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Blastocystis: unravelling potential risk factors and clinical significance of a common but neglected parasite

C. R. STENSVOLD[1]*, H. C. LEWIS[2], A. M. HAMMERUM[3], L. J. PORSBO[4], S. S. NIELSEN[1], K. E. P. OLSEN[1], M. C. ARENDRUP[1], H. V. NIELSEN[1] AND K. MØLBAK[2]

[1] *Department of Bacteriology, Mycology and Parasitology, Statens Serum Institut, Copenhagen, Denmark*
[2] *Department of Epidemiology, Statens Serum Institut, Copenhagen, Denmark*
[3] *National Centre for Antimicrobials and Infection Control, Statens Serum Institut, Copenhagen, Denmark*
[4] *National Food Institute, Technical University of Denmark, Søborg, Denmark*

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SUMMARY

Two independent studies were conducted to describe symptoms and potential risk factors associated with *Blastocystis* infection. Isolates were subtyped by molecular analysis. In the NORMAT study (126 individuals randomly sampled from the general population) 24 (19%) were positive for *Blastocystis*. *Blastocystis* was associated with irritable bowel syndrome ($P=0{\cdot}04$), contact with pigs ($P<0{\cdot}01$) and poultry ($P=0{\cdot}03$). In the Follow-up (FU) study (follow-up of 92 *Blastocystis*-positive patients), reports on bloating were associated with subtype (ST) 2 ($P<0{\cdot}01$), and blood in stool to mixed subtype infection ($P=0{\cdot}06$). ST1 was more common in FU individuals (32%) than in NORMAT individuals (8%), whereas single subtype infections due to ST3 or ST4 were seen in 63% of the NORMAT cases and 28% of the FU cases. Only FU individuals hosted ST7, and ST6/7 infections due to ST7 or ST9 were characterized by multiple intestinal symptoms. The data indicate subtype-dependent differences in the clinical significance of *Blastocystis*.

Key words: Gastrointestinal infections, molecular epidemiology, parasites, parasitic disease epidemiology and control.

INTRODUCTION

Blastocystis is a common, single-celled, enteric endosymbiont of man and animals [1–5]. Often, *Blastocystis* is the only potential disease-causing agent found in faecal specimens from patients with diarrhoea or other gastrointestinal (GI) symptoms; however, *Blastocystis* is also common in asymptomatic individuals [6–11]. For eradication of *Blastocystis* infections, metronidazole is considered the first-line therapy [1, 12].

Based on molecular analysis of the small subunit (SSU) rRNA gene, *Blastocystis* isolates from man, mammals and birds can be assigned to one of at least ten subtypes [2, 13], the genetic distance between some of which is >7% [14, 15], which is more than that seen between homologous genes of pathogenic and non-pathogenic species of *Entamoeba* [14].

So far, studies seeking to unveil subtype-specific pathogenicity have produced conflicting conclusions

* Author for correspondence: Dr C. R. Stensvold, Department of Bacteriology, Mycology and Parasitology, Statens Serum Institut, Artillerivej 5, DK-2300 Copenhagen S, Denmark.
(Email: RUN@ssi.dk)

[6–11, 16]. However, such studies have not consistently included testing for other enteropathogens, e.g. *Dientamoeba fragilis* which is often a co-infection with *Blastocystis* [17, 18]. Moreover, the identification of *Blastocystis* carriers and non-carriers has been unconvincing due to the use of insensitive diagnostic methods [19].

Taamasri *et al*. [20] and Li *et al*. [21] investigated risk factors associated with *Blastocystis* carriage; however, neither study provided data on the health status of the study population.

Aims of the present investigation included (i) molecular characterization of *Blastocystis* isolates from Danish symptomatic and asymptomatic individuals, (ii) identification of potential risk factors for infections due to *Blastocystis*, and (iii) description of symptoms and identification of potential differences in pathogenicity in the distinct subtypes.

MATERIALS AND METHODS

Study types, study populations, specimen collection and parasitological examination

Two independent studies were included in the investigation. Both studies involved the collection of stool samples for the testing of pathogens and administration of a structured questionnaire to capture demographic and clinical data, as well as information on possible risk factors for GI pathogens.

NORMAT study

The NORMAT study was initiated in 2002 as part of DANMAP (Danish Integrated Antimicrobial Resistance Monitoring and Research Programme) to monitor antimicrobial drug resistance in commensal bacteria obtained from volunteers in the general population without acute gastroenteritis. Participants were identified through the Danish Civil Registry, a continuously updated registry of all residents in Denmark. A selection algorithm was developed to generate a sample of residents, representative of the age and gender distribution of the Danish population which took into account the differential participation rates of various demographic groups. Individuals who consented to participate were mailed a questionnaire and a faecal sampling test tube. Faecal samples were analysed for *Salmonella*, *Campylobacter*, *Yersinia enterocolitica*, *Shigella*, *Vibrio*, *Aeromonas*, *Plesiomonas shigelloides*, *Clostridium difficile* and diarrhoeagenic *Escherichia coli* using previously published methods including multiplex PCR [22]. Specimens were processed by the formol ethyl acetate concentration technique (FECT) and examined for parasites. Faecal concentrates were also stained by the modified Ziehl–Neelsen technique and evaluated for oocysts of sporozoa. From each fresh specimen about 200 mg was submitted to DNA extraction. Samples analysed in the present study were collected until the end of 2005.

Follow-up (FU) study

The FU study was a follow-up of patients, referred by general practitioners (GPs), diagnosed with *Blastocystis* between January and October 2005 by the use of FECT. The patients were contacted 3–6 weeks following diagnosis, after informed consent from their respective GPs. Patients were asked to complete a questionnaire, which was different from the questionnaire used in the NORMAT study, and to submit stool samples for follow-up investigation. Stools were cultured and examined for *Blastocystis* after 48–72 h as described previously [23]. The remainder of the specimen was submitted for DNA extraction, stool concentration and microscopy for parasites as above.

DNA extraction, *Blastocystis* PCR, (pyro)sequencing and subtype identification

DNA was extracted from faecal samples as described previously [23]. All study individuals were screened for *Blastocystis* by PCR. Three *Blastocystis* PCR protocols were applied to detect and characterize patient isolates: Since the nested PCR protocol described in previous papers [5, 24], based on the RD3/RD5 primers [14] and the F1/R1 primers [6], was insensitive regarding detection of *Blastocystis* sp. ST3, patients' samples were also analysed by the PCR method described previously [23]. Dideoxy sequencing was as described by Stensvold *et al*. [24]. Finally, 48 samples (15 NORMAT samples and 33 FU samples) were submitted to PCR and pyrosequencing as described previously [24]. Table 1 displays the primers used in the study for PCR amplification of *Blastocystis*-specific DNA.

Blastocystis sp. subtype identification

Sequence chromatograms and pyrograms were manually interpreted, nucleotide sequences edited, aligned and phylogenetically analysed as described previously [24] to identify subtypes. *Blastocystis* sp. subtype terminology according to Stensvold *et al*. [13] was used.

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Table 1. *Primers targeting the small subunit rRNA gene of* Blastocystis *used in the study*

Primer set	Primer sequence	Ref.
bl1400ForC	5′-GGAATCCTCTTAGAGGGACACTATACAT-3′	[23]
bl1710RevC	5′-TTACTAAAATCCAAAGTGTTCATCGGAC-3′	[23]
RD3	5′-CGGATCCTGATCCTTCCCCAGGTTCACCTAC-3′	[14]
RD5	5′-GGAAGCTTATCTGGTTGATCCTGCCAGTA-3′	[14]
F1	5′-GGAGGTAGTGACAATAAATC-3′	[6]
R1	5′-CGTTCATGATGAACAATTAC-3′	[6]
RSBHpyro2F	5′-GCGAAAGCATTTACCAAGGATGT-3′	[24]
RSBHpyro2R	5′-Biotin-CCGGAACCCAAAGACTTTGAT-3′	[24]
RSBHpyro2S	5′-TAGATACCCTCGTAGTCTTA-3′	[24]

PCR for *D. fragilis*

Faecal, genomic DNA from all study individuals was also screened for *D. fragilis* by PCR using the primers *D. fragilis*F 5′-CGGAGGTGGTAATGACCAGTTAT-3′ and *D. fragilis*R 5′-TTGCAGAGCTGGAATTACCG-3′, with standard PCR reagents and conditions [25]. Internal process controls were incorporated to identify cases of inhibition.

Questionnaires – data collection

The NORMAT questionnaire covered information on gender, age and employment, travel in the 3 months before diagnosis, medications, GI diseases and symptoms during the most recent month, other symptoms, food consumption in the month before diagnosis, and contact to animals 7 days prior to diagnosis. Participants were asked to complete the questionnaire as soon as possible after collecting the faecal sample. Upon receipt of the sample in the laboratory, investigators contacted participants for a telephone interview based on their written responses.

Individuals in the FU study were invited to complete a questionnaire which included information on demographic factors, travel activity, regular contact with animals, dietary preferences, symptoms at the time of diagnosis and at follow-up, chronic conditions, hospital admission and chemotherapeutic treatment.

For both studies, individuals were excluded from the study if a potential or known enteric pathogen other than *Blastocystis* or *D. fragilis* was isolated from the sample.

Statistical analysis

Questionnaire data from both studies were entered into Excel spread sheets and imported into the Stata 9.0/SE software program (Stata Corporation, USA) using StatTransfer 7.0 (Circle Systems, USA).

NORMAT study

As the dataset consisted of an unselected group from the general population with individuals both positive and negative for *Blastocystis* and *D. fragilis*, it was analysed retrospectively as a cohort study. Separate analyses were undertaken for *Blastocystis* and *D. fragilis*. Relative risks (RR) of infection associated with potential risk factors, and 95% confidence intervals (CI) were calculated using Fisher's exact test. In addition, the RR of various outcomes (symptoms and conditions) potentially associated with infection and 95% CI were calculated using Fisher's exact test.

Multivariable logistic regression was used to model potential risk factors for *Blastocystis* and *D. fragilis* selecting variables based on a P value of $<0{\cdot}2$ from the univariate analysis. Stepwise exclusion was used in the reduction of the model using the likelihood ratio test.

FU study

As all individuals in the dataset were positive for *Blastocystis*, we examined for association between risk factors or symptoms and distinct subtypes by Fisher's exact test. Multivariable analysis included variables with a P value $<0{\cdot}2$ and possible confounders where relevant. A non-parametric test, Kruskal–Wallis one-way ANOVA, was used to examine the difference in age in the subtypes.

Ethical considerations

The Scientific Ethics Committee for the Copenhagen and Frederiksberg municipalities approved the

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Table 2. *Subtypes of* Blastocystis *cases from the NORMAT group with and without a history of gastrointestinal (GI) symptoms*

Blastocystis sp. subtype	No. of infections in individuals with GI symptoms	No. of infections in individuals without GI symptoms	Total
ST1	0	2	2
ST2	0	3	3
ST3	2	4	6
ST4	2	7	9
ST9	1	0	1
MSI	0	3	3
Total	5	19	24

MSI, Mixed subtype infections: ST2 and ST3, ST2 and ST4, ST2 and ST4 and ST6.

protocols for the NORMAT study (KF 01-006/02) and the FU study (KF 01-188/04). Written consent was obtained from all participants who were ensured confidentiality.

RESULTS

NORMAT study

Microbiological and demographic data

The study comprised 134 study individuals. Seven individuals were excluded due to the presence of *Clostridium difficile*, enteroaggregative- or verocytotoxin-producing *Escherichia coli* and one was excluded due to *Giardia duodenalis* infection, which left 126 individuals in the dataset. By PCR, 24 (19%) individuals were shown to host *Blastocystis*, 14 (58%) of whom had been negative for *Blastocystis* by microscopy of faecal concentrates.

Co-infection with *D. fragilis* was seen in four cases, and in total, 16 (13%) persons had *D. fragilis*. Ninety individuals were negative for all pathogens tested for. The median ages of solely *Blastocystis*- and *Dientamoeba*-infected individuals were 42 years (range 4–76 years) and 41 years (range 2–71 years), respectively. The median age of pathogen-free individuals was 46 years (range 1–81 years). In total, 16 individuals, four of whom had *Blastocystis*, one *D. fragilis*, and one dual infection, reported having experienced GI symptoms in the month before stool submission. The subtypes of *Blastocystis* cases with and without GI symptoms are given in Table 2. Mixed *Blastocystis* subtype infection (MSI) was seen in three individuals. The most common subtypes (ST) were ST3 and ST4, isolated from 75% of the persons, most of whom were asymptomatic (78%) (Table 2).

Risk analysis of separate variables

When comparing the 24 *Blastocystis*-positive individuals to 102 individuals negative for *Blastocystis*, a significant association was seen between irritable bowel syndrome (IBS) and *Blastocystis* infection (Table 3). However, due to the small number of *Blastocystis*-positive individuals ($n=5$), IBS could not be correlated to any particular subtype. *Blastocystis* carriage was not associated with reports on GI symptoms (Table 3), and neither *Blastocystis* nor *D. fragilis* infections were associated with chronic diseases such as gastric ulcer ($n=0$), enteric cancer ($n=0$), intestinal radiation damage ($n=0$), inflammatory bowel disease ($n=0$), Crohn's disease ($n=0$), other chronic intestinal inflammation ($n=0$) or other enteric disease ($n=1$).

Fifty-five persons were asked to provide information on conditions considered to be sequelae of infection, and joint pain was associated with *Blastocystis* infection (RR 3·20, 95% CI 1·03–9·97, $P=0·07$). All *Blastocystis*-infected individuals complaining of joint pains ($n=4$) reported pain in multiple joints, each describing pain in the knees.

Contact to pigs or poultry was strongly associated with *Blastocystis* infection (Table 4). Three *Blastocystis*-positive individuals reporting contact with pigs hosted ST2 and ST3 (one case had a mixed ST2/3 infection), and those five reporting contact with poultry had ST1, ST2, ST3 and ST4 (one case had ST2 and ST3). Drinking unpasteurized milk (RR 2·11, 95% CI 0·79–5·59, $P=0·18$) and contact with rabbits (Table 4) had P values $<0·2$. Due to co-linearity and small numbers, a multivariable logistic regression model could not be built.

Travel 3 months prior to diagnosis was found not to be a risk factor for *Blastocystis* infection in the univariate analysis (RR 1·00, 95% CI 0·47–2·15, $P=1·00$).

Fifteen individuals reported having rabbits as pets, and five of these were positive for *D. fragilis* (data not shown). Contact with poultry and game had P values $<0·2$ and were therefore also included in a logistic regression model; contact with rabbits remained as the only independent variable (RR 3·60, 95% CI 1·42–9·10, $P=0·02$).

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Table 3. *Univariate analysis of* Blastocystis *infection, gastrointestinal (GI) symptoms and irritable bowel syndrome (IBS) in the NORMAT group*

Clinical condition	*Blastocystis* positive ($n=24$)	*Blastocystis* negative ($n=102$)	RR (95% CI)	*P* value
GI symptoms ($n=16$)	5 (21%)	11 (11%)	1·89 (0·73–4·94)	0·19
IBS ($n=11$)	5 (21%)	6 (6%)	3·51 (1·17–10·54)	0·04

RR, Relative risk; CI, confidence interval.

Table 4. *Univariate analysis of* Blastocystis *infection and animal contact 7 days before diagnosis in the NORMAT group*

	Blastocystis cases (including those co-infected with *Dientamoeba fragilis*)			
Variable	Cases in those exposed (%)	Cases in those not exposed (%)	RR (95% CI)	*P* value
Pig	3/3 (100%)	21/123 (17%)	5·86 (3·97–8·65)	<0·01
Poultry	5/11 (45%)	19/115 (17%)	2·88 (1·33–6·24)	0·03
Rabbit	5/15 (33%)	19/111 (17%)	2·00 (0·87–4·59)	0·15
Cat	11/44 (25%)	13/82 (16%)	1·63 (0·78–3·37)	0·23
Horse	1/14 (7%)	23/112 (21%)	0·35 (0·05–2·38)	0·30
Cattle	2/6 (33%)	22/120 (18%)	1·82 (0·55–5·99)	0·32
Dog	12/64 (19%)	12/62 (19%)	1·15 (0·52–2·51)	0·81
Sheep or goat	1/7 (14%)	23/119 (19%)	0·73 (0·12–4·67)	1·00
Wild birds	0/1 (0%)	0/125 (0%)	0·00 (—)	n.a.
Game	0/1 (0%)	0/125 (0%)	0·00 (—)	n.a.
Other animals and birds	0/7 (0%)	0/119 (0%)	0·00 (—)	n.a.

RR, Relative risk; CI, confidence interval; n.a., not applicable.

FU study

Microbiological and demographic data

The study included 111 patients. The average number of stool specimens submitted per patient for follow-up examination was 2·8; no patient submitted more than three stool samples. A total of 93 (83·8%) patients were *Blastocystis*-positive at the time of follow-up by PCR. The remaining 18 were negative by microscopy, culture and PCR and were therefore excluded as was one patient positive for *Cryptosporidium*; this left 92 cases in the dataset. Fifty-six patients had pure *Blastocystis* infections, whereas 32 (34·8%) were co-infected with *D. fragilis*. In four cases, *D. fragilis* PCR analysis was inconclusive. The subtype distribution of *Blastocystis* isolates related to symptoms is listed in Table 5. Overall, taking single and MSI together, ST3 was the most common subtype detected in 35 (38%) of the patients, followed by ST2 and ST1 in 33 (36%) and 29 (32%), respectively. *Blastocystis* sp. subtype was not associated with *D. fragilis* co-infection ($P=0·26$, Fisher's exact test). Age was significantly associated with discrete subtypes ($P=0·02$, Kruskal–Wallis test), those having ST3, ST4 and ST7 being older (median ages 60, 54 and 46 years, respectively) compared to ST1 (27 years).

Analysis of Blastocystis *infection outcomes*

Reported symptoms of FU study individuals are listed in Table 6, with diarrhoea being the most common symptom (70%). At follow-up, 74% still had symptoms. Eighteen (20%) reported having one or more chronic disease(s): One had Crohn's disease, one had diverticulosis, seven had IBS, one had celiac disease, five suffered from allergic diseases, three had gastric ulcer, one had urticaria and four reported of arthritis. The subtype distribution of *Blastocystis* in IBS patients was independent of symptoms of IBS ($P=0·97$, Fisher's exact test) and patients with chronic diseases did not harbour specific subtypes (data not shown).

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Table 5. Blastocystis *sp. subtype distribution related to symptoms in the Follow-up group* ($n=92$)

Blastocystis sp. subtype (ST)	Any symptoms (%)	No symptoms (%)
Single subtype infections		
ST1	18 (21)	1 (14)
ST2	17 (20)	2 (29)
ST3	13 (15)	2 (29)
ST4	10 (12)	1 (14)
ST7	5 (6)	0 (0)
Mixed subtype infections		
ST2, ST3	10 (12)	0 (0)
ST1, ST2	1 (1)	0 (0)
ST1, ST2, ST3	1 (1)	0 (0)
ST1, ST3	6 (7)	0 (0)
ST1, ST3, ST4	2 (2)	0 (0)
ST2, ST4	2 (2)	0 (0)
ST3, ST7	0 (0)	1 (14)
Total	85 (99)*	7 (100)

* Percentages only add up to 99% due to rounded figures.

Table 6. *Reported gastrointestinal (GI) symptoms in* Blastocystis *cases with (+) or without (−)* Dientamoeba fragilis *(DF) in the Follow-up group*

Symptom	All cases (± DF) ($n=92$)	Cases without DF ($n=56$)
GI symptoms*	80 (87%)	49 (88%)
Diarrhoea	64 (70%)	42 (75%)
Abdominal pain	57 (62%)	34 (61%)
Flatulence	40 (43%)	26 (46%)
Bloating	35 (38%)	24 (43%)
Anorexia	30 (33%)	22 (39%)
Weight loss	28 (30%)	21 (38%)
Nausea/vomiting	26 (28%)	19 (34%)
Slime in stool	22 (24%)	12 (21%)
Constipation	13 (14%)	9 (16%)
Joint pain	13 (14%)	7 (13%)
Erythema	9 (10%)	4 (7%)
Fever	7 (8%)	6 (11%)
Blood in stool	7 (8%)	4 (7%)
Symptoms at follow-up	68 (74%)	42 (75%)
None of the above symptoms	7 (8%)	4 (7%)

* Diarrhoea, nausea/vomiting or abdominal pain.

The most common reported symptoms lasting for $\geqslant$1 month were diarrhoea (25/92, 27%) and abdominal pain (21/92, 23%). ST1 or ST2 were hosted by >50% of patients with long-term diarrhoea ($n=14$) or abdominal pain ($n=11$). Of six patients infected with ST7, five (83%) complained of multiple GI symptoms, all describing diarrhoea and abdominal pain.

Reports on bloating at follow-up were associated with subtype ($P=0{\cdot}02$, Fisher's exact test), with most case-patients having ST2 ($n=12$, $P<0{\cdot}01$). Seven patients reported blood in stool, four of whom had MSI ($P=0{\cdot}06$, Fisher's exact test).

A total of 68 (74%) of the patients had been travelling in the year before diagnosis, which left very few cases for an analysis of risk factors in Denmark. Analysis of all cases, irrespective of whether they travelled or not, could not establish any significant risk factor associated with a particular subtype (data not shown).

Of those with any symptoms ($n=85$), 40 (47·0%) received medical treatment because of their symptoms. Of those treated with standard drugs 23/31 continued to have symptoms or experienced the return of symptoms after treatment. A total of 17 took metronidazole treatment as prescribed, 10 (59%) of whom reported reoccurrence of symptoms at the time of follow-up.

DISCUSSION

The present investigation is among the most comprehensive with regard to subtype analysis of *Blastocystis* from human hosts, and the first to generate data from risk-factor analysis of *Blastocystis* in symptomatic and asymptomatic individuals. Moreover, it is the first study to identify potential risk factors for *D. fragilis* infection.

The subtype distribution detected in the studies reflects the distribution reported in studies from Eurasian countries [11], the most frequent subtypes found in humans being ST1–ST4. Various studies identified ST3 as the most common subtype [6, 11, 19, 21, 23, 26] accounting for up to 75·9% and 70·5% of subtypes detected in Turkey [11] and China [21], respectively.

Whereas ST1 was rare in NORMAT individuals, it was present in 32% of the patients in the FU study. Conversely, the share of single subtype infections due to ST3 and ST4 was much larger (63%) in the NORMAT group than in the FU group (28%). ST7 isolates were found only in FU individuals. These findings may be central in the understanding of the transmission and relative pathogenicity of separate subtypes and partly reinforce trends from other

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studies in which ST1 has been identified as 'pathogenic' and ST3 'non-pathogenic' [8, 10].

ST5–ST9, occurring infrequently in humans, could be mainly zoonotically transmitted [2]. In the present study, ST7 and ST9 isolates were seen in seven of the individuals, six (86%) of whom were symptomatic. It should be investigated to what extent such potentially zoonotic subtypes are correlated with disease, and clearly, more studies should aim at clarifying issues related to transmission routes and host specificity.

MSI were more prevalent in the FU individuals (26% *vs.* 12% in the NORMAT group) and most patients reporting blood in stool had MSI. Therefore, the data might indicate that MSI are more prevalent in individuals seeking medical advice due to GI symptoms, and this should be explored in future studies. Taking both studies together, the proportion of MSI was remarkably high compared to previous reports, but this may be due to the fact that 2–3 PCR assays were used in concert to characterize each isolate, and preferential amplification of discrete subtypes by different primers has previously been accounted for [24, 26]. Hitherto, MSI has been detected in up to 14·3% of analysed cases [10]. A high prevalence of MSI could indicate that super-infection occurs frequently and/or that infections persist for a long time if left untreated.

The NORMAT study saw a strong association between IBS and *Blastocystis* infection. IBS could result in an increased susceptibility of *Blastocystis*, or a tendency for longer carriage. It has also been speculated whether *Blastocystis* could be (partly) responsible for the development of IBS [27–30], and it should be investigated whether certain subtypes of *Blastocystis* could be linked to IBS. It is suggested, that the focus on resolving differential diagnostic aspects of IBS is increased, and therefore patients suspected of IBS should be examined for *Blastocystis*. Moreover, future studies should include large-scale molecular analyses of *Blastocystis* isolates from IBS patients and controls.

Reports on joint pain were associated with *Blastocystis* infection. A few studies have reported *Blastocystis*-related reactive arthritis [31–33], suggesting that also this topic requires further research. In the NORMAT study, travel was found not to be a risk factor for *Blastocystis* infection, thus endorsing previous data indicating that *Blastocystis* infection is of endemic occurrence in Denmark [18].

A recent study [21] showed that pig ownership was a risk factor for *Blastocystis* infection. Similarly, in the NORMAT study, risk factors included contact with pigs or poultry within 7 days prior to stool submission. This is not surprising considering accumulating data from molecular studies of *Blastocystis* from potential reservoir hosts [3–5, 34–37]. No particular subtype was associated with pig or poultry contact, and individuals reporting such contact were shown to host ST1, ST2, ST3 and/or ST4, which are the subtypes usually isolated from humans. However, pigs have been shown to host ST1, ST2, ST3 and ST5 (Stensvold *et al.*, unpublished observations). Contact with rabbits was a risk factor for *D. fragilis* infection. To date there have been no reports on *D. fragilis* in animals, except for pigs [38] and gorillas [39].

The overall prevalence of *Blastocystis* and *D. fragilis* in the NORMAT group was surprisingly high, almost comparable to the prevalence in symptomatic individuals reported in a previous study [18], which might be explained by the fact that the response rate in the NORMAT study was little more than 10%. This could indicate that individuals particularly predisposed for activities related to this study participated, e.g. persons with suspected, current or previous (intestinal) disease.

In the FU study, 92 (84%) of the patients were still positive for *Blastocystis* at the time of follow-up. Unless these patients resolved infection spontaneously or by treatment and then became re-infected before follow-up, this suggests that *Blastocystis* infections typically persist for at least several weeks.

Although considered the drug of choice for eradication of *Blastocystis*, there have been several reports on the failure of metronidazole treatment [16, 29, 40]. In the FU study, 59% of patients taking metronidazole treatment as prescribed by their GP experienced a recurrence of symptoms, and all patients were still positive for *Blastocystis* at follow-up. It is possible that parasite load is reduced during metronidazole treatment below the detection limit of microscopy methods [16]. While these data are strongly indicative of limited efficacy of metronidazole treatment, randomized controlled treatment studies are needed to further elucidate this.

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DECLARATION OF INTEREST

None.

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