

Distribution of *Cryptosporidium* species, genotypes and *C. parvum* subtypes in cattle in European countries

Kálmán Imre[✉], Gheorghe Dărăbuș

Banat University of Agricultural Sciences and Veterinary Medicine, Faculty of Veterinary Medicine, Department of Parasitology and Parasitic Diseases, Calea Aradului 119, Timișoara 300645, Romania.

Correspondence: Tel. +40256494023, Fax +40256200296, E-mail kalman_imre27@yahoo.com

Abstract. The present review reports the prevalence studies carried out in bovine cryptosporidiosis in European countries in the last decade, including the molecular background when available. In foreground, the distribution of *Cryptosporidium* species, genotypes and *C. parvum* subtype families (alleles) in cattle are described in the twenty countries where molecular studies have been conducted. Surveys carried out in bovines have shown that eight species (*C. parvum*, *C. andersoni*, *C. bovis*, *C. ryanae*, *C. felis*, *C. hominis*, *C. meleagridis* and *C. suis*) and three genotypes (*Cryptosporidium* deer-like genotype, *C. suis*-like and *Cryptosporidium* pig genotype) were responsible for cryptosporidial infections. Mainly the first four enumerated *Cryptosporidium* species were found with a varied prevalence. The DNA sequence analysis of the 60 kDa glycoprotein (GP60) gene in *C. parvum* has revealed three subtype families (IIa, IIc and III) of the eleven alleles (IIa-IIk) known to be present in cattle. The epidemiological survey results suggest that cattle are a potential source and an important reservoir of *C. parvum* infection for both humans and animals in Europe.

Keywords: *Cryptosporidium*; Cryptosporidiosis; Molecular epidemiology; Cattle; Europe.

Received 03/12/2010. Accepted 02/03/2011.

Background

Members of genus *Cryptosporidium* are unicellular parasites that infect epithelial cells of the gastrointestinal and respiratory tract of a variety of vertebrate hosts (Ondráčková et al., 2009).

Bovines are the most important animal group known to be infected with *Cryptosporidium*, probably because of the large amount of studies performed on them and because of their economic importance.

Bovine cryptosporidiosis was first reported four decades ago by Panciera et al. (1971) in an eight month old heifer with chronic diarrhea (Dărăbuș and Imre, 2010).

In the last years, in many European countries different kinds of molecular diagnostic tools were developed for the identification and differentiation of *Cryptosporidium* species, genotypes and *C. hominis* and *C. parvum* subtypes. In endemic and epidemic areas these methods are intensively used in epidemiological surveys of cryptosporidiosis (Xiao, 2010).

Molecular epidemiology studies from cattle have reported the identification of eight species (*C. parvum*, *C. andersoni*, *C. bovis*, *C. ryanae*, *C. felis*, *C. hominis*, *C. meleagridis* and *C. suis*) and three genotypes (*Cryptosporidium* deer-like genotype, *C. suis*-like and *Cryptosporidium* pig genotype) (Bornay-Llinares et al., 1999; Smith et al., 2005; Geurden et al., 2007; Langkjæer et al., 2007; Mendonça et al., 2007; Plutzer and Karanis, 2007; Santín and Trout, 2007; Dărăbuș and Imre, 2010; Xiao, 2010). Usually, cattle have been considered as major hosts for the first four enumerated *Cryptosporidium* species.

The data of European researchers have contributed greatly to the knowledge of the molecular epidemiology of bovine cryptosporidiosis (Thompson et al., 2007; Geurden et al., 2007; Misić and Abe, 2007; Plutzer and Karanis, 2007; Wielinga et al., 2007; Broglia et al., 2008; Quilez et al., 2008; Soba and Logar, 2008; Brook et al., 2009; Duranti et al., 2009; Imre et al., 2010; Silverlås et al., 2010). The molecular data confirmed in 31 (91.2%) out of 34 publications that calves are an important source of zoonotic cryptosporidiosis in humans. Generally, *C. parvum* is found in pre-weaned calves (<2 months), *C. bovis* and *C. ryanae* in post-weaned calves (2 months to 1 year) and *C. andersoni* in yearling heifers and mature cows.

The continuous screening of the genetic properties is the most important in defining and describing *Cryptosporidium* genetic diversity. The aim of the present review is to report the current status of the distribution of *Cryptosporidium* species, genotypes and *C. parvum* subtypes in cattle from Europe and their zoonotic importance.

Species and genotypes distribution

Although many genes can be used for *Cryptosporidium* species and genotypes differentiation, the most widespread is the small subunit (SSU) rRNA gene. This ribosomal genomic gene can be replicated in multiple copies. Other genes, such as the oocyst wall protein (COWP), 70-kDa heat shock protein (HSP70) and actin genes are more rarely used.

Especially for the bovine-origin specimens, nested PCR analysis of the SSU rRNA gene was

used followed by restriction fragment length polymorphism (RFLP) analysis of the secondary PCR products with *SspI* and *VspI* enzymes. For genotype identifications *MboII* and *DdeI* enzymes have been successfully used. Each of these techniques tends to obtain a clear and visible image of the final PCR product in agarose gel.

Recently, the development of the *MboII*-RFLP analysis of the SSU rRNA gene to differentiate the three intestinal bovine species (*C. parvum*, *C. bovis* and *C. ryanae*) facilitated new studies in bovine cryptosporidiosis (Geurden et al., 2007; Soba and Logar, 2008; Follet et al., 2009; Ondráčková et al., 2009). Moreover, this method may be especially useful in identifying species in mixed infections. In order to distinguish *C. parvum* from *C. bovis* or the *Cryptosporidium* deer-like genotype, the sequencing can be successfully used but the procedure is more expensive. This technique can also offer more reliable information than PCR-RFLP.

Several studies investigated the presence of *Cryptosporidium* in dairy and beef cattle in Europe. The molecular data on species diversity are shown in table 1. It is evident that the most common and spread species in all age categories and in most regions is *C. parvum*. Other common species/genotypes are *C. bovis*, *C. andersoni* and the deer genotype. Infections with *C. parvum* have been reported in dairy and beef cattle, in many parts of Europe, but the prevalence data have often varied significantly. The high occurrence of *C. parvum* in cattle has been shown more recently in Belgium (Geurden et al., 2007), England (Brook et al., 2009), France (Paraud et al., 2009), Germany (Broglia et al., 2008), Italy (Grana et al., 2006; Duranti et al., 2009; Mangili et al., 2009; Merildi et al., 2009), Netherlands (Wielinga et al., 2007), Poland (Majewska et al., 2004), Portugal (Alves et al., 2003, 2006; Mendonça et al., 2007), Romania (Imre et al., 2009), Serbia and Montenegro (Misić and Abe, 2007), Slovenia (Soba and Logar, 2008) and Spain (Quilez et al., 2008). Several studies conducted in Italy confirmed that *C. parvum* was the only species responsible for bovine cryptosporidiosis (Cacciò et al., 2000; 2001; Wu et al., 2003; Cencioni, 2005; Grana et al., 2006; Duranti et al., 2009; Mangili et al., 2009).

Table 1. *Cryptosporidium* species/genotypes and subtypes reported from bovines in Europe based on molecular data

Country	Examined/ positive	Species or genotype (%)	Subtypes ^a	Reference
Belgium	832 ^b /115	<i>C. parvum</i> (90.5) <i>C. bovis</i> (7.9) <i>C. suis</i> (0.8) NA (0.8)	IlaA15G2R1 (98.4), IlaA16G2R1 (3.3), IlaA13G2R1 (1.1), IlaA14G2R1 (1.1), IIdA22G1 (1.1)	Geurden et al., 2007
	695 ^c /382	<i>C. andersoni</i> ^c (100%)	-	Kváč and Vítovec, 2003
Czech Republic	995 ^d /44	<i>C. andersoni</i> (93.2) <i>C. bovis</i> (4.5) <i>C. parvum</i> (2.3)	IlaA16G1R1 (100)	Ondráčková et al., 2009
	2 [*] /2	<i>C. andersoni</i> (50) <i>C. bovis</i> (50)	-	Hajdušek et al., 2004
Denmark	1150 ^d /154	<i>C. parvum</i> (50.6) <i>C. bovis</i> (37.0) Deer-like genotype (7.8) <i>C. suis</i> -like genotype (1.9) Pig genotype II (0.7) Atypical isolates (1.9)	-	Langkjær et al., 2007
	292 ^c /100	<i>C. andersoni</i> (69) <i>C. parvum</i> (31)	-	Enemark et al., 2002
England	215/60	<i>C. parvum</i> (92.6) <i>C. bovis</i> (5.5) Deer-like genotype (1.9)	IlaA15G2R1 (68.6), IlaA17G2R1 (13.8), IlaA16G3R1 (7.8), IlaA18G1R1 (3.9), IlaA19G1R1 (3.9), IlaA14G2R1 (2)	Brook et al., 2009
	2 [*] /2	<i>C. parvum</i> (100)	IlaA20G3R1 (100)	Chalmers et al., 2005
France	42 [*] /3	<i>C. parvum</i> (66.6) <i>C. bovis</i> (33.3)	-	Paraud et al., 2009
	142 ^c /100	<i>C. parvum</i> (49.7) <i>C. ryanae</i> (25.8) <i>C. bovis</i> (24.5)	-	Follet et al., 2009
Germany	[*] /134	<i>C. parvum</i> (100)	IlaA15G2R1 (81.1), IlaA14G2R1 (3.8), IlaA17G2R1 (3.8), IlaA18G2R1 (3.7), IlaA21R1 (1.9), IlaA22G1R1 (1.9), IlaA16G1R1 (1.9), IIdA22G1 (1.9)	Broglia et al., 2008
Hungary	79 [*] /22	<i>C. parvum</i> (27.8) Deer-like genotype (1.2)	IlaA16G1R1 (71.4), IlaA17G1R1 (14.2), IlaA18G1R1 (4.8), IIdA19G1 (4.8), IIdA22G1 (4.8)	Plutzer and Karanis, 2007
Ireland	288 ^c /21	<i>C. parvum</i> (45.5) <i>C. andersoni</i> (54.5)	-	Moriarty et al., 2005
	[*] /1	<i>C. parvum</i> (100)	IlaA13G2R1 (100)	Wu et al., 2003
	[*] /29	<i>C. parvum</i> (100)	-	Cacciò et al., 2000; 2001
	[*] /3	<i>C. parvum</i> (100)	-	Cencioni, 2005
Italy	[*] /15	<i>C. parvum</i> (100)	-	Grana et al., 2006
	2024 ^b /101	<i>C. parvum</i> (100)	IlaA15G2R1 (54.8), IlaA18G2R1 (16.1), IlaA17G2R1 (14.5), IlaA14 (8.1), IlaA13 (6.5)	Duranti et al., 2009
	90 [*] /10	<i>C. parvum</i> (70) <i>C. hominis</i> (30)	-	Merildi et al., 2009
Netherlands	1454 [*] /48	<i>C. parvum</i> (100)	-	Mangili et al., 2009
	[*] /160	<i>C. parvum</i> (100)	IlaA15G2R1 (69.0), IlaA17G1R1 (10.9), IlaA16G3R1 (4.7), IlaA13G2R1 (1.5), IlaA14G2R1 (1.5), IlaA17G2R1 (1.5), IlaA18G4R1 (1.5), IlaA18R1 (1.5), IlaA19G2R1 (1.5), IlaA11G2R1 (0.8), IlaA12G2R1 (0.8), IlaA16G1R1 (0.8), IlaA16G2R1 (0.8), IlaA18G3R1 (0.8), IlaA19G1R1 (0.8), IlaA21G3R1 (0.8), IIIA24R2 (0.8)	Wielinga et al., 2007

Table 1 (cont). *Cryptosporidium* species/genotypes and subtypes reported from bovines in Europe based on molecular data

Country	Examined/positive	Species or genotype (%)	Subtypes ^a	Reference
Northern-Ireland	779 ^a /224	<i>C. parvum</i> (95.1) <i>C. bovis</i> (3.6) Deer-like genotype (1.3)	IlaA18G3R1 (56.1), IlaA15G2R1 (13.1), IlaA17G2R1 (8.9), IlaA19G4R1 (7.0), IlaA20G3R1 (2.8), IlaA17G3R1 (2.4), IlaA19G3R1 (2.4), Ila-unknown (1.9), IlaA20G5R1 (1.4), IlaA18G2R1 (1.0), IlaA20G2R1 (1.0), IlaA16G3R1 (0.4), IlaA17G1R1 (0.4), IlaA18R1 (0.4), IlaA19G2R (0.4), IlaA20G4R1 (0.4)	Thompson et al., 2007
Poland	26 [*] /8	<i>C. parvum</i> (100)	-	Majewska et al., 2004
	[*] /76	<i>C. parvum</i> (100)	IlaA15G2R1 (84.7), IlaA16G2R1 (9.7), IlaA17G1 (5.6)	Alves et al., 2003; 2006
Portugal	467 ^b /82	<i>C. parvum</i> (100) <i>C. andersoni</i> (1.2) <i>C. meleagridis</i> (1.2)	-	Mendonça et al., 2007
Romania	[*] /22	<i>C. parvum</i> (100)	IlaA15G2R1 (53.3), IlaA16G1R1 (33.3), IlaA22G2R1 (13.4)	Imre et al., 2009; 2010
Scotland	^b /411	<i>C. parvum</i> (99.5) <i>C. bovis</i> (0.5)	-	Smith et al., 2005
Serbia and Montenegro	103 ^d /62	<i>C. parvum</i> (100)	IlaA16G1R1 (33.3), IIIA16 (22.3), IlaA18G1R1 (11.1), IlaA20G1R1 (11.1), IlaA18G1b (11.1), IIIA17 (11.1)	Misic and Abe, 2007
Slovenia	[*] /51	<i>C. parvum</i> (88.2) <i>C. bovis</i> (5.9) Deer-like genotype (5.9)	IlaA15G2R1 (60.0), IlaA16G1R1 (13.4), IlaA13R1 (11.1) IlaA16R1 (6.7), IIIA16 (4.4), IIIA18 (4.4)	Soba and Logar, 2008
	[*] /4	<i>C. parvum</i> (100)	IlaA17G1R1 (100)	Stantic-Pavlinic et al., 2003
Spain	287 ^d /149	<i>C. parvum</i> (98.6) <i>C. bovis</i> (1.4)	IlaA15G2R1 (75.7), IlaA16G3R1 (10.0), IlaA18G3R1 (5.7), IlaA16G2R1 (2.9), IlaA17G2R1 (2.9), IlaA19G3R1 (1.4), IlaA23G1 (1.4)	Quilez et al., 2008
Sweden	50 ^b /2	<i>C. parvum</i> new genotype (100)	-	Björkman and Mattson, 2006
	1202 ^d /110	<i>C. bovis</i> (75.5) <i>C. parvum</i> (13.6) <i>C. ryanae</i> (9.1) <i>C. andersoni</i> (1.8)	IlaA18G1R1 (15.4), IlaA20G1e (15.4), IlaA23G1 (15.4), IlaA21G1R1 (15.4) IlaA15G1R1 (15.4), IlaA16G1R1 (7.7), IlaA22G1c (7.7), IlaA16G1 (7.6),	Silverlås et al., 2010
Wales	101 ^d /7	<i>C. andersoni</i> (100)	-	Robinson et al., 2006

^a - numbers in parantheses are percents from the total number of *C. parvum* GP60 subtyped;

^b - the sample origin was mixed dairy and beef cattle;

^c - the sample origin was from beef cattle;

^{c'} - the bovines were monitored only for the presence of *C. andersoni*;

^d - the sample origin was from dairy cattle;

* - unspecified data;

NA - no amplification.

In Belgium, the study carried out by Geurden et al. (2007) showed that *C. parvum* is the main cause of bovine cryptosporidiosis. Feces from 499 dairy and 333 beef calves were collected and tested for *Cryptosporidium* by DFA microscopy, followed by molecular characterization of positive samples. The overall prevalence was 13.8%. *Cryptosporidium parvum*, *C. bovis* and *C. suis* were identified in 104, 9 and respectively 1 sample using PCR. The identity of one species remained uncertain.

In a long term study from Northern Ireland, of the 779 cattle examined cattle, 224 were positive for *Cryptosporidium*. Of these, *C. parvum* was identified in 213 (95.1%), *C. bovis* in eight (3.6%), and *Cryptosporidium* deer-like genotype in three (1.3%) specimens.

Similar results were published by Brook et al. (2009) in England, who found *C. parvum* in 92.6% followed by *C. bovis* (5.5%) and the deer-like genotype (1.9%).

Broglia et al. (2008) carried out a study to determine if *Cryptosporidium* infections are present in pre-weaned calves from different German Federal States. In 134 fecal samples *Cryptosporidium* species were identified using *SspI*, *MboII* and *RsaI* endonucleases for the digestion of 18S rDNA and COWP amplified fragments. In all samples, restriction patterns corresponding to *C. parvum* were identified.

The same picture was described in Serbia and Montenegro by Misić and Abe (2007) from 10 farms in the Belgrade area. Of 103 examined dairy calves, 60.2% were positive for *Cryptosporidium* infection by PCR. All of them were identified as *C. parvum* by PCR-RFLP of SSU rRNA and COWP genes. Stool samples from 160 *Cryptosporidium* infected cattle were submitted to PCR-RFLP analysis in Netherlands. All of the cattle isolates were infected with *C. parvum*. In Hungary, *C. parvum* was also found to be the primary species in pre-weaned calves with diarrhea from 52 farms in nine counties (Plutzer and Karanis, 2007). Other studies, conducted in France (Paraud et al., 2009), Scotland (Smith et al., 2005), Slovenia (Soba and Logar, 2008) and Spain (Quilez et al., 2008) have reported *C. parvum* as the most common species in young dairy calves, followed by *C. bovis*. In Romania, outbreaks of cryptosporidiosis produced by *C. parvum* were responsible for diarrhea in 22 pre-weaned dairy calves (Imre et al., 2009). In other countries such as Poland (Majewska et al., 2004) or Portugal (Alves et al., 2003; 2006), infections with *C. parvum* appear to be the most common.

High rates of *C. andersoni* infection in beef cattle were confirmed in various parts of Europe (Enemark et al., 2002; Kváč and Vítovec, 2003; Moriarty et al., 2005; Robinson et al., 2006). This species infects the abomasums of juvenile and mature cattle. In the Ireland, fecal samples from 218 beef cattle were submitted for detection of *Cryptosporidium*. *Cryptosporidium andersoni* (5.04%) and *C. parvum* (4.58%) were detected in 11 and 10 fecal samples, respectively (Moriarty et al., 2005). In Denmark, 69 (23.6%) and 31 (10.6%) out of 292 beef cattle fecal samples were positive for *C. andersoni* and *C. parvum* (Enemark et al., 2002).

In southwestern Bohemia (Czech Republic) a long term study of a selected breed of beef cattle has been conducted. Feces from 599 beef calves and 96 cows were collected and tested for *Cryptosporidium*. Based on molecular methods, *C. andersoni* was identified in 340 fecal samples from beef calves and 42 samples from cows (Kváč and Vítovec, 2003). In this study the prevalence of *C. andersoni* was very high compared with prevalence values reported in other studies. Another studies in dairy cattle from Czech Republic reported high rates of *C. andersoni* (Hajdušek et al., 2004; Ondráčková et al., 2009).

In Wales, of the 101 samples collected from dairy cattle, 7 (6.9%) were positive for *C. andersoni* (Robinson et al., 2006). In addition, *C. andersoni* infections have been reported to be found in dairy cattle in Sweden (Silverlås et al., 2010) and Portugal (Mendonça et al., 2007). Natural infections with *C. bovis* have been found in various regions of Europe, such as Belgium, Czech Republic, Denmark, England, France, Northern Ireland, Scotland, Slovenia, Spain and Sweden (Hajdušek et al., 2004; Smith et al., 2005; Thompson et al., 2007; Geurden et al., 2007; Langkjær et al., 2007; Quilez et al., 2008; Soba and Logar, 2008; Brook et al., 2009; Follet et al., 2009; Ondráčková et al., 2009; Paraud et al., 2009; Silverlås et al., 2010). In Sweden, feces from dairy cattle were processed by PCR followed by sequencing of the 18S rRNA gene. Of 1202 fecal samples examined 110 (9.15%) had oocysts. *C. bovis*, *C. parvum*, *C. ryanae* and *C. andersoni* were identified in 83, 15, 10 and 2 samples, respectively (Silverlås et al., 2010). The genetic diversity of *Cryptosporidium* from dairy cattle in Denmark was determined by Langkjær et al. (2007). According to the molecular characterization, out of 1150 cattle, originating from 50 dairy herds, 154 were amplified and sequenced successfully. Overall, *C. parvum*, *C. bovis*, the deer-like genotype, *C. suis*-like genotype, the pig genotype II, and atypical isolates were diagnosed in 78, 57, 12, 3, 1 and 3 samples, respectively.

Bovine cryptosporidiosis caused by *C. ryanae* was detected in France and Sweden (Follet et al., 2009; Silverlås et al., 2010). In a study on beef cattle carried out by Follet et al. (2009) in

France, the prevalence of *C. ryanae* (17.6%) was higher than reported in Sweden (Silverlås et al., 2010).

Other *Cryptosporidium* species naturally infecting calves are rarely reported. The porcine-specific *C. suis* was identified in one beef calf in Belgium (Geurden et al., 2007); the avian-related species, *C. meleagridis* was found in one adult cattle in Northern Portugal (Mendonça et al., 2007). The anthroponotic *C. hominis* was recently diagnosed in three cattle in Italy by Merildi et al. (2009). Infections with the deer-like genotype have been found sporadically in England (Brook et al., 2009), Denmark (Langkjær et al., 2007), Hungary (Plutzer and Karanis, 2007), Northern Ireland (Thompson et al., 2007) and Slovenia (Soba and Logar, 2008). *Cryptosporidium suis*-like genotype was found in three cattle in Denmark and the pig genotype II in a cow (Langkjær et al., 2007).

***C. parvum* subtype families and their distribution**

The anthroponotic *C. parvum* subtyping tools have been based on the 60-kDa glycoprotein gene (GP60). The GP60 gene (also known as Cpgp15/45) is the most polymorphic marker of *Cryptosporidium* genome. It is similar to a microsatellite sequence repeated in tandem at the end 5' and is encoded by TCA, TCG and TCT trinucleotides. The subtype allele families differ from each other by the number of repeated trinucleotides (Xiao, 2010). Moreover, *C. parvum* Ila-IIk is classified according on GP60 sequence data (Soba and Logar, 2008).

Currently, sequencing availability varies in different parts of Europe. In the last eight years (2003-2010), 926 subtypes of *C. parvum* in 17 scientific works from 14 countries were reported. Geographic distribution of *C. parvum* subtypes in bovines based on GP60 subtyping is shown in table 1.

In Europe, Ila zoonotic subtypes were dominant (96.7%) followed by IId (2.1%) and III (1.2%) (misnamed as IIj in some publications) subtypes which were found occasionally in some countries.

Of 931 *C. parvum* subtypes, 515 (55.3%) were confirmed as IlaA15G2R1. This subtype was predominantly identified in Belgium (Geurden et al., 2007), England (Brook et al., 2009), Germany (Broglia et al., 2008), Italy (Duranti et al., 2009), Netherlands (Wielinga et al., 2007), Portugal (Alves et al., 2006), Romania (Imre et al., 2010), Slovenia (Soba and Logar, 2008) and Spain (Quilez et al., 2008).

Several other subtypes were found dominant in most areas studied. For instance, IlaA16G1R1 is a major subtype in Hungary (Plutzer and Karanis, 2007) and Serbia and Montenegro (Misic and Abe, 2007). In Northern Ireland the most common subtype was IlaA18G3R1 (Thompson et al., 2007).

High subtype diversity of *C. parvum* was reported in many European countries. In Netherlands, the study carried out by Wielinga et al. (2007) found 17 subtypes. In Northern Ireland, GP60 sequence analysis of 214 bovine samples revealed 15 subtypes from the family Ila (table 1). Subtyping of *C. parvum* isolates in Germany indicated the presence of 7 alleles of Ila subtypes, along with the 1 subtype IIdA22G1 (Broglia et al., 2008). Similar results were published in Belgium (Wielinga et al., 2007), Hungary (Plutzer and Karanis, 2007) and Spain (Quilez et al., 2008) where IId subtypes were found only occasionally. In England, subtype IlaA15G2R1 was responsible for 69.6% of *C. parvum* infections in calves. The second most common and widely distributed subtype was IlaA17G2R1 (13.2%) followed by IlaA16G3R1 (7.5%), IlaA18G1R1 (3.8%), IlaA19G1R1 (3.8%), IlaA20G3R1 (3.8%) and IlaA14G2R1 (1.9%), respectively (Chalmers et al., 2005; Brook et al., 2009).

Interesting results have been published recently in Sweden by Silverlås et al. (2010), where sequencing of the GP60 gene was successful in 13 of the *C. parvum* samples. Eight different subtypes from two subtype families (Ila and IId) had approximately equal distribution (table 1). Three subtypes within *C. parvum* Ila (IlaA16G1R1, IlaA18G1R1, and IlaA20G1R1) were diagnosed in Serbia and Montenegro. *Cryptosporidium parvum* III was identified in six bovine isolates (IIIA16 in four cattle and IIIA17 in two specimens,

respectively). The presence of IIdA18G1b subtype was also reported (Misić and Abe, 2007). The finding of the III zoonotic subtype family has been confirmed from Netherlands (Wielinga et al., 2007) and Slovenia (Soba and Logar, 2008). In Slovenia, three and two subtypes were diagnosed within IIa and III, respectively.

The subtype diversity of *C. parvum* in cattle can be considered low in Portugal (Alves et al., 2003; 2006), Romania (Imre et al., 2010) Czech Republic (Ondráčková et al., 2009) and Slovenia (Stantić-Pavlinić et al., 2003). Three alleles were identified in Portugal (IIaA15G2R1, IIaA16G2R1 and IIdA17G1) and Romania (IIaA15G2R1, IIaA16G1R1, IIdA22G2R1) corresponding to two subtype families.

The subtyping proved to be very useful in tracking the source of infection and can be the main epidemiology tool of cryptosporidiosis worldwide (Xiao, 2010).

Conclusions

Undoubtedly, the molecular epidemiology studies results suggest that cattle can be an important reservoir of *Cryptosporidium parvum* infection for both humans and animals in Europe. The distribution of *Cryptosporidium* species and genotypes can be associated with age; four species (*C. parvum*, *C. andersoni*, *C. bovis* and *C. ryanae*) and one genotype (the deer-like genotype) were found more frequently. On the other hand, the spread and segregation of *C. parvum* subtypes is not yet clear.

Several molecular epidemiology studies on human cryptosporidiosis in various countries showed that the most prevalent zoonotic variants were *C. parvum* subtypes. Indeed, much higher genetic diversity of *C. parvum* was recorded among farms in different geographic regions. Except for III, the other two subtypes found in calves (IIa and IId) in these areas were previously found in humans and vice versa. These findings about subtyping of *C. parvum* isolates suggest that some subtype families are zoonotic and some are only anthroponotic.

Given the importance of this zoonosis, the number of molecular and genetic research is increasing gradually. If the epidemiology, subtyping and genomic studies continue, there are chances that new *Cryptosporidium* species, genotypes and subtypes will be found in cattle.

Acknowledgements

This work was published within "Postdoctoral School of Agriculture and Veterinary Medicine POSDRU/89/1.5/S/ 62371", co-financed by the European Social Fund through the Sectoral Operational Programme for the Human Resources Development 2007-2013.

References

- Alves M., Xiao L., Antunes F., Matos O. 2006. Distribution of *Cryptosporidium* subtypes in humans, domestic and wild ruminants in Portugal. *Parasitol. Res.* 99:287-292.
- Alves M., Xiao L., Sulaiman I., Lal A.A., Matos O., Antunes F. 2003. Subgenotype analysis of *Cryptosporidium* isolates from humans, cattle and zoo ruminants in Portugal. *J. Clin. Microbiol.* 41:2744-2747.
- Björkman C., Mattson J.G. 2006. Persistent infection in a dairy herd with an unusual genotype of bovine *Cryptosporidium parvum*. *FEMS Microbiol. Lett.* 254:71-74.
- Bornay-Llinares F.J., da Silva A.J., Moura I.N., Myjak P., Pietkiewicz H., Kruminis-Lozowska W., Graczyk T.K., Pieniazek N.J. 1999. Identification of *Cryptosporidium felis* in a cow by morphologic and molecular methods. *Appl. Environ. Microbiol.* 65:1455-1458.
- Brogliani A., Reckinger S., Cacciò S.M., Nöckler K. 2008. Distribution of *Cryptosporidium parvum* subtypes in calves in Germany. *Vet. Parasitol.* 154:8-13.
- Brook E.J., Anthony Hart C., French N.P., Christley R.M. 2009. Molecular epidemiology of *Cryptosporidium* subtypes in cattle in England. *Vet. J.* 179:378-382.
- Cacciò S., Homan W., Camilli R., Traldi G., Kortbeek T., Pozio E. 2000. A microsatellite marker reveals population heterogeneity within human and animal genotypes of *Cryptosporidium parvum*. *Parasitology* 120:237-244.
- Cacciò S., Spano F., Pozio E. 2001. Large sequence variation at two microsatellite loci among zoonotic (genotype C) isolates of *Cryptosporidium parvum*. *Int. J. Parasitol.* 31:1082-1086.

- Cencioni V. 2005. Applicazione della PCR per la diagnosi e la tipizzazione di *Cryptosporidium* spp. in isolati umani e animali. Degree Thesis AA, 2005-2006.
- Chalmers R.M., Ferguson C., Cacció S.M., Gasser R.B., Abs El-Osta Y.G., Heijnen L., Xiao L., Elwin K., Hadfield S., Sinclair M., Stevens M. 2005. Direct comparison of selected methods for genetic categorisation of *Cryptosporidium parvum* and *Cryptosporidium hominis* species. *Int. J. Parasitol.* 35:397-410.
- Dărăbuș G., Imre K. 2010. Criptosporidioza. [Cryptosporidiosis] [in Romanian]. Editura Agroprint, Timișoara, Romania, 322 pp.
- Duranti A., Cacció S.M., Pozio E., Di Egidio A., De Curtis M., Battisti A., Scaramozzino P. 2009. Risk factors associated with *Cryptosporidium parvum* infection in cattle. *Zoonoses Public Health.* 56:176-182.
- Enemark H.M., Ahrens P., Lowery C.J., Thamsborg S.M., Enemark J.M.D., Bille-Hansen V., Lind P. 2002. *Cryptosporidium andersoni* from a Danish cattle herd: identification and preliminary characterisation. *Vet. Parasitol.* 107:37-49.
- Follet J., Guyot K., Leruste H., Follet-Dumoulin A., Dei-Cas E., Halama P. 2009. Prevalence of *Cryptosporidium* species in young beef cattle in France. III-rd International *Giardia* and *Cryptosporidium* Conference, Orvieto, Italy, Abstract Book 83.
- Geurden T., Berkvens D., Martens C., Casaert S., Vercruyse J., Claerebout E. 2007. Molecular epidemiology with subtype analysis of *Cryptosporidium* in calves in Belgium. *Parasitology* 134:1981-1987.
- Grana L., Lalle M., Habluetzel A., Silvestri S., Traldi G., Tonanti D., Pozio E., Cacció S.M. 2006. Distribution of zoonotic and animal specific genotypes of *Cryptosporidium* and *Giardia* in calves of cattle farms in the Marche region. *Parassitologia* 48:208.
- Hajdušek O., Ditrich O., Šlapeta J. 2004. Molecular identification of *Cryptosporidium* in animal and humans hosts from the Czech Republic. *Vet. Parasitol.* 122:183-192.
- Imre K., Matos O., Dărăbuș G., Mederle N., Oprescu I., Morariu S., Ilie M.S., Hotea I., Imre M. 2009. First genetic identification of *Cryptosporidium* spp. in cattle in Romania. *Lucr. Șt. Med. Vet. Timișoara* 52:26-30.
- Imre K., Dărăbuș G., Mederle N., Oprescu I., Morariu S., Ilie M., Hotea I., Imre M., Indre D., Balint A., Sorescu D. 2010. Intraspecific characterization of some *Cryptosporidium parvum* isolates from calves and lambs in Western Romania using molecular techniques. *Sci. Parasitol.* 11:47-50.
- Kváč M., Vítovec J. 2003. Prevalence and pathogenicity of *Cryptosporidium andersoni* in one herd of beef cattle. *J. Vet. Med. B Infect. Dis. Vet. Public Health* 50:451-457.
- Langkjær R.B., Vigre H., Enemark H.L., Maddox-Hyttel C. 2007. Molecular and phylogenetic characterization of *Cryptosporidium* and *Giardia* from pigs and cattle in Denmark. *Parasitology* 134:339-350.
- Majewska A.C., Jędrzejewski Sz. Słodkiewicz-Kowalska A., Solarczyk P., Werner A. 2004. Outbreak of cryptosporidiosis on dairy farm. *Wiad. Parazytol.* 50(suppl):71.
- Mangili P., D'Avino N., Venditti G., Centellini M., Filippini G., Pezzotti G., Grelloni V. 2009. Detection of *Cryptosporidium* spp. in faeces of calves affected by enteric disorders in Central Italy. III-rd International *Giardia* and *Cryptosporidium* Conference, Orvieto, Italy, Abstract Book 88.
- Mendonça C., Almeida A., Castro A., de Lurdes Delgado M., Soares S., da Costa J.M., Canada N. 2007. Molecular characterization of *Cryptosporidium* and *Giardia* isolates from cattle from Portugal. *Vet. Parasitol.* 147:47-50.
- Merildi V., Mancianti F., Lamioni H., Passantino A., Papini R. 2009. Preliminary report on prevalence and genotyping of *Cryptosporidium* spp. in cattle from Tuscany (Central Italy). III-rd International *Giardia* and *Cryptosporidium* Conference, Orvieto, Italy, Abstract Book 84.
- Misic Z., Abe N. 2007. Subtype analysis of *Cryptosporidium parvum* isolates from calves on farms around Belgrade, Serbia and Montenegro, using the 60 kDa glycoprotein gene sequences. *Parasitology* 134:351-358.
- Moriarty E.M., McEvoy J.M., Lowery C.J., Thompson H.P., Finn M., Sheridan J.J., Blair I.S., McDowell D.A., Duffy G. 2005. Prevalence and characterisation of *Cryptosporidium* species in cattle faeces and on beef carcasses at slaughter. *Vet. Rec.* 156:165-168.
- Ondráčková Z., Kváč M., Sak B., Květoňová L., Rost M. 2009. Prevalence and molecular characterization of *Cryptosporidium* spp. in dairy cattle in South Bohemia, the Czech Republic. *Vet. Parasitol.* 165:141-144.
- Paraud C., Guyot K., Chartier C. 2009. Prevalence and molecular characterization of *Cryptosporidium* spp. infection in calves, lambs and goat kids reared in a same farm in France. III-rd International *Giardia* and *Cryptosporidium* Conference, Orvieto, Italy, Abstract Book 83.
- Plutzer J., Karanis P. 2007. Genotype and subtype analyses of *Cryptosporidium* isolates from cattle in Hungary. *Vet. Parasitol.* 146:357-362.

- Quilez J., Torres E., Chalmers R.M., Robinson G., Del Cacho E., Sanchez-Acedo C. 2008. *Cryptosporidium* species and subtype analysis from dairy calves in Spain. *Parasitology* 135:1613-1620.
- Pancieria R., Thomassen R., Gamer F. 1971. Cryptosporidial infection in a calf. *Vet. Pathol.* 8:479-484.
- Robinson G., Thomas A.L., Daniel R.G., Hadfield S.J., Elwin K., Chalmers R.M. 2006. Sample prevalence and molecular characterisation of *Cryptosporidium andersoni* within a dairy herd in the United Kingdom. *Vet. Parasitol.* 142:163-167.
- Santín M., Trout J.M. 2007. Livestock. In: Fayer R., Xiao L. (Eds.), *Cryptosporidium* and cryptosporidiosis. CRC Press, Boca Raton, Florida, pp. 451-483.
- Silverlås C., Näslund K., Björkman C., Mattson J.G. 2010. Molecular characterisation of *Cryptosporidium* isolates from Swedish dairy cattle in relation to age, diarrhoea and region. *Vet. Parasitol.* 169:289-295.
- Smith H.V., Nichols R.A., Mallon M., Macleod A., Tait A., Reilly W.J., Browning L.M., Gray D., Reid S.W., Wastling J.M. 2005. Natural *Cryptosporidium hominis* infections in Scottish cattle. *Vet. Rec.* 156:710-711.
- Soba B., Logar J. 2008. Genetic classification of *Cryptosporidium* isolates from humans and calves in Slovenia. *Parasitology* 135:1263-1270.
- Stantic-Pavlinic M., Xiao L., Glaberman S., Lal A.A., Oražen T., Rataj-Verglez A., Logar J., Berce I. 2003. Cryptosporidiosis associated with animal contacts. *Wien. Klin. Wochenschr.* 115:125-127.
- Thompson H.P., Dooley J.M., Kenny J., McCoy M., Lowery C.J., Moore J.E., Xiao L. 2007. Genotypes and subtypes of *Cryptosporidium* spp. in neonatal calves in Northern Ireland. *Parasitol. Res.* 100:619-624.
- Wielinga P.R., De Vries A., van der Goot T.H., Mank T., Mars M.H., Kortbeek L.M., van der Giessen J.W. 2007. Molecular epidemiology of *Cryptosporidium* in humans and cattle in The Netherlands. *Int. J. Parasitol.* 38:809-817.
- Wu Z., Nagano I., Boonmars T., Nakada T., Takahashi Y. 2003. Intraspecies polymorphism of *Cryptosporidium parvum* revealed by PCR-restriction fragment length polymorphism (RFLP) and RFLP-single-strand conformational polymorphism analyses. *Appl. Environ. Microbiol.* 69:4720-4726.
- Xiao L. 2010. Molecular epidemiology of cryptosporidiosis: An update. *Exp. Parasitol.* 124:80-89.