Prevalence and identification of Cryptosporidium species in immunocompetent paediatric patients with diarrhoea in the department of Seine-Maritime, upper-Normandy (North western, France)

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Prevalence and identification of *Cryptosporidium* species in immunocompetent paediatric patients with diarrhea in the Department of Seine-Maritime, Upper-Normandy (north-western France)

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CONTEXT

• Cryptosporidiosis caused by protozoan parasites of the genus *Cryptosporidium* is a worldwide spread diarrheal disease primarily affecting children and immunocompromised patients, recognized by the WHO's Neglected Diseases Initiative. Seroprevalence rates of 25-35% have been reported in industrialized countries and reaches 42% in China, 57% and 64% in Latin America. The prevalence of Cryptosporidium infection is lower in Europe (1-2%) than in developing areas. According to a 2012 report from the ECDC, an increase in *Cryptosporidium* infections has been observed in several European countries. Human cryptosporidiosis worldwide is caused by 2 major species, *C. parvum* and *C. hominis*. *C. hominis* is transmitted only between humans whereas *C. parvum* infections can result from either zoonotic or anthroponotic transmission. *C. parvum* infects a wide range of vertebrates, including cattle which acts as the major disease reservoir for human transmission. There is no reliable information on the prevalence of cryptosporidiosis in France and data on the prevalence and incidence of cryptosporidiosis are needed. Sporadic cases of cryptosporidiosis are not reported at regional or national level (it is not a notifiable disease). Moreover, we have insufficient information on infecting genotypes. For this reason, cryptosporidiosis is still considered a rare disease and remains underdiagnosed.

• Acute gastroenteritis is an extremely common problem in childhood, particularly in the first 3 years of life however, few data on the role of *Cryptosporidium* as etiologic agent is unknown. This study was conducted to assess the role of *Cryptosporidium* in the etiology of childhood diarrhea in immunocompetent children in Upper-Normandy, France.

MATERIALS and METHODS

Study design

During the 34-months period study (between January 2007 and November 2014), stool specimens from children (aged from 6 months to 16 years old) with acute gastroenteritis, were prospectively screened for the presence of *Cryptosporidium* oocysts.

Children were attending :

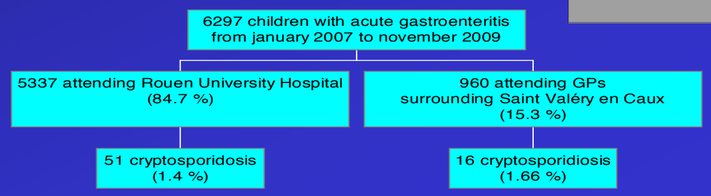
• either the Pediatric emergency service of Rouen University Hospital, Upper-Normandy (North-western France). Stools were subsequently tested at the Parasitology laboratory of the University hospital of Rouen

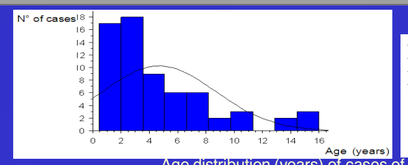
• either general practitioners around Saint Valéry en Caux, 60 kms from Rouen. Stools were subsequently tested at a private clinical laboratory in Saint Valéry en Caux.

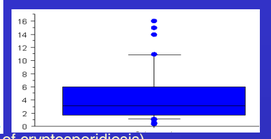
Cryptosporidium oocyst detection and species and subgenotype identification

The presence of *Cryptosporidium* was assessed by microscopy after Heine staining of faecal thin smears. *Cryptosporidium* species identification was based on polymerase chain reaction with PCR targeting the Hsp70 and/or the 18S rRNA genes followed by 18S rRNA gene fragment sequencing (Rochelle *et al.* Appl. Environ Microbiol 1997; Johnson *et al.* Appl Environ Microbiol 1995; Dalle *et al.* J Clin Microbiol. 2003). For subgenotyping analysis of *Cryptosporidium parvum* isolates, DNA samples were subject to amplification of a 850bp fragment of the GP60 gene using a nested PCR. Subgenotypes were identified by sequence analysis as described by Alves M *et al.* (J Clin Microbiol 2003; 41: 2744–2747).

RESULTS

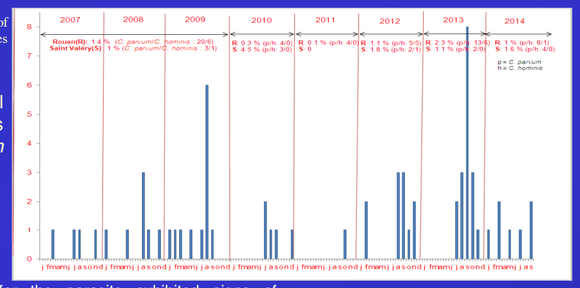


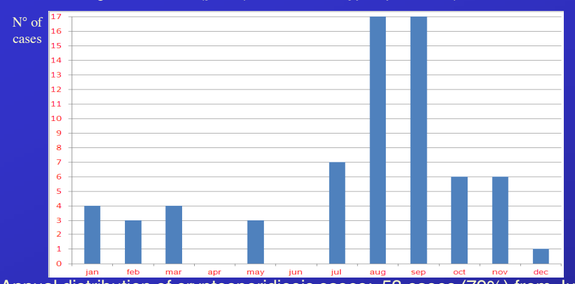




Age distribution (years) of cases of cryptosporidiosis)

Monthly total numbers of cases of *Cryptosporidium*





Annual distribution of cryptosporidiosis cases: 53 cases (79%) from July to November, 32 ases (48 %) in August and September

• None of the children positive for the parasite exhibited signs of immunodeficiency or had received immunosuppressive drugs

• 4 patients were co-infected : 1 by an adenovirus and 3 by *Campylobacter*

• Hospitalization was necessary for 46/51 patients who attended Rouen Hospital and 1/16 who was seen in Saint Valéry en Caux

Clinical expression of cases of cryptosporidiosis

All children :

- diarrhea (mostly liquid stool) : 64/67 (95.5%) for 6.1 days [3 - 20] before diagnostic
- vomiting : 42/67 (63%)
- fever : 31/67 (46%) (61% for *C. hominis*, 43% for *C. parvum*)

Out of hospitalized children :

- vomiting : 39/46 (85%)
- abdominal pains: 15/46 (32%)
- dehydration (moderate to severe)/weight loss: 25/46 (54%)

Species involved	Home location (cases not associated with travel abroad)	Direct contact with farm animals (cattle)	Contact with recreational waters	Travel abroad within 15 days before the onset of disorders
C. parvum: 49/67 (73%) *C. hominis*: 18/67 (27%)	rural : 18/54 semi-rural: 7/54 urban : 29/54	8/67 (12%)	3/67 (4.5%)	12/67 (18%) 3 *C. parvum* 9 *C. hominis*

Subgenotyping of *C. parvum* isolates

Analysis of the Gp60 gene performed on 21 isolates, apparently acquired locally, in Normandy, revelead two *C. parvum* allele families (IIa, IId) and five distinct subgenotypes, IIaA15G2R1 (for 16 distinct isolates), IIaA17G2R1 (2 isolates), IIaA18G1R1 (1 isolate), IIdA23G1 (1 isolate), IIaA10G2R1 (1 isolate)

DISCUSSION

Our data on prevalence are in the range of published data : 0-3 % in Europe (Guarino *et al.* J Pediatr Gastroenterol Nutr 2008), 1.4% in England (Baxby & Hart, 1986), 1.6 % in West Germany (Freidank & Kist 1987), 0.4-1% in the USA (Rose & Slifko 1999). The predominance of *C. parvum* is in contrast to findings from many other industrial and developing countries, where *C. hominis* often dominates (Chalmers *et al.* Epidemiol Infect 2011; Gatei *et al.* Am J Trop Med Hyg 2006; Ng *et al.* Exp Parasitol 2010). In Europe, the two species are rather evenly distributed, with *C. parvum* being more prevalent in some reports (Chalmers *et al.* Epidemiol Infect 2011; Zintl *et al.* Epidemiol Infect 2009) and *C. hominis* in others (Chalmers *et al.* Eurosurveillance 2009; Wielinga *et al.* Int J Parasitol 2008). A primarily zoonotic transmission route of domestic *C. parvum* infection was indicated, because all such isolates belonged to zoonotic allele families IIa and IId. Infections with *C. parvum* have probably been linked to contact with farms and farm animals, and infections with *C. hominis* with travel abroad and/or contact with other individuals with diarrhea. In an urban area, where contact with farm animals is minimal, other transmission routes like consumption of contaminated food or water or contact with recreational waters might possibly have been more important. The predominance of cases in late summer and autumn has also been noted in reports from the USA and other European countries including France (Semenza *et al.* Eurosurveillance 2007; McLauchlin *et al.* J Clin Microbiol 2000; Lake *et al.* Eur J Epidemiol 2007; ANOFEL Network. Eurosurveillance 2010), except for the UK and Ireland, where a spring peak, mainly due to *C. parvum* infections, was noted (Zintl *et al.* Epidemiol Infect 2009). Many children were hospitalized and in need of intravenous rehydration. There were only slight differences in the intensity of clinical symptoms by species in the present study, in agreement with reports from the UK and France (Chalmers *et al.* Epidemiology and Infection 2011; ANOFEL Network. Eurosurveillance2010). *C. parvum* cases in this study all belonged to zoonotic allele families IIa and IId. Little is known about *Cryptosporidium* prevalence, species and subtype distribution and zoonotic potential in French animals. However, subtype IIaA15G2R1, frequently identified in domestic cases in this study, was common in calves. We also identified 3 other subtypes: IIaA17G2R1 isolated from 2 children, one has been in contact with farm animals; IIdA23G1 isolated from a 2 year old boy seriously ill with hepatobiliary disease; IIaA10G2R1 isolated from a 6 year old girl with fever, abdominal pain and vomiting leading to severe dehydration.