WORLDWIDE DISTRIBUTION OF CRYPTOSPORIDIUM SPECIES IN BOVINES

By

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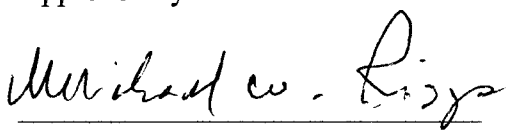

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Abstract

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; Rzeżutka et al. 2013; Cacciò 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 (Cacciò 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 (Cacciò 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).

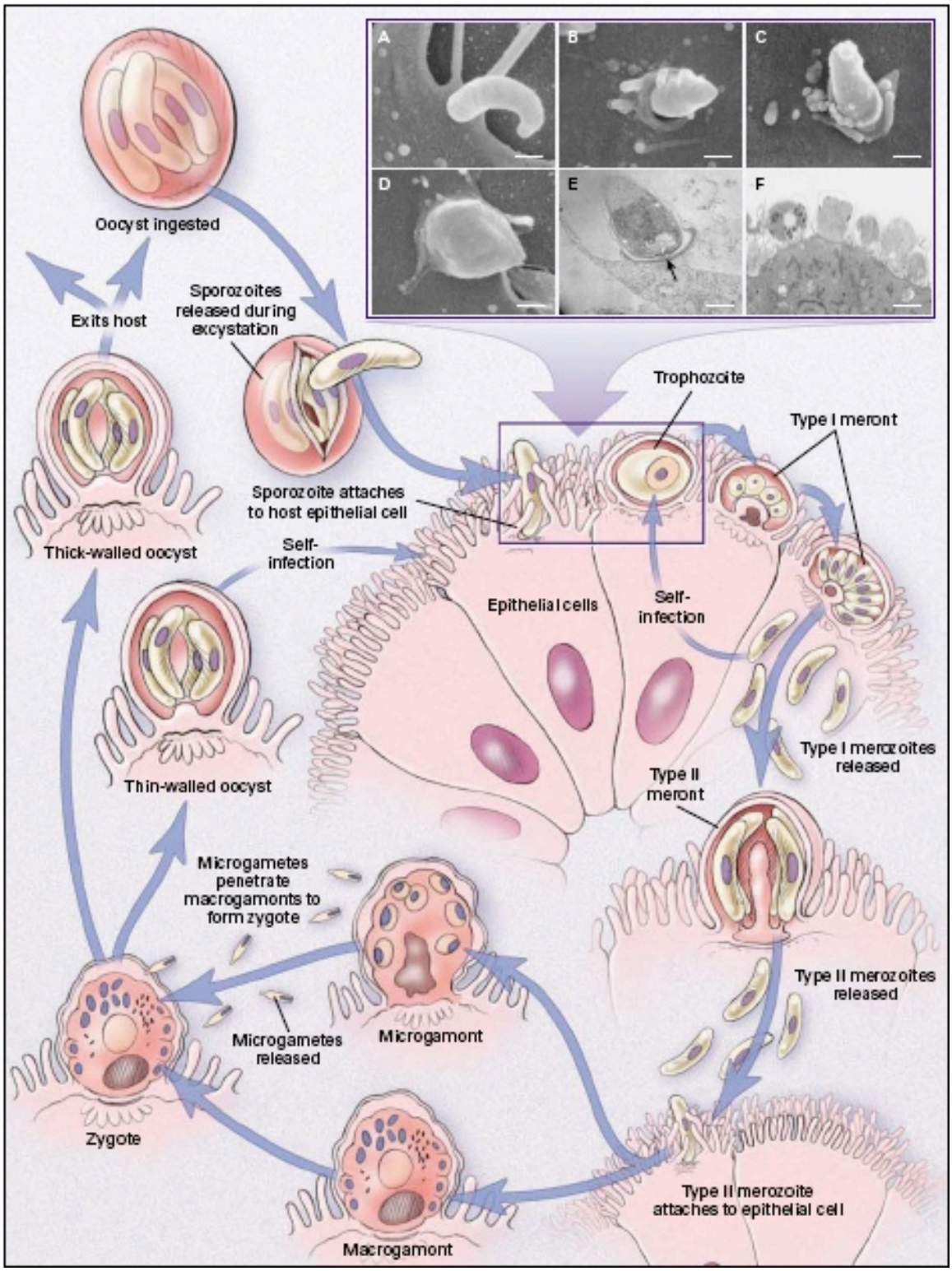
Attachment and Invasion: Once sporozoites emerge from the cyst they immediately attach themselves to the surface of host gastrointestinal epithelial cells. Like other apicomplexan parasites, *Cryptosporidium* contains an apical complex formed by organelles such as a polar ring, conoid, rhoptry and micronemes (Cacciò 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 *Cyptosporidium,* 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 (Cacciò 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 (Cacciò 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 (Cacciò 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-infectous 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. xiaoi, 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

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

			Large intestine	2004b	
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 Cacciò and Widmer (2014)

Cryptosporidiosis in Bovines

Farmed bovines, including cattle, water buffalo and zebu, are amongst the most important livestock species worldwide (Cacciò 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 (Cacciò 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		
Bovine	***Cryptosporidium* Species**	**Clinical Notes**
Bovines, including cattle (*Bos Taurus and Bos indicus*), water buffalo (*Bubalus bubalis*), and yaks (*Bos grunnies*)	*C. andersoni*	Commonly found in older post-weaned calves, yearlings and adults; some decrease in milk production has been reported
	C. bovis	Commonly found in post-weaned calves; no documented pathogenicity available
	C. parvum	Commonly found in pre-weaned calves; acute onset of symptoms and diarrhea
	C. ryanae	Commonly found in post-weaned calves

Adapted from Cacciò 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 (Cacciò 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 (Cacciò 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 immunodeficienct (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% (Cacciò 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; Santı´n et al. 2004; Smith et al. 2005; Geurden et al. 2006; Langkjaer et al. 2007; Chen and Huang 2012; (Cacciò 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 3: Prevalence of *Cryptosporidium species* in Cattle in Recent Studies (2004-2013)

Location	Age	Number of Animals/ Farm or Location	Prevalence	Identification Method/ Molecular Identification	Species Identified	References
Australia, NSW	Calves	196/20 herds	74%	Molecular/ 18S rRNA and GP60	*C. parvum* 59% *C. bovis* 20% *C. ryanae* 10% Mixed inf. 10% Not identified 1%	Ng et al. 2012
Brazil	≤ 30 days	196/dairy	11%	Molecular/ 18S	Not identified 42%	Meireles et

		herds		rRNA and GP60	*C. parvum* 33% *C. ryanae* 10% *C. andersoni* 10% *C. bovis* 5%	al. 2011
Canada	< 2 months 2-6 months >6 months	752/20 dairy herds	17% 14% 15%	Microscopy/ 18S rRNA	*C. bovis* 51% *C. andersoni* 27% *C. ryanae* 17% *C. parvum* 5%	Buda-Amoako et al. 2012a
Canada	≤ 6 months >6 months	739/20 beef herds	18% 15%	Microscopy/ 18S rRNA and HSP70	*C. andersoni* 49% *C. parvum* 24% *C. bovis* 20% *C. ryanae* 7%	Buda-Amoako et al. 2012b
Czech Republic	20-60 days	750/24 dairy herds	21%	Microscopy/ 18S rRNA RFLP	*C. parvum* 86% *C. andersoni* 13% *C. bovis* 2%	Kváč et al. 2011
China	0 to >48 months	2,056/14 dairy herds	19%	Microscopy/ 18S rRNA	*C. parvum* 48% *C. andersoni* 29% *C. bovis* 16% *C. hominis* 6% *C. serpentis* 1%	Chen and Huang 2012
China	0-8 weeks	801/8 herds	21%	Microscopy/ 18S rRNA and GP60	*C. bovis* 38% *C. parvum* 31% Mixed inf. 12% *C. ryanae* 11% *C. andersoni* 7%	Wang et al. 2011b
England and Wales	≤ 3 months	229 dairy or beef calves/ diagnostic lab	45%	Microscopy/ 18S rRNA	*C. parvum* 91% Not identified 7% *C. bovis* 2%	Featherstone et al. 2010a
England and Wales	Pre-weaned Immature Adults	116/11 herds	81% 58% 19%	Microscopy/ 18S rRNA	*C. parvum* 77% *C. andersoni* 16% *C. bovis* 5% Not identified 2%	Smith et al. 2010
Egypt	< 6 weeks	96/2 dairy herds	30%	Microscopy/ 18S rRNA and COWP	*C. parvum* 93% *C. andersoni* 7%	Amer et al. 2010
Egypt	1d -3 months >3 months-1y	593	30% 13% 13% 5%	Other/ 18S rRNA and GP60	*C. parvum* 65% Mixed inf. 17% *C. ryanae* 14% *C. bovis* 4%	Helmy et al. 2013

	>1-2 years >2 years					
Hungary	Pre-weaned	79 diarrheic/ 52 herds	49%	Microscopy/ 18S rRNA and GP60	*C. parvum* 95% *C. ryanae* 5%	Plutzer and Karanis 2007
India	< 3 months	461/ various	16%	Microscopy/ 18S sRNA	*C. parvum* 100%	Maurya er al. 2013
India	0-2 months 3-12 months >12 months	180/ 2 dairy herds	20% 14% 4%	Microscopy/ 18S rRNA	*C. bovis* 38% *C. parvum* 29% *C. ryanae* 14% *C. andersoni* 14% *C. suis*-like 5%	Khan et al. 2010
Iran	1-20 weeks	272/15 dairy herds	19%	Microscopy/ 18S rRNA	*C. parvum* 73% *C. andersoni* 18% *C. bovis* 8% Atypical isolates 2%	Keshavarz et al. 2009
Italy	0d to < 12 mo	2.024/248 dairy and beef herds	8%	ELISA and Microscopy/ COWP and GP60	*C. parvum* 100%	Duranti et al. 2009
Japan	3-48 days	80 diarrheic	75%	Molecular/ 18S rRNA	*C. parvum* 53% Not identified 45% *C. bovis* 2%	Karanis et al. 2010
Malaysia	1d to ≤ 4.5mo >4.5-12 mo	250/16 herds	31% 23%	Molecular/ 18S rRNA and GP60	*C. bovis* 25% *C. andersoni* 20% *C. parvum* 17% Not identified 17% *C. ryanae* 15% Mixed inf. 6%	Muhid et al. 2011
Nigeria	2-365 days	194/20 herds	16%	Molecular/ 18S rRNA	*C. bovis* 45% *C. ryanae* 26% *C. andersoni* 16% Mixed inf. 13%	Maikai et al. 2011
Northern Ireland	< 1 month	779 diarrheic/ diagnostic lab	37%	Microscopy/ 18S rRNA	*C. parvum* 95% *C. bovis* 4% *C. ryanae* 1%	Thompson et al. 2007
Romania	1-30 days	258 diarrheic/ 9	25%	Microscopy/ 18S rRNA and	*C. parvum* 100%	Imer et al. 2011

		dairy herds		GP60		
Spain	Neonatal Heifers Cows	649	61% 15% 8%	Microscopy/ 18S rRNA	*C. parvum* 56% *C. andersoni* 23% Not identified 21%	Castro-Hermida et al. 2011a
Spain	≤ 21 days	61 diarrheic/ 27 herds	49%	Microscopy/ 18S rRNA and GP60	*C. parvum* 100%	Díaz et al. 2010a
Sweden	≤2 months 4-12 months Cows	1,202/ 50 dairy herds	52% 29% 6%	Microscopy/ 18S rRNA and GP60	*C. bovis* 75% *C. parvum* 14% *C. ryanae* 9% *C. andersoni* 2%	Silverlås et al. 2009b; Silverlås et al. 2010b
USA (7 states)	5d -2 months 3-11 months	971/15 dairy herds	50% 20%	Microscopy/ 18S rRNA	*C. parvum* 50% *C. bovis* 28% *C. ryanae* 16% *C. andersoni* 6% Mixed inf. 10% Not identified 1%	Santín et al. 2004
USA (7 states)	12-24 months	571/14 dairy herds	12%	Molecular/ 18S rRNA	*C. andersoni* 43% *C. bovis* 35% *C. ryanae* 15% *C. parvum* 6% *C. suis* 1%	Fayer et al. 2006
USA (20 states)	6-18 months	819/49 beef herds	20%	Molecular/ 18S rRNA	*C. andersoni* 68% *C. bovis* 23% *C. ryanae* 9%	Fayer et al. 2010

Adopted from Cacciò 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 (Cacciò 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 (Cacciò and Widmer 2014). Clinical signs most prominently found with bovine cryptosporidiosis caused by *C. partum* include presentation of pasty to watery diarrhea with or without lethargy, inappetence, fever, dehydration and/or weight loss (Cacciò and Widmer 2014). Both malabsorption and increased fluid secretion in the ileum and proximal portion of the large intestine leads to these clinical signs (Cacciò 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 (Cacciò 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 (Cacciò and Widmer 2014; Uga et al. 2000). Recovery in these animals varies largely. Individuals most often recover spontaneously within one to two weeks (Cacciò 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; Cacciò 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 (Cacciò 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 (Cacciò 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 $8x10^6$ 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 (Cacciò 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 (Cacciò 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 (Cacciò 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%[c]; 88%[d]; 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
References	DuPont et al.	Okhuysen et al.	Okhuysen et al.	Okhuysen et al.

	(1995)	(1999)	(1999)	(1999)

[a] Clinical= symptoms reported

[b] Microbiological= oocysts detected in stool

[c] 30 oocyst dose given

[d] >300 oocyst dose given

Adopted from Cacciò and Widmer (2014)

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 (Cacciò 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 (Cacciò and Widmer 2014). Severely infected calves may require supportive care as described above including administration of medication for pain management (Cacciò 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 (Cacciò 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 (Cacciò 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 (Cacciò 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 (Cacciò 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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