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Isolation and Identification of Cryptosporidium Spp. From Raw Meat Samples Sold In Open Markets of the City of Kolkata.

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Abstract: *The present investigation was undertaken to detect the carriage rate of Cryptosporidium species in the raw meat samples collected from open markets of the city of Kolkata. In the present investigation 200 raw meat samples (100 chickens and 100 muttons) were utilized. The incidence of Cryptosporidium in meat sample is very sparse in India. In view of very limited literature, the present study had been carried out in the city of Kolkata where the meat samples from the open markets were processed. In this study Cryptosporidium spp. was found to be 6% in the meat samples. The poor sanitary measures and hygienic practices were prevailing in most of the places from where samples were collected which may cause infection to the human beings, if the meat is not handled properly. Again, cross contamination, particularly to those foodstuffs which are eaten raw may be another factor which may give rise to infection in human beings.*

Keywords: *Cryptosporidium, Kolkata, Meat, open market, Raw.*

I. Introduction

Meat is animal flesh that is eaten as food. Humans are omnivorous [1], and have hunted and killed animals for meat since prehistoric times [2]. Meat is the richest source of B vitamins, particularly thiamine, riboflavin and niacin. It also contains a good amount of fat soluble vitamin A, phosphorus, iron and copper. [3] Study suggested that diets that contain an average of 30 g per day of protein from animal sources will be adequate in quality regardless of the source of the remainder of the protein. Meat is highly susceptible to microbial contaminations, which can cause its spoilage and food borne infections in human, resulting in economic and health losses [4]. Protozoa are a diverse group of eukaryotic, typically unicellular, Microorganisms. The majority of protozoa are free-living organisms that can reside in fresh water and pose no risk to health. However, some protozoa are pathogenic to humans. These protozoa comprises of two functional groups: enteric parasites and free-living protozoa. Human infections caused by the free living protozoa are generally the result of contact during domestic usage of water other than drinking. Enteric protozoa, on the other hand, have been associated with the diseases transmitted through drinking water, food etc. In developing countries, the disease probably exerts most of its impact on pediatric health. In addition to the occurrence of diarrhoea, cryptosporidiosis has been attributed to malnutrition and stunted growth [5]. In developed countries, waterborne outbreaks of cryptosporidiosis have a significant economic impact. Cryptosporidium parvum, mostly considered to be the second common pathogen in young diarrheic calves, is a common pathogenic protozoan of the gastrointestinal tract of humans and animals [6]. A higher significant rate of diarrhoea was detected in calves infected with C. parvum than non infected calves suggesting that C. parvum is the likely cause of diarrhoea [7].Cryptosporidium species are apicomplexan parasites that infect the microvillus border of the gastrointestinal epithelium of a wide range of vertebrate hosts, including humans. Infected individuals show a wide spectrum of clinical presentations, but the pathogenicity of Cryptosporidium varies with the species of parasites involved and the type, age, and immune status of the host [8]. Direct transmission via the faecal/oral route is likely to be the most common route of transmission whether zoonotic or direct person to person [9]. However, indirect transmission, where infection results through the mechanical transmission of oocysts on, for example, flies [10] or other animals such as dogs or livestock, or by the contamination of food or water sources [11] poses a significant threat, particularly in the developing world. In the immune deficient patient, C. parvum infections were not always confined to the gastrointestinal tract, and additional clinical symptoms have been associated with the extra intestinal infections including a variety of respiratory problems, cholecystitis, hepatitis, and pancreatitis.[12]During the rainy season, prevalence of Cryptosporidium was 4% in river and sea water samples, but none in summer season[13]

Different species of Cryptosporidium that are currently considered valid include C. andersoni (cattle), C. baileyi (chicken and some other birds), C. canis (dogs), C. felis (cats), C. galli (birds), C. hominis (humans), C. meleagridis (birds and humans), C. molnari (fish), C. muris (rodents and some other mammals), C. parvum (ruminants and humans), C. wrairi (guinea pigs), C. saurophilum (lizards and snakes), and C. serpentis (snakes

and lizards) [14] . Therefore considering the importance of this species of the present work was undertaken to elucidate the isolation and identification of this species in raw meat.

II. Materials And Methods

In present study, 200 meat samples (100 chickens and 100 muttons) were collected from different open markets in and around Kolkata for detection of Cryptosporidium spp. Most of the samples were collected from road side meat shop. After that meat sample were homogenized and transferred to centrifuge tube and rotated at 3000 RPM for 5 minutes. The supernatant was discarded and the sediment is transferred to a clean grease free micro slide for detection of Cryptosporidium. It was detected by an in-house acid-fast procedure. The conventional methods for the detection of Cryptosporidium include concentration of the sample and staining of the faecal smear. The floatation method is the most suitable method for the detection from samples containing a small number of Cryptosporidium oocysts. Study [15] suggested that the results of the sugar floatation methods were reliable for samples including more than $1.0x10^3$ oocysts/ml.

III. Result And Discussion

The parasitic Zoonoses are a major burden on public health and wellbeing. The magnitude and scope of this burden varies for each of the parasites. Enteric protozoa like Cryptosporidium spp. is of major public health concern as they have been reported to have zoonotic potential. Domestic animals living in close contact with the human beings serve as the main source of infection of these protozoa to the human beings. In this study Cryptosporidium spp. was found to be 6% in the meat samples (Table.1). Different studies show that diarrheal disease was the third leading cause of death in low-income countries in 2004, causing 6.9% of deaths overall (WHO, 2009) and fourth in the year 1990 (2.9 million) [16]. Parasitic nematodes are one of the most important causes of production losses in most cattle-producing countries of the world [17].The enteric protozoan parasite, Cryptosporidium, was first recognized as a human parasite in 1976, when it was reported as a causative agent of diarrhea in a three-year-old child with self-limiting enterocolitis. It was not until the emergence of the HIV pandemic in the 1980's that Cryptosporidium became widely recognized as an important human pathogen. The first case of cryptosporidiosis in a homosexual man with AIDS was reported in 1982. Since then, numerous reports worldwide have identified cryptosporidiosis as a significant pathogen in HIV/AIDS. There are multiple routes of transmission of cryptosporidiosis in humans, including person-to-person, waterborne, foodborne and zoonotic. In 1993, Cryptosporidium sparked great public health interest after a very large waterborne outbreak in Milwaukee, Wisconsin, which resulted in 403,000 people being affected, with 5,000 confirmed cases of Cryptosporidiosis and 100 fatalities, mostly involving immune compromised individuals. Currently, Cryptosporidiosis is commonly reported in HIV-infected individuals and is listed as an AIDS-defining illness (Clinical Category C) by the US Centers for Disease Control and Prevention. The infection in individuals with HIV/AIDS is persistent and life threatening and often involves infections of the entire gastrointestinal tract in addition to hepatobiliary and respiratory tract infections. The most common presentation of cryptosporidiosis in patients is chronic or watery diarrhea, resulting in severe dehydration, abdominal pain, vomiting, nausea, low-grade fever, malnutrition and significant weight loss. Prolonged infections contribute to severe morbidity and mortality in HIV/AIDS patients. The infection rates were found to be 1.5% and 0.15% for Cryptosporidium in HIV positive patients receiving antiretroviral therapy and HIV negative controls respectively in Shanghai, China [18]. High mortality rate and case fatality rate of 35.2 and 44.4% were observed in young calves between 0 and 30 days of age [19].

Modes of transmission of Cryptosporidium include mechanical transport through soil and insects such as cockroaches and houseflies [20].Although data on prevalence and molecular characterization are currently available from various countries in regions such as Africa, Asia, Europe, North America and South America, there has been little effort in collating these data and placing information into perspective for future work, and the actual global status of the species, genotypes and sub genotypes of Cryptosporidium in HIV-infected individuals is currently difficult to assess. Therefore, the aims of the present article were to review the current global knowledge in terms of prevalence and molecular characterization of cryptosporidiosis in HIV-infected individuals and to offer recommendations for an improved understanding of Cryptosporidium infections and its global epidemiology, and control measures in HIV-infected individuals.

Cryptosporidium was detected in 2.8% of the faecal sample, from animals in nine Melbourne Water reservoir areas, collected over a period of two-years [21]. 6% of fecal samples from wild and domestic animals tested during 2007 to 2010 were positive for Cryptosporidium oocysts in California [22]. In another study, 59.1% domestic animals and 14.5% of humans were found to have Cryptosporidium spp. infection in Shushtar district from Khuzestan Province, south- west of Iran [23]. Diagnostic PCR revealed a prevalence of Cryptosporidium of 32.4% in humans, 11.1% in non-human primates, and 2.2% in livestock in Uganda [24] The overall prevalence of Cryptosporidial infection in human samples was 10.8% in Iran, but the prevalence (25.6%)

in diarrheic humans was higher than that (3.7%) in non-diarrheic humans. Oocysts of Cryptosporidium spp. were detected in the feces of 21.4%, 9.3%, 8.8%, 6.7% and 5.7% of different age groups respectively [25]. 9·9% of the samples collected in Yemen, from patients who attended a hospital in Sana'a city, were positive for Cryptosporidium of which, 97% were identified as C. parvum while 3% was caused by C. hominis [26]. The incidence of Cryptosporidiosis ranges from 5-62.4% in various species of animals across the world. The prevalence of Cryptosporidiosis in cattle, buffaloes, dogs in Calcutta were 11.32%, 12.97% and 0 % respectively and that in animal handlers were found to be 3%, [27].

According to the literature, available so far, regarding the incidence of Cryptosporidiosis in India, studies were mainly carried out on the isolation of Cryptosporidium spp. from the faecal matters from different animals and also from the animal handlers. The present study probably depicts the first study where the incidence of carriage of Cryptosporidium spp. in meat samples, available in open markets in the city of Kolkata, had been done. The poor sanitary measures and hygienic practices were prevailing in most of the places from where samples were collected which may cause infection to the human beings if the meat is not handled properly. Again, cross contamination, particularly to those foodstuffs which are eaten raw may be another factor which may give rise to infection in human beings.

IV. Conclusion

In the present study probably depicts the first study where the incidence of carriage of Cryptosporidium spp. in meat samples, available in open markets in the city of Kolkata, had been done. The poor sanitary measures and hygienic practices were prevailing in most of the places from where samples were collected which may cause infection to the human beings if the meat is not handled properly. Again, cross contamination, particularly to those foodstuffs which are eaten raw may be another factor which may give rise to infection in human beings.

References

[1]. Robert, E. C., Wildman, D. M. M., Advanced Human Nutrition. CRC Press.2000, 37.
[2]. **M**cArdle, John. "Humans are Omnivores". Vegetarian Resource Group. Retrieved October 6, 2013.
[3]. Altschul A. M., Proteins: The chemistry and politics. Basic Books, New York. 1965.
[4]. Komba E. V. G., Komba, E. M. Mkupasi, A. O. Mbyuzi, S. Mshamu, D. Luwumbra, Z. Busagwe and A. Mzula,Sanitary practices and occurrence of zoonotic conditions in cattle at slaughter in Morogoro Municipality, Tanzania: implications for public health. Tanzania Journal Health Research, 14 (2),2012,
[5]. Mølbak, K., Andersen, M., Aaby, P., Højlyng, N., Jakobsen, M., Sodemann, M., & Da Silva, A. P., Cryptosporidium infection in infancy as a cause of malnutrition: a community study from Guinea-Bissau, West Africa. The American journal of clinical nutrition, 65(1), 1997, 149-152.
[6]. Noordeen, F., Rajapakse, R. P. V. J., Faizal, A. C. M., Horadagoda, N. U., & Arulkanthan, A., Prevalence of Cryptosporidium infection in goats in selected locations in three agroclimatic zones of Sri Lanka. Veterinary parasitology, 93(2), 2000, 95-101.
[7]. Soliman, N., Cryptosporidium infection among primary school children in a rural area in Alexandria. The Journal of the Egyptian Public Health Association, 67(3-4), 1992, 501.
[8]. Xiao, L., Fayer, R., Ryan, U., & Upton, S. J., Cryptosporidium taxonomy: recent advances and implications for public health. Clinical Microbiology Reviews, 17(1), 2004, 72-97.
[9]. Thompson, R. C. A., & Smith, A., Zoonotic enteric protozoa. Veterinary parasitology, 182(1), 2011, 70-78.
[10]. Szostakowska, B., Kruminis-Lozowska, W., Racewicz, M., Knight, R., Tamang, L., Myjak, P., & Graczyk, T. K.,Cryptosporidium parvum and Giardia lamblia recovered from flies on a cattle farm and in a landfill. Applied and environmental, 70 (6), 2004, 3742-3744.
[11]. Karanis, P., Kourenti, C., & Smith, H., Waterborne transmission of protozoan parasites: a worldwide review of outbreaks and lessons learnt. Journal of Water and Health, 5(1), 2007, 1-38.
[12]. Fayer, R., & Ungar, B. L.,Cryptosporidium spp. and cryptosporidiosis. Microbiological reviews, 50(4), 1986, 458.
[13]. Koompapong, K., & Sukthana, Y.,Seasonal variation and potential sources of Cryptosporidium contamination in surface waters of Chao Phraya River and Bang Pu Nature Reserve pier, Thailand. The Southeast Asian journal of tropical medicine and public health, 43(4), 2012, 832-840.
[14]. Fayer, R., Santín, M., & Trout, J. M.,Cryptosporidium ryanaen. sp. (Apicomplexa: Cryptosporidiidae) in cattle (Bos taurus). Veterinary parasitology, 156(3), 2008, 191-198.
[15]. Fujino, T., Matsuo, T., Okada, M., & Matsui, T., Detection of a small number of Cryptosporidium parvum oocysts by sugar flotation and sugar centrifugation methods. The Journal of veterinary medical science/the Japanese Society of Veterinary Science, 68(11), 2006, 1191.
[16]. Murray, C. J., & Lopez, A. D.,Global mortality, disability, and the contribution of risk factors: Global Burden of Disease Study. Lancet, 349(9063), 1997, 1436-1442.
[17]. Huang, C. C., Wang, L. C., Pan, C. H., Yang, C. H., & Lai, C. H., Investigation of gastrointestinal parasites of dairy cattle around Taiwan.Journal of Microbiology, Immunology and Infection.44(6);2012.
[18]. Wang, L., Zhang, H., Zhao, X., Zhang, L., Zhang, G., Guo, M. & Xiao, L., Zoonotic Cryptosporidium species and Enterocytozoon bieneusi genotypes in HIV- positive patients on antiretroviral therapy. Journal of clinical microbiology, 51(2), 2013, 557-563.
[19]. Singh, B. B., Sharma, R., Kumar, H., Banga, H. S., Aulakh, R. S., Gill, J. P. S., & Sharma, J. K.,Prevalence of Cryptosporidium parvum infection in Punjab (India) and its association with diarrhoea in neonatal dairy calves. Veterinary parasitology, 140(1), 2006, 162-165.
[20]. Dillingham, R. A., Lima, A. A., & Guerrant, R. L., Cryptosporidiosis: epidemiology and impact. Microbes and Infection, 4(10), 2002, 1059-1066.

[21]. Nolan, M. J., Jex, A. R., Koehler, A., Haydon, S. R., Stevens, M. A., & Gasser, R. B., Molecular-based Investigation of Cryptosporidium and Giardia from Animals in Water Catchments in Southeastern Australia. Water research. Norway. Journal of Food Protection. 64(11),2012,1793–1798

[22]. Oates, S. C., Miller, M. A., Hardin, D., Conrad, P. A., Melli, A., Jessup, D. A., & Miller, W. A., Prevalence, environmental loading, and molecular characterization of Cryptosporidium and Giardia isolates from domestic and wild animals along the central California coast. Applied and environmental microbiology, 78(24), 2012, 8762-8772.

[23]. Heidarnegadi, S. M., Mohebali, M., Maraghi, S., Babaei, Z., Farnia, S., Bairami, A., & Rezaeian, M., Cryptosporidium Spp. Infection in Human and Domestic Animals. Iranian Journal of Parasitology, 7(1), 2012, 53-58.

[24]. Salyer, S. J., Gillespie, T. R., Rwego, I. B., Chapman, C. A., & Goldberg, T. L., Epidemiology and Molecular Relationships of Cryptosporidium spp. in People, Primates, and Livestock from Western Uganda. PLoS Neglected Tropical Diseases, 6(4), 2012, 1597.

[25]. Mirzaei, M., Prevalence of Cryptosporidium sp. infection in diarrheic and nondiarrheic humans in Iran. The Korean journal of parasitology, 45(2), 2007, 133-137.

[26]. Alyousefi, N. A., MAHDY, M., LIM, Y., Xiao, L., & Mahmud, R., First molecular characterization of Cryptosporidium in Yemen. Parasitology, 2013, 1-6.

[27]. Chattopadhyay, U. K., Chowdhury, D., Dasgupta, C. K., & Pramanik, A. K., Prevalence of cryptosporidiosis in man and animals in an around Calcutta. Journal of Veterinary Parasitology,14,2000,167-168.

Table 1: Distribution of Cryptosporidium in different zones of Kolkata .

Nature of sample	Total Positive	Zones				
		Central	East	West	North	South
Chicken (N=100)	7	2	1	1	2	1
Mutton (N=100)	5	1	1	2	1	0
Total = 200	12	3	2	3	3	1