. The data collected was used to calculate the Z- scores and then compared with the NCHS reference values. Z- score value relates the relative position of each child's age and weight value to the distribution of the age and weight values of the NCHS/WHO reference population.

Results

The study involved collection of blood and stool samples from both HIV positive and HIV negative children suffering from diarrhea within a period of six months. A total of 93 children were involved in the study, where 50 were HIV negative and 43 HIV positive.

Prevalence of HIV/AIDS among children presenting with diarrhea

The prevalence of children suffering from HIV and had diarrhea was 46.3% while those who were HIV negative and suffered diarrhea was 53.7%. This was determined by collection of whole blood samples and screening the blood using test kit as mentioned in methodology above. However CD4 and CD8 cell counts were not done due to unavailability of Florescence Activated Cell sorter (FACS) machine.

Anthropometric measurements

The anthropometrics measurements were obtained by weighing the child using the standard weighing machine for those who were 1 month up to and including 1 year.

Those who were 1 year and above were weighed using Avery weighing scale. The children total body weights were obtained without clothing. The age of the child was calculated from percentile card information (current date of observation – date of birth) and recorded. The data obtained for Age/Weight was used to calculate the nutrition status of the child.

Nutrition status of the children

The anthropometrics data obtained was used to calculate the Z- score using the formulae:

WAZ- score = W – MW/SD

W = weight of child, MW = Median weight of reference child (same age and sex), SD =Absolute value of standard deviation, WAZ = Weight for Age Z-score.
SD = Median of references–the required standard (+/-).
Classification
Above – 1.00. S.D = Normal (1)
1.00 _ - 1.99 S.D = Mild malnourished (2)
2.00_-2.99 S.D=Moderately malnourished (3)
3.00 S.D and below severely malnourished (4).

The following are the results obtained for children who had *C. parvum* oocyte infection as shown in Table 1 and 2 below.

Table 1. Correlation data of *Cryptosporidium parvum* oocyte count and Nutrition status in immunocompetent children with diarrhea in Western Kenya

Sexes [a]	Age Month	Body Weight (kg)	Standard body weight (kg)	N/status per Z-score classification	Degree count of *C. parvum* oocytes /gm (stool)
M (1)	12	9.5	10.2	1	+ (6)
M (1)	24	7.8	12.3	4	+ (3)
M (1)	26	13.0	12.7	1	+ (6)
F (1)	36	13.0	14.1	1	+ (3)
M (1)	48	10.0	16.7	4	+ (6)

[a] = Number of observed children within the same age (months)
(+) = Degree /Number of *Cryptosporidium parvum* oocysts observed per focus/stool

Table 2. Correlation data of *Cryptosporidium parvum* oocyte count and Nutrition status in immunocompromised children with diarrhea in Western Kenya

Sexes [a]	Age Month	Body Weight (kg)	Standard body weight (kg)	N/status per Z-score classification	Degree count of *C. parvum* Oocytes /gm (feces)
M (1)	14	9.7	10.2	2	+ (6)
M (1)	24	8.0	12.3	4	+ (3)

[a] = Number of observed children within the same age (months)
(+) = Degree /Number of *Cryptosporidium parvum* oocysts observed per focus/stool

Table 3. Relationship between *Cryptosporidium parvum* infection and Nutrition status.

C. parvum Oocyte Grouping	*C. parvum* Oocyte count /gram (stool)	Nutrition status per Z-score classification
1 - 4	20	1
5 - 9	40	2
10 - 14	60	3
15 - 19	80	4
20 – 24 >	100 >	4 >

The regression analysis of observed body weight versus nutrition status was r = 0.703, P = 0.186, t = 8.471 in immunocompetent children with diarrhea. The regression analysis for observed body weight versus nutrition status was r =1.0, P 0.0 Pearson correlation = -1.00, P = 0.01; body weight versus *C. parvum* infection Pearson correlation = 1.00 regression r =1, P =0.0 while for nutritional status versus *C. parvum*, Pearson correlation = 1.00 P = 0.01, regression r = 1, P = 0.0 in immunocompromised children. The obtained data show that *Cryptosporidium* is more prevalent in well-nourished immunocompetent children than in malnourished children with diarrhea. All observed immunocompromised children infected with *Cryptosporidium* were malnourished. In total the observed immunocompromised and immunocompetent children with *Cryptosporidium parvum* infection, 42.8% were severely malnourished, 42.8% had a normal nutrition status, and 14.2% had mild malnutrition.

Prevalence of *Cryptosporidium parvum* in stool

Only 4.7% (n = 2/43) of the immuno-compromised children observed had *Cryptosporidium parvum* oocyte infection of which 67% (n =29) were males, only 2 (6.9%) male children had *Cryptosporidium parvum* infection (in essence all the *Cryptosporidium parvum* positive children were male). The mean of observed weights after infection and dehydration due to diarrhea was 9.9 kilograms as compared to the expected mean standard weights of the normal healthy children of similar age group and sex (11.8 kg). This was 1.9 kg difference in weight from normal children as compared to NCHS (1979) WHO data. In immunocompetent children the incidence of *Cryptosporidium parvum* was 10% (n=5/50) with most of the infected children having had diarrhea for not less than 3 consecutive days. Out of the 50 cases, 42% (n =21/50) were male children and 19% (n = 4/50) immunocompetent male children had *Cryptosporidium parvum* infection. The regression analysis of observed body weight versus *C. parvum* infection in immuno-competent children was r =0.104 P = 0.868 with Pearson correlation = – 0.167, t = 2.485. 58% (n = 29/50) of the total immunocompetent children were female and only 2% (n = 1/50) of this suffered from *Cryptosporidium parvum* oocyst infection. This represents only 3.4% of the total immunocompetent female children infected by *Cryptosporidium parvum*. The regression analysis was r = 0.167, P = 0.789 t = 0.371, Pearson correlation analysis = - 0.167, P = 0.789.

Out of 14 immunocompromised female children, none suffered from *C. parvum* oocyst infection. The same trend on reduction in weight in immunocompromised children was also observed in immunocompetent children who had a mean weight of 10.6 kg as compared to the expected mean standard weight of normal healthy children of similar age group and sex (11.8 kg). However there was increased weight loss in immunocompromised children than in immununocompetent children.

The Pearson correlation statistics of *C. parvum* to diarrhea duration was 1.0 in immunocompetent and 0.611 in immunocompromised. This was significant at 5% level of significance. Therefore the two groups have equal chances of *C. parvum* infection.

In imunocompromised children, the peak incidence of *C. parvum* infection was between age 1.1 and 2 years while in immunocompetent it was 1 and 4 years. It is also extrapolated that more male children suffered from *C. parvum* infection in both the study groups.

Discussion

The low incidence *of C. parvum* in immunocompromised children can be attributed to small sample size, and also limitations on the methodology used. Also the fact that it is an intracellular parasite, its elimination in the system requires the interplay of cell mediated immunity where the T°differentiate to Th_1 and Th_2 under the influence of interleukin 1 (IL-1), T_1 and cytokines.

The Th_1 secretes IL - 12 and IL -2 which stimulates Natural Killer Cells (NK) and cytotoxic T cells that are involve in killing of *C. parvum*. Th_1 also secretes IL-2 and IFN-γ that acts on microphages, which produce Tumor Necrosis Factor (TNF), reactive nitrogen radical (RNT), reactive oxygen radical (ROI) that are involved in the killing of infected cell (Delayed Type Hypersensitivity-DTH). The Th_2 secretes IL-4, IL-6, IL-5 that acts on B cells which eventually secretes immunoglobulin D, G, M, A, E. IL-5 also triggers eosinophils that act directly in the elimination of parasites by binding on them after they have been processed by antigen processing cells (APC) thus forming antigen -antibody complex that is eventually phagocytosed. The interplay of these immune effectors mechanisms could have lead to the observation of low numbers of *C. parvum* oocytes in immunocompetent individuals where the disease is self-limiting. Though the cytokine levels and lymphocyte counts were not done, the rate of recovery from diarrhea was high in immunocompetent children than in immuncompromised children. However, in immunocompromised children, where the virus affects the immune system, the presence of the immune effecter mechanisms was low or absent. This should lead to a higher oocyte count; but this was not the case. It is speculated that since *C. parvum* is an enterocyte parasite it attaches on to the intestinal villi for it to penetrate the epithelial cells. However, this cells are eroded by other organisms e.g. *Enterobacterieceae* that are known to be opportunists in HIV and thus rendering no attachment sites for *C. parvum*. Also the

IgA, which is mostly found within the intestinal mucosal region, could be playing a role in lowing the population of *C. parvum*.

The proliferation of CD4 cells in HIV positive situations is very high in order to combat the virus. The high CD4 cell titer in the circulatory system of these HIV infected patients needed for fighting HIV elimination could have counteracted the parasite leading to low *C. parvum* detection in stool and consequential effects. This phenomenon is not experienced by immunocompetent children who do not have the virus in their system to fight, and therefore normal CD4 T-lymphocyte cell count leading to reduced parasite elimination. However this was not studied. The low *C. parvum* infection in HIV positive children of age 1- 5 years is also confirmed by Tzipori *et al*., (1999) - where the prevalence rates of *Cryptosporidiosis* in diarrhea illness ranged from a few percent in cooler, more developed countries, (0.1 -2% overall, perhaps twice this number in children) to 0.5 - 10% in warmer, less developed countries with a peak incidence in children aged 1-5 years.

Smit *et al.,* 2000 have also documented low percentage of *Cryptosporidium* species (3%; (N = 50) in stool specimen collected from pediatrics. The low percentage of *C. parvum* infection in immunocompromised children (Table 2) could also be attributed to activation of the immune system by existing intestinal microflora thus resulting into interferon-γ (IFN γ) production by natural killer cells (NK). This could eventually lead to parasite elimination and resistance to infection (Tzipori, 1999). Anorexia and fatigue resulting from disease or medication can also lead to "poor eating habits " thus resulting to prolonged diarrhea duration that eventually lead to reduction in entire body weight. The pathological effects of cytokines produced by the immune system to protect the individual could also lead to reduction in weight as documented by Arditti *et al.,* (1993). Gastrointestinal pathology without evidence of infection as documented by Crews *et al*., (1994) has raised the possibility that other mechanisms such as malabsorption of disaccharide's or other nutrients may lead to malabsorptive diarrhea. This malabsorptive phenomenon leads to malnourished individuals who may develop acquired immunological deficiencies, such as T cell depletion (T - helper lymphocytes) (Crews, 1994). This may play a role in gastrointestinal dysfunction depriving the body of essential growth components thus the low body weight. Weight loss has also been reported to be due to a cascaded of secondary effects perpetuated by HIV produced cytokines via anorexia and hypermetabolism (Cimoch, 1997). This is supported by the fact that HIV can infect gastrointestinal cells directly with a subsequent immune- mediated cellular atrophy which thus may account for chronic diarrhea leading to low / less nutrient absorption with the enterocyte that eventually results to malnutrition and loss of body weight (Mok, 1995). Underweight, due chronic under nutrition or wasting or both, affects fewer children globally than stunting. However, underweight is still very widespread. In the year 2000 it was estimated that 27% of preschool children in developing countries were underweight (<-2 SD weight-for-age). Both the prevalence and number of underweight children have declined steadily since 1980. Both Western and Eastern Africa have lower prevalence (37% and 36% respectively) of underweight children than South Central Asia, but the situation is deteriorating. Countries of Eastern Africa are experiencing a rise in underweight of 0.55 percentage points per year, or a full 5% point increase between 1990 and 2000. In Sub-Saharan Africa, 8.2 million more children are underweight now than in 1990 (Sub-Committee on Nutrition (ACC/SCN January 2000). The anthropometric index weight-for-age represents body mass relative to age. Weight-for-age is influenced by the height and weight of a child and is thus a composite of stunting and wasting, making interpretation of this indicator difficult. In the absence of wasting, both weight-for-age and height-for-age reflect the long-term nutrition and health experience of the individual or population (Sub-Committee on Nutrition (ACC/SCN January 2000)). It was observed from this study that most children who were undernourished fell within the age of 1-2 years, and they presented with a long diarrhea period. Nutritional results obtained in this study show that most HIV positive children had mild malnutrition during the onset of diarrhea and as diarrhea duration progressed the nutrition status advanced to moderate then severe (Table 2). As a result they recorded a slightly reduced low body weight as compared to normal standard body weight of the same age. It is suspected that this malnutrition condition could be due to impaired absorption of nutrient from the gastrointestinal tract as a result of damaged villous enterocytes that are largely responsible for nutrient absorption. The villous enterocytes cells are damaged by enteropathogens, which further take advantage of the decrease in the number of CD4 positive cells in the intestinal mucosa. This lead to significant abnormalities in the mucosal immune function including defects both in cell- mediated immunity and in the secretory immunoglobin A (IgA) system that are involved with pathogen phagocytosis and elimination. These condition could also be enhanced by the effects of Protein Calories Malnutrition (PCM) that is associated with a decrease in thymic size, diminished natural killer cell activity, and altered migration of gut mucosal lymphocytes- decrease in secretory IgA and total lymphocyte count (particularly $CD4^+$ T lymphocytes), diminished delayed hypersensitivity, lower complement

C_3 levels, and reduced bactericidal capacity (Cimoch, 1997). Lima *et al.,* 1997 documents that patients with Cryptosporidial diarrhea develop almost 6–fold higher Lactulose: Mannital (L: M) excretion ratio than those without diarrhea (P< 0.001) and nearly 3– fold higher than those with non-Cryptosporidial diarrhea (P = 0.02). HIV patients with Cryptosporidiosis have greater disruption of intestinal barrier function with potentially important nutritional consequence (Lima, 1997). Eventually these adversely affect the overall nutritional status of HIV- infected individuals, which is expressed as ratio of weight for age.

The effect of nutrition not only on growth and physical development, but also on cognitive and social development is well documented. A malnourished child is more vulnerable to disease and cognitive development will be in peril, especially during the first three years of life. In children, malnutrition manifests itself as growth failure, which in this case lead to retarded growth, effect on psychomotor hence their physical appearance – like an old aged person.

Stunted physical growth is closely linked to reduced mental development. With a distorted intake of nutrients-- to little, too much, or unsafe-- or with too great a loss of nutrients.

The low *Cryptosporidium parvum* incidence rate in the study unlike the literature can be attributed to small sample size, sample collection time and diagnostic technique used. Samples for *Cryptosporidium parvum* diagnosis should be collected between 6 – and – 9 am and probably 11 pm when the parasite is out of lamina propria of the bowel. Also *Cryptosporidium parvum* infection has gone unnoticed by many laboratory technicians due to improper diagnostic technique using Kinyoun's technique (Modified Zeihel - Nelsen Technique) and this could have lead to low parasites low counts, therefore a more precise and over- the counter method should be developed. Polymerase Chain Reaction (PCR) should be used to confirm the Modified Zeihel – Nelsen results within medical facilities. This calls for training of personnel by the Ministry of Health.

Intervention studies using larger sample size (> 300) should be done to determine *C. parvum* infection and its effect on nutrition status in HIV positive children with diarrhea as presenting sign since the survey has shown the existence of the parasite within the population

In conclusion, both immunocompromised and immunocompetent children have equal chance of infection with *Cryptosporidium parvum*. However, infection rate was higher in immunocompetent (Table 1) than in immunocompromised (Table 2) children which is contrary to literature.

Infection by *Cryptosporidium* affect the nutrition status of an individual as can be seen in this study (Table 3) with the effect being more on the immunocompromised than in immunocompetent children. Also the infection rate is centralized within age group 12 - 24 months in both the study cohorts.

References

Arditti. D, M.N., ARNP 1993 *The Wasting syndrome in HIV infected individuals*. Nutritional management in HIV Lecture notes. South Africa.

Baker. J F., Silverton. E.R., 1978. *Staining techniques*. Introduction to Medical Laboratory Technology 5th ed. Pp 234.

Clark D. P. and Sears C. L. 1996 *The pathogenesis of Cryptospordiosis.* Parasitology Today Vol 12. No. 6 1996. Elsevier Science Ltd pp221-225.

Cimoch. P.J., FACP, 1997. Director of Medical Services, Center for Special Immunology, Irvine, California - *Treatment of Nutritional Health: Prevention and HIV- Associated Malnutrition*: A case Management's Guide. Suggested citation.

Crew D.E., and Garruto R.M., 1994 Perspectives on human variation over the life span- Nutrition and aging. Biological anthropology and ageing pp242- 248.

Ebrahim G. J. 1993. Diarrhea -Pediatric practice in developing Countries. 2nd ed pp125.

Fayer, R., Speer, C.A. and Dubey, J.P. 1997. The general *Cryptosporidium*. In: *Cryptosporidium* and Crypto-sporodiosis (R. Fayer, ed), PP 1–41. Boca Raca Raton, FL, CRC Press.

Lihua X., Una .M., Josef. L., Ananias .E., Arrow .M., Shullow .W., Thompson. R.C.A., Fayer .R, and Alfat. And Lal. I. 1999 Genetic Diversity within *Cryptosporidium parvum* and related Cyptosporidium species. Journal of Clinical Microbiology 1999.

Lima AAM., Silva . TMJ., Gifoni AMR., Barrett. LJ., McAuliffe. IT., Bao. Y., Fox. JW., Fedorko. DP., Guerrant. RL.1997. Mucosal injury and disruption of intestinal barrier funtion in HIV – infected individuals with and without diarrhea and Cryptosporidiosis in North East Brazil. AMJ Gastroenterol. 1997 Oct, 92 (50); 1861-6. 46 ref, Eng

Mok. Y. Q.J., and Marie-Louise Newell., 1995. A guide to practical Management HIV infection in children; Pp104.

National AIDS/STDs Control Programme active NASCOP 2001. *Social and economic impacts of AIDS.* AIDS IN KENYA – Background. Projections. Impact. Interventions. Policy. Pp. 1-12.

National AIDS/STDs Control Programme active NASCOP 1999 *Social and economic impacts of AIDS.* AIDS IN KENYA – Background. Projections. Impact. Interventions.

NCHS 1979 WHO data. National Census for Health Statistics U.S.A

Ochieng .W., Otsyula. M., Mulaa. F., Ogoyi. D., Ogola. S., D'Agostino. A., 2000 *Pathogenesis of HIV Disease in children perinatally infected with HIV –1*. Proceedings of 21st African Health Sciences Congress 24th –28th April 2000. Pp. 26.

Paget S., Martin .B. M. C., Michael .P., and Tony .W., 1991. 4th ed -*Diseases of the Children in the Subtropics and Tropics*. Pp462 -3, 730, 981.

Pelletier. D.L., Frongillo E.A. jr., Schroeder D.G., Habicht. J. P., 1995. *The effects of malnutrition on child mortality in developing countries*. Division of Nutritional Sciences, Cornell University, Ithaca, New York 14853, USA.

Peng M..M., Xiao. L., Freema. A.R. n., Arrowood .M.J., Escalant .e A.A. , Weltman. C., Ong. C.S.L., MacKenzie. W.R., Lal. A.A., and Beard C.B., 1997. Vol 3 No. 4 - *Genetic*

Polymorphism among Cryptosporidium parvum isolates. Evidence of two District Human transmission cycles. Suggested publication

Smit .T. K., Shilumane .K. C., Mehlape. S.F., and Steele. A.D., 2000. - *MRC Diarrhea Pathogens Research Unit*, MEDUNSA. 15. Sub-Committee on Nutrition (ACC / SCN; 4th Report on The World Nutrition Situation January 2000. Nutrition throughout the life cycle).

Tzipori. S., 1999. *Cryptosporidium. Molecular Basis of Host - parasite interaction, Vol 40*. Advanced Parasitology, Opportunistic protozoa in humans. PP 8, 158-173,190,224-238. Academic Press-San Diego London Boston New York Sydney Tokyo Toronto.

Una. M., Morgan W. R., Lihua X., Irhad S., Andrew T.R.C., Wangeci N., Altaf Lal., Anne M. and Peter .D., March 2000., *Molecular characterization of Cryptosporidium isolates obtained from Human immunodeficiency virus-infected individuals living in Switzerland, Kenya and the United States. Journal* of *Clinical Microbiology* Mar. 2000, pp 1180-1183 Vol. 38 No. 3, 2000