WORLDWIDE DISTRIBUTION OF CRYPTOSPORIDIUM SPECIES IN BOVINES

By

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Abstract

The following research-based paper will provide a detailed list of all *Cryptosporidium* species known, to date, to infect bovine populations worldwide. Prevalence data from around the world, including Australia, Brazil, Canada, China, England, Egypt, India, Italy, Japan, Spain, Sweden, the United States of America and others, are detailed and tabularly presented.

*Cryptosporidium parvum*, *C. andersoni*, *C. bovis* and *C. ryanae* are the major relevant species of *Cryptosporidium* that readily infect bovines. Information on infection duration, oocyst shedding volumes and symptomology on each major species is encompassed. The current genomics and subtypes of this organism are detailed and discussed. Treatment including vaccination, chemotherapy and immunotherapy are reviewed. Lastly, the future developments in the bovine *Cryptosporidium* field are touched upon.
Introduction

Cryptosporidiosis is a life threatening diarrheal disease of immunocompromised individuals, such as AIDS patients, who lack the ability to mount a sufficient immune response to rid themselves of the disease (Amer et al. 2009). The causative agent, known as Cryptosporidium, is an Apicomplexa protozoan parasite which has been discovered to be a major cause of human and animal diarrheal disease worldwide (Amer et al. 2009; Zambriski et al. 2013). At this time there is no effective chemotherapeutic agent available to treat this infection in either humans or animals (Zambriski et al. 2013). Currently, nitazoxanide is the only FDA approved drug for treating human cryptosporidiosis however, it is only marginally effective. To date, this genus is comprised of 25 genetically unique and valid species including those with wide range mammalian host specificity and known zoonotic transfer (Amer et al. 2009; Zambriski et al. 2013; Rzeńutka et al. 2013; Cacció and Widmer 2014). The most common zoonotic transmission of Cryptosporidium is C. parvum from infected bovines to susceptible humans via direct contact or consumption of contaminated water (Amer et al. 2009). Many Cryptosporidium species are able to infect and are responsible for bovine diarrheal disease worldwide including C. parvum, C. hominis, C. bovis, C. andersoni and C. ryanae (Castro-Hermida et al. 2011a; Chen and Huang 2012; Helmy et al. 2013; Imer et al. 2011; Keshavarz et al. 2009; Khan et al. 2010; Meireles et al. 2011; Santín et al. 2004).

Discovery of Cryptosporidium

In 1907, the American physician Ernest Edward Tyzzer became the first person to clearly describe the protozoan parasite known as Cryptosporidium (Tyzzer 1907; Dubey et al. 1990). While conducting cancer research he frequently found this organism in large numbers colonizing
the gastric glands of his laboratory mice (Tyzzer 1907). During his investigation, Tyzzer described this new organism’s life cycle including its sexual and asexual reproduction stages, spore (oocyst) formation, possible autoinfectious stages as well as fecal transmission. Knowing this organism had no previous taxonomy, Tyzzer named this new parasite Cryptosporidium muris, after the mouse species he was working with, Mus musculus (Fayer 1997; Dubey et al. 1990). At that time, transmission of C. muris to other mice was successful, but attempts to infect a rat were unsuccessful (Caccio and Widmer 2014). In 1989, nearly 80 years later, Iseki et al. reported that C. muris (strain RN66) oocysts obtained from a rat was capable of infecting other specific pathogen–free (SPF) laboratory rats, mice, guinea pigs, rabbits, cats and dogs (Caccio and Widmer 2014). Infection of each of these species was based on presence of excreted oocysts from the host and developmental stages in the stomach (Iseki et al. 1989). In addition to the previously stated species, C. muris has been documented to infect pigs, giraffes, camels, seals and nonhuman primates among others (Fayer 2010; Feng et al. 2011). There have also been many reports of human infection (Katsumata et al. 2000; Guyot et al. 2001; Gatei et al. 2002b, 2003, 2006; Tiangtip and Jongwutiwes 2002; Palmer et al. 2003; Muthusamy et al. 2006; Al-Brikan et al. 2008). Due to this, C. muris is known for having broad host specificity and zoonotic activity.

Life Cycle

Cryptosporidium is an intracellular, apicomplexan, protozoan parasite. Unlike other parasites, this organism is monoxenous and can complete its entire life cycle within a single host. This includes both the asexual and sexual phases of replication. The cycle is composed of seven stages beginning with ingestion of the organism’s oocyst spore form and ending in excretion of
new oocysts from the host. A color illustration of this organism’s complete life cycle is included below in this section.

Ingestion: To acquire a Cryptosporidium infection the infectious form of the parasite, known as the oocyst, must be orally ingested or inhaled. This is referred to as a fecal-oral transmission due to the infectious oocyst residing in the fecal material of a previously infected host. This can be accomplished by consuming contaminated items such as: drinking water (filtered and unfiltered), unwashed or uncooked food, and recreational water. Other mechanisms of infection include transfer to the mouth of the individual by contaminated hands through a variety of occupational and personal activities. The infectious dose of oocysts required to produce infection in 50 percent of experimental subjects (ID$_{50}$) is significantly unpredictable and differs significantly with each Cryptosporidium species and species isolate. ID$_{50}$ values for Cryptosporidium parvum isolates range from 9 to 2,788 oocysts, whereas ID$_{50}$ values for the C. hominis isolate TU502 range from 10 to 83 oocysts in humans (Okhuysen et al. 1999; Chappell et al. 2006).

Excystation: After ingestion of an infectious dose of oocysts by a susceptible host, excystation of the organism(s) occur which is the first step towards infection. Excystation is the process by which Cryptosporidium sporozoites, and other Apicomplexan parasites, are released from the oocyst. Each Cryptosporidium oocyst contains four, naked, motile, infectious sporozoites. Transit though the host’s stomach and into the small intestine triggers oocyst excystation. A comprehensive list of mechanisms involved in and required for Cryptosporidium excystation is not yet available. However, in vitro experiments suggest these banana-shaped sporozoites are released following exposure to bile salts, body temperature (37°C), pH fluctuations, proteases and trypsin (Smith et al. 2005). It should be noted however, that
Cryptosporidium species such as *C. muris*, that infect the stomach of the host, respond more readily to these conditions compared to those species infecting the intestinal tract of the host (Widmer et al. 2007).

Attachment and Invasion: Once sporozoites emerge from the cyst they immediately attach themselves to the surface of host gastrointestinal epithelial cells. Like other apicomplexan parasites, *Cryptosporidium* contains an apical complex formed by organelles such as a polar ring, conoid, rhoptry and micronemes (Caccio and Widmer 2014). These structures aid in parasitic attachment and cellular invasion of the gastrointestinal epithelium.

Asexual Replication: After internalization the sporozoite differentiates into the asexual, spherical trophozoite form. Unique to *Cryptosporidium*, both of the following asexual and sexual replication stages develop within a parasitophorous vacuole in the epithelial cell that is intracellular yet extracytoplasmic. Asexual replication of this organism is known as merogony. Merogony is characterized by nuclear division resulting in the production of multinucleated schizonts that mature into merozoites. *Cryptosporidium parvum* is unique in the fact that it has two types of meronts; type I and type II (Caccio and Widmer 2014). Type I merozoites released from their initial epithelial cells can travel one of two pathways. One path is to infect neighboring epithelial cells and undergo a second round of asexual replication producing further type I merozoite progeny. The second path is formation of a type II meront. Each type II meront matures into four type II merozoites each of which proceed to initiate the sexual stage of replication once released (Chen et al. 2002).

Sexual Replication: Once released, type II merozoites attach to and invade new epithelial cells where initiation of sexual replication, also known as gametogony, begins. Each individual merozoite differentiates into either a microgamont or macrogamont (Chen et al. 2002).
Microgamonts undergo nuclear division leading to the production and release of numerous microgametes, parasitic sperm cells, from the parasitophorous vacuole (Caccio and Widmer 2014). Macrogamonts, parasitic ova, are then fertilized by these microgametes. This product of fertilization known as the zygote, develops into an oocyst.

Sporogony: During sporogony the newly formed zygote differentiates into four sporozoites within the oocyst structure, constituting a fully sporulated oocyst (Caccio and Widmer 2014). Fully formed oocysts are then excreted from the initial host with the prospect of transmission to a new host.
Chen et al. 2002
Excretion: Unlike other parasites such as *Eimeria* and *Hammondia*, once these oocysts are excreted they are fully sporulated and immediately infectious to the next host (Dubey et al. 1990; Fayer 1997). Sporulated oocysts contain either a thick or thin oocyst wall. Thick walled oocysts are released into the intestinal lumen and are expelled from the host through fecal excretion. These oocysts are more environmentally resistant then their thin walled counterpart. Thin walled oocysts are responsible for auto-infection of the parasitized host by releasing their sporozoites within the intestinal lumen. These oocysts are largely retained within the host, undergoing merogony, gametogony and sporogony again. For this reason treatment with anti-diarrheals and anticholinergics such as Imodium, Bentyl and Anaspaz are contra-indicated for *Cryptosporidium* infections. These drugs trap auto-infectious oocysts in the gastrointestinal tract resulting in hyperinfection of the host.

*Cryptosporidium* has the ability to produce a massive amount of infectious oocysts during a single host infection. It has been reported that infected persons can shed $10^7$-$10^8$ infectious oocysts in a single bowel movement (Yoder et al. 2010; Yoder et al. 2012). Others studies show that infectious oocysts can continue to be shed up to 60 days after gastrointestinal symptoms have ceased (Yoder et al. 2012).

**Cryptosporidium in Mammals**

There are currently 18 species of *Cryptosporidium* that are known to infect mammals. These species include: *C. parvum, C. hominis, C. bovis, C. muris, C. canis, C. felis, C. andersoni, C. ryanae, C. tyzzeri, C. viatorum, C. wrairi, C. suis, C. fayeri, C. xiaoii, C. macropodum, C. cuniculus, C. scrofarum, and C. ubiquitum* (Cacciò and Widmer 2014). In 2008 and 2010, Fayer reported that over 150 species, belonging to twelve mammalian Orders, are
hosts of Cryptosporidium species. Table 1 provides a brief overview of Cryptosporidium species known to infect mammalian hosts.

Table 1: Mammalian Cryptosporidium Species

<table>
<thead>
<tr>
<th>Species</th>
<th>Host(s)</th>
<th>Approximate Oocyst size (mm)</th>
<th>Infection Site</th>
<th>References</th>
<th>GenBank accession number (18SrRNA)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. andersoni</em></td>
<td>Cattle</td>
<td>7.4 x 5.5</td>
<td>Abomasum</td>
<td>Lindsay et al. 2000</td>
<td>AF093496</td>
</tr>
<tr>
<td><em>C. bovis</em></td>
<td>Cattle</td>
<td>4.89 x 4.63</td>
<td>Small intestine</td>
<td>Fayer et al. 2005</td>
<td>AY741305</td>
</tr>
<tr>
<td><em>C. canis</em></td>
<td>Dogs</td>
<td>4.95 x 4.75</td>
<td>Small intestine</td>
<td>Fayer et al. 2001</td>
<td>AF112576</td>
</tr>
<tr>
<td><em>C. cuniculus</em></td>
<td>Rabbits</td>
<td>5.98 x 5.38</td>
<td>Small intestine</td>
<td>Robinson et al. 2010; Inman and Takeuchi 1979</td>
<td>FJ262725</td>
</tr>
<tr>
<td><em>C. fayeri</em></td>
<td>Marsupials</td>
<td>4.9 x 4.3</td>
<td>Small intestine</td>
<td>Ryan et al. 2008</td>
<td>AF159112</td>
</tr>
<tr>
<td><em>C. felis</em></td>
<td>Cats</td>
<td>4.5 x 5.0</td>
<td>Small intestine</td>
<td>Iseki 1979</td>
<td>AF108862</td>
</tr>
<tr>
<td><em>C. hominis</em></td>
<td>Humans</td>
<td>4.8 x 5.2</td>
<td>Small intestine</td>
<td>Morgan-Ryan et al. 2002</td>
<td>AF108865</td>
</tr>
<tr>
<td><em>C. macropodum</em></td>
<td>Marsupials</td>
<td>4.9 x 5.4</td>
<td>Small intestine</td>
<td>Power and Ryan 2008</td>
<td>AF513227</td>
</tr>
<tr>
<td><em>C. muris</em></td>
<td>Rodents</td>
<td>6.1 x 8.4, 8.1 x 5.9</td>
<td>Stomach</td>
<td>Tyzzer 1907</td>
<td>AB089284</td>
</tr>
<tr>
<td><em>C. parvum</em></td>
<td>Cattle, Sheep, Humans</td>
<td>4.9 x 4.4</td>
<td>Small intestine</td>
<td>Tyzzer 1912</td>
<td>AF308600</td>
</tr>
<tr>
<td><em>C. ryanae</em></td>
<td>Cattle</td>
<td>3.16 x 3.73</td>
<td>Small intestine</td>
<td>Fayer et al. 2008</td>
<td>AY587166</td>
</tr>
<tr>
<td><em>C. scrofarum</em></td>
<td>Pigs</td>
<td>5.16 x 4.83</td>
<td>Small intestine</td>
<td>Kvač et al. 2013</td>
<td>EU331243</td>
</tr>
<tr>
<td><em>C. suis</em></td>
<td>Pigs</td>
<td>4.6 x 4.2</td>
<td>Small and</td>
<td>Ryan et al.</td>
<td>AF115377</td>
</tr>
</tbody>
</table>
C. tyzzeri  Mice  4.64μm x 4.19  Small intestine  Ren et al. 2012  AF112571

C. ubiquitum  Sheep, Wildlife  5.19 x 4.87  Small intestine  Fayer et al. 2010  AF262328

C. viatorum  Humans  5.35 x 4.72  Small intestine  Elwin et al. 2012b  HM485434

C. wrairi  Guinea pigs  4.6 x 5.4  Small intestine  Vetterling et al. 1971  AF115378

C. xiaoi  Sheep  3.94 x 3.44μm  Small intestine  Fayer and Santín 2009  EU408314

Adapted from Caccio and Widmer (2014)

Cryptosporidiosis in Bovines

Farmed bovines, including cattle, water buffalo and zebu, are amongst the most important livestock species worldwide (Caccio and Widmer 2014). These animals provide the human population with various commodities; including meat, organs, milk, and other dairy products. Current estimates place these animals at 1.5 billion head worldwide. This is a ratio of approximately 3 animals per 14 people (Caccio and Widmer 2014). The first report of bovine cryptosporidiosis was in 1971 by Panciera et al. and described an eight-month-old, bovine leukemia virus (BLV) positive heifer presenting with chronic diarrhea. Since then, *Cryptosporidium* infection in bovines, especially cattle, has been identified and documented worldwide. The four major *Cryptosporidium* species known to infect cattle are: *C. andersoni*, *C. bovis*, *C. parvum*, and *C. ryanae*. Table 2 provides a brief overview of these four *Cryptosporidium* species known to readily infect bovine hosts.
Table 2: Overview of Major *Cryptosporidium* species of Bovines

<table>
<thead>
<tr>
<th>Bovine</th>
<th><em>Cryptosporidium</em> Species</th>
<th>Clinical Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bovines, including cattle (<em>Bos Taurus</em> and <em>Bos indicus</em>), water buffalo (<em>Bubalus bubalis</em>), and yaks (<em>Bos grunnies</em>)</td>
<td><em>C. andersoni</em></td>
<td>Commonly found in older post-weaned calves, yearlings and adults; some decrease in milk production has been reported</td>
</tr>
<tr>
<td></td>
<td><em>C. bovis</em></td>
<td>Commonly found in post-weaned calves; no documented pathogenicity available</td>
</tr>
<tr>
<td></td>
<td><em>C. parvum</em></td>
<td>Commonly found in pre-weaned calves; acute onset of symptoms and diarrhea</td>
</tr>
<tr>
<td></td>
<td><em>C. ryanae</em></td>
<td>Commonly found in post-weaned calves</td>
</tr>
</tbody>
</table>

Adapted from Caccio and Widmer (2014)

*Cryptosporidium parvum*

*Cryptosporidium parvum* was first described by Tyzzer in 1912 as being a coccidian infecting the small intestine of the common mouse. Measuring 4.9 x 4.4mm, *C. parvum* is smaller than *C. muris* and develops only in the small intestine, rather than the stomach of its host (Tyzzer 1912; Dubey et al. 1990; Fayer 1997). *Cryptosporidium parvum* primarily infects the small intestine of cattle, sheep and humans but has a very broad host range with the ability to infect most mammals (Caccio and Widmer 2014). Subsequent testing revealed *C. parvum* infectivity to over 150 mammals. Infection was confirmed by microscopic identification of *C. parvum* oocysts in host feces (Fayer 2010).

Bovine infection with *Cryptosporidium parvum* is most commonly found in pre-weaned calves presenting with acute intestinal discomfort and diarrhea (Caccio and Widmer 2014). Due to its high infectivity in neonatal calves, resulting in exponential oocyst production, this is
currently the most widely researched and studied *Cryptosporidium* species (Cacciò and Widmer 2014). It is through research that there is a hope of developing an effective chemotherapeutic treatment and vaccine for mammalian cryptosporidiosis.

*Cryptosporidium andersoni*

*Cryptosporidium andersoni* is most commonly found to infect the abomasum of older post-weaned calves, yearlings and adults (Cacciò and Widmer 2014). Measuring 7.4 x 5.5 mm *C. andersoni* oocysts are slightly smaller than those of *C. muris* and were originally mistaken in cattle as *C. muris* infections (Cacciò and Widmer 2014). This was corrected in 2000, when it was identified as a new species based on infection location, host specificity, and genetic differences (Lindsay et al. 2000). In this study it was determined that *C. andersoni* was not infectious to chickens, goats, inbred, outbred, immunocompetent or immunodeficient mice (Lindsay et al. 2000).

*Cryptosporidium andersoni* has more recently been identified to occasionally infect camels, sheep, goats and humans (Leoni et al. 2006; Morse et al. 2007; Fayer 2010; Feng et al. 2011; Waldron et al. 2011). A novel *C. andersoni* genotype from Japan has also been reported to infect severe combined immunodeficient (SCID) mice (Satoh et al. 2003, Matsubayashi et al. 2005).

*Cryptosporidium hominis*

*Cryptosporidium hominis* is primarily an infection of humans and was originally referred to as *C. parvum* human genotype, genotype 1 and genotype H (Cacciò and Widmer 2014). Through molecular and biological differentiation of isolates from human and bovine sources, *C. hominis* was classified as a new species.
Under experimental conditions, *Cryptosporidium hominis* has been deemed infective to calves, lambs and piglets. However, it is non-infectious to rats, mice, dogs, cats, goats, cattle under the same conditions (Morgan-Ryan et al. 2002; Xiao et al. 2002b). Natural infections with this *Cryptosporidium* species have also been reported in bovine, goats, marsupials, and dugong (marine mammal) but are uncommon (Morgan et al. 2000c; Park et al. 2006; Fayer 2010; Abeywardena et al. 2012; Ryan and Power 2012).

*Cryptosporidium bovis*

*Cryptosporidium bovis* is commonly found in post-weaned calves and considered to be less pathogenic than *C. parvum*. This *Cryptosporidium* species was originally referred to as *Cryptosporidium* genotype bovine B (Xiao et al. 2002b). After experimental infection revealed this genotype was unable to be transmitted to neonatal BALB/c mice and lambs, it was designated a new species. Genetic differentiation of the 18S rRNA, HSP70 and actin loci were also used to confirm this new species (Fayer et al. 2005).

*Cryptosporidium ryanae*

*Cryptosporidium ryanae* was previously referred to as *Cryptosporidium* deer-like genotype and designated a new species after transmission and genetic differentiation was determined in 2008 (Fayer et al. 2008). The term “deer-like” was used not because it had ever been found in a deer but due to the similarity in 18s rRNA sequence between it and the deer genotype (Xiao et al. 2002a).

*Cryptosporidium ryanae* is closely related to *Cryptosporidium bovis* and shares many similar characteristics. Together these two species are responsible for the majority of *Cryptosporidium* infections in post-weaned calves. Similar to *C. bovis*, *C. ryanae* has been experimentally determined to be unable to infect BALB/c mice and lambs (Fayer et al. 2008).
Prevalence of Cryptosporidiosis in Bovines

Numerous reports of *Cryptosporidium* infections in bovines have been published over the years indicating it is common worldwide. Estimates of prevalence in these reports vary considerably ranging from 0 to 100% (Caccioà and Widmer 2014). In addition to expected *Cryptosporidium* species infection, sporadic natural infection with *C. felis*, *C. hominis*, *C. scrofarum*, *C. serpentis*, *C. suis* and *C. suis*-like genotype have been reported in cattle (Bornay-Llinares et al. 1999; Santìn et al. 2004; Smith et al. 2005; Geurden et al. 2006; Langkjaer et al. 2007; Chen and Huang 2012; (Caccioà and Widmer 2014). Whether these findings reflect natural *Cryptosporidium* infections or simply accidental infection remains unknown. In this context incidental infection refers to the passage of ingested oocysts through the gastrointestinal tract absent of excystation and epithelial cell invasion.

Table 3 provides information on prevalence rates of infection in both dairy and beef herds, identification methods used and species breakdown. The information presented below is from 2004 to 2013 and for this reason a complete worldwide distribution of *Cryptosporidium* infection is not provided.

<table>
<thead>
<tr>
<th>Location</th>
<th>Age</th>
<th>Number of Animals/Farm or Location</th>
<th>Prevalence</th>
<th>Identification Method/ Molecular Identification</th>
<th>Species Identified</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Australia, NSW</td>
<td>Calves</td>
<td>196/20 herds</td>
<td>74%</td>
<td>Molecular/ 18S rRNA and GP60</td>
<td><em>C. parvum</em> 59%</td>
<td>Ng et al. 2012</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>C. bovis</em> 20%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>C. ryanae</em> 10%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mixed inf. 10%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Not identified 1%</td>
<td></td>
</tr>
</tbody>
</table>

<p>| Brazil          | ≤ 30 days | 196/dairy                          | 11%        | Molecular/ 18S                               | Not identified 42% | Meireles et         |
|                 |           |                                    |            |                                               |                    |                     |</p>
<table>
<thead>
<tr>
<th>Country</th>
<th>Age Group</th>
<th>Herds/Lab</th>
<th>Methodology</th>
<th>Genotypes</th>
<th>Al.</th>
</tr>
</thead>
</table>
| Canada           | 0-6 months| 752/20    | Microscopy/18S rRNA | C. parvum 33%  
C. ryanae 10%  
C. andersoni 10%  
C. bovis 5% | Buda-Amoako et al. 2012a |
| Czech Republic   | 20-60 days| 750/24    | Microscopy/18S rRNA RFLP | C. parvum 86%  
C. andersoni 13%  
C. bovis 2% | Kváč et al. 2011 |
| China            | 0-6 months| 2,056/14  | Microscopy/18S rRNA | C. parvum 48%  
C. andersoni 29%  
C. bovis 16%  
C. hominis 6%  
C. serpents 1% | Chen and Huang 2012 |
| China            | 0-8 weeks | 801/8     | Microscopy/18S rRNA and GP60 | C. parvum 91%  
Not identified 7%  
C. bovis 2% | Wang et al. 2011b |
| England and Wales| ≤ 3 months| 229       | Microscopy/18S rRNA | C. parvum 77%  
C. andersoni 16%  
C. bovis 5%  
Not identified 2% | Featherstone et al. 2010a |
| England and Wales| Pre-weaned Immature Adults | 116/11 | Microscopy/18S rRNA | C. parvum 93%  
C. andersoni 7% | Smith et al. 2010 |
| Egypt            | < 6 weeks | 96/2      | Microscopy/18S rRNA and COWP | C. parvum 65%  
Mixed inf. 17%  
C. ryanae 14%  
C. bovis 4% | Amer et al. 2010 |
| Egypt            | 1d -3 months  
>3 months-1y | 593       | Other/ 18S rRNA and GP60 | C. parvum 33%  
C. ryanae 14%  
C. bovis 5% | Helmy et al. 2013 |
<table>
<thead>
<tr>
<th>Country</th>
<th>Age Group</th>
<th>Number of Herds</th>
<th>Diagnosis Method</th>
<th>Species Identified</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hungary</td>
<td>&gt;1-2 years</td>
<td>79 diarrheic/ 52 herds</td>
<td>Microscopy/ 18S rRNA and GP60</td>
<td>C. parvum 95%</td>
<td>Plutzer and Karanis 2007</td>
</tr>
<tr>
<td></td>
<td>&gt;2 years</td>
<td></td>
<td></td>
<td>C. ryanae 5%</td>
<td></td>
</tr>
<tr>
<td>India</td>
<td>&lt; 3 months</td>
<td>461/ various</td>
<td>Microscopy/ 18S sRNA</td>
<td>C. parvum 100%</td>
<td>Maurya et al. 2013</td>
</tr>
<tr>
<td>India</td>
<td>0-2 months</td>
<td>180/ 2 dairy herds</td>
<td>Microscopy/ 18S rRNA</td>
<td>C. bovis 38%</td>
<td>Khan et al. 2010</td>
</tr>
<tr>
<td></td>
<td>3-12 months</td>
<td></td>
<td></td>
<td>C. parvum 29%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt;12 months</td>
<td></td>
<td></td>
<td>C. ryanae 14%</td>
<td></td>
</tr>
<tr>
<td>Iran</td>
<td>1-20 weeks</td>
<td>272/15 dairy herds</td>
<td>Microscopy/ 18S rRNA</td>
<td>C. parvum 73%</td>
<td>Keshavarz et al. 2009</td>
</tr>
<tr>
<td>Italy</td>
<td>0d to &lt; 12 mo</td>
<td>2.024/248 dairy and beef herds</td>
<td>ELISA and Microscopy/ COWP and GP60</td>
<td>C. parvum 100%</td>
<td>Duranti et al. 2009</td>
</tr>
<tr>
<td>Japan</td>
<td>3-48 days</td>
<td>80 diarrheic</td>
<td>Molecular/ 18S rRNA</td>
<td>C. parvum 53%</td>
<td>Karanis et al. 2010</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Not identified 45%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C. bovis 2%</td>
<td></td>
</tr>
<tr>
<td>Malaysia</td>
<td>1d to ≤ 4.5mo</td>
<td>250/16 herds</td>
<td>Molecular/ 18S rRNA and GP60</td>
<td>C. bovis 25%</td>
<td>Muhid et al. 2011</td>
</tr>
<tr>
<td></td>
<td>&gt;4.5-12 mo</td>
<td></td>
<td></td>
<td>C. ryanae 20%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C. parvum 17%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Not identified 17%</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C. ryanae 15%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mixed inf. 6%</td>
<td></td>
</tr>
<tr>
<td>Nigeria</td>
<td>2-365 days</td>
<td>194/20 herds</td>
<td>Molecular/ 18S rRNA</td>
<td>C. bovis 45%</td>
<td>Maikai et al. 2011</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C. ryanae 26%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C. andersoni 16%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mixed inf. 13%</td>
<td></td>
</tr>
<tr>
<td>Northern Ireland</td>
<td>779 diarrheic/ diagnostic lab</td>
<td>Microscopy/ 18S rRNA</td>
<td>C. parvum 95%</td>
<td>Thompson et al. 2007</td>
<td></td>
</tr>
<tr>
<td>Romania</td>
<td>1-30 days</td>
<td>258 diarrheic/ 9</td>
<td>Microscopy/ 18S rRNA and</td>
<td>C. parvum 100%</td>
<td>Imer et al. 2011</td>
</tr>
<tr>
<td></td>
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</tr>
<tr>
<td>Country</td>
<td>Herd Type</td>
<td>Sample Size</td>
<td>GP60</td>
<td>Cryptosporidium Species</td>
<td>Reference</td>
</tr>
<tr>
<td>--------------</td>
<td>----------------------------------------</td>
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<td>------</td>
<td>-------------------------</td>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>Spain</td>
<td>Neonatal Heifers / Cows</td>
<td>649</td>
<td>GP60</td>
<td>C. parvum 56%</td>
<td>Castro-Hermida et al. 2011a</td>
</tr>
<tr>
<td>Spain</td>
<td>≤21 days</td>
<td>61 diarrheic/27 herds</td>
<td>GP60</td>
<td>C. parvum 100%</td>
<td>Díaz et al. 2010a</td>
</tr>
<tr>
<td>Sweden</td>
<td>≤2 months 4-12 months Cows</td>
<td>1,202/50 dairy herds</td>
<td>GP60</td>
<td>C. parvum 50%</td>
<td>Silverlås et al. 2009b; Silverlås et al. 2010b</td>
</tr>
<tr>
<td>USA (7 states)</td>
<td>5d -2 months 3-11 months</td>
<td>971/15 dairy herds</td>
<td>GP60</td>
<td>C. bovis 75%</td>
<td>Santín et al. 2004</td>
</tr>
<tr>
<td>USA (7 states)</td>
<td>12-24 months</td>
<td>571/14 dairy herds</td>
<td>GP60</td>
<td>C. andersoni 43%</td>
<td>Fayer et al. 2006</td>
</tr>
<tr>
<td>USA (20 states)</td>
<td>6-18 months</td>
<td>819/49 beef herds</td>
<td>GP60</td>
<td>C. andersoni 68%</td>
<td>Fayer et al. 2010</td>
</tr>
</tbody>
</table>

Adopted from Caccio and Widmer (2014)

**Association with Clinical Disease**

Disease caused by infection with pathogenic *Cryptosporidium* species typically increases in severity when the organism reaches the small intestine (Caccio and Widmer 2014). Infection in both humans and animals results in a significant amount of anatomical and functional gastrointestinal disturbances. The architecture of the crypts, villi and microvilli of the small intestine are the most significantly affected. Under certain conditions complete villous atrophy and compensatory crypt hyperplasia are seen (Argenzio et al. 1990; Genta et al. 1993; Phillips et
al. 1992). These alterations are the result of the uncontrolled influx of inflammatory cells to the intestinal mucosa, specifically lamina propria and epithelium, leading to a decrease in enzymatic activity (aminopeptidases and disaccharidases) and surface area of this tissue (Farthing 2000). The decline of enzymatic activity is thought to be due, in part, to microvilli damage during the infection and most severe in association with parasitic epithelial attachment (Bird and Smith 1980; Farthing 2000).

In cattle, *Cryptosporidium parvum* and *Cryptosporidium andersoni* are the two major species associated with clinical disease (Caccio and Widmer 2014). Clinical signs most prominently found with bovine cryptosporidiosis caused by *C. parvum* include presentation of pasty to watery diarrhea with or without lethargy, inappetence, fever, dehydration and/or weight loss (Caccio and Widmer 2014). Both malabsorption and increased fluid secretion in the ileum and proximal portion of the large intestine leads to these clinical signs (Caccio and Widmer 2014).

It is recommended that during the first 12 hours of life all newborn calves ingest an adequate amount of colostrum to help prevent infection with common enteropathogens (Caccio and Widmer 2014). However, it has been discovered normal colostrum from cows has negligible activity against *Cryptosporidium* infection. In young calves, infection with *C. parvum* is considered the major cause of clinical cryptosporidiosis and diarrhea (Blanchard 2012; Radostits et al. 2007). It is not uncommon for calves to be infected during the first week of life and others to present with clinical symptoms up to six weeks of age (Caccio and Widmer 2014; Uga et al. 2000). Recovery in these animals varies largely. Individuals most often recover spontaneously within one to two weeks (Caccio and Widmer 2014). For others the infection can be fatal; especially if co-infection with other enteropathogens occurs (Fayer et al. 1998). For those that do
recover, a decrease in growth rate may be seen for several weeks; however no long-term effects on growth or performance have been reported (Klein et al. 2008; Cacciò and Widmer 2014).

Diarrhea in calves is invariably more likely to be due to *Cryptosporidium parvum* infection than *C. bovis* (Silverlås et al. 2013; Starkey et al. 2006; Cacciò and Widmer 2014). However, recent identification of high levels of *C. bovis* oocysts have been reported in diarrheic calves (Silverlås et al. 2010a, b, 2013). This is slightly surprising considering that *C. bovis* was not previously shown to cause diarrhea in calves and more commonly considered to be a widespread subclinical infection of older animals and even non-pathogenic in some cases (Cacciò and Widmer 2014). To date, only one experimental infection trial with *Cryptosporidium bovis* in calves has been performed (Fayer et al. 2005). Here, oral inoculation of three calves under one to eight weeks of age resulted in development of subclinical infection in two of the three calves. It should be noted that both of these animals had previously been infected with *Cryptosporidium parvum* and cross-protective immunity could not be ruled out.

*Cryptosporidium ryanae* predominately infects older calves and has yet to be reported in association with clinical disease (Cacciò and Widmer 2014). In 2008, Fayer et al performed an infectivion trial with *C. ryanae* using two colostrum-deprived calves 17-18 days of age. Neither showed any clinical signs of infection; however, both started excreting oocysts eleven days after inoculation (Fayer et al. 2008).

Unlike the other species, *C. andersoni* infects the abomasum of its bovine host and as a result does not cause diarrhea. However, it has been associated with maldigestion causing moderate to severe weight gain impairment (failure to thrive) in young animals post infection and reduced milk production in cows (Esteban and Anderson 1995; Lindsay et al. 2000; Cacciò and Widmer 2014). In all cases of cryptosporidiosis, simultaneous infection with other pathogens
such as rotavirus, coronavirus and K99+ *Escherichia coli* (enteropathogenic) can prolong illness duration and clinical signs (Blanchard 2012; Caccio and Widmer 2014).

**Incubation Period and Oocyst Excretion Levels**

Calves infected with *Cryptosporidium parvum* begin excreting oocysts 2-6 days after natural inoculation and subsequently continue shedding for 1-13 days (Fayer et al. 1998; Tzipori et al 1983). During the first two weeks of infection, a single calf can produce and excrete millions of infectious oocysts and over $10^{13}$ if experimentally hyperinfected (Fayer et al. 1998; Uga et al. 2000). In nature, this results in heavy environmental contamination and efficient parasitic dissemination within the herd (Caccio and Widmer 2014). Fecal samples obtained from naturally infected, symptomatic calves containing $10^6$-$10^8$ *C. parvum* oocysts per gram of feces are common (Silverlås et al. 2013). In herds exhibiting an established *C. parvum* infection, most calves excreted oocysts between 2 and 4 weeks of age (O’Handley et al. 1999; Santín et al. 2008; Uga et al. 2000). A sampling study revealed that not only was there a prevalence of 100% in dairy calves before they were three weeks of age, but that *C. parvum* oocysts were also found in a calf 16 weeks of age and another at six months of age (Santín et al. 2008). This suggests that it is possible for *C. parvum* oocysts to be shed intermittently over a long period of time after the initial infection. Alternatively, it is more likely that these individuals acquired new *C. parvum* infections after failing to develop fully protective immunity after the initial infection (Caccio and Widmer 2014).

Calves experimentally infected with *Cryptosporidium bovis* begin excreting oocysts 10-12 days after inoculation and subsequently continue shedding for 1-18 days (Fayer et al. 2005). Calves experimentally infected with *Cryptosporidium ryanae* begin excreting oocysts 11 days
after inoculation and subsequently continue shedding for 15-17 days (Fayer et al. 2008). Oocyst excretion levels were not determined in either of these experimental infections. Shorter prepatent periods for both *C. bovis* and *C. ryanae* have been documented in naturally acquired infections (Silverlås et al. 2010b; Silverlås and Blanco-Penedo 2013). Calves naturally infected with *C. bovis* have been reported to excrete 300 to 8×10⁶ oocysts per gram of feces (Silverlås and Blanco-Penedo 2013). Calves naturally infected with *C. ryanae* have been reported to excrete 100 to 835,000 oocysts per gram of feces (Silverlås and Blanco-Penedo 2013). Exact values regarding incubation period and excretion levels of *C. andersoni* infected bovines are not currently available. However, adult cattle remain persistently infected for years related to anatomic localization of the infection in the abomasum.

**Zoonotic Transmission**

Zoonotic transmission of *Cryptosporidium parvum* is not an uncommon event in the general population. There have been numerous reports of human cryptosporidiosis arising after contact with infected calves (Caccio and Widmer 2014). Those affected, may be exposed both occupationally and recreationally. Reported outbreaks often involve veterinarians, veterinary students, those working on farms (Gait et al. 2008; Kiang et al. 2006; Pohjola et al. 1986; Robertson et al. 2006) and young children after visiting farms or petting zoos (Gormley et al. 2011; Smith et al. 2004). Ample evidence concludes that contact with calves is a risk factor for infection and that *C. parvum* can be transmitted from calves to humans through direct contact or contamination (Caccio and Widmer 2014; Hunter et al. 2004; Robertson et al. 2002; Roy et al. 2004). The risk of zoonotic transmission is highest in areas where diarrheic calves, due to *Cryptosporidium* infection, are physically located or have previously been housed (Caccio and
Widmer 2014). Prevention includes good hygiene practices and avoidance of potentially infected, symptomatic calves and other livestock when acceptable.

Of all Cryptosporidium species infecting cattle, Cryptosporidium parvum is by far the most common zoonotic agent due to its wide range of host infectivity and large number of animal reservoirs (Cacciò and Widmer 2014). It was initially thought that all human Cryptosporidium parvum infections were of zoonotic origin and calves deemed the main source of oocyst isolates/infectious oocysts (Cacciò and Widmer 2014). Subsequent studies have found this to not be the case. Instead, it is likely that a majority of the C. parvum infections are anthroponotic, transmitted from man to animal, or of human-to-human transmission with human adapted C. parvum subgenotypes (Grinberg et al. 2008; Mallon et al. 2003). The occasional finding of C. hominis in cattle highlights the fact that transmission (direct, foodborne and/or waterborne) can not only be from cattle to humans, but also from humans to cattle (Chen and Huang 2012; Smith et al. 2005).

**Cryptosporidium Subtypes**

Cryptosporidium subtyping aims to investigate the genetic diversity within and between Cryptosporidium species. Subtyping also provides information on transmission dynamics and sources of infection. Subtypes of C. parvum and C. hominis are currently of most importance due to their role and prevalence in human cryptosporidiosis. The most common genetic marker used for subtype identification and differentiation is the 60 kDa glycoprotein (gp60 or gp40/15) gene (Cacciò and Widmer 2014). There are multiple genes that can be used for isolate identification and to date there is no single uniformly applied subtyping method (Cacciò and Widmer 2014).
Table 4 provides clinical information on three *C. parvum* subtypes, IOWA, TAMU and UCP, including ID$_{50}$ levels, prepatent, incubation and patent period lengths.

| Table 4: Infection Characteristics and Clinical Features Associated with *Cryptosporidium parvum* isolates in Humans |
|--------------------------------------------------|------------------|------------------|------------------|------------------|
| *C. parvum* Isolate | IOWA | IOWA | TAMU | UCP |
| Source of *C. parvum* isolate | Calf | Calf | Foal | Calf |
| ID$_{50}$ | 132$^{a,b}$ | 87$^a$, 74.5$^b$ | 9$^a$, 125$^b$ | 1,042$^a$, 2788$^b$ |
| Experimental Oocyst Dose Given | 30-100; 300-500; >1,000 | > 1,000 | > 1,000 | > 1,000 |
| Percent of Subjects Developing Symptoms | 20%; 88%; 100% | 52% | 86% | 59% |
| Presence of Asymptomatic Shedding | Yes | Yes | Yes | Yes |
| Prepatent Period (mean days) | 10; 9; 6 | 7.7 | 4 | 7 |
| Incubation period (mean; median days) | 9; 6.5 | 9; 7 | 5; 5 | 11; 6 |
| Patent Period; Shedding Duration (mean days) | 2; 10; 12 | 8.4 | 3.4 | 3.3 |
| Diarrhea Duration (mean hours) | 74 | 64.2 | 94.5 | 81.6 |
| Severity (mean unformed stools) | 12.7 | 7 | 9 | 8 |
Clinical Management of Cryptosporidiosis

Current treatment of *Cryptosporidium* infection in both humans and animals is limited. Symptomatic infection is characterized by diarrhea which, if not treated can result in extreme dehydration (Caccio and Widmer 2014). Therefore, management begins with rehydration. This can be accomplished orally, which is the most preferable method, although intravenous and subcutaneous administration may be required in more severe cases. Oral or intravenous restoration of electrolytes is also important in decreasing infection severity and decreasing recovery time.

Calves infected with *Cryptosporidium* and symptomatic should be isolated from other calves to prevent spreading infectious oocysts and housed in a clean, warm, and dry environment until they have fully recovered (Caccio and Widmer 2014). Severely infected calves may require supportive care as described above including administration of medication for pain management (Caccio and Widmer 2014). It is advised such calves be given small amounts of milk several times daily to optimize digestion and minimize weight loss during the duration of the infection (Caccio and Widmer 2014).
**Future Areas of Drug Development**

To date, no widely effective vaccines or drug-based intervention strategies are available to treat human or animal cryptosporidiosis (Caccio and Widmer 2014). Many drug targets have been proposed over the years however none have come to fruition. Reasons for this include lethal host drug toxicity, inefficacy in clinical trials or animal models or even an absence of the presumed drug target (Caccio and Widmer 2014). This is thought to be the case with nitazoxanide. Nitazoxanide is the only FDA approved drug, marginally effective, for treating human cryptosporidiosis. A short list of future areas of drug development includes: flavonoids, bisphosphonates, polyamine biosynthesis, fatty acid synthase, anti-tubulin agents, dihydrofolate reductase (DHRF) inhibitors, inosine monophosphate dehydrogenase inhibitors, pyrimidine salvage enzymes, and protein kinases (Caccio and Widmer 2014). It is thought these therapies possess great promise due to their in vivo efficacy, in vitro efficacy, highly selective target and/or unique approach. A better understanding of of host-parasite interactions, Cryptosporidium immunity and continued increase in genome sequencing is expected to aid in the identification of new vaccines and drug therapies.
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