PREVALENCE OF *CRYPTOSPORIDIUM PARVUM* W 6-60 MONTH OLD

CHILDREN PRESENTING WITH DIARRHOEA AT KENYATTA NATIONAL

HOSPITAL, NAIROBI, KENYA.

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DECLARATION: I hereby declare that this dissertation is my original work and has not been presented for a degree-m any other university.

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<u>DEDICATION</u>

To my wife Aileen, for the continuous encouragement she gave me and for keeping the home going during my long absence, and to my sons Michael, David, Jesse and Daniel for enduring all that time without their daddy.

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12. Most of all, I thank God, whose hand I saw throughout the study period.

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LIST OF ABBREVIATIONS

KNH   Kenyatta National Hospital

HIV   Human Immunodeficiency Virus

AIDS--....... Acquired Immunodeficiency Syndrome

PEM   Protein Energy Malnutrition

CDC   Centre for Disease Control

C. parvum— Cryptosporidium parvum

W/A   Weight for Age

W/v   Weight for volume   (

V/v   Volume for volume

PFC   Paediatric Filter Clinic

Study Definitions

1. Diarrhoea: Passage of 3 or more watery or loose stools in 24 hours

2. Caretaker: The person who spends most of the daytime with the child

3. Slum: An area of residence in a town with small poorly built and crowded houses and with no organized communication network or waste disposal system and poor sanitary conditions <

SUMMARY

Background: Diarrhoea remains one of the most important causes of morbidity and mortality in the tropics. Since the discovery of HIV-1 in 1982, *C. parvum* has become an increasingly important cause of diarrhea, especially in immunocompromised patients. It has been associated with large outbreaks of diarrhoea from treated water supplies. Data on prevalence in children is mainly from industrialized countries and ranges from 1% to 3.6%, with only scanty data from the African region ranging from 5.2% to 26%. The prevalence in Kenya is 18% in malnourished children.

Objectives'. To determine the prevalence of *C.parvum* in 6-60 month old children presenting with diarrhoea and to relate this td social, demographic and clinical correlates of *C.parvum* in these children.

Design: Cross sectional study.

Setting: Kenyatta National Hospital, Paediatric Filter Clinic

Subjects: 6-60 month old children presenting with diarrhoea.

Alain outcome measures: Prevalence of *C. parvum* is 14.5%. Low caretakers level of education is associated with increased prevalence of *C. parvum* diarrhoea. *C. parvum* infection is more prevalent among children with HIV seropositivity (37%) than HIV seronegative children (10%).

INTRODUCTION AND LITERATURE REVIEW

Diarrhoea is one of the most important causes of morbidity and mortality in the tropics

C. parvum is recognized as an important cause of diarrhoea in both animals and

humans worldwide. In the last 30 years, the disease cryptosporidiosis has changed from

a rare, largely asymptomatic disease, to an important cause of gastrointestinal disease

worldwide. In animals, cryptosporidiosis is a major cause of calfhood diarrhoea, and in

humans it accounts for up to 20% of all cases of childhood diarrhoea in developing

countries and is a potentially fatal complication of AIDS (1,2). Factors that have

led to the emergence of cryptosporidiosis in animals include lack of effective treatment

and prevention and environmental contamination. In humans, the zoonotic nature of the

infection and the increased vulnerable population have contributed to increasing the

prevalence of cryptosporidiosis (2).

Cryptosporidia are unicellular protozoan parasites belonging to the phylum *Sporozoa*.

These coccidian parasites are found worldwide and are widely distributed in reptiles,

birds, fish and mammals (3,6,5). First described by Tyzzer in 1907 in the gastric

mucosa of laboratory mice, Cryptosporidia were considered non pathogenic until 1955

when a fatal diarrhoea syndrome in turkeys was described (4,7). In 1971, a similar fatal

diarrhoea outbreak was described in calves (7). The first case in humans was described

in 1976 by Nime et al (8). Between 1976 and 1981 7 cases, most of these (5) being in

HIV positive patients, were described. In 1981-82, a further 47 cases were reported by

CDC in Atlanta, most of these cases being in immunodefecient patients. Clinically, the

disease may be asymptomatic or present with a self limiting diarrhoea associated with a

low-grade fever, weight loss and malnutrition if prolonged. In the

immunocompromised, a chronic course may be taken (9,10,11).

C. parvum has come to be of public health importance due to its ability to cause waterborne infection as a result of certain qualities it possesses. These include: a hardy oocyst which is able to resist light chlorination and even full strength household 5.24% bleach (12,14), its tiny size which enables it to escape filtration from processed drinking water supplies, even when filtration is working optimally (13), low infection dose of as few as 30 oocysts (14), fully infectious development when shed, and its zoonotic potential making it a real threat in drinking and recreational water, contaminated food, day care centres, hospitals and in persons exposed to animals e.g. veterinarians and dairy or cattle farm workers (13,14,15).

In April 1993, the largest outbreak of infectious diarrhoea in the USA occurred, in the greater Milwaukee, Wisconsin area, following contamination of the city's water supply by C.parvum. This affected an estimated 403,000 residents who experienced gastrointestinal illness (16,17,18). It is unclear whether local waterborne disease outbreaks leading to diarrhoeal disease may or may not be related to cryptosporidiosis, which has been poorly investigated., .

Prevalence of infection in children in general decreases with age, peak age being 1 to 5 years (7, 19-22). Prevalence studies in children are mainly from the west with only scanty data from the African continent. Reported rates have varied from 1 to 3.6% in industrialized countries and 1-13% in China (7).

In Africa, Narco and other workers in Burkina Fasso examined stool samples of 1,392 children aged less than 36 months in a hospital set up. Of these, 756 had diarrhoea and the rest (629) did not have diarrhoea. He found the overall prevalence of *C. parvum* to be 5.2 % (72 ou^of 1,392) and 7.8 % (59 out of 756) in the children with diarrhoea

respectively. Peak age was in the age group 6-23 months and no infection was noted in the less than 6 months age group (20).

In Nigeria Kwara State, Nwabuisi examined stool samples of 198 0-14 years old children with diarrhoea and found the prevalence of *C. parvum* to be 15.1 %, with 86.7 % of oocyst secretion occurring in the 0-2 years age group and no secretion occurring after the age of 4 years.(21) Also in Nigeria, Okafor and Okunji in Enugu State looked at stool samples of 373 primary school children of whom 38 had diarrhoea and 335 had no diarrhoea and found oocysts in 15 of the 38 (39 %) diarrhoeal stool samples and in 81 of the 335 (24 %) non diarrhoeal stool samples (22).

Duong and other workers in Gabon studied 288 children aged 0-2 years and got a Cryptosporidia prevalence of 28% in those jvith acute diarrhoea and 14.8% in those without diarrhoea (23).

In Zambia, Nchito and others examined stool samples of 222 children from four crowded townships, and got a Cryptosporidia prevalence of 18% (24).

In Addis Ababa, Asefa et al sampled stool from 214 under fives who were attending a clinic for diarrhoea. He reported Cryptosporidia oocysts in 12 of them, the prevalence being 2.5% (19).

Locally, in 1990, Kitili studied cryptosporidiosis in malnourished children at Kenyatta National Hospital. After examining stools from 285 children aged from 2 weeks to 5 years, she found that 99 (38.7 %) had diarrhoea and 157 (61.3%) did not have diarrhoea. The prevalence of *C. parvum* in both groups was the same, 18%. Oocysts were found in the age group 8-37 months. The prevalence of *C. parvum* was lower in those that tested positive for HI^ compared to those that tested negative for the same. No association was found between *C. parvum* and chronic diarrhoea. The study found *C. parvum* to be most

prevalent in children with marasmic kwashiorkor (21.7%), followed respectively by the underweight (20.3%), kwashiorkor (18.4%) and marasmus groups (10.5%) (25). While Kitili's study focused on *C. parvutn* in malnourished children, this study's focus is on *C. parvutn* in children with diarrhoea.

Cryptosporidiosis has been associated with various social, demographic and clinical factors. Saredi and Bava studied 553 hospitalized paediatric patients in Argentina and found 21 (3.8%) to be positive for C. *parvum.* In his analysis, he found that in children less than 18 months, *Cryptosporidium parvum* occurred more frequently in those who had diarrhoea, were immunodefecient, and were from low socioeconomic background and were living in poor sanitary conditions with no potable running water (26).

In the Burkina Fasso study, positive correlations with malnutrition (p<0.01) and Rotavirus infection (p<0.05) were noted (23).

Duong's study showed striking differences in infection rates between the malnourished (31.8%) and eutrophic children (16.8%) (p<0.01) (26).

In HIV infected children, *C. parvumyws* found to be the most common intestinal parasite (26%) in a study of 200 children aged 6-24 months in Lusaka Zambia by Amadi et al (30). The same was demonstrated by Leelayoova et al in Bangkok Thailand (28). Pratt et al in Barcelona found *C. parvum* to be the most frequent cause of gastroenteritis in HIV infected children (29).

While Okafor's study did not find any significant difference in occurrence rates in urban and rural school children, he observed that oocyst occurrence was significantly higher in watery stool than formed stool, an observation also noted by Narco and Duong (20,22).

Study Justification

C. parvum has been implicated in large water borne outbreaks of diarrhoea in the West where prevalence levels are relatively low. Locally, prevalence levels are expected to be higher, taking into account the AIDS scourge, increasing poverty, and the poor hygienic and living conditions, especially amongst the urban poor.

No local data on prevalence or correlates of diarrhoea due to *C. parvum* is available. Since no specific treatment for cryptosporidiosis is available yet, primary prevention is of paramount importance and involves, amongst other measures: more stringent quality control at water treatment plants; protection of such water sources as wells, pools and springs from human and animal contamination; health education to vulnerable populations. Though feasible as public health measures, such preventive measures would be implemented with even more justification if the magnitude of cryptosporidiosis has first been ascertained and relevant demographic data availed.

Major Objective

To determine the prevalence and correlates of *C. parvum* in 6 to 60 month old children

presenting with diarrhoea at Kenyatta National Hospital.

Specific Objectives

1. To determine prevalence of *C. parvum* infection in 6 to 60 month old children

2. To determine socio-demographic and clinical correlates of *C. parvum* diarrhoea in 6 to
month old children

METHODOLOGY

Study Design: Cross sectional Study

Sample Size Estimation:

The sample size was calculated using formula below: (30)

$$N = \frac{(Z1 - oc)^2 \cdot P \cdot (1-P)}{F}$$

oc   = Level of significance = 5%

P   = Prevalence of C. parvum from other studies (18%)

d   = Degree of precision ± 5%

$\frac{(Z1 - oc)^2}{2}$ = 1.96 (from tables of standard normal distribution) corresponds to 95% confidence interval

.*. Minimum sample size required

$$\frac{1.96^2 \times 0.18 \times 0.82}{0.05^2} = 226$$

Study Area

This was a hospital based study conducted in the Paediatric Filter Clinic (PFC) of Kenyatta

National Hospital Nairobi. Kenyatta National Hospital is a tertiary referral and teaching

hospital. It also serves as a primary health facility for patients from Nairobi and its environs.

An average of 9700 children are seen per month in the PFC of which 1400 are admitted.

Of the 1400 admissions, those with diarrhoea comprise 155.

There are 4 paediatric wards each admitting at least twice a week. The admitting doctors

comprise a Senior House Officer (Registrar) and a Resident Doctor (Intern) assisted by clinical

officers.

Study Population

6 to 60 month old children with diarrhoea presenting at the PFC.

Inclusion Criteria   * "

1. 6 to 60 month old children with diarrhoea

2. Informed consent by parent/guardian.

3. Availability of stool sample

t   .   •

Exclusion Criteria

1. Unconsenting parent/guardian

2. Failure to obtain stool sample

Sampling Procedure

Eligible children were sequentially recruited into the study, until the sample size was

achieved

Clinical Procedures

The investigator reviewed 6 to 60 months old children with diarrhoea presenting at the PFC. Their parents or guardians were each briefed on the study and its aims, and was requested to have their children recruited into the study. Those consenting signed a written consent, and were sequentially enrolled into the study.

Demographic data (name, age, sex, residence and water source) and history of the current diarrhoeal illness including its duration, frequency and the characteristics of the stool ie. colour, consistency and presence or absence of mucus or blood and presence of other associated symptoms were recorded in a precoded questionnaire.

The child's temperature was taken after which he/she was weighed using regularly calibrated Healthometer™ paediatric weighing scales manufactured by Healthometer

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Inc. Bridgeview, Illinois, USA. The weighing scales comprised a spring type (model 322) with a capacity of 16 Kg for the younger children (up to 16 Kg), and bar type with a capacity of 150 Kg for the older children who could stand. The child's weight percentage of the standard value and classification of nutritional status using the

Wellcome Classification (Appendix **III)** was then determined.

Laboratory Methods

Stool samples were collected in plastic polypot containers provided to the parent/guardian after reviewing the child. In cases where the stool was too watery, a swab was used to get a specimen from the rectum. The samples were taken to the parasitology laboratory of Kenyatta National Hospital within 1 hour of collection. Each stool sample was subjected to two staining techniques, Safranin-Methylene blue technique for cryptosporidial oocysts and the formol ether concentration technique for ova and cysts of other intestinal parasites (Appendix IV, V) (31,32)

HIV 1 & 2 testing was done using the rapid immunoassay method, which employed two different tests that were run in parallel namely, the Uni-Gold™ HIV test kit from Trinity Biotech PLC, Ireland and the Determine™ test from Abbott Laboratories Inc. USA (Appendix VI, VII). For the Uni-Gold™ test, whole blood obtained from the tip of the finger by needle prick aspirated using a pipette was used. Two drops 60fil of blood were put into the sample port. Two drops of the reagent were then added. The result was read after 10 minutes. A line of any intensity forming in the test region, plus a line forming in the control region, indicated a positive result. A line in the control region only indicated a negative result. For the Determine™ test, 50 il of whole blood aspirated by pipette from the same needle prick site as the first test, was put into the sample pad. After the chase buffer was then put into the blood containing pad and the final result read after 15 minutes. Interpretation was as for the Uni-Gold™ test.

Ethical Considerations

1. A written consent sought from the Ethical and Research committee of the
   Kenyatta National Hospital, approving the study, was obtained before
   embarking on the study.

2. A written consent from the parent or guardian after being briefed about the study
   protocol was obtained.

3. No benefit or treatment was given or withdrawn as a result of inclusion.

4. All information and results obtained remained confidential and were only
   used for the study.

5. Results useful in the child's treatment were communicated to the attending
   doctors .

6. HIV related pretest and post-test counseling was done.

Data Management and Analysis

All raw data was coded and entered into a computer using the SPSS-10 package (Statistical
Programme for Social Sciences). Data was summarized into frequency tables, charts and graphs.
The prevalence of *C. parvum* was computed among children with diarrhoea. Frequency
distributions and statistical differences between various correlates were done. Characteristics of
various groups were compared using 2-tailed t-test when and where data was continuous and chi
square (x^2) where data was categorical. Multiple logistic regression analysis was done for those
variables found to be significantly associated with *C. parvum* diarrhoea.

RESULTS

<u>Description of population studied</u>

During the period December 2003 and March 2004, a total of 220 six month to 5 year old children presenting with diarrrhoea were recruited into the study. The children comprised 128 (58%) males and 92 (41) females, Male: Female ratio was 1.4:1.

The mean age was 16.5 months with 180 (81%) being under 24 months
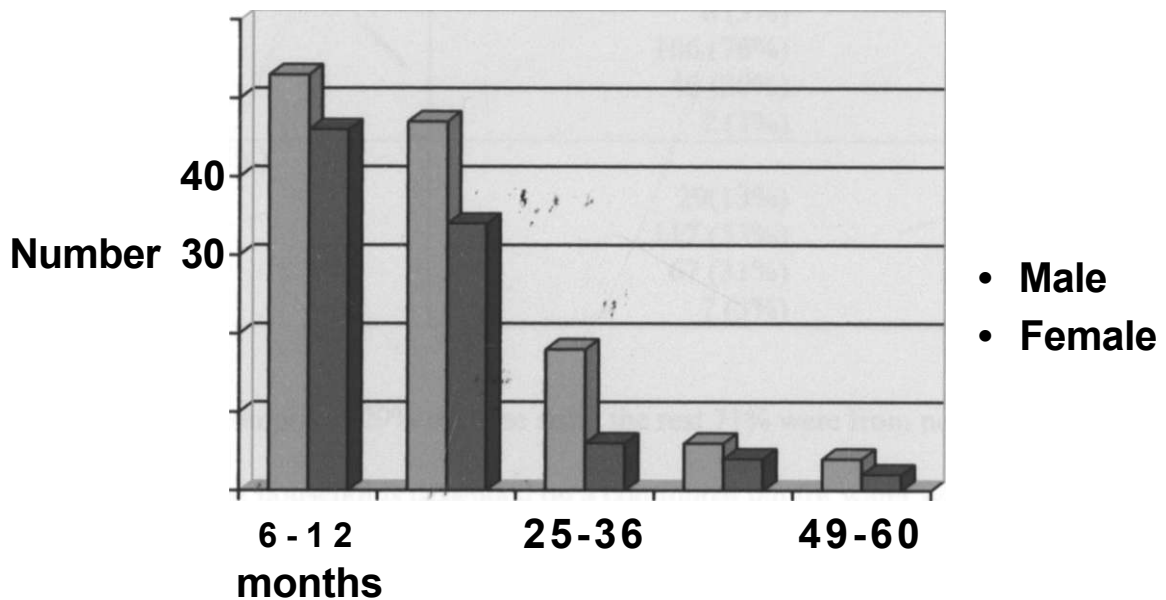
Figure 1: Age and sex distribution of study population

The number of children seen presenting with diarrhoea decreased with increasing age, from 99 (45%) in the 6 to 12 month age group to 6 (3%) in those aged 49 to 60 months. Majority of the children seen were under 24 months and there was a male predominance in all age categories (figure 1).   -

Table 2: Sociodemographic characteristics of study population

Characteristic	Number (%)
Residence (Slum)	64 (29%)
Water Source	
Communal Tap	157(71%)
Non tap	63 (29%)
Primary Caretaker- mother	187 (85%)
Age of Caretaker (yrs)	
<18	8 (4%)
19-30	180 (82%)
>30	31 (14%)
Education of Caretaker (yrs)	
Nil	6 (3%)
1-8	166 (76%)
9-12	46 (20%)
>12	2(1%)
Monthly Income (KSh.)	
<1,000	< , , 29(13%)
1,000-5,000	117(53%)
5,001-10,000	67 (31%)
>10,000	7 (3%)

Residents of slums comprised 29% of those seen, the rest 71% were from non-slum areas. Majority 157 (71%) of households depended on a communal tap for water while the remaining 63 (29%) depended on water from vendors, wells etc. Majority of the children had their mother as caretaker (85%). Majority of the caretakers were aged 19-30 years (82%) and had received between 1 and 8 years education (76%). Most households (66%) had monthly incomes of less than KSh.5,000 (Table 2).

Table 3: Clinical and stool characteristics of study population

Clinical / Stool Characteristic	Number (%)
Dehydration	179 (81%)
Vomiting	160 (73%)
Abd. Discomfort	112(51%)
Fever (>38°C)	95 (43%)
Cough	47 (21%)
HIV status Positive	38(17%)
Nutritional status Malnourished	110(50%)
Stool characteristic Watery Mucoid Bloody	189 (86%) 143 (65%) 25(11%)
Stool frequency 3-4 Bouts/ day > 5 bouts/ day	86 (39%) 134(61%)
Diarrhoea duration < 14days (acute) > 14 days (persistent)	208 (95%) 12 (5%)

Dehydration followed respectively by vomiting, abdominal discomfort, fever and cough were the most common clinical signs. 110 (50%) children were suffering from various degrees of malnutrition, 38 (17%) tested positive for HIV, 208 (95%) had acute diarrhoea and 134 (61%) had had more than 5 bouts in the preceding 24 hours. Majority 189 (86%) of the stool samples were watery, 143 (65%) were mucoid while 25(11%) were bloody. Some stool samples had more than one of the characteristics (Table 3).
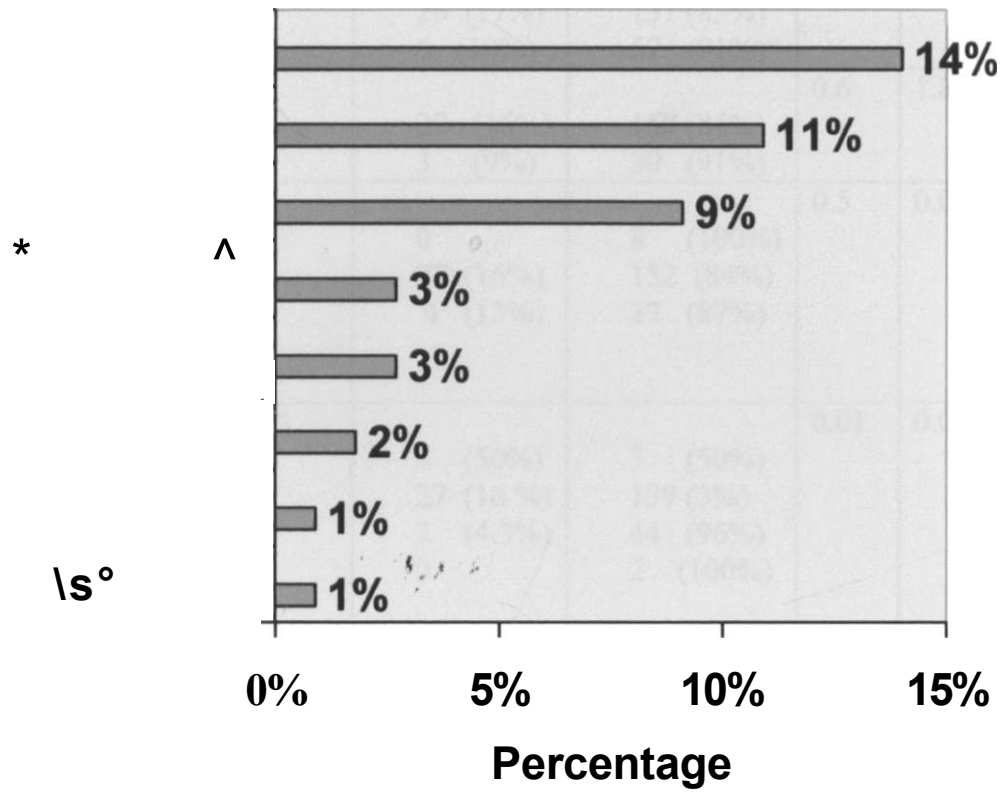
Figure 2   Frequency distribution of parasites seen in stool of study population

. Cryptosporidium (14%) followed respectively by Hookworm (10%), Amoeba (9%), Giardia

(3%) and taenia (3%) were the parasites most frequently found (figure 2).

Table 4 Association between *C. parvum* and sociodemographic characteristics

Characteristics	*C. parvum* Present (%)	*C. parvum* Absent (%)	P-value	OR	95%CI
Water Source			0.1	1.9	0.7-5.4
Communal Tap	26 (17%)	131 (83%)			
Others	6 (10%)	57 (91%)			
Primary Caretaker			0.6	1.8	0.5-8.1
Mother	29 (16%)	158 (84%)			
Others	3 (9%)	30 (91%)			
Age of Caretaker (Yrs)			0.5	0.0	0.0-4.1
< 18	0	8 (100%)			
19-30	27 (16%)	152 (84%)			
>30	4 (13%)	27 (87%)			
Education of caretaker (Yrs)			0.01	0.0	0.0-0.4
Nil	3 (50%)	3 (50%)			
1-8	27 (16%)	139 (3%)			
9-12	2 (4.3%)	44 (96%)			
>12		2 (100%)			
> t					
Residence:			0.05	2.2	0.9-4.9
Slum	14(22%)	50 (78%)			
Non- slum	18(12%)	138 (88%)			
> i >					
Monthly Income of Household (Kshs)			0.02	2.5	0.7-10.7
<1,000	8 (28%)	21 (72%)			
1,000-5,000	14 (12%)	103 (88%)			
5,000-10,000	7 (10%)	60 (90%)			
>10,000	3 (43%)	4 (57%)			

Of those using the communal tap, 26 (17%) had *C. parvum* infection compared to 6 (10%) in those that depended on other water sources. *C. parvum* infection decreased with increasing level of education, from 50% to 16% to 13% in caretakers with no education, Ito 8 years, 9-12 years and more than 12 years* df education respectively. *C. parvum* infection was twice as common in stool of slum dwellers (22%) than non-slum dwellers (12%).

The prevalence of *C. parvum* infection was inversely associated with the number of years of education of the caretaker (OR=0.0) and monthly income (OR=1.23). Children from the slum were significant more likely to have *C.parvum* (22%) than children from non-slum neighborhoods

(OR=2.2). There was no association between *C.parvum* and source of water, primary caretaker or age of caretaker (Table 4).

Table 5: Association between *C. parvum* and clinical characteristics

Characteristics	*C. parvum* Present (%)	*C. parvum* Absent (%)	p-value
Dehydration			0.1
Present	29 (16%)	150 (84%)	
Absent	3 (7%)	38 (93%)	
Vomiting			0.1
Present	20(13%)	140 (88%)	
Absent	12 (20%)	48 (80%)	
Abdominal Discomfort			0.5
Present	16 (14%) <	, 96(86%)	
Absent	16(15%)	92 (86%)	
Temperature		»˙	0.2
<38° C	15 (12%)	110(88%)	
>=38° C	17(18%) - -	78 (82%)	
Cough			0.01
Present	13 (27%)	34 (72%)	
Absent	19 (11%)	154 (89%)	
Nutritional status			0.02
Eutrophic	10(9%)	100 (91%)	
Malnourished	22 (20%)	88 (80%)	
HIV status			<0.001
Positive	14 (37%)	24 (63%)	
Negative	18(10%)	164 (90%)	

13(27%) of children presenting with cough had *C. parvum* infection compared to 19(11%) who did not have cough. Twice as many malnourished children (20%) had C. *parvum* infection compared to eutrophic children (9%). 14 (37%) of HIV seropositive children had concurrent C. *parvum* infection compared to 18 (10%) in those that were HIV seronegative.

C. parvum infection was 4 times more frequent among HIV seropositive children than HIV

seronegative children (p<0.001). Malnourished children were twice as likely to have *C. parvum*

than well nourished children (p=0.02). Children with cough had a higher prevalence of

C. parvum than those without cough (p=0.01). No associations were found between *C. parvum*

and dehydration, vomiting, abdominal discomfort or temperature (Table 5).

Table 6: Association between *C. parvum* and stool characteristics

	C. parvum Present (%)	*C. parvum* Absent (%)	p-value
Frequency			0.5
3-4 bouts	30 (15%)	70 (85%)	
>5 bouts	19(14%)	115 (86%)	
Duration (Days)		>f	0.02
<14 (acute)	27(13%)	181 (87%)	
>14 (Persistent)	5 (42%)	- ... 7 (58%)	
Consistency			0.7
Watery	28 (15%)	161 (85%)	
Formed	3(10%)	27 (90%)	
Mucoid			0.3
Yes	19 (13%)	124 (86%)	
No	13(17%)	64 (83%)	
Blood Stained			0.5
Yes	4(16%)	21 (84%)	
No	28(14%)	167 (86%)	

5 (42%) of children with persistent diarrhoea had C. *parvum* infection in stool compared to27

(13%) in those who had acute diarrhoea.C. *parvum* infection was 3 times more frequent in

children with persistent diarrhoea than those with acute diarrhoea (p=0.02). Other stool

characteristics i.e. frequency of stool, stool consistency, mucoid or blood stained stool were not

significantly associated with *C. parvum* infection (Table 6).

Table 7: Association between *C. parvum* and other parasites in stool

	C. parvum Present (%)	*C. parvum* Absent (%)	p-value
Hookworm			0.04
Present	7 (29%)	17(71%)	< • >
Absent	25(13%)	171 (87%)	
Amoeba			0.2
Present	31 (62%)	19(38%)	
Absent	1 (1%)	169 (99%)	⟨d
Giardia			0.2
Present	2 (33%)	4 (67%)-	
Absent	30(14%)	184(86%)	
Taenia			0.2
Present	2 (33%)	4 (67%)	
Absent	30(14%)	183 (86%)	
Isospora			0.3
Present	1 (50%)	1 (50%)	
Absent	31 (14%)	187(86%)	

7 (29%) of children who had hookworm in their stool also had *C. parvum* compared to 25

(13%) in those that did not have *C. parvum* infection.

Children with hookworm in stool were twice as likely to also have *C. parvum* in their stool than

children withoilf hookworm (p=0.04). None of the other parasites identified in the stool were

found to be significantly associated with *C. parvum* infection (Table 7).

Logistic regression

Multivariate logistic regression analysis was performed in order to find out which of the variables were independently significantly associated with *C. parvum* diarrhoea. In order to do this, all variables with significant p-values on bivariate testing and which were not correlated were considered for the logistic regression model. Those eventually included in the model were: duration of diarrhoea, residence, and education of caretaker, HIV status and cough.

Table 8: Logistic regression analysis

Variable	p.value*	OR*	95% CI*
Duration of diarrhoea	0.07	0.2	0.1-1.1
Residence	0.1	0.5	0.2-1.2
Educ. of caretaker	0.04	5.2	1.1-25.9
HIV status	0.004	0.2	•0.1-0.6
Cough	0.08	0.5	0.2-1.1

*Sig. - significance, *OR- Odds ratio, *CI - confidence interval

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Table 8 shows results of multivariate analysis of factors found to be significantly associated with *C.parvum* infection. After analysis, low education of the caretaker and HIV infection were found to be independently significantly associated with *C. parvum* diarrhoea with p-values of 0.04 (OR=5.2) and 0.004 (OR= 0.2) respectively after adjusting for the other variables in the model. There was a trend for association between C. parvum infection and persistent diarrhoea and slum residence in adjusted analysis (Table 8).

Logistic regression

Multivariate logistic regression analysis was performed in order to find out which of the variables were independently significantly associated with *C. parvum* diarrhoea. In order to do this, all variables with significant p-values on bivariate testing and which were not correlated were considered for the logistic regression model. Those eventually included in the model were: duration of diarrhoea, residence, and education of caretaker, HIV status and cough.

Table 8: Logistic regression analysis

Variable	p.value*	OR*	95% CI*
Duration of diarrhoea	0.07	0.2	0.1-1.1
Residence	0.1	0.5	0.2-1.2
Educ. of caretaker	0.04	5.2	1.1-25.9
HIV status	0.004	0.2	«. •0.1-0.6
Cough	0.08	0.5	0.2-1.1

*Sig. - significance, *OR- Odds ratio, *CI - confidence interval

Table 8 shows results of multivariate analysis of factors found to be significantly associated with *C. parvum* infection. After analysis, low education of the caretaker and HIV infection were found to be independently significantly associated with *C. parvum* diarrhoea with p-values of 0.04 (OR=5.2) and 0.004 (OR= 0.2) respectively after adjusting for the other variables in the model. There was a trend for association between C. parvum infection and persistent diarrhoea and slum residence in adjusted analysis (Table 8).

DISCUSSION

The prevalence of *C. parvum* in the 220 children seen in this study was 14.5 %. This figure falls within the range 5.2% - 26 % found in other African studies (22-28), it lies slightly above the upper limit of prevalence range reported in the Chinese studies (1% -13 %) (7) and is way above that for western countries of 1% - 3.6 %. These differences may be explained by the different socio-economic status settings that are known to influence the prevalence of *C. parvum* in the different areas (29).

There was a slight male preponderance 1.2: 1 (M: F) similar to other studies, and a left skewed age distribution with 78 % of the children with C. parvum oocysts in stool being less than 18 months. The numbers of *C. parvum* positive stools decreased with increasing age. This is consistent with the general findings that peak prevalence in children is 1 to 5 years. This is also

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consistent with peak prevalence findings of Narco in Burkina Faso (6- 23 months), Nwabuisi in Nigeria (0-2 years) and Kitili locally (8-37months), (23,24,28). Molbak et al, in his study on infant and childhood mortality from cryptosporidiosis in Guinea Bissau, found that cryptosporidiosis was associated with excess mortality in children who had the infection in infancy and this was not attributable to malnutrition, adverse socioeconomic factors, poor hygienic conditions or inappropriate breastfeeding practice (33). The implications of these findings in this study are that the infants who were infected with *C.parvum* and who comprised 50% of all the infected children are at increased risk of death and thus re-emphasises the importance of early identification and appropriate intervention to avert mortality. According to that study, such interventions would reduce mortality by 18% (33).

Children residing in slum areas most probably had other factors that were significantly associated with *C. parvum* infection such as the low education of the caretaker, low monthly income and malnutrition. In univariate analysis, residing in the slums was significantly

associated with *C. parvum* infection and a trend for association in multivariate analysis. Socioeconomic status is known to have an inverse relationship with cryptosporidial infection (26). Although this study revealed an inverse association between cryptosporidiosis and the monthly income of the household after limitation, the small number of subjects in the above Ksh. 10,000/= may have had an influencing effect on the outcome of the analysis. In addition, monthly income itself may not have been a sufficient enough pointer to the socioeconomic status of the household.

The communal tap, a water tap placed outside the house but within the plot (compound) and shared by 2 to 10 families residing in the plot, was the most the most common source of water in the study population (71.4%). Water obtained from a communal tap is collected in jerry cans of varying sizes depending on who is collecting the water, and stored in the house for immediate or later use, for drinking, cooking or washing laundry. Hygienic handling of the water e.g. washing of hands and water containers prior to water collection and subsequent storage in the house is doubtful, paving way for water contamination and subsequent disease transmission. Though infection rates with C. *parvum* were higher in those depending on water from communal taps than those depending on other sources, the differences were not significantly so. Contaminated piped water supplies are an important source of infection (24,26). This study did not look at the level of contamination of piped water by *C. parvum* and thus how much this mode of transmission contributes to infection in children. No local data is available.

Cryptosporidiosis can be prevented by measures such as improving domestic and personal hygiene. A strong correlation between education of the caretaker and *C. parvum* infection was found both during univariate and multivariate analysis, unlike in the Zambian study (24), which did not show ari# association. Public health intervention in the form of health education talks in affected areas would go a long away in reducing infection rates.

The stool characteristics of this study are similar to those described by other workers (1,7,19,20, 23). The clinical presentation of the subjects of this study is similar to the standard description of *C. parvum* (1,7). Pneumonitis is a known systemic complication of cryptosporidiosis (10,11). Clinically this may present with cough and fast breathing. Cough and *C. parvum* were found to be associated in this study during univariate analysis. After controlling for confounders in multivariate analysis however, the association became less strong, suggesting that the association between cough and *C.parvum* may partly be explained by HIV infection and education level of parent (by other factors adjusted for in the model).

The relationship between *C. parvum* and immunosuppressive states such as malnutrition and HIV is firmly established in various studies (27,28,29) and was confirmed in this study of Kenyan children.

The study had two limitations; the first limitation is tjiat the subjects seen came from the lower and middle social class. There was no representation from the upper class subjects who most probably sought treatment in private fee paying hospitals. This made the sample unrepresentative of the general population. The second limitation was that the inability to make a definitive diagnosis of HIV in children less than 18 months may have mis-classified some HIV uninfected children in the HIV group, thus confounding the results.

<u>Conclusions:</u>

1. The prevalence of cryptosporidiosis in 6-60 months old children presenting with diarrhoea is 14.5%

2. Low caretakers level of education is associated with increased prevalence of C. *parvum* diarrhoea.

3. *C. parvum* infection is more prevalent among children with HIV seropositivity (37%) than HIV seronegative children (10%)

<u>Recommendations:</u>

1. Need for routine examination of stool for *C. parvum* among children less than 2 years.

2. Need to carry out a case control study to determine the prevalence of *C. parvum* in children with and without diarrhoea from the community with a view of planning public health interventions

3. Need for centralized reporting of all positive cases, as an unusual increase in numbers may be   the only indication of an epidemic.

4. Need to carry out a similar study in private hospitals to determine C. parvum prevalence among children from the middle and upper socio-economic classes.

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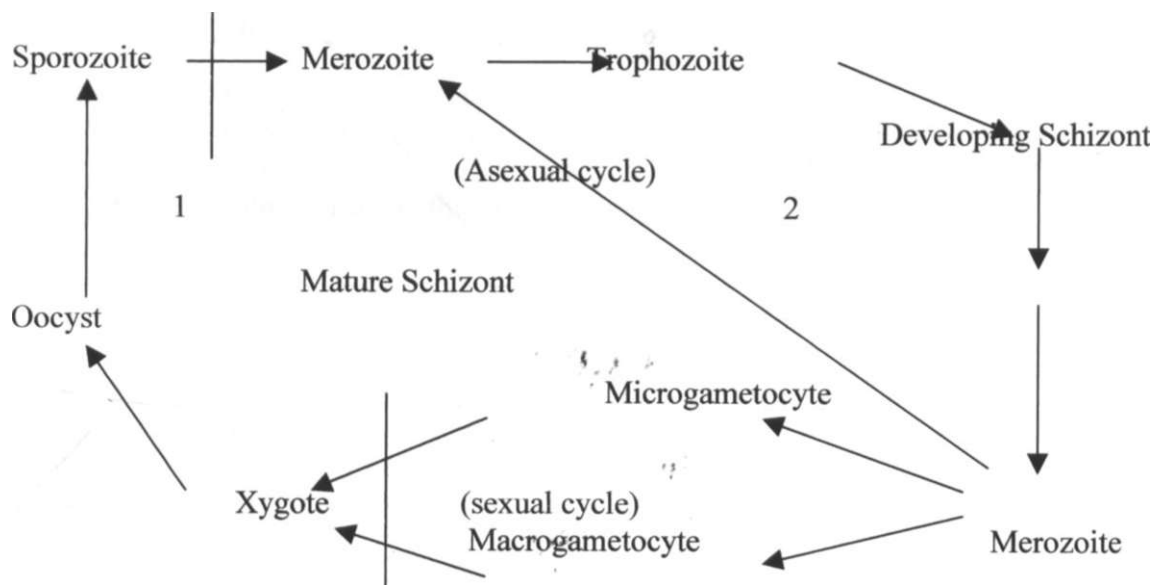
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APPENDIX

Appendix 1

Life Cycle of *C. parvum*

Sporozoite ———→ Merozoite ——— Trophozoite

Developing Schizont

(Asexual cycle)

1   2

Mature Schizont

Oocyst

Microgametocyte

Xygote   (sexual cycle)
                Macrogametocyte   Merozoite

1 - Outside Parasitophorous vacuole

2 - Inside Parasitophorous vacuole

Appendix II

Country Prevalence Rates of *C. parvum* in children with diarrhoea

	YEAR	LOCATION OF STUDY	PREVALENCES)	REF
1.	2001	Kwara State Nigeria	15.1	21
2.	1996	Enugu State Nigeria	25.1	22
3.	1998	Burkina Fasso	5.2	20
4.	1996	Addis Ababa (Ethiopia)	5.6	19
5.	1995	Gabon	24	23
6.	2001	Zambia	26	24
7.	1997	Bangladesh	3.5	33
8.	1996	Nepal	6.8	34
9.	2000	Israel	3.4-7.4	35
10.	2000	Melbourne (Australia)	0.4	36
11.	1996	Spain (day care centre)	10	37
12.	1996	Spain (Aragon region)	1.93	38

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Appendix III

Work Sheet

STUDY NUMBER:

A: HISTORY

    1. Date of interview :   \   \
    2. Name
    3. IP No:
    4. Sex: '
    5. Age (months):
    6. Residence (village/Estate):
    7. Drinking Water source: Tap (0) Other(specify)(l) ( )
    8. Age of mother: <18 yrs (0), 19-30 yrs **(f),** >30 yrs (2) ( )
    9. Primary caretaker: Mother (0), Other (1) ( )
    10. (e.g. Maid, auntie, sibling, etc)
    11. Education of caretaker: Nil (0), 1-8 yrs (1), 9-12 yrs (2), >12yrs (3) ( )
    12. Monthly Income KSh.: < 1,000 (0), 1,000-5,000 (1), 5,000-10,000 (2), >10,000 (3)

              ( )

    13. Characteristics of stool:

        a) Colour: Green (1) Yellow (2) White (3) Other-specify (4) ( )

        b) Mucoid: Yes (0) No(1)
        c) Watery: Yes (0) No(1)
        d) Blood stained: Yes (0) No (1)
        e) Duration: <2weeks(0) >2 weeks (1)
           ( )
        f) Frequency of stool per 24 hrs.

9. Other associated symptoms: present (1)   absent (0)
              •   Vomiting: ( )
              •   Fever: ( )
              •   Dehydration: ( )
              •   Weight loss: ( )
              •   Abdominal pain: ( )
              •   Others (specify): ( )

10. Other household members with diarrhoea: Yes (1) No (0) ( )

CLINICAL EVALUATION

1. Weight (Kgs):

2. Weight for Age (%):

3. Nutritional Status:
   (See guide below)

4. Temperature (°C):

Wellcome Classification of Nutritional Status

* » -

0: Normal (W/A >80%)

1: Underweight (W/A 60-80%)

2: Kwashiokor (W/A 60-80% + edema)

3: Marasmus (W/A <60%)

/   / •

4: Marasmic Kwashiokor (W/A <60% + edema)

LABARATORY DATA   -

1. *C. parvum* present: Yes(1) No(0)   ( )

2. HIV Test: (Positive (1) Negative (0)   ()

3. Other Parasites seen: Present (1) Absent (0)

a. Ascaris	( )
b. Taenia	( )
c. Hookworm	( )
d. Isospora	( )
e. Giardia	( )
f. Cyclospora	( )
g. Others(specify)	( )

Appendix IV

Safranin methylene blue staining technique (31)

1. Prepare a thin smear of fresh formalin fixed stool on a slide

2. Air dry smear. Briefly pass slide,smear uppermost, once through the flame of a spirit

   lamp or Bunsen burner

3. Fix the smear in 3% v/v hydrochloric acid in absolute methanol for 3-5 minutes

4. Wash with clean tap water

5. Stain the smear with hot 1% w/v aqueous safranin solution for one minute

6. Wash off the stain with clean tap water

7. Counterstain with 1% w/v methylene blue for 30 seconds (other counterstains do not

   give as reliable results as methylene blue).

8. Wash with water, wipe the back of the slide, clean and place it in a draining rack for

   smear to dry

9. Examine the smear microscopically for oocysts under oil immersion (xlOO)

   - Look for small round to oval orange to pink bodies measuring about 5(im in

     diameter.

   - The sporozoites within the oocyst stain slightly darker and are sometimes

     arranged around the periphery

   - Yeasts, fecal debri and cysts of other protozoa stain blue. Bacterial spores stain

     red bit they are generally smaller and do not have the same appearance as

     C. parvum oocysts, and the red staining sporocysts of *Sarcocystis hominis*

     are much larger

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Appendix V

Formol ether concentration technique (32)

1. Using a rod or stick, emulsify an estimated 1 gram of faeces in about 4mls of 10% formol water contained in a screw cap bottle or tube.

2. Add a further 3-4 mis of 10% v/v formol water, cap the bottle, and mix by shaking for about 20 seconds.

3. Sieve the emulsified faeces, collecting the sieved suspension in a beaker.

4. Transfer the suspension to a conical (centrifuge) tube made of strong glass or polypropylene. Add an equal volume of ether, i.e. 3-4 mis.

5. Stopper the tube and mix for 1 minute. If using a vortex mixer, leave the tube unstoppered and mix for about 15 seconds.

6. With a tissue or piece of cloth wrapped round the top of the tube, loosen the stopper. Centrifuge immediately at 750-1000 g (approx. 3000 rpm) for 1 minute>

7. Using a stick on the stem of a plastic bulb pipette, loosen the layers of faecal debris from the side of the tube and rapidly invert the tube to discard the ether, fecal debris and formol water. The sediment will remain.

8. Return the tube to its upright position and allow the fluid from the side to drain to the bottom of the tube.

9. Mix the sediment using a plastic bulb pipette or Pasteur pipette. Transfer all the sediment to a slide, and cover with a covet glass.

10. Examine microscopically the entire preparation using lOx objective with the condenser iris closed sufficiently Jo give good contrast. Use the 40x objective to identify the small cysts and eggs. If cysts are present, run a small drop of iodine under the cover glass to confirm their identity.

Consent Form

I, Dr. P. Mandi of the Dept. of Paediatrics, University of Nairobi, am conducting a study to find out the magnitude of *C. parvum*, in children admitted to Kenyatta National Hospital with diarrhoea and aged 6 months to 5 years. *C. parvum* is a parasite found in stool and is an important cause of diarrhoea in children. I am also studying its relation with other conditions that your child may or may not have such as fever, state of nutrition, HIV status, age, and factors in the child's environment such as area of residence, type of water used and the caretakers of the child. When compiled together the results of the study will be useful in planning interventional measures against *C. parvum.*

All answers to questions asked will be filled in a questionnaire. The child will then be examined. A stool sample in a container and a blood sample from a needle prick on the finger, which may cause slight temporary discomfort, for laboratory testing will be required.

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I am requesting for permission to collect the above samples. If you would not want to know the results of any/either of the tests, I will remove the identifying labels on the sample in your presence and they (stool and/or blood) will be tested as anonymous.

The information you give and results obtained shall be treated with strict confidence and used only for the study. Any useful results for his/her treatment shall be communicated to the attending doctors

You may opt not to participate in the study and the treatment of your child will not be altered with in any way.

Do you wish to be part of the study?

Name & Signature (Parent/Guardian)_____child's name

Name of witness_____Signature
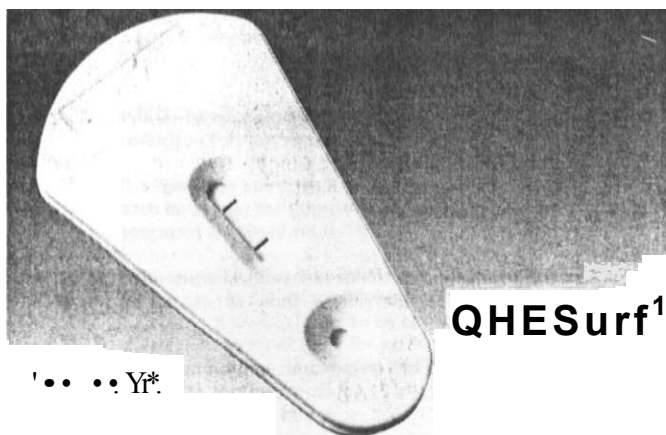
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QHESurf[1]

Intended Use   rw*r?r-   I —   in   umm

The Trinity Biotech Uni Gold'" HIV test is a single reagent assay for the detection of antibodies to human immunodeficiency virus types 1 and 2 in serum, plasma or wholeblood

Summary and Explanation dEfflfTlWW^""

Human Immunodeficiency Virus (HIV> has been recognised as the etiological agent of the acguired immunodeficiency syndrome (AIDS)

The Trinity Biotech Uni-Gold.M HIV test is a rapid immunoassay based on the immunochromatographic sandwich principle.

Principles of the Procedu

Recombinant proteins representing the immunodominant regions of the envelope proteins of HIV 1 and HIV 2, glycoprotein gp41, gp120 (HIV 1) and glycoprotein gp36 (HIV 2) respectively are immobilised at the test region of the nitrocellulose strip These proteins are also linked to colloidal gold and impregnated below the test region of the device. A narrow band of the nitrocellulose membrane is also sensitised as a control region.

During testing two drops of serum, plasma or whole blood is applied to the sample port, followed by two drops of wash buffer and allowed to react. Antibodies of any immunoglobulin class, specific to the recombinant HIV 1 or HIV-2 proteins, will react with the colloidal gold linked antigens. The antibody protein colloidal gold complex moves chromatographically along the membrane to the test and control regions of the test device.

A positive reaction is visualised by a pink/red band in the test region of the device.   ,

A negative reaction occurs in the absence of human immunoglobulin antibodies to HIV in the analysed specimen. Conseguently no visually detectable band develops in the test region of the device.

Excess conjugate forms a second pink/red band in the control region of the device. The appearance of this band indicates proper performance of the reagents in the kit.

Kit Contents   ~~   r-
a) 20 Test Devices
Each test device contains colloidal gold labelled with recombinant HIV proteins, recombinant HIV proteins as test zone, and a control line.

b) Wash Reagent (2 ml)

Single reagent for whole blood, serum or plasma.

c) 20 Disposable Pipettes

d) Package Insert

Materials required but not provided.
* Timer or stopwatch
* Rlood collection devices (i.e., lancets, capillary tubes/ test tubes)

The Trinity Rioterh Uni Gold.M HIV test device and wash solution ran he stored at 2 7l C.

No kit components should he used after the kit expiry date.

- The Trinity Biotech Uni Gold.M HIV test is for professional use only.
- The package insert instructions must be followed to ensure optimum test performance
- The Trinity Biotech Uni Gold'" HIV test is intended for in vitro use.
- Controls where supplied have been certified virus free. As with all screening assays, any results should be considered presumptive until confirmatory assays have been preformed according to local practice or WHO guidelines.

Safety Precautions
- Standard precautions for handling infectious agents should be observed whpn using this kit.
- Wear protective clothing such as lab coat and disposable gloves when handling specimens and assay reagents.
- Wash hands thoroughly after use.
- In rase of contact with eyes, rinse immediately with plenty of water and seek medical advice

Appropriate biosafety practices should be used when handling specimens and reagents These precautions include, but are not limited to the following

- Do not smoke, eat, drink, apply cosmetics or handle contacts lenses in areas in which specimens are handled.
- Dispose of all specimens, used devices and pipettes as though they are capable of transmitting infection. The preferred methods of disposal are by autoclaving at 12TC for a minimum of 60 minutes or by incineration.
- When disposing of wash buffer, avoid contact with acid to prevent liberation of a toxic gas.
- All spills should be wiped thoroughly using a suitable disinfectant such as a sodium hypochlorite solution.
- Use a separate disposable pipette and device for each specimen tested
- Do not pipette by mouth.
- In a small number of cases it has been noted that the control or the test line may appear "broken ". While this does not effect the test result it is recommended that the testing of that sample is repeated on another Uni-Gold'M HIV test device.

Handling Procedures
Do not use any device if the pouches have been perforated.

Each device is for single use only.

Do not mix reagents from different kits.

Do not use the kit past the expiration date

Whole blood, serum or plasma may be used.

Whole Blood: If fingerstick whole blood is used, drops of blood produced should be taken up from the finger tip by the pipette supplied and dropped from the pipette onto the device. Blood droplets should not be dropped directly from the fingertip onto the device as their size may vary.

Whole blood specimens should be used within ten minutes of collection for optimum performance.

If a specimen has started to dot, do not remix before testing. In such instances, the clear serum should be pipetted off the clotted specimen and used for analysis.

If an anticoagulant has been used in the blood sample, whole blood can be used directly on the device using the pipette supplied. If testing is not to be carried out immediately, samples should be stored at 2 8'C for up to three days, or preferably, the sample should be centrifuged and the plasma retained for future testing.

Serum or Plasma:
Serum or plasma may be kept for seven days at 2 8°C. Samples should be frozen for longer storage. Avoid repeated freezing and thawing of samples.

Quality Contro

Good Laboratory Practice necessitates the use of control specimens to ensure proper device performance at least once daily.

A built in procedural control on the test device indicates that the test is functioning correctly. A pink/red band should alwavs appear at the control window.

Please note certain commerical controls designed for ELISA may not preform properly with the Trinity Biotech Uni Gold'" kit. For further information please contact Trinity Biotech.

1. If any reagent/sample has been in refrigerated storage, remove and allow to stand for at least 20 minutes to reach room temperature.

2. Remove the required number of Trinity Biotech Uni Gold·ᴹ HIV test devices from their protective wrappers

3. Label each test with the appropriate patient information.

4. Using one of the disposable pipettes supplied, fill with sample (serum/plasma/whole blood).

5. Holding the pipette over the sample port add two drops of sample (approx. 60 pi) carefully.

6 Add 2 drops (approx. 60 pi) of the wash reaqent to sample port.

7. Allow 10 minutes from the time of wash reagent addition for reaction to occur. The result should be read immediately after the end of the 10 minute incubation time but is stable for a further 10 minutes after the incubation time. Do not read results after 20 minutes following'Sample additon.

10.   **Interpretation of Test Results**   Rim1EP?

NEGATIVE
A iine in the control region only indicates a negative test result

POSITIVE
A line of any intensity forming in the test region, plus a lino forming.fn the control region, indicates a positive result.

INCONCLUSIVE
No line appears in the control region. The test should be repeated with a fresh device. Irrespective of a line developing in the test region.

The Trinity Biotech Uni-Gold·ᴹ HIV test procedure and interpretation of results must be followed closely when testing for the presence of HIV antibodies In serum, plasma or whole blood.

The Trinity Biotech Uni Gold·ᴹ HIV test is intended for the testing of undiluted samples only. Samples should not be diluted before testing.

Immunosuppressed or Immunocompromised individuals Infected with HIV 1 or HIV 2 may not produce antibodies to the virus. Testing with any kit designed to detect antibodies may give negative results and would not be a reliable test method for such patients.

Infants may receive antibodies from an infected mother or they may not produce antibodies in response to an infection, therefore, it is necessary to exercise great care In interpreting their results.

A negative result with Uni Gold'" HIV does not exclude the possibility of infection with HIV. A false negative result can occur in the following circumstances:
   low levels of antibody (e.g., early seroconversion specimens) are below the detection limit of the test
- infection with a variant of the virus that is less detectable by the Uni Gold'" HIV assay configuration
- HIV antibodies in the patient that do not react with specific antigens utilized in the assay configuration
   specimen handling conditions which result in the loss of HIV antibody multivalency

Performance

Uni Gold^HIV has been evaluated by a number of Independent organizations.

Evaluation Performed by	Sensitivity	Specificity	NPV	PPV	Test Efficiency
Caribbean Epidexuiology Centre* CAREC/PAHO/ WHO	100%	99.70%	99%	100%	99.40%
WHO Evaluation '(Phase 1)" (Draft report)	100%	100%	100%	100%	N/A

* 4/1 sera including 102 HIV positive and 369 HIV negative sera which were in the main collected from antenatal clinics. Along with 100 stored plasma samples collected from confirmed HIV women.
**250 whole blood specimes were evaluated compared to the reference test.

13.   **References**

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Catibbean Epidemiology Centre
Evaluation of Three Rapid HIV Assays.

WHO Evaluation
Evaluation (Phase 1) of the Uni-Gold'"HIV using Whole Blood Specimens.

tetermine
IIV-1/2

>is package insert must be read carefully prior to use Package Inserl Instructions must be lol1o*ed accordingly Reliability assay results cannot be guaranteed H there are deviations from the instructions m this package insert

ME AND INTENDED USE

i Abbott Determine'" HIV 1/2 is an *In Vitro*, visually read, guatitative immunoassay for the detection of antibodies to HIV nd HIV 2 in human serum, plasma or whole blood The lest is Intended as an aid to detect antibodies to HIV-1/HIV 2 liom tried Individuals

MMARY AND EXPLANATION OF THE TEST

>S (Acquired Immunodeficiency Syndrome) is characterized by changes In the population of T-ce»l lymphocyies In an cted Individual, the virus causes depletion of helper T cells. which loaves the person susceptible to opportunistic Elections I some malignancies The virus that causes AIDS eilsts as two related types known as HIV t and HIV 2 The presence of AIDS virus elicits the production of specific antibodies to either HIV 1 or HIV-2

ILOGICAL PRINCIPLES OF THE PROCEDURE

ermine HIV 1/2 1s an immunochromalographic lest for the qualitative detection of antibodies to HIV-1 and HIV-2

nple Is addod to the sample pad As the sample migrates through the conjugate pad. H reconstitutes and mues with the >nlum colloid antigen con)ugale This mliture conlinues to migrate thro«gh the solid phase lo the Immobilized recombinant gens and synthetic peptides at the patient window site

ntibodies to HIV 1 and/or HIV 2 are present in the sample, the antibodies bind to the antigen selenium colloJd and lo the gen at the patient window, forming a red line at the patient window site

ntibodios lo HIV 1 and/or HIV-2 are absent, the antigen selenium colloid flows past the patient window and nn red line Is aed at H·Ml patient window site

nsure assay validity a procedural control bar Is Incorporated In the assay device

NTENTS

•otl Determine HIV 1/2 Serum/Plasma Assay (List No 7023 13), 100 Tasts

Determine HIV-1/2 Test Card. 10 cards (10 tests/card), HIV-1/2 recombinant antigen and synthetic peptide coated

oft Determine HIV-1/2 Whole Blood Assay (List No 7023-33), 100 Ttts

Determine HIV 1/2 Test Card. 10 cards (10 tests/card). HIV 1/2 recombinant antigen and synthetic peptide coated

1 Bottle (2.5 ml) Chase Puffer (List No 7D22-/TJ prepared in phosphate buffer Preservatives Antimicrobial Agents

:ESSORIES (required but not provided)

		Serum/Plasma or Whole Blood		
		(venipuncture assay)	Whole Blood (fingerstick assay)	
Pipette		No 7022 51	Lancets*	No 7D22 31
Pipette Tips		No 7022 61	EDTA Capillary Ttibes	No 7D22 21

'Not available in European Union countries

HNINGS ANO PRECAUTIONS

In Vitro Diagnostic U M.

ITION:

ropriate blosafety practices" should be uaed when handling specimens and reagents These precautions Include, but •Kit limited to the following:

- Wear gloves
- Do not plpette by mouth
- Do not eat. drink, smoke, apply cosmetics, or hnndle contact lenses In areas where these materials are handled
- Clean and disinfect all spills of specimens or reagents using a suitable disinfectant, such as 0 5% sodium hypochlo-rite"
- Decontaminate and dispose of all specimens, reagents, and other potentially contaminated materials In accordance with local regulations ''

RAGE

Abbott Determine HIV 1? Test Cards and Chase Buffer must be stored at 2-30 C until expiration dale

omponents are stable until evpiration dale when handtod and stored as directed Do not use kit components beyond ation date

CIMEN COLLECTION

im. Plasma, and Whole Blood Collection by Venipuncture

an sorum. plasma, and whole blood collected by venipuncture should be collected asoplieaMy in such a way as lo avoid olysls

E: For whole blood and plasma specimens. EDTA collection tubes must be used,

le Blood Collection by Fingerstick""

'e collecting a fingorstick specimen, place an EDTA capillary tube on a clean dry surlace
Choose the fingertip of the middle, ting, or Index fingor (whichever Is the least callused) lor adults and i needed with a warm, moist towel or warm water lo Increase

llean lingertip with alcohol, allow lo air dry PosWon the hand palm side up

Jse a new lancet for each person Place the lancet off center on the fingertip rimrly press the lancet against ^e finger and puncture the skin Dispose of the lancet In an appropriate biohazard sharps container

Vipe away the first drop of blond with a sterile gauze pad

)TA Capillary Tubes (No 7D22 21) will be used, fin the tube with blood between the 2 marked lines

:IMEN STORAGE

enjm and plasma specimens should be stored at 2 8°C If the test is to be run within 7 days of collection If testing is delayed •ore than 7 days. Ihe specimen should be frozen (20°C or colder)

/hole blood collected by venipuncture should be stored at 2-8'C If the test is lo be run within 7 days of collection Do not eeze whole blood specimens

/hole Mood collected by fingerstick should be tested immediately

PROCEDURE

lesired number of test units from the 10-test card can be removed by bending and tearing at the perforation

!: Removal of the test units should start from the right side of the test card to preserve the lot number which appears
• left side of the test card.

emove the protective foH cover from each test

ur serum or plasma samples

) Apply 50 pL of sample (precision pipotte) to the sample pad (marked by the arrow symbol)
> Wait a minimum of 15 minutes (up to 60 mlnules) and read result

3r whole blood (venipuncture) samples

i Apply 50 pL of sample (precision pipotte) lo the sample pad (marked by the arrow symbol)
i Walt one minute, then apply one drop of Chase Buffer lo Ihe sample pad
Walt a minimum of 15 minutes (up to 60 minutes) and read result

>r whole blood (fingerstick) samples

i Apply 50 pL ol sample (by EDTA capHlary tube) to Ihe sample pad (marked by the arrow symbol)
Walt until blood Is absorbed Into the sample pad. then apply one drop of Chase Buffer to the sample pad
Walt a minimum of 15 minutes (up to 60 minutes) and read result

rry CONTROL

ure assay validity, a procedural control is incorporated in the device and Is labeled Control" It the control bar does not KJ by assay completion, the test result Is Invalid and the sample should be retested
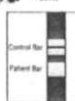
IPRETATION OF RESULTS*

TVE (Two Bars)

ars appear In both the control window (labeled 'Control") and Ihe patient window KJ "Patient") of the strip Any visible red color In the patient window should be inter l as positive

TIVE (One Bar)

id bar appears in tt»e control window of the strip (labeled "Control"), and no red bar rs In the patient window of the strip (labeled "Patient")

ID (No Bar)

Is no red bar in the control window of the slrip. and even If a red bar appears m fhe window of the strip the result is invalid and should be repeated If the problem s. contact your local Abbott Customer Servic^yind Support Center

I: •
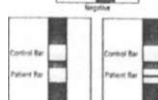
(»lest resufl Is positive even Il the patient bar appears tighter or darker than the control

m invalid lest resuft occurs repeatedty. or lor technical assistance, contact your local bott Customer Service and Support Center

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LIMITATIONS OF THE PROCEDURF

- The Abbott Determine HIV   1/2 test I« designed to detect antibodies to HIV   I and HIV   2 In human sonjm. plasma, and whole bk»od Other body fluids or poo'ed 'pncirnens may not Qtve accurate results
- The Intensity of the patient bar does not ne^essanly correlate to the titer of antibrxty In the specimen
- A negative rosuH with Determine HIV does not eicfcide the possibly of infection with HIV A lake negative result can occur In the following cirr unistances

low levels o« antibody (e Q early seroconversion specimens! are below the detection limit ol the lest
infection with a variant of the virus thai is less detectable by the Determine HIV assay configuration
HIV antHmrtins in »lie patient lii.it   net r« »rl *ifh "|M>r tftr antigens utilized in the assay rnnftquratinn
specimen handling rondilions whu.h result in loss of HIV antibody rniittivalenry

For these reasons care should be taken in interpreting n*aai've results Other riuural data ie q symptoms or risk factors) Should be used in conjunction with the test results

Hills should be evaluated In light ol the overall

- Whole Wood or plasma specimens containing anticoagulants other than f DTA may i orrecl results

PERFORMANCE CHARACTERISTICS

SPECIFICITY

A to'al ol 1.594 serum and plasma specimens Irom Asia. West Afnca and North America wen tested by Abbott Determine HIV 1/2 and a commercially available test (Table I)

Table I
Specilicity ol Abbott Oetermine II1V1/2

	Number of Specimens Tested	Negative by Abbott Determine HIV-1/2	Negative by a Commeicially Available Test"
Seionegalive			
	908	907*908 (99 89'.)	908 908 (100 00%)
Plasm*	403	403/403 (100 00%l	403403 (100 00%)
Pregnant Females	58 '	57/57 (100 00*.)	57/57 (100 00%)
west Aincans	49	4 ft/49 (97 96'.)	48/49 (97 96%)
Disease Slate*: Other than HIV and Potentially Interfering Substances	1 76 •	173/175 (98 Bfi-.I	174/175 (99 45'.)
ratal	1.594 ••	1.568/1.592 (99 75".)	1.59a 1.59? (99 87%)

One specimen from a pregnant female and sn HCV positive patient were positive by both Abbott Determine and the commercially available test Both specimens confirmed positive by HIV 1 Western Blot
'* 456 specimens were Irom North America 1089 specimens were trom Asia and 40 specimens were from Alrica
••* The reference method of a commercially available tost is particle agglutmatmn

A Total ol 3663 seronegative serum and plasma specimens from North America Asia, and Africa *ere tested by Abbott Determine HIV 1/2 and commercially available tosls (Table II) The speomoes Irom No»th America. Asia, and 49 of 2116 specimens from Africa (referred to as West Africans m Table I) were included in TaNe I Discordant specimens were confirmed negative by either Western Not or HIV t PCR assays

Table II
A Comparison ol Abbott Determine HIV 1/2 Specificity by Geographic Area

	Number of Specimens Tested	Negative by Abbott Determine HIV 1/2	Negative by Commercially Available Tests'
North Amen A 511	1089*	451/454 (99 34 ,)	453/454 (99 78'.)
		1089 1089 (100 00'.)	1080 1089 (10000%)
Africa	21 IB	2079/2118 (98 16'«|	2100'2118 <99 15*.)

ally available tosts are particle agglutination enzyme immunoassay and chemilu

A total of 368 seronegative whole blood specimens Irom Thailand were tested with paired serum and plasma by Abbott Determine HIV 1/2 Thirty nine of ttie whole Wood %pjrirnens were collected by both venipuncture and fingerstick (Table III)

Table HI
A Comparison of Abbott Determine HIV-1/2 Specificity In Seronegative Whole Blood and Paired Serum and Plasma Specimens

Specimen Type	Number of Specimens Tested	Negative by Abbott Determine HIV 1/2
Setum	368	308 368 (10000%)
Plasma	368	368 368 (100 00·.)
Whole Blood (venipuncture)	368	368/368 (100 00%)
Whole Blood (tingerslicM	39	39.39 (100 00%)

SENSITIVITY

A total ol 869 HIV t and HIV 2 antitbdy positive serum and plasma specimens trom Asia Alnca. Noith and South America were teslod by Abbott Determine HIV 1/2 and a commercially available lesl (Table IV)

Table IV
Sensitivity ol Abbott Determine HIV 1/?

Population	Number of Specimens Tested	Positive by Abbott Determine HIV-172	Positive by • Commercially Available Teal"
HIV 1 Positive	521 •	521/521 (10000%)	521/521 (100 00-.)
HIV 2 Positive	114*	I14'114 (100 00 .)	114'114 (100 00*.1
HIV 1 Subtypes A G	222	222/222 (10000%l	Not Tested Not Tested
HIV-1 Group O	12	12/12 (100 00%)	Not rested Not tested
Total	869	869/869 (100 00*.)	635/635 (100 001.)

228 specimens were from North America 29f. specimens were from Asia and 111 specimens were f»om Africa
The reference method of a commercially available test is particle agglutination

A Total of 1653 seropositrve serum and plasma specimens from North America. Asia and Africa were tested by Abbott Determine HIV-1/2 and commercially available tests (TaNe V) The specimons Irom North America. Asia, and 111 of 1129 specimens from Africa (referred to as HIV 2 Positive m Table IV) were included in Table IV Discordant specimens were confirmed HIV-1 positive by either Western Not or HIV 1 PCR assays

Table V
A Comparison of Abbott Determine HIV-1/2 Sensitivity by Geographic Area

	Number of Specimens Tested		Positive by Commercially Available Tests"
North America	228	228-220 (100 00*.)	228 228 (100 00--)
Asia	296	296/296 (10O00%1	296/296 (100 00%)
Alrica	1129	1128 /1129 (99 91%)	1129/1129 (100 00 .)

One negative specimen by Determine HIV-1/2 conlirmed positive by HIV 1 PCR
** The relerence methods of commercially available tests are particle agglutination enzyme immunoassay and chemWu-minescent immunoassay

A total of 102 seropositive whole blood specimens from lhailand wore tested with paired serum and plasma by Abbott Determine HIV 1/2 Thirty two of the whole Nood specimens were collected by both venipuncture and fingerstick (Table VI)

Table VI
A Comparison of Abbotl Determine HIV-1/2 Sensitivity In Seropositive Whole Blood and Paired Serum and Plasma Specimens

	Number ol Specimens Tested	Positive by Abbotl Determine HIV-1/2
Sorum	102	102/102 (100 00'".)
Plasma	102	102/102 (100 00-.)
Whole Blood (venipunrlurel	102	102/102 (100 00%)
Whole Blood (fingerstick)	32	32/32 (100 00%)

BIBLIOGRAPHY (See Back Page)