

Original Article

Molecular identification of *Balantioides coli* in asymptomatic pigs from Norwegian farmsLuz Aurora Martinez-Contreras^{a,b}, M. Rey Toleco^a, Yohannes Seyoum^a, Marit Gaastra Maaland^c, Marianne Oropeza-Moe^c, Mark van der Giezen^{a,d,e,*},¹^a University of Stavanger, Department of Chemistry, Bioscience, and Environmental Engineering, Norway^b Facultad de Bioanálisis, Region Veracruz, Universidad Veracruzana, Mexico^c Norwegian University of Life Sciences, Faculty of Veterinary Medicine, Department of Production Animal Clinical Sciences, Sandnes, Norway^d University of Exeter, Biosciences, Exeter, United Kingdom^e Research Department, Stavanger University Hospital, Stavanger, Norway

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ABSTRACT

Balantioides coli is a ciliated intestinal parasite of pigs with known zoonotic potential. Although Norway maintains high biosecurity standards and restricts live animal imports, the prevalence of *B. coli* in Norwegian pig herds has not been formally evaluated. We investigated the occurrence of *B. coli* in faecal samples from 125 pigs across eight commercial farms and one research facility. Microscopic examination revealed trophozoites and cysts in 48 % of wet-mount preparations and 28 % of McMaster flotation samples. PCR targeting the 18S rRNA gene identified *B. coli* in 70.4 % of animals, with prevalence ranging from 33 % to 100 % across farms. All four age groups tested positive, with the highest detection rate in finisher pigs (93.5 %) and the lowest in suckling piglets (16.7 %). Sequencing confirmed the identity of the PCR products, and phylogenetic analysis clustered all samples within the previously described Type II group associated with domestic pigs. No clinical signs of infection were observed, consistent with the generally asymptomatic nature of *B. coli* in pigs. However, its high prevalence, even under strict biosecurity measures, indicates that the parasite is endemic in Norwegian pigs. The potential impact on animal welfare and productivity remains uncertain. This initial survey provides a baseline for continued surveillance and the standardisation of detection methods to better understand the impact of *Balantioides coli* on animal welfare and productivity in intensive pig production systems.

1. Introduction

Enteric protozoan infections are a significant and persistent challenge to swine health and productivity worldwide (Symeonidou et al., 2020). Among these, *Balantioides coli* (formerly known as *Balantidium coli*) is a ubiquitous ciliate protozoan recognised for its presence in swine populations. The infection is typically asymptomatic but can occasionally contribute to enteric disturbances (Ponce-Gordo and García-Rodríguez, 2021). Although human infections are relatively rare, many cases have been reported worldwide, often associated with occupational exposure to pigs or contaminated environments (Ponce-Gordo and García-Rodríguez, 2021; Schuster and Ramirez-Avila, 2008; Solaymani-Mohammadi and Petri Jr, 2006).

Balantioides coli is thought to be present on all continents except Antarctica (Robertson et al., 2019). Recent studies, such as a report from neighbouring Denmark, have indicated a high prevalence of *B. coli* exceeding 50 % in domestic pig populations (Stensvold et al., 2021). Similar prevalences have been reported in intensive production systems, including Switzerland (Schubnell et al., 2016), Italy (Allievi et al., 2025; Giarratana et al., 2021), and Spain (Dashti et al., 2022), indicating that *B. coli* is widespread throughout Europe. Given the close geographical proximity between Denmark and Norway, similar epidemiological patterns might be anticipated. However, Norway maintains exceptionally strict biosecurity measures and import controls for live animals, reflecting its national commitment to safeguarding its favourable animal health status and preventing the introduction of infectious diseases into

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the country. This includes tightly controlled live animal imports, with a principal absence of live pig imports for production purposes, often favouring the import of genetic material to mitigate disease risks (Årsmeldinger, 2024; KOORIMP og KIF, 2025).

Although wild boar serves as hosts, domestic pigs are considered the principal reservoir of *B. coli* due to the high density and close contact of animals under intensive housing conditions (Santos-Silva et al., 2023; Solaymani-Mohammadi and Petri Jr, 2006). While wild boar populations are expanding in parts of Norway (Vitenskapskomiteen for mat og miljø, 2018), they are notably absent from the southwestern region where this study was conducted. Considering these unique epidemiological factors, data on *B. coli* prevalence in pigs in Norway are limited. *B. coli* has previously been mentioned in symptomatic pigs in Norway (Gjerde, 2011) based on unpublished observations. However, no methodological details were provided, and the detection method, whether microscopic or molecular, was not specified. In the absence of supporting data, the extent of *B. coli* infection in pigs in Norway remains unclear. Currently, there is no universally standardised diagnostic method for *B. coli*. Depending on the study, detection relies on sedimentation, flotation, or molecular assays, or a combination thereof, contributing to the wide variation in reported prevalences worldwide.

This study aimed to determine the occurrence and molecular confirmation of *Balantioides coli* in domestic pigs from Rogaland, the principal pig-rearing region of Norway, and to evaluate its prevalence and potential implications for swine and public health.

2. Materials and methods

2.1. Sample collection

This study included 125 faecal samples from apparently healthy pigs raised on farms in Rogaland, Norway. Samples were collected directly from the rectum of sows and grower/finisher pigs for further analysis. For piglets, faecal samples were collected immediately after defaecation without touching the ground. The pig samples were categorised as suckling piglets (1–5 weeks old), weaner piglets (5–10 weeks old), growers/finishers (10–21 weeks old), and sows (>52 weeks old). Faecal samples were obtained between June 2022 and September 2022 and between November 2023 and January 2024 from 125 pigs based at the Sandnes Education and Research Center (SEARCH) and eight farms in Rogaland, Norway. All participating farms were intensive, indoor pig production systems operating under standard Norwegian conditions. Norwegian pig production generally maintains a high health status, free of e.g. Porcine Reproductive and Respiratory Syndrome Virus (PRRSV), *Mycoplasma hyopneumoniae*, Porcine Epidemic Diarrhea Virus (PEDV), and Aujeszky's disease, underpinned by strict biosecurity measures, active surveillance and rapid response systems. Faecal samples were collected from animals raised at both SPF-status farms (free of *Actinobacillus pleuropneumoniae*, *Sarcoptes scabiei* var. *suis*, toxin-producing *Pasteurella multocida*, and *Brachyspira hyodysenteriae*) and conventional farms.

B. coli visualisation and morphological analysis were performed on 25 samples using wet-mount slide preparations with buffered saline and the McMaster technique employing a sugar-salt flotation solution with a specific gravity of 1.27 g/mL. For the McMaster procedure, 4 g of faecal sample was suspended in 56 mL of water and mixed thoroughly. After standing for 30 min, the suspension was remixed to ensure complete homogenisation, passed through a sieve, and 10 mL of the filtrate was centrifuged at 300 × g for 5 min. The supernatant was discarded, and the sediment was resuspended in the sugar-salt flotation solution. Samples were examined using a McMaster counting chamber. A sample was considered positive if at least one cyst or trophozoite of *B. coli* was observed in either preparation under the microscope.

2.2. DNA isolation and molecular biology

DNA was extracted from all 125 faecal samples for molecular analysis using the ZymoBIOMICS DNA Miniprep Kit (Zymo Research, USA) according to the manufacturer's recommendations. The quality of the extracted DNA in each sample was checked by electrophoresis, using 1 μL of DNA loaded into a 1 % agarose gel. The DNA concentration in each sample was measured using a NanoDrop One Microvolume UV-Vis Spectrophotometer (Thermo Fisher Scientific). All DNA samples were stored at −20 °C until further analysis. Forward primer SSUf (5'-CGCAAATCGGATTTTGTGCGC-3') and reverse primer SSUrBB (5'-AAATACATAGTCCCTCTAAGAAGTC-3') were used to amplify a 1047 bp fragment from *B. coli* 18S SSU rRNA gene. The method described by Pomajbíková et al. (2013) was followed: 10 min at 94 °C, 35 cycles of 1 min at 94 °C, 1 min at 59 °C, 1 min at 72 °C, and 5 min at 72 °C. The PCR products were checked by electrophoresis using 10 μL of the PCR reaction mixture, loaded into a 1.5 % agarose gel. PCR products were visualised by staining with GelRed and UV transillumination.

Amplicons of expected size (1047 bp) were excised from the gels and purified using the QIAEX II Gel Extraction Kit (Qiagen) or E.Z.N.A. Gel Extraction kit (Omega Bio-Tek) according to the manufacturer's recommendations. Purified DNA fragments of 19 samples were sequenced using the Sanger method at Microsynth AG (Germany). Nucleotide sequences were queried against the NCBI GenBank database using the Basic Local Alignment Search Tool (BLASTn).

2.3. Phylogenetic analysis

The obtained partial 18S rRNA sequences were aligned with available homologues from various ciliates from GenBank using MUSCLE (Edgar, 2004), and the alignment was curated using Gblocks (Castresana, 2000). Data were analysed using maximum likelihood and Bayesian inference. Maximum likelihood topologies were computed using a gamma-corrected GTR model with a discrete gamma distribution to account for rate variation across sites, as implemented in PhyML (Dereeper et al., 2008; Guindon and Gascuel, 2003). For the Bayesian phylogenetic analysis, MrBayes was used with a GTR model with six substitution types and four rate categories (Dereeper et al., 2008; Huelsenbeck and Ronquist, 2001).

3. Results

As part of routine screening, 25 pig faecal samples were microscopically investigated using wet mount slide preparations and the McMaster technique. Large ciliated trophozoites and cysts resembling *B. coli* (Supplementary Fig. 1) were observed in 48 % (12 out of 25) of the wet-mount slide preparations and 28 % (7 out of 25) of the McMaster flotation samples (Table 1).

To confirm whether these were *B. coli*, we used an 18S-based PCR

Table 1
Detection rate (%) of *B. coli* in Norwegian domestic pigs by microscopy and PCR techniques, and by age-groups category (PCR results).

Detection Method	Detection rate (%)	Positive/Total samples
Direct microscopy	48	12/25
McMaster Technique	28	7/25
18S rRNA PCR	70.4	88/125
Detection rate by age group (18S rRNA PCR)		
Age-Groups	Detection rate (%)	Positive/Total samples
Suckling piglets	16.7	2/12
Weaner piglets	71.9	23/32
Sows	68	34/50
Grower/Finisher	93.5	29/31

approach to identify the cells (Pomajbíková et al., 2013). PCR amplification of the *B. coli* 18S rRNA gene using the primer set described by Pomajbíková et al. (2013) resulted in 88 animals testing positive out of the 125 animals tested, a prevalence of 70.4 % (Supplementary Fig. 2). When considering age categories, the prevalence was 16.7 % for suckling piglets, 71.9 % for weaner piglets, 68.0 % for sows, and 93.5 % for finisher pigs (Table 1). All tested farms were positive for *B. coli*, with detection rates ranging from 33 to 100 %.

To confirm that these PCR amplicons were indeed *B. coli*, a subset (21 %, $N = 19$) of the samples were subjected to Sanger sequencing, representing all farms and production categories and reduce sequencing costs. After checking the sequences using BLAST (Altschul et al., 1990), all sequences were confirmed to be *B. coli* (GenBank accession numbers PV910461–PV910479). Both maximum likelihood and Bayesian phylogenetic analyses resulted in an identical tree topology (not shown) with all Norwegian samples in a clade with other *B. coli* samples from various locations (Fig. 1). All Norwegian samples clustered in the previously designated Type II *B. coli* cluster (Stensvold et al., 2021).

4. Discussion

Intestinal parasites are found in pigs worldwide; however, improved on-farm hygiene and biosecurity have reduced their prevalence (Pinto Jimenez et al., 2023; Roepstorff et al., 1998). Mitigating the parasite load not only improves animal welfare but also enhances sustainability and productivity, leading most countries to prioritise the management of the disease burden in production animals. Implementing hazard-control measures in food systems that recognise the interdependence of all components involved in producing safe food will facilitate the development of more comprehensive and sustainable practices. This approach, referred to as ‘One Food’ management, operationalises the One Health concept for integrated, multi-hazard food-system design (Bremner et al., 2023). Norway has a high animal health status and has introduced strict biosecurity measures and import controls for live animals to maintain this status. However, only a few studies have assessed the prevalence of intestinal parasites in pig herds in Norway (Hannes et al., 2007; Roepstorff et al., 1998), and of these, only *Cryptosporidium* spp., *Giardia intestinalis*, and *Eimeria* spp. have been tested as parasitic protozoa. To the best of our knowledge, apart from an unpublished

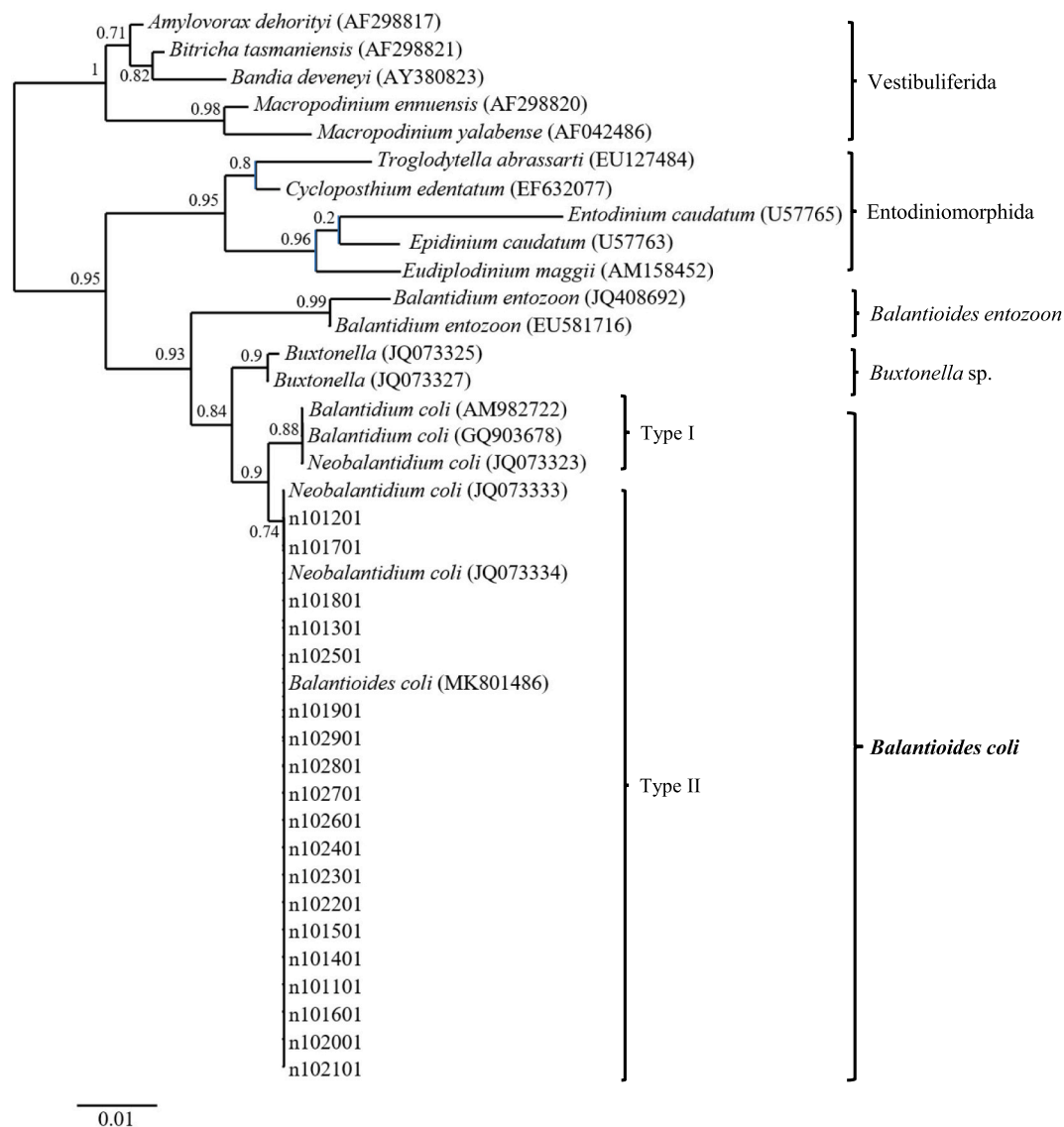


Fig. 1. Phylogenetic analysis of 19 Norwegian *Balantioides coli* 18S rRNA sequences. An unrooted maximum likelihood phylogenetic tree is presented, showing bootstrap values as determined using PhyML (Guindon and Gascuel, 2003). All Norwegian samples were recovered as part of a well-supported monophyletic clade of *B. coli* sequences and were found in the recently designated Type II group (Stensvold et al., 2021). The scale bar represents 10 changes per 100 positions.

observation from 2000 (Gjerde, 2011), no prevalence information is available for *Balantioides coli* in Norwegian pigs (nor from pigs in neighbouring Sweden (Pettersson et al., 2021)). In contrast, research conducted in Denmark, another Scandinavian nation, has documented a widespread and high prevalence, with rates ranging from 57 to 100 % (Hindsbo et al., 2000; Stensvold et al., 2021).

Recent studies from other European countries confirm the widespread occurrence of *B. coli* in domestic pigs and highlight how methodological and management factors influence the reported prevalence. In Switzerland, Schubnell et al. (2016) reported *B. coli* as the most frequently detected intestinal protozoan in pigs, with prevalence increasing from 5.1 % in suckling piglets to 50 % in fatteners, and approximately 43 % of herds testing positive by copromicroscopy. In southern Italy, Giarratana et al. (2021), using copromicroscopy, documented an overall prevalence of 46.9 %, with significantly higher infection rates in commercial hybrid pigs (up to 64.8 %) compared to local breeds (up to 27.9 %), and in intensive systems compared to extensive ones, emphasizing the role of husbandry practices in parasite transmission. More recently, Allievi et al. (2025) conducted a large-scale survey in northern Italy, detecting *B. coli* in 92.4 % of pigs when using sedimentation techniques, while flotation methods showed much lower sensitivity, underlining the strong effect of diagnostic approach on prevalence estimates. Similarly, a study in Spain by Dashti et al. (2022) identified *B. coli* using PCR in approximately 45–50 % of pigs across different breeds and production systems, again confirming the high endemicity of this protozoan in European herds. Collectively, these findings indicate that *B. coli* infection rates in European pig populations typically range between 40 and 90 %, depending on production intensity, diagnostic sensitivity, and age group sampled. Despite such high prevalences, overt clinical disease appears uncommon, supporting the view that *B. coli* behaves largely as a commensal organism with opportunistic pathogenic potential under suboptimal conditions. Here, we report similar *B. coli* prevalence across eight commercial farms and one research farm (33–100 %), in line with findings worldwide (Ahmed et al., 2020).

The large ciliated trophozoites and size of *B. coli* cysts make microscopic detection generally straightforward (Ponce-Gordo and García-Rodríguez, 2021). Microscopy has long been the standard diagnostic approach and remains widely used, especially in resource-limited settings. However, reported detection rates vary greatly depending on the specific technique and flotation solution employed, since cysts are dense and may not float efficiently in media with lower specific gravity (da Silva Barbosa et al., 2017; Dryden et al., 2005; Vadlejch et al., 2011). Consequently, inter-laboratory differences in technique and reagent composition likely contribute to the wide range of prevalences reported worldwide, from below 1 to over 90 % (Ahmed et al., 2020). In our study, direct wet-mount examination detected *B. coli* in 48 % of samples, whereas the McMaster flotation method yielded 28 %, probably reflecting both methodological and preservation effects related to the flotation medium. In comparison, PCR-based screening detected *B. coli* in 70.4 % of animals, confirming that molecular assays provide greater analytical sensitivity and species-level specificity than microscopic observation (Nilles-Bije and Rivera, 2010; Robertson et al., 2019).

Previous studies have suggested the presence of genetic variation within *B. coli* based on ITS and rRNA sequence data (Pomajbíková et al., 2013; Ponce-Gordo et al., 2011). More recently, Stensvold et al. (2021) suggested that 18S rRNA data might be able to distinguish two subtypes which they termed Type I and Type II. Our phylogenetic analyses, both maximum likelihood analyses using PhyML (Guindon and Gascuel, 2003) and Bayesian inference using MrBayes (Huelsenbeck and Ronquist, 2001), clearly identified two *B. coli* clades. All samples from Norwegian domestic pigs clustered in Type II, in agreement with a previous observation that this clade was dominated by *B. coli* sequences from domestic pigs (Stensvold et al., 2021). As it is now becoming clear that there are different sequence types of *B. coli*, it may be important to consider this from a public health perspective, considering the zoonotic

potential of this intestinal parasite.

B. coli DNA was detected in samples from all four age categories included in our study. The prevalence of *B. coli* was 16.7 % in sucking piglets, 71.2 % in weaner piglets, 68.0 % in sows, and 93.5 % in finisher pigs. The correlation between *B. coli* infection and different age groups of pigs has been evaluated previously (Damriyasa and Bauer, 2006; Nakauchi, 1991; Pakandl, 1994). In agreement with these studies, we detected higher *B. coli* infection rates in older pigs and lower infection rates in suckling piglets, with increased detection in weaners. None of the pigs in the present study exhibited any clinical signs of infection. Clinical manifestations of *B. coli* in pigs occur more frequently in overcrowded farms and among pregnant or lactating animals, or those with nutritional disorders or concurrent diseases (Nishi et al., 2000). The pig industry in Rogaland and Norway relies on small-scale family-owned farms, where the agricultural concession framework imposes restrictions on herd size.

All farms studied had *B. coli* infections in pigs. The highest occurrence rates of *B. coli* infection are mainly related to the sanitary conditions of the host habitat in tropical and subtropical areas (Zaman and Cox, 2010). However, intestinal protozoa may occur even in properties with good management practices (Sangioni et al., 2017). As *B. coli* has a cosmopolitan distribution and swine are its natural reservoir, even in countries with high biosecurity standards, such as Norway, a vigilant monitoring programme might be required for this zoonotic parasite.

Although *B. coli* is generally asymptomatic in pigs, it may still affect animal welfare and productivity. Our study, along with previous research, suggests that pigs are rarely free of infection. This raises the question of whether all animals might actually be less productive, as most would be carrying *B. coli*, an organism that has been linked to opportunistic infections in pigs (Lai et al., 2011; Schuster and Ramirez-Avila, 2008). A robust follow-up study comparing the growth and productivity parameters of infected and non-infected animals may allow for a proper assessment of the health and sustainability aspects of high levels of *B. coli* in commercial pig herds worldwide.

The results obtained in this study identified and detected *B. coli* in Norwegian pigs with a high prevalence, despite high biosecurity measures and high farm hygiene standards. The presence of *B. coli* in Norwegian pigs likely predates current animal welfare measures, and *B. coli* must be assumed to be endemic in Norwegian pigs. Once the true effect of *B. coli* on animal productivity has been assessed, it will be possible to evaluate its broader implications for animal welfare, herd management, and sustainable production systems.

This exploratory baseline study was initiated following incidental detection of *B. coli* during routine faecal screening, as previous reports lacked methodological detail. The work was therefore designed to confirm its presence in Norwegian herds rather than to test specific hypotheses. The study was limited by the number of farms included, the lack of detailed metadata, and the absence of quantitative comparison of diagnostic sensitivities. A formal risk-factor analysis (for example, by age category) was not feasible because sampling was unbalanced across age groups and clustered within nine farms, providing insufficient power for robust statistical modelling. While these constraints do not affect the validity of the findings, they underline the need for larger, systematically designed studies in the future.

5. Conclusion

This study provides the first molecular confirmation of *Balantioides coli* in Norwegian pigs and aligns with findings from other European countries, showing that the organism is endemic despite stringent biosecurity measures. Although infections are usually asymptomatic, the parasite's persistence warrants continued surveillance and standardisation of detection methods to assess possible effects on welfare and productivity. This initial survey therefore establishes an essential baseline for future studies aimed at evaluating the impact of *B. coli* within intensive, indoor pig production systems.

CRedit authorship contribution statement

Luz Aurora Martínez-Contreras: Writing – review & editing, Writing – original draft, Methodology, Investigation. **M. Rey Toleco:** Writing – review & editing, Methodology, Investigation. **Yohannes Seyoum:** Writing – review & editing, Methodology, Investigation. **Marit Gaastra Maaland:** Writing – review & editing, Methodology, Investigation. **Marianne Oropeza-Moe:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Mark van der Geizen:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Conceptualization.

Ethical statement

All work conducted has been done according to all relevant local legislation and guidance and the manuscript has been written according to academic standards and procedures.

Declaration of generative AI and AI-assisted technologies in the writing process

Statement: During the preparation of this work, the author(s) used ChatGPT to improve the readability and language of the manuscript. After using this tool/service, the author(s) reviewed and edited the content as needed and took full responsibility for the content of the published article.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.vprsr.2025.101401>.

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