ADVANCES IN BIORESEARCH, Vol 1 [1] JUNE 2010: 34 - 40

Society of Education, India
http://www.soeagra.com
ISSN 0976-4585

RESEARCH ARTICLE

Diagnosis of Cryptosporidiosis in Africa: Prospects and Challenges

Okangba, C.C[1], Oyibo, W.A[1], *Obi, R.K[2], Nwanebu, F.C[2], Mgbemene, I.C[3], Ojuromi, T[4], Orji, N.M[5]

[1]WHO/TDR Malaria Specimen Bank Collection Site, Department of Medical Microbiology and Parasitology, College of Medicine, University of Lagos, Nigeria.

[2]Department of Microbiology, Federal University of Technology, Owerri, PMB 1526, Owerri, Imo State, Nigeria.

[3]Department of Biotechnology, Federal University of Technology, Owerri, PMB 1526, Owerri, Imo State, Nigeria.

[4]Department of Zoology, Lagos State University, Lagos, Nigeria

[5]Department of Biological Sciences, Anambra State University, Uli, P.O.Box 02, Uli, Anambra State, Nigeria

Email: robertobi_2003@yahoo.ca

ABSTRACT

Cryptosporidium parvum is an opportunistic parasite capable of causing gastrointestinal illness in both immunocompetent and immunocompromised patients as well as in children less than 2 years old particularly in developing countries. The parasite causes diarrhoea that is self-limiting in immunocompetent individuals and another that is potentially life-threatening in immunosuppressed individuals. Transmission of the parasite could be direct through faecal-oral route or indirect via contaminated water supply, food or environment. Methods of diagnosis of infection due to the parasite, involve use of polymerase chain reaction (PCR), immunological based detection methods, and microscopy. These diagnostic procedures in Africa are however confronted with several challenges ranging from constant lack of power supply and lack of experienced technologists, to poor funding from government for periodic trainings, seminars and workshops for laboratory workers as well as their technical complexity. Given the poor state of health care infrastructure in Africa, brain drain of skilled personnel, low allocation of resources to health care etc; the inclusion of immediate work-ups for the prompt diagnosis of Cryptosporidiosis is imperative if not urgent.

KEYWORDS: *Polymerase chain reaction, Microscopy, Cryptosporidium, Diarrhoea, Faecal oral.*

INTRODUCTION

Cryptosporidiosis is a diarrhoeal disease caused by parasites of the genus *Cryptosporidium*, which measure between 4 6µm in size [1,2]. Diarrhoea caused by parasites account for more than 3.1 million deaths each year among children less than 15years of age, mostly in developing countries [3]. Cryptosporidiosis causes chronic and life threatening diarrhoea among immunocompromised individuals as well as asymptomatic diarrhoea in immunocompetent individuals [4]. Infection by the parasite accounts for up to 6% of all diarrhoeal diseases in immunocompetent persons and presents in up to 24% of persons with both AIDS and diarrhoea worldwide [5].

Cryptosporidiosis is normally initiated by ingestion of *Cryptosporidium* oocysts from contaminated untreated drinking water, recreational water and contaminated food. Following ingestion, oocysts excyst in the gastrointestinal tract and release infective sporozoites, which attach to the apical membrane of the host epithelial cell. When the sporozoites mature, they undergo asexual reproduction to produce merozoites which are released into the intestinal lumen. The merozoites can either infect other epithelial cells or mature into gametocytes which are the sexual forms of the parasite. The gametocytes later release the oocysts (infective stages) which are excreted via diarrhoeic stool into the environment to start another life cycle [6].

The major routes of transmission include drinking and recreational water (swimming pools). Other routes are through food handlers, animal handlers and day care centre attendants. Most instances of transmission occur by the feacal-oral route and several recreational outbreaks have been traced to individuals who had recently recovered

from *Cryptosporidium*- related diarrhoea and who were thought to be shedding oocysts at the time [7-8].
Clinically, cryptosporidiosis could result in life threatening diarrhoea, while the entire population could be seriously affected due to the ability of the oocyst to survive the usual water treatment procedures. Severe outbreaks affecting a larger population have also been reported and the tendency of animals to contribute to the spread of the parasite has also been confirmed [9]. This clearly shows the immense role accurate diagnosis could play both for patient management and safeguarding the population.
Diagnosis of cryptosporidiosis has progressed from histologic identification in intestinal biopsies to microscopic examination of faecal specimens for infective oocysts, enzyme immunoassay (EIA) for parasite antigens and nucleic acid amplification assay as well as use of molecular techniques such as Polymerase Chain Reaction (PCR) [10,11].
Given the poor state of health infrastructure in Africa therefore, brain drain of skilled personnel, low allocation of resources to health etc; the inclusion of immediate work-ups for the prompt diagnosis of cryptosporidiosis is imperative if not urgent. Furthermore as the hall mark of managing cryptosporidiosis lies on accurate diagnosis, (both for patient and environmental samples), it is important to elucidate on the available diagnostic methods and recommend for the inclusion of *Cryptosporidium* in routine testing for diarrhoeal pathogens.

DIAGNOSIS OF CRYPTOSPORIDIOSIS

There are different methods used in detecting *C. parvum* oocysts in clinical and environmental samples. These include microscopic techniques, polymerase chain reaction (PCR) and enzyme immunoassay methods [12-14].
Microscopic diagnosis involves the morphological identification of the parasite in any specimen. This depends totally on the skill, patience and experience of the microscopist. PCR is sensitive and has shown potential for accurate diagnosis in patients unaware of the cause of their diarrhoea since it is highly sensitive. The enzyme immunoassay methods are the simplest and least labour intensive of the available immunoassays and rapid tests that can be done by relatively unskilled operators with little training. Conventional microscopic method with acid fast stain was however found in a study to be a more reliable diagnostic tool than immunological methods because its affordability and ease of usage [15].

Microscopy

Historically, the use of electron or light microscope has been the method of choice to diagnose *Cryptosporidium species* oocysts. Failure to recognize *Cryptosporidium* oocyst as a cause of human disease until 1976 was, in large part, associated with the use of inadequate staining techniques to identify oocysts in faeces and environmental samples of human and animals. Microscopic analysis of stained faecal smears is however the most widely used method for screening stool samples for *Cryptosporidium* in clinical diagnostic laboratories [16].
Microscopy method involves concentration of the samples and staining to obtain accurate diagnosis. Today diagnosis is achieved by demonstrating the presence of oocysts, the environmentally resistant forms, in stool samples. *C. parvum* oocysts in infected persons can be identified by staining of faecal smear with an acid-fast stain (Ziehl-Neelsen). The oocysts measures 4-6µm and stain bright red with refractile round thick capsules when observed under high power light microscope. Acid fast stain technique is more widely used than other staining techniques such as Giemsa and Metheinamine silver stains [7,14]. Microscopy has several advantages including provision of a single procedure for the detection of *C. parvum,* inexpensive nature; absence of false negative results; freedom from inhibitors; and absence of technical complexity. The system is however not without challenges as is made manifest by its time consuming procedures, tediousness, requirement of experienced microscopists for parasite identification, lack of constant power supply especially in developing countries hampering use of light microscopes, and insensitive to bulk processing since the Technologist is required to spend a minimum of five minutes per slide irrespective of the number of samples to be screened [17,18].

Immunological-based detection methods

Immunofluorescence assay (IFA)

Considerable experience is often required with the concentration and staining methods to obtain an accurate diagnosis in microscopy method. For this reason, immunoflourescent assay (IFA) procedures, employing *Cryptosporidium* specific polyclonal or monoclonal antibodies was developed to aid in the identification of oocysts in stool and environmental samples [10].
Antibodies specific to *Cryptosporidium* have been detected, using IFA procedure, in a sera obtained from persons who recovered from confirmed infections. The IFA can either be direct or indirect. In direct IFA, the sample is fixed onto a microscope slide, then a drop of pathogen specific antibody labelled with a suitable fluorochrome e.g.

fluoescein isothiocyanate (FITC) is applied and incubated. If the antibody binds to antigen, the sample will fluoresce against a green background while any unbound conjugates would be rinsed off when washed with water; the slide is then examined under an epifluorescent microscope [10]. In the case of indirect IFA, the pathogen's specific-antibody is detected by a second anti-immunoglobulin antibody labelled with FITC. IFA technique is not required to distinguish between *C. parvum* oocyst and non-*C. parvum* oocysts, but all the reviewed, commercially available diagnostic tests will give a positive result for non-*C. parvum oocysts*. This calls for a need for better diagnostic techniques that would reliably differentiate *C. parvum* species. The direct IFA has proved to be highly specific and reasonably sensitive for human clinical samples.

IFA is important since it can be done in combination with membrane exclusion / permeability dyes to estimate oocysts viability. However important challenges facing the usefulness of this method in Africa is that it is time consuming, allows only few samples to be examined per day, and the necessity for an expert or experienced personnel which may not be available, to interpret the results [19,20].

Enzyme immunoassay (antigen detection)

Traditional method (microscopy) may be insufficient to demonstrate the presence of *Cryptosporidium* species oocysts. Fortunately, over the past decade, a variety of highly sensitive and specific enzyme immunoassays have been developed that detect the antigens of *Cryptosporidium* species [21]. These enzyme immunoassays have become widely accepted as methods for screening stool samples for the detection of *Cryptosporidium* and other parasitic organisms [22].

There are two commercially available enzyme immunoassays namely:

a. Prospect *Cryptosporidium*-micro plate assay
b. Color PAC *Cryptosporidium* rapid assay.

These two assays are used in detecting *C. parvum* found in stool specimens which is fixed in 10% formalin. The techniques combine the specimen antibody with the amplification of antibody-antigen interaction by enzyme catalysis, and therefore, can detect very small amounts of pathogen. Many assays are done in wells of microtitre plates in which the reactants are immobilized. Antigen in the sample may or may not be bound by specific antibody immobilized on the surface [23].

Specific anti-*Cryptosporidium* IgG and/or IgM was also detected by an enzyme-linked immunoabsorbent assay (ELISA), in the sera of 95% of patients with cryptosporidiosis at the time of medical presentation and in 100% of patients with cryptosporidiosis in 2 weeks of presentation [24]. Several serological surveys have reported that more than 50% of persons with no known infection may have anti-*Cryptosporidium* IgG, suggesting recent exposure to the parasite [25].ELISA can be more sensitive in formalin fixed samples.

Antigen based ELISA have several significant advantages over other methods currently used for diagnosis of *Cryptosporidium* oocysts including excellent sensitivity and specificity, use of 96 well plate formats which enhances their potential as large scale screening tools in epidemiological studies such as water borne outbreak situations [24], widely used and simple to perform with minimal labour. However the expensive nature of the enzyme immunoassay kits used for ELISA places them above the reach of many laboratories in developing countries and this may hamper their routine usage in diagnostic services in these parts of the world [26,27].

Polymerase chain reaction (PCR) based method

Genetic methods for detecting *Cryptosporidium* oocysts have recently been developed. The method identifies and amplifies *Cryptosporidium* nucleic acid using the PCR [28,29]. Oocysts could be detected by PCR in waste water, surface water, stool sample and drinking water; however the sensitivity of the PCR assay could be inhibited by uncharacterized components in the samples. A study carried out by Dixon *et al*, [30] however showed that flow cytometry, dot blot and magnetic antibody methods could improve the sensitivity of the PCR assay.

Several factors could complicate the PCR-based detection of *C. parvum* in stool samples; for example, standard fixation in 10% buffered formalin could reduce the sensitivity of PCR, particularly if fixation occurs over an extended period. Also, extended formalin fixation may alter the buoyancy of

C. parvum oocysts from frozen stool. Similarly the sensitivity of PCR could also be reduced, when *C. parvum* oocysts are examined in frozen stool, probably due to rupture of oocysts during thawing. A study carried out by Morgan *et al*., [31] showed that faecal constituents such as bilirubin, bile salts and complex polysaccharides could reduce PCR sensitivity and inhibit it even when they are present at low concentrations. To eliminate these problems one method for oocyst purification from stool was developed for routine use in research laboratories and it involves density gradient centrifugation of stools [32,33]. Direct use of PCR does not distinguish between live and dead

oocysts, since oocyst DNA is apparently preserved for at least a week after cell death. Viability of oocysts is determined by detecting and amplifying a viable sporozoite DNA fragment. These protocols are adequate to detect low numbers of viable oocysts in drinking water and environmental samples [34].

Morgan *et al.*, [31] in their contribution noted the importance of PCR in processing clinical as well as environmental specimens suspected to be contaminated with *Cryptosporidium* species. The workers posited that the developed PCR primers specific for *Cryptosporidium* and random amplified polymorphic DNA (RAPD) are simpler approaches for developing diagnostic primers, since most of the products generated by RAPD-PCR are frequently species specific. In a similar contribution Balatbat *et al.*, [35] developed a nested PCR assay for detection of *C. parvum* oocysts directly from stool specimens by using a nested primer. This test can detect few oocysts ranging from asymptomatic infections to monitoring response to therapy.

For clinical as well as environmental samples, PCR has the ability to detect pathogens reliably in addition to the important function of determining the numbers of pathogens. PCR is sensitive and has the potential for accurate diagnosis in patients who do not presently know the reason for their diarrhoea. This could have considerable advantage in the treatment of immunosuppressed individuals, allowing for early diagnosis before the onset of symptoms. PCR technique is rapid and accurately obtained results are easy to interpret [36]. Furthermore PCR test is capable of directly differentiating between human and animal derived genotypes of *Cryptosporidium* on the basis of size of the PCR product [6].

PCR technique cannot however be used for routine diagnosis of *Cryptosporidium* oocysts due to the following challenges including its technical complexity and interference of results by inhibitors, its expensive nature, and time-consuming procedures [37,38].

Concentration techniques for detection of oocysts in water

Flow cytometry method

Flow-cytometry is very sensitive in detecting *Cryptosporidium* oocysts in environmental samples [30]. Flow-cytometry with fluorescence activated cell sorter (FACS) method is used routinely in the U.K and Australia for detection of *Cryptosporidium spp* and *Giardia spp* in environmental samples . Flow cytometry is a laser-based instrument that analyzes particles in a liquid suspension on a particle-by-particle basis. It can differentiate and physically separate particles based on their size, internal complexity and fluorescence. Its advantages are varied amongst which are increased sensitivity, less labor intensivity, and less-time demanding processes. On the other hand, initial expense of the instrument and the level of flow cytometry expertise required are some of the challenges hampering the usage of this diagnostic procedure in African countries [39].

Immunomagnetic separation (IMS)

Immunomagnetic separation is used to separate oocysts following a filtration step. IMS concentrates *Cryptosporidium* oocysts by using a magnetic bead coated with an *anti-Cryptosporidium* antibody. Following elution, the sample is incubated with magnetic beads that bind the oocysts. The solution is inserted into a magnetic particle concentrator that binds the magnetic bead *Cryptosporidium* complex. After the supernatant is decanted, the beads are released from the magnet. Oocysts are dissociated from the magnetic particles using an acid wash, neutralized with base, and then discarded [40]. However, the efficiency of the IMS method could be decreased by high turbidity water samples while at the same time recognizing their efficacy in purifying pathogens from water samples [40].

Fluorescence activated cell sorter (FACS)

Cells stained with fluorescein isothiocyanate (FITC), antibody conjugate can be identified and separated from other components of a suspension in an instrument known as 'fluorescence activated cells sorter' (FACS). The process involves passage of the suspension through a laser beam with the cells being separated on the basis of fluorescence intensity. The sorted cells could then be examined microscopically. This technique is mainly used to identify *Cryptosporidium* oocysts in water samples.

In another studies carried out by Hoffman *et al.,* [41], with a variety of environmental samples showed that FACS detected almost three times more *Cryptosporidium*-positive samples than membrane immunofluorescence (94.1% versus 35.3%) respectively.

CHALLENGES OF CRYPTOSPORIDIOSIS DIAGNOSIS IN AFRICA

The current goal of the World Health Organization is to assist each country in Africa to develop her ability to provide laboratory diagnosis of diseases [42]. Many serious diseases in developing countries go undetected due to improper diagnosis, since they could not afford the type of standard diagnostic tests available in developed countries.

Consequently African nations have been called upon to prepare for a more proactive system in which cryptosporidiosis diagnosis would be included in routine tests. To achieve this aim, there is the need for African countries to train skilled public health workers, and nationalize regional laboratories for diagnosis and research communication systems [42].

(1) Capacity of the technologist: for the technologist to acquire enough experience, funds have to be made available for the attendance of seminars, workshops and training courses to equip them with new techniques in the diagnosis of parasites. Lack of this exposure could eventually lead to inefficiency of the technologists and loss of confidence in the services rendered. It is indeed a fact that African countries are confronted with the problems of not being compliant with advances in the field of diagnostic testing, thus necessitating the need for a constant upgrade of the curriculum in order to acquire the requisite advanced technical skills applicable to modern scientific research [39].

(2) Infrastructure: African countries are in addition, faced with the challenge of inadequate facilities and equipment for diagnosis of cryptosporidiosis. This makes research impracticable. The reason for this may not be unconnected with the expensive nature of diagnostic equipment such as a good microscope, which places them beyond the reach of the technologists. Furthermore, lack of good and conducive working environments are other factors contributing to the challenge of efficient and accurate diagnosis of Cryptosporidiosis in Africa [42].

(3) Inadequate and inefficient power supply common among African countries have contributed in no small way to the problem confronting reliable diagnosis of *C. parvum* infection in the continent. Inadequate power supply renders specimens and samples stored in laboratory fridges and freezers completely inadequate for future use [39].

(4) **Availability of materials such as kits, primers and reagents for diagnosis of cryptosporidiosis:** the use of kits and reagents enhances accurate diagnostic results. These kits are however not readily available in Africa due to their high cost and lack of knowledge to produce one. In many developing countries, the lack of proper equipment and kits makes it difficult to diagnose and treat patient's illness accurately [42].

(5) In addition to the points mentioned above, **technological development**, **poverty** and **lack of established reference laboratories** in Africa contributes in no small way to the problem of accurate diagnosis of cryptosporidiosis in the continent [43].

CONCLUSION

Government should invest in regular power supply so as to enable researchers and technologists carry-out proper diagnosis of cryptosporidiosis and other parasitic infections, which is hampered by absence and inadequate power supply. In addition, training and periodic re-training of microscopists and other laboratory staff involved in diagnosis of cryptosporidiosis should be encouraged since this would enable them interpret laboratory results and findings correctly. Furthermore there is the need for capacity building in developing countries. To this effect the governments should raise funds, create time and train experts in order to effectively address the problem facing diagnosis of cryptosporidiosis. Several additional elements should be put-in place to provide the wherewithal for effective and timely disease control and prevention efforts. Similarly, there is the need for establishment of reference laboratories that would distribute diagnostic tests and perform comprehensive surveillance for cryptosporidiosis. Finally, enhanced public education on simple health measures in developing countries should be instituted as adequate creation of awareness on the mode of transmission and severity of the disease could assist in reducing the incidence of cryptosporidiosis in Africa.

REFERENCES

[1] Morgan UML, Xiao RF, Lai AA, and Thompson RCA (1999). Variation within *Cryptosporidium*: towards a taxonomic revision of the genus. *Int. J. Parasitol*; **29**: 1733-1751.

[2] Robertson LJ, Hermansin L, Gjerde BK (2006). Occurence of *Cryptosporidium* oocyst *Giardia* cyst in sewage in Norway. *Appl Env Microbiol* **72**: 5297-5303

[3] Colford JS, Wade T, Wright C, Charles S, and Eisenberg J. (2005). A pilot randomized, controlled trial of an in-home drinking water intervention among HIV positive persons **3**(2):173-184.

[4] Paul R H. and Gordon. N. (2002).Epidemiology and clinical features of *Cryptosporidium* infection in immunocompromised patients . *J. Clin.Microbiol*. **15**:145-154

[5] Bialek AJ, Ives NJ, Gazzard BG and Easterbrook PJ (2002). The changing pattern of AIDS defining illness with the introduction of (HAART) in a London clinic. *Journal infection* **42**(2): 134 139.

[6] Morgan UR, Weber L, Xiao I, Sulaiman RC, Thompson WN, Lab A, Moore A, and Deplazes P (2002) Molecular

characterization of *Cryptosporidium* isolates obtained from human immunodeficiency virus-infected individuals living in Switzerland, Kenya, and the United States. *J. Clin. Microbiol*. **38**: 1180-1183.

[7] Xiao L, Bern J, Limor RH, Lol AA (2002). Identification of 5 types of *Cryptosporidium* parasite in children in Lima, Peru. *J. Infect. Dis*. **183**: 492-497.

[8] Karanis P, Kourent C, Smith H (2007). Waterborne transmission of Protozoan parasites: a world wide review of outbreaks and lessons learnt . *J Water health* **5**: 1-38

[9] Geurden T, Goma FY, Siwila J, Phiri IT, Mwanza AM, Gabriel S, Claerebout E, Vercruysse J (2006). Prevalence and Genotype of Cryptosporidium in three cattle husbandry systems in Zambia *Vet Parasitol* **5**:237-245

[10] Garcia LS and Bruckner DA (1998). *Diagnostic Medical Parasitology*, 3rd ed. *A.S.M Press* incomplete PROVIDE PAGE AND TOWN OF PUBLISHERS

[11] Phillip J.L, Rebecca C.L, Robinson M, Okhuysen PC, and Chappell L C (2008) Cryptosporidium muris in Texas cainin population. *Am J. Trop Med. Hyg* **78** (6): 917-921

[12] AL Braiken A.F, Amin A, Beeching N.J, Hommel M and Hart C.A (2003). Detection of *Cryptosporidium* amongst diarrhoeic and asymptomatic children in Jeddah, Saudi Arabia. *Annals of Tropical Medicine and Parasitology vol 97* No 5 505-510

[13] Coupe S, Sarfati C, Hamame S, Derouin F, 2005. Detection of *Cryptosporidium* and identification to the species level by nested PCR and restriction fragment length polymorphism. *J Clin Microbiol 43:* 10171023

[14] Pelayo L, Nunez F. A, Rojas L, Wilke H, Furuseth H.E, Mulder B, Cyerde B and Robertson L (2008). Molecular and Epidemiological investigation of cryptosporidiosis in Cuban children. *Annals of Tropical Medicine and Parasitology.* **102** (8): 659-669.

[15] Tzipori S, and Ward H (2002). Cryptosporidiosis biology, pathogenesis and disease. *Microbes infect*; **4**:1047-1058.

[16] Tzipor S, Rolerton D (1986). Remission of diarrhoea due to Cryptosporidiosis in an Immunodeficient child treated with hyperimmune bovine colostrums. *B. Med. J:* **29**:1276-1277.

[17] Areeshi M, Dove W, Papaventsis D, Gatei W, Combe P, Grosjean P, Leatherbarrow H, and Hart C.A (2008). *Cryptosporidium speciecs* causing acute diarrhea in children in Antananarivo, Madagascar. *Annals of Trop Medicine and Parasitology.* **102 (**4): 309-315

[18] Essid R, Mousli M, Aoun K, Rim A, Mellouli F, Fakher K, Francis D and Aida B (2008). Identification of Cryptosporidium species infecting Human in Tunisia. Am J Trop Med Hgy 79 (5) pp 702-705.

[19] Chappell CL, Okhuysen PC, 2002. Cryptosporidiosis. *Curr Opin Infect Dis 15:* 523527.

[20] Nair P, Mohammed J A, H erbert L, Jose F. F, Carlin L.G, Jiang Zhi-Dong, Jaime B, Francisco G, and Okhuysen C (2008). Epidemiology of cryptosporidiosis in North America Travelers to Mexico. *Am J Trop Med Hyg* **79** (2): 210-214.

[21] Shimizu SRJ, Deckelbaum II, Schmid S, Harlap MB and Spora DT (2000). *Cryptosporidium*, nutrition, and chronic diarrhoea in children. *Am. J. Dis. Child* **142**: 312-315.

[22] Pieniazek NJ, da Silva AJ, Moura IN, and Addiss DG (2002). New *Cryptosporidium* genotypes in HIV infected persons. *Emerg. Infect. Dis.***5**: 444-449.

[23] Marques FR, Cardoso LV, Cavasini CE, Almeida MC, Bassi NA, Almeida MT, Rossit AR, Machado RL, (2005). Performance an immunoenzymatic assay for *Cryptosporidium* diagnosis of fecal samples. *Braz J Infect Dis* **9***:* 35.

[24] Ungar L, Morgan UM, Fayer R, Thompson RCA and Lal AA (1988) *Cryptosporidium* systematic and implications for public health. *Parasitol Today* **16**: 287-292.

[25] Ungar S, Kunaruk N, Rai SK, and Watanabe M (1994). *Cryptosporidium* infection in HIV-seropositive and seronegative populations in Southern Thailand. *Southeast Asia J. Trop. Med. Public Health*. **29**:100-104.

[26] Warren KS, Swan RA, MorganRyan UM, (2003). *Cryptosporidium muris* infection in bilbies (*Macrotis lagotis*). *Aust Vet J* **81**: 739741.

[27] Miller WA, Gardner IA, Atwill ER, Leutenegger CM, Miller MA, Hedrick RP, Melli AC, Barnes NM, Conrad PA, (2006). Evaluation of methods for improved detection of *Cryptosporidium* spp. in mussels (*Mytilus californianus*). *J Microbiol Methods* **65***:* 367379.

[28] Johnson DW, Pieniazek NJ, Griffin DW, Misener L, and Rose JB (1995). Development of a PCR protocol for sensitive defection of *Cryptosporidium* oocysts in water samples. *Appl. Environ Microbol*. **61**:3849 3855.

[29] Abe N, Kimata I, Iseki M (2002). Comparative study of PCR-based *Cryptosporidium* discriminating techniques. *J Parasitol* **76** (10): 869-81

[30] Dixon B R, Fayer R, Parrington M, (2002) Cryptosporidiosis surveillance and water borne outbreaks in Europe. *Euro Surv*; **12**:611-711

[31] Morgan UM, Constance CC, Forbes DA, Thompson RC (1998). Differentiation between *C. parvum* using rDNA sequencing and direct PCR analysis. *J. Parasitol*; **36**:995-998

[32] Tzipori S, and Griffiths JK (1998). Natural history and biology of *Cryptosporidium parvum .Ave Parasitol*; **40**:6-36.

[33] de Otazu S, Caccio C, Williams KY, Won EK, Nace CW, Pieniazek NJ, and Eberhard ML (2004). Molecular characterization of a *Cryptosporidium* genotype identified in Lemurs. *Vet. Parasitol*. **111**: 297-307.

[34] Semenza JC, Nicholas G (2007). Cryptosporodiosis surveillance and waterborne outbreaks in *Europe Euro Surv*; **12**: 611-711
[35] Balatbal ABG, Jordan W, Tang YJ, Silva JJ (1996). Detection of *Cryptosporidium parvum* DNA in Human feces by nested PCR. *J Clin Microbiol*: **34**: 1767-1772.
[36] Keegan FM, Warhurst DC, McDonald V (2003). Detection and species identification of *Cryptosporidium* oocyst using a system based on PCR technique. *Parasitology* **109**: 19-22.
[37] Sulaiman I. M, Hira P. R, Zhou L, Al-Ali F. M, Al-Shelahi F.A, Shweiki H.M, Iqbal J, Khaid N, and Xiao L (2005). Unique endemicity of cryptosporidiosis in children in kuwait. *Journal of Clinical Microbiology,* **43**: 2805-2809.
[38] Gatei MH, Hayes EB, Matte TD, and Rose JB (2006). Large community out break in Milwaukee of *Cryptosporidium* infection transmitted through the public water supply *N. Engl J Med* **331**:161-167.
[39] WHO (2005).Report Geneva: WHO
[40] Fontaine KL and Guillot JA (2003).Threshold of detection of *Cryptosporidium* oocysts in human stool specimens:evidence for low sensitivity of current diagnostic methods. *J Clin Microbiol*, **29**:1323-7.
[41] Hoffman JK, Theodos C, Paris M, and Tzipori S (1997). The gamma interferon gene knock-out mouse: a highly sensitive model for evaluation of therapeutic agents against *Cryptosporidium parvum*. *J. Clin Microbiol*: **36**: 2503-2508.
[42] WHO (2004). Report Geneva: WHO
[43] Okpala ER, Nwabuisi KA and Okunji ET (2005). A survey of the incidence of intestinal parasites among government workers in Lagos, Nigeria. *W. Africa. Med. Journal.* **10**:148-157.