



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

Stéphanie Greige, Dima El Safadi, Salma Khaled, Nausicaa Gantois, Martha Baydoun, Marianne Chemaly, Sadia Benamrouz-Vanneste, Magali Chabe, Marwan Osman, Gabriela Certad, et al.

### **► To cite this version:**

Stéphanie Greige, Dima El Safadi, Salma Khaled, Nausicaa Gantois, Martha Baydoun, et al.. First report on the prevalence and subtype distribution of *Blastocystis* sp. in dairy cattle in Lebanon and assessment of zoonotic transmission. *Acta Tropica*, 2019, 194, pp.23-29. 10.1016/j.actatropica.2019.02.013 . hal-02399502

**HAL Id: hal-02399502**

**<https://hal.science/hal-02399502>**

Submitted on 12 Dec 2019

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

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

Stéphanie Greige<sup>a,b,1</sup>, Dima El Safadi<sup>a,b,1</sup>, Salma Khaled<sup>a,b,1</sup>, Nausicaa Gantois<sup>a</sup>, Martha  
Baydoun<sup>a</sup>, Marianne Chemaly<sup>c</sup>, Sadia Benamrouz-Vanneste<sup>a</sup>, Magali Chabé<sup>a</sup>, Marwan  
Osman<sup>b</sup>, Gabriela Certad<sup>a,d</sup>, Monzer Hamze<sup>b</sup>, Eric Viscogliosi<sup>a\*</sup>

<sup>a</sup> Université de Lille, CNRS, Inserm, CHU Lille, Institut Pasteur de Lille, U1019 – UMR  
8204 – CIIL – Centre d’Infection et d’Immunité de Lille, 1 rue du Professeur Calmette, BP  
245, 59019 Lille cedex, France

<sup>b</sup> Laboratoire Microbiologie Santé et Environnement (LMSE), Ecole Doctorale des Sciences  
et de Technologie, Faculté de Santé Publique, Université Libanaise, Rue Mitein, Tripoli,  
Lebanon

<sup>c</sup> ANSES, Ploufragan-Plouzané Laboratory, Hygiene and Quality of Poultry and Pig Products  
Unit, Bretagne-Loire University, Ploufragan, France

<sup>d</sup> Département de la Recherche Médicale, Groupement des Hôpitaux de l’Institut Catholique  
de Lille, Faculté de Médecine et Maïeutique, Université Catholique de Lille, 51 Boulevard de  
Belfort, 59000 Lille, France

\* Corresponding author. Tel.: +33 3 20 87 79 61; fax: +33 3 20 87 78 88

E-mail address: eric.viscogliosi@pasteur-lille.fr (E. Viscogliosi)

<sup>1</sup> Equal contributors

## 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.

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

50

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

53

## 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  $36 \pm 10$  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  $24 \pm 12$  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.

#### **Acknowledgments**

378           The authors would like to thank all those who participated in the study, the staff of  
379   Hamidi Medical Centre in Tripoli, breeders for their assistance in sample collection and  
380   processing, and veterinary doctor Rabah Outour. This work was supported by grants from the  
381   Programme Orientations Stratégiques of the University of Lille 2, the Partenariat Hubert  
382   Curien (PHC) France Lebanon CEDRE 2015 Project n°32684NM from the Ministère des  
383   Affaires Etrangères et du Développement International and the Ministère de l'Éducation  
384   Nationale, de l'Enseignement Supérieur et de la Recherche, the Centre National de la  
385   Recherche Scientifique, the Institut Pasteur of Lille, the Lebanese University and the Catholic  
386   University of Lille. S.G. and S.K. were supported by Ph.D. fellowships from the Azm &  
387   Saade Association of Lebanon.

## References

- Abe, N., Nagoshi, M., Takami, K., Sawano, Y., Yoshikawa, H., 2002. A survey of *Blastocystis* sp. in livestock, pets, and zoo animals in Japan. *Vet Parasitol.* 106, 203-212.
- Ajjampur, S.S., Tan, K.S., 2016. Pathogenic mechanisms in *Blastocystis* spp. - Interpreting results from *in vitro* and *in vivo* studies. *Parasitol Int.* 65, 772-779.
- Alfellani, M.A., Taner-Mulla, D., Jacob, A.S., Imeede, C.A., Yoshikawa, H., Stensvold, C.R., Clark, C.G., 2013a. Genetic diversity of *Blastocystis* in livestock and zoo animals. *Protist.* 164, 497-509.
- Alfellani, M.A., Stensvold, C.R., Vidal-Lapiedra, A., Onuoha, E.S., Fagbenro-Beyioku, A.F., Clark, C.G., 2013b. Variable geographic distribution of *Blastocystis* subtypes and its potential implications. *Acta Trop.* 126, 11-18.
- Andersen, L.O., Bonde, I., Nielsen, H.B., Stensvold, C.R., 2015. A retrospective metagenomics approach to studying *Blastocystis*. *FEMS Microbiol Ecol.* 91, fiv072.
- Badparva, E., Sadraee, J., Kheirandish, F., 2015. Genetic diversity of *Blastocystis* isolated from cattle in Khorramabad, Iran. *Jundishapur J Microbiol.* 8, e14810.
- Bart, A., Wentink-Bonnema, E.M.S., Gilis, H., Verhaar, N., Wassenaar, C.J.A., van Vugt, M., Goorhuis, A., van Gool, T., 2013. Diagnosis and subtype analysis of *Blastocystis* sp. in 442 patients in a hospital setting in the Netherlands. *BMC Infect Dis.* 13, 389.
- Cian, A., El Safadi, D., Osman, M., Moriniere, R., Gantois, N., Benamrouz-Vanneste, S., Delgado-Viscogliosi, P., Guyot, K., Li, L.L., Monchy, S., Noël, C., Poirier, P., Nourrisson, C., Wawrzyniak, I., Delbac, F., Bosc, S., Chabe, M., Petit, T., Certad, G., Viscogliosi, E., 2017. Molecular epidemiology of *Blastocystis* sp. in various animal groups from two French zoos and evaluation of potential zoonotic risk. *PLoS One* 12, e0169659.

413 Clark, C.G., van der Giezen, M., Alfellani, M.A., Stensvold, C.R., 2013. Recent  
414 developments in *Blastocystis* research. *Adv Parasitol.* 82, 1–32.

415 El Safadi, D., Gaayeb, L., Meloni, D., Cian, A., Poirier, P., Wawrzyniak, I., Delbac, F.,  
416 Dabboussi, F., Delhaes, L., Seck, M., Hamze, M., Riveau, G., Viscogliosi, E., 2014.  
417 Children of Senegal River Basin show the highest prevalence of *Blastocystis* sp. ever  
418 observed worldwide. *BMC Infect Dis.* 14, 164.

419 El Safadi, D., Cian, A., Nourrisson, C., Pereira, B., Morelle, C., Bastien, P., Bellanger, A.-P.,  
420 Botterel, F., Candolfi, E., Desoubeaux, G., Lachaud, L., Morio, F., Pomares, C.,  
421 Rabodonirina, M., Wawrzyniak, I., Delbac, F., Gantois, N., Certad, G., Delhaes, L.,  
422 Poirier, P., Viscogliosi, E., 2016. Prevalence, risk factors for infection and subtype  
423 distribution of the intestinal parasite *Blastocystis* sp. from a large-scale multi-center  
424 study in France. *BMC Infect Dis.* 16, 451.

425 Fayer, R., Santin, M., Macarisin, D., 2012. Detection of concurrent infection of dairy cattle  
426 with *Blastocystis*, *Cryptosporidium*, *Giardia*, and *Enterocytozoon* by molecular and  
427 microscopic methods. *Parasitol Res.* 111, 1349-1355.

428 Forsell, J., Granlund, M., Samuelsson, L., Koskiniemi, S., Edebro, H., Evengard, B., 2016.  
429 High occurrence of *Blastocystis* sp. subtypes 1-3 and *Giardia intestinalis* assemblage  
430 B among patients in Zanzibar, Tanzania. *Parasit Vectors.* 9, 370.

431 Franco Moura, R.G., de Oliveira-Silva, M.B., Pedrosa, A.L., Nogueira Nascentes, G.A.,  
432 Cabrine-Santos, M., 2018. Occurrence of *Blastocystis* spp. in domestic animals in  
433 Triangulo Mineiro area of Brazil. *Rev Soc Bras Med Trop.* 51, 240-243<;

434 Greige, S., El safadi, D., Bécu, N., Gantois, N., Pereira, B., Chabé, M., Benamrouz-Vanneste,  
435 S., Certad, G., El Hage, R., Chemaly, M., Hamze, M., Viscogliosi, E., 2018.  
436 Prevalence and subtype distribution of *Blastocystis* sp. isolates from poultry in  
437 Lebanon and evidence of zoonotic potential. *Parasit Vectors.* 11, 389.

438 Lee, H., Lee, S.-H., Seo, M.-G., Kim, H.-Y., Kim, J. W., Lee, Y.6R., Kim, J. H., Kwon, O.-  
 439 D., Kwak, D., 2018. Occurrence and genetic diversity of *Blastocystis* in Korean cattle.  
 440 Vet Parasitol. 258, 70-73.

441 Lepczynska, M., Chen, W.C., Dzika, E., 2015. Mysterious chronic urticarial caused by  
 442 *Blastocystis* spp.? Int J Dermatol. 55, 259-266.

443 Maloney, J.G., Lombard, J.E., Urie, N.J., Shivley, C.B., Santin, M., 2019. Zoonotic and  
 444 genetically diverse subtypes of *Blastocystis* in US pre-weaned dairy heifer calves.  
 445 Parasitol Res. 118, 578-582.

446 Masuda, A., Sumiyoshi, T., Ohtaki, T., Matsumoto, J., 2018. Prevalence and molecular  
 447 subtyping of *Blastocystis* from dairy cattle in Kanagawa, Japan. Parasitol Int. 67, 702-  
 448 705.

449 Noël, C., Dufernez, F., Gerbod, D., Edgcomb, V.P., Delgado-Viscogliosi, P., Ho, L.C.,  
 450 Singh, M., Wintjens, R., Sogin, M.L., Capron, M., Pierce, R., Zenner, L., Viscogliosi,  
 451 E., 2005. Molecular phylogenies of *Blastocystis* isolates from different hosts:  
 452 implications for genetic diversity, identification of species, and zoonosis. J Clin  
 453 Microbiol. 43, 348-355.

454 Osman, M., Bories, J., El Safadi, D., Poirel, M.-T., Gantois, N., Benamrouz-Vanneste, S.,  
 455 Delhaes, L., Hugonnard, M., Certad, G., Zenner, L., Viscogliosi, E., 2015. Prevalence  
 456 and genetic diversity of the intestinal parasites *Blastocystis* sp. and *Cryptosporidium*  
 457 spp. in household dogs in France and evaluation of zoonotic transmission risk. Vet  
 458 Parasitol. 214, 167-170.

459 Osman, M., El Safadi, D., Cian, A., Benamrouz, S., Nourrisson, C., Poirier, P., Pereira, B.,  
 460 Razakandrainibe, R., Pinon, A., Lambert, C., Wawrzyniak, I., Dabboussi, F., Delbac,  
 461 F., Favennec, L., Hamze, M., Viscogliosi, E., Certad, G., 2016. Prevalence and risk  
 462 factors for intestinal protozoan infections with *Cryptosporidium*, *Giardia*, *Blastocystis*

463 and *Dientamoeba* among schoolchildren in Tripoli, Lebanon. PLoS Negl Trop Dis.  
 464 10, e0004496.

465 Poirier, P., Wawrzyniak, I., Albert, A., El Alaoui, H., Delbac, F., Livrelli, V., 2011.  
 466 Development and evaluation of a real-time PCR assay for detection and quantification  
 467 of *Blastocystis* parasites in human stool samples: prospective study of patients with  
 468 hematological malignancies. J Clin Microbiol. 49, 975–983.

469 Quilez, J., Sanchez-Acedo, C., Clavel, A., Causapé, A.C., 1995. Occurrence of *Blastocystis*  
 470 sp. in cattle in Aragon, northeastern Spain. Parasitol Res. 81, 703-705.

471 Rajah Salim, H., Suresh Kumar, G., Vellayan, S., Mak, J.W., Khairul Anuar, A., Init, I.,  
 472 Vennila, G.D., Saminathan, R., Ramakrishnan, K., 1999. *Blastocystis* in animal  
 473 handlers. Parasitol Res. 85, 1032-1033.

474 Ramirez, J.D., Sanchez, L.V., Bautista, D.C., Corredor, A.F., Florez, A.C., Stensvold, C.R.,  
 475 2014. *Blastocystis* subtypes detected in humans and animals from Colombia. Infect  
 476 Genet Evol. 22, 223-228.

477 Ramírez JD, Sánchez A, Hernández C, Florez C, Bernal MC, Giraldo JC, Reyes, P., Lopez,  
 478 M.C., Garcia, L., Cooper, P.J., Vicuna, Y., Mongi, F., Casero, R.D., 2016. Geographic  
 479 distribution of human *Blastocystis* subtypes in South America. Infect Genet Evol. 41,  
 480 32-35.

481 Santin, M., Gomez-Munos, M.T., Solano-Aguilar, G., Fayer, R., 2011. Development of a new  
 482 PCR protocol to detect and subtype *Blastocystis* spp. from humans and animals.  
 483 Parasitol Res. 109, 205-212.

484 Stensvold, C.R., Clark, C.G., 2016. Current status of *Blastocystis*: A personal view. Parasitol  
 485 Int. 65, 763-771.

486 Stensvold, C.R., Suresh, G.K., Tan, K.S., Thompson, R.C., Traub, R.J., Viscogliosi, E.,  
 487 Yoshikawa, H., Clark, C.G., 2007. Terminology for *Blastocystis* subtypes a  
 488 consensus. Trends Parasitol. 23, 93-96.

489 Stensvold, C.R., Alfellani, M.A., Norskov-Lauritsen, S., Prip, K., Victory, E.L., Maddox, C.,  
 490 Nielsen, H.V., Clark, C.G., 2009. Subtype distribution of *Blastocystis* isolates from  
 491 synanthropic and zoo animals and identification of a new subtype. Int J Parasitol. 39,  
 492 473-479.

493 Tan, K.S.W., 2004. *Blastocystis* in humans and animals: new insights using modern  
 494 methodologies. Vet Parasitol. 126, 121–144.

495 Tan, K.S.W., 2008. New insights on classification, identification, and clinical relevance of  
 496 *Blastocystis* spp. Clin Microbiol Rev. 21, 639–665.

497 Tan, K.S.W., Mirza, H., Teo, J.D.W., Wu, B., MacAry, P.A., 2010. Current views on the  
 498 clinical relevance of *Blastocystis* spp. Curr Infect Dis Rep. 12, 28-35.

499 Udonsom, R., Prasertbun, R., Mahittikorn, A., Mori, H., Changbunjong, T., Komalamisra, C.,  
 500 Pintong, A., Sukthana, Y., Popruk, S., 2018. *Blastocystis* infection and subtype  
 501 distribution in humans, cattle, goats, and pigs in central and western Thailand. Infect  
 502 genet Evol. 65, 107-111.

503 Wang, W., Cuttall, L., Bielefeldt-Ohmann, H., Inpankaew, T., Owen, H., Traub, R.J., 2013.  
 504 Diversity of *Blastocystis* subtypes in dogs in different geographical settings. Parasit  
 505 Vectors. 6, 215.

506 Wang, W., Owen, H., Traub, R.J., Cuttall, L., Inpankaew, T., Bielefeldt-Ohmann, H., 2014.  
 507 Molecular epidemiology of *Blastocystis* in pigs and their in-contact humans in  
 508 Southeast Queensland, Australia, and Cambodia. Vet Parasitol. 203, 264-269.

509 Wang, J., Gong, B., Yang, F., Zhang, W., Zheng, Y., Liu, A., 2018. Subtype distribution and  
 510 genetic characterization of *Blastocystis* in pigs, cattle, sheep and goats in northeastern  
 511 China's Heilongjiang Province. Infect Genet Evol. 57, 171-176.  
 512 Wawrzyniak, I., Poirier, P., Viscogliosi, E., Meloni, D., Texier, C., Delbac, F., El Alaoui, H.,  
 513 2013. *Blastocystis*, an unrecognized parasite: an overview of pathogenesis and  
 514 diagnosis. Ther Adv Infect Dis. 1, 167-178.  
 515 Yan, Y., Su, S., Ye, J., Lai, X., Lai, R., Liao, H., Chen, G., Zhang, R., Hou, Z., Luo, X.,  
 516 2007. *Blastocystis* sp. subtype 5 : a possibly zoonotic genotype. Parasitol Res. 101,  
 517 1527-1532.  
 518 Yoshikawa, H., Koyama, Y., Tsuchiya, E., Takami, K., 2016. *Blastocystis* phylogeny among  
 519 various isolates from humans to insects. Parasitol Int. 65, 750-759.  
 520 Zhu, W., Tao, W., Gong, B., Yang, H., Li, Y., Song, M., Lu, Y., Li, W., 2017. First report of  
 521 *Blastocystis* infections in cattle in China. Vet Parasitol. 246, 38-42.  
 522



**Table 1**Prevalence of *Blastocystis* sp. infection and ST distribution in animal and human cohorts in

North Lebanon

Infection	Akkar dairy cattle ( <i>n</i> = 157)	North dairy cattle ( <i>n</i> = 97)	North breeders ( <i>n</i> = 40)	North hospital patients ( <i>n</i> = 40)
% positive	78.3% (123)	39.2% (38 <sup>a</sup> )	52.5% (21 <sup>b</sup> )	57.5% (23 <sup>c</sup> )
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 <sup>d</sup>	39	8	0	0

<sup>a</sup>Mixed infections by 2 or 3 STs identified in 9 selected samples resulting in the molecular characterisation of a total of 41 isolates

<sup>b</sup>Mixed infections by 2 STs identified in 3 samples resulting in the molecular characterisation of a total of 24 isolates

<sup>c</sup>Mixed infections by 2 STs identified in 3 samples resulting in the molecular characterisation of a total of 26 isolates

<sup>d</sup>MI, Mixed infections with unidentified STs

534 **Table 2**

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

536

537	Country	No. of isolates identified	<i>Blastocystis</i> sp. STs											Reference
538		by sequencing												
539			ST1	ST2	ST3	ST4	ST5	ST6	ST7	ST10	ST12	ST14	Others	
540														
541	Japan	45	0	0	0	0	0	0	0	1	0	44	0	Masuda et al., 2018
542	Japan <sup>a</sup>	8	1	0	1	0	6	0	0	0	0	0	0	Yoshikawa et al., 2004
543	Japan <sup>a</sup>	3	1	0	2	0	0	0	0	0	0	0	0	Abe et al., 2003
544	Korea	30	6	0	0	0	5	0	0	9	0	10	0	Lee et al., 2018
545	China	14	0	0	2	0	0	0	0	10	0	2	0	Wang et al., 2018
546	China	54	0	0	0	2	1	0	0	41	0	10	0	Zhu et al., 2017
547	Thailand	6	0	0	0	0	0	0	0	2	4	0	0	Udonsom et al., 2018
548	Iran <sup>a</sup>	17	0	0	4	0	11	2	0	0	0	0	0	Badparva et al., 2015
549	Libya	10	0	0	0	0	2	0	0	6	0	2	0	Alfellani et al., 2013a

550	Lebanon	125	9	10	1	0	3	0	1	55	0	46	0	Present study
551	UK	5	1	0	0	0	1	0	0	3	0	0	0	Alfellani et al., 2013a
552	Denmark	25	0	0	0	0	3	0	0	22	0	0	0	Stensvold et al., 2009
553	Colombia	20	12	0	8	0	0	0	0	0	0	0	0	Ramirez et al., 2014
554	USA	7	0	0	0	0	0	0	0	7	0	0	0	Santin et al., 2011
555	USA	11	0	0	0	0	0	0	0	8	0	3	0	Fayer et al., 2012
556	USA	76	0	0	4	18	27	0	0	5	0	8	14 <sup>b</sup>	Maloney et al., 2019
557	Total	456	30	10	22	20	59	2	1	169	4	125	14	

558

559 <sup>a</sup>PCR using ST-specific primers allowing the detection of only ST1 to ST7

560 <sup>b</sup>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.

