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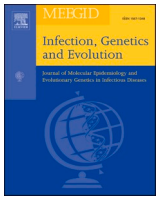

Complete sequencing of the *Cryptosporidium suis gp60* gene reveals a novel type of tandem repeats—Implications for surveillance

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ABSTRACT

Cryptosporidiosis is an infectious enteric disease caused by species (some of them zoonotic) of the genus *Cryptosporidium* that in many countries are under surveillance. Typing assays critical to the surveillance of cryptosporidiosis typically involve characterization of *Cryptosporidium* glycoprotein 60 genes (*gp60*). Here, we characterized the *gp60* of *Cryptosporidium suis* from two samples—a human and a porcine faecal sample—based on which a preliminary typing scheme was developed. A conspicuous feature of the *C. suis gp60* was a novel type of tandem repeats located in the 5′ end of the gene and that took up 777/1635 bp (48%) of the gene. The *C. suis gp60* lacked the classical poly-serine repeats (TCA/TCG/TCT), which is usually subject to major genetic variation, and the length of the tandem repeat made a typing assay incorporating this region based on Sanger sequencing practically unfeasible. We therefore designed a typing assay based on the post-repeat region only and applied it to *C. suis*-positive samples from suid hosts from Norway, Denmark, and Spain. We were able to distinguish three different subtypes; XXVa-1, XXVa-2, and XXVa-3. Subtype XXVa-1 had a wider geographic distribution than the other subtypes and was also observed in the human sample. We think that the present data will inform future strategies to develop a *C. suis* typing assay that could be even more informative by including a greater part of the gene, including the tandem repeat region, e.g., by the use of long-read next-generation sequencing.

1. Introduction

Cryptosporidium oocysts detected in domestic pigs were first reported as *Cryptosporidium parvum* pig variant (Morgan et al., 1999; Xiao et al., 1999); later, when a second genotype (*Cryptosporidium* pig genotype II [*Cryptosporidium scrofarum*]) was discovered in pigs, it was renamed *Cryptosporidium* pig genotype I (Ryan et al., 2003), and in 2004, it gained species status and was redescribed as *Cryptosporidium suis* (Ryan et al., 2004). Although *C. suis* naturally infects suid host species, including domestic pigs and wild boars (Dashti et al., 2022; De Felice et al., 2020; Němejc et al., 2012; Němejc et al., 2013; Pettersson et al., 2020), it has also been found sporadically in other animals such as cattle, chimpanzees, deer, raccoon dogs, and rodents (Bodager et al., 2015; Danisova et al., 2017; Dashti et al., 2023; Parsons et al., 2015; Perec-Matysiak et al., 2023). These occasional reports were most likely the product of spurious (i.e., mechanical carriage) rather than true infections. It should be noted that some DNA sequences in the NCBI database have been annotated as *C. suis*, although these are in fact *Cryptosporidium occultus*, a species that was described only in 2018 (Kvac et al., 2018). It is thus likely that some of the *C. suis* sequences identified in non-suid hosts

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correspond to *C. occultus*.

Little is known about the pathogenic potential of *C. suis* in pigs. Whereas clinical anorexia and watery diarrhoea have been described in pigs experimentally infected with *C. suis* at 4–6 days post-infection (Enemark et al., 2003) and in nursing piglets (Mišić et al., 2003), most epidemiological studies proposing a link between *Cryptosporidium* infection and diarrhoea in pigs lack molecular data at the species/genotype level (Quilez et al., 1996) and/or did not consider the potential influence of concomitant infections with other primary diarrhoeagenic agents, such as rotavirus (Sanford, 1987). Indeed, most previously published literature concurs that natural infection with *C. suis* in pigs generally does not result in the development of diarrhoea (Lindsay et al., 2019).

The first human case of *C. suis* infection was detected in 2001 (Xiao et al., 2002), and since then, at least 16 more cases of human infection with *C. suis* have been reported from different countries in Europe, South America, Asia, and Africa (Supplementary Table 1).

Cryptosporidium subtyping is important for surveillance and control of cryptosporidiosis, including the understanding of transmission patterns and potential zoonotic sources. Subtyping tools targeting the 60-kilodalton (kDa) glycoprotein (*gp60*) gene have substantially enhanced our understanding of the transmission of *C. hominis* and *C. parvum* (Feng et al., 2018; Lebbad et al., 2021; Yang et al., 2021). Methods based on the *gp60* gene were originally mainly used for the *C. hominis*/*C. parvum* complex (Strong et al., 2000). Recently, however, specific primers were developed for other species including *Cryptosporidium bovis* (Wang et al., 2021), *Cryptosporidium canis* (Jiang et al., 2021), *Cryptosporidium fayeri* (Power et al., 2009), *Cryptosporidium felis* (Rojas-Lopez et al., 2020), *Cryptosporidium meleagridis* (Stensvold et al., 2014), *Cryptosporidium mortiferum* (previously *Cryptosporidium* chipmunk sp. genotype I) (Guo et al., 2015), *Cryptosporidium ryanae* (Yang et al., 2020), *Cryptosporidium ubiquitum* (Li et al., 2014), *Cryptosporidium viatorum* (Stensvold et al., 2015), and *Cryptosporidium xiaoi* (Fan et al., 2021). However, a subtyping tool for *C. suis* is still missing. In this article we describe the *gp60* gene of *C. suis* and attempts to design a subtyping tool.

2. Materials and methods

2.1. Samples used in the primary investigation of the C. suis gp60 gene

In a previous *Cryptosporidium* study (Lebbad et al., 2021), a two-year-old boy adopted from Lithuania in 2014 was diagnosed with a *C. suis* infection upon arrival to Sweden. He had loose stools, but no other symptoms were reported. Approval (registration number 2013/201–31/4) was obtained from the ethics committee of Karolinska Institutet, Stockholm, Sweden.

DNA from the boy's stool sample (Swec705) was used for the initial amplification of the full *C. suis gp60* gene. This sample was also subject to small subunit rRNA (*ssu* rRNA), actin, and 70-kDa heat shock protein (*hsp70*) gene sequencing (Lebbad et al., 2021) (Supplementary Table 1). In addition, a Swedish pig sample (SwePigD_211) positive for *C. suis* was included in the primary investigations.

2.2. Samples used in the subtyping study

To evaluate primers developed for typing of *C. suis*-specific *gp60* genes (see below), DNA preparations from 41 animal faecal samples initially considered positive for *C. suis* at the *ssu* rRNA locus were included; 29 from domestic pigs, 11 from wild boars, and one from a deer (Table 1, Supplementary Table 2). The samples originated from previous/ongoing studies, and DNA extraction and species detection by PCR/sequencing of *ssu* rRNA genes were performed as previously described (Dashti et al., 2022; Marti-Marco et al., 2023; Rivero-Juarez et al., 2020; Stensvold et al., 2021).

Table 1
Origin and *gp60* subtype data of *Cryptosporidium suis* specimens* used in this study.

Host species (no. of animals)	Country	No. of *C. suis*-positive samples	No. of samples positive at the *gp60* locus (%)	*gp60* subtype (no. of samples)	References
Red deer (1)	Spain	1	0	NA	(Dashti et al., 2023)
Domestic pig (17)	Denmark	17	14 (82)	XXVa-2 (12) XXVa-1 (2)	(Stensvold et al., 2021)
Domestic pig (2)	Norway	2	2 (100)	XXVa-1 (2)	Ongoing study
Large white pig (9) Iberian pig (1)	Spain	10	4 (40)	XXVa-1 (4)	(Dashti et al., 2022)
Wild boar (5)	Spain	5	5 (100)	XXVa-1 (4) XXVa-3 (1)	(Marti-Marco et al., 2023)
Total		35	25 (71)	XXVa-1 (12) XXVa-2 (12) XXVa-3 (1)	

* Six *C. occultus* samples initially considered positive for *C. suis* were excluded from the table. NA = not applicable.

2.3. Molecular characterization of the gp60 gene and subtyping of samples

Initially, a ~ 2000-bp DNA sequence encompassing the *gp60* locus of *C. suis* was generated from the DNA sample 'Swec705' using the primers described for subtyping of the *C. mortiferum gp60* gene (Guo et al., 2015). For sequencing the product, the reverse primer R2 from the original PCR and several additional primers (data not shown) were used. The original F2 primer could not be used for sequencing as it produced low-quality sequences due to the presence of a poly (A) track shortly after the primer sequence. The sequence obtained from sample Swec705 (1682 bp), covered the entire open reading frame (ORF) plus 47 additional bases.

The sequence from Swec705 (GenBank accession number MH187875) was used to design primers for a nested PCRs targeting the post-repetitive region, for which amplification was performed using the primers CsuisN1F (5'–TGC TGT TGC TAC TGA AGC TAG TGG–3′) and CsuisN1R (5′–GAA GAA CGC GGC GAA AAT TG–3′) for primary reactions and CsuisN2F (5'–CTA CTG ATG ATA CAA AGA GTG C–3′) and CsuisN2R (5′–GGA TGG AAT GAC ATA TCT AAG–3′) for secondary reactions, with the PCR products expected to comprise 685 and 632 bp, respectively.

Nested PCR was carried out in 25-μL reactions, using 12.5 μL of Extract-N-Amp PCR ReadyMix (Sigma-Aldrich, Søborg, Denmark), 1 μL of each primer (10 μM), 2.0 μL template and 8.5 μL water. Cycling conditions for PCR I consisted of initial denaturation (3 min at 94 °C) followed by 35 amplification cycles (1 min at 94 °C, 1 min at 58 °C, and 1 min at 72 °C) followed by a final extension (5 min at 72 °C). Cycling conditions for PCR II consisted of initial denaturation (3 min at 94 °C) followed by 35 amplification cycles (1 min at 94 °C, 1 min at 50 °C, and 1 min at 72 °C) followed by a final extension (5 min at 72 °C).

PCR products were subjected to bidirectional sequencing (Eurofins, Germany) using the primers employed in the secondary PCR. DNA sequences were aligned and edited using BioEdit version 7.2.5 (http://www. mbio.ncsu.edu/bioedit) and Staden Package (Staden

et al., 2000), and phylogenetic analysis was performed using MEGA 11.0.13 software (www.megasoftware.net/). Tandem repeats in the gene were identified using the Tandem Repeat Finder (http://www.tandem.bu.edu/ trf/trf).

2.4. Protein sequence analysis

The ORF of the sequenced *gp60* gene was predicted using the NCBI database (https://www.ncbi.nlm.nih.gov/orffinder/). *gp60* amino acid (aa) sequences were aligned with MUSCLE using the MEGA 11.0.13 software or sequence analysis tools services from EMBL-EBI (https://www.ebi.ac.uk/jdispatcher/msa/muscle) (Madeira et al., 2022). Signal peptide predictions were performed using SignalP 6.0 (https://services.healthtech.dtu.dk/services/SignalP-6.0/) (Teufel et al., 2022). Transmembrane domain regions in the sequences were predicted using Phobius available at https://phobius.sbc.su.se/instructions.html. Furin proteolytic cleavage sites were predicted using the ProP v.1.0b server available at https://services.healthtech.dtu.dk/services/ProP-1.0/ with a score cut-off ≥0.425 (Duckert et al., 2004). N-glycosylated sites and O-glycosylated sites were predicted using PROSITE available at https://prosite.expasy.org/ (Sigrist et al., 2013) and NetOGlyc 4.0 (https://services.healthtech.dtu.dk/services/NetOGlyc-4.0/) (Steentoft et al., 2013), respectively. For aa sequence alignment, sequences from the following species were included (with GenBank accession numbers in parentheses): *C. parvum* (AAF82349), *C. hominis* (ACQ82740), *C. ubiquitum* (XP_028874367), *Cryptosporidium* sp. skunk genotype (ASU09672), *C. suis* Swec705 (AYA71611), and *C. suis* SwePigD211 (AYA71610).

2.5. Nucleotide sequence accession numbers

Representative nucleotide sequences were deposited in GenBank under the accession numbers MH187872 (actin), MH187873 (*hsp70*) MH187874, MH187875, PP467566–PP467568 (*gp60*) and MH187876, MH187877 (*ssu* RNA).

3. Results

3.1. gp60 gene analysis

The complete *gp60* ORF was available for Swec705 (AYA71611) and comprised 1635 bp, encoding 544 aa, which makes it one of the longest *gp60* genes identified to date. At the aa sequence level, the *gp60* gene of *C. suis* has greatest overall similarity to *C. ubiquitum* (NCBI BLASTP, 64% identity to the sequence with accession number AJW72318). Two *C. suis* aa sequences were included for analysis (Fig. 1).

The extreme regions characteristic of the *Cryptosporidium gp60* gene were observed, including a 21-aa sequence coding for the signal peptide and a 19-aa sequence coding for a transmembrane domain. The polyserine domain often seen in *Cryptosporidium gp60* sequences was also present, although relatively short, and of particular note was the fact that the short serine repeat in *C. suis* was not made up by the triplets ('TCA','TCG' or 'TCT') usually seen in the polyserine tract, but of 'AGT' and 'AGC' triplets. Multiple O-glycosylation sites and one potential N-glycosylation site could be predicted. Of note, no furin cleavage site could be predicted.

What makes *C. suis* rather unique is the insertion of the many 21-bp repeats (aa, "G(G/D/V)Q(E/G)NAQ") almost immediately after the signal peptide cleavage site. Hence, in the 5′ region of the *gp60* gene of Swec705, no less than 37 repeats with a length of 21 bp, with polymorphisms observed at the 5th, 11th and 18th bases (GGT G[A/G/T]T CAA G[A/G]G AAT GC[C/T] CAG) were observed (MH187875) (Fig. 2). Isolate SwePigD_211 had 39 similar repeats, with double peaks appearing at seven places either in the 5th or the 11th position (MH187874). Both isolates were identical in the post-repetitive region. The subtype family was designated XXVa in concordance with the established nomenclature of *gp60* subtype families (Xiao and Feng, 2017), and consequently, the subtype for Swec705 was XXVaR37 and for SwePigD_211 XXVaR39.

Supporting the observations from aa sequence analysis, the phylogenetic analysis showed maximum bootstrap support for *C. suis* clustering together with *C. ubiquitum* in a major clade that included sequences from *C. viatorum*, *C. mortiferum*, and *Cryptosporidium* sp. skunk genotype (Fig. 3) and that was separated by maximum bootstrap support from the other major clade, which holds *C. meleagridis*, *C. parvum*, and *C. hominis* among others.

3.2. gp60 gene-based typing assay: PCR targeting the post-repetitive region

Six of the samples from wild boar originally included in the study were excluded as re-evaluation of the *ssu* RNA sequences and additional analyses of the actin gene showed that they were *C. occultus* (Supplementary Table 2), and none of them produced bands with the *gp60* PCR.

The expected PCR product of ~630 bp was obtained from 25 out of 35 remaining DNA samples with nested PCR (Table 1), resulting in an overall amplification rate of 71%. Two of these 25 samples were first unsuccessful (Table 1, Supplementary Table 2), but repeated PCR gave positive results, and so these two were included in the data set. DNA from the only non-porcine sample (from a Spanish red deer) failed to produce a PCR product.

Subsequent sequence analysis was successful for all 25 *C. suis gp60* PCR products, and the sequences were available for intra-species comparative analysis. The sequences were almost identical, with only three single nucleotide polymorphisms (SNP) in the post-repetitive region. As the subtypes could not be named using the subtype nomenclature based on the number of other repeats (R) as mentioned earlier, we propose that they could be called XXVa-1 (PP467566), XXVa-2 (PP467567), and XXVa-3 (PP467568), a nomenclature similar to what is used for e.g. *C. canis* (Jiang et al., 2021). Subtype XXVa-1 was identical in the post-repetitive area to the reference sequence Swec705 while XXVa-2 had a nonsynonymous mutation causing an amino acid shift from alanine to proline. The SNP in subtype XXVa-3 did not cause any amino acid shift.

Subtype XXVa-1 (n = 12) was identified in domestic pigs from Denmark, Norway, and Spain as well as in wild boars from Spain, and were also observed in the human sample from Lithuania and the Swedish pig sample analysed initially. Subtype XXVa-2 (n = 12) was only found at one sampling location in Denmark, and the only sample producing the sequence XXVa-3 originated from a wild boar from Spain.

4. Discussion

4.1. Study incentive and observations on the gp60 gene of Cryptosporidium suis

Multiple species of *Cryptosporidium* are known to infect pigs, but their relative contribution to porcine health and disease remains unclear. Although the role of pigs as source of human disease is considered limited (Lindsay et al., 2019), the risk of occupational infection for people working with these animals (e.g., farmers, veterinarians) should not be overlooked. Immunosuppressed individuals appear particularly vulnerable, as at least 11 out of 17 globally reported *C. suis* cases were observed in HIV-positive individuals (Supplementary Table 1).

Typing assays are key elements in the surveillance of cryptosporidiosis, and the *gp60* gene currently is the most used genetic marker for subtyping *Cryptosporidium* spp. It is well known that the extensive sequence variation in the *gp60* gene among various *Cryptosporidium* species may reduce the ability of PCR primers that were designed based on *C. parvum* and *C. hominis* sequences to amplify species genetically different to those species (Ryan et al., 2021).

In this study, we used the *gp60* PCR primers originally designed for

Fig. 1. Multiple alignment of amino acid (aa) sequences of *gp60* ORFs of different *Cryptosporidium* species including *C. suis.* Predicted signal peptides are indicated in yellow, and predicted transmembrane domain regions of complete *gp60* ORFs are indicated in green. Predicted furin cleavage sites are highlighted in blue; note that no furin cleavage sites could be predicted in *C. suis, Cryptosporidium* sp. skunk genotype (subtype family XVIa), or *C. ubiquitum gp60* sequences. Predicted O-glycosylation sites are underlined, and N-glycosylation sites are shaded in black. Dashes (––) represent gaps, and asterisks (*) indicate residues identical in all sequences in the alignment. A colon (:) indicates that conserved (i.e., having similar characteristics) substitutions have been observed, and a period (.) indicates that semiconserved (i.e., having similar shape) substitutions have been observed. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

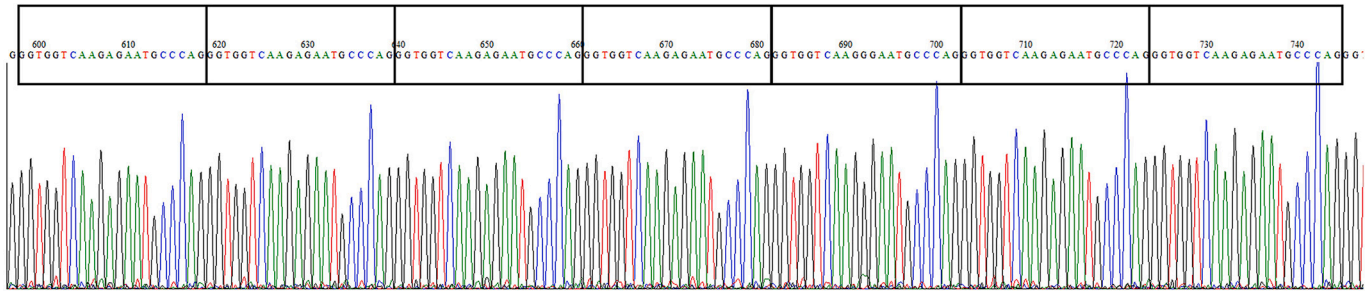

Fig. 2. A section of a *gp60 Cryptosporidium suis* chromatogram from sample 'Swec705' including 7 repeats of 21 bp. Each square represents one repeat of 21 bp.

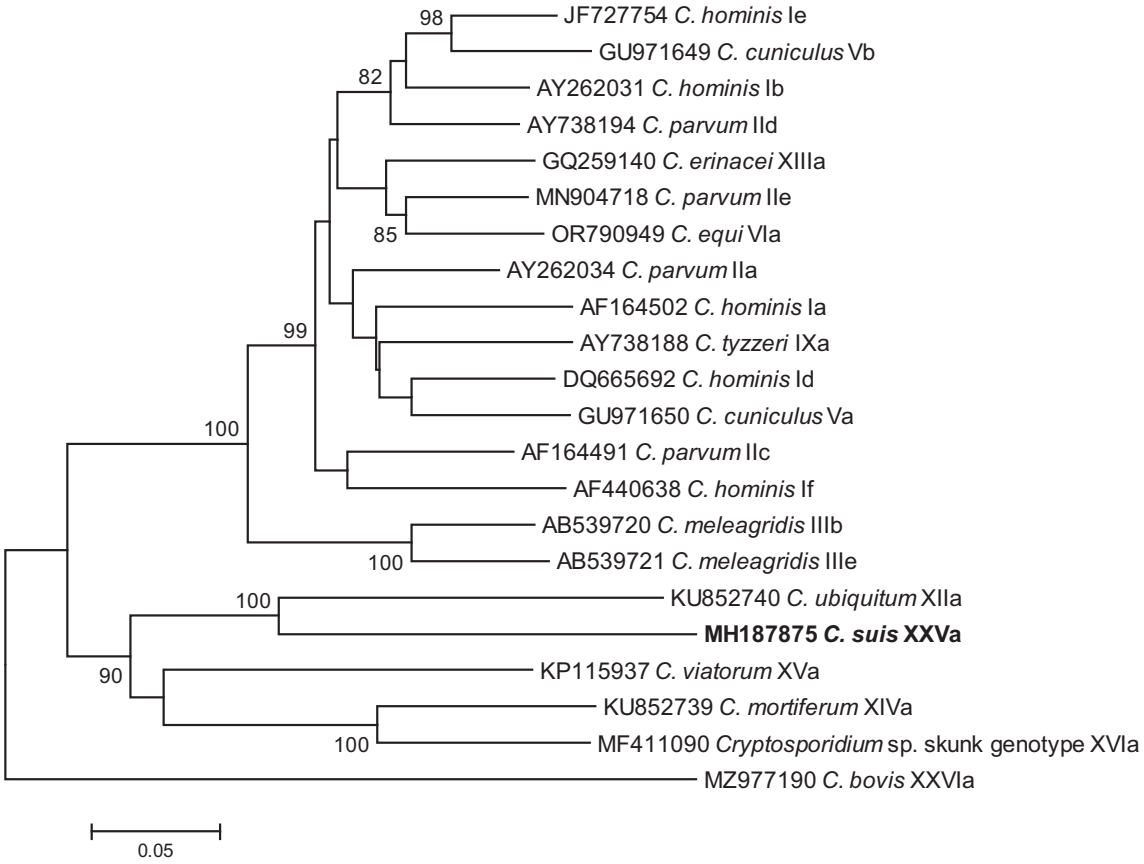

Fig. 3. Phylogenetic analysis of the *Cryptosporidium suis* glycoprotein 60 kDa DNA sequence MH187875 (highlighted in bold) with relevant reference sequences from GenBank. The analysis was based only on the post-repeat region and included 480 positions. The evolutionary history was inferred using the Neighbor-Joining method using MEGA11. The evolutionary distances were computed using the Kimura 2-parameter method (1000 bootstraps) and are in the units of the number of base substitutions per site. Only bootstrap values of 70 or higher were included. The analysis involved 22 nucleotide sequences. Scale bar indicates nucleotide substitutions per site.

C. mortiferum (Guo et al., 2015) to amplify the full sequence of the *C. suis gp60* gene. Those primers were designed based on conserved nucleotide sequences flanking several *gp60* genes including *C. ubiquitum* and *C. mortiferum*, and they are currently also used to subtype the *gp60* gene of *Cryptosporidium* sp. skunk genotype (Yan et al., 2017). Analysis of the *C. suis gp60* sequence obtained in this study revealed genetic relatedness to exactly these three species (Fig. 3). However, the length of the *gp60* gene of *C. suis* was 1635 bp, which is much longer than the *gp60* genes of *C. mortiferum* (963 bp), *C. ubiquitum* (945 bp), and *Cryptosporidium* sp. skunk genotype (1089 bp). The occurrence of a high number of long repeats in the 5′ region (37 repeats with a length of 21 bp each, equal to 777 bp) explained the unusual length of the gene. The SwePigD_211 isolate had an even longer repeat stretch (39 × 21 bp, equal to 819 bp). The double peaks observed in the repeat stretch of the pig isolate might indicate mixed subtypes with different number of repeats.

The trinucleotide repeats of TCA/TCG/TCT encoding a polyserine tract at the 5′ end of the *gp60* gene and widely used to differentiate subtypes, were absent in the *gp60* sequence of *C. suis*; a circumstance that *C. suis* has in common with other *Cryptosporidium* species such as *C. bovis* (Wang et al., 2021), *C. felis* (Rojas-Lopez et al., 2020), *C. ryanae* (Yang et al., 2020), *C. ubiquitum* (Li et al., 2014), and *C. xiaoi* (Fan et al., 2021). On the other hand, the occurrence of 21-bp repeats in the 5′ region of the gene appears to be a feature specific to *C. suis*, since it has not been observed in any of the complete or near-complete *gp60* genes sequenced to date, to the knowledge of the authors.

4.2. Amino acid features of the gp60 gene of Cryptosporidium suis

The most remarkable feature of the *C. suis gp60* gene was the long unique 21-bp repeat. A reminiscent feature of a unique insert was observed recently in *C. canis gp60* genes, and the authors speculated that the long insertions and major sequences differences could contribute to the canine-adapted nature of *C. canis* (Jiang et al., 2021). Nevertheless, both *C. suis* and *C. canis* with their unique *gp60* genes, which are involved in host cell attachment and invasion, are able to infect other hosts, including humans.

The absence of a furin cleavage site has not only been observed in *C. ubiquitum* (Li et al., 2014), its closest relative identified to date, but also within most subtype families of *Cryptosporidium* sp. skunk genotype (XVIa, XVIc and XVId); only the XVIb subtype family codes for the furin cleavage site (Yan et al., 2017). The furin cleavage site has been suggested to be necessary for the cleavage of the *gp60* precursor protein into gp15 and gp40 during the invasion process (Wanyiri et al., 2009; Yan et al., 2017), and it remains unclear how this process happens in those species or specific subtype families where furin cleavage sites cannot be predicted.

4.3. Subtyping method and geographical distribution of subtypes of Cryptosporidium suis

The finding of different number of repeats in the 5′ region in two isolates initially appeared to be a promising discriminatory marker for *C. suis gp60*. However, efforts to develop a PCR-based typing assay covering the whole repeat region plus a considerable post-repetitive fragment were unsuccessful due to several circumstances. First, most efforts produced no PCR bands or blurry bands/smears, and secondly, if a band was produced, the length of the repeat stretch (around 800 bases) was difficult to cover in one single sequence using Sanger sequencing. Due to the high number of almost identical 21-bp repeats, it was not possible to let shorter sequences overlap; the whole repeat area would have to be covered in one read.

The next step was to then try and develop a PCR assay covering only the post-repetitive area. This worked well, but the discriminatory power was limited, as this region was quite similar in all isolates tested so far and resulted in only three different subtypes. One subtype, XXVa-1 was seen in domestic pigs from several European countries; Denmark, Norway, Sweden, and Spain, and in the patient who became infected in Lithuania. Subtype XXVa-2 was only seen in domestic pigs from a single sampling site in Denmark, and subtype XXVa-3 in a wild boar from Spain, which could indicate more restricted geographical distribution of subtypes XXVa-2 and XXVa-3. The remaining Spanish wild boars harboured subtype XXVa-1, indicating that wild boars and pigs in Spain share subtype XXVa-1. Although still preliminary and in need of confirmation in future studies analysing larger panels of *C. suis* isolates, our data suggest the occurrence of potential geography-restricted subtype distribution patterns, which could reflect differences in sources of infection and transmission pathways.

Some of the samples tested with the *gp60* assay failed to produce PCR products and by re-evaluating and reinvestigating them, some of these samples were in fact confirmed to be positive for *C. occultus* instead of *C. suis*. This means that the *gp60* primers that we developed for *C. suis* do not amplify *gp60* genes of *C. occultus*, at least not the *C. occultus* that we found in the wild boar samples tested here. To this end, it should be mentioned that no primers for *C. occultus gp60* typing have yet been published.

4.4. Study limitations and future work

This study has some limitations. First, low parasite DNA concentration in combination with repeated freezing and thawing of the DNAs might be the explanation of the low amplification rate for the domestic pig samples from Spain (40%; 4/10). A low amplification rate might also reflect genetic variation in primer regions; data so far available is too scarce to be able to evaluate this.

Secondly, the subtype method showed a relatively low discriminatory power, as the repetitive region, which would have been a useful marker, was not covered. Hopefully, future work would result in a test that would cover both the length of the repeats and the post-repetitive area, leading to a higher discriminatory power. This might be achieved using a different polymerase (that enables amplification of large PCR products) and long-read next-generation sequencing. In those laboratories where this would not be an option, fragment size analysis of the *gp60* gene might be the solution (Ramo et al., 2014).

Finally, all tested samples originated from Europe, which might explain the low sequence variation. More research is required to better elucidate the epidemiology of *C. suis* in its natural host species.

5. Conclusions

In this study, *C. suis gp60* genes were amplified and sequenced from faecal samples of human and porcine origin. A primer set was developed for subtyping purposes that was able to differentiate at least three separate subtypes of *C. suis* among 25 samples yielding the specific PCR product. The assay, however, did not cover the unique multiple 21-bp repeat region and therefore might miss information that could otherwise have been made available using bespoke next-generation sequencing methods and that could have conveyed more resolution to the assay. Still, the *C. suis gp60* data provided here could be important to inform future research and development in this field.

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Ethical considerations

Institutional Review Board Statement: Approval (registration number 2013/201–31/4) was obtained from the Ethical Review Board at Karolinska Institutet, Stockholm, Sweden, including approval to publish the data (date of approval: 27 February 2013).

CRediT authorship contribution statement

Marianne Lebbad: Writing – original draft, Writing – review & editing, Visualization, Validation, Supervision, Software, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Jana Grüttner:** Writing – review & editing, Visualization, Validation, Software, Methodology, Investigation, Formal analysis. **Jessica Beser:** Writing – review & editing, Visualization, Validation, Software, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation. **Victor Lizana:** Writing – review & editing, Supervision, Project administration, Methodology, Investigation, Formal analysis, Data curation. **Maria Auxiliadora Dea-Ayuela:** Writing – review & editing, Resources, Methodology, Investigation, Formal analysis, Data curation. **Marianne Oropeza-Moe:** Writing – review & editing, Visualization, Validation, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **David Carmena:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Investigation, Formal analysis, Data curation. **Christen Rune Stensvold:** Writing – review & editing, Visualization, Validation, Supervision, Software, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability statement

The data used for the analyses were publicly available in the NCBI Database (GenBank) and/or in the journals mentioned in the reference list.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.meegid.2024.105614.

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