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Panmictic Structure of the *Cryptosporidium parvum* Population in Irish Calves: Influence of Prevalence and Host Movement

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In total, 245 *Cryptosporidium parvum* specimens obtained from calves in 205 Irish herds between 2003 and 2005 were subtyped by sequencing the glycoprotein gene *gp60* and performing multilocus analysis of seven markers. The transmission dynamics of *C. parvum* and the influence of temporal, spatial, parasitic, and host-related factors on the parasite (sub)populations were studied. The relationship of those factors to the risk of cryptosporidiosis was also investigated using results from 1,368 fecal specimens submitted to the veterinary laboratories for routine diagnosis during 2005. The prevalence was greatest in the northwest and midwest of the country and on farms that bought in calves. The panmixia (random mating) detected in the *C. parvum* population may relate to its high prevalence, the cattle density, and the frequent movement of cattle. However, local variations in these factors were reflected in the *C. parvum* subpopulations. This study demonstrated the importance of biosecurity in the control of bovine cryptosporidiosis (e.g., isolation and testing of calves before introduction into a herd). Furthermore, the zoonotic risk of *C. parvum* was confirmed, as most specimens possessed GP60 and MS1 subtypes previously described in humans.

Cryptosporidium parvum is a major cause of enteric disease, which is sometimes fatal, in calves and humans (1, 2). In Ireland, the parasite has been identified in over 25% of diarrheic calves examined in the Regional Veterinary Laboratories (RVLs) (3). Calves with cryptosporidiosis excrete large numbers of infective oocysts (4). Several disease outbreaks in humans have been due to contact with infected calves or ingestion of food and water contaminated by oocysts of bovine origin (2). Since 2004, there has been a high incidence rate of cryptosporidiosis among humans in Ireland (5). In recent years, several waterborne outbreaks of cryptosporidiosis have occurred in different Irish cities, and in some of them, *C. parvum* was identified (6). A comparison of three agricultural river catchments in Ireland demonstrated that higher *C. parvum* infection rates among calves were correlated to higher oocyst contamination of surface waters (6). Prevention of waterborne cryptosporidiosis poses a major challenge because of the difficulty of protection of surface water and treatment of contaminated drinking water (7). Thus, it is important that *C. parvum* strains in cattle be identified and their transmission patterns be elucidated for the control of zoonotic cryptosporidiosis.

Methods for subtyping, such as sequencing of the polymorphic sporozoite surface glycoprotein gene (*gp60*), have made it possible to track the source of infection and examine the population genetics of *C. parvum* (8). To date, all *C. parvum* specimens from Irish cattle have been classified in the zoonotic subtype family IIa (9, 10). The predominant subtype in humans in the Republic of Ireland was also common in cattle in Northern Ireland (5, 9).

On account of the potential for sexual recombination, the population structure of the parasite is best studied using multilocus subtyping techniques (11). The combined results obtained with polymorphic micro- and minisatellite markers are highly discriminatory for studying transmission patterns and the population structure of the parasite in relation to the host, location, and time (12–14).

The main objective of the present study was to assess the transmission dynamics of *C. parvum* in Irish calves using *gp60* gene sequencing and a multilocus subtyping approach. Spatial, temporal, and host-related factors were identified and investigated for their influence on the parasite population. An additional study using results from the diagnostic service of the RVLs was performed to determine the prevalence and risk factors associated with cryptosporidiosis on Irish farms.

MATERIALS AND METHODS

Microscopic examination and data acquisition. Each spring (March to April) from 2003 to 2005, fecal samples from neonatal diarrheic calves submitted to the RVLs for routine diagnostic purposes were examined for enteric pathogens using standard viral, bacteriological, and parasitological procedures. The modified Ziehl-Neelsen staining method or an immunofluorescent-antibody test kit (Bio-X Diagnostics Sprl, Belgium) was used on fecal smears to stain *Cryptosporidium* species oocysts for microscopic detection. Specimens positive for *Cryptosporidium* spp. were sent to the Central Veterinary Research Laboratory (CVRL) for molecular typing.

Two data sets were compiled. The first one recorded the results of molecular typing performed at the CVRL for the specimens received from

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the RVLs between 2003 and 2005. This data set was completed with information obtained from the databases of the Department of Agriculture, including the date of sampling, date of birth, breed, sex, animal identification number, herd number, herd size (on 1 January of the year that the sample was collected), number of calves born in each herd, herd type (dairy, mixed, or beef), herd movement (number of calves bought in and sold from each herd during the year of testing, number of adult cattle bought in and sold from each herd during the same year), and location of the various parcels of land used by the farmer.

The second data set recorded the microscopic results from neonatal fecal samples obtained at the RVLs during the spring of 2005. The microscopic results from 2003 and 2004 were not available. Additional information was included in this data set, as described above.

In addition, information on herd size, location, and the number of animals bought and sold was obtained from 1,000 other randomly selected herds from the registry at the Department of Agriculture for comparison with the information on the cryptosporidiosis-positive herds.

Molecular methods. DNA was extracted from 500 μg of feces with a commercial kit (FastDNA Spin kit for soil; Qbiogene Inc.) (10). *Cryptosporidium* species were identified using a nested PCR that amplified a segment of the small subunit rRNA gene followed by restriction fragment length polymorphism (RFLP) analysis using the enzymes SspI, VspI, and MboII (New England BioLabs) (15, 16). Any ambiguous species/genotypes were confirmed by sequencing of the amplicons using primers from the secondary PCR. Purification and sequencing of the amplicons were carried out by a commercial company (MWG Biotech AG, Germany). Sequences were aligned with reference sequences from GenBank using Lasergene software (DNAStar, Inc., Madison, WI).

All samples identified as *C. parvum* were further characterized using a nested PCR that amplified a fragment of the *gp60* gene (17). Secondary PCR products were sequenced in both directions (MWG Biotech AG, Germany). The subtypes were categorized using the nomenclature proposed by Sulaiman et al. (18).

Cryptosporidium parvum samples were also characterized using a multilocus subtyping method targeting the seven markers GP15, TP14, MM5, MM18, MM19, MS1, and MS5 (see Table S1 in the supplemental material) (12, 13, 19). The primers and amplification conditions had previously been described by Mallon et al. (19) and Drumo et al. (13). A total of 5 μl of diluted amplicon (diluted 1:50 using DNase-RNase-free water) was mixed with 5 μl of a solution of a standard ladder (ET900-R size standard; GE Healthcare Life Sciences, United Kingdom), which was diluted with formamide (1:20). After centrifugation at 111 × *g* for 2 min and heating at 95°C for 2 min 30 s, the size of each amplicon was determined by separation on a capillary-based sequencer (MegaBACE 377; Applied Biosystems) coupled with the software Genetic Profiler (version 2.2; GE Healthcare Life Sciences, United Kingdom). Alleles were coded with a three-digit number indicating their size (in base pairs). To confirm the estimated size, each allele was purified using the ExoSAP-IT reagent (Affymetrix UK Ltd., United Kingdom) and sequenced by a commercial company (Source Bioscience, Ireland). Any sample with an additional peak(s) of more than 90% of the height of the main peak for a specific locus was considered to have a mixed infection; otherwise, only the main peak was considered. The combination of the alleles at each of the seven loci defined the multilocus subtype (MLS) of each sample.

Statistical analysis. (i) Risk factors, including spatial analysis. The data set recording each sample tested for the presence of *Cryptosporidium* oocysts at the RVLs was analyzed with STATA/MP (version 10.0) software (Stata Corporation, College Station, TX) for variables that may be associated with the presence of cryptosporidiosis in a herd. Univariable analysis using random-effect logistic regression was performed on each variable, such as herd location, herd type, herd size, animal movement, and month of sampling. Those with *P* values of <0.2 were considered for inclusion in the multivariable model. The multivariable model was built keeping variables with *P* values of <0.05 in the final model and assessed for confounding variables, interactions, and goodness of fit (20). The model fit was assessed using the Hosmer-Lemeshow statistic on the final model without the random effect. In addition, a comparison between the parameters of models with 7 and 20 quadrature points was done (21).

A spatial analysis was performed to evaluate possible clustering of infected herds during 2005 using the average nearest-neighbor analysis (ArcGIS, version 9, and ArcMap, version 9.2; Environmental Systems Research Institute Inc.) performed within a square window representing most of the country and using the spatial scan statistics (SaTScan, version 9.1.1, software for the spatial and space-time scan statistics; M. Kulldorff, Department of Ambulatory Care and Prevention, Harvard Medical School and Harvard Pilgrim Health Care [http://www.satscan.org, accessed August 2012]) (22, 23). A herd was considered infected with *Cryptosporidium* spp. if at least one sample submitted from that herd was positive.

(ii) Population genetic analysis. The data set recording results for each *C. parvum* specimen subtyped at the CVRL was used for the population genetic analysis of the parasite. The genetic diversity of *C. parvum* was estimated using the following parameters in FSTAT (version 2.9.3; J. Goudet, Department of Ecology and Evolution, Lausanne, Switzerland [http://www2.unil.ch/popgen/softwares/fstat.htm; accessed September 2012]): number of alleles per locus and gene diversity per locus and per region.

Smith et al. (24) described three population structures: panmixia, where random mating occurred, leading to free genetic exchange; clonality, where there is relative genetic isolation leading to limited genetic exchange; and, between those extremes, the epidemic structure, defined by a rapid expansion of particular genetic types masking underlying genetic exchange. To identify population structure, the presence of linkage disequilibrium across loci for each (sub)population was assessed by measuring the standardized index of association (I_A^S) and by testing the null hypothesis of linkage equilibrium with the software LIAN (version 3.5; B. Haubold and R. R. Hudson, Department of Evolutionary Genetics, Max Planck Institute for Evolutionary Biology, Plön, Germany [http://adenine.biz.fh-weihenstephan.de/cgi-bin/lian/lian.cgi.pl; accessed September 2012]) using the Monte Carlo method with 10,000 allele randomizations (25). The index of association has a value of 0 for panmixia and a positive value if linkage disequilibrium is detected. In addition, the values of the variance of pairwise differences (V_D) and the 95% critical value for V_D (L) were compared. The analysis was performed at three levels, i.e., inclusion of all MLSs sampled within each (sub)population, inclusion of unique MLSs within each (sub)population per herd (i.e., replicate clones within each herd were removed), and inclusion of unique MLSs per (sub)population [i.e., replicate clones within each (sub)population were removed].

The parasite population structure was explored by visual assessment of the single-locus variants network constructed using eBURST software (version 3; Department of Infectious Disease Epidemiology, Imperial College London, London, United Kingdom [http://eburst.mlst.net; accessed September 2012]), which allows identification of the clonal nature of the subtypes (26).

Population subdivision was further analyzed without *a priori* definition of subpopulation boundaries by estimating the pairwise shared allele distance (DSA) between individual *C. parvum* MLSs. The DSA was estimated using the *adegenet* (version 1.3-4) package (propShared function) implemented in R software (R Foundation for Statistical Computing, Vienna, Austria [http://www.R-project.org; accessed August 2012]) (27). These pairwise distances were visualized using a classical multidimensional scaling plot. If at least two clusters were identified in the plot, they were investigated visually for association with temporal, spatial, host-related, or parasite-related factors.

Finally, the population structure was investigated using *a priori* defined subpopulations, such as regions, years of sampling, and herd types. The fixation index (F_{ST}) was estimated in FSTAT (version 2.9.3) software using its unbiased equivalent θ (28). The parameter was measured for all subpopulations and by pairwise comparison between them. The significance of θ was tested using 10,000 permutations (not assuming Hardy-

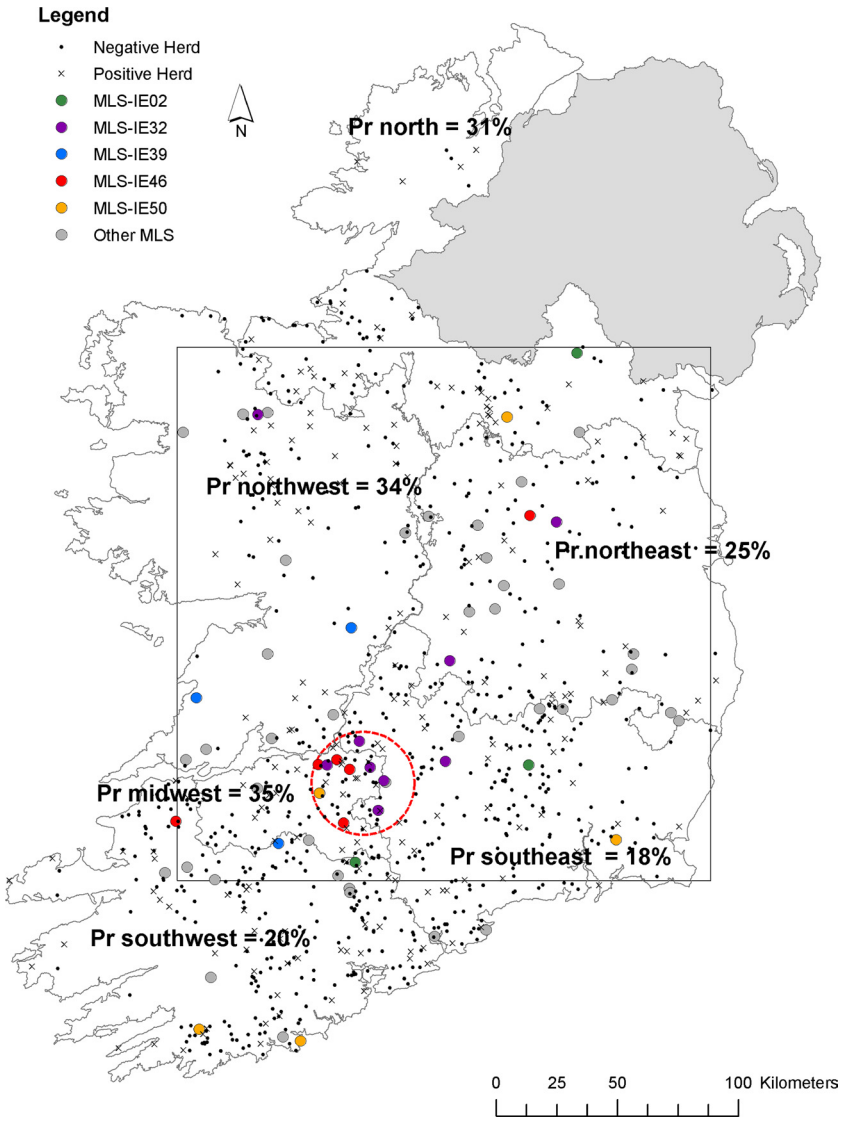

FIG 1 Spatial distribution of 944 Irish herds with or without cryptosporidiosis cases during 2005 and presentation of the MLSs identified among the *C. parvum*-positive herds subtyped in 2005 ($n = 68$). The distribution of *Cryptosporidium*-negative (·) and -positive (×) Irish herds detected by microscopic assay in 2005 and the apparent prevalence (Pr) for each region are shown. Localization of a primary cluster (dashed red circle) of MLS IE46 was detected by SaTScan. The average nearest-neighbor analysis was performed on the samples collected from calves in farms in the area demarcated by the square window.

Weinberg equilibrium). The data set was also analyzed by the discriminant analysis of principal components (DAPC) using the *adegenet* (version 1.3-4) package for R software, and the results of DAPC analysis are presented in scatterplots (29). This multivariate method (i.e., DAPC) aims to describe clusters of genetically related individuals by maximizing the between-population variability while minimizing the within-population variability.

Nucleotide sequence accession numbers. The sequence of subtype IIaA23G3R1, identified here for the first time, was deposited in the GenBank database under accession number JX441324. Nucleotide sequences generated by multilocus subtyping were deposited in the GenBank database under accession numbers JX413498 to JX413510.

RESULTS

Prevalence and risk factors of cryptosporidiosis in 2005. *Cryptosporidium* species oocysts were found in 25% of the 1,368 fecal samples from neonatal calves submitted to the RVLs during the spring of 2005. At least one animal positive for *Cryptosporidium* oocysts was detected in 29% of the 944 herds from which fecal samples were examined. The prevalence of cryptosporidiosis was the highest (35%) in the midwest of Ireland and was the lowest (18%) in the southeast (Fig. 1).

A total of 283 of the 338 samples positive for *Cryptosporidium* spp. were also tested for other common neonatal enteropathogens. Thirty-nine percent of the samples were coinfected with other enteropathogens, mainly rotavirus, which was present in 25% of the samples, in addition to coronavirus (2.9%), *Salmonella* spp. (1.8%), and *Escherichia coli* (1.4%).

Eighty-nine fecal samples with a presumptive diagnosis of cryptosporidiosis were received for molecular typing at the CVRL. In total, 84 specimens were identified as *C. parvum* by RFLP. Of these, the sequencing of 14 randomly selected amplicons confirmed 100% sequence identity with the sequence with

TABLE 1 Multivariable random-effect logistic regression model showing the variables associated with *Cryptosporidium* species excretion in calves

Predictor	Coefficient	*P*	95% confidence interval	
			Upper limit	Lower limit
At least one calf bought in	0.479	0.027[a]	0.053	0.905
Month of sampling	0.476	0.001[a]	0.203	0.749
Southeast vs southwest	−0.242	0.425	−0.836	0.352
Northwest vs southwest	0.945	0.007[a]	0.261	1.630
Midwest vs southwest	1.183	0.002[a]	0.428	1.937
Northeast vs southwest	0.308	0.367	−0.361	0.976
North vs southwest	0.672	0.067	−0.047	1.392

[a] Significant *P* value of <0.05.

TABLE 2 Number of alleles for each locus and gene diversity by locus and by spatial population (region of sampling) for 245 *Cryptosporidium parvum* samples

Locus	No. of alleles ($n = 245$)	Gene diversity[a]				
		Northwest ($n = 29$)	Midwest ($n = 67$)	Southwest ($n = 69$)	Southeast ($n = 55$)	Northeast ($n = 23$)
GP15	10	0.618	0.636	0.468	0.640	0.794
MM5	4	0.527	0.522	0.517	0.536	0.549
TP14	6	0.488	0.524	0.535	0.529	0.482
MM19	6	0.406	0.370	0.486	0.443	0.668
MM18	6	0	0.116	0.140	0.072	0.249
MS1	2	0	0	0	0.036	0
MS5	1	0	0	0	0	0
Mean		0.291	0.310	0.307	0.322	0.392

[a] The gene diversity of the samples collected in the north ($n = 2$) was not included on account of the small sample size. *n*, number of samples.

GenBank accession number AB441687 (http://www.ncbi.nlm.nih.gov/GenBank). Four samples collected in 2005 were identified as *C. bovis* (100% sequence identity with the sequence with GenBank accession number AY741305) and one sample was identified as *C. ryanae* (100% sequence identity with the sequence with GenBank accession number AY587166).

The mean size of the herds with cryptosporidiosis in at least one calf was higher than the mean size of the herds randomly selected for comparison, with 156 cattle (standard deviation [SD], 132; range, 4 to 1,323) and 60 cattle (SD, 69; range, 1 to 663), respectively. A higher mean number of herds with bought-in calves was observed in the east than in the other regions; this was observed in both data sets (i.e., herds with cryptosporidiosis and herds randomly selected).

Univariable models returned four variables associated with cryptosporidiosis. The risk of cryptosporidiosis was the lowest in the southwest. Calves in cow-calf beef herds were more at risk of infection than dairy calves. The risk increased if at least one calf was bought in to the herd, and the risk increased progressively during the calving period (from March to May). Location, time, and introduction of young animals were significant predictors in the final multivariate model (Table 1). The Hosmer-Lemeshow statistic did not indicate a lack of fit ($P = 0.913$), and there was no difference between the parameter estimates of models with 7 and 20 quadrature points, indicating robustness of the model.

The predictors identified above were not associated with specific subtypes of *C. parvum*. On the basis of the average nearest-neighbor analysis, the herds subtyped in 2005 ($n = 56$) were randomly distributed on the island, with a value of 1.4 obtained for the ratio of the observed mean distance to the expected mean distance (*Z* score = 0.62 SD). The spatial clustering analysis identified a cluster of four herds with MLS IE46 with a radius of circa 25 km using both the Poisson model ($P = 0.038$) and the Bernoulli model (relative risk = 33.33, $P = 0.016$), with 6 herds infected with the *C. parvum* MLS IE46 and 50 herds infected with other MLS subtypes (Fig. 1).

Population genetics of *Cryptosporidium parvum*. A total of 277 *C. parvum* specimens tested by microscopy in RVLs and confirmed by molecular methods in CVRL were included in the population genetics analysis, i.e., 143, 50, and 84 specimens from 2003, 2004, and 2005, respectively. With the exception of 11 specimens unsuccessfully sequenced and one ambiguous sequence, all the *gp60* amplicons that were sequenced ($n = 265$) belonged to 17 *C. parvum* subtypes of the family IIa. The majority (58%) of the samples were subtyped as IIaA18G3R1. A further 15%, 8%, and 5% of the samples were identified as subtypes IIaA15G2R1, IIaA20G3R1, and IIaA19G3R1, respectively. One subtype, IIaA23G3R1, was identified for the first time, and its sequence was deposited in the GenBank database.

The 277 *C. parvum* specimens were further characterized by multilocus subtyping for seven markers. A total of 32 samples were removed from the analysis for various reasons, such as an insufficient quantity of DNA to perform testing ($n = 21$) and a lack of information on the sample origin ($n = 11$). The remaining 245 samples had been collected from 205 herds. The number of alleles at each locus ranged from 1 for MS5 to 10 for GP15 (Table 2). Ten samples had a biallelic profile at one locus (TP14, MM5, or MM18), and another sample had biallelic results at three loci (TP14, MM5, and GP15) (see Table S2 in the supplemental material). The lowest genetic diversity was obtained in the northwest, and the highest value was obtained in the northeast (Table 2). A total of 78 MLSs were identified. Thirty-three herds were sampled at least twice over the 3-year period, and 11 of them had calves harboring different MLSs. Without taking into account the samples collected in the north ($n = 2$), the highest ratio of distinct MLSs for the number of samples collected was observed in the northeast, with a ratio of 0.78, compared to ratios of 0.53, 0.52, 0.43, and 0.41 in the southeast, northwest, midwest, and southwest, respectively (Table 3).

Overall, the population of *C. parvum* in Ireland had a panmictic structure with a value of 0.007 for I_A^S (Table 3). Departures from panmixia were observed when the analysis was performed with all MLSs present in the region in the southeast ($I_A^S = 0.023$, $P = 0.043$, $V_D > L$) and the southwest ($I_A^S = 0.021$, $P = 0.030$, $V_D > L$) and also in the southwest with inclusion of one MLS per herd in the region ($I_A^S = 0.023$, $P = 0.031$, $V_D > L$).

The eBURST network (Fig. 2) with a star-like phylogeny showed a central founder (MLS IE32; bootstrap confidence = 89%) and 18 linked single-locus variants. Five MLSs (IE02, IE32, IE39, IE46, and IE50) were represented in 45% of the samples and were present in dairy, mixed, or beef herds in the southern regions (midwest, southwest, and southeast) during the 3 years of the study (Fig. 2). Using DSA analysis, four clusters of parasites were identified that were associated with the four most abundant MLSs (i.e., IE32, IE39, IE46, and IE50) and their single-locus variants

TABLE 3 Number of MLSs and I_A^S for the *Cryptosporidium parvum* populations collected from calves in Ireland and six spatial subpopulations[a]

(Sub)population	Sample level[b]		Herd level[c]		(Sub)population level[d]	
	No. of MLSs	I_A^S	No. of MLSs	I_A^S	No. of MLSs	I_A^S
Ireland	245	0.007	194	0.006	78	−0.021
Northwest	29	0.032	27	0.023	15	0.005
Midwest	67	0.006	48	0.006	30	−0.024
Southwest	69	0.021[e]	60	0.023[e]	28	0.002
Southeast	55	0.023[e]	40	0.013	29	−0.021
Northeast	23	0.014	17	−0.024	18	−0.011
North	2	ND[f]	2	ND	2	ND

[a] The six spatial subpopulations comprised those from the northwest, midwest, southwest, southeast, northeast, and north.
[b] Analysis of I_A^S with inclusion of all MLSs sampled within each (sub)population.
[c] Analysis of I_A^S with inclusion of one MLS per herd within each (sub)population; 11 of the 205 herds had calves harboring different MLSs, and those were removed.
[d] Analysis of I_A^S with inclusion of one MLS per (sub)population.
[e] Significant linkage disequilibrium ($P < 0.05$) and $V_D > L$ (where V_D is the variance of pairwise differentiation and L is the 95% critical value for V_D).
[f] ND, not done due to insufficient sample size.

TABLE 4 θ values and associated *P* values obtained by pairwise analysis on spatial populations of 243[a] *Cryptosporidium parvum* samples

(Sub)population	θ or *P* value[b]				
	Northwest	Midwest	Southwest	Southeast	Northeast
Northwest		0.005	0.001	−0.008	0.050[c]
Midwest	0.077		0.008[d]	−0.011	0.024[c]
Southwest	0.333	0.043		0.001	0.064[c]
Southeast	0.223	0.807	0.093		0.019
Northeast	0.003	0.003	0.003	0.127	

[a] Data for the samples collected in the north ($n = 2$) are not presented.
[b] The θ values are given in the upper triangle, and the *P* values are given in the lower triangle.
[c] Significant at the 5% level following Bonferroni correction for multiple testing.
[d] Significant at the 5% level.

(see Fig. S1 in the supplemental material). Those clusters were not related to location, year of sampling, or host-related factors.

The overall θ for the entire data set was low (0.005). After the Bonferroni correction, the pairwise analysis indicated a low but significant genetic differentiation between the northeast and the western regions (Table 4). Similar results were obtained with or without the samples with biallelic profiles. The scatterplots of the DAPC also showed a slight separation of the northeast population from the other subpopulations (Fig. 3).

Because, for the same target, the *gp60* sequencing increased the discriminatory power compared with that obtained by the *gp15* sizing method (see Table S2 in the supplemental material), all the analyses were repeated with *gp60* (instead of *gp15*) in the MLS and showed similar results (not presented).

DISCUSSION

The sample of the *C. parvum* population collected from Irish neonatal calves presented a panmictic structure, suggesting random mating regardless of genetic or environmental factors. This population structure of the parasite was also recently reported with

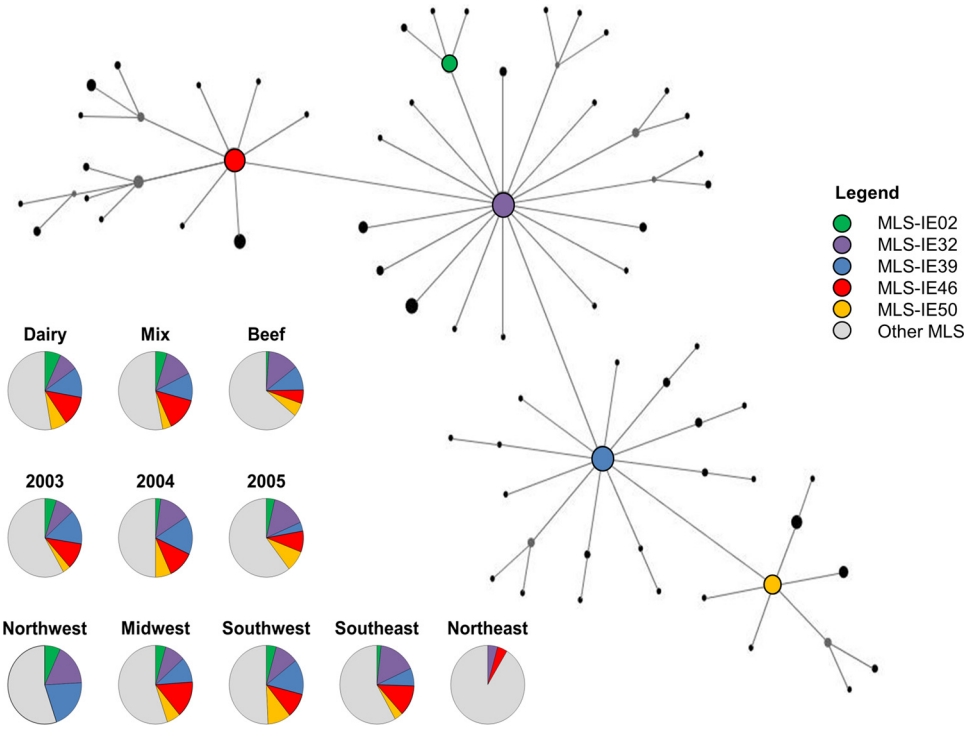

FIG 2 Single-locus variant eBURST network for *Cryptosporidium parvum* population in Ireland (IE). Each MLS is represented by a dot. The dot diameter is proportional to the number of isolates. Single-locus variants are connected by lines. Pie charts represent the proportion of the MLS for each herd type (dairy, mix, and suckler beef herd), each year of sampling (2003, 2004, and 2005), and each Irish region sampled (northwest, midwest, southwest, southeast, northeast). Note that only two samples were subtyped from the north, and therefore, the results from this region were not presented in the pie chart.

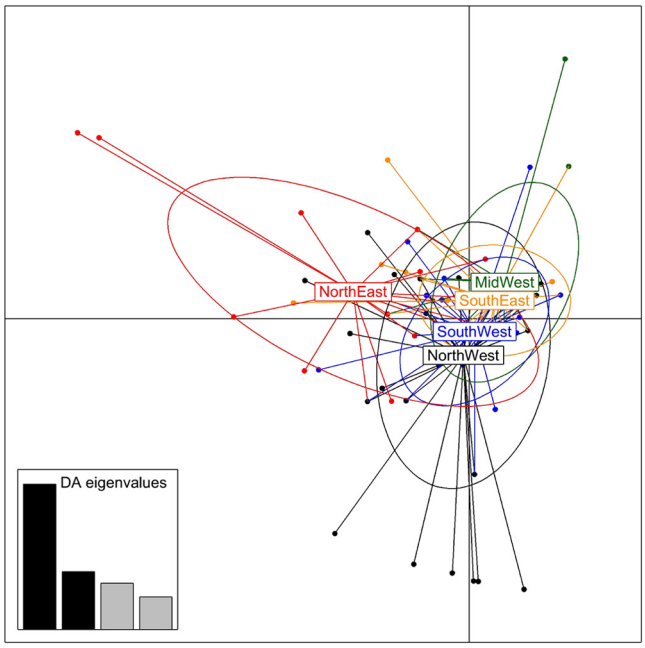

FIG 3 Scatterplots of the discriminant analysis (DA) of principal components of *Cryptosporidium parvum* samples collected from calves in different regions of Ireland. This scatterplot shows the first two principal components of the DAPC of regional *C. parvum*, using regions of sampling as prior clusters. Groups are shown by different colors and inertia ellipses, representing northwest, midwest, southwest, southeast, and northeast, while dots represent individual strains. The percentages of variation were 25% and 21% for the first and second axes, respectively. Note that the samples with biallelic profiles were not included.

specimens collected from calves in the Upper Midwest United States (30). In contrast, the parasite population from cattle in Italy and Scotland presented a departure from panmixia, with evidence of subpopulation structure variations (12, 13). The Italian study included *C. parvum* specimens from humans and livestock (calves, sheep, and goats) and showed low indexes of association and a high level of diversity, suggesting that both clonal expansion and some genetic exchange may occur (13). In addition, the parasite population suggested the existence of host-related subpopulations (goats) (13). The panmixia observed in Ireland may be due to the high prevalence of this parasite, host-related factors, or a combination of these two. A previous report stated that panmixia occurred in countries where *C. parvum* is endemic (31). In agreement with surveillance findings in Ireland, the present study reported a high prevalence of cryptosporidiosis in calves, especially that caused by the zoonotic organism *C. parvum* (3). The MLS typing revealed four abundant subtypes and their single-locus variants, which were also grouped into four genetic clusters, unrelated to location, year of sampling, or host-related factors. This indicated a high degree of spatial and temporal genetic exchange in the *C. parvum* population regardless of herd size and type. A high prevalence of more than one strain of the parasite on the island would increase the likelihood of coinfection with genetically heterogeneous parasites and recombination, resulting in a panmictic structure. However, it was surprising to find a smaller number of mixed infections than in other European studies (13, 19, 32). This might be due to the geographical isolation of Ireland.

The structure of the *C. parvum* population may also be linked to host-related factors, such as the size of the cattle population (approximately 6.5 million cattle in 70,273 km^2) and the distance and frequency of cattle movements, especially of young calves, between herds (33). Ashe et al. (34) illustrated that there was a substantial dispersal of cattle throughout Ireland. A recent study over a 4-year period showed that 60% of Irish cattle were likely to be moved between herds, with the majority being moved within their first year of life (35). The same authors observed a peak in calf movements within the first 12 weeks of life in dairy herds and at between 21 and 36 weeks of age in beef herds. Young calves are considered to be at greater risk of *C. parvum* infection than older cattle (36). In addition, some infected calves might present no clinical signs (37). The present study showed that the frequency of calf introduction was higher in herds with cryptosporidiosis than in uninfected or randomly selected herds.

Differences in the prevalence of the parasite and host-related factors at the local level were reflected in the subpopulations of the parasite. Notwithstanding the sampling bias created by only sampling calves with enteritis, the prevalence of bovine neonatal cryptosporidiosis in 2005 varied from region to region. The lowest prevalence was recorded in the most southern region of Ireland, where a departure from panmixia was observed. As reported for other protozoa, *C. parvum* utilizes mixed mating, in which outbreeding occurs in regions with high rates of transmission, while inbreeding predominates in areas with low rates of transmission (38). Variation in the parasite structure was also observed in Scotland, with an epidemic structure in Thurso and Orkney and a panmictic structure in Aberdeenshire and Dumfriesshire (12). The spatial cluster of MLS IE46 detected in County Limerick may be explained by its topographic isolation, being surrounded by a river and mountains, and/or the low number of bought-in calves. Spatial clustering of other subtypes had previously been reported in the United Kingdom and Denmark (39, 40). In agreement with previous reports (12, 41), increased calf introduction accounted for the greater genetic diversity in the northeast (34). In this regard, the southwest of Ireland contains a high density of dairy herds, which only occasionally introduce new young animals, while the majority of farms in the northeast buy in young cattle for beef production.

Although sequencing increased the discriminatory power, replacing the MLS *gp15* sizing with *gp60* sequencing results did not affect the interpretation of the results. The *C. parvum* specimens in this study belonged to the potentially zoonotic subtype family IIa, with the most common subtype being IIaA18G3R1, as previously reported (9, 10). This subtype had previously been identified in Australia, Canada, Italy, and The Netherlands (8). In Ireland, this common bovine subtype, IIaA18G3R1, is also the predominant *gp60* strain detected in humans and has been implicated in a waterborne outbreak in Northern Ireland (5, 42). In addition, 7 of the 17 subtypes found in calves in this study had been reported in humans. Studies of *C. parvum* in humans have recently included two other markers, ML1 and MS1 (5). As in the present study, one predominant MS1 subtype (117 out of 121 specimens) was observed, and the comparison of some sequences from human specimens with those from cattle revealed 100% sequence identity. The high prevalence of *C. parvum* in calves and the predominance of subtypes which have been identified in humans in Ireland indicated that there is a risk of zoonotic transmission, notwithstanding the fact that only two markers were used to compare *C. parvum* from both cattle and human populations. However, both

humans and animals can contribute to the contamination of surface waters and, hence, continuously infect each other (5). In addition to calves, sheep, goats, and wild animals infected with *C. parvum* could also contaminate the environment (43).

Two nonzoonotic species, *C. bovis* and *C. ryanae*, were also identified. Since their pathogenesis in cattle has not been elucidated, their role in neonatal enteritis is unknown.

In conclusion, the population structure of *C. parvum* in Irish calves was panmictic. This structure may be due to the high prevalence of the parasite, the host density, and the practice, in some parts of the country, of buying in very young calves for on-site rearing. The application of standard farm biosecurity principles, including isolation and testing of calves before introduction into a herd, may reduce the risk of cryptosporidiosis. Future studies using additional polymorphic markers are required for a greater understanding of the transmission dynamics of this zoonotic parasite in humans and cattle. Furthermore, it would be interesting to compare the *C. parvum* population from Ireland with that from other countries, especially those from which cattle were imported to Ireland.

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