



Molecular identification of *Cryptosporidium* species infecting Wisconsin dairy calves



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Objective

➔ Determine the species identity of *Cryptosporidium* parasites that infect Wisconsin dairy calves

Introduction

Cryptosporidium is a genus of protozoan parasites that infect the gastrointestinal tract of many vertebrate hosts including livestock, wildlife, and humans.

Neonatal calf diarrhea has long been associated with *Cryptosporidium* and has caused economic losses for Wisconsin dairy farms.

Wisconsin consistently experiences a high number of human cryptosporidiosis cases (Figure 1).

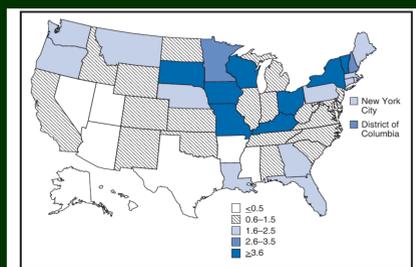


Figure 1. Cases of human cryptosporidiosis in the United States, 2005. (Yoder, 2007)

At least three species of *Cryptosporidium* infect cattle. However, only *Cryptosporidium parvum* is zoonotic, meaning it can infect both people and animals. The various species of *Cryptosporidium* are identical in a microscopic exam (Figure 2).

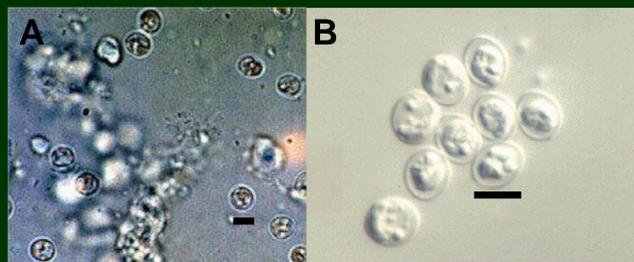


Figure 2. *Cryptosporidium* oocysts. A) *C. andersoni* B) *C. parvum* (size bars = 5µm)

Genetic techniques are necessary to discover the species identity of *Cryptosporidium* isolated from cattle. The purpose of this project was to determine what species of *Cryptosporidium* infect young Wisconsin dairy calves.

Acknowledgements

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Methods

Specimen Collection

Fecal samples were collected rectally from dairy calves. Specimens were purified by cesium chloride centrifugation

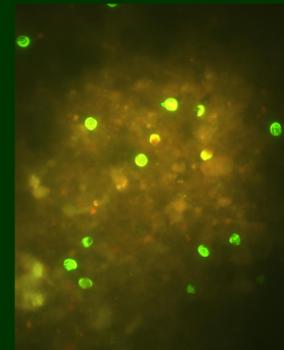


Figure 3. *Cryptosporidium* stained with fluorescent antibodies

Immunofluorescent Stain

Purified fecal sample was stained with antibodies specific to *Cryptosporidium* cell wall proteins (Figure 3).

DNA Amplification

DNA was extracted infected samples. A nested polymerase chain reaction (PCR) was used to amplify the 18S rRNA gene.

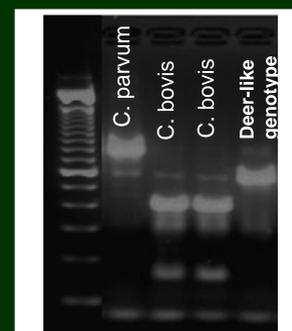


Figure 4. Digestion of 18S PCR products with *Mbol*I. (From: Feng et al., 2007)

Restriction Fragment Analysis

PCR products were digested with restriction enzymes and separated by electrophoresis (Figure 4).

Results

We have successfully amplified the 18S gene of *Cryptosporidium* from 25 different infected calves.

Digestion of 10 PCR products with *Ssp*I produced 450, 270, and 110 base pair fragments (Figure 5). These results are consistent with reports of *C. parvum* (Feng et al., 2007).

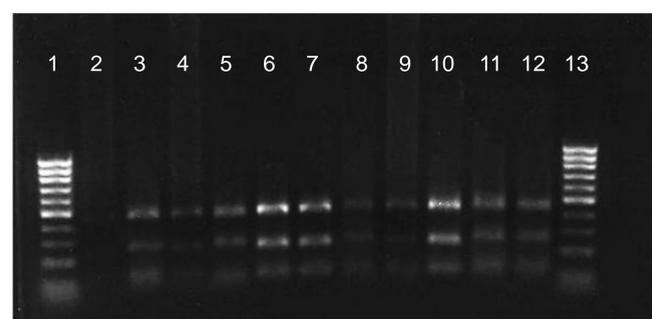


Figure 5. Digestion of 18S PCR products with *Ssp*I. Lanes 1 and 13: 100 bp weight markers. Lanes 2-11: *C. parvum*

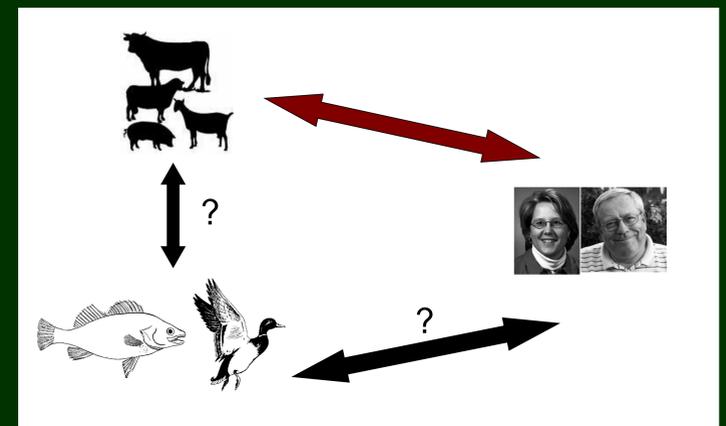
Discussion

Our results demonstrate the presence of *C. parvum* in Wisconsin dairy calves. Feltus et al. (2006) found that this species also commonly infects humans here.

One of the specimens included in this study was isolated from a Jersey calf. This is the first report of *C. parvum* in this breed which was previously thought to only host *C. bovis* (Starkey et al., 2006).

Young dairy calves appear to be involved in the zoonotic transmission of *Cryptosporidium*. While calf manure is only a small volume of the slurry that is spread as fertilizer, it can contain a large number of oocysts.

Many other animal hosts may play an important role in the transmission of *Cryptosporidium*. The parasite has been identified in reptiles, birds, and over 150 species of mammals. Few studies have described the molecular epidemiology of cryptosporidiosis in other domestic animals or wildlife.



C. parvum is found in both humans and cattle. Further research is required to examine the transmission of the parasite between these two groups of vertebrates.

In future work, we will characterize these specimens on the basis of another gene. This analysis will provide additional clues about the transmission cycle.

References

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