Pakistan J. Zool., vol. 49(4), pp 1455-1461, 2017. DOI: http://dx.doi.org/10.17582/journal.pjz/2017.49.4.1455.1461

Cryptosporidium: An Emerging Zoonosis in Southern Khyber Pakhtunkhwa (KPK), Pakistan

Naimat Ullah Khan[1,2], Muhammad Hassan Saleem[1], Aneela Zameer Durrani[1], Nisar Ahmad[3], Ayesha Hassan[1], Sultan Ayaz[2], Sajid Umar[4], Muhammad Luqman Sohail[5,*], Muhammad Shafee[6], Ikramullah Khan[7], Mumtaz Ali Khan[1], Azmat Ullah Khan[8], Naimat Ullah Malik[1] and Abdul Razzaq[9]

[1]*Department of Clinical Medicine and Surgery, University of Veterinary and Animal Sciences, Lahore, Pakistan*

[2]*College of Veterinary Sciences and Animal Husbandry, Abdul Wali Khan University Mardan, Pakistan*

[3]*Department of Parasitology, University of Veterinary and Animal Sciences, Lahore, Pakistan*

[4]*Department of Pathobiology, Faculty of Veterinary Sciences, Pir Meher Ali Shah Arid Agriculture University, Rawalpindi, Pakistan*

[5]*University College of Veterinary and Animal Sciences, The Islamia University of Bahwalpur, Pakistan*

[6]*Centre for Advanced Studies in Vaccinology and Biotechnology, University of Balochistan, Quetta, Pakistan*

[7]*Department of Livestock Management, Animal Breeding and Genetics, The University of Agriculture, Peshawar, Pakistan*

[8]*Department of Livestock and Dairy Development, The University of Agriculture, Peshawar, Pakistan*

[9]*Department of Veterinary Anatomy and Histology, Sindh Agriculture University, Tandojam, Pakistan*

Article Information
Received 21 March 2017
Revised 11 April 2017
Accepted 13 May 2017
Available online 25 July 2017

Authors' Contribution
HS, AZD, NA, AH and SA designed the study plan. NUK, MS, IUK, MAK, NUM and AK collected samples and executed the experimental work. AR, MLS and SU recorded and analyzed the data and wrote the article.

Key words
Children, *Cryptosporidium*, epidemiology, Livestock.

ABSTRACT

Zoonotic *Cryptosporidium* is chief cause of diarrhea in both developing and developed countries, causing millions of deaths each year. This study was designed to estimate the zoonotic potential of *Cryptosporidium* in three districts of Khyber Pakhtunkhwa (KPK), *i.e.* Bannu, Lakki Marwat and Kohat. For this purpose 360 stool samples were collected from the District Headquarter Hospitals of all the three districts and were screened through microscopy. Results yielded an overall prevalence of 11.11%, with significant difference ($P<0.05$) among different study areas. Prevalence was highest in district Bannu (11.66%), followed by district Lakki Marwat and Kohat with 10% in each district. Statistical analysis revealed significant difference ($P<0.05$) among children of different age groups with highest disease prevalence in children of less than 5 years of age (21.40%) and lowest in children of 11-15 years of age (15.99%). *Cryptosporidium* was significantly higher ($P<0.05$) in study subjects having livestock animal contact and persistent diarrhea, highlighting its zoonotic potential from livestock animals to human beings in the study area. This is first study addressing the one of the main cause of neonatal mortality in Southern KPK and its zoonotic potential.

INTRODUCTION

Diarrheal diseases are second leading cause of mortality among young children around the globe. It causes 9% of child deaths worldwide (Bhutta and Black, 2013). *Cryptosporidium* is among the top four causes of moderate to severe diarrheal disease in young children in developing countries and is a major opportunistic co-infection with HIV (Bodager *et al*., 2015). It is ranked 5th among the 24 most important food borne parasites globally (Aniesona and Bamaiyi, 2014). It is mostly prevalent in hot and humid weather during the year, with worldwide distribution. (Jafari *et al*., 2013). *Cryptosporidium* infection has been reported in humans from 3 days to 95 years of age, but

* Corresponding author: vet.luqman@gmail.com
0030-9923/2017/0004-1455 $ 9.00/0

data suggest that young children are most susceptible to infection (Fayer, 2004), along with immunocompromised hosts (Sow *et al*., 2016). Epidemiologic studies have demonstrated that *Cryptosporidium* is more prevalent in developing countries (5% to 10%) than in developed countries (1% to 3%) (Iqbal *et al*., 1999).

After identification of *Cryptosporidium* in 1907, its zoonotic potential was revealed in late 1970s (Shoultz *et al*., 2016). The most noteworthy symptom in immunologically healthy persons is diarrhea, usually voluminous and watery. Abdominal discomfort, anorexia, nausea, vomiting, weight loss, fever, fatigue and respiratory problems may accompany diarrhea (Masood *et al*., 2013). Prolonged diarrhea (7-14 days) and persistent diarrhea (≥14 days) is also reported during *Cryptosporidiosis* (Moore *et al*., 2010). Which may lead to malnutrition, hospitalizations and even death (Kotloff *et al*., 2013). Fecal shedding of *Cryptosporidium* oocysts can continue for weeks after clinical illness resolves. Since *Cryptosporidium* oocysts tolerate chlorination, waterborne outbreaks also occur in industrialized countries (Shirley *et al*., 2012).

For epidemiological studies, serological tests might also be used but microscopy is an important diagnostic method and because of the low cost of reagents, it is well practiced in lower or middle income countries (Checkly *et al*., 2015).

To the best of our knowledge, no data is available regarding the prevalence of cryptosporidium in children of Southern KPK, exposed to livestock animals and still is a neglected area of study. This paper will provide the information about the epidemiological study and related risk factors responsible for occurrence of the *Cryptosporidium* in children. Limited access of human population to health care facilities will further aggravate the issue if left, unreported.

MATERIALS AND METHODS

Study area

The present study was conducted in three selected districts of Southern Khyber Pakhtunkhwa, district Bannu, Lakki Marwat and Kohat. Coordinates of the study areas were determined by global positioning system (GPS). Bannu is located at 32.99° North latitude, 70.61° East longitude and have an elevation of 371 meters from the sea level. Lakki Marwat, the neighboring district of Bannu, have coordinates 32° 36’ 27” North, 70° 54’ 45” East. Kohat district lies between North latitude 32° 47’ and 33° 53’ and East longitude 70° 34’ and 72° 17.

Experimental site

All the samples were processed at Diagnostic Laboratory, Department of Clinical Medicine and Surgery and in the Parasitology Laboratory, Department of Parasitology, University of Veterinary and Animal Sciences (UVAS) Lahore, Pakistan.

Inclusion criteria

Individuals visiting District Headquarter Hospitals of study area with abdominal anomalies *i.e.,* acute diarrhea (three loose stools within last 24 h prior to hospital visit) or persistent diarrhea, vomiting (once in last 24 h before hospital visit) and abdominal cramps were included in the study. All the participants had exposure to livestock and other animals.

All the participants were informed about the study’s purpose and procedures. Written informed consent was obtained from the parents or the guardian on behalf of the study children prior to study enrolment.

Sample size and collection

Fecal samples from 360 individuals, satisfying the inclusion criteria were collected through convenient sampling with the assistance of Registered Medical Practitioners (RMP) of Pakistan Medical and Dental Council (PMDC) from Outdoor Patients Department (OPD) of three District Headquarter Hospitals (DHQ’s) of district Bannu, Lakki Marwat and Kohat from January to June, 2016.

All the collected samples were preserved in 10% formalin (2:3). The samples were collected into sterile wide mouthed plastic bottles, properly labeled and transported to the experimental site till further analysis. Collected samples were examined microscopically and screened out for the presence of *Cryptosporidium* oocysts.

The purpose of this study was to determine the prevalence of *Cryptosporidium* infection in children and adults due to ignorance, poor literacy rate and lack of information about the zoonotic aspect of the cryptosporidiosis in South KPK, Pakistan.

Laboratory analysis of fecal samples

The samples were concentrated by formalin-ethyl-acetate sedimentation method and stained using the modified Ziehl-Neelsen technique (MZN) for the detection of cryptosporidiosis (Shafieyan *et al*., 2016). Briefly, Smears were fixed using absolute methanol and were stained by carbol fuchsin solution for 15 min. Non-*Cryptosporidium* materials were decolorized by acid alcohol solution for 2 min. Then, the slides were treated with malachite green solution for one minute, washed, dried and examined for presence of the oocysts under light microscopy.

Identification of Cryptosporidium *oocysts*

Cryptosporidium oocysts appeared as bright red granules on a blue-green background in MZN stained fecal smears and were identified on the basis of morphology, size and other key features as described by Wantanabe *et al*. (2005).

A fecal sample was considered positive if at least one, clearly identifiable oocyst was recognized. The total number of oocysts per gram (OPG) of feces was calculated by multiplying the total number of oocysts on slide by 50.

Statistical analysis

The data collected was fed in the Microsoft Excel version 2013 and was exported to the Statistical Product and Service Solutions (SPSS) version 20.0 to analyze associations between prevalence of *Cryptosporidium* and the risk factors at 95% level of confidence. The prevalence was the proportion of positive individuals out of the total number of individuals analyzed and was presented in percentage (%). Statistical differences in prevalence and other variables were determined using Chi-square test (X^2). All values at statistical difference $P < 0.05$ were considered significant.

Table I.- Prevalence of *Cryptosporidium* in adults and children in three districts of Southern KPK.

Factor	Infected / Total (Prevalence)			
	Bannu	Lakki Marwat	Kohat	Overall
Adult Humans	3/60 (5%)	3/60 (5%)	2/60 (3.33%)	10/180 (5.55%)
Children	11/60 (18.33%)	9/60 (15%)	10/60 (16.66%)	30/180 (16.66%)

Statistically mean values that are carrying same superscript are differ from each other non-significantly (P>0.05) while those statistical mean values having different superscripts are differ significantly (P< 0.05).

RESULTS

Results showed significantly higher prevalence (P < 0.05) of *Cryptosporidium* in children (30/180) 16.66% as compared to adults (10/180) 5.55%. Similar findings were recorded in all the three study areas. In Bannu, children (18.33%) had higher prevalence as compared to adults (5%). Similarly, in Lakki Marwat prevalence in children was 15% and in adults it was 5%. In Kohat 16.66% of children had *Cryptosporidium* whereas adults had 3.33%.

Table II shows age wise prevalence of *Cryptosporidium* in children having abdominal disturbances. Highest prevalence (5%) was recorded in children in less than 5 years of age (21.42%), followed by children of age 6-10 years (17.30%) and the lowest prevalence was recorded in children from 11-15 years of age (10.34%). Statistical analysis revealed significant difference (P < 0.05) among the study subjects of different ages.

Higher prevalence was recorded in children having close contact with domestic animals (20%) as compared to those having no contact with domestic animals (12.94%). Statistical analysis revealed significant difference between the two study groups (P < 0.05) (Table III).

Table II.- Prevalence of *Cryptosporidium* according to age in young children in three districts of Southern KPK.

Age	Infected / Total (Prevalence)			
	Bannu	Lakki Marwat	Kohat	Overall
0 -5 years	5/23 (21.73%)	5/21 (23.80%)	5/26 (19.23%)	15/70 (21.40^{a}%)
6-10 years	3/17 (17.64%)	3/18 (16.66%)	3/17 (17.64%)	9/52 (17.29^{b}%)
11-15 years	3/20 (15%)	1/21 (4.76%)	2/17 (11.76%)	6/58 (10.33^{c}%)

Statistically mean values that are carrying same superscript are differ from each other non-significantly (P>0.05) while those statistical mean values having different superscripts are differ significantly (P< 0.05).

Table III.- Prevalence of *Cryptosporidium* according to animal contact in three districts of South KPK.

Contact with animals	Infected / Total (Prevalence)			
	Bannu	Lakki Marwat	Kohat	Overall
Domestic animals	7/34 (20.58%)	6/29 (20.68%)	6/32 (18.75%)	19/95 (20^{a}%)
Others	4/26 (15.38%)	3/31 (9.67%)	4/28 (14.28%)	11/85 (12.94^{b}%)

Statistically mean values that are carrying same superscript are differ from each other non-significantly (P>0.05) while those statistical mean values having different superscripts are differ significantly (P< 0.05).

In Bannu, the higher prevalence (20.58%) was recorded in those children having direct contact with domestic animals as compared to children with no contact with domestic animals (15.58%). Similar results were observed in other two study areas (Lakki Marwat and Kohat).

Results showed an overall higher prevalence (25.84%) in those children having persistent diarrhea as compared to the children suffering from acute diarrhea (7.69%). On statistical analysis significant difference (P < 0.05) was found on the basis of nature of diarrhea (acute or persistent) among study subjects (Table IV).

Table IV.- Prevalence of *Cryptosporidium* according to nature of diarrhea in three districts of South KPK.

Nature of diarrhea	Infected/Total (Prevalence)			
	Bannu	Lakki Marwat	Kohat	Overall
Acute	2/24 (8.33%)	3/35 (8.57%)	2/32 (6.25%)	7/91 (7.69[a]%)
Persistent	9/36 (25%)	6/25 (24%)	8/28 (28.57%)	23/89 (25.84[b]%)

Statistically mean values that are carrying same superscript are differ from each other non-significantly (P>0.05) while those statistical mean values having different superscripts are differ significantly (P< 0.05).

DISCUSSION

Prevention is the only way out to protect masses from getting infection as no vaccine is currently available against *Cryptosporidium* infection and limited understanding of the its specific biology and challenges encountered in vaccine development, masks the availability of its vaccine in near future (Ryan and Hijjawi, 2015). Hence, to design effective disease control and prevention strategies a comprehensive understanding of various risk factors contributing to the spread of disease among human and animal population is unavoidable (Collinet-Adle *et al*., 2015).

Cryptosporidium infects a wide range of livestock animals and humans, causing substantial economic losses and serious public health concern (Li *et al*., 2016). Various molecular techniques are used for oocysts detection, which are costly, time consuming, laborious and require a range of equipment, rendering them not the best candidate for screening studies, so for large scale investigations, microscopy of stained fecal smears is more useful. Taking these aspect of diagnosis in consideration modified Ziehl–Neelsen method is arguably a useful tool for *Cryptosporidium* diagnosis (Shafieyan *et al*., 2016). Despite of the improved sensitivity of molecular technique, microscopy remains the gold standard in different labs (Helmy *et al*., 2013). To the best of our knowledge, this communication is the first documented study on zoonotic aspect of *Cryptosporidium* in population of Southern KPK, Pakistan.

Exposure to animals, poor sanitation and hygienic conditions are the factors responsible for transmission of the parasitic diseases (Zambrano *et al*., 2014) and these factors are highly prevalent in the study area, where poor sanitation, open toiletries, shared water sources of animals and humans fuel the fire of its spread among the human population.

This debut study on human beings of Southern KPK yielded higher prevalence (%) of *Cryptosporidium* in children (16.66%). Similar findings were reported from the other regions of the country, reporting 20.8% prevalence in Sakurdu (Khushdil *et al*., 2016). A hospital based study in an urbanized city of similar province yielded comparatively lower prevalence (9%), which can be attributed to better health care facilities in urban areas as compared to our study area (Mumtaz *et al*., 2010).

An extensive range of prevalence of the disease have also been described in children from other countries, 1.9% in Philippine, 25.3% in Uganda (Natividad *et al*., 2008; Tumwine *et al*., 2003). Dissemination of the parasite in each community and country depends on extent of contamination of water and food, animal contact and health measurements (Sharbatkhori *et al*., 2015).

Age of children was found to be significant risk factor for the occurrence of *Cryptosporidium* in Southern KPK. Similar principle age pattern of *Cryptosporidium* prevalence (less than 5 years of age) was reported by literature from developing countries (Helmy *et al*., 2013). Similar higher prevalence in pre-school children were reported by Eibach *et al*. (2015), supporting studies from various other tropical countries. This high incidence of the disease in children may be related to the lack of pre-existing immunity, as older people may get exposed to *Cryptosporidium* infection in their lifetime. Moreover, children were more exposed to water during playing, increasing the chance of getting infected, and there was a more frequent attendance of diarrheic children than adults at DHQ's (El-Badry *et al*., 2015). *Cryptosporidium* in early age in the study area can be related to malnutrition which has been described as potential risk factor for the diarrhea due to *Cryptosporidium* (Checkly *et al*., 2015). According to Mehmood *et al*. (2016) more than 50% of children in this study area are victim of malnutrition, which further aggravate the situation.

Diarrheal diseases are one of the most leading cause of morbidity and mortality in children under 5 years of age throughout of the world (Walker *et al*., 2013). Present study reports significantly higher prevalence in study subjects having persistent diarrhea, when compared to people having acute diarrhea. One of the pathological hallmarks of intestinal cryptosporidiosis is villous atrophy (villi became stunted and shortened), with a diffuse shortening or loss of brush border microvilli (Huang and White, 2006). The imbalance of absorption and secretion is likely a major contributor to disease manifestation, specially diarrhea (Zhang *et al*., 2016).

According to another model, prostaglandins altered NaCl transport which primarily stimulates the enteric nervous system. Because of the profuse secretory diarrhea experienced by some patients, it has been hypothesized that *Cryptosporidium* produces an enterotoxin (Fayer,

2004).

Exposure to animals was concluded to be a significant risk factor for the occurrence of *Cryptosporidium* in humans because of its zoonotic transmission. Adamu *et al*. (2014) also reported livestock animals as a major reservoir for the zoonotic transmission of disease to both immunocompetent and immunocompromised individuals. Kinross *et al*. (2015) reported infected calves and other livestock animals as the potential source of transmission of infection to human beings. Another possible route of transmission can be the water bodies in the study area, shared by animals and humans, as *Cryptosporidium* has been found in water sources of different regions of KPK province in previous studies (Akbar *et al*., 2015).

CONCLUSION

This study warns about the zoonotic transmission of *Cryptosporidium*, one of four main causes of morbidity in pre-school children. It provides an evidence of *Cryptosporidium* transmission in young children from livestock animals. Furthermore, poor sanitation and contaminated water and environment fuel the fire of its spread. Due to limited treatment options available and poor health care facilities in study areas, situation can aggravate if remain unnoticed.

Statement of conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

Adamu, H., Petros, B., Zhang, G., Kassa, H., Amer, S., Ye, J., Feng, Y. and Xiao, L., 2014. Distribution and clinical manifestations of *Cryptosporidium* species and subtypes in HIV/AIDS patients in Ethiopia. *PLoS Negl. Trop. Dis*., **8**: e2831. https://doi.org/10.1371/journal.pntd.0002831

Akbar, N.U., Ayaz, S., Khan, M.A., Rahman, S., Khan, S., Khan, S.N., Waqar, M., Raza, F., Shagufta, B.I., Ali, S. and Saeed, S., 2015. Molecular detection of *Cryptosporidium parvum* in different water sources of District Peshawar, Pakistan. *Adv. biol. Res*., **9**: 344-349.

Aniesona, A.T. and Bamaiyi, P.H., 2014. Retrospective study of cryptosporidiosis among diarrheic children in the arid region of North-Eastern Nigeria. *Zoon. Publ. Hlth*., **61**: 420-426. https://doi.org/10.1111/zph.12088

Bhutta, Z.A. and Black, R.E., 2013. Global maternal, newborn, and child health—so near and yet so far. *N. Engl. J. Med.,* **369**: 2226–2235. https://doi.org/10.1056/NEJMra1111853

Bodager, J.R., Parsons, M.B., Wright, P.C., Rasambainarivo, F., Roellig, D., Xiao, L. and Gillespie, T.R., 2015. Complex epidemiology and zoonotic potential for *Cryptosporidium* suis in rural Madagascar. *Vet Parasitol*., **207**: 140-143. https://doi.org/10.1016/j.vetpar.2014.11.013

Checkley, W., White, A.C., Jaganath, D., Arrowood, M.J., Chalmers, R.M., Chen, X.M., Fayer, R., Griffiths, J.K., Guerrant, R.L., Hedstrom, L. and Huston, C.D., 2015. A review of the global burden, novel diagnostics, therapeutics, and vaccine targets for *Cryptosporidium*. *Lancet Infect. Dis*., **15**: 85-94. https://doi.org/10.1016/S1473-3099(14)70772-8

Collinet-Adler, S., Babji, S., Francis, M., Kattula, D., Premkumar, P.S. and Sarkar, R., 2015. Environmental factors associated with high fly densities and diarrhea in Vellore, India. *Appl. environ. Microbiol*., **81**: 6053–6058. https://doi.org/10.1128/AEM.01236-15

Eibach, D., Krumkamp, R., Al-Emran, H.M., Sarpong, N., Hagen, R.M., Adu-Sarkodie, Y., Tannich, E. and May, J., 2015. Molecular characterization of *Cryptosporidium* spp. among children in rural Ghana. *PLoS Negl. Trop. Dis*., **9**: e0003551. https://doi.org/10.1371/journal.pntd.0003551

El-Badry, A.A., Al-Antably, A.S., Hassan, M.A., Hanafy, N.A. and Abu-Sarea, E.Y., 2015. Molecular seasonal, age and gender distributions of *Cryptosporidium* in diarrhoeic Egyptians: distinct endemicity. *Eur. J. clin. Microbiol. Infect. Dis*., **34**: 2447-2453. https://doi.org/10.1007/s10096-015-2502-y

Fayer, R., 2004. *Cryptosporidium*: A water-borne zoonotic parasite. *Vet Parasitol*., **126**: 37-56. https://doi.org/10.1016/j.vetpar.2004.09.004

Helmy, Y.A., Krücken, J., Nöckler, K., von Samson-Himmelstjerna, G. and Zessin, K.H., 2013. Molecular epidemiology of *Cryptosporidium* in livestock animals and humans in the Ismailia province of Egypt. *Vet Parasitol*., **193**: 15-24. https://doi.org/10.1016/j.vetpar.2012.12.015

Huang, D.B. and White, A.C., 2006. An updated review on *Cryptosporidium* and *Giardia*. *Gastroenterol. Clin. N. Am*., **35**: 291-314. https://doi.org/10.1016/j.gtc.2006.03.006

Iqbal, J., Munir, M.A. and Khan, M.A., 1999. Cryptosporidium infection in young children with diarrhea in Rawalpindi, Pakistan. *Am. J. Trop. Med. Hyg*., **60**: 868-870. https://doi.org/10.4269/ajtmh.1999.60.868

Jafari, R., Maghsood, A.H. and Fallah, M., 2013. Prevalence of *Cryptosporidium* infection among livestock and humans in contact with livestock in Hamadan District. *Iran J. Res. Hlth. Sci*., **13**: 86-89.

Khushdil, A., Murtaza, F. and Chattha, M.N., 2016. Cryptosporidiosis among children of district Skardu, Pakistan. *J Ayub med. Coll. Abbottabad*, **28**: 575-577.

Kinross, P., Beser, J., Troell, K., Silverlås, C., Björkman, C., Lebbad, M., Winiecka-Krusnell, J., Lindh, J. and Löfdahl, M., 2015. *Cryptosporidium parvum* infections in a cohort of veterinary students in Sweden–Corrigendum. *Epidemiol. Infect*., **143**: 2757-2757. https://doi.org/10.1017/S0950268814003318

Kotloff, K.L., Nataro, J.P., Blackwelder, W.C., Nasrin, D., Farag, T.H. and Panchalingam, S., 2013. Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. *Lancet*, **382**: 209–222. https://doi.org/10.1016/S0140-6736(13)60844-2

Li, P., Cai, J., Cai, M., Wu, W., Li, C., Lei, M., Xu, H., Feng, L., Ma, J., Feng, Y. and Xiao, L., 2016. Distribution of *Cryptosporidium* species in Tibetan sheep and yaks in Qinghai, China. *Vet. Parasitol*., **215**: 58-62. https://doi.org/10.1016/j.vetpar.2015.11.009

Masood, S., Maqbool, A., Khan, U.J., Chaudhry, Z.I. and Anjum, A.A., 2013. Anti-*Cryptosporidium* activity of albendazole, metronidazole and paromomycin in experimentally infected cattle. *Pakistan J. Zool*., **45**: 935-940.

Mehmood, Y., Ahad, B., Gul, R. Khan, T.A., 2016. Nutritional status of children under 5 years of age in three tertiary care hospitals of Peshawar. *J. Islamabad Med. Dent. Coll.*, **5**: 50-53.

Moore, S.R., Lima, N.L. and Soares, A.M., 2010. Prolonged episodes of acute diarrhea reduce growth and increase risk of persistent diarrhea in children. *Gastroenterology*, **139**: 1156–1164. https://doi.org/10.1053/j.gastro.2010.05.076

Mumtaz, S., Ahmed, J. and Ali, L., 2010. Frequency of cryptosporidium infection in children under five years of age having diarrhea in the North West of Pakistan. *Afr. J. Biotech*., **9**: 1230. https://doi.org/10.5897/AJB09.1278

Natividad, F.F., Buerano, C.C., Lago, C.B., Mapua, C.A., de Guzman, B.B., Seraspe, E.B., Samentar, L.P. and Endo, T., 2008. Prevalence rates of Giardia and Cryptosporidium among diarrheic patients in the Philippines. *Southeast. Asian J. Trop. Med. Publ. Hlth*., **39**: 991-999.

Ryan, U. and Hijjawi, N., 2015. New developments in *Cryptosporidium* research. *Int. J. Parasitol*., **45**: 367–373. https://doi.org/10.1016/j.ijpara.2015.01.009

Shafieyan, H., Alborzi, A., Hamidinejat, H., Tabandeh, M.R. and Hajikolaei, M.R.H., 2016. Prevalence of *Cryptosporidium* spp. in ruminants of Lorestan province. *Iran J. Parasit. Dis.*, **40**: 1165-1169. https://doi.org/10.1007/s12639-014-0642-0

Sharbatkhori, M., Mojarad, E.N., Taghipour, N., Pagheh, A.S. and Mesgarian, F., 2015. Prevalence and genetic characterization of *Cryptosporidium* spp. in diarrheic children from Gonbad Kavoos City, Iran. *Iran J. Parasitol*., **10**: 441.

Shirley, D.A., Moonah, S.N. and Kotloff, K.L., 2012. Burden of disease from cryptosporidiosis. *Curr. Opin. Infect. Dis.,* **25**: 555–563. https://doi.org/10.1097/QCO.0b013e328357e569

Shoultz, D.A., de Hostos, E.L. and Choy, R.K., 2016. Addressing *Cryptosporidium* infection among young children in low-income settings: the crucial role of new and existing drugs for reducing morbidity and mortality. *PLoS Negl. Trop. Dis*., **10**: e0004242. https://doi.org/10.1371/journal.pntd.0004242

Sow, S.O., Muhsen, K., Nasrin, D., Blackwelder, W.C., Wu, Y., Farag, T.H., Panchalingam, S., Sur, D., Zaidi, A.K., Faruque, A.S. and Saha, D., 2016. The burden of cryptosporidium diarrheal disease among children< 24 months of age in moderate/high mortality regions of sub-Saharan Africa and South Asia, utilizing data from the Global Enteric Multicenter Study (GEMS). *PLoS Negl. Trop. Dis*., **10**: e0004729. https://doi.org/10.1371/journal.pntd.0004729

Tumwine, J.K., Kekitiinwa, A., Nabukeera, N., Akiyoshi, D.E., Rich, S.M., Widmer, G., Feng, X. and Tzipori, S., 2003. *Cryptosporidium parvum* in children with diarrhea in Mulago Hospital, Kampala, Uganda. *Am. J. Trop. Med. Hyg*., **68**: 710-715.

Walker, C.L.F., Rudan, I., Liu, L., Nair, H., Theodoratou, E., Bhutta, Z.A., O'Brien, K.L., Campbell, H. and Black, R.E., 2013. Global burden of childhood pneumonia and diarrhea. *Lancet*, **381**: 1405–1416. https://doi.org/10.1016/S0140-6736(13)60222-6

Watanabe, Y., Yang, C.H. and Ooi, H.K., 2005. Cryptosporidium infection in livestock and first identification of *Cryptosporidium parvum* genotype in cattle feces in Taiwan. *Parasitol. Res*., **97**: 238-241. https://doi.org/10.1007/s00436-005-1428-1

Zambrano, L.D., Levy, K., Menezes, N.P. and Freeman, M.C., 2014. Human diarrhea infections associated with domestic animal husbandry: A systematic review and meta-analysis. *Trans. R. Soc. Trop. Med. Hyg*., **108**: 313–325. https://doi.org/10.1093/trstmh/tru056

Zhang, X.T., Gong, A.Y., Wang, Y., Chen, X., Lim, S.Y.S., Dolata, C.E. and Chen, X.M., 2016. *Cryptosporidium parvum* infection attenuates the *ex vivo* propagation of murine intestinal enteroids. *Physiol. Rep*., **4**: e13060. https://doi.org/10.14814/phy2.13060