First report on the prevalence and subtype distribution of Blastocystis sp. in dairy cattle in Lebanon and assessment of zoonotic transmission

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Abstract

Blastocystis sp. is frequently identified in a wide range of animal hosts, including bovids. Because of its burden and zoonotic potential, this parasite has been sought in domestic cattle from various countries, since this livestock may also represent a possible reservoir of human infection. However, epidemiological data regarding the prevalence and ST distribution of *Blastocystis* sp. in this animal group is lacking in Lebanon. Therefore, faecal samples were collected from a total of 254 dairy cattle raised on 55 farms located in the North Lebanon region and screened for the presence of the parasite by quantitative real-time PCR. The overall prevalence of *Blastocystis* sp. was shown to reach 63.4% in cattle livestock. Sequence analysis of positive samples indicated the presence of seven STs, with predominance of ST10 (44.0%) and ST14 (36.8%) and lower proportions of ST2 (8.0%), ST1 (7.2%), ST5 (2.4%), ST3 and ST7 (0.8% each). This survey was the first conducted worldwide reporting ST2 and ST7 in domestic cattle and confirmed that ST10 and ST14 represent cattle-adapted STs in view of their high prevalence. Faecal samples from in-contact dairy farmers and patients hospitalised in the same Lebanese governorate who reported no contact with cattle livestock were also analysed for the presence of *Blastocystis* sp. The same three STs were identified in both human cohorts, with predominance of ST3, followed either by ST1 or ST2 depending of the group. No other STs, including ST10 or ST14, have been reported. Moreover, even though ST1, ST2 and ST3 were found to be common to dairy cattle and farmers cohorts, only one ST3 isolate showed 100% sequence identity between both hosts. Consequently, the presence and low prevalence of ST1, ST2, ST3, ST5 and ST7 identified herein in domestic cattle, most of which exhibit low host specificity, could be derived from occasional direct exposure to faecal material from human and non-human hosts or by ingestion of contaminated drinking water or food in the enclosure of the farms.

Together with the absence of ST10 and ST14 in the human population, these data suggest that cattle play a negligible role as zoonotic reservoirs of *Blastocystis* sp.

Keywords: *Blastocystis* sp., Dairy cattle, Intestinal parasite, Molecular epidemiology, Transmission, Zoonosis

1. Introduction

Blastocystis sp. is currently the most common single-celled intestinal parasite found in humans, since its prevalence can greatly exceed 50% in developing countries (El Safadi et al., 2014; Forsell et al., 2016) and reach an average of around 20% in industrialised regions of Europe (Bart et al., 2013; El Safadi et al., 2016). This protozoan has also been frequently found in many groups of animals, including non-human primates and various groups of mammals, birds, reptiles, amphibians and insects (Tan, 2004; Stensvold and Clark, 2016; Yoshikawa et al., 2016; Cian et al., 2017). The principal mode of transmission of *Blastocystis* sp. is the faecal-oral route, essentially through consumption of water or food contaminated by environmentally resistant cystic forms of the parasite (Tan, 2008). Due to the very common asymptomatic carriage of the parasite in the human population worldwide, its pathogenic potential and clinical significance is remained uncertain (Andersen and Stensvold, 2016). However numerous recent *in vitro* studies have identified the mechanisms and molecules involved in the virulence of the parasite (Clark et al., 2013; Wawrzyniak et al., 2013; Ajjampur and Tan, 2016; Stensvold and Clark, 2016).. *Blastocystis* sp. infection would thus be associated with various gastrointestinal disorders (Tan, 2008; Tan et al., 2010) and/or urticaria (Lepczynska et al., 2015) in numerous human clinical cases.

An extensive genetic diversity has been demonstrated within the genus *Blastocystis* based on the comparison of small subunit (SSU) rRNA gene sequences (Noël et al., 2005; Stensvold et al., 2007). Indeed, 17 subtypes (STs) have been identified so far in mammalian and avian hosts (Alfellani et al., 2013a), ten of which infect humans with varying prevalence (Alfellani et al., 2013b; Clark et al., 2013; Ramirez et al., 2016; Stensvold and Clark, 2016). Briefly, more than 90% of human isolates belong to ST1 to ST4, in large part likely related to human-to-human transmission (Alfellani et al., 2013b; Clark et al., 2013). Most other STs found in the human population are considered to be of animal origin, with low host

specificity and are potentially zoonotic. For instance, ST5 frequently infects pigs (Wang et al., 2014), whereas ST6 and ST7 are predominant in birds (Cian et al., 2017). Based on large-scale epidemiological surveys, these last STs were considered to be pig- and avian-adapted STs respectively, and corresponding isolates were shown to be possibly transmitted from these animals to their in-contact workers at intensive commercial piggeries and poultry slaughterhouses (Wang et al., 2014; Greige et al., 2018). In addition, ST8, which is common in non-human primates, was identified with unexpected high prevalence in their keepers (Stensvold et al., 2009). In view of these data, it became necessary to determine the infection rate and ST distribution of *Blastocystis* sp. in other animal groups, such as bovids and particularly livestock cattle. In the main recent molecular epidemiological surveys including a large number of specimens (Zhu et al., 2017; Lee et al., 2018; Masuda et al., 2018; Wang et al., 2018; Maloney et al., 2019), the prevalence of the parasite in domestic dairy/beef cattle varied between 2.9% and 54.1%, depending on the country. The potential significant occurrence of *Blastocystis* sp. in livestock cattle could thus generate a risk of zoonotic transmission, especially in dairy farmers. Moreover, the same molecular studies and a few others identified ST10 and ST14 as the predominant STs in cattle, likely reflecting bovids as natural hosts for both STs (Cian et al., 2017; Zhu et al., 2017; Masuda et al., 2018). Strikingly, ST10 and ST14 have never yet been reported in human infections, suggesting minimal risk of zoonotic transmission (Cian et al., 2017; Wang et al., 2018). However, neither of these STs has so far been sought in cattle and their in-contact farmers to confirm this hypothesis.

The objectives of this study was thus to obtain the first molecular data regarding the prevalence and ST distribution of *Blastocystis* sp. in dairy cattle in Lebanon. A particular purpose was to confirm that bovids represent natural hosts of ST10 and ST14. In addition, the potential zoonotic transmission of the parasite was assessed through a comparative analysis

of the ST distribution of isolates identified in dairy cattle and in-contact farmers, as well as in individuals reporting no contact with these animals.

2. Materials and methods

2.1. Ethics statement

The protocol of this study was approved by the Lebanese Minister of Public Health with reference number 2014/4/39716 and by the research ethics committee of Hamidi Medical Centre in Tripoli, Lebanon, with reference number Hamidi-7-2017. Oral and written informed consent was obtained from all subjects included in this study after a clear explanation of the research objectives prior to enrolment. The subjects' data were collected anonymously (with encryption of the identity of individuals). This study was conducted in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki). All dairy cattle samples were collected under the supervision of the farmers. No approval from the Institutional Animal Care and Use Committee or ethics committee was necessary, as no invasive sampling approach was performed.

2.2. Study sites and sample collection

The study was conducted in the two governorates comprising the North Lebanon region, namely Akkar and North governorates, the latter including the regional capital Tripoli (Figure 1). All healthy adult dairy cattle included in this study were older than 12 months and were of the Holstein-Friesian breed (*Bos taurus*). In Akkar governorate, 157 faecal specimens were randomly collected from 22 villages and 36 farms from July to November 2016. In order to obtain epidemiological data from dairy cattle in different geographical areas in Lebanon, 97 samples were also randomly obtained from 15 villages and 19 farms from July to November 2017 in North governorate, for an overall total of 254 cattle samples screened in

this survey (one specimen per animal). Briefly, faecal samples were collected in the early morning with the assistance of the breeders and veterinarians. Specimens were either collected directly from the rectum of the animal with a gloved hand or directly from the ground immediately after defecation to prevent potential contamination between animals. Each fresh faecal sample was recovered in a sterile plastic container, respecting the conditions of asepsis and hygiene. The containers were stored in ice and then immediately transported to the Laboratoire Microbiologie Santé et Environnement (LMSE) at the AZM Centre in Tripoli. To evaluate the zoonotic potential of *Blastocystis* sp. isolates identified in dairy cattle, human stool samples were obtained during the same period from 40 farmers working at the same 19 dairy cattle farms located in North governorate and from 40 individuals followed up for different pathologies or who presented for routine medical check-ups at Hamidi Medical Centre in Tripoli who reported no contact with cattle (control population). A standardised questionnaire was designed to collect data of interest, such as age, sex, residency and gastrointestinal symptoms (abdominal pain, bloating, constipation, diarrhoea and/or vomiting) for each volunteer, as well as number of years as in-contact worker with cattle for the cohort of dairy farmers.

2.3. DNA extraction

Total genomic DNA was extracted directly from approximately 250 mg of animal or human faecal samples using the QIAamp DNA Stool Mini Kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's recommended procedures. The DNA was eluted in 200 µl of elution buffer and stored at -20°C at the LMSE in Tripoli. The DNA samples were then transported to the Pasteur Institute in Lille (France) for molecular screening and subtyping of *Blastocystis* sp.

2.4. Detection and molecular subtyping of *Blastocystis* sp. isolates

SSU rRNA gene detection of the parasite was performed by quantitative real-time PCR (qPCR) using 2 µl of extracted DNA and the *Blastocystis*-specific primer pair BL18SPPF1 / BL18SR2PP, as described previously by Poirier et al. (2011). DNA extraction controls (isolation of DNAs without stool and from a *Blastocystis* sp.-negative stool) subsequently used in qPCR assays and positive (DNA obtained from *Blastocystis* sp. ST7 strain B axenic culture maintained in the laboratory) and negative (DNA matrix replaced by water) qPCR controls were performed. The qPCR product from each positive sample was purified and sequenced in both strands by Genoscreen (Lille, France). For 3 human samples obtained from breeders and 3 others collected from patients followed at Hamidi Medical Centre in Tripoli, sequence chromatogram analysis revealed the presence of double traces, suggesting mixed infection by different STs. Mixed signals were also generated for numerous qPCR products obtained from dairy cattle samples. All 6 human samples and a selection of 9 animal samples corresponding to mixed infections were thus re-analysed by non-qPCR using the same primer pair as for qPCR. Non-qPCR amplification, as well as purification and cloning of the non-qPCR product, were performed as described previously (Cian et al., 2017). Briefly, the purified non-qPCR product cloned in the T-vector, pCR 2.1-TOPO (Invitrogen, Carlsbad, USA) was amplified in *Escherichia coli* One Shot TOP10 competent cells and minipreparations of plasmid DNA were done using the NucleoSpin Plasmid kit (Macherey-Nagel, Düren, Germany). Five positive clones containing inserts of the expected size were selected arbitrarily and sequenced on both strands. The SSU rRNA gene sequences obtained in this study were deposited in GenBank under accession numbers MH883051 to MH883225. The sequences obtained were compared with all *Blastocystis* sp. homologous sequences available from the National Centre for Biotechnology Information (NCBI) using the nucleotide Basic Local Alignment Search Tool (BLAST) program. The STs were identified

by determining the exact match or closest similarity to all known mammalian and avian *Blastocystis* sp. STs according to the most recent classification of the parasite (Alfellani et al., 2013a).

2.5. Statistical analyses

Statistical analyses were performed using GraphPad Prism software, version 6 (GraphPad Software, La Jolla, CA, USA). The chi-squared test was used to compare the *Blastocystis* sp. prevalence between the Akkar and North governorate dairy cattle groups. Fisher's exact test was used to compare the ST distribution of the parasite between the two human cohorts, as well as those of the two animal groups. All tests were two-sided and the general significance level was set at a *P* value of below 0.05.

3. Results and discussion

A total of 254 single dairy cattle samples collected from 37 villages and 55 farms located in two governorates of North Lebanon were screened in this study (Table 1). Of these samples, 157 were obtained at Akkar governorate from cattle raised on 36 different farms. Of these 157 samples, 78.3% (n = 123) were identified as positive for *Blastocystis* sp. by qPCR. Of the 97 dairy cattle samples collected from 19 different farms in North governorate, 38 (39.2%) were also positive for the parasite by qPCR. The statistically significant difference of *Blastocystis* sp. prevalence in cattle between the two geographical regions (chi-squared test, $Z = 4.498$, $P < 0.05$) may possibly be due to the lower quality hygiene and sanitary conditions at farms in the rural governorate of Akkar, thus facilitating the transmission of the parasite between livestock. By combining the molecular data obtained from the two governorates, the prevalence of *Blastocystis* sp. reached 63.4% (161/254) among dairy cattle samples. This high infection rate in Lebanon was roughly similar to that observed using molecular methods

in other countries, including Japan (54.1%) (Masuda et al., 2018), Thailand (50.0%) (Udonsom et al., 2018), Colombia (80.0%) (Ramirez et al., 2014) and Libya (41.7%) (Alfellani et al., 2013a) but was significantly higher than that reported in Korea (6.7%) (Lee et al., 2018), China (9.5% and 10.3%) (Zhu et al., 2017; Wang et al., 2018, respectively), Iran (9.6%) (Badparva et al., 2015), the United Kingdom (22.6%) (Alfellani et al., 2013a) and the United States (19.1% and 2.9%) (Fayer et al., 2012; Maloney et al., 2019, respectively). Additional surveys also revealed a prevalence of 71.0%, 21.4% and 1.8% in Japan (Abe et al., 2002), Brazil (Franco Moura et al., 2018) and Spain (Quilez et al., 1995) respectively, using direct light microscopy of faecal smears, in the knowledge that this identification method has been shown to be less sensitive than PCR (Poirier et al., 2011). Although the prevalence of the parasite in domestic cattle may vary between geographical areas, it is nevertheless common worldwide, thus generating a potential risk of transmission to in-contact humans.

To start assessing the zoonotic potential of the parasite, single stool samples were collected from a total of 80 volunteers divided into two cohorts (Table 1). The first cohort included 40 individuals (26 males and 14 females), all working on dairy cattle farms in North governorate for a period of between 6 months and 30 years, with a median of 8 years, indicating a potentially long period of contact with animals. The age of the individuals in this cohort was between 22 and 65 years (mean age of 36 ± 10 years) and 21 of these 40 breeders (52.5%) were shown to be infected with *Blastocystis* sp. by qPCR. Moreover, only 5 breeders presented gastrointestinal symptoms and 3 of them were infected by the parasite. The second cohort (or control group) consisted of 40 patients (12 males and 28 females) aged 21 to 70 years (mean age of 24 ± 12 years), hospitalised at Hamidi Medical Centre in Tripoli for various pathologies or medical check-ups, who reported no contact with cattle. 62.5% of the patients (25/40) presented one or more gastrointestinal symptoms. Using qPCR, the

prevalence of *Blastocystis* sp. in this second human cohort was not significantly different to that of the dairy farmers, i.e. 57.5% (23/40). In addition, 52.0% of symptomatic patients (13/25) were infected by the parasite. Interestingly, the infection rate observed in each of these two groups (52.5% and 57.5% respectively) was very similar to that recently reported in North Lebanon region in a large cohort of schoolchildren (63.0%) (Osman et al., 2016) and two groups of individuals working in a poultry slaughterhouse (56.0%) or hospitalised at Hamidi Medical Centre in Tripoli (54.0%) (Greige et al., 2018). On the other hand, the similar prevalence observed between all these Lebanese groups of individuals suggested that people working closely with animals, including for instance domestic cattle or poultry (Greige et al., 2018) but also pigs (Wang et al., 2014), did not systematically have a higher risk of acquiring *Blastocystis* sp. infection as previously proposed (Rajah Salim et al., 1999), without affecting the possibility that some STs are of zoonotic origin.

In a second step, the genetic diversity of *Blastocystis* sp. isolates was explored in dairy cattle and human cohorts. In this study, all partial SSU rRNA gene sequences obtained from animal or human samples showed 99 to 100% identity with homologous sequences available in databases allowing the direct subtyping of the corresponding isolates (Table 1). Regarding the 123 positive dairy cattle samples identified in Akkar governorate, 84 corresponded to single infections by either ST1 (n = 4, 3.3%), ST5 (n = 3, 2.4%), ST7 (n = 1, 0.8%), ST10 (n = 40, 32.5%) and ST14 (n = 36, 29.3%). The 39 remaining positive dairy cattle samples (31.7%) from this governorate presented mixed infections with at least 2 unidentified STs according to the resulting sequence chromatograms. Of the 38 positive animal specimens identified in North governorate, 21 corresponded to single infections with the detection of ST2 (n = 1, 2.6%), ST3 (n = 1, 2.6%), ST10 (n = 12, 31.6%) and ST14 (n = 7, 18.4%) and the 17 other samples (44.8%) to mixed infections. Given the large number of mixed infections identified in animal samples collected in both governorates, only a selection of them was thus

254 re-analysed by non-qPCR to have a partial overview of the STs found in these specimens.
255 Therefore, one sample from each farm presenting mixed infection in North governorate was
256 selected. Cloning of the non-qPCR products was performed from these 9 representative
257 animal specimens for subtyping, and 5 positive clones were selected arbitrarily and
258 sequenced for each cloning. Seven of the 9 selected samples showed mixed infections with
259 two different STs (ST2/ST1, *n* = 4; ST2/ST10, *n* = 1; ST2/ST14, *n* = 2) whereas the two
260 remaining samples harboured 3 different STs (ST1/ST2/ST10 and ST2/ST10/ST14
261 respectively). Taking into account these 9 mixed infections consisting of either two or three
262 different STs, the ST distribution in dairy cattle from this governorate was as follows: ST1 (*n*
263 = 5), ST2 (*n* = 10), ST3 (*n* = 1), ST10 (*n* = 15) and ST14 (*n* = 10). The major difference
264 observed between these two animal cohorts in terms of distribution of STs was the presence
265 of ST2 only in the group of North dairy cattle, while it was absent from the Akkar group of
266 animals (Fisher's exact test, 24.4% vs. 0%, $P < 0.05$). By merging all molecular data
267 obtained from dairy cattle samples in North Lebanon region, a total of 125 isolates were
268 subtyped and belonged to 7 different STs with, in order of prevalence, ST10 (55/125, 44.0%),
269 ST14 (46/125, 36.8%), ST2 (10/125, 8.0%), ST1 (9/125, 7.2%), ST5 (3/125, 2.4%), ST3 and
270 ST7 (1/125 each, 0.8%). Herein, ST10 and ST14 were thus largely predominant by
271 accounting for more than 80% of the subtyped isolates. As shown in Table 2 reporting all the
272 isolates subtyped from domestic cattle in various geographical areas, ST10 and ST14 also
273 globally represented the most widely distributed STs (64.5% of total isolates). This was
274 clearly the case, for instance, in Denmark (Stensvold et al., 2009), Japan (Masuda et al.,
275 2018), Korea (Lee et al., 2018), China (Zhu et al., 2017) and Libya (Alfellani et al., 2013a).
276 Strikingly, ST10 and ST14 were the only two STs identified in cattle in two studies
277 conducted in the USA (Santin et al., 2011; Fayer et al., 2012). Therefore, all of these data
278 confirmed that domestic cattle would be natural hosts for *Blastocystis* sp. ST10 and ST14. In

addition to these two predominant STs, five minor STs (ST1, ST2, ST3, ST5 and ST7) were identified in the livestock investigated in this study. Briefly, it was the first report of the presence of ST2 and ST7 in domestic cattle, while the three other STs had previously been detected with varying prevalence in the same animal group in different countries (Table 2).

Regarding the dairy farmers cohort, 18 individuals presented single infections by the parasite, and 3 others had mixed infections with two STs. With the addition of these mixed infections, a total of 24 isolates was subtyped. As shown in Table 1, ST3 was predominant (12/24, 50.0%), followed by ST2 (7/24, 29.2%) and ST1 (5/24, 20.8%). In the case of the second human cohort comprising patients enrolled at Hamidi Medical Centre in Tripoli, 20 and 3 of them presented single and mixed infections with two STs respectively. Therefore, a total of 26 isolates was subtyped and three different STs were identified as follows: ST3 (13/26, 50.0%), ST1 (8/26, 30.8%) and ST2 (5/26, 19.2%). Interestingly, no other ST was identified in these two human groups. Moreover, although the number of samples analysed in each cohort remained limited, the distribution of the different STs was not significantly different between the two human groups (Fisher's exact test, $P = 0.67$). Strikingly, this distribution with predominance of ST3 followed by either ST1 or ST2 was also similar to that observed in previous studies conducted recently among patients at the same hospital in Tripoli, staff members working in poultry slaughterhouses in North Lebanon (Greige et al., 2018) and among schoolchildren in Tripoli (Osman et al., 2016).

By analysing all the molecular data obtained herein, the zoonotic potential of *Blastocystis* sp. was evaluated through the comparison of the ST distribution reported in animal and human cohorts in North Lebanon. Despite the high prevalence of ST10 and ST14 in cattle livestock, neither ST was identified especially in dairy farmers despite these workers having intimate, long-term and repeated contact with these animals. To our knowledge, these two STs have also never been documented in human infections worldwide (Clark et al., 2013;

304 Stensvold and Clark, 2016), suggesting that humans would not be susceptible to their
305 infection. Another hypothesis that remains to be further confirmed could be that these two
306 STs produce non-resistant cystic environmental forms, thus limiting possible transmission of
307 the parasite to another host through indirect contact. ST5 was also infrequently identified in
308 dairy cattle, as in previous studies conducted in various countries (Table 2) and herein only in
309 animals raised at Akkar governorate. Recently, ST5 was proposed as a pig-adapted ST (Wang
310 et al., 2014) and consequently cannot likely be assigned to domestic cattle at this time. Its
311 zoonotic potential was confirmed through the frequent infection of piggery staff by this ST
312 (Wang et al., 2014) and pig-rearing villagers (Yan et al., 2007). However, despite the large
313 number of samples screened, ST5 has not yet been identified in this study as in other
314 Lebanese human cohorts (Osman et al., 2016; Greige et al., 2018). The only known case of
315 ST7 infection in cattle was identified in this study at a farm located at Akkar governorate.
316 This ST was proposed as an avian-adapted ST with potential zoonotic risk (Alfellani et al.,
317 2013a; Cian et al., 2017) and its sporadic prevalence in cattle livestock likely reflected
318 opportunistic infection through contact with bird faeces. Again, ST7 has never been yet
319 identified in the Lebanese human population (Osman et al., 2016; Greige et al., 2018), as
320 confirmed herein. Interestingly, three STs (ST1, ST2 and ST3) were found in common in
321 dairy cattle and their in-contact farmers in North governorate, suggesting potential
322 transmission between the hosts. However, only six animal isolates belonging to these STs and
323 as part of single or mixed infections were identified on 4 different farms at which dairy
324 farmers were infected by the same STs. By comparing the corresponding dairy cattle and in-
325 contact farmer isolate sequences, 100% identity was shown for only one ST3 isolate infecting
326 both hosts. Since ST3 is known to be predominantly harboured by the human population
327 (Clark et al., 2013; Stensvold and Clark, 2016) including Lebanon and not frequently found
328 in domestic cattle as shown in Table 2, this case of *Blastocystis* sp. ST3 infestation in

livestock can likely be explained by reverse zoonosis from human handlers to animals, as suggested in previous studies focused on various animal hosts (Ramirez et al., 2014) and pigs (Wang et al., 2014). Regarding the two others, ST1 and ST2, considered to be STs linked to human infection (Clark et al., 2013; Stensvold and Clark, 2016), their origin in Lebanese domestic cattle remains uncertain according to our sequence data. Globally, the low prevalence of ST1, ST2, ST3, ST5 and ST7 observed in the Lebanese cattle population suggests that these animals are unlikely to be natural hosts of these STs and are potentially opportunistically infected through direct exposure to faecal material from human and non-human hosts or by ingestion of contaminated drinking water or food in their environment, as proposed in both stray and household dogs (Wang et al., 2013; Osman et al., 2015). The difference in the prevalence of ST2 observed between dairy cattle raised in the two Lebanese governorates, while this ST is known to infect humans and numerous animal groups (Clark et al., 2013; Stensvold and Clark, 2016; Cian et al., 2017), reinforced this hypothesis by highlighting different infection sources in two different Lebanese geographical areas. However, since domestic cattle carry potential zoonotic STs, these animals are thus capable of shedding the corresponding isolates and may therefore represent secondary zoonotic reservoirs of infection.

4. Conclusion

This study presents the first molecular epidemiological data on *Blastocystis* sp. infection in cattle livestock in Lebanon. Through both the high number of samples screened and isolates subtyped, it provided new insights into the prevalence and ST distribution of the parasite in this animal host. This survey conducted in North Lebanon demonstrated that dairy cattle were frequently infected by *Blastocystis* sp., with an overall prevalence of the parasite exceeding 60%. This clearly raised the question of the impact of this infection in the bovid

sector, which is of major importance in this country. The variety of STs colonising this animal group was also highlighted, with a total of seven STs identified, including ST1, ST2, ST3, ST5, ST7, ST10 and ST14. ST10 and ST14 were largely predominant in this animal population, confirming that both STs could be considered cattle-adapted STs. Others STs were all identified with low prevalence and their presence was suggested to be related to opportunistic contamination from various animal or even human and environmental sources. Finally, by comparing the ST distribution of the parasite in cattle and in-contact dairy farmers, as well as in individuals without contact with bovids, the risk of zoonotic transmission of *Blastocystis* sp. was highlighted to be likely minimal. However, further large-scale studies should be conducted to fully understand the exact role of domestic cattle in the epidemiology of *Blastocystis* sp. and as potential sources of transmission of the parasite to humans, at least as secondary reservoirs.

Conflicts of interest

The authors declare that there are no competing interests.

Authors' contributions

D.E.S., M.O., G.C., M.H. and E.V. conceived the study and designed the experiments. S.G., D.E.S., S.K., N.G. and M.O. collected the samples and/or performed the experiments. M.B. performed the statistical analyses. S.G., D.E.S., S.K., S.B.V., M.Che, M.Cha, G.C., M.H. and E.V. participated in the interpretation of data. S.G., D.E.S., S.K. and E.V. wrote the manuscript. All authors have read and approved the final version of the manuscript.

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522

Table 1

Prevalence of *Blastocystis* sp. infection and ST distribution in animal and human cohorts in North Lebanon

Infection	Akkar dairy cattle (*n* = 157)	North dairy cattle (*n* = 97)	North breeders (*n* = 40)	North hospital patients (*n* = 40)
% positive	78.3% (123)	39.2% (38[a])	52.5% (21[b])	57.5% (23[c])
ST1	4	5	5	8
ST2	0	10	7	5
ST3	0	1	12	13
ST5	3	0	0	0
ST7	1	0	0	0
ST10	40	15	0	0
ST14	36	10	0	0
MI[d]	39	8	0	0

[a]Mixed infections by 2 or 3 STs identified in 9 selected samples resulting in the molecular characterisation of a total of 41 isolates

[b]Mixed infections by 2 STs identified in 3 samples resulting in the molecular characterisation of a total of 24 isolates

[c]Mixed infections by 2 STs identified in 3 samples resulting in the molecular characterisation of a total of 26 isolates

[d]MI, Mixed infections with unidentified STs

Table 2

ST distribution of *Blastocystis* sp. in domestic cattle around the world

Country	No. of isolates identified by sequencing	*Blastocystis* sp. STs											Reference
		ST1	ST2	ST3	ST4	ST5	ST6	ST7	ST10	ST12	ST14	Others	
Japan	45	0	0	0	0	0	0	0	1	0	44	0	Masuda et al., 2018
Japan[a]	8	1	0	1	0	6	0	0	0	0	0	0	Yoshikawa et al., 2004
Japan[a]	3	1	0	2	0	0	0	0	0	0	0	0	Abe et al., 2003
Korea	30	6	0	0	0	5	0	0	9	0	10	0	Lee et al., 2018
China	14	0	0	2	0	0	0	0	10	0	2	0	Wang et al., 2018
China	54	0	0	0	2	1	0	0	41	0	10	0	Zhu et al., 2017
Thailand	6	0	0	0	0	0	0	0	2	4	0	0	Udonsom et al., 2018
Iran[a]	17	0	0	4	0	11	2	0	0	0	0	0	Badparva et al., 2015
Libya	10	0	0	0	0	2	0	0	6	0	2	0	Alfellani et al., 2013a

Lebanon	125	9	10	1	0	3	0	1	55	0	46	0	Present study
UK	5	1	0	0	0	1	0	0	3	0	0	0	Alfellani et al., 2013a
Denmark	25	0	0	0	0	3	0	0	22	0	0	0	Stensvold et al., 2009
Colombia	20	12	0	8	0	0	0	0	0	0	0	0	Ramirez et al., 2014
USA	7	0	0	0	0	0	0	0	7	0	0	0	Santin et al., 2011
USA	11	0	0	0	0	0	0	0	8	0	3	0	Fayer et al., 2012
USA	76	0	0	4	18	27	0	0	5	0	8	14[b]	Maloney et al., 2019
Total	456	30	10	22	20	59	2	1	169	4	125	14	

[a]PCR using ST-specific primers allowing the detection of only ST1 to ST7

[b]Including STs 17, 21, 23, 24, 25 and 26 proposed by Maloney et al., 2019

Figure captions

Fig. 1. Study area in Lebanon and description of the animal and human cohorts screened in this epidemiological survey.