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Catalina Avendaño, Ana Ramo, Claudia Vergara-Castiblanco, Caridad Sánchez-Acedo & Joaquín Quílez

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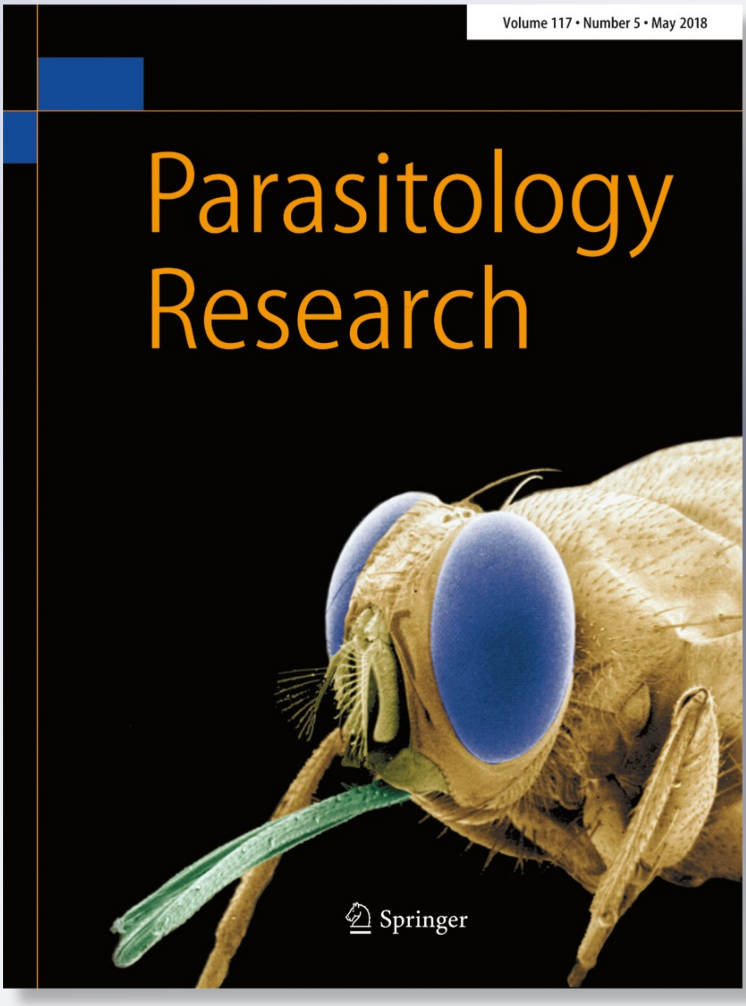

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ORIGINAL PAPER

Genetic uniqueness of *Cryptosporidium parvum* from dairy calves in Colombia

Catalina Avendaño[1] · **Ana Ramo**[2] · **Claudia Vergara-Castiblanco**[2] · **Caridad Sánchez-Acedo**[2] · **Joaquín Quílez**[2]

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Abstract

Fecal specimens from 432 pre-weaned calves younger than 35 days were collected over a 2-year period (2010–2012) from 74 dairy cattle farms in the central area of Colombia. These samples were microscopically examined for the presence of *Cryptosporidium* oocysts, and positive specimens were selected for molecular examination. Microscopy revealed that 115 calves (26.6%) from 44 farms (59.5%) tested positive. Oocyst shedding was recorded in calves aged 3-day-old onwards, although the infection rate peaked at 8–14 days (40.7%). Infection rates were higher in diarrheic (52.2%) than in non-diarrheic calves (19.9%) ($p < 0.0001$, χ^2), and infected calves had up to seven times more probability of having diarrhea than non-infected calves. *Cryptosporidium* species and subtypes were successfully identified in 73 samples from 32 farms. Restriction and sequence analyses of the *SSU rRNA* gene revealed *C. parvum* in all but two isolates identified as *Cryptosporidium bovis*. Sequence analyses of the 60-KDa glycoprotein (*gp60*) gene revealed eight subtypes within the IIa family. An unusual subtype (IIaA18G5R1) was the most prevalent and widely distributed (more than 66% specimens and 68% farms) while the subtype most frequently reported in cattle worldwide (IIaA15G2R1) was found in less than 13% of specimens and 16% farms. The remaining subtypes (IIaA16G2R1, IIaA17G4R1, IIaA20G5R1, IIaA19G6R1, IIaA20G6R1, and IIaA20G7R1) were restricted to 1–3 farms. This is the first large-sample size study of *Cryptosporidium* species and subtypes in Colombia and demonstrates the genetic uniqueness of this protozoan in cattle farms in this geographical area.

Keywords *Cryptosporidium* species · *gp60* subtypes · Dairy calves · Colombia

Introduction

Cryptosporidium is a major cause of diarrhea in humans and livestock worldwide. The genus consists of multiple genetically distinct species and genotypes whose identification relies on molecular methods since oocysts are morphologically indistinguishable. Thirty-one *Cryptosporidium* species have been reported to date, although only two are responsible for most human infections, including the anthroponotic species *C. hominis* and the zoonotic species *C. parvum* (Ryan et al. 2016). The latter is widely endemic and one of the most common causes of profuse watery diarrhea in pre-weaned calves which are considered to be the major zoonotic reservoir for humans (Chalmers and Katzer 2013). Infections in post-weaned calves, heifers, or adult cattle are mostly due to other ruminant-adapted *Cryptosporidium* spp., including *C. ryanae*, *C. bovis*, and *C. andersoni* (Ryan et al. 2014). The latter two species have occasionally been reported in humans, although they do not significantly contribute to zoonotic cryptosporidiosis (Zahedi et al. 2016).

Molecular analysis using the highly polymorphic 60-kDa glycoprotein (*gp60*) gene has identified human-specific, animal-specific, and zoonotic *C. parvum* subtypes. At least 14 subtype families have been identified to date among *C. parvum* isolates from humans and animals (IIa to IIo) as well as several subtypes within each family (Ryan et al. 2014). Some families (especially IIc and IIe) have so far only been found in humans, thereby indicating anthroponotic transmission, but other families such as IIa and IId are found in both humans and ruminants and cause zoonotic cryptosporidiosis.

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✉ Joaquín Quílez
jquilez@unizar.es

[1] Faculty of Animal Sciences, University of Applied and Environmental Sciences, U.D.C.A, Bogotá, Colombia

[2] Department of Animal Pathology, Faculty of Veterinary Sciences, University of Zaragoza, Miguel Servet 177, 50013 Zaragoza, Spain

The IIa family (particularly the major zoonotic subtype IIaA15G2R1) is the most frequently reported in cattle worldwide (Ryan et al. 2014).

Cryptosporidiosis is a significant public health problem in South American countries where high infection rates are usually reported in children and immunocompromised patients, and *Cryptosporidium* oocysts have been detected in water and food (Putignani and Menichella 2010). The protozoan has also been recognized as a cause of neonatal diarrhea in calves in Brazil, Venezuela, Argentina, and Chile; molecular studies have been carried out in some of these countries (Surumay-Vílchez and Alfaro 2000; Del Coco et al. 2008, 2014; Meireles et al. 2011; Mercado et al. 2015). *Cryptosporidium* oocysts have been detected in human drinking water and water from dairy farms in Colombia (Alarcón et al. 2005; Rodríguez et al. 2012; Lora-Suarez et al. 2016; Triviño-Valencia et al. 2016). Infection rates ranging from 10.4 to 29% have been reported in HIV+ patients, and several *Cryptosporidium* species and subtypes have been identified in Colombian humans, including *C. hominis* (IdA19 and IaA12R8), *C. parvum* (IIcA5G3c), *C. felis*, and *C. viatorum* (Flórez et al. 2003; Navarro-i-Martínez et al. 2006; Velasco et al. 2011; Sánchez et al. 2017).

Cryptosporidium oocysts have also been identified in fecal specimens from both diarrheic and asymptomatic neonatal calves in Colombia, and anti-*Cryptosporidium* antibodies have been detected in serum samples from adult cattle in the Andean region (Vergara-Castiblanco et al. 2001; Pardo and Oliver 2012; Hernández-Gallo and Cortés-Vecino 2012; Cadavid-Betancur et al. 2014; Pulido-Medellin et al. 2014). However, data on *Cryptosporidium* species and subtypes infecting cattle are much more limited, just a single reference reporting the presence of *C. parvum* in calves (Ocampo et al. 2012). It is worth noting that cattle-breeding is the most widespread agricultural activity in Colombia which has the fourth largest stock in South America and is among the top 11 countries having the highest dairy cow population in the world (https://www.ciwf.org.uk/media/5235182/Statistics-Dairy-cows.pdf). The current study was designed to provide data on the occurrence, age distribution, and contribution of *Cryptosporidium* to neonatal diarrhea in cattle farms in Colombia. The potential public health significance of zoonotic *C. parvum* subtypes from pre-weaned dairy calves was also investigated.

Materials and methods

Sample collection

Fresh fecal specimens were collected over a 2-year period (2010 to 2012) from the rectum of 432 diarrheic and non-diarrheic calves (*Bos taurus*) younger than 35 days from 74 dairy cattle farms. Calves in most farms (71/74) were reared under a semi-extensive system. The farms were located in 20 municipalities in four departments in Colombia's central area: Antioquia (2 farms/1 municipality), Boyacá (21/3), Cundinamarca (50/15), and Meta (1/1). One to 31 samples were collected from each farm (mean 5.8 ± 6.4). The population was stratified into five age groups: ≤ 7 days (*n* 53), 8–14 days (*n* 118), 15–21 days (*n* 102), 22–28 days (*n* 104), and > 28 days (*n* 55) (Table 1). Carbol fuchsin negative staining of direct fecal smears was used for detecting *Cryptosporidium* oocysts (Heine 1982); microscopy-positive fecal samples were selected for molecular characterization.

DNA extraction

Oocysts were concentrated from 2 g of positive feces using a previously described saturated sodium chloride flotation method (Elwin et al. 2001). Floating material containing oocysts was washed with distilled water to remove salt residue; the oocysts were then suspended in 1 ml of distilled water. Oocyst suspensions were stored at 4 °C until required. A QIAamp DNA mini kit (Qiagen, Hilden, Germany) was used for total DNA extraction from 200 µl oocyst suspensions, according to the manufacturer's instructions. An initial step involving three freeze-thaw cycles (freezing in liquid nitrogen for 1 min and heating at 100 °C for 5 min) followed by incubation at 56 °C for 30 min in lysis buffer containing proteinase K was incorporated in the protocol. DNA was stored at − 20 °C.

Molecular characterization

Cryptosporidium oocysts were identified at species level by a previously described nested PCR of a small-subunit (*SSU*) *rRNA* gene fragment and restriction fragment length polymorphism (RFLP) analysis with *Ssp*I, *Vsp*I, and *Mbo*II endonucleases (Fermentas Life Sciences, EU) (Xiao et al. 2001; Feng et al. 2007). Primary PCR step involved a reaction containing 5 µl DNA template, 1× PCR buffer, 6 mM $MgCl_2$, 200 µM of each deoxynucleoside triphosphate (dNTP), 0.2 µM of each primer, and 2.5 U Taq polymerase in 50 µl total reaction volume. Thirty-five cycles were performed, each consisting of 94 °C for 45 s, 55 °C for 45 s, and 72 °C for 1 min; initial denaturation was done at 94 °C for 3 min and a final extension step at 72 °C for 7 min. The secondary PCR mixture and cycling conditions were identical to those used in the primary PCR, except for 3 mM $MgCl_2$ concentration and 5 µl primary PCR product. PCR products were separated on 1% agarose gels and restriction products on 2% gels and stained with GelRed nucleic acid gel stain (Biotium, Hayward, CA). A subset of 10 representative isolates (including samples which had produced a banding pattern different from that for *C. parvum* with the conventional restriction enzymes) were selected to confirm RFLP results by DNA sequence analysis.

Table 1 Occurrence of *Cryptosporidium* infection in pre-weaned calves according to the age range and presence of diarrhea

Age group (days)	Infected/studied (%)			
	Diarrheic	Non-diarrheic	p^a	Total calves
≤ 7	6/11 (54.5%)	6/42 (14.3%)	0.0045	12/53 (22.6%)
8–14	23/28 (82.1%)	25/90 (27.8%)	< 0.0001	48/118 (40.7%)
15–21	8/18 (44.4%)	22/84 (26.2%)	NS	30/102 (29.4%)
22–28	4/21 (19%)	9/83 (10.8%)	NS	13/104 (12.5%)
≥ 29	6/12 (50%)	6/43 (13.9%)	< 0.0001	12/55 (21.8%)
Total	47/90 (52.2%)	68/342 (19.9%)	< 0.0001	115/432 (26.6%)

NS not significant

[a] *p* value obtained after comparison of infection rates between diarrheic and non-diarrheic calves at each age group

Samples containing *C. parvum* were subtyped by nested PCR and direct sequencing of a 60-kDa glycoprotein (*gp60*) gene fragment (~ 850 bp) as described by Alves et al. (2003). The PCR mixture consisted of 1 μl DNA template (for primary PCR) or 1 μl primary PCR product (for secondary PCR), 1× PCR buffer, 3 mM $MgCl_2$, 200 μM of each dNTP, 0.2 μM of the forward and reverse primers, and 5 U Taq polymerase in a 50-μl reaction mixture. Each PCR involved 40 cycles consisting of 95 °C for 45 s, 52 °C for 45 s, and 72 °C for 1 min, with an initial denaturation step at 95 °C for 3 min and a final extension at 72 °C for 10 min. Selected *SSU rRNA* products, as well as all *gp60* products, were purified with ExoSAP-IT (Thermo Fisher Scientific, Vilnius, Lithuania) and subjected to bi-directional sequencing on a 3500xL Genetic Analyzer (Applied Biosystems, Life Technologies, Halle, Belgium) according to the manufacturer's instructions. ClustalW was used for editing nucleotide sequences and Bioedit (version 7.0.9) (http://www.mbio.ncsu.edu/BioEdit/bioedit.html) for aligning reference sequences. Sense and anti-sense strands' consensus sequences were analyzed using a BLAST search in NCBI databases (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Subtypes were named based on the number of TCA (A), TCG (G), and ACATCA (R) repeats as described by Sulaiman et al. (2005). Nucleotide sequences generated in this study were deposited in the GenBank database under accession numbers MF142032 to MF142044.

Statistical analysis

Chi-squared or two-tailed Fisher's exact tests were used for evaluating association between *Cryptosporidium* infection and animals' age group or those having diarrhea. R software (version 3.1.3) (R Development Core Team 2013) was used for analysis; $a < 0.05$ *p*-value was required for establishing significance. Potential risk factors were computed using Win Episcope 2.0 (Thrusfield et al. 2001). Odds ratios (ORs) and 95% confidence intervals (95% CIs) for *Cryptosporidium* infection in the different age groups were calculated using each age group as reference. The risk of infection was considered significant if 95% CI for OR did not include 1.0 (Fletcher et al. 1996).

Results

Occurrence of *Cryptosporidium*

Cryptosporidium oocysts were identified by microscopy in the feces of 115 calves (26.6%) from 44 farms (59.5%). Infected farms were distributed throughout the four departments sampled. Oocysts were found in calves as young as 3 days old; age was associated with the odds of shedding *Cryptosporidium* oocysts. Infection rates were significantly higher in calves aged 8–14 days (40.7%) than in the other age groups ($p < 0.05$, χ^2) (Table 1). Calves younger than 21 days were 1.6 to 4.3 times more likely to be infected (90/273 = 32.9%) than those older than 21 days (25/159 = 15.7%) (OR 2.64; 95% CI 1.62–4.28).

Diarrhea was reported in 90 calves (20.8%) from 36 farms. *Cryptosporidium* infection rates were higher in diarrheic (52.2%) than in non-diarrheic (19.9%) calves. Statistically significant differences for *Cryptosporidium* occurrence between calves with and without diarrhea were found for calves younger than 14 days and those older than 29 days ($p < 0.05$) (Table 1). The probability of diarrhea was significantly higher for calves shedding *Cryptosporidium* oocysts (47/115 = 40.8%) than for those that did not excrete the parasite (43/317 = 13.6%) ($p < 0.001$, χ^2). Calves positive for *Cryptosporidium* had 2.7 to 7 times more odds of suffering diarrhea than non-infected calves (OR 4.40; 95% CI 2.75–7.05).

Cryptosporidium species and subtype identification

Seventy-three *Cryptosporidium*-positive samples were successfully amplified at the *SSU rRNA* locus. Restriction analysis yielded banding patterns indicative of *C. parvum* for 71 specimens. These isolates originated from 32 farms in 16 municipalities. Eight of them were sequenced and had 100% similarity with the *C. parvum* reference sequence AF093490 (Xiao et al. 1999). The remaining two *Cryptosporidium* isolates came from two different farms and had 100% sequence identity with *C. bovis* AY741305 (Fayer et al. 2006). *C. bovis* was identified in 14- and 19-day-old calves, respectively. Concurrent infection with mixed species was not found.

All 71 *C. parvum* isolates were successfully amplified and sequenced at the *gp60* locus. Aligning the sequences obtained with reference sequences downloaded from GenBank showed that isolates belonged to eight subtypes within *C. parvum* family IIa (Table 2). Three subtypes (IIaA19G6R1, IIaA20G6R1, and IIaA20G7R1) differed from reference sequences regarding the amount of TCA and/or TCG repeats and were considered novel *C. parvum* subtypes. Subtype IIaA18G5R1 was identified in most specimens (> 66%) and farms (> 68%) in 12 municipalities and was by far the most prevalent subtype in calves. The remaining subtypes were geographically restricted to 1–5 farms in 1–2 municipalities, including subtype IIaA15G2R1 which was the second most common in this study. A single subtype was identified on most farms where two or more calves were sampled (11/15), each of the remaining farms harboring two different subtypes. Subtype distribution comparing diarrheic to non-diarrheic calves showed that four subtypes occurred more commonly in the first group (IIaA15G2R1, IIaA16G2R1, IIaA17G4R1, and IIaA18G5R1) whereas the remaining four subtypes (IIaA19G6R1, IIaA20G5R1, IIaA20G6R1, and IIaA20G7R1) were only seen in non-diarrheic calves; nonetheless, such differences were not statistically significant.

Discussion

This study has highlighted *Cryptosporidium* as a common and widespread pathogen for pre-weaned dairy cattle in Colombia's central area. The parasite was detected in more than 26% of calves and 59% of farms throughout the four departments, thereby agreeing with other studies on South American cattle farms. The occurrence of *Cryptosporidium* infection in calves in Venezuela, Argentina, or Brazil has ranged from 10 to 29.3% as detected by microscopic methods (Surumay-Vílchez and Alfaro 2000; Del Coco et al. 2008; Meireles et al. 2011; Do Couto et al. 2014). Great variability regarding *Cryptosporidium* infection rate has been reported on Colombian dairy farms, ranging from 4.9% in pre-weaned calves in the Bogota savanna's north-western region to 48% reported in cattle farms in the Boyacá Department, although occurrence in calves younger than 12 months increased to 90% in this Department (Hernández-Gallo and Cortés-Vecino 2012; Pulido-Medellin et al. 2014).

Cryptosporidium oocysts were detected in calves as young as 3 days of age, and more than 22% of calves excreted oocysts during the first week of age, indicating that many became infected immediately after birth. This observation is consistent with the duration of the parasite's lifecycle which has been estimated as being around 4 days, also suggesting heavy environmental contamination in the calving area (Santín and Trout 2008). The percentage of calves shedding oocysts peaked at 8–14 days of age (40.7%), and the probability of becoming infected was significantly reduced in calves older than 21 days. These results were similar to other point prevalence and longitudinal studies concluding that cryptosporidiosis in calves normally becomes established during the initial 2 weeks of life (Castro-Hermida et al. 2002; Trotz-Williams et al. 2007; Santín et al. 2008).

It is worth mentioning that the infection rates detected in this study may have been underestimated since direct fecal smears have been recognized as being less sensitive than other microscopic techniques with stool concentration or molecular methods (Smith 2008). The sensitivity of Heine staining on non-concentrated feces regarding a direct immunofluorescence antibody test on diethyl ether concentrated feces has been estimated to be 76.6%, although this figure increases to 90% for samples containing more than 10,000 oocysts per gram (Chartier et al. 2013). Similarly, a PCR analysis targeted at the *SSU rRNA* gene was more sensitive than microscopic or immunological methods for the detection of *Cryptosporidium* oocysts in cattle samples (Ezzaty Mirhashemi et al. 2015). Negative staining in the current study could thus have favored the detection of calves having heavy infection (i.e., calves having diarrhea compared to non-diarrheic calves) and those infected by some *Cryptosporidium* species associated with higher oocyst shedding intensity (Santín and Trout 2008). Feng et al. (2007) reported that *C. bovis* in calves were concealed by the overwhelming *C. parvum* infection.

The role of *Cryptosporidium* in the etiology of diarrhea in pre-weaned calves has been well documented. Most studies worldwide have found that calf diarrhea has a multifactorial etiology, rotavirus and *Cryptosporidium* being the two most common enteropathogens (Meganck et al. 2015). A previous study on diarrheic calves in Colombia's central area using an antigen ELISA test also identified *Cryptosporidium* (38% of samples) and rotavirus (19%) as being the most prevalent pathogens (Pardo and Oliver 2012). A similar conclusion was reported using an analogous test in Colombia's northern highlands where the occurrence of both microorganisms (89 and 47% for *Cryptosporidium* and rotavirus, respectively) was even higher than that mentioned above (Cadavid-Betancur et al. 2014). Neither bacterial nor viral infections were excluded in calves in this study, but the protozoan was associated with a significantly higher probability of calves having diarrhea. The *Cryptosporidium* infection rate in diarrheic calves younger than 7 days and those aged 8–14 days exceeded 54 and 82%, respectively; infected calves had up to seven times more likelihood of suffering diarrhea than non-infected calves. All the above findings suggest that this protozoan should be considered one of the major enteropathogens associated with neonatal diarrhea in calves in Colombia's central region.

Molecular analysis revealed *C. parvum* as the major *Cryptosporidium* spp. infecting pre-weaned calves in this area of Colombia since it was reported in all but two specimens that were identified as *C. bovis*. These findings are consistent with *Cryptosporidium* spp. distribution reported in dairy and beef cattle in Europe, North America, Australia, and New Zealand, where *C. parvum* is responsible for most infections in pre-weaned calves, whereas *C. bovis* and *C. ryanae* are found predominantly in 3-month-old to 2-year-old cattle and *C. andersoni* is much more prevalent in cows older than 2 years (Trotz-Williams et al. 2006; Fayer et al. 2007; Broglia et al. 2008; Quílez et al. 2008; Brook et al. 2009; Ng et al. 2012; Rieux et al. 2013; Smith et al. 2014; Al Mawly et al. 2015). *C. parvum* has also been the single species identified in pre-weaned calves in Argentina and Brazil (Tomazic et al. 2013; Del Coco et al. 2014; Do Couto et al. 2014) and among 11 *Cryptosporidium*-positive specimens from cattle farms in a municipality of Colombia (Ocampo et al. 2012). However, other studies with specimens from pre-weaned calves have reported *C. bovis* as the most common *Cryptosporidium* species in Sweden (54/73), China (65/172), Canada (7/12), or Ethiopia (7/10) (Silverlås et al. 2010; Wang et al. 2011; Budu-Amoako et al. 2012; Wegayehu et al. 2016).

Sequence analysis of the *gp60* gene revealed significant genetic diversity with the presence of eight subtypes all belonging to the IIa subtype family, which is the major *C. parvum* zoonotic family found in cattle worldwide (Santín and Trout 2008). Three subtypes (IIaA19G6R1, IIaA20G6R1, IIaA20G7R1) have not been identified previously anywhere and should thus be considered novel subtypes. Four subtypes (IIaA15G2R, IIaA16G2R1, IIaA17G4R1, IIaA20G5R1) have been identified in humans and cattle in other studies and should thus be considered potential zoonotic subtypes (Trotz-Williams et al. 2006; Waldron et al. 2009; Zintl et al. 2009; Chalmers et al. 2011; Waldron et al. 2011a, b; Mercado et al. 2015). No relationship between *C. parvum* subtypes and diarrhea was found, although the above-mentioned novel subtypes and IIaA20G5R1 were only seen in non-diarrheic calves.

Subtype distribution revealed the uniqueness of *C. parvum* isolates infecting cattle in Colombia. An unusual subtype, IIaA18G5R1, was responsible for more than 66% of *C. parvum* infection in calves in 12/16 municipalities. This subtype had 100% sequence identity with the *C. parvum* NINC1 isolate from a calf used by Strong et al. (2000) for *gp60* gene cloning and sequence analysis (GenBank accession number AF022929). Surprisingly, no other reports of natural infections by this subtype have yet been documented in cattle or humans (Xiao et al. 2007). A secondary role has been assigned to subtype IIaA15G2R1 which is overwhelmingly the dominant subtype in calves and one of the major subtypes responsible for zoonotic cryptosporidiosis in many parts of the world (Ryan et al. 2014). Subtype IIaA15G2R1 was the second most common *C. parvum* in this study, but it was seen in only 9/71 calves from 5/32 farms. Two subtypes (IIaA20G5R1 and IIaA16G2R1) have been deposited in GenBank (accession numbers MF142043 and MF142044, respectively) and had 100% sequence similarity to *C. parvum* sequences from humans or cattle in Canada, Australia, Ireland, and/or the UK (Trotz-Williams et al. 2006; Waldron et al. 2009; Zintl et al. 2009; Chalmers et al. 2011). The sequence of isolates subtyped as IIaA17G4R1 (GenBank accession number MF142039) differed by four and six nucleotide polymorphisms regarding *C. parvum* isolates from humans and cattle in Australia, respectively (Waldron et al. 2011a).

Reports on *C. parvum* molecular subtyping in cattle in South America are limited and few studies have involved large-scale sampling. Subtype IIaA15G2R1 was the only variant found among a few *C. parvum* isolates from young calves in Brazil (Meireles et al. 2011; Silva et al. 2013), although a subsequent report involving a more significant amount of samples has revealed the presence of up to eight different subtypes: IIaA14G2R2, IIaA16G3R2, IIaA18G1R1, IIaA18G2R2, IIaA19G2R1, IIaA19G2R2, IIaA20G2R1, and IIaA20G2R2 (Do Couto et al. 2014). The concurrent presence of three different subtypes (IIaA17G4R1, IIaA16G4R1, and IIaA15G4R1) has been reported in Chile after cloning the *gp60* amplicon of a single calf isolate originally subtyped as IIaA17G4R1 (Mercado et al. 2015). Relatively high genetic variability has also been found in pre-weaned calves in Argentina. The first study reported six subtypes

Table 2 Distribution of *Cryptosporidium parvum gp60* subtypes in diarrheic and non-diarrheic calves younger than 35 days from dairy farms in the central area of Colombia

Subtype	No. of samples (%) (*n* 71)	Diarrheic (*n* 28)	Non-diarrheic (*n* 43)	No. of farms (*n* 32)	No. of municipalities (*n* 16)
IIaA15G2R1	9 (12.7%)	5 (17.8%)	4 (9.3%)	5 (15.6%)	2 (12.5%)
IIaA16G2R1	3 (4.2%)	2 (7.1%)	1 (2.3%)	1 (3.1%)	1 (6.3%)
IIaA17G4R1	2 (2.8%)	1 (3.6%)	1 (2.3%)	1 (3.1%)	1 (6.3%)
IIaA18G5R1	47 (66.2%)	20 (71.4%)	27 (62.8%)	22 (68.7%)	12 (75%)
IIaA19G6R1	2 (2.8%)	0	2/43 (4.6)	2 (6.2%)	2 (12.5%)
IIaA20G5R1	3 (4.2%)	0	3/43 (6.9)	3 (9.3%)	2 (12.5%)
IIaA20G6R1	4 (5.6%)	0	4/43 (9.3)	1 (3.1%)	1 (6.3%)
IIaA20G7R1	1 (1.3%)	0	1/43 (2.3)	1 (3.1%)	1 (6.3%)

(IIaA17G1R1, IIaA18G1R1, IIaA20G1R1, IIaA21G1R1, IIaA22G1R1, and IIaA23G1R1) from 45 calves in dairy and beef farms located in the provinces of Buenos Aires, Santa Fe, and Cordoba (Tomazic et al. 2013). Most of these subtypes were also reported in a second study which identified up to seven subtypes (IIaA16G1R1, IIaA18G1R1, IIaA19G1R1, IIaA20G1R1, IIaA21G1R1, IIaA22G1R1, IIaA23G1R1) from 73 calves in dairy farms from Buenos Aires province (Del Coco et al. 2014). It is worth mentioning that a single subtype (IIaA18G1R1) found in the above-mentioned investigations was shared by livestock from different South American countries, and only two of them (IIaA15G2R1, IIaA17G4R1) were seen in the current study, highlighting the geographic isolation of *C. parvum* strains infecting cattle in South America. This is the first large-scale surveillance of *Cryptosporidium* species and subtypes in Colombia. Further research is needed to confirm whether the genetic distinctiveness of *C. parvum* isolates infecting calves in Colombia's central region can be extrapolated to cattle farms in other areas of the country.

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