AA-007

NDSU

Zoonotic transmission of *Cryptosporidium parvum*

Grant Herges, Christopher Pennil, Catherine Giddings, and John McEvoy
North Dakota State University
Department of Veterinary and Microbiological Sciences

Contact Information:
Grant Herges
North Dakota State University
Van Es 132
Fargo, ND 58102
(701)-231-8591
Grant.Herges@ndsu.edu

Abstract

Background: *Cryptosporidium* causes cryptosporidiosis, an infectious diarrheal disease, which can become chronic and life-threatening in immunocompromised individuals. *Cryptosporidium parvum* and *C. hominis* are the primary cause of human cryptosporidiosis. Of these species, *C. hominis* only infects humans while *C. parvum* additionally infects ruminants, in particular neonatal calves. A key knowledge gap remains in our understanding of the role of zoonotic transmission in human cryptosporidiosis. We hypothesize that zoonotic transmission of *C. parvum* is significant in the Midwest United States. To test this hypothesis, we used multilocus genotyping to characterize the relationship between *C. parvum* isolates from humans and neonatal calves in the region. **Methods:** We examined 216 isolates from cases of human cryptosporidiosis in Minnesota and Wisconsin and 39 isolates from diarrheic calves in Minnesota and North Dakota. Each isolate was genotyped at eight micro- or minisatellite loci using either fragment size analysis or sequencing. Genotypes from each locus were combined to give a multilocus type (MLT) for each isolate. **Results:** Eight MLTs were common to cattle and humans including the most common MLT identified. Two of these MLTs accounted for 23 % of typed isolates. **Conclusion:** The finding of a significant commonality of *C. parvum* genotypes between cattle and humans supports the hypothesis of zoonotic transmission in the Midwest United States.

Introduction

Cryptosporidiosis is an intestinal disease caused by a number of species within the genus *Cryptosporidium* (7). While the disease is normally self-limiting, it can become chronic and life threatening in individuals with a compromised immune system. There is currently no effective treatment for cryptosporidiosis (7). Data from our lab (2 and unpublished) has shown that *C. parvum* is responsible for most human cryptosporidiosis cases in Wisconsin and Minnesota. *C. parvum* infects humans and ruminants, particularly neonatal cattle, and may be transmitted directly from animal to human or human to human, and indirectly via contaminated food and water. Wisconsin and the neighboring states of Minnesota and North Dakota consistently have among the highest incidences of cryptosporidiosis in the nation (4). Therefore, understanding the role that zoonotic transmission plays in human cryptosporidiosis will lead to more effective control of contamination reservoirs.

Objective

To understand the transmission dynamics of *C. parvum* in the upper Midwest states of Wisconsin, Minnesota, and North Dakota

Materials and Methods

Isolate source

216 *C. parvum* isolates were obtained from separate cases of human cryptosporidiosis in Minnesota and Wisconsin. Thirty nine *C. parvum* isolates were obtained from separate cases of calf cryptosporidiosis in Eastern North Dakota and Minnesota. All isolates had previously been confirmed as *C. parvum* by PCR-RFLP analysis of the 18s rRNA gene.

Targets for the multilocus typing of *C. parvum* isolates

Seven regions of the *C. parvum* genome (GP60, CP47, MS5, MS9, MSC 6-7, DZ-HRGP and TP14) were identified based on the presence of microsatellites and previous reports of their polymorphism (1, 5, 6). A sixth region (GRH) was located using data from CryptoDB (www.cryptodb.org) and primers were designed using CLC Main Workbench 4 (CLC bio).

Sequence analysis

The amplified fragment of the GP60 gene was sequenced in both directions. Sequences were assembled and edited using SeqMan (DNAStar) and aligned using the Clustal W algorithm in MegAlign (DNAStar).

Fragment size analysis

The sizes of fragments amplified from CP47, MS5, MS9, GRH, MSC 6-7, DZ-HRGP and TP14 loci was determined using a fluorescence based fragment sizing approach. Briefly, the forward primer in each PCR reaction (or secondary reaction in the case of a nested PCR) was labeled at the 5' end with FAM (a fluorescent dye). The resulting fluorescent product following PCR amplification was separated on a 3730xl DNA Analyzer (Applied Biosystems) with a ROX labeled size standard. Target fragment sizes were called using Peak Scanner v1.0 (Applied Biosystems).

Multilocus typing

Alleles at each of the eight loci were assigned a number. For each isolate, alleles from eight loci were combined to give a multilocus type.

Results

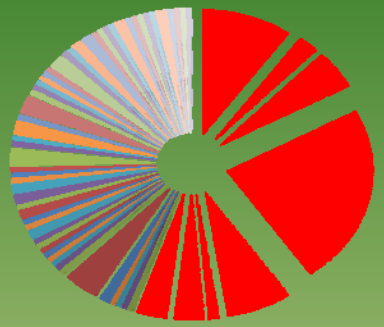

Figure 1

Frequencies of each MLT from cattle and humans. Each segment represents a unique MLT. Bright red segments are common to cattle and humans

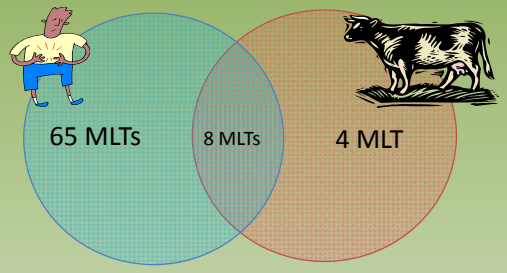

Figure 2

Distribution of MLTs among humans and cattle

- 77 multilocus types were identified.
- 10% of MLTs were common to humans and cattle.
- One MLT was identified in 19% of isolates, and was common to cattle and humans
- There was more diversity among isolates from humans (65 unique MLTs) than cattle (4 unique MLT)
- GP60 was the most polymorphic region typed with 23 different alleles, compared with 3 for MS5, 4 for MSC 6-7, 5 for CP47, 7 for TP14, DZ-HRGP and MS9, and 10 for GRH.

Discussion

GP60 was highly polymorphic relative to the other loci examined. The GP60 region has been useful in previous investigations of *C. parvum* transmission dynamics due to its high variability. One factor which may have contributed to the greater variability observed at this site was the selection of sequence analysis rather than fragment size analysis for genotyping. Sequence analysis has the advantage of providing information on nucleotide composition in addition to length. It can therefore detect single nucleotide polymorphisms that would not affect fragment length.

The finding of a significant number of MLTs that are common to cattle and humans is strong evidence of zoonotic *C. parvum* transmission. Based on our data, it is clear that two MLTs are responsible for most cryptosporidiosis cases in humans and cattle in the area. These MLTs were very similar, with variance at only one loci. The reason for the high prevalence of a select few strains in humans and cattle is unknown. One explanation would be that these stains are more virulent.

Human isolates were more diverse than cattle isolates as evidenced by the higher number of MLTs. This may be explained in part by the higher number of human isolates in the study. Alternatively, humans may be exposed to *C. parvum* from a number of sources and from geographically diverse areas. We did not have patient histories and therefore could not exclude travel associated disease.

Conclusions

Zoonotic *C. parvum* transmission appears likely in the region

***C. parvum* is considerably more diverse in humans than cattle**

References

1. Alves, M., L. Xiao, I. Sulaiman, A. A. Lal, O. Matos, and F. Antunes. 2003. Subgenotype analysis of *Cryptosporidium* isolates from humans, cattle, and zoo ruminants in Portugal. J.Clin.Microbiol. 41:2744-2747.
2. Feltus, D. C., C. W. Giddings, B. L. Schneck, T. Monson, D. Warshauer, and J. M. McEvoy. 2006. Evidence supporting zoonotic transmission of *Cryptosporidium* in Wisconsin. J. Clin. Microbiol. 44: 4303-8.
3. Gatei, W., P. Das, P. Dutta, A. Sen, V. Cama, A. A. Lal, and L. Xiao. 2007. Multilocus sequence typing and genetic structure of Cryptosporidium hominis from children in Kolkata, India. Infect.Genet.Evol. 7:197-205.
4. Hlavsa, M. C., J. C. Watson, and M. J. Beach. 2005. *Cryptosporidiosis* surveillance--United States 1999-2002. MMWR.Morb.Mortal.Wkly.Rep. 54:1-8.
5. Mallon, M. E., A. MacLeod, J. M. Wastling, H. Smith, and A. Tait. 2003. Multilocus genotyping of *Cryptosporidium parvum* Type 2: population genetics and sub-structuring. Infection, Genetics and Evolution 3:207-218.
6. Tanriverdi, S., M. O. Arslan, D. E. Akiyoshi, S. Tzipori, and G. Widmer. 2003. Identification of genotypically mixed *Cryptosporidium parvum* populations in humans and calves. Mol.Biochem.Parasitol. 130:13-22.
7. Thompson, R. C., M. E. Olson, G. Zhu, S. Enomoto, M. S. Abrahamsen, and N. S. Hijjawi. 2005. *Cryptosporidium* and cryptosporidiosis. Adv.Parasitol. 59:77-158.

Acknowledgements

The authors acknowledge NIH NIAID (Project number: A1067284-01A1) for support. The contents are solely the responsibility of the authors and do not necessarily represent the official view of NIAID or the NIH.