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Human Cryptosporidiosis: An insight into Epidemiology, Modern Diagnostic Tools and Recent Drug Discoveries

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Abstract

Cryptosporidiosis is an emerging food and water borne zoonotic disease, which is caused by genus *Cryptosporidium*. The first *Cryptosporidium* spp. was isolated from mice in 1907 and gained importance when it was found in an HIV positive patient. It usually causes self-limiting diarrhea in young children and immunocompetent patients. However, it may lead to chronic diarrhea with life threatening condition in immunocompromised patients. Other complications related to this transmittable infection may include respiratory problems, skin rashes and headache. HIV/AIDS patients are highly susceptible host for this parasite. *Cryptosporidium parvum* and *Cryptosporidium hominis* are the known pathogenic species, prevalent among humans and they are being transmitted through contaminated food and water. Usually, the diagnosis of *Cryptosporidium* spp. is dependent on microscopic technique in many countries, which has a low sensitivity and specificity leading to false positive results. However, for a step forward to successful epidemiological studies, advanced techniques (Serological and DNA-based) provide us the better ways of diagnosis with more sensitivity and specificity. Furthermore, no antiparasitic drug has found to be effective against *Cryptosporidium* spp. except Nitazoxanide which is FDA-approved and effective only when administered along with antiretroviral therapy. In this regard, present review summarizes the various epidemiological studies conducted around the globe along with modern diagnostic tools and the suitable treatment available now a days. This systemized review will help the scientists to better understand all the aspects of cryptosporidiosis at one platform which may help in designing surveillance studies through selection of sensitive diagnostic techniques. The new drugs mentioned in this review may also help to better control this parasite in humans, especially immunocompromised individuals.

Introduction

Cryptosporidium spp., an opportunistic intracellular protozoan parasite, belong to Phylum Apicomplexa and causes high economic losses in case of morbidity and mortality by affecting the gastrointestinal tract of sheep, goat, pig, cattle and human [1]. The first specie was isolated from gastric juice of mice, named as *Cryptosporidium muris,* in 1907 by Tyzzer [2]. Later, *Cryptosporidium* was also detected, from human stool sample in 1976 and became most emergent protozoa due to its zoonotic importance. Despite, *Cryptosporidium* spp. are host specific, there are 26 recognized species causing chronic diarrhea, dysentery and malabsorption in humans as well as in farm animals. Most prevalent species in humans are *C. parvum* and *C. hominis*. However, new species or new strains are going to be discovered due to rapid genetic recombination as i) two or more species are affecting the same host at the same time ii) it undergoes both sexual and asexual production leading to genetic variation [3].

Chances of infection; chronic diarrhea/dysentery, are more in immunocompromised patients like HIV patients as compared to Immunocompetent patients; who suffer with a short-term and self-limiting diarrhea [4,5]. In the past, *Cryptosporidium* was considered to be linked with childhood diarrhea, malnutrition. Situation became worse in 1993 when 4 million people got *Cryptosporidium* infection in USA and Milwaukee. Despite of the evidence, cryptosporidiosis was under-diagnosed, and the treatment was only symptomatic [6].

Nevertheless, new diagnostic tools and literature has improved the estimate of global burden. However, there are still gaps which need to be filled, particularly in the fields of epidemiological studies, diagnosis and proper cure for cryptosporidiosis. Moreover, there is a deficiency of well-organized and comprehensive reviews covering all the aspects of cryptosporidiosis i.e. disease outbreaks, modern diagnostic tools and therapeutic controls especially in HIV patients. Such detailed reviews are very important for the young parasitologists and researchers for better understanding the current scenarios regarding the disease epidemics, modern diagnostic tools and the latest drug discoveries. Considering all the above-mentioned prospects, following review was planned to summarize a detailed information about the disease burden of *Cryptosporidium* infection in many countries, modern diagnostic tools and the new drugs available against *Cryptosporidiosis.*

Methods

Literature Search Strategy and Selection Criteria

The main objective of writing this review was to summarize recent advancements in some aspects of cryptosporidiosis important for controlling the disease, including epidemiology and recent disease outbreaks in various countries, virulent factors, latest diagnostic techniques and recently available drugs to cure *Cryptosporidium* infection, particularly in HIV/AIDS patients. Doing so, a massive search was carried out on google scholar by providing different terms like "Cryptosporidiosis", "Disease outbreaks of *Cryptosporidium parvum*", "Epidemiological studies on *Cryptosporidium parvum*", "*Cryptosporidium* infection is HIV/AIDS patients", "*Cryptosporidium* infection in children", "diagnostic techniques in *Cryptosporidium* infection", "modern techniques for the identification of *Cryptosporidium parvum*", "DNA based techniques for the identification of *Cryptosporidium* in humans", "Mortality rate in immunocompromised patients due to *Cryptosporidium* spp.", "how to treat *Cryptosporidium* infection in humans", "latest drug discoveries against *Cryptosporidium* infection", "FDA approved drugs for cryptosporidiosis". A total of 65 citations were reviewed after screening according to the contents of this review.

Discussion

Epidemiology

Early epidemiological studies have revealed the prevalence of *Cryptosporidium* to be 1% in developed countries while 5-10% in poor countries [6]. However, results of recent studies, based on serological and molecular techniques, revealed that the previous studies have underestimated the frequency of *C. parvum* infection indicating the prevalence rate of 15-25% in diarrheal patients [6].

Disease burden

Numerous researches have been conducted on Cryptosporidiosis throughout the world due to its zoonotic and opportunistic behavior. Regarding the global disease burden published in 2010, highest prevalence rate of 3.5-35.8% has been documented in America, followed in order by Africa (2.6-21.3%), Europe (0.1-14.4%) and Asia (1.3-13.1%) [7]. However,

a brief history of current occurrence of cryptosporidiosis documented in various hosts along with various diagnostic techniques is listed in Table 1.

Transmission and Pathogenesis

C. parvum mostly transmit either by direct or indirect ways. Direct transmission occurs when humans drink contaminated water or food with infected fecal material or by direct touching the infected person. Chances of disease spread are higher in day care centers and schools, where children get exposed to the contaminated surface and mud while playing. Meanwhile, a person can also get infection with sexual practices. Moreover, indirect transmission may also occur by drinking or eating contaminated water and food, respectively. It is clear with evidence that *C. parvum* can easily be passed through water filtrations plants due to its very small size of one micron. Furthermore, there is no effect of chlorination on these parasites during water treatment. So, drinking water is a main source of its transmission [13,41,42].

Pathologically, *Cryptosporidium* cause inflammatory damage to the epithelium of small intestine, which leads to the malabsorption and increased intestinal secretions resulting in diarrhea, dysentery, vomiting and nausea which may last for more than seven days. Situation may lead to death due to sever and persistent diarrhea in very young children and immunocompromised/HIV patients. By entering the host, they attach with intestinal epithelium with the help of gp900 and gp60 (glycoproteins) and start proliferating with the help of micronemes and rhoptries, resulting in the formation of parasitophorous vacuoles where they undergo sexual and asexual reproduction. The whole situation is called cryptosporidiosis [42]. Some studies have shown that *Cryptosporidium* may also affect the respiratory tract and cause some respiratory disorders along with skin rashes and headache [4,7,43]. Several virulenec factors cause damage to the host cells are mentioned in Table 2 [1].

Associated risk factors

It is evident from various studies that many associated risk factors may likely to be linked with occurrence of cryptosporidiosis in humans. Some of them found associated with varied prevalence of cryptosporidiosis have been listed below:

Seasonal variations

Cryptosporidiosis has been found endemic throughout the year in various parts of the world, but infection is most likely to be happened in rainy season due to water contamination [44]. In a critical study, another aspect was highlighted when *Cryptosporidium* along with *Giardia* and *Entamoeba* showed clear seasonal variation. The prevalence was stable before rainy season but during the rainy season, an increased rate of infection was noticed. However, this analysis does not support the pattern in case of cattle host.

Age

As a result of cryptosporidiosis, different immune responses can be observed which are dependent on age variation of host. Cryptosporidiosis is found to be more prevalent in children of age less than 24 months as they have less developed immune system as compared to adults [11]. Some supporting results were obtained in a research conducted at a goat farm in Brazil. This study showed more prevalence of *Cryptosporidium* in juvenile (age less than 12 months) as compared to adults (age more than 12 months) as all 4.8% prevalence was observed in juvenile animals [45]. To observe the prevalence of cryptosporidiosis, a farm level study was performed which revealed the risk to be high in calves of age 8-21 weeks in comparison with 0-7 days aged calves. During the same year, similar results were found in buffalo calves which showed high risk of prevalence of infection in calves of age 1-15 days [46]. In another farm level study, PCR study disclosed cryptosporidiosis to be more prevalent in pre-weaned calves as compared to post weaned calves. In human, *Cryptosporidium* was comparatively more prevalent in children of age 7-15 days than children of 3-12 years.

Gender

Occurrence of cryptosporidiosis is higher in male population as compared to female population. However, it may vary due to the exposure level of males and females. In china, It has been observed that the prevalence of *Cryptosporidium* infection was 3 times higher in males (12.6%) as compared to those of females (4.4%) [47].

Socio-economics behavior

Socio-economic behavior of host directly affects the transmission of *Cryptosporidium*. *Cryptosporidium* is transmitted through ingestion of parasites which could be by means of waterborne, person to person and food borne whereas the middle one is occasional. The cryptosporidium mostly affects children with immunosuppression while suppression is more prominent in malnourished children as compared to children aged less than 6 months which are breastfed [48]. In a study, it was observed that *cryptosporidium* spp., were more prevalent in rural areas especially in the persons exposed to public toilets and infected with HIV [49]. In another investigation, samples from 1731 drug abuser rehabilitation patients were collected to record prevalence of *Cryptosporidium* spp., infection and the results showed 19.6% persons to be positive with infection.

Diagnostic Tools

- **Conventional Method**

Traditionally, *Cryptosporidium* oocysts detection in environment, food, water, tissue and fecal sample has greatly relied on microscopic examination. But when it comes to the identification of *Cryptosporidium*, morphological characters are few. Therefore, identification based on light microscopy is time consuming and unreliable. Several staining techniques are used to identify *Cryptosporidium* oocysts. The least simple stains include modified Ziehl–Neelsen, Kinyoun, dimethyl sulphoxide carbol fuchsin and safranin–methylene blue. However, samples containing less oocysts number can suffer low sensitivity and/or specificity. Moreover, negative staining methods are also available. For example, by using chemicals like malachite green and green perbromide, background of slide is stained leaving the oocysts unstained. These latter staining techniques are time consuming, laborious and require perfection. Moreover, none of the techniques is helpful to identify *Cryptosporidium* species. For the identification of *Cryptosporidium* oocysts, modified Ziehl-Neelsen staining and wet mount preparation methods are also commonly used [36]. Another method for the identification and detection of *Cryptosporidium* oocysts is flow cytometry. To identify *Cryptosporidium* in sewage samples and water, Vesey used a skytron Argos 100-5 instrument spiked with oocysts stained with an FITC-C-mAb. The outcome of this study is encouraging by means of flow cytometry because it detects as few as 1000 oocysts/L.

- **Immunological Assays**

For detection and identification, Immunological methods have several advantages over microscopic techniques. For instance, direct fluorescent antibody (DFA) and fluorescence microscopy assay use fluorescence in isothiocyanate-conjugated anti-Cryptosporidium monoclonal antibody (FITC-C-mAb), which identify epitopes on the surface of *Cryptosporidium* oocysts with high sensitivity (98.5–100%) and specificity (96–100%) for *Cryptosporidium* oocysts detection in environmental samples and fecal smears. FITC-C, commercially available monoclonal antibody, is used for enumerating and detecting *Cryptosporidium* oocysts routinely in environmental and fecal samples. DFA assays which are based on monoclonal antibody can differ in their sensitivity and specificity of diagnosis which depends on many factors including 'biophysics' detection system, antibody conjugated reporter (enzyme fluorochrome), *Cryptosporidium* antigen used to develop mAb and the avidity and subclass/class of the antibody. IgG monoclonal antibody seems to have better avidity to *Cryptosporidium* oocyst surface antigens as compared to IgM resulting in better recoveries with immuno-magnetic separation (IMS) within water samples of high turbidity. Both molecular interaction and fluorochrome selection are the critical aspects and are responsible for false positive results due to autofluorescence in emission and excitation spectra. Though different monoclonal antibodies have been developed but commercially existing mAbs cannot identify *Cryptosporidium* specifically. Monoclonal antibodies have been developed with lesser number of *Cryptosporidium parvum* oocysts [50].

In the last few years, identification and diagnostic trends have been greatly changed towards EIA (Enzyme immunoassays), IC (Immuno-chromography) and ELISA (Enzyme Linked Immunosorbent Assay) for the detection of *Cryptosporidium* oocyst antigens. Specificity of these assays is reported to be high (98–100%). Advantage of these assays is that they detect infections when oocysts are not being excreted in feces [51]. while limitation of some kits may be seen because they mainly depend upon visual inspection which enable subjective interpretation of test results.

Region	Animal species	Technique used for detection	Total samples	Positive samples	Prevalence (%)	Reference
Africa						
Nigeria	Human	PCR	500	222	44.4	[8]
Egypt	Human	Microscopy	1275	214	17	[9]
Kenya	Human	Microscopy	541	187	34.5	[10]
	Human	ELISA	1778	196	11	[11]
Zambia	Sheep & Goat	ELISA	250	107	42.8	[12]
Ghana	Human	Microscopy	320	63	19.7	[13]
Europe						
Brazil	Horse	Microscopy	71	25	35.21	[14]
Canada	Human	PCR	108	17	15.7	[15]
Jamaica	Human	PCR	35	25	71.45	[16]
Mexico	Sheep	Microscopy	37	12.8	34.3	[17]
Italy	Dogs	Microscopy	140	5	3.5	[18]
Spain	Sheep	Microscopy	583	344	59	[19]
New Zealand	Cattle	Microscopy	554	109	19.7	[20]
	Possum	Microscopy	39	5	12.8	[21]
	Rats	Microscopy	25	5	20	
	Cattle	Microscopy	1190	31	2.6	[22]
USA						
California	Pig	Microscopy	221	12	5.4	[23]
Colorado	Dog	PCR	129	9	7	[24]
Texas	Human	ELISA	196	279	70.2	[25]
Southeastern USA	Deer	Microscopy	360	32	8.8	[26]
Asia						
Bangladesh	Human	PCR	3646	1349	37	[27]
	Human	qPCR, ELISA Quick Check,	832	145	17.4	[5]
India	Bovine	Microscopy	455	26	5.71	[28]
	Buffalo	Microscopy	162	62	38.3	[29]
	Human	Microscopy, ELISA	637	97	15.23	[30]
Iraq	Human	Microscopy	600	203	19.17	[31]
	Equines	Microscopy	107	29	27.10	[32]
Jammu and Kashmir	Sheep	Microscopy	120	88	73.33	[33]
Nepal	Human	Microscopy	75	8	10.6	[34]
Pakistan (Lahore)	Cattle	Microscopy	250	68	27.2	[35]
East west Pakistan	Human	Microscopy	200	18	9	[36]
Thailand	Mussels	Microscopy	56	7	12.5	[37]
Australia						
New South Wales	Human	PCR	261	154	59	[38]
Sydney	Kangaroo	Microscopy	3557	239	6.5	[39]
Western Australia	Pig	Microscopy	646	39	6.03	[40]

Table 1: Worldwide Prevalence (%) of *Cryptosporidium* spp. with respect to diagnostic tools.

Virulence factors	Functions
Aminopeptidase, Serine proteases	Excystation
Glycoprotein; gp900, gp60, P30, Cp47, TRAP-C1, CpMIC1, P23	Adhesion, Invasion
Hemolysin H4	Membrane lysis
CpABC	Nutrient transport
CpATPase2	Biomembrane heavy metal transport
CpATPase3	Ion or phospholipid transport
HSP70/90	Stress protection
Cysteine protease	Cytokine modulation
Acetyl CoA synthetase	Fatty acid metabolism

Table 2: Some virulence factors of *Cryptosporidium* spp.

Furthermore, these assays like many other immunological approaches do not diagnose *Cryptosporidium* genotype or species. So far, no such immunological tool is present which makes a distinction among oocysts of dissimilar species. One effort has been made with the development of PCR-EIA which differentiates between *C. hominis* and *C. parvum*. In a recent study, results have shown the 100% specificity and 86.6% sensitivity of antigen ELISA for *Cryptosporidium* [52].

- **DNA Based Approaches**

Molecular techniques are available to find out species, genotypes and sub-genotypes of *Cryptosporidium* to differentiate among human and animal pathogens. Nucleic acid-based methods have been evaluated for species identification in animals and human from environmental, water and fecal samples. Some methods are based on in-situ hybridization of probes within oocysts to specific genetic loci, while most methods are based on specific amplification of loci from genomic DNA by PCR. These applications have led to understanding *Cryptosporidium* species regarding biology, ecology, epidemiology, systematic and population genetics for control and prevention of cryptosporidiosis in animals and humans. DNA profiles from human isolates showed two different genotypes, *C. hominis* (anthroponotic) and *C. parvum* zoonotic genotype. Analysis of tri-nucleotide sequence has shown twelve subtypes of *Cryptosporidium hominis* and seven subtypes of *Cryptosporidium parvum* [53].

FISH (Fluorescent In-situ Hybridization) utilizes probes (fluorescently-labelled oligonucleotides) by targeting RNA or DNA sequences. For identification of *Cryptosporidium,* most of FISH assays are employed on RNA hybridization rather DNA by making target on a small subunit of nuclear rRNA of variable region [50,54]. Because within cell, small subunit (SSU) has a high copy number considered as rich target. Some limitations with the use of FISH probes depend upon the viability of oocysts as SSU-rRNA decay during cell death making useless the FISH technique. Degradation varies with environmental conditions including RNase contamination, pH, salinity and temperature etc.

- **Advanced molecular assays**

Different methods are available regarding PCR and many of them have been applied to *Cryptosporidium* in the past. A variety of techniques have been evaluated, but currently, only those methods are needed which are easy to perform, universally applicable and effective in approach.

- **Fingerprinting**

Different techniques are available which permit genetic fingerprint to be developed for parasite sample. Such techniques based on genome(s) screening for distinction in organization and sequence. Positive approach of some methods is that no earlier genomic information is needed for a sample which is to be characterized. However, the disadvantage is that pooled organism's genetic fingerprinting show organism's population rather than individual, therefore, individual markers may not signify all population. However, these techniques have valuable applications. RAPD (Random Amplification of Polymorphic DNA) or AP-PCR (Arbitrarily Primed-Polymerase Chain Reaction) are developed, based on DNA fragments amplification usually using single primers (10-mer) following separation of amplicons by polyacrylamide or agarose gel electrophoresis. This technique has the benefit of being easy, fast and efficient. Band profiles reproducibility can be raised by using dissimilar thermal cyclers, primer sensitivity, DNA quality and template concentration.

- **AFLP (Amplified fragment length polymorphism)**

AFLP has been used in parentage analysis, forensic science, for genetic investigation and disease diagnosis in humans and animals. This technique is based on (i) DNA digestion with two restriction enzymes (ii) ligation to 5′ and 3′- end with specific adapters of restriction fragments (iii) use of primers for amplifying restriction fragments (iv) analysis of subsequent restriction fragments through electrophoresis. AFLP has the advantage of being used to composite DNA of every origin. PCR can be applied with high stringency that can attained high discrimination level. Disadvantage of this technique is that it takes more time than other fingerprinting methods. Occasionally, in band profiles a high variability can limit the AFLP performance, but this technique has been utilized to find out the genotype of different organisms, including protozoa but has not been utilized to *Cryptosporidium* [55]. Some researchers have focused on satellite DNA utilization for

genetic make-up analysis of parasite populations. Microsatellites and Minisatellites have been used as ubiquitous and profuse in all eukaryotic genomes. They are usually non-transcribed and sustain polymorphism because of alteration in repeat number. On gel electrophoresis, this change let the alleles to be scored by size and characterization is done by variability in alleles. Thus, it is utilized to investigate genetic mapping and genetic structure of organism's populations. PCR is an excellent technique for the amplification of repeat region that can be examined by gel electrophoresis and visualized by auto-radiography or staining. Analysis with multi-locus satellite has been used to explore population structures and to identify genetic exchange role in *Cryptosporidium* [56]. Multi-locus satellite technique has also been utilized to investigate zoonotic threat from several protozoa including *Cryptosporidium* [57]. Fingerprinting method has also been utilized to explore differences between *C. parvum* and *C. hominis*. In electrophoretic band profiles, a similar method revealed the substantial heterogeneity in clinical isolates taken from sporadic diseases and homogeneity among clinical isolates from an outbreak. Based on these studies, it is now clear that these diagnostic tools have given useful information on population structure and diversity in *C. hominis* and *C. parvum* and similar methods are required for other *Cryptosporidium* species.

- **PCR-based sequencing and restriction fragment length**

Different PCR-based methods, utilizing selective pairs of primers for the amplification of genetic loci followed by sequencing or enzymatic cleavage, have been employed to classify and characterize the *Cryptosporidium* species genotypes [4]. Several loci (key markers) comprise rRNA genes and spacers, COWP (Cryptosporidium oocyst wall protein), HSP70 (70 kDa heat shock protein), TRAP (Thrombospondin-related adhesive protein) genes and GP60 (genes and the 60 kDa glycoprotein). The SSU-rRNA gene gave a valuable genetic marker for *Cryptosporidium* identification having great interspecific and low intraspecific sequence differences [7,58]. Markers allow identification at species level include the hsp70 and actin gene which have been used together with SSU (small subunit) in phylogenetic (systematic) analysis of *Cryptosporidium,* giving basic structure for members classification in the genus [59]. The ITS (internal transcribed spacers) of ribosomal DNA are helpful for revealing variability because their sequences possess great intraspecific variation rather than rRNA gene regions. Extremely variable loci may have repetitive microsatellite regions including gp60, ML1 (microsatellite locus 1) and ML2 (microsatellite locus 2) have been used to explore population genetics of *Cryptosporidium* especially for *C. parvum* and *C. hominis* [60].

- **PCR-RFLP (PCR-based restriction fragment length polymorphism)**

It has been used to classify *Cryptosporidium* spp. genetics in many researches. PCR-RFLP based genotypic analysis of gene fragment 18s rRNA has shown 82% isolates from *C. hominis,* while 18% from *C. parvum* in children [53]. Moreover, PCR-RFLP analysis for the gene (SSU rRNA) explains that native breeds of cattle do not transmit human *Cryptosporidium* in Nigeria (Kaduna State). In an advance study, four species of *Cryptosporidium*, *C. bovis*, *C. andersoni, C. parvum* and *C. ryanae* were explored by analyzing the SSU rRNA (18S) and COWP genes in polish breed cattle. This method does not identify all the sequence length variations in amplicons because endonuclease/s, which is utilized, identifies a limited number of variable sites. One of the gold standard methods remains the direct DNA sequencing to identify polymorphism or genetic variation and can be used for multi-copy (provided there is no sequence heterogeneity in copies) as well as single-copy genes. DNA sequence data can be employed for comparative genetic analysis and suitable for phylogenetic studies. But direct DNA sequencing have some restrictions, as small quantity of DNA from single oocyst of *Cryptosporidium* is not of practical approach for PCR amplification; amplicons are produced always from that isolate which represents oocysts population. If considerable polymorphism or heterogeneity present in that isolate as in first (ITS-1) and second (ITS-2), ITS regions may not be able to develop a precise sequence from an amplicon [61]. However, analysis of polymorphic amplicons can be done by mutation scanning methods.

- **Electrophoretic scanning**

Some restrictions in the investigation of sequence variation can be minimized by high resolution electrophoretic methods. These methods are comprised of mutation scanning techniques, for instance, DGGE (denaturing gradient gel electrophoresis), TGGE (temperature gradient gel electrophoresis), SSCP (single strand conformation polymorphism) and hetero duplex analysis are being used. Particularly, SSCP is a valuable method that is based on the principle which states that electrophoretic movement of ssDNA molecule in non-denaturing gel is dependent mainly on two things, its structure and size, enabling the method to verify mutation at a single point in 500bp amplicons [18]. Thus, PCR-based SSCP has exhibited a useful diagnostic tool for identification and genotypes of *Cryptosporidium* species to verify genetic variation among and within large quantity of samples. In *Cryptosporidium*, this has also been utilized for exhibiting sequence variation in the regions of nuclear gene (SSU and hsp70) with lesser number of samples from proper oocyst DNA.

New drug discoveries

Several therapeutics have been tested against cryptosporidiosis such as macrolide Paromomycin aminoglycoside, ionophores and immunotherapy. However, Nitazoxanide was the only anti-protozoal drug against cryptosporidiosis, which was approved in 2006 by the U.S. food and drug administration. But this drug is only effective in immunocompetent patients. In immunocompromised patients, combination of medicine; paromomycin + protease inhibitors along with antiretroviral therapy is recommended [62-65]. The new drugs recently approved for the treatment of cryptosporidiosis along with their limitations have been mentioned in table 3.

Conclusion

Cryptosporidium spp. is an emerging zoonotic protozoan parasite which is being transmitted through contaminated food, raw vegetables and drinking water which may be a leading cause of gastrointestinal illnesses in humans. *Cryptosporidium parvum* and *Cryptosporidium hominis* are most prevalent parasites among all kind of hosts. These species cause chronic diarrhea and dysentery in young children and particularly, in immunocompromised patients or HIV/AIDS patients. Recovery chances from this parasitic infection are very rare in HIV patients. Even, no antiparasitic drug against these virulent protozoa is efficacious in HIV infected/ AIDS patients until a suitable anti-retroviral medicine is given in combination. Nitazoxanide is a drug of choice against *Cryptosporidium* spp. and is recently approved by FDA. Moreover, the most appropriate option to avoid cryptosporidiosis is to adopt better hygienic and sanitation conditions.

Drug	Status	Limitations	Reference
Nitazoxanide	Approved for use for cryptosporidiosis but not with HIV co-infection	Efficacy 56–96% in healthy hosts. Not effective in patients with advanced AIDS. Costly and low availability.	[63]
Paromomycin	Approved for use for other indications	Limited efficacy in patients with AIDS. No controlled data in other groups.	[62]
Azithromycin	Approved for use for other indications	Not effective in patients with advanced AIDS. Anecdotes of efficacy in combination in patients with AIDS.	[62]
Rifaximin	Approved for use for other indications	Anecdotes of responses in patients with AIDS.	[62]
Rifabutin	Approved for use for other indications	Effective at prevention of cryptosporidiosis in studies of *Mycobacterium avium* prophylaxis.	[62]
HIV protease inhibitors	Approved for use for HIV treatment	Associated with resolution of cryptosporidiosis in patients with AIDS. Partial efficacy against *Cryptosporidium parvum* in mouse models.	[62]

Table 3: Some chemotherapeutic drugs against cryptosporidiosis

Conflict of Interest Statement

The authors declare that there is no conflict of interest regarding the publication of this paper.

References

1. Vanathy K, Parija SC, Mandal J, Hamide A, Krishnamurthy S. Cryptosporidiosis: A mini review. Tropical parasitology, (2017); 7(2): 72.
2. Jirku M, Valigurová A, Koudela B, Krízek J, Modrý D, *et al.* New species of Cryptosporidium Tyzzer, 1907 (Apicomplexa) from amphibian host: morphology, biology and phylogeny. Folia parasitologica, (2008); 55(2): 81.
3. Feng Y, Ryan UM, Xiao L. Genetic diversity and population structure of Cryptosporidium. Trends in parasitology, (2018).
4. Xiao L, Fayer R, Ryan U, Upton SJ. Cryptosporidium taxonomy: recent advances and implications for public health. Clinical microbiology reviews, (2004); 17(1): 72-97.
5. Kabir M, Ahmed E, Hossain B, Alam M, Ahmed S, *et al.* Giardia/Cryptosporidium QUIK CHEK® Assay is more Specific than qPCR for Rapid Point-of-care Diagnosis of

Cryptosporidiosis in Infants in Bangladesh. Clinical Infectious Diseases, (2018); 67(12): 1897-1903.
6. Checkley W, White Jr AC, Jaganath D, Arrowood MJ, Chalmers RM, *et al.* A review of the global burden, novel diagnostics, therapeutics, and vaccine targets for cryptosporidium. The Lancet Infectious Diseases, (2015); 15(1): 85-94.
7. Fayer R. Taxonomy and species delimitation in Cryptosporidium. Experimental parasitology, (2010); 124(1): 90-97.
8. Akinbo FO, Okaka CE, Omoregie R, Dearen T, Leon ET, *et al.* Molecular characterization of Cryptosporidium spp. in HIV-infected persons in Benin City, Edo State, Nigeria. Fooyin Journal of Health Sciences, (2010); 2(3-4): 85-89.
9. Abdel-Messih IA, Wierzba TF, Abu-Elyazeed R, Ibrahim AF, Ahmed SF, *et al.* Diarrhea associated with Cryptosporidium parvum among young children of the Nile River Delta in Egypt. Journal of Tropical Pediatrics, (2005); 51(3): 154-159.
10. Mbae CK, Nokes DJ, Mulinge E, Nyambura J, Waruru A, *et al.* Intestinal parasitic infections in children presenting with diarrhoea in outpatient and inpatient settings in an informal settlement of Nairobi, Kenya. BMC Infectious Diseases, (2013); 13(1): 243.
11. Delahoy MJ, Omore R, Ayers TL, Schilling KA, Blackstock AJ, *et al.* Clinical, environmental, and behavioral characteristics associated with Cryptosporidium infection among children with moderate-to-severe diarrhea in rural western Kenya, 2008–2012: The Global Enteric Multicenter Study (GEMS). PLoS neglected tropical diseases, (2018); 12(7): e0006640.
12. Goma F, Geurden T, Siwila J, Phiri I, Gabriël S, *et al.* The prevalence and molecular characterisation of Cryptosporidium spp. in small ruminants in Zambia. Small Ruminant Research, (2007); 72(1): 77-80.
13. Mensah G, Bosompem K, Ayeh-Kumi P, Oppong J, Niampoma S. Epidemiology of Cryptosporidium Sp Upstream the Water Treatment Plants in Kpong and Weija, Ghana. Ghana Journal of Science, (2018); 585-11.
14. Marques SMT. Cryptosporidiosis in Horses of Urban Areas of Porto Alegre, Rio Grande do Sul, Southern Brazil. Journal of equine veterinary science, (2010); 30(7): 356-358.
15. Iqbal A, Goldfarb DM, Slinger R, Dixon BR. Prevalence and molecular characterization of Cryptosporidium spp. and Giardia duodenalis in diarrhoeic patients in the Qikiqtani Region, Nunavut, Canada. International journal of circumpolar health, (2015); 74(1): 27713.
16. Gatei W, Barrett D, Lindo JF, Eldemire-Shearer D, Cama V, *et al.* Unique Cryptosporidium population in HIV-infected persons, Jamaica. Emerging Infectious Diseases, (2008); 14(5): 841.
17. Alonso-Fresan M, Garcia-Alvarez A, Salazar-Garcia F, Vázquez-Chagoyán J, Pescador-Salas N, *et al.* Prevalence of Cryptosporidium spp. in asymptomatic sheep in family flocks from Mexico State. Journal of Veterinary Medicine, Series B, (2005); 52(10): 482-483.
18. Giangaspero A, Iorio R, Paoletti B, Traversa D, Capelli G. Molecular evidence for Cryptosporidium infection in dogs in Central Italy. Parasitology Research, (2006); 99(3): 297-299.
19. Causape A, Quilez J, Sanchez-Acedo C, Del Cacho E. Prevalence of intestinal parasites, including Cryptosporidium parvum, in dogs in Zaragoza city, Spain. Veterinary parasitology, (1996); 67(3-4): 161-167.
20. Quilez J, Sanchez-Acedo C, Del Cacho E, Clavel A, Causape A. Prevalence of Cryptosporidium and Giardia infections in cattle in Aragon (northeastern Spain). Veterinary Parasitology, (1996); 66(3-4): 139-146.
21. Chilvers B, Cowan P, Waddington D, Kelly P, Brown T. The prevalence of infection of Giardia spp. and Cryptosporidium spp. in wild animals on farmland, southeastern North Island, New Zealand. International Journal of Environmental Health Research, (1998); 8(1): 59-64.
22. Winkworth C, Matthaei C, Townsend C. Prevalence of Giardia and Cryptosporidium spp in calves from a region in New Zealand experiencing intensifi cation of dairying. New Zealand veterinary journal, (2008); 56(1): 15-20.
23. Atwill ER, Sweitzer RA, Pereira M, Gardner IA, Van Vuren D, *et al.* Prevalence of and associated risk factors for shedding Cryptosporidium parvum oocysts and Giardia cysts within feral pig populations in California. Applied and Environmental Microbiology, (1997); 63(10): 3946-3949.
24. Wang A, Ruch-Gallie R, Scorza V, Lin P, Lappin MR. Prevalence of Giardia and Cryptosporidium species in dog park attending dogs compared to non-dog park attending dogs in one region of Colorado. Veterinary Parasitology, (2012); 184(2-4): 335-340.
25. Leach CT, Koo FC, Kuhls TL, Hilsenbeck SG, Jenson HB. Prevalence of Cryptosporidium parvum infection in children along the Texas-Mexico border and associated risk factors. The American journal of tropical medicine and hygiene, (2000); 62(5): 656-661.
26. Rickard LG, Siefker C, Boyle CR, Gentz EJ. The prevalence of Cryptosporidium and Giardia spp. in fecal samples from free-ranging white-tailed deer (Odocoileus virginianus) in the southeastern United States. Journal of Veterinary Diagnostic Investigation, (1999); 11(1): 65-72.
27. Petri Jr WA, Haque R, Mondal D, Karim A, Molla IH, *et al.* Prospective case-control study of the association between common enteric protozoal parasites and diarrhea in Bangladesh. Clinical Infectious Diseases, (2009); 48(9): 1191-1197.
28. Mallinath HK, G Chikkachowdappa P, K Javare Gowda A, E D'Souza P. Studies on the prevalence of cryptosporidiosis in bovines in organized dairy farms in and around Bangalore, South India. Veterinarski arhiv, (2009); 79(5): 461-470.
29. Bhat S, Juyal P, Singla L. Prevalence of cryptosporidiosis in neonatal buffalo calves in Ludhiana district of Punjab, India. Asian J Anim Vet Adv, (2012); 7(6): 512-520.
30. Ghoshal U, Jain V, Dey A, Ranjan P. Evaluation of enzyme linked immunosorbent assay for stool antigen detection for the diagnosis of cryptosporidiosis among HIV negative immunocompromised patients in a tertiary care hospital of northern India. Journal of infection and public health, (2018); 11(1): 115-119.
31. Rahi AA, Magda A, Al-Charrakh AH. Prevalence of Cryptosporidium parvum among children in Iraq. American Journal of Life Sciences, (2013); 1(6): 256-260.
32. Butty E. Detection of Cryptosporidium and Giardia doudenalis in equines in Nineveh, Iraq. Iraqi Journal of Veterinary Sciences, (2011); 25(2): 43-46.

33. Ahamed I, Yadav A, Katoch R, Godara R, Saleem T, *et al.* Prevalence and analysis of associated risk factors for Cryptosporidium infection in lambs in Jammu district. Journal of parasitic diseases, (2015); 39(3): 414-417.
34. Ghimire P, Sapkota D, Manandhar SP. Cryptosporidiosis: opportunistic infection in HIV/AIDS patients in Nepal. J Trop Med Parasitol, (2004); 277-10.
35. Nasir A, Avais M, Khan M, Ahmad N. Prevalence of Cryptosporidium parvum infection in Lahore (Pakistan) and its association with diarrhea in dairy calves. Int J Agric Biol, (2009); 11(2): 221-224.
36. Mumtaz S, Ahmed J, Ali L. Frequency of cryptosporidium infection in children under five years of age having diarrhea in the North West of Pakistan. African Journal of Biotechnology, (2010); 9(8); 1230-1235.
37. Srisuphanunt M, Karanis P, Charoenca N, Boonkhao N, Ongerth JE. Cryptosporidium and Giardia detection in environmental waters of southwest coastal areas of Thailand. Parasitology research, (2010); 106(6): 1299-1306.
38. Waldron LS, Dimeski B, Beggs PJ, Ferrari BC, Power ML. Molecular epidemiology, spatiotemporal analysis, and ecology of sporadic human cryptosporidiosis in Australia. Applied and environmental microbiology, (2011); 77(21): 7757-7765.
39. Power M, Shanker S, Sangster N, Veal D. Evaluation of a combined immunomagnetic separation/flow cytometry technique for epidemiological investigations of Cryptosporidium in domestic and Australian native animals. Veterinary parasitology, (2003); 112(1-2): 21-31.
40. Ryan U, Samarasinghe B, Read C, Buddle J, Robertson I, *et al.* Identification of a novel Cryptosporidium genotype in pigs. Applied and Environmental Microbiology, (2003); 69(7): 3970-3974.
41. Karanis P, Kourenti C, Smith H. Waterborne transmission of protozoan parasites: a worldwide review of outbreaks and lessons learnt. Journal of water and health, (2007); 5(1): 1-38.
42. Thompson RA, Colwell DD, Shury T, Appelbee AJ, Read C, *et al.* The molecular epidemiology of Cryptosporidium and Giardia infections in coyotes from Alberta, Canada, and observations on some cohabiting parasites. Veterinary parasitology, (2009); 159(2): 167-170.
43. Abrahamsen MS, Templeton TJ, Enomoto S, Abrahante JE, Zhu G, *et al.* Complete genome sequence of the apicomplexan, Cryptosporidium parvum. Science, (2004); 304(5669): 441-445.
44. Muchiri JM, Ascolillo L, Mugambi M, Mutwiri T, Ward HD, *et al.* Seasonality of Cryptosporidium oocyst detection in surface waters of Meru, Kenya as determined by two isolation methods followed by PCR. Journal of water and health, (2009); 7(1): 67-75.
45. Bomfim T, Huber F, Gomes R, Alves L. Natural infection by Giardia sp. and Cryptosporidium sp. in dairy goats, associated with possible risk factors of the studied properties. Veterinary parasitology, (2005); 134(1-2): 9-13.
46. El-Khodery SA, Osman SA. Cryptosporidiosis in buffalo calves (Bubalus bubalis): prevalence and potential risk factors. Tropical animal health and production, (2008); 40(6): 419-426.
47. Tian L-G, Chen J-X, Wang T-P, Cheng G-J, Steinmann P, *et al.* Co-infection of HIV and intestinal parasites in rural area of China. Parasites & vectors, (2012); 5(1): 36.
48. Adjei AA, Armah H, Rodrigues O, Renner L, Borketey P, *et al.* Cryptosporidium spp., a frequent cause of diarrhea among children at the Korle-Bu Teaching Hospital, Accra, Ghana. Japanese journal of infectious diseases, (2004); 57(5): 216-219.
49. Wumba R, Longo-Mbenza B, Menotti J, Mandina M, Kintoki F, *et al.* Epidemiology, clinical, immune, and molecular profiles of microsporidiosis and cryptosporidiosis among HIV/AIDS patients. International journal of general medicine, (2012); 5603.
50. Smith H (2007) Diagnostics. Cryptosporidium and Cryptosporidiosis, Second Edition: CRC Press. pp. 181-215.
51. Smith H, Caccio S, Cook N, Nichols R, Tait A. Cryptosporidium and Giardia as foodborne zoonoses. Veterinary parasitology, (2007); 149(1-2): 29-40.
52. Uppal B, Singh O, Chadha S, Jha AK. A comparison of nested PCR assay with conventional techniques for diagnosis of intestinal cryptosporidiosis in AIDS cases from northern India. Journal of parasitology research, (2014); 2014.
53. Nyamwange CI (2013) Epidemiology and Molecular Characterization of Cryptosporidium Species among Children and HIV Infected Individuals in the North Rift Region of Kenya. PhD Thesis. Jomo Kenyatta University of Agriculture and Technology
54. Bednarska M, Bajer A, Sinski E, Girouard AS, Tamang L, *et al.* Fluorescent in situ hybridization as a tool to retrospectively identify Cryptosporidium parvum and Giardia lamblia in samples from terrestrial mammalian wildlife. Parasitology research, (2007); 100(3): 455-460.
55. Blake D, Smith A, Shirley M. Amplified fragment length polymorphism analyses of Eimeria spp.: an improved process for genetic studies of recombinant parasites. Parasitology research, (2003); 90(6): 473-475.
56. Mallon M, MacLeod A, Wastling J, Smith H, Reilly B, *et al.* Population structures and the role of genetic exchange in the zoonotic pathogen Cryptosporidium parvum. Journal of Molecular Evolution, (2003); 56(4): 407-417.
57. Grinberg A, Lopez-Villalobos N, Pomroy W, Widmer G, Smith H, *et al.* Host-shaped segregation of the Cryptosporidium parvum multilocus genotype repertoire. Epidemiology & Infection, (2008); 136(2): 273-278.
58. Jex AR, Whipp M, Campbell BE, Cacciò SM, Stevens M, *et al.* A practical and cost-effective mutation scanning-based approach for investigating genetic variation in Cryptosporidium. Electrophoresis, (2007); 28(21): 3875-3883.
59. Xiao L. Molecular epidemiology of cryptosporidiosis: an update. Experimental parasitology, (2010); 124(1): 80-89.
60. Chalmers RM, Katzer F. Looking for Cryptosporidium: the application of advances in detection and diagnosis. Trends in parasitology, (2013); 29(5): 237-251.
61. Schindler AR, EL-Osta YGA, Stevens M, Sinclair MI, Gasser RB. Capillary electrophoretic analysis of fragment length polymorphism in ribosomal markers of Cryptosporidium from humans. Molecular and cellular probes, (2005); 19(6): 394-399.
62. Cabada MM, White Jr AC. Treatment of cryptosporidiosis: do we know what we think we know? Current opinion in infectious diseases, (2010); 23(5): 494-499.
63. Rossignol JF, Kabil SM, El–Gohary Y, Younis AM. Effect of nitazoxanide in diarrhea and enteritis caused by Cryptosporidium species. Clinical Gastroenterology and Hepatology, (2006); 4(3): 320-324.
64. Wang RJ, Li JQ, Chen YC, Zhang LX, Xiao LH. Widespread occurrence of Cryptosporidium infections in patients with

HIV/AIDS: Epidemiology, clinical feature, diagnosis, and therapy. Acta tropica, (2018); 187:257-263.

65. Waywa D, Silpasakorn S, Phungthaisong A, Suputtamongkol Y. Intestinal Parasitic Infections in Thai Patients: Five-Year Experiences at Siriraj Hospital. Siriraj Medical Journal, (2018); 58(11): 1107-1109.