Chapter 4
Cryptosporidium parvum: The Veterinary Perspective

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Abstract Apicomplexan parasites of the genus Cryptosporidium are increasingly considered as pathogens of zoonotic and animal health impact. However, many questions related to important aspects such as epidemiology, pathogenesis, immunology and efficient control measures remain to be answered. Advanced and conventional tools are available to study these parasites in depth which opens the door to multidisciplinary approaches combining expertise from different scientific disciplines. This short review highlights several aspects where particularly veterinary parasitologists are committed to provide relevant contributions to cryptosporidiosis research.

Cryptosporidium parvum is a **zoonotic protozoan parasite** (Table 4.1) that is increasingly regarded as an important intestinal pathogen in animals, particularly young ruminants. It is well documented that, in contrast to most other monoxenous apicomplexa, *C. parvum* is not restricted to a single host but may dwell in the intestines of a large number of vertebrate species. In spite of many attempts to develop pharmaceuticals or vaccines to allow control of cryptosporidiosis in animals and man the respective options are very limited. Thus, it will remain a challenge for the future to evolve efficient control options. Suitable experimental models are inevitable for this means. Considering these aspects *C. parvum* may serve as an excellent example to comment on the general tasks of veterinary medicine in current research on parasites which are:

1. To reduce the risk of zoonotic transmission of parasitic pathogens
2. To prevent and/or cure parasitic disease in animals

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Table 4.1 Case numbers for notifiable parasitic zoonotic infections in Germany

Year	2001	2002	2003	2004	2005	2006	2007
Giardiosis	3,894	3,097	3,216	4,621	4,519	3,661	3,654
Cryptosporidiosis	1,481	816	885	935	1,309	1,204	1,459
Toxoplasmosis (prenatal)	38	18	19	16	18	10	20
Echinococcosis	46	41	85	97	125	124	93
Trichinellosis	5	10	3	5	0	22	10

Source of data: Yearly report of Robert Koch Institute (http://www.rki.de/cln_160/nn_196658/DE/Content/Infekt/Jahrbuch/jahrbuch__node.html?__nnn=true)

3. To maintain productivity of livestock farming
4. To develop and provide models suitable to evaluate for example host–parasite interaction or parasite control options

This short review highlights these topics with particular emphasis on the author's personal experience in the area of *C. parvum* research and does not claim to comprehensively reflect the current state of knowledge.

In short, the **monoxenous life cycle** of *C. parvum* is relatively simple. After oral ingestion of sporulated oocysts from the environment each oocyst releases four sporozoites that invade the brush border of the intestinal lining and subsequently transform to trophozoites. Following asexual multiplication (merogony) the parasite undergoes sexual differentiation (gamogony). After fertilization the zygote develops into the oocyst stage. It is typical for cryptosporidia that oocysts are sporulated already before they are passed with the faeces into the environment, and thus they are fully infective upon excretion. The life cycle may be completed within as few as 3–7 days and infected animals may shed tremendous amounts of oocysts within a short time. Oocysts may survive, depending on the environmental conditions, for several weeks or even months, thus imposing a continuing infection risk to susceptible hosts. Surface water is believed to be an efficient carrier for oocysts and epidemic outbreaks of human cryptosporidiosis (the most cited reported from Milwaukee by MacKenzie et al. 1995) are explained by ingestion of contaminated water. In fact, several studies from different continents documenting oocysts in surface water have shown that zoonotic genotypes were present in the respective samples (e.g. Ono et al. 2001; Ward et al. 2002; Xiao et al. 2001).

Originally only two *Cryptosporidium* spp. were believed to represent the respective genus, namely *C. muris* (Tyzzer 1907) and *C. parvum* (Tyzzer 1912). Further on, intestinal infections with cryptosporidia were described for many animal species and were exclusively attributed to *C. parvum* which thus appeared to lack host specificity. Later it became evident, mainly based on oocyst morphology but also on host and/or site preference, that the genus is more divergent than previously thought.

For instance, other host-specific species of *Cryptosporidium* (e.g. *C. felis* in cats, *C. meleagridis* in poultry) may be zoonotic under certain circumstances (Morgan et al. 2000). Two *C. parvum* types, one being zoonotic and mainly found in animals and the other obviously adapted to humans, were described (Awad-El-Kariem et al. 1998) and the latter is meanwhile regarded as a different species, named *C. hominis*. Many other *C. parvum* genotypes have been defined and may or may not reach species status in the future. The various *C. parvum* genotypes are more or less adapted to certain host species, some representing zoonotic genotypes while others

appear to be of less or no zoonotic relevance. Immunocompromized persons, for example those infected with HIV, are particularly susceptible to cryptosporidia infection and may suffer from severe, potentially life-threatening disease. This may be less dramatic in industrialized countries due to the high level of medical care, for example improved treatment options for AIDS, however, in less developed areas and under conditions of poor hygiene cryptosporidiosis will remain a severe threat to exposed persons, particularly those belonging to a risk group. Unfortunately, our knowledge on the epidemiology and relevance of cryptosporidiosis in developing countries is very limited (Rahman et al. 1985; Jex and Gasser 2010).

Other cryptosporidia species being less important as zoonotic pathogens may occasionally infect humans. For instance, *C. felis* and *C. meleagridis* were found in AIDS patients (Morgan et al. 2000). Close contact to pet animals increases the risk of zoonotic transmission of *Cryptosporidium* spp. (Joachim and Daugschies 2004) such as *C. felis* or *C. canis*. The molecular taxonomy of the genus *Cryptosporidium* and particularly of *C. parvum* is still in progress (Jex and Gasser 2010) and new respective insights will have profound consequences for the elucidation of epidemiological questions, for example regarding the source of human infection and transmission routes.

In Germany **human cryptosporidiosis** belongs to the group of **notifiable infectious diseases** and appears to be more prevalent than other parasitic and potentially zoonotic infections in humans (Table 4.1). Since the clinical course is not very specific and disease is typically transient in immune-competent persons the prevalence estimated on the basis of notified cases (less than 1,500 per year in Germany) is without doubt extremely underestimated. The infectious dose for humans is low (30 oocysts) whereas one infected calf may shed as much as 10^{11} oocysts during patency (Joachim and Daugschies 2004) and thus it appears very likely, considering that *C. parvum* is a ubiquitous pathogen in ruminant herds, that livestock contribute to the contamination of surface water (Fayer 2004; Karanis et al. 2006) and that every person in the vicinity of young ruminants (e.g. animal keepers, veterinarians) will attract *C. parvum* infection sooner or later. However, data are fragmentary and mainly reflect the situation in developed countries whereas very little is known on the zoonotic transmission in economically less developed countries where the problems are supposed to be much more dramatic (Jex and Gasser 2010).

Wildlife may also serve as hosts for cryptosporidia including *C. parvum* as the host range of the latter is extremely wide (Fayer 2004; Jex and Gasser 2010). European hedgehogs are often kept during the winter season under human care if they are found to be in a condition (e.g. young or diseased hedgehog) that is related to a risk of nonsurvival. Around 20–40% of these animals excrete oocysts of different genotypes of *C. parvum*. Further genotyping of GP60, HSP70 and actin revealed three subtypes, namely IIa (bovine), IIc (human) and a proposed new genotype VIIa (Dyachenko et al. 2010). The former two indicate the possibility of zoonotic transmission by hedgehogs whereas the latter one remains to be studied further.

Besides its role as an agent of **zoonotic disease** *C. parvum* in particular attracts considerable attention as a cause of **enteritis** in animals. Mainly young animals such as suckling calves or lambs are susceptible to cryptosporidiosis which displays as transient watery diarrhoea, sometimes resulting in rapid dehydration and death. However, most affected animals survive and build up protective immunity but may show sustained reduction of productivity, although this aspect is not well documented.

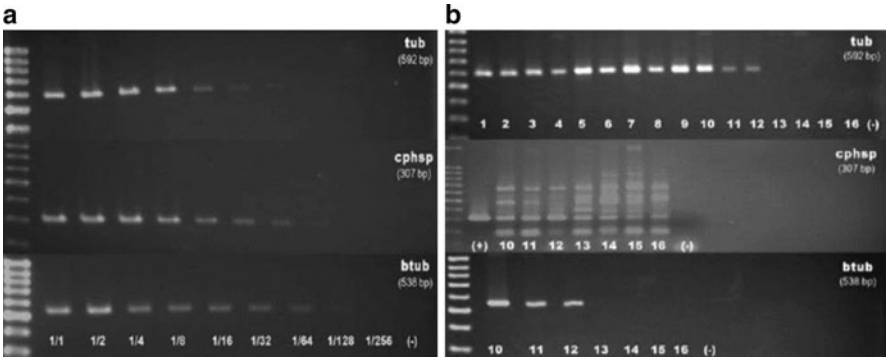

Fig. 4.1 Banding pattern of amplicons after PCR with various primer pairs (tub: Caccio et al. 1999; cphsp: Rochelle et al. 1997; btub: Widmer et al. 1998). (**a**) Pure oocyst suspension; (**b**) amplification from faecal samples

Diagnosis of cryptosporidiosis in animals is classically done by microscopic evaluation of oocysts. Because diseased animals tend to shed tremendous amounts of oocysts, stained (e.g. carbol fuchsine, Ziehl Neelsen stain) or unstained faecal smears are generally suitably sensitive for diagnostic purposes. Alternatively, immunological test kits are commercially available. PCR is very sensitive, however, not all primer combinations published are suited for direct detection of parasite DNA in faecal samples. In our experience, primers targeted against the β-tubulin gene are specific in faecal specimen whereas the heat shock protein gene locus (HSP70) resulted in many unspecific bands (Fig. 4.1). Calves experimentally infected with *C. parvum* were positive by conventional microscopy from 4 days postinfection (dpi) until 12 dpi whereas PCR delivered respective signals from 4 dpi until 14 dpi (Kar et al. 2010). Thus, molecular methods are without doubt powerful and useful tools in epidemiological research on cryptosporidiosis but it appears that the additional technological effort and costs related to PCR are not justified for routine diagnostic purposes in veterinary medicine.

It has long been questioned whether cryptosporidia are primary pathogens or whether they exert relevant insult only under certain conditions (e.g. altered immunity or in combination with other pathogens). Meanwhile it is clear that infections with only *C. parvum* induce diarrhoea in otherwise healthy young calves or lambs under experimental conditions as well as in livestock herds. Of course, the severity and course of disease depend on other factors in field situations, for example sufficient access to colostrum or exposure to other germs. For instance, lambs shed more pathogenic *E. coli* if raised colostrum-deprived and simultaneously infected with *C. parvum* (La Ragione et al. 2006). It has been estimated that the prevalence of *C. parvum* in calves ranges between 20% and 40% in Europe, which in the light of own experience appears to be a rather conservative approximation. Farmers (and veterinarians) tend to accept diarrhoea in young calves as long as this is not related to considerable mortality, or the self-limiting disease is attributed to other causes (e.g. bacterial infection, feeding). Consequently, specific

diagnosis is often neglected and awareness is low. Therefore, more epidemiological data on *C. parvum* in ruminant livestock herds are urgently needed.

Attempts to protect young ruminants by vaccination have been published. Because clinical cryptosporidiosis may already develop in the first week after birth application of a *C. parvum* vaccine to exposed new-born animals appears not to be a promising option. Goat lambs were reported to be well protected against *C. parvum* challenge when their mothers were treated with a plasmid vaccine in the 3rd/4th month of gestation (Sagodira et al. 1999). However, commercial vaccines are still not available.

Cleaning and disinfection of areas where susceptible (young) animals are kept are obviously necessary measures to keep the infection pressure at an acceptable level. Chemical disinfectants that are able to inactivate oocysts are available on the market and represent cresol-based products in most cases (Shahiduzzaman et al. 2010). However, in a conventional cow herd chemical disinfection with a cresol alone did not efficiently control cryptosporidiosis in calves. In this herd prevalence was 100% and thus the level of contamination with oocysts was probably very high. Considering the low oocyst dose necessary to induce infection and short prepatent period of *C. parvum* it appears that reduction of environmental contamination by increased hygiene alone to a level preventing cryptosporidiosis may be an exhausting challenge in farms with high initial prevalence (Keidel 2004).

Only **few drugs** have been registered for control of cryptosporidiosis in animals worldwide. Halofuginone is on the market in several European countries (Halocur^{R}, Intervet). Calves need to be treated with this drug on 7 consecutive days starting early (within 24 h) after birth to achieve metaphylactic control. Even under these laborious conditions oocyst excretion is not completely suppressed within groups of naturally exposed calves (Fig. 4.2) and some may still present diarrhoea (Keidel 2004). Interestingly, the combination of cresol disinfection and halofuginone

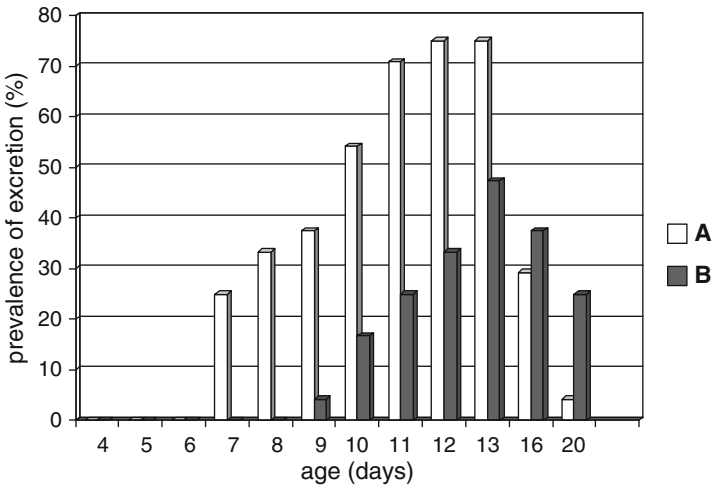

Fig. 4.2 Oocyst excretion by calves ($n = 24$) treated with placebo (**a**) or halofuginone (**b**) 100 μg/kg body weight daily over 7 days starting within 24 h after birth)

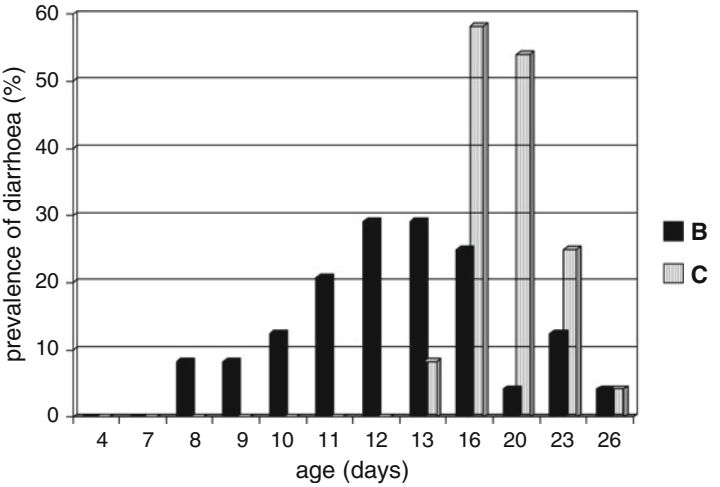

Fig. 4.3 Diarrhoea prevalence in calves ($n = 24$) treated with halofuginone (**b**, **c**) 100 µg/kg body weight daily over 7 days starting within 24 h after birth) and kept in crates either disinfected (**c**) with a cresol-based product or not chemically disinfected (**b**)

treatment almost completely controlled cryptosporidiosis during the period of particular risk in conventional calves, i.e. the first and second week after birth. The protected calves were more susceptible thereafter than calves that experienced cryptosporidiosis before, indicating insufficient immune stimulation in the former group (Fig. 4.3). Although older animals suffering from cryptosporidiosis appear to be less prone to develop a severe disease than younger ones it remains to be evaluated whether optimal protection of young animals is a worthwhile strategy in terms of economic benefit and reduced oocyst excretion.

Despite significant steps forward major gaps in knowledge on *Cryptosporidium* still exist. This includes taxonomy and epidemiology, risk analysis, host–parasite interaction and immunology and, particularly important from a veterinary point of view, control options. To assess most of these aspects advanced molecular tools are now available and the genome of *C. parvum* is completely sequenced (Abrahamsen et al. 2004).

To evaluate new control options animal infection models, particularly in mice, are commonly used in laboratories all over the world whereas studies in experimentally infected natural hosts such as calves are subject to practical constraints. *Cryptosporidium parvum* can be cultured in vitro in permanent cell lines and HCT-8 cells appear to be particularly suitable. They are easily grown to confluence in a simple medium (RPMI-1640 supplemented with fetal calf serum, L-glutamate, sodium pyruvate and antibiotics) at 37°C and 5% CO_2. Recently a combination of *C. parvum* in vitro culture and quantitative PCR ("cc-qPCR") to screen drugs and inactivation measures (e.g. chemical disinfection) at a laboratory scale has been published. The system is well standardized and results are reproducible thus allowing quantification of reproduction (viability, infectivity) of the parasite under strictly defined conditions (Fig. 4.4, Shahiduzzaman et al. 2009a, b, 2010).

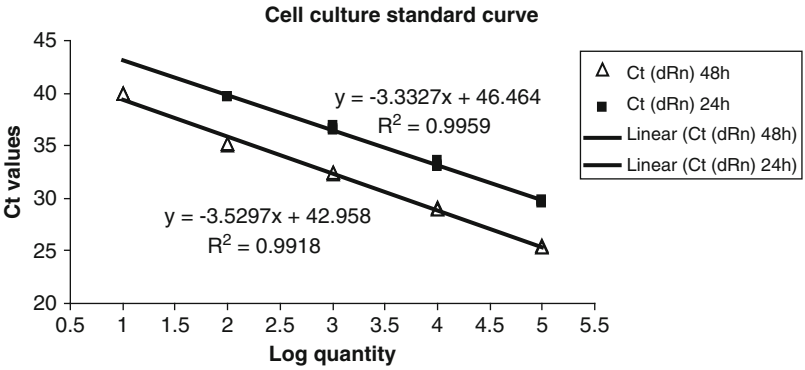

Fig. 4.4 Linear association of infection dose of *C. parvum* oocysts and Ct values in cc-qPCR

Table 4.2 Comparison of conventional assessment of oocyst inactivation by commercial chemical disinfectants (incubation period: 2 h) according to DVG-guideline (in vivo, *E. tenella*) and cc-qPCR

		Inactivation (%)	
	Product concentration (%)	cc-qPCR (*C. parvum*)	In vivo (*E. tenella*)
	3	99.40	92.9
	4	99.93	96.8
	3	99.11	90.6
Neopredisan® 135-1	4	99.91	95.2
Aldecoc® TGE	4	99.91	95.5
	3	99.86	95.5
KokziDes®	4	99.87	99.93
Threshold value		≥99.5%	≥95%

In Germany anti-parasitic chemical disinfectants have to be tested according to guidelines published by the German Veterinary Association (DVG) to obtain respective certification (see www.dvg.net). The ability of chemical disinfectants to inactivate coccidia oocysts is currently being evaluated with *Eimeria tenella* oocysts in a chicken infection model (Daugschies et al. 2002). Excretion of oocysts by experimentally infected birds is assessed and results for birds infected with treated/untreated oocysts are compared. Inactivation is considered sufficient if oocyst excretion in birds infected with disinfected oocysts is reduced by at least 95%. When *C. parvum* oocysts were treated with the same disinfectants in an identical manner (time, concentration) a 99.5% reduction of viability was always recorded in cc-qPCR. On the other hand, insufficient inactivation in the chicken model (<95%) was paralleled by <99.5% inactivation in cc-qPCR (Table 4.2, Shahiduzzaman et al. 2010). Therefore, it is proposed to replace the standard animal infection model by cc-qPCR and respective further testing is currently being performed.

A similar in vitro model has been applied to test curcumin (herbal extract isolated from *Curcuma longa*) for its potential as an anti-cryptosporidial drug. It was clearly

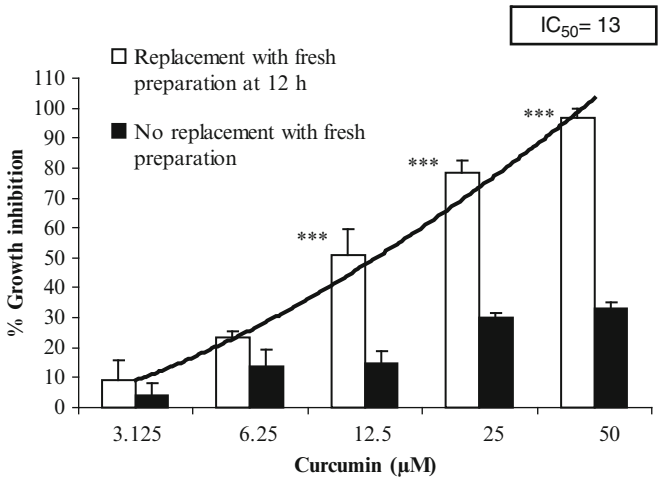

Fig. 4.5 Suppression of multiplication of *C. parvum* in HCT-8 cells by 24 h incubation with curcumin

seen, that curcumin inhibits *C. parvum* multiplication in a dose-dependent way with an IC_{50} of 13 µM provided that the drug-containing medium was replaced every 12 h (Fig. 4.5). This indicates that the herbal extract is not very stable under the given conditions, however, further exploration of curcumin may be rewarding (Shahiduzzaman et al. 2009b).

The demand for new drugs that may hopefully allow better control of *C. parvum* infections in animals and man has stimulated the search for respective targets. For instance, calcium-dependant protein kinases (CDPK) are not expressed in mammalian cells but have been demonstrated in *C. parvum*. They appear to be constitutive in the parasite and treatment of *C. parvum* cultures with specific antiserum inhibits parasite replication dose dependently. Although the functions of CDPK remain to be characterized in *C. parvum* it appears that this may be a promising drug target (Etzold et al. 2009).

Altogether, cryptosporidia and particularly *C. parvum* are a rewarding area for research in many respects. Veterinary parasitologists have the duty to improve animal health and livestock productivity, to protect man from zoonotic transmission and to make use of any modern and traditional tools to achieve these ambitious goals. The multiple aspects to be considered demand inter-disciplinary networks based on mutual respect and understanding thus strengthening collaboration between researchers from the fields of veterinary, medical, biological and other sciences.

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