Gastroenterology and Hepatology From Bed to Bench

EDITORIAL

The importance of subtype analysis of *Cryptosporidium* spp. in epidemiological investigations of human cryptosporidiosis in Iran and other Mideast countries

Ehsan Nazemalhosseini-Mojarad[1], Yaoyu Feng[2], Lihua Xiao[3]

[1] *Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

[2] *School of Resource and Environmental Engineering, East China University of Science and Technology, Shanghai, China*

[3] *Division of Foodborne, Waterborne and Environmental Diseases, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, Public Health Services, Atlanta, U.S.A*

Despite the clinical and public health importance of *Cryptosporidium parvum*, little is known about its transmission dynamics in cattle and other farm animals, especially in Iran and other Mideast countries. Currently, the maintenance of the parasites on cattle farms and the role of herd-to-herd transmission in cryptosporidiosis epidemiology are not clear (1).

Recent molecular epidemiologic studies of cryptosporidiosis have helped researchers to better understand the transmission of cryptosporidiosis in humans and the public health significance of *Cryptosporidium* spp. in animals and the environment (2- 3).

Because of the ability of *Cryptosporidium* spp. to infect humans and a wide variety of animals, and because of the ubiquitous presence of *Cryptosporidium* oocysts in the environment, humans can acquire *Cryptosporidium* infections through several transmission routes, such as direct contact with infected persons (person-to-person transmission) or animals (zoonotic transmission), and ingestion of contaminated food (foodborne transmission) and water (waterborne transmission). The relative importance of these transmission routes in the epidemiology of cryptosporidiosis is not entirely clear, largely due to the fact that traditional diagnostic tools do not have the ability to differentiate sources of parasites (3).

The use of molecular tools has been helpful in assessing the zoonotic potential of various *Cryptosporidium* species and the sources of human infection; it has begun to play a significant role in the characterization of transmission dynamics in different areas and in the determination of host specificity of various *Cryptosporidium* spp. The 60 kDa glycoprotein (gp60, also known as Cpgp15/45) gene encodes a precursor protein that is proteolytically cleaved to yield mature surface glycoproteins gp45 and gp15 (also known as Cp17), both of which are implicated in the attachment and invasion of enterocytes by sporozoites and merozoites.

Received: 1 October 2011 *Accepted*: 18 December 2011
Reprint or Correspondence: Lihua Xiao, DVM, PhD. Division of Foodborne, Waterborne and Environmental Diseases, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, Public Health Services, Atlanta, U.S.A
E-mail: lxiao@cdc.gov

Table 1. Distribution of *Cryptosporidium* spp. in humans in Mideast countries

Population	N	*C. hominis*	*C. parvum*	*C. meleagridis*	*C. felis*	*C. canis*	Mixed species	Reference
Iran	15	4	12					(9)
Iran	24	17	7					(10)
Iran	21	15	6					(11)
Iran	25	3	22					(12)
Turkey	4		4					(19)
Kuwait	62	3	58				2	(5)
Kuwait	83	22	61					(20)
Jordan	44	20	22	1				(21)
Saudi Arabia	31	13	15	1			1	(22)
Saudi Arabia	53	9	43				1	(23)
Egypt	36	24	10	2				(24)
Egypt	15	9	3					(25)

Table 2. Distribution of *C. parvum* subtypes in humans and cattle in Iran.

subtype families	Subtype	No. of isolates	Source of Samples	Accession number
IIa	IIa A16G3R1	1	Cattle	AB560739
IId	IId A15G1	2	Cattle	AB560740
IIa	IIa A15G2R1	22	Cattle	AB560741
IId	IId A26G1	1	Children with diarrhea	AB560742
IId	IId A18G1	3	Children with diarrhea	AB560743
IIa	IIa A16G3R1	1	Children with diarrhea	AB560744
IId	IId A20G1a	9	Children with diarrhea	AB560745
IId	IId A21G1a	1	Children with diarrhea	AB560746
IIa	IIa A15G2R1	6	Children with diarrhea	AB560747
IId	IId A15G1	1	Children with diarrhea	AB560748

An important feature of this gene is its high degree of sequence polymorphism in *C. hominis*, *C. parvum*, and *C. meleagridis* isolates. Several subtype families have been identified in these species: 7 subtype families in *C. hominis* (Ia–Ig), 2 zoonotic (IIa, IId) and 10 non-zoonotic (IIb, IIc, IIe–IIl) subtype families in *C. parvum*, and 6 subtype families in *C. meleagridis* (4). Within each subtype family, there are multiple subtypes based primarily on the number of tri-nucleotide repeats coding for the amino acid serine, as suggested by Sulaiman et al. (2005)(5).

The use of gp60 subtyping has allowed the identification of geographic and temporal differences in the transmission dynamics of cryptosporidiosis, the role of zoonotic infections in epidemiology, better appreciation of the public health significance of *Cryptosporidium* species/genotypes in ruminants and significance of parasite subtypes/strains in clinical manifestations and outbreak potentials, and the tracking of infection and contamination sources during outbreak and endemic investigations (1-5).

To our knowledge, there are several molecular epidemiological studies that have documented the presence of *C. parvum* and *C. hominis* In Iran (Table 1) (6-12). However, the distribution of subtypes of the two species in humans, animals and environmental is unclear. In the first characterization of *Cryptosporidium* subtypes in humans and cattle in Iran by sequence analysis of the gp60 gene, 47 samples of *C. parvum* (22 from children and 25 from cattle) and three of *C. hominis* (all from children) were characterized. Nine subtypes (two of *C. hominis* and seven of *C. parvum*) belonging to four subtype families were found. Cattle were mainly infected with *C. parvum* IIa subtypes and humans mostly with *C. parvum* IIa and IId subtypes (Table 2). The predominance of IIa and IId subtypes underlines

Table 3. Distribution of *C. parvum* subtype families in humans in Mideast countries

Location	N	Subtype Family						Reference
		IIa	IIc	IIb	IId	IIe	Other	
Kuwait	59	28	2	0	29	0	1	(5)
Kuwait	61	29	10	0	19	0	3	(20)
Saudi Arabia	37	1	2	0	34	0	0	(23)
Jordan	13	3	2	0	8	0	0	(21)
Iran	22	7	0	0	15	0	0	(13,14,15)

the importance of zoonotic *Cryptosporidium* transmission in Iran. Thus, cattle could be a source of human infection with *C. parvum* IIa in Iran (13-15). Although the source of IId subtypes in humans is not yet clear, IId subtypes are known to be common in sheep and goats in some countries such as Spain (16) and in dairy cattle in some other countries such as Egypt (17) and China (18). Further molecular study in humans and animals is needed in order to determine the extent and animal source of zoonotic transmission of cryptosporidiosis in Iran.

The dominance of *C. parvum* and wide occurrence of IId *C. parvum* subtypes in humans in Iran is similar to the situation seen in other Mideast countries(9-12, 19-25) (Tables 1 and 3). Children in the Kuwait City are almost exclusively infected with IIa and IId subtypes, although they have little contact with farm animals. As the city uses desalinized sea water as drinking water, the *C. parvum* transmission appears to be anthroponotic in origin (5, 20). IId subtypes are also common in children in Saudi Arabia and Jordan (Table 3). In many industrialized nations in other areas, *C. parvum* infections are much less common in humans than *C. hominis* infections, with the exception of European countries and New Zealand, where both *C. parvum* and *C. hominis* are commonly seen in humans. In these industrialized nations, most *C. parvum* infections are caused by the IIa subtypes commonly found in cattle, indicating zoonotic transmission plays a significant role in cryptosporidiosis epidemiology. In contrast, humans in developing countries are much less commonly infected with *C. parvum* and most of the few *C. parvum* infections are caused by the anthroponotic IIc subtype family (2).

In conclusion, preliminary molecular epidemiological studies have revealed some unique features of cryptosporidiosis transmission in humans in Iran and other Mideast countries. As the *C. parvum* subtype family IId was the dominant family causing cryptosporidiosis in humans in Iran (13-15), zoonotic transmission could possibly be involved. However, more extensive sampling of both humans and farm animals, especially sheep and goats, and collection of epidemiological data in case-control and longitudinal studies are needed for a better understanding of the sources of *C. parvum* infections in humans in Iran and other Mideast countries.

Acknowledgment

This work was supported in part by the National Natural science Foundation of China (grant no. 31110103901). The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

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