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

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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.
The importance of subtype analysis of Cryptosporidium spp. in epidemiological investigations

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              | IIa A15G1   | 2               | Cattle                    | AB560740         |
| III              | IIa A15G2R1 | 22              | Cattle                    | AB560741         |
| III              | IIa A26G1   | 1               | Children with diarrhea    | AB560742         |
| III              | IIa A18G1   | 3               | Children with diarrhea    | AB560743         |
| III              | IIa A16G3R1 | 1               | Children with diarrhea    | AB560744         |
| III              | IIa A20G1a  | 9               | Children with diarrhea    | AB560745         |
| III              | IIa A21G1a  | 1               | Children with diarrhea    | AB560746         |
| III              | IIa A15G2R1 | 6               | Children with diarrhea    | AB560747         |
| III              | IIa 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, Ile–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  | IIa | IIc | IIb | IId | IIe | Other | Reference |
|---------------|----|-----|-----|-----|-----|-----|-------|-----------|
| 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 desalinated 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. 311110103901). 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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