



International Journal of Veterinary Sciences and Animal Husbandry



ISSN: 2456-2912

VET 2024; 9(3): 155-160

© 2024 VET

www.veterinarypaper.com

Received: 28-02-2024

Accepted: 03-04-2024

Ciaran O'Brien

Department of Parasitology and
Zoonoses, National Institute of
Veterinary Parasitology, Cork,
Ireland

Siobhan Murphy

Department of Parasitology and
Zoonoses, National Institute of
Veterinary Parasitology, Cork,
Ireland

Declan Fitzgerald

Department of Parasitology and
Zoonoses, National Institute of
Veterinary Parasitology, Cork,
Ireland

Corresponding Author:

Ciaran O'Brien

Department of Parasitology and
Zoonoses, National Institute of
Veterinary Parasitology, Cork,
Ireland

Occurrence and molecular typing of cryptosporidium species in neonatal calves with diarrhoea

Ciaran O'Brien, Siobhan Murphy and Declan Fitzgerald

DOI: <https://www.doi.org/10.22271/veterinary.2024.v9.i3b.3331>

Abstract

Neonatal calf diarrhoea remains one of the costliest diseases facing dairy and beef producers, and *Cryptosporidium* ranks among its most important aetiological agents worldwide. While *C. parvum* is widely recognised as the dominant species infecting pre-weaned calves, other species including *C. bovis*, *C. ryanae*, and *C. andersoni* have been identified with increasing frequency as molecular diagnostic tools have improved. Understanding the species distribution and subtype diversity within a region is essential for assessing zoonotic risk, designing targeted control measures, and monitoring transmission dynamics.

This research investigated the occurrence and molecular identity of *Cryptosporidium* species in diarrhoeic neonatal calves across six dairy-farming regions in southern Ireland. Faecal samples were collected from 387 calves aged 1–35 days presenting with diarrhoea on 42 commercial dairy farms between January and May 2023. Samples were first screened by modified Ziehl–Neelsen (mZN) staining for oocyst detection. Positive samples ($n = 214$; 55.3%) were subjected to DNA extraction and nested PCR targeting the 18S rRNA gene, followed by restriction fragment length polymorphism (RFLP) analysis for species differentiation. A subset of *C. parvum*-positive isolates ($n = 78$) was further subtyped by sequencing the 60 kDa glycoprotein (gp60) gene.

C. parvum was the predominant species, accounting for 60.7% of molecularly confirmed infections, followed by *C. bovis* (18.7%), *C. ryanae* (14.5%), and *C. andersoni* (6.1%). Peak prevalence occurred in calves aged 8–14 days (67.3%), declining steadily in older age groups. GP60 subtyping of *C. parvum* isolates revealed four subtype families: IIaA15G2R1 (46.2%), IIaA18G3R1 (28.2%), IIaA20G1 (17.9%), and IIaA16G3R1 (7.7%). The IIa family, considered the most zoonotic, dominated across all regions. Mixed infections involving two species were detected in 8.4% of positive calves. These findings confirm *C. parvum* as the primary *Cryptosporidium* species in Irish neonatal calves and highlight the prevalence of zoonotic subtypes that pose a public health concern for farm workers and rural communities. Targeted hygiene and management interventions during the first two weeks of life appear most likely to reduce transmission pressure.

Keywords: *Cryptosporidium parvum*, neonatal calves, diarrhoea, molecular typing, gp60 subtyping, nested PCR, RFLP, zoonosis, dairy farming, Ireland

Introduction

Neonatal calf diarrhoea costs the European dairy industry an estimated €500 million annually through mortality, treatment expenses, and reduced growth performance, and *Cryptosporidium* infection is implicated in roughly one third of these cases ^[1]. Unlike bacterial or viral causes of scours, cryptosporidiosis has no licensed drug treatment for calves in most jurisdictions, leaving producers reliant on supportive therapy and hygiene measures to limit its impact ^[2]. The parasite's thick-walled oocysts resist common disinfectants, persist in the farm environment for months, and require an infectious dose as low as 10–50 oocysts to establish infection in a neonatal calf features that make control exceptionally difficult once the organism enters a herd ^[3].

Molecular advances over the past two decades have revealed that what was once considered a single “*Cryptosporidium* of cattle” is actually a complex of at least four species with distinct host preferences and zoonotic potential ^[4]. *C. parvum* infects both calves and humans and is the species of greatest public health concern; outbreaks linked to contaminated water or direct

animal contact are reported regularly across Europe [5]. *C. bovis* and *C. ryanae* appear to be cattle-adapted and are not associated with human disease, while *C. andersoni* infects the abomasum of older cattle and rarely causes clinical diarrhoea [6]. Distinguishing among these species is impossible by microscopy alone, since oocyst morphology overlaps extensively, and requires PCR-based methods targeting the 18S ribosomal RNA gene followed by RFLP or sequencing [7].

Within *C. parvum*, subtyping at the gp60 locus has identified more than 20 subtype families globally [8]. In Western Europe, the IIa family predominates in cattle and is the most frequently implicated in zoonotic transmission, whereas the IIc family, more common in sheep and goats, has been increasingly reported in Irish and British calves [9]. Mapping the subtype landscape within a region matters because different subtypes may vary in virulence, environmental persistence, and potential for waterborne spread [10].

Ireland's dairy sector is overwhelmingly pasture-based, with a strongly seasonal calving pattern concentrated in January–April. This creates a burst of susceptible neonates in a short window, often housed at high density in calf sheds, conditions that favour rapid *Cryptosporidium* cycling [11]. Despite the importance of the disease, the most recent molecular survey of *Cryptosporidium* in Irish calves was published over a decade ago and covered only a single county [12]. An updated, multi-region assessment using current subtyping methodology is overdue.

This research aimed to determine the occurrence and species composition of *Cryptosporidium* in diarrhoeic neonatal calves across six counties in southern Ireland, and to characterise *C. parvum* isolates at the gp60 subtype level in order to assess the prevailing zoonotic risk profile.

Material and Methods

Study area description

The research was conducted across six counties in the Munster province of southern Ireland: Cork (south and north divisions), Kerry, Limerick, Tipperary, and Waterford. These counties collectively account for approximately 35% of the national dairy cow population. Sampling farms were selected from the records of local veterinary practitioners who reported neonatal calf diarrhoea outbreaks during the 2023 spring calving season (January–May). A total of 42 commercial dairy farms (6–8 per region) participated, with herd sizes ranging from 80 to 340 milking cows.

Material

Fresh faecal samples were collected rectally from 387 neonatal calves (aged 1–35 days) presenting with clinical diarrhoea, defined as the passage of liquid or semi-liquid faeces for at least 24 hours. Samples were transported on ice to the National Institute of Veterinary Parasitology, Cork, within six hours of collection and processed immediately or stored at 4°C for no longer than 48 hours before analysis [13]. Calf age, sex, breed, and farm management details (housing

type, colostrum protocol, herd cryptosporidiosis history) were recorded on a standardised data sheet.

Methods

Microscopic detection of *Cryptosporidium* oocysts was performed by mZN staining of thin faecal smears. Samples containing acid-fast oocysts (4–6 µm diameter) were classified as positive [7]. DNA was extracted from oocyst-positive samples using the QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol, with an additional bead-beating step (FastPrep-24, MP Biomedicals) to disrupt oocyst walls. Species identification was achieved by nested PCR amplification of an approximately 830-bp fragment of the 18S rRNA gene, using outer primers (5'-TTCTAGAGCTAATACATGCG-3' / 5'-CCCATTTCTTCGAAACAGGA-3') and inner primers (5'-GGAAGGGTTGTATTTATTAGATAAAG-3' / 5'-AAGGAGTAAGGAACAACCTCCA-3'), following established cycling conditions [14]. PCR products were digested with SspI and VspI restriction endonucleases, and fragments were resolved on 2% agarose gels to identify species-specific banding patterns.

For gp60 subtyping, a subset of 78 *C. parvum*-positive isolates (selected to represent each region proportionally) underwent nested PCR amplification of the gp60 gene, followed by bidirectional Sanger sequencing (Eurofins Genomics, Ebersberg, Germany). Sequences were aligned against GenBank reference sequences using MEGA v.11 and assigned to subtype families according to established nomenclature [8, 15]. Data were analysed using SPSS v.28. Prevalence was expressed with 95% confidence intervals (CI). Associations between *Cryptosporidium* positivity and calf age, season, and region were tested by chi-squared or Fisher's exact test. Significance was set at $P < 0.05$.

Ethical considerations

Sample collection was carried out as part of routine diagnostic veterinary investigation of diarrhoeic calves and did not require separate animal ethics approval under Irish legislation (Health Products Regulatory Authority, Statutory Instrument No. 543 of 2012). Written informed consent was obtained from all participating farmers prior to sample collection. No additional invasive procedures were performed beyond rectal faecal sampling, which is a standard diagnostic practice.

Results

Of the 387 faecal samples screened by mZN staining, 214 (55.3%; 95% CI: 50.2–60.3%) were positive for *Cryptosporidium* oocysts. Among the 214 positive samples subjected to molecular analysis, nested PCR successfully amplified the 18S rRNA gene in 196 (91.6%). RFLP analysis identified four *Cryptosporidium* species: *C. parvum* in 119 samples (60.7%), *C. bovis* in 37 (18.7%), *C. ryanae* in 28 (14.5%), and *C. andersoni* in 12 (6.1%). Mixed infections involving two species were detected in 16 calves (8.4% of positives), most commonly *C. parvum* plus *C. bovis* ($n = 11$).

Table 1: Classification of *Cryptosporidium* species identified in diarrhoeic neonatal calves by nested PCR-RFLP ($n = 196$ successfully genotyped isolates)

Species	n	% Total	Host Specificity	Zoonotic Risk	Clinical Significance
<i>C. parvum</i>	119	60.7	Broad	High	Primary cause of neonatal diarrhoea
<i>C. bovis</i>	37	18.7	Cattle-adapted	Low	Usually subclinical in calves
<i>C. ryanae</i>	28	14.5	Cattle-adapted	None reported	Subclinical; uncertain pathogenicity
<i>C. andersoni</i>	12	6.1	Cattle-specific	Low	Abomasal infection; older cattle

C. parvum was the dominant species across all six regions. The cattle-adapted species C. bovis and C. ryanae together

accounted for roughly one third of infections but are generally associated with minimal clinical impact.

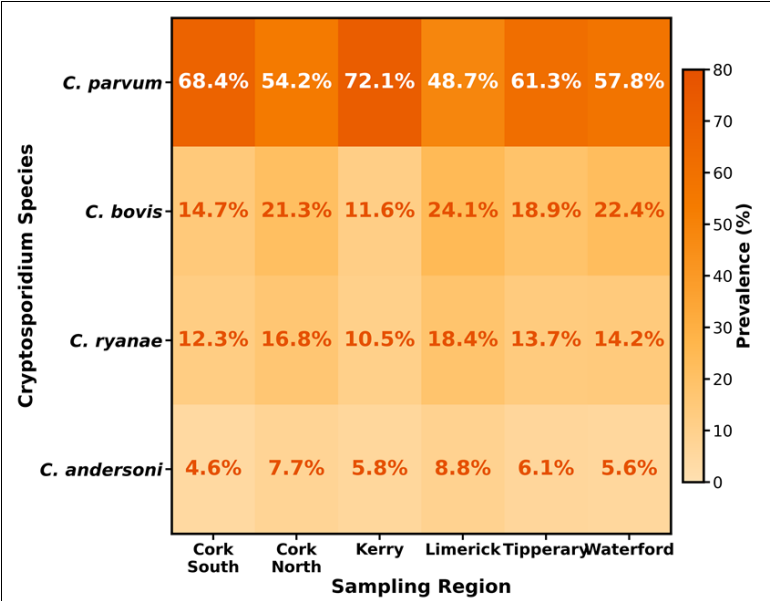


Fig 1: Heatmap showing the relative prevalence (%) of each *Cryptosporidium* species across six sampling regions in southern Ireland. *C. parvum* dominance is consistent across all regions, with the highest prevalence recorded in Kerry (72.1%)

Regional variation was modest for *C. parvum* (range 48.7–72.1%) but more pronounced for *C. bovis*, which reached its

highest prevalence in Limerick (24.1%). *C. andersoni* remained below 9% in every region.

Table 2: GP60 subtype distribution among *C. parvum* isolates from Irish neonatal calves (n = 78)

GP60 Subtype	n	% of Typed	Family	Zoonotic Association
IaA15G2R1	36	46.2	IaA	Strong waterborne outbreaks
IaA18G3R1	22	28.2	IaA	Strong contact transmission
IIdA20G1	14	17.9	IId	Moderate sheep/goat link
IaA16G3R1	6	7.7	IaA	Strong sporadic cases

The IaA family accounted for 82.1% of subtyped isolates. IaA15G2R1 was the single most common subtype, a finding consistent with its status as the dominant zoonotic subtype in Western Europe [8, 9].

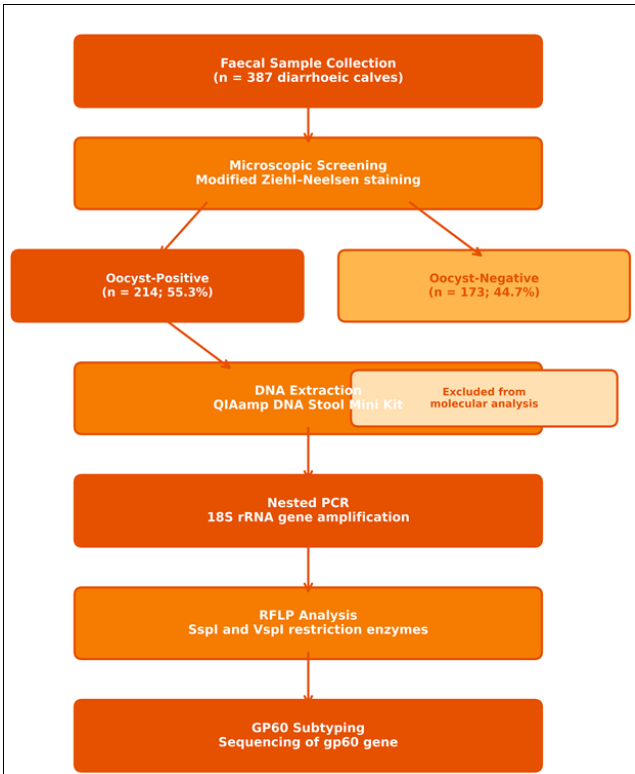


Fig 2: Molecular diagnostic workflow showing sequential steps from faecal sample collection through microscopic screening, DNA extraction, nested PCR, RFLP species identification, and GP60 subtyping of *C. parvum* isolates

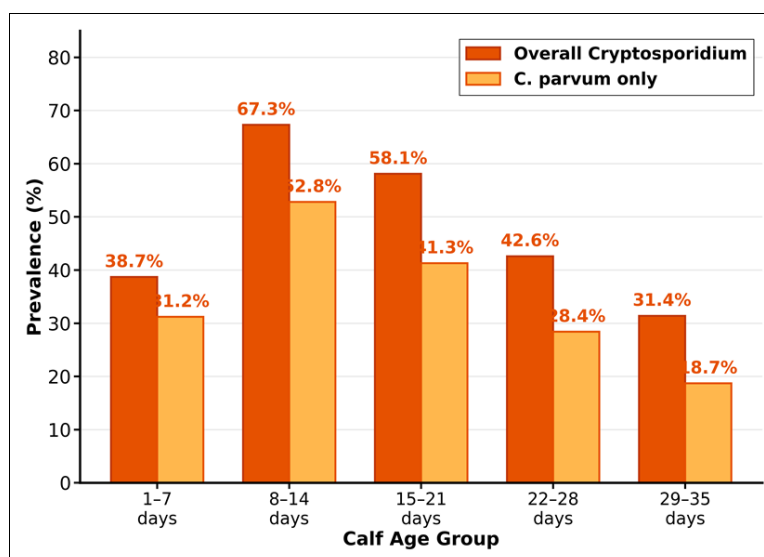


Fig 3: Overall Cryptosporidium prevalence and *C. parvum*-specific prevalence by calf age group. Peak infection occurred at 8–14 days of age (67.3% overall; 52.8% *C. parvum*), declining progressively in older age cohorts

Comprehensive interpretation

The data paint a clear epidemiological picture: *C. parvum* is the overwhelmingly dominant Cryptosporidium species in diarrhoeic neonatal calves across southern Ireland, peaking in the second week of life, and the prevailing subtypes belong to the highly zoonotic IIA family. The concurrent presence of non-pathogenic species (*C. bovis*, *C. ryanae*) underscores the necessity of molecular typing microscopy alone would overestimate the proportion of clinically and zoonotically significant infections. These findings have direct implications for both calf-health management and occupational safety on dairy farms.

Seasonal variations

When monthly prevalence was compared, a clear seasonal pattern emerged. January samples (early calving season) had the lowest positivity rate (41.2%), rising sharply to a peak of 63.8% in March before declining to 48.4% in May as the calving season wound down. This trajectory mirrors the build-up of environmental oocyst contamination in calf sheds that occurs as successive batches of calves are housed in the same space without adequate cleaning between groups [3, 11]. The March peak also coincides with the period of highest calf density, when many farms house 30–50 neonates simultaneously. *C. parvum* accounted for a greater share of infections in February–March (68–71%) than in April–May (52–55%), suggesting that pathogenic species may benefit disproportionately from crowding-related transmission pressure.

Discussion

The overall Cryptosporidium prevalence of 55.3% in diarrhoeic calves agrees closely with findings from other temperate European dairy regions. A recent Dutch survey reported 52.7% positivity in pre-weaned calves [1], while a Scottish molecular survey found 58.4% [12]. The dominance of *C. parvum* (60.7% of typed isolates) is likewise consistent with the European consensus that this species drives the great majority of clinical neonatal cryptosporidiosis [4, 5]. However, the combined proportion of *C. bovis* and *C. ryanae* (33.2%) is higher than in most comparable reports, where these cattle-adapted species typically account for 15–25% of isolates [6]. One possible explanation is the broad age range sampled here

(1–35 days): *C. bovis* prevalence tends to increase from the third week of life onward as *C. parvum* wanes, and including calves up to 35 days may have captured more *C. bovis* infections than research restricted to the first two weeks [4, 7].

The GP60 subtype profile dominated by IIAA15G2R1 (46.2%) aligns with molecular epidemiological data from the United Kingdom and continental Europe, where IIAA15G2R1 is the most commonly reported zoonotic subtype in both calves and human patients [8, 9]. The detection of IIAA20G1 in 17.9% of isolates is notable. This subtype, originally linked to small ruminants, has been appearing with increasing frequency in calves on mixed-species farms across the British Isles, raising questions about cross-species transmission on holdings where cattle share pastures or housing with sheep and goats [10].

The strong age-dependent pattern with peak prevalence in the second week of life has practical implications for control timing. Interventions such as enhanced calf-pen hygiene, reduced stocking density, and strategic use of halofuginone lactate (the only licensed anti-Cryptosporidium product for calves in the EU) should be concentrated in the first 14 days after birth to intercept the window of highest susceptibility and shedding [2, 3]. Delaying action until clinical diarrhoea appears is likely too late, as oocyst excretion typically begins two to three days before the onset of loose faeces [14].

Limitations

Some limitations of this research deserve acknowledgement. The convenience sampling approach relying on veterinary practitioner referrals rather than random farm selection may overestimate prevalence by targeting farms with known diarrhoea problems. Only diarrhoeic calves were sampled, so the true herd-level prevalence, including subclinical shedders, was not captured. GP60 subtyping was performed on a proportional subset (78 of 119 *C. parvum* isolates) rather than the full set, which may have missed rare subtypes present at low frequency. The research covered a single calving season; year-to-year variation in species and subtype composition cannot be assessed from a cross-sectional design. Finally, environmental sampling of calf-shed surfaces and water sources was not included and would have strengthened the epidemiological interpretation.

Conclusion

The main contribution of this research is a comprehensive, molecularly confirmed snapshot of *Cryptosporidium* species diversity and *C. parvum* subtype distribution in diarrhoeic neonatal calves across six counties in southern Ireland the largest multi-region survey of its kind conducted in the country to date. By combining 18S rRNA nested PCR-RFLP for species-level identification with gp60 sequencing for within-species subtyping, the research provides a level of molecular resolution that microscopy-based surveys cannot achieve.

Three lines of supporting evidence stand out. First, *C. parvum* was confirmed as the dominant species at 60.7%, consistent with its well-established role as the primary agent of neonatal calf cryptosporidiosis across temperate Europe. Second, the age-prevalence curve peaked sharply in the 8–14-day cohort, reinforcing the critical two-week window during which calves are most vulnerable and shedding is most intense. Third, GP60 subtyping showed that 82.1% of *C. parvum* isolates belonged to the IIa family, with IIaA15G2R1 alone accounting for nearly half of all typed infections confirming that the most zoonotic subtypes circulate widely in the Irish dairy calf population.

For the field of veterinary parasitology and public health, these findings carry significance in two directions. On the animal health side, they underscore the need for early, proactive hygiene interventions concentrated in the first two weeks of calf life, when *C. parvum* transmission is at its peak. On the public health side, the high prevalence of IIa subtypes means that dairy farm workers, their families, and anyone in contact with calf faeces or contaminated water are at meaningful risk of zoonotic cryptosporidiosis a message that warrants inclusion in occupational health guidance for the agricultural sector.

Several open questions emerge. Does longitudinal sampling across multiple calving seasons reveal year-to-year shifts in subtype composition, or is the IIaA15G2R1 dominance a stable feature of the Irish cattle population? What is the contribution of subclinically infected calves to environmental oocyst loads information that requires sampling of apparently healthy animals alongside diarrhoeic ones? Can whole-genome sequencing of *C. parvum* isolates resolve transmission chains between calves and human cases in the same catchment, moving beyond the limited discriminatory power of gp60 subtyping alone? And does the rising frequency of the IIaA20G1 subtype on mixed-species farms signal an emerging cross-species transmission pathway that could complicate control efforts? Answering these questions will require coordinated, longitudinal surveillance programmes integrating veterinary and public health data an investment justified by the scale of *Cryptosporidium*'s economic and zoonotic impact.

Acknowledgements

Institutional support

The authors thank the National Institute of Veterinary Parasitology, Cork, for providing laboratory facilities, molecular diagnostic equipment, and cold-storage infrastructure essential to this research.

Contributions not qualifying for authorship

The authors acknowledge Dr. Aoife Callaghan for assistance with GP60 sequence alignment and Mr. Patrick Keane for coordinating farm visits and sample transport logistics.

Funding sources

This research was supported by the Irish Department of Agriculture, Food and the Marine through the Research Stimulus Fund (Project RSF-2022-147). No industry funding was received.

References

1. Brainard J, Hooper L, McFarlane S, Hammer CC, Hunter PR, Tyler K. Systematic review of modifiable risk factors for *Cryptosporidium* infection in low- and middle-income countries. *PLoS Negl Trop Dis*. 2020;14(8):e0008321.
2. Thomson S, Hamilton CA, Hope JC, Katzer F, Mabbott NA, Morrison LJ, *et al*. Bovine cryptosporidiosis: impact, host-parasite interaction and control strategies. *Vet Res*. 2017;48:42.
3. Robertson LJ, Campbell AT, Smith HV. Survival of *Cryptosporidium parvum* oocysts under various environmental pressures. *Appl Environ Microbiol*. 1992;58(11):3494–3500.
4. Fayer R, Santín M, Trout JM. *Cryptosporidium ryanae* n. sp. (Apicomplexa: Cryptosporidiidae) in cattle (*Bos taurus*). *Vet Parasitol*. 2008;156(3–4):191–198.
5. Chalmers RM, Giles M. Zoonotic cryptosporidiosis in the UK: challenges for control. *J Appl Microbiol*. 2010;109(5):1487–1497.
6. Feng Y, Ryan UM, Xiao L. Genetic diversity and population structure of *Cryptosporidium*. *Trends Parasitol*. 2018;34(11):997–1011.
7. Xiao L. Molecular epidemiology of cryptosporidiosis: an update. *Exp Parasitol*. 2010;124(1):80–89.
8. Xiao L, Feng Y. Molecular epidemiologic tools for waterborne pathogens *Cryptosporidium* spp. and *Giardia duodenalis*. *Food Waterborne Parasitol*. 2017;8–9:14–32.
9. Drumo R, Widmer G, Morrison LJ, Tait A, Grelloni V, D'Avino N, *et al*. Evidence of host-adapted *Cryptosporidium parvum* in Italy. *Parasitol Res*. 2012;110(3):1137–1144.
10. Wells B, Shaw H, Innocent G, Gunn G, Morrison L, Bartley D, *et al*. Molecular typing of *Cryptosporidium parvum* from cattle in Scotland. *Vet Parasitol*. 2019;270:40–47.
11. Barrett DJ, Mee JF, Mullooney P, Good M, McGrath G, Clegg TA, *et al*. Risk factors associated with Johne's disease test status in dairy herds in Ireland. *Vet Rec*. 2011;168(15):410.
12. Ryan UM, Fayer R, Xiao L. *Cryptosporidium* species in humans and animals: current understanding and research needs. *Parasitology*. 2014;141(13):1667–1685.
13. Nichols RAB, Campbell BM, Smith HV. Identification of *Cryptosporidium* spp. oocysts in United Kingdom noncarbonated natural mineral waters and drinking waters by using a modified nested PCR-restriction fragment length polymorphism assay. *Appl Environ Microbiol*. 2003;69(7):4183–4189.
14. Santín M, Trout JM, Xiao L, Zhou L, Greiner E, Fayer R. Prevalence and age-related variation of *Cryptosporidium* species and genotypes in dairy calves. *Vet Parasitol*. 2004;122(2):103–117.
15. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: molecular evolutionary genetics analysis across computing platforms. *Mol Biol Evol*. 2018;35(6):1547–1549.
16. Holzhausen I, Tanner S, Heinz E, Troll H, Kaplan R, Gilman RH, *et al*. Effects of livestock management and

seasonality on *Cryptosporidium* infection. One Health. 2022;14:100368.

17. Shaw HJ, Innes EA, Morrison LJ, Katzer F, Wells B. Long-term production effects of clinical cryptosporidiosis in neonatal calves. Int J Parasitol. 2020;50(5):371–376.

How to Cite This Article

O'Brien C, Murphy S and Fitzgerald D. Occurrence and molecular typing of cryptosporidium species in neonatal calves with diarrhoea. International Journal of Veterinary Sciences and Animal Husbandry. 2024; 9(3): 155-160.

Creative Commons (CC) License

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International (CC BY-NC-SA 4.0) License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.