One Health 18 (2024) 100682

Contents lists available at ScienceDirect

One Health

journal homepage: www.elsevier.com/locate/onehlt

# Rodent-adapted *Cryptosporidium* infection in humans: Seven new cases and review of the literature

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ARTICLE INFO

*Keywords:*
Protozoa
Zoonosis
Surveillance
One Health
Molecular methods
Parasites
Rodents

ABSTRACT

Cases of cryptosporidiosis in humans have been reported with strong indication of transmission from rodents. Here, we report seven new human cases of cryptosporidiosis involving rodent-adapted species (*Cryptosporidium ditrichi* [*n* = 1], *Cryptosporidium mortiferum* [*n* = 4; previously known as *Cryptosporidium* chipmunk genotype I], *Cryptosporidium tyzzeri* [*n* = 1], and *Cryptosporidium viatorum* [n = 1]) and review cases of human infection caused by these four species published to date. The seven new cases were detected in Denmark within a period of twelve months from 2022 to 2023. Only the *C. tyzzeri* and *C. viatorum* cases were associated with travel outside Denmark. The total number of human cases of cryptosporidiosis due to *C. ditrichi* and *C. tyzzeri* documented to date globally are still limited (4 and 7, respectively), whereas cases involving *C. viatorum* and *C. mortiferum* have been detected to a larger extent (43 and 63 cases, respectively). The four new cases of *C. mortiferum* were all of the XIVaA20G2T1 subtype, which is the only subtype identified so far in Scandinavia, and which is a subtype not yet found outside of Scandinavia. The new *C. viatorum* case was identified as the XVaA3g subtype. The *C. tyzzeri* case was subtyped as IXbA6. No subtype data were produced for *C. ditrichi* due to lack of a subtype assay. Review of existing data suggests the presence of *C. ditrichi* and *C. mortiferum* primarily in northern countries and *C. tyzzeri* and *C. viatorum* primarily in warmer climates. While our data may further support the role of *Cryptosporidium* as a cause of zoonotic disease, case descriptions should be obtained where possible to determine if *Cryptosporidium* species primarily adapted to rodents are the likely cause of symptoms or just an incidental finding.

## 1. Introduction

*Cryptosporidium* is a single-celled intestinal parasite that can infect a broad variety of hosts, including humans. Symptoms associated with cryptosporidiosis include diarrhoea and other gastrointestinal symptoms. The disease might be lengthy and severe in immunocompromised individuals, is difficult to treat, and no vaccine is available [1]. Hence, *Cryptosporidium* is one of the most common protozoa to produce hardly curable disease in both humans and animals which is also why a One Health approaching to detecting, differentiating, preventing, and controlling cryptosporidiosis is of high relevance.

With increased awareness of *Cryptosporidium*, technological advances, and adherence to the In Vitro Diagnostic Regulation (IVDR – EU 2017/746), there is potential for standardized and more accurate detection and surveillance of cryptosporidiosis in EU countries where cryptosporidiosis was previously overlooked [2].

Until very recently, the epidemiology of *Cryptosporidium* infections in Denmark remained largely unknown. However, in preparation for national surveillance of cryptosporidiosis in Denmark, which has been mandatory in Denmark since November 2023, molecular characterization of *Cryptosporidium* identified in stool samples at local CMD throughout the country has become a priority at the Laboratory of

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https://doi.org/10.1016/j.onehlt.2024.100682
Received 17 November 2023; Accepted 19 January 2024
Available online 20 January 2024

Parasitology, Statens Serum Institut (SSI), Copenhagen. DNA sequence analysis is useful for epidemiological purposes and infectious disease monitoring, including identification of outbreaks, routes of transmission, changes in transmission patterns, differences in virulence/clinical significance, etc. The genetic markers used to differentiate species of *Cryptosporidium* include the SSU rRNA and actin genes, and higher resolution can be obtained by especially DNA sequence analysis of glycoprotein 60 (gp60) genes, which enables identification at subtype level; however, gp60 typing schemes are not available for all species of *Cryptosporidium* identified to date.

Cryptosporidiosis is a well-known zoonosis [3]; however, most cases of zoonotic cryptosporidiosis appear to involve *Cryptosporidium parvum*, which is a parasite especially observed in young cattle. We here report on findings from the current surveillance work at SSI that indicate transmission of *Cryptosporidium* from rodents to humans. We moreover provide an overview of the number of human cases of cryptosporidiosis linked to rodent reservoirs identified to date based on a review of current data and literature.

## 2. Materials and methods

### 2.1. Molecular characterization and phylogenetic analysis

Generally, when the Laboratory of Parasitology at SSI receives sample material from CMD that was locally identified positive for *Cryptosporidium*, the material is first subject to DNA extraction (eMAG, bioMérieux) and real-time PCR for *Cryptosporidium* and *Giardia* [4]. If *Cryptosporidium*-positive by real-time PCR targeting the SSU rRNA gene [4], the DNAs are subject to sequencing of the gp60 gene using nested PCR [5]. In those cases where no PCR product is produced for the gp60 gene, PCR for SSU ribosomal RNA [6], and actin [7] genes are carried out with subsequent Sanger sequencing in order to identify the species, and more specific gp60-based PCR and sequencing methods may be used as relevant. Specifically, in the present study, the protocols for *C. mortiferum* [8] and *C. viatorum* [9] were followed.

Sanger sequencing services offered by Eurofins (Ebersberg, Germany) are employed, and the resulting sequences from bidirectional sequencing are assembled and analysed. Finally, the sequences are compared with reference sequences by multiple sequence alignment; here, both manual alignment with reference sequencing and BLAST queries in the National Center for Biotechnology (NCBI) Database are used.

Phylogenetic analyses of actin, SSU rDNA, and gp60 DNA sequences were done using Neighbor-Joining algorithm.

### 2.2. Data retrieval from databases

In order to identify sequences from cases of human cryptosporidiosis that would possibly reflect transmission from rodents, three different approaches were taken: The first approach involved sourcing data pertaining to *C. viatorum*, *C. tyzzeri* (and *Cryptosporidium* mouse genotype I, which was the previous name for *C. tyzzeri*), *C. ditrichi*, and *Cryptosporidium* chipmunk genotype I (which was the previous name for *C. mortiferum* [10]). This was done using the filtering and export functions available at the 'Nucleotide' page of NCBI (https://www.ncbi.nlm.nih.gov/nucleotide/). Only sequences up to 3000-bp length were searched for to restrict the search to those sequences that would typically be a result of molecular characterization of the genes typically involved in *Cryptosporidium* surveillance, namely the SSU rRNA, actin, hsp70, cowp, and gp60 genes. The second approach involved manual BLAST queries of a selection of sequences and sequence regions with a view to picking up sequences that might have been annotated in a way so that they would not be detected by the filtering algorithm. The third approach involved a literature search on Google Scholar and PubMed (https://pubmed.ncbi.nlm.nih.gov/) for studies on human cases of cryptosporidiosis caused by any of the species mentioned above for which DNA sequences were not submitted to the NCBI Database.

### 2.3. Metadata for cases

Adhering the general data protection regulation applicable to the Danish health care system, we were not able to provide full demographic information on the cases. All seven patients had actively contacted their doctors due to intestinal symptoms, and information on travel was available for all.

### 2.4. Submission of sequences to the NCBI database

Representative DNA sequences were submitted to the NCBI Database under accession numbers OR750464-OR750468 (actin DNA sequences) and OR750469-OR750474 (gp60 DNA sequences), and OR794336 (SSU rDNA sequence).

## 3. Results

A total of seven new cases of human cryptosporidiosis likely reflecting transmission from rodents were identified in the period from mid-September 2022 to mid-September 2023, including one case of *C. ditrichi*, one case of *C. tyzzeri*, one case of *C. viatorum*, and four cases of *C. mortiferum* infection (Tables 1 and 2; Fig. 1). In that period, a total of approximately 200 samples had been successfully typed to subtype level.

All seven cases had been detected using QIAstat-Dx® Gastrointestinal Panel, and all cases apart from the *C. viatorum* case which was detected at the CMD in Slagelse, Denmark had initially been confirmed *Cryptosporidium*-positive at the CMD in Herlev-Gentofte, Denmark and later also by real-time PCR at SSI [4].

Actin sequences were obtained for all seven cases and used in a phylogenetic analysis (Fig. 1). SSU rDNA sequences were available for 5 cases; however, only a stretch of 200 bp of the SSU rDNA sequence for *C. tyzzeri* could be called with confidence; this part by itself was insufficient to distinguish it from other species of *Cryptosporidium*. Since no gp60-based typing scheme is yet available for *C. ditrichi*, gp60 data could only be produced for *C. tyzzeri*, *C. mortiferum* and *C. viatorum*. For the *C. tyzzeri* case, subtype IXbA6 was identified, and the four cases with *C. mortiferum* were all subtype XIVaA20G2T1. The *C. viatorum*-positive sample was categorized as subtype XVaA3g.

Phylogenetic analyses of the gp60 genes sequenced in the study are available in Figs. 2, 3, and 4.

Based on these seven new cases and our various searches in the literature and NCBI database, we were able to identify documentation for a total of four human cases positive for *C. ditrichi*, seven cases positive for *C. tyzzeri* (including one case with mixed *C. tyzzeri* subtype infection in the presence of a *C. parvum* infection), 43 cases positive for

**Table 1**
Summary of human cases of cryptosporidiosis caused by rodent-adapted species of *Cryptosporidium* (*C. ditrichi, C. mortiferum, C. tyzzeri,* and *C. viatorum*) documented to date according to geography.

| | *C. ditrichi* | *C. mortiferum* | *C. tyzzeri* | *C. viatorum* | TOTAL |
|---|---|---|---|---|---|
| Boreal regions | | | | | |
| Europe | 4 | 33 | 1 | 0 | 38 |
| North America | 0 | 30 | 0 | 0 | 30 |
| Peri-equatorial and Meridional/Austral regions | | | | | |
| Central/South America | 0 | 0 | 0 | 3 | 3 |
| Africa | 0 | 0 | 1 | 20 | 21 |
| Asia | 0 | 0 | 5 | 19 | 24 |
| Oceania | 0 | 0 | 0 | 1 | 1 |
| TOTAL | 4 | 63 | 7 | 43 | 117 |

**Table 2**
Comprehensive overview of cases of human cryptosporidiosis caused by rodent-adapted species of *Cryptosporidium* (*C. ditrichi* [C. d.], *C. mortiferum* [C. m.], *C. tyzzeri* [C. t.], and *C. viatorum* [C. v.]) documented to date and with extensive referencing. The seven new cases presented in this study are highlighted in boldface. NA = not available/applicable.

| Species | ID | Number of cases/ individuals | Origin of infection | SSU rRNA | Actin | COWP | hsp70 | GP60 | GP60 subtype | Publication(s) |
|---|---|---|---|---|---|---|---|---|---|---|
| C. d. | Swec1089 | 1 | Sweden | MN065795 | MN065792 | MN065794 | MN065793 | NA | NA | [11] |
| C. d. | Swec795 | 1 | Sweden | MH187880 | MH187878 | NA | MH187879 | NA | NA | [11] |
| C. d. | Swec449 | 1 | Sweden | KU892562 | KU892569 | NA | KU892575 | NA | NA | [11] |
| **C. d.** | **69485** | **1** | **Denmark** | **OR794336** | **OR750465** | **NA** | **NA** | **NA** | **NA** | **present study** |
| C. m. | WH33 | 2 | USA/ Wisconsin | DQ640638 | NA | NA | NA | NA | NA | [12] |
| C. m. | NA | 1 | France | NA | NA | NA | NA | NA | NA | [13] |
| C. m. | Swec176/ 39971 | 1 | Sweden | JX978272 | JX978270 | NA | JX978276 | Identical to KU852739 | XIVaA20G2T1 | [8,14] |
| C. m. | Swec096/ 39970 | 1 | Sweden | Identical to JX978272 | Identical to JX978270 | JX984442 | JX978275 | Identical to KU852739 | XIVaA20G2T1 | [8,14] |
| C. m. | 39969 | 1 | USA/Maine | NA | NA | NA | NA | NA | XIVaA16G2T2 | [8] |
| C. m. | 37187 | 1 | USA/ Vermont | NA | NA | NA | NA | KP099081 | XIVaA17G2T3 | [8] |
| C. m. | 37189 | 1 | USA/ Vermont | NA | NA | NA | NA | NA | XIVaA16G2T2 | [8] |
| C. m. | 37555 | 1 | USA/ Vermont | NA | NA | NA | NA | KP099080 | XIVaA16G2T2 | [8] |
| C. m. | 37763 | 1 | USA/ Vermont | NA | NA | NA | NA | KP099078 | XIVaA14G2T2 | [8] |
| C. m. | 37764 | 1 | USA/ Vermont | NA | NA | NA | NA | KP099079 | XIVaA15G2T3 | [8] |
| C. m. | 41602 | 1 | USA/ Vermont | NA | NA | NA | NA | NA | XIVaA15G2T3 | [8] |
| C. m. | 41604 | 1 | USA/ Vermont | NA | NA | NA | NA | NA | XIVaA16G2T2 | [8] |
| C. m. | 39974 | 1 | USA/ Wisconsin | NA | NA | NA | NA | NA | XIVaA16G2T1b | [8] |
| C. m. | 39975 | 1 | USA/ Wisconsin | NA | NA | NA | NA | KP099092 | XIVaA16G2T1 | [8] |
| C. m. | 40694 | 1 | USA/ Minnesota | NA | NA | NA | NA | NA | XIVaA16G2T1 | [8] |
| C. m. | 40693 | 1 | USA/ Minnesota | NA | NA | NA | NA | KP099087 | XIVaA17G2T2 | [8] |
| C. m. | 40695 | 1 | USA/ Minnesota | NA | NA | NA | NA | NA | XIVaA20G2T2 | [8] |
| C. m. | 40696 | 1 | USA/ Minnesota | NA | NA | NA | NA | KP099083 | XIVaA20G2T2 | [8] |
| C. m. | 40697 | 1 | USA/ Minnesota | NA | NA | NA | NA | KP099085 | XIVaA19G2T2a | [8] |
| C. m. | 40702 | 1 | USA/ Minnesota | NA | NA | NA | NA | KP099088 | XIVaA14G2T1 | [8] |
| C. m. | 40703 | 1 | USA/ Minnesota | NA | NA | NA | NA | KP099086 | XIVaA18G2T1b | [8] |
| C. m. | 40705 | 1 | USA/ Minnesota | NA | NA | NA | NA | NA | XIVaA18G2T1b | [8] |
| C. m. | 40709 | 1 | USA/ Minnesota | NA | NA | NA | NA | KP099084 | XIVaA19G2T2b | [8] |
| C. m. | 39972 | 1 | Sweden | NA | NA | NA | NA | NA | XIVaA20G2T1 | [8] |
| C. m. | 40136 | 1 | Sweden | NA | NA | NA | NA | KP099089 | XIVaA20G2T1 | [8,15] |
| C. m. | NA | 3 | USA/ Nebraska | NA | NA | NA | NA | NA | NA | [16] |
| C. m. | Swec641 | 1 | Sweden | Identical to JX978272 | NA | NA | NA | KU852739 | XIVaA20G2T1 | [15] |
| C. m. | Swec644 | 1 | Sweden | Identical to JX978272 | NA | NA | NA | identical to KU852739 | XIVaA20G2T1 | [15] |
| C. m. | Swec732 | 1 | Sweden | Identical to JX978272 | NA | NA | NA | identical to KU852739 | XIVaA20G2T1 | [15] |
| C. m. | Swec750 | 1 | Sweden | Identical to JX978272 | NA | NA | NA | identical to KU852739 | XIVaA20G2T1 | [15] |
| C. m. | Swec950 | 1 | Sweden | MW179499 | NA | NA | NA | NA | NA | [17] |
| C. m. | Swec1412 | 19 | Sweden | MW179500 | NA | NA | NA | MW177562 | XIVaA20G2T1 | [17] |
| C. m. | 57019, 42679, 48984 | 4 | USA/New York | OP935202, OP935211, OP935212 | NA | NA | NA | NA | NA | [18] |
| C. m. | Patient 93 | 1 | USA? | AF133842 | NA | NA | NA | NA | NA | Submitted as *'C. parvum'*. Unpublished Pieniazek 1999 |
| C. m. | CHIP_Ih | 1 | USA | OQ627027 | OQ632464 | OQ632472 | OQ632485 | OQ632477 | XIVaA18G2T2 | [10] |

(*continued on next page*)

**Table 2** (*continued*)

| Species | ID | Number of cases/ individuals | Origin of infection | SSU rRNA | Actin | COWP | hsp70 | GP60 | GP60 subtype | Publication(s) |
|---|---|---|---|---|---|---|---|---|---|---|
| **C. m.** | **15689** | **1** | **Denmark** | **Identical to JX978272** | **OR750467** | **NA** | **NA** | **OR750469** | **XIVaA20G2T1** | **Present study** |
| **C. m.** | **62398** | **1** | **Denmark** | **Identical to JX978272** | **NA** | **NA** | **NA** | **OR750471** | **XIVaA20G2T1** | **Present study** |
| **C. m.** | **51246** | **1** | **Denmark** | **Identical to JX978272** | **OR750468** | **NA** | **NA** | **OR750470** | **XIVaA20G2T1** | **Present study** |
| **C. m.** | **64857** | **1** | **Denmark** | **NA** | **NA** | **NA** | **NA** | **OR750472** | **XIVaA20G2T1** | **Present study** |
| C. t. | 7490 | 1 | Kuwait | NA | NA | NA | NA | AY738188 | IXaA6 | [19] |
| C. t. | RPHN* | 1 | China | DQ898158 | NA | NA | DQ898162 | NA | NA | Unpublished? |
| C. t. | M805A and M805B | 1 | Czech Republic | NA | NA | NA | NA | JX445924 and JX445925 | IXbA6 and IXaA8 | [20] |
| C. t. | 15753 | 1 | New Zealand | NA | NA | NA | NA | MT265681 | IXbA6 | [21] |
| C. t. | 14314 | 1 | New Zealand | NA | NA | NA | NA | MT265682 | IXbA6 | [21] |
| C. t. | 11758 | 1 | New Zealand | NA | NA | NA | NA | MT265683 | IXbA5 | [21] |
| **C. t.** | **537** | **1** | **Subsaharan Africa** | **NA** | **OR750464** | **NA** | **NA** | **OR750473** | **IXbA6** | **Present study** |
| C. v. | PAR/ CV_010/ 2017 | 1 | India | NA | NA | NA | MT341769 | NA | NA | [22] |
| C. v. | PAR/ CV_031/ 2018 | 1 | India | NA | NA | NA | OP420532 | NA | NA | Unpublished |
| C. v. | AM83/ Mo66 | 1 | Mozambique | MW563970.1 | NA | NA | NA | MW574004 | XVaA3a | [23] |
| C. v. | CFZ01 | 1 | Malawi | MW147226 | NA | NA | NA | MW159770 | XVaA3 | [24] |
| C. v. | NA | 1 | Australia | NA | NA | NA | NA | MK165991 | XVaA3g | [25] |
| C. v. | GX800 | 1 | China | MH807495 | NA | NA | NA | MH807494 | XVaA3h | [26] |
| C. v. | MD171 | 1 | Myanmar | MW014315 | NA | NA | NA | MW014316 | XVcA2G1c | [27] |
| C. v. | W14532 | 1 | India | NA | NA | NA | NA | KP115936 | XVaA3a | [9,28,29] |
| C. v. | SC322 | 1 | Kenya | NA | NA | NA | NA | KP115937 | XVaA3b | [9] |
| C. v. | Swec066 | 1 | Guatemala | NA | NA | JX984441 | JX978274 | KP115938 | XVaA3c | [9,30] |
| C. v. | Swec025 | 1 | Kenya | JX978271 | JX978269 | NA | JX978273 | KP115939 | XVaA3d | [9,30] |
| C. v. | W23461 | 1 | Nepal | NA | NA | NA | NA | KP115940 | XVaA3e | [9,28] |
| C. v. | W25284 | 1 | India | NA | NA | NA | NA | KP115941 | XVaA3f | [9,28] |
| C. v. | W25244 | 1 | India | JN846705 | JN846707 | NA | JN846706 | NA | XVaA3a | [9,28] |
| C. v. | W24580 | 1 | India | JN846708 | NA | NA | NA | NA | XVaA3a | [9,28] |
| C. v. | W16767 | 1 | Pakistan | NA | NA | NA | NA | NA | XVaA3a | [9,28] |
| C. v. | W22242 | 1 | Bangladesh | NA | NA | NA | NA | NA | XVaA3a | [9,28] |
| C. v. | W25121 | 1 | India | NA | NA | NA | NA | NA | XVaA3a | [9,28] |
| C. v. | W25123 | 1 | India and Dubai | NA | NA | NA | NA | NA | XVaA3a | [9,28] |
| C. v. | W25259 | 1 | India | NA | NA | NA | NA | NA | XVaA3a | [9,28] |
| C. v. | W27485 | 1 | India | NA | NA | NA | NA | NA | XVaA3a | [9] |
| C. v. | W27691 | 1 | NA | NA | NA | NA | NA | NA | XVaA3d | [9] |
| C. v. | W29326 | 1 | Barbados | NA | NA | NA | NA | NA | XVaA3d | [9] |
| C. v. | 35465 and more | 9 | Ethiopia | NA | NA | NA | NA | NA | XVaA3d | [9,31] |
| C. v. | NA | 2 | Ethiopia | Identical to JX644908 | NA | NA | NA | NA | NA | [32] |
| C. v. | W27718 | 1 | India | NA | NA | NA | NA | NA | XVaA3f | [9] |
| C. v. | Ess33 | 1 | Kenya | MZ435786 | NA | NA | NA | NA | NA | [33] |
| C. v. | 31332 | 1 | Nigeria | JX644908 | NA | NA | NA | NA | NA | [34] |
| C. v. | NA | 1 | Colombia | RFLP | NA | NA | NA | NA | NA | [35] |
| C. v. | NA | 1 | Nigeria | NA | NA | NA | NA | NA | NA | [36] |
| C. v. | 44 and 148 | 2 | Madagascar | NA | NA | NA | NA | NA | NA | [37] |
| C. v. | SSU-rRNAIS20 | 1 | India | KX174309 | NA | NA | NA | NA | NA | [38] |
| **C. v.** | **47630** | **1** | **India** | **NA** | **OR750466** | **NA** | **NA** | **OR750474** | **XVaA3g** | **Present study** |

* This isolated was passed through a mouse, so, theoretically, this sequence could be of mouse origin rather than human origin.

*C. viatorum*, and no less than 63 human cases positive for *C. mortiferum* at the time of writing (Tables 1 and 2).

The hosts reported to date for all four *Cryptosporidium* species are provided in Suppl. Table 1.

## 4. Discussion

Many species of *Cryptosporidium* have been observed in stool, and the zoonotic potential of the genus appears to be extensive [3]. The disease toll of *Cryptosporidium* infection in humans might depend on several parameters, including the species involved, and it remains unclear to which extent asymptomatic carriage of rodent-adapted species of *Cryptosporidium* exists in humans.

All seven patients contacted the health care system in order to obtain a stool-diagnostic workup. Since we did not have clinical data for all of the cases, we consider it beyond the scope of the present study to go into a deeper discussion of the clinical impact of the four species. We will therefore be focusing on basic epidemiological aspects of the species, such as host specificity and genetic diversity, based on the data published to date.

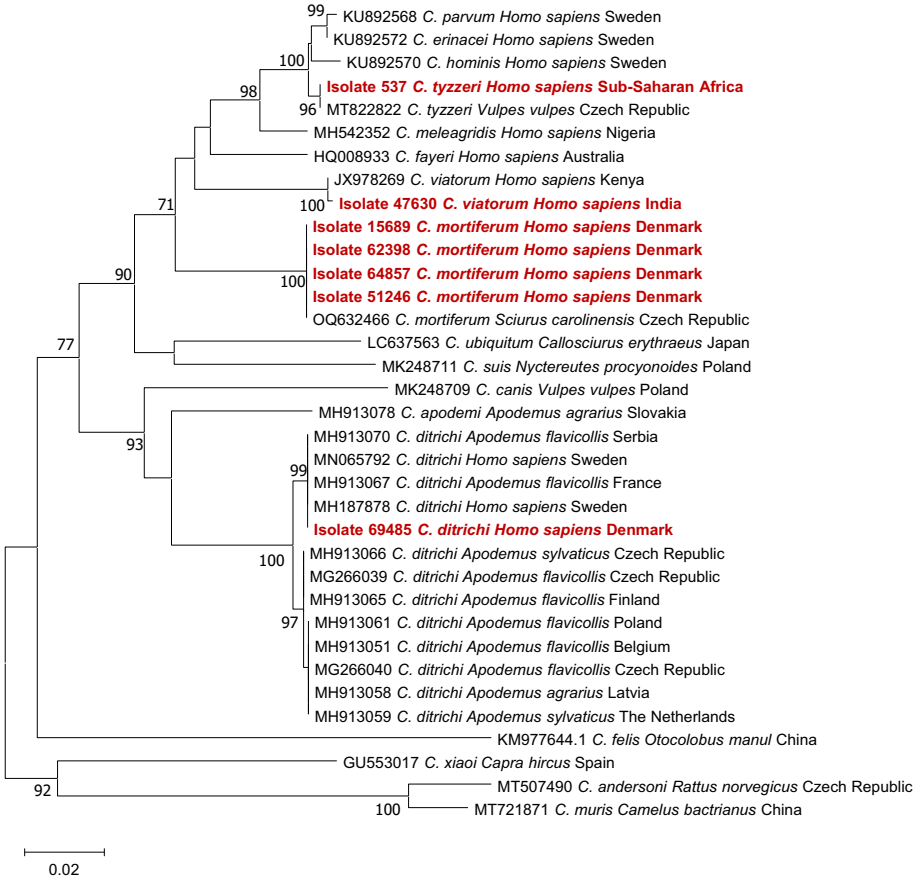


**Fig. 1.** Phylogenetic analysis of actin DNA sequences obtained in the present study (highlighted in red and bold) and representative references sequences. Information on host and geographic location is indicated. The analyses involved 35 nucleotide sequences. All ambiguous positions were removed for each sequence pair. There were a total of 812 positions in final dataset. Bootstrap values above or equal to 70% are shown.

### 4.1. *Cryptosporidium ditrichi*

*Cryptosporidium ditrichi* was described quite recently, in 2018 [39]. Human infection with *C. ditrichi* was first observed only a couple of years back [11] in three unrelated patients who were likely to have contracted infection in Sweden. We did not identify any other reports on human *C. ditrichi* infection, so the present case is only the fourth human case ever reported. All four human cases observed to date were found in Scandinavia.

The vast majority of *C. ditrichi* sequences in the NCBI Database are from various species of *Apodemus*, typically, *Apodemus flavicollis* sampled all over Europe from Latvia and Finland to France and Spain [40,41] (Suppl. Table 1), and it was by far the most common species detected in murids and cricetids sampled in the wild in Denmark in a recent survey [42]. *C. ditrichi* was also found in a beech marten sampled in Poland [43].

Two clades can be distinguished for *C. ditrichi* actin sequences (Fig. 1). The four sequences found in humans are in the same clade, which could indicate cryptic host specificity of *C. ditrichi*. Still, sequences from both clades have been identified in faeces from *Apodemus flavicollis* in the Czech Republic. Analysis of *C. ditrichi* SSU rDNA sequences available in NCBI Database (Suppl. Table 1) reveals several clusters (Suppl. Fig. 1). The four sequences generated to date from human samples all fall in the same clade with sequences from rodent hosts.

Interestingly, the SSU rDNA sequence MG266031 from *A. flavicollis* clusters with human sequences while its corresponding actin sequence (MG266040) does not (Fig. 1). To investigate intrageneric diversity in *C. ditrichi* further, it could be useful to sequence hsp70 genes, something which has been done for only a couple of samples until now [11].

### 4.2. *Cryptosporidium mortiferum*

*Cryptosporidium mortiferum*, previously known as *Cryptosporidium* chipmunk genotype I, which typically infects various rodents such as squirrels, chipmunks, and deer mouse [8] in their natural habitat, has emerged as the third-most common *Cryptosporidium* species in humans samples in Sweden [17].

Both human and non-human cases of *C. mortiferum* are yet to be reported from continents other than Europe and North America. A total of 30/63 (48%) of all reported human cases of *C. mortiferum* are from the US, while 32/63 (51%) are from Scandinavia; the only European case reported outside of Scandinavia was from France (Table 2).

All subtypes of *C. mortiferum* identified to date belong to one single subtype family, XIVa, and at least 16 subtypes have already been identified; the majority of these were observed in the US. Most of these subtypes have been detected either in humans or animals; however, data from Sweden suggest that zoonotic transmission can occur, as the same subtype, XIVaA20G2T1, was detected both in humans and red squirrels (*Sciurus vulgaris*) [17]. Interestingly, all four cases of *C. mortiferum* detected in the present study involved XIVaA20G2T1, which is the same and only subtype reported in Scandinavia so far (Table 2; Fig. 2); moreover, XIVaA20G2T1 has only been detected in Scandinavia to date. It should be mentioned that the only subtype apart from XIVaA20G2T1 that has been identified in both humans and non-human hosts is XIVaA18G2T2, but here, the human and animal cases were from two

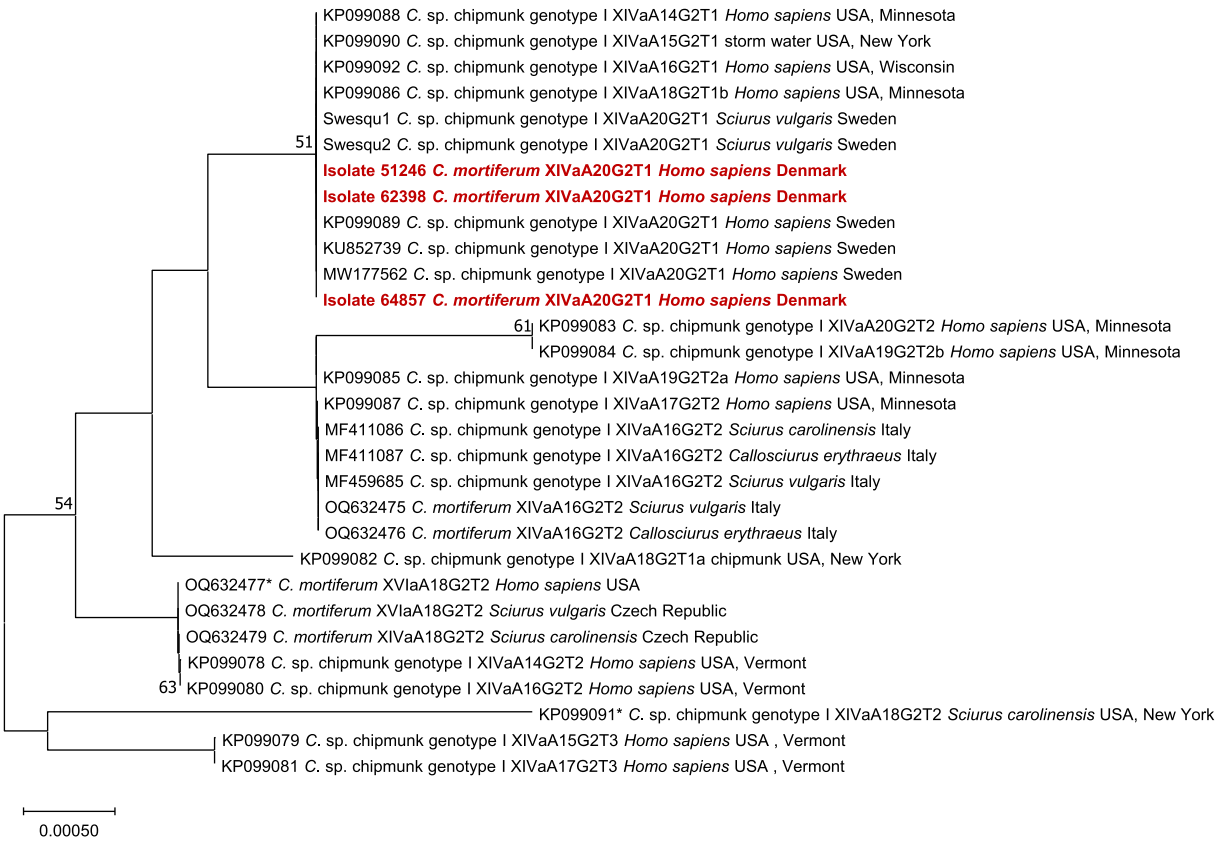


**Fig. 2.** Phylogenetic analysis of gp60 DNA sequences of *Cryptosporidium mortiferum.* Information on host and geographic location is indicated. The analyses involved 30 nucleotide sequences. All ambiguous positions were removed for each sequence pair. There were a total of 850 positions in final dataset. Bootstrap values above or equal to 50% are shown. *OQ632477 is not identical to KP099091.

different continents, representing a much weaker epidemiolocal link. It should also be noted, that XIVaA18G2T2 holds cryptic genetic diversity, with KP099091 being different to OQ632477–OQ632479 (OQ632477 is from a human, and OQ632478 and OQ632479 are from animals) (Fig. 2) due to shuffling of the trinucleotide repeats, a phenomenon observed mainly in the *C. parvum* IIa subtype family.

Another subtype, XIVaA16G2T2, has been reported from four human cases in the US and five rodents in Europe; however, the sequences from the human hosts differ from those from the rodent hosts by one single nucleotide polymorphism, indicating a different subtype, which also explains the different topology of the samples in Fig. 2.

It has been hypothesized that *C. mortiferum* was introduced to Europe from North America during the last century by their natural host, grey squirrels [44], but as only red squirrels are found in Denmark and Sweden, this statement requires further investigations. For now, it appears that no other sequence produced from sampling in Europe clusters with the ones from Scandinavia (Fig. 2).

### 4.3. *Cryptosporidium tyzzeri*

*Cryptosporidium tyzzeri* was described in 2012 by Ren and colleagues [45], but the first case of a human *C. tyzzeri* infection might have been the one reported by Sulaiman and colleagues in 2005 [19], originally reported as *C. parvum* IIfA6. Since then, at least six additional human cases have been documented, including the one from this study (Tables 1 and 2).

Human cases have been detected both in- and outside of Europe, although only one of the cases would appear to reflect local transmission within Europe, namely the case from the Czech Republic [20], in which case two different subtypes of *C. tyzzeri* were observed (Table 2). The case observed in Denmark and included in the present study had most likely contracted the infection in Sub-Saharan Africa.

*Cryptosporidium tyzzeri* has been detected in wild murids and cricetids in Germany, Poland, Czech Republic, and Spain, including the Canary Islands (Suppl. Table 1) but was not detected in a few dozens of murids and cricetids recently sampled in the wild in Denmark (Rotovnik et al., submitted). Most *C. tyzzeri* DNA sequences in the NCBI Database are from *Mus musculus*, but the parasite has also been detected in other rodents, including *Apodemus flavicollis*, *Microtus arvalis*, and *Clethrionomys glareolus* [46], other species of *Apodemus* [40], and rats [47].

Maybe not surprisingly, *C. tyzzeri* has been detected in faecal DNA from snakes and other predators. Xiao and colleagues found it in a corn snake (*Elaphe guttata guttata*) [48], and it was also found in *Lampropeltis getula* (Kingsnake) at the Lille Zoo (France) [49]; in both cases the species was called on the basis of SSU rDNA sequence analysis only. Pedraza-Diaz and colleagues sequenced the hsp70 and SSU loci of *C. tyzzeri* found in a python (*Python regius*)[50]. Very recently, Lobao and colleagues identified *C. tyzzeri* in quite a few different species of venomous pit vipers in Rio de Janeiro, Brazil [51]. Other animals praying on rodents include the red fox (*Vulpes vulpes*), in which *C. tyzzeri* was found based on SSU, actin, and gp60 DNA analysis [52]. Finally, *C. tyzzeri* has been reported in synanthropic mammals, such as horse [53] and cattle (Suppl. Table 1).

Gp60 data is available for most human cases and for quite a few non-human samples. In the absence of a consensus terminology for *C. tyzzeri* subtypes, we have based our terminology only on the number of TCA repeats and not on other types of repeats (Fig. 3). This entails that *C. tyzzeri* subtypes with identical names may have genetically dissimilar gp60 DNA sequences, and therefore the development of a more structured and detailed terminology appears relevant for molecular epidemiological purposes.


**Fig. 3.** Phylogenetic analysis of gp60 DNA sequences of *Cryptosporidium tyzzeri.* Information on host and geographic location is indicated. The analyses involved 28 nucleotide sequences. All ambiguous positions were removed for each sequence pair. There were a total of 713 positions in final dataset. Bootstrap values above or equal to 70% are shown.

### *4.4. Cryptosporidium viatorum*

This species was first described in 2012 by Elwin and colleagues [28] and a common feature of the ten human cases then described was the fact that they had all been returning from India. Incidentally, the new case included in the present study had also been returning from India prior to receiving parasitological workup.

Forty-three cases of human *C. viatorum* infection have been documented to date (Tables 1 and 2), and more than half have been found in Australasia. The remaining cases were observed in Sub-Saharan Africa/Madagascar and peri-equatorial America, including Barbados. Of the four subtype families identified to date, the XVa family has been predominating in human stool samples.

Initially considered a parasite of humans, the finding of *C. viatorum* in an Australian swamp rat published in 2018 [54] was the first indication of *C. viatorum*-associated cryptosporidiosis being a potential zoonosis; however, the subtype found in the swamp rat (XVbA2G1) was different to those that had been found in humans, and so it was only in 2019 that the zoonotic potential was finally confirmed by the finding of XVaA3g in both a human and several rats [25,55]. Interestingly, the subtype found in the present study was also XVaA3g.

It should be mentioned that two sequences, MH807494 and MN614416 from human and rat, respectively, have been allocated the same subtype name (XVaA3h); however, the two sequences are not identical (Fig. 4) and therefore not the same subtype.

Although there is a single observation of *C. viatorum* in a cow [37], which may be an accidental finding, the overall data still suggest that the primary reservoir is made up by rodents, apparently rats in particular, and it is notable that the species was found in rat in Corsica [56], which could indicate its presence in Europe.

## 5. Conclusion

Human cases of cryptosporidiosis involving other species of *Cryptosporidium* adapted to rodents include the one attributable to *Cryptosporidium wrairi* reported by Hernandez-Castro and colleagues [57], those attributable to *Cryptosporidium occultus* [26,58], and several cases caused by *Cryptosporidium muris*, some of which were included in a recent review by Hancke and Suárez [59].

A limitation of our study was the fact that we were not able to contribute metadata such as clinical information and demographics due to local GDPR policies.

It is known from several surveys of rodents that species of *Cryptosporidium* frequently infect these hosts. There is a tendency that *C. tyzzeri* is mainly seen in species of *Mus*, whereas *C. ditrichi* is mainly seen in yellow-necked mice; however, some overlap in host specificity has been observed. Likewise, the reservoir for *C. viatorum* may typically be rats in Africa and Australasia, and for *C. mortiferum*, mainly squirrels in the northern part of the boreal hemisphere appear to be the main group of reservoir hosts.

For both C. *mortiferum* and *C. viatorum* more samples from humans than non-human hosts have been subtyped, while for *C. tyzzeri*, the vast majority of the subtyped samples are from rodent hosts. For *C. mortiferum,* studies of this species in squirrels sampled especially in the northern part of Central Europe (Poland, Germany) would be of interest to see whether these are more similar to the *C. mortiferum* seen in Scandinavia or those seen in the rest of Central and Southern Europe.

Our data add to the evidence that these species may be found in faecal samples from humans who contact the health care system with a view to identifying causes of gastrointestinal symptoms. Although these data could further substantiate the role of *Cryptosporidium* as a zoonotic

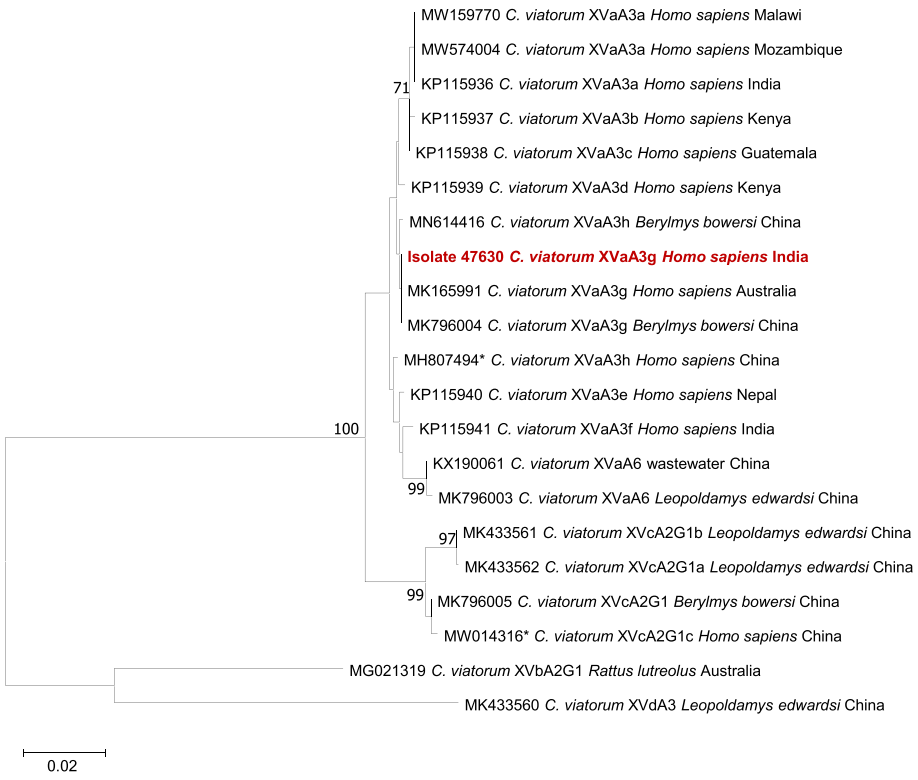


**Fig. 4.** Phylogenetic analysis of gp60 DNA sequences of *Cryptosporidium viatorum.* Information on host and geographic location is indicated. The analyses involved 21 nucleotide sequences. All ambiguous positions were removed for each sequence pair. There were a total of 783 positions in final dataset. Bootstrap values above or equal to 70% are shown. *Two sequences (MN614416 and MH807494) are not identical and should not have same subtype names; see text for details.

disease, it is important – where possible – to include case descriptions in order to identify whether the species primarily adapted to rodents of *Cryptosporidium* are the likely cause of the disease or merely an incidental finding and to identify exposures and opportunity for prevention.

## CRediT authorship contribution statement

**Christen Rune Stensvold:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Tine Graakjær Larsen:** Conceptualization, Investigation, Methodology, Project administration, Resources, Writing – review & editing. **Jana Grüttner:** Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – review & editing. **Lene Nielsen:** Data curation, Formal analysis, Project administration, Supervision, Writing – review & editing. **Jørgen Engberg:** Conceptualization, Data curation, Methodology, Project administration, Supervision, Writing – review & editing. **Marianne Lebbad:** Conceptualization, Data curation, Formal analysis, Investigation, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – review & editing.

## 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

DNA sequence data has been made available in GenBank.

## Acknowledgements

Lis Lykke Wassmann is thanked for excellent laboratory assistance. The authors TGL and JG are fellows of the ECDC Fellowship Programme, supported financially by the European Centre for Disease Prevention and Control. The views and opinions expressed herein do not state or reflect those of ECDC. ECDC is not responsible for the data and information collation and analysis and cannot be held liable for conclusions or opinions drawn.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.onehlt.2024.100682.

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