DOI: 10.14260/jemds/2014/3331

ORIGINAL ARTICLE

INVESTIGATIONS OF CRYPTOSPORIDIUM IN HUMAN AND ANIMAL POPULATION IN AND AROUND LONI VILLAGE

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HOW TO CITE THIS ARTICLE:
Apoorva Tripathi, Atul R. Rukadikar. "Investigations of Cryptosporidium in Human and Animal Population in and around Loni Village". Journal of Evolution of Medical and Dental Sciences 2014; Vol. 3, Issue 41, September 04; Page: 10239-10248, DOI: 10.14260/jemds/2014/3331

ABSTRACT: INTRODUCTION: Cryptosporidium was first reported as a human pathogen in 1976. In the 1980s it emerged worldwide as a common cause of acute, self-limiting gastroenteritis in otherwise healthy individuals especially children and of severe or life threatening infection in the severely immuno compromised patients. **MATERIAL ANDMETHODS:** A total of 376 Human and 100 animal faecal samples were collected and processed as per standard microbiological procedure. Oocyst of cryptosporidium was detected by modified Kinyoun acid fast staining **RESULTS:** Maximum cases of cryptosporidium detected in patients with symptoms out of which 7 in immuno competent and 1 in immunocompromised. In immunocompetent subjects, the symptomatic patients showed increased incidence of cryptosporidium while in immunocompromised subjects only 1 case of cryptosporidium was detected. Out of 7 detected case of cryptosporidium in immunocompetent with symptoms 4(57.1%) were males and 3(42.9%) were females. In the same category in the group without symptoms cryptosporidium was detected in 1 subject out of 75 and that too was a female (100%). In immunocompetent subjects with symptoms. **CONCLUSION:** In conclusion, routine screening of faecal specimens should be encouraged for detection of cryptosporidium. Water reservoirs should be prohibited for animal use since they are widely present in animal population and can cause outbreaks of cryptosporidial diarrheoa.

KEYWORDS: Cryptosporidium.

INTRODUCTION: Acute diarrheal diseases constitute an important cause of morbidity and mortality throughout the world particularly in infants and children in the developing countries. Acute diarrheal diseases vary in severity from an inconvenience to rapidly fatal flux.[1]

A large number of microorganisms are now known to cause diarrhoea. These microorganisms can be bacteria, viruses, protozoa and fungi.[1]

In 1980, an obligate intracellular parasite known as cryptosporidium parvum emerged worldwide as a common cause of acute self-limiting gastroenteritis in otherwise healthy subjects and severe life threatening infection in severely immunocompromised especially those with AIDS.[2]

This parasite was first discovered by Tyzzer in 1912 in mice[2]. First case in man was reported in 1976. Its identification in patients with AIDS was achieved in 1980's casting it firmly in the role of an opportunist.[3]

The clinical picture of this infection in immunocompromised patients shows a self-limiting gastroenteritis with a variety of symptoms. Diarrhoea is the most common symptom. Stool is usually watery, greenish in color containing mucus and very offensive. Vomiting is commonly associated.

Other symptoms may be colicky abdominal pain, anorexia, nausea and abdominal distension. Frequency of passing stool may be up to 20 times a day but usually 3-6 times. In immunocompromised patients diarrhoea may be frequent, profuse and watery like cholera and fluid

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loss may be up to 20 liters a day or more. In AIDS patients it is one of the common causes of mortality.[3]

Incidence in India was reported from 1.3-13 percent. Carriage stage is also reported. With this aim study was done to investigate the incidence of cryptosporidium parvum in human and animal population in rural area in and around Loni, in Ahmednagar district, Maharashtra state.

MATERIAL AND METHODS: The present study was conducted between January 1998 to September 1999 in the Department of Microbiology Rural Medical College, Loni.

In Human: A total of 376 faecal samples were collected from the patients visiting hospital. Out of 376,250 specimens were collected from the patients suffering from acute or chronic diarrhoea. A total 51 samples were collected from cancer patients on chemotherapy.

In Animal: A total of 100 faecal specimens animals like cow, buffalo, goat, dog, cat were collected randomly. Processing of the sample was carried out by standard microbiological procedure. Oocyst of cryptosporidium was detected by modified Kinyoun acid fast staining.

RESULTS: As explained from Table no 1 to Table no 15.

DISCUSSION: Crytosporidium parvum has been reported as a causative agent of diarrhea from immunocompetent as well as immunocompromised subjects. It is also linked to gastrointestinal diseases in many species of animals. (Harish Khubnani et al).[4] Therefore the study was undertaken to find out the incidence and carrier rate of cryptosporidium parvum in human being and animals in rural area.

Incidence: In the present study the incidence in symptomatic was 2.95% compared to 0.95% in asymptomatic. In symptomatic the incidence was more in immunosuppressed i.e., 4.76% as compared to immunocompetents i.e., 2.8% indicating that it produces symptoms in immunocompromised more easily than immunocompetent, which also proved by absence of cryptosporidium parvum in immunosuppressed without symptoms subjects while in immunocompetent without symptoms subjects it is 1.33%.

The incidence of cryptosporidium parvum is 1-3% in industrialized world and up to 17% in developing countries.[3] From china the incidence reported was 1-13% while in Indian study in immunocompetent subjects highest incidence reported was from Vellore i.e 13% (Mathan et al)[5] followed by Mumbai 5.6% (Saraswathi et al),[6] Chandigarh 5% (Kaur et al),[7] West Bengal 4.5% (Das et al)[8], Mumbai 4.4.% (Pherwani et al),[9] Bangalore 4% (Macaden et al),[10] Rural Maharashtra 1.3% (Khubnani et al)[4] and Chandigarh 1.3% (Malla et al).[11]

While in immunocompromised subjects among cancer subjects on chemotherapy the incidence of cryptosporidium parvum was reported from Mexico by Gaur et al[12] was 3%, from Egypt Hammoundla et al[13] reported it as 13.3%. From Bangalore Sreedharan et al[14] reported the incidence as 1.3%, while Rudrapatna et al[15] reported the incidence as 0.3%.

Age: In the present study, in immunocompetent subjects with and without symptoms, the highest incidence of cryptosporidium was in the age group of 0-5yrs i.e., 4% and 2.8% respectively.

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While in both groups the incidence was 0% in above 35 years of age. In immunosuppressed subjects there was only 1 positive case which was in above the 35 years age group. But as the cases in other age group were very few, the statistical significance is doubtful. In the present study, incidence is more in children which may be because the immune system usually not fully developed in infants therefore they are more prone to infection and in children, the exposure need not be as repeated as in adults.

Malla et al[11] from Chandigarh in their study of 375 subjects below 12 years of age reported the incidence 1.3% all of them below 2yrs of age. Saraswathi et al[6] from Mumbai reported the incidence of 5.6% again from the subjects below 2 years of age. Pherwani et al[9] from Mumbai in their study showed the maximum incidence of cryptosporidium parvum between 5-35yrs of age.

Sex: In the present study, in immunocompetent group of symptomatic subjects the incidence of Cryptosporidium parvum in male was little more (57.7%) than in females (42.9%). In immunocompetent group of asymptomatic it was positive only in 1 case i.e. in female but in immunosuppressed group with symptoms it was positive with 1 case which was a male patient. It indicates that there is no significant difference between the sexes. This was also supported by Casemore DP.[16]

Clinical Features: In immunocompetent subjects the commonest complaint associated with diarrhea was abdominal pain (60%) followed by vomiting (54.4%) but weight loss was in only 2.8% cases. In contrast in immunocompromised subjects the commonest symptom was weight loss (100%) followed by anorexia (90.4%) and abdominal pain (85.7%).The reason for this could be that the immunosuppressed subjects selected were cancer patients on chemotherapy and symptoms like weight loss and anorexia may be because of cancer rather than parasitic infection.

In the cryptosporidial infection the commonest symptoms associated with diarrhea was abdominal pain (50%), followed by vomiting (37.5%), weight loss (25%) while steatorrhoea and fever was least common i.e., 12.5% each. This was supported by Casemore et al[12] where they reported diarrhea in 88% followed by abdominal pain (66%), vomiting (55%), anorexia (43%), fever (12%). Giacometti et al in their study also supported these findings.

Parasitic Incidence: In the present study, out of 9 human subjects positive for cryptosporidium parvum 1 was also infected with Giardia lamblia and Ascaris lumbricoides in the other. In the remaining 7 no other parasite was detected.

Seasonal Variation: In the present study, the maximum incidence was in the month of Jan to Sept (Rainy Season) i.e. 55.55% which may be because of polluted rain water contaminating drinking water in ponds, unprotected wells etc. Similar findings were reported from Bangladesh[17], Brazil,[18] and Central America.[19]

Duration of Diarrhoea: The maximum duration of diarrhea was more than 1 month in 1 case which was in the immunosuppressed subject, 3 cases had diarrhea for less than 7 days (all in immunocompetent cases) and 4 had diarrhea for between 7-14 days. (all immunocompetent).

Water Supply: In the present study out of 9 positive cases, 6 had history of consumption of water from pipeline and the remaining 3 were drinking water from wells. Isolation from water was not attempted.

Animals: 9 cases of cryptosporidiosis had a history of keeping animals. Oocysts of cryptosporidium was detected from 1 buffalo out of 5, belonging to 1 patient positive for C.parvum.

In the animal study, out of a total of 100 animals 3 cows, 3 buffaloes, 2 cats and 1 dog yielded cryptosporidium in their faeces. Sargant et al and Lappin et al from Australia[20] and USA[21] in their studies detected Cryptosporidium in 1.2% and 2.4% respectively. Cawsap et al[22] from Spain in their study reported the incidence as 4.7% in dogs.

Olson et al[23] from British Columbia, Maldenabo et al[24] from Mexico and Fuente et al[25] from central Spain in their study in dairy cows reported the incidence as 59%, 25%, 52.3% respectively. Munoz et al[26] from Spain in his study reported positivity in goat kids 42% while 45% positivity in lambs. Khubnani et al[4] from India reported Cryptosporidium 10.89% in different animals.

CONCLUSION: In conclusion, routine screening of faecal specimens should be encouraged for detection of cryptosporidium. Water reservoirs should be prohibited for animal use since they are widely present in animal population and can cause outbreaks of cryptosporidial diarrhea.

REFERENCES:

1. Ananthnarayan R, Panikar CKJ. Acute Diarrhoeal Diseases in Text Book Of Microbiology, Orient Longman, 4th Ed 1990; 608-18.
2. Casemore DP, Warrel DA. Crytosporidium and Cryptosporidiosis. In Oxford Textbook of Med. 1996, 3rd Ed. (1): 869-876.
3. Michel JG, Farthing; Ana-Maria and Paul Kelly.Intestinal Protozoa in Manson's Tropical Diseases. Gordan C. ELBS. 28th Ed, 1996; 1283-98.
4. Khubnani H, Sivarajan K, Khubnani A. Study of Crytosporidiosis in a Rural Area of Maharashtra. Indian J. Pathol. Microbiol. 1997; (1): 33-36.
5. Mathan MM, Venkateshan S, George R, Mathew M and Mathan VI. Crytosporidiosis and Diarhoea In South Indian Children. Lancet II (8465) 1985; 1172-5.
6. Saraswathi K, Pandit DV, Deodhar LP and Bichik LS. Prevalence of Crytosporidiosis in Patients with Diarrhoea in Mumbai. IJMR, March 1988; 87: 121-224.
7. Kaur N and Diwan N. Cryptosporidiosis in North Indian Children. Indian J. Med Science 1991; 45 (6): 143-145.
8. Das P, Pal S, Dutta D, Bhattacharya MK et al. Crytosporidiosis in Bengali Children with Acute Diarrhoea. Trans.Roy Soc. Trop Med Hyg 1987; 81: 241.
9. Pherwani AV, Bhave SY, Bijm AM and Desai AG. Prevalence of Cryptosporidium in Children with Acute diarrhea. Indian J. Paediatric. 1989; 56: 133-135.
10. Macaden R, Sriniwas S and Bhat P. Preliminary Study on Cryptosporidiosis in Bangalore. Proceedings X National Congress of IAMM, Bangalore 1986; 83.
11. Malla N, Sehgal R, Ganguli NK and Mahajan RC. Crytosporidiosis in Children in Chandigarh. Indian J. Med Res. 1987; 86: 722-725.

12. Guar J, Matilde NJ et al. Frequency of Intestinal Parasites in Adult Cancer Patients in Mexico. Arch Med Res 1997 Summer; 28 (2): 219-22.
13. Hammounda NA, Sadaka Ha, El-Gebaly WM et al. Opportunistic Intestinal Protozoa in Chronic Diarrhoeic Immunosuppressed Patients. J. Egypt Soc Parasitol 1996 Apr; 26:143-53.
14. Sreedharan A, Jayshree RS, Sridhar H. Cryptosporidiosis among Cancer Patients: An Observation. J. Diarrhoeal Dis Res 1996 Sep; 14 (3): 211-113.
15. Rudrapatna JS, Kumar V, Sridhar H. Interstitial Parasitic Infection in Patients with Malignancy. J. Diarrhoel Dis Res 1997 Jun; 15 (2): 71-4.
16. Casemore DP. Epidemiological Aspects of Human Crytosporidiosis. Epidemol Infect 1990;104: 1-28
17. Rehman ASMH, Sanyal Sc, Al Mahmud KA, Sobhan A. Cryptosporidial diarrhea in calves and their Handlers In Bangladesh. Ind J. Med Res 1983; 82: 510-16
18. Gennari CML et al. Cryptosporidium Sp. In Children Suffering from Acute diarrhea at Ubert Andia City, State of Minas, Brazil, Mem Inst Oswaldo Cauz 1996 Sept-Oct; 91(5): 551-4
19. Casemore DP. Human Cryptosporidiosis: Recent Advances In Infection-3, Edited By Reeves DS, Geddes AM, Churchill Livingstone 1983;209-36
20. Sargent KD, Morgan UM, Elliot A, Thompson RC. Morphological and Genetic Characterisation of Cryptosporidium Oocysts fron Domestic Cats. Vet Parasitol 1998 Jun 30;70(4): 221-7
21. Lappin MR, Ungar B et al. ELISA for the detection of Cryptosporidium parvum IgG in the serum of Cats. J. Parasitol 1997 Oct; 83 (5):957-60
22. Causape AC, Quilez J, Sanchez AC. Prevalence of Intestinal Parasites Including C. Parvum in Dogs in Zaragoza City in Spain. Vet Parasitol 1996 Dec 31; 67(3-4):161-7
23. Olson ME, Guselle NJ, O Handley RM et al. Giardia and Cryptosporidium in Dairy Calves in Br. Columbia. Can Vet J 1997 Nov; 38 (11): 703-6
24. Maldonado CS, Atwill ER, Saltijeral OA et al. Prevalence of and risk factors for shedding of C. parvun in Holstein Freisian Dairy Calves in Central Mexica. Prev. Vet Med 1998 Aug; 36 (2): 95-107.
25. Funte R, Garcia A, et al. Proportional Morbidity Rates of enteropathogenes among Diarrhoeic Dairy Calves in Central Spain. Prev Vet Med 1998 Aug 7; 36 (2): 145-52.
26. Munoz M, Alvarez M, Lanza J et al. Role of enteric pathogens in the aetiology of neonatal diarrhea in lambs and goat kids in spain. Epidemol infect 1996 Aug; 117 (1): 203-11.

Sr. No.	Status	With Symptoms	Without Symptoms	Total
1	Immunocompetent	250	75	325
2	Immunocompromised	21	30	51
	Total	**271**	**105**	**376**

TABLE NO. 1: IMMUNE STATUS OF SYMPTOMATIC AND ASYMPTOMATIC PATIENTS

DOI: 10.14260/jemds/2014/3331

Age	Immunocompetents		Immunosuppressed	
	With symptoms n=250	Without symptoms n=75	With symptoms n=21	Without symptoms n=30
0-5yrs	100	35	02	02
6-35yrs	90	30	06	09
>35yrs	60	10	13	19

TABLE NO. 2: AGE WISE DISTRIBUTION OF SUBJECTS

Sr. No.	Immunocompetents		Immunosuppressed	
	With symptoms n=250	Without symptoms n=75	With symptoms n=21	Without symptoms n=30
Male	127	40	13	19
Female	123	35	08	11
Total	**250**	**75**	**21**	**30**

TABLE NO. 3: SEX WISE DISTRIBUTION OF SUBJECTS

Symptoms	Immunocompetent Subjects n=250	Immunosuppressed Subjects n=21
Abdominal Pain	150 (60%)	18 (85.7%)
Vomiting	136 (54.4%)	15 (71.42%)
Anorexia	115 (46%)	19 (90.4%)
Steatorrhoea	25 (10%)	08 (38.09%)
Fever	13 (5.2%)	12 (57.14%)
Flatulence	13 (5.2%)	12 (57.14%)
Weight loss	07 (2.8%)	21 (100%)

TABLE NO. 4: DISTRIBUTION OF SYMPTOMS ASSOCIATED WITH DIARRHEA

Sr. No.	Immunocompetents		Immunosuppressed		Total
	With symptoms	Without symptoms	With symptoms	Without symptoms	
Parasite detected	44 (17.6%)	5 (6.66%)	5 (23.8%)	1 (3.33%)	55 (14.63%)
No Parasite isolated	206 (82.4%)	70 (93.4%)	16 (76.2%)	29 (96.3%)	321 (85.37%)
Total	**250**	**75**	**21**	**30**	**376**

TABLE NO. 5: IMMUNE STATUS WISE DETECTION OF PARASITES (CRYPTOSPORIDIUM WITH OTHER PARASITES)

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Sr. No.	Immunocompetents		Immunosuppressed		Total
	With symptoms	Without symptoms	With symptoms	Without symptoms	
Single Parasite detected	30 (68.18%)	4 (80%)	4 (80%)	1 (100%)	39 (70.9%)
More than one Parasite	14 (31.2%)	1 (20%)	1 (20%)	0 (0%)	16 (29.09%)
Total	**44**	**5**	**5**	**1**	**55**

TABLE NO. 6: DISTRIBUTION OF PARASITE IN SUBJECTS ON THE BASIS OF IMMUNE STATUS

Parasites (n=72)	Immunocompetents		Immunosuppressed	
	With symptoms	Without symptoms	With symptoms	Without symptoms
Giardia lamblia	15 (25.42%)	1 (16.67%)	2 (33.33%)	0 (0%)
Entamoeba histolytica	9 (15.25%)	1 (16.67%)	1 (16.67%)	1 (100%)
Cryptosporidium parvum	7 (11.86%)	1 (16.67%)	1 (16.67%)	1 (100%)
Ancylostoma duodenale	7 (11.86%)	0 (0%)	0 (0%)	0 (0%)
Hymenolepis nana	6 (10.16%)	0 (0%)	0 (0%)	0 (0%)
Iodamoeba butschlii	5 (8.47%)	1 (16.67%)	0 (0%)	0 (0%)
Entamoeba coli	5 (8.47%)	2 (33.33%)	1 (16.67%)	0 (0%)
Ascaris lumbricoides	3 (5.1%)	0 (0%)	1 (16.67%)	0 (0%)
Taenia species	1 (1.69%)	0 (0%)	0 (0%)	0 (0%)
Enterobius vermicularis	1 (1.69%)	0 (0%)	0 (0%)	0 (0%)
Total parasites	59 (81.94%)	6 (8.33%)	6 (8.33%)	1 (1.38%)

TABLE NO. 7: DISTRIBUTION OF DIFFERENT PARASITES IN STUDY SUBJECTS

Sr. No.	Status	With Symptom	Without Symptom	Total
1	Immunocompetent	07	01	08
2	Immunocompromised	01	00	01
	Total	08	01	09

TABLE NO. 8: DETECTION OF CRYPTOSPORIDIUM IN DIFFERENT IMMUNE STATUS

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Age	Immunocompetents		Immunosuppressed	
	With symptoms n=250	Without symptoms n=75	With symptoms n=21	Without symptoms n=30
0-5yrs	04 (4%)	01 (2.8%)	00 (0%)	00 (0%)
6-35yrs	03 (3.33%)	00 (0%)	00 (0%)	00 (0%)
>35yrs	00 (0%)	00 (0%)	1 (7.7 %)	00 (0%)
Total	**7**	**1**	**1**	**0**

TABLE NO. 9: AGEWISE DETECTION OF CRYTOSPORIDIUM

Sr. No.	Immunocompetents		Immunosuppressed	
	With symptoms	Without symptoms	With symptoms	Without symptoms
Male	4 (57.1%)	0 (0%)	1 (100%)	0
Female	3 (42.9%)	1 (100%)	0 (0%)	0
Total	**7**	**1**	**1**	**0**

TABLE NO. 10: SEXWISE INCIDENCE OF CRYTOSPORIDIUM ISOLATION

Sr. No.	Immunocompetents		Immunosuppressed	
	With symptoms	Without symptoms	With symptoms	Without symptoms
Feb – May (Summer)	2 (28.5%)	0 (0%)	1 (100%)	0
June – Sept (Rainy)	4 (57.14%)	1 (100%)	0 (0%)	0
October – January (Winter)	1 (14.28%)	0 (0%)	0 (0%)	0
Total	**7**	**1**	**1**	**0**

TABLE NO. 11: SEASON WISE DETECTION OF CRYPTOSPORIDIUM

Sr. No.	Age in years	Immune Status	Chief Complaints	Other parasites	History of pets	Water Supply
1	1 yrs 6 months/M	Immunocompetent	Diarrhoea	Nil	Yes	P
2	2 yrs 3 months/M	Immunocompetent	Diarrhoea	Nil	No	P
3	2 yrs 4 months/M	Immunocompetent	Diarrhoea	Ova- A. lumbricoides	Yes	W
4	3 yrs 6 months/F	Immunocompetent	Diarrhoea	Nil	Yes	P
5	5 yrs 4 months/F	Immunocompetent	Diarrhoea	Giardia lamblia	Yes	W

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6	6 yrs/M	Immunocompetent	Diarrhoea	Nil	No	P
7	19 yrs/F	Immunocompetent	Diarrhoea	Nil	Yes	P
8	3 yrs/ F	Immunocompetent	Asymptomatic	Nil	Yes	W
9	65 yrs/M	Immunocompetent	Diarrhoea	Nil	No	P

TABLE NO. 12: CLINICAL DATA OF CASES POSITIVE WITH CRYPTOSPORIDIUM

Immune Status	< 7 days	< 14 days	15 days – 1month	> 1 month
Immunocompetent n=7	3	4	0	0
Immunosuppressed n=1	0	0	0	1

TABLE NO. 13: DURATION OF DIARRHOEA IN CRYTOSPORIDIOSIS

Symptoms	n=8
Diarrhoea	8 (100%)
Abdominal pain	4 (50%)
Vomiting	3 (37.7%)
Anorexia	2 (25%)
Weight loss	2 (25%)
Fever	1 (12.25%)
Steatorrhoea	1 (12.25%)

TABLE NO. 14: DISTRIBUTION OF SYMPTOMS IN EIGHT SYMPTOMATIC PATIENTS

Sr. No.	Animals	Total animals studied	Cryptosporidium detected	Percentage
1	Cow	23	3	13%
2	Buffalo	43	3	7%
3	Goat	10	0	0%
4	Cat	14	2	14.3%
5	Dog	10	1	10%

TABLE NO. 15: DETECTION OF CRYPTOSPORIDIUM IN DIFFERENT ANIMALS

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Date of Submission: 20/08/2014.
Date of Peer Review: 21/08/2014.
Date of Acceptance: 27/08/2014.
Date of Publishing: 02/09/2014.