

# Genetic diversity of caprine *Blastocystis* from Peninsular Malaysia

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**Abstract** *Blastocystis* sp. is a common intestinal parasite found in humans and animals. The possibility of zoonotic transmission to humans from livestock especially goats led us to investigate the genetic diversity of caprine *Blastocystis* sp. obtained from five different farms in Peninsular Malaysia. Moreover, there is a lack of information on the prevalence as well as genetic diversity of *Blastocystis* sp. in goat worldwide. Results showed that 73/236 (30.9 %) of the goats were found to be positive for *Blastocystis* infection. The most predominant *Blastocystis* sp. subtype was ST1 (60.3 %) followed by ST7 (41.1 %), ST6 (41.1 %), and ST3 (11.0 %) when amplified by PCR using sequenced-tagged site (STS) primers. Four farms had goats infected only with ST1 whereas the fifth showed mixed infections with multiple STs. The proximity of the fifth farm to human dwellings, nearby domesticated animals and grass land as opposed to a sterile captive environment in the first four farms may account for the multiple STs seen in the fifth farm. Since ST1, ST3, ST6 and ST 7 were previously reported in human infection worldwide in particular Malaysia, the potential of the zoonotic transmission of blastocystosis should not be disregarded. The implications of different farm management systems on the distribution of *Blastocystis* sp. STs are discussed.

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## Introduction

*Blastocystis* sp. is a common intestinal parasite found in humans and animals (Tan 2008). *Blastocystis* sp. is a unicellular parasite which exists in various morphotypes such as vacuolar, multivacuolar, avacuolar, granular, amoeboid and cystic forms (Tan 2008; Suresh et al. 2009). The transmission of *Blastocystis* is via the faecal–oral route where it commonly inhabits the host's large intestine (Tan 2008).

*Blastocystis* sp. are currently classified into 13 distinct subtypes (STs; ST1–13) that have been isolated from humans, mammalian, avian, reptilian, amphibian and insect hosts (Belova and Krylov 1998; Abe et al. 2002; Noël et al. 2003, 2005; Yoshikawa et al. 2004a; Parkar et al. 2010). ST1 is commonly found in monkeys, pigs, cattle, birds, pheasants, chickens, dogs and non-human primates. ST7 has been isolated from chickens, quails, geese and birds (Duda et al. 1998; Abe et al. 2003a; Yoshikawa et al. 2004b; Noël et al. 2005). Recently, Parkar et al. (2010) reported that *Blastocystis* ST11, ST12 and ST13 were host specific in infecting elephants, giraffes and quokkas, respectively.

Sequence and phylogenetic analyses of partial ssu rDNA of *Blastocystis* sp. from a human, pig and horse revealed a common subgroup and the isolates from the pig and horse were monophyletic and closely related to the *Blastocystis* sp. isolated from humans (Thathaisong et al. 2003). Due to its low host specificity, transmission between humans and livestock like goats warrant serious attention. Malaysia is an important livestock export market for the Australian goat industry. Hence, the more important implication can be that animal to human transmission can take place among those who handle goats at the farms and at the slaughter houses. Therefore, our aims in this study were to determine the genetic diversity of *Blastocystis* sp. harboured by locally reared goats from farms by PCR amplification using sequenced-tagged site (STS) primers.

## Materials and methods

### Sample collection and preparation of genomic DNA

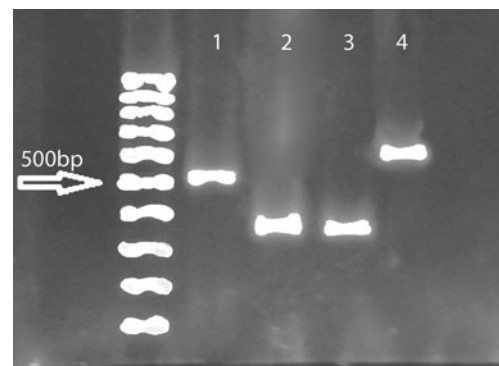
A total of 236 goat faecal samples were collected from five different farms in Peninsular Malaysia namely UPM1, UPM2, UPM3, Kuala Klawang and Ulu Langat. The breeds comprised of Jamnapari, Saanen and Boer. The diarrheic symptom of the goats was recorded by the animal handlers at the respective farms. Genomic DNA from fresh faecal samples were extracted using the QIAmp® DNA stool mini kit (Qiagen, Valencia, CA) according to the manufacturer's protocol.

### Genotyping by PCR with STS primers

PCR was performed using the seven pairs of STS primers (SB83, SB155, SB227, SB332, SB340, SB336 and SB337) to genotype *Blastocystis* sp. as employed by Yoshikawa et al. (2004c). PCR conditions consisted of 1 cycle of initial denaturing at 94 °C for 3 min, followed by 30 cycles including denaturing at 94 °C for 30 s, annealing at 56.3 °C for 30 s, and extension at 72 °C for 1 min, and an additional 10-min chain elongation cycle at 72 °C (Thermocycler Eppendorf, Germany). The amplicons were electrophoresed in 1.5 % agarose gels (Promega, USA) with Tris–borate–EDTA buffer. Gels were stained with ethidium bromide and photographed using a ultraviolet gel documentation system (Uvitec, UK). The PCR amplification for each primer pair was repeated thrice. While the primers of Yoshikawa et al. (2004c) were used, the results were reported in ST format which is the consensus terminology for *Blastocystis* subtypes as reported by Stensvold et al. (2007).

## Results

All the goats did not show any diarrheic symptoms. *Blastocystis* sp. infection was detected in 30.9 % of goats. The most predominant *Blastocystis* sp. subtype was ST1 (60.3 %), followed by ST7 (41.1 %), ST6 (41.1 %) and ST3 (11.0 %). The gel electrophoresis photo for each subtype is presented in Fig. 1. Although direct PCR amplification of faecal samples were employed, we did inoculate a subset of the faecal samples into Jones' medium and it gave rise to the vacuolar forms of *Blastocystis* sp. as seen in Fig. 2. The occurrence of *Blastocystis* sp. isolates from goats in UPM1, UPM2, UPM3, Kuala Klawang and Ulu Langat farms are listed in Table 1. The highest occurrence was detected in the goats from the Ulu Langat farm, while the lowest was from UPM3. The most predominant *Blastocystis* sp. STs was ST1, occurring exclusively in four of the five farms sampled (Table 1). Mixed infection with ST1, ST3,

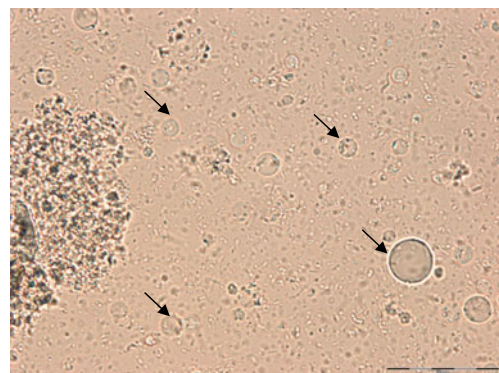


**Fig. 1** PCR amplification reaction for faeces obtained from goats infected with *Blastocystis* sp. using sequenced-tagged primers SB227 (lane 1; 526 bp) indicating ST3, SB83 (lane 2; 351 bp) indicating ST1, SB332 (lane 3; 338 bp) indicating ST6 and SB155 (lane 4; 650 bp) indicating ST7

ST6 and ST7 were detected only at the Ulu Langat farm, with the combination of ST6 and ST7 being the most common (70 %) form of mixed infection. When the results from the farms were pooled, there was no significant effect of gender on the occurrence of *Blastocystis* infection. The highest percentage of infection (46.4 %) was detected in the older goats (more than 2 years old).

## Discussion

*Blastocystis* sp. is known to infect a wide array of animals including mammals, birds and amphibians, with high prevalence (Table 2). The present work represents the first successful attempt to determine the occurrence as well as the genetic diversity of *Blastocystis* sp. from goats in Malaysia although Abe et al. (2002) previously screened goats in Japan but were unable to detect *Blastocystis* in faecal samples collected. This could be due to the small sample size as only six animals were screened. In addition, Snowden et al. (2000) amplified only one *Blastocystis* sp. isolate from a single faecal sample from a goat in the USA. In view of the



**Fig. 2** Vacuolar forms of *Blastocystis* sp. (arrow) upon in vitro cultivation of faeces obtained from goat in Jones' medium

**Table 1** Occurrence of the various subtypes of *Blastocystis* sp. in relation to (A) the sampling location (farm) and (B) the signalment (sex and age) of the goats

A		N (M/F)	P ( $P_M/P_F$ )	% Subtypes (ST)						
				1	3	6	7	6+7 <sup>a</sup>	1+6+7 <sup>a</sup>	3+6+7 <sup>a</sup>
Farm	UPM1	74 (0/74)	29.7 (0/29.7)	100	–	–	–	–	–	–
	UPM2	21 (21/0)	28.6 (28.6/0)	100	–	–	–	–	–	–
	UPM3	48 (13/35)	18.8 (15.4/20.0)	100	–	–	–	–	–	–
	K. Klawang	21 (0/21)	28.6 (0/28.6)	100	–	–	–	–	–	–
	Ulu Langat	72 (9/63)	41.7 (55.6/39.7)	–	–	–	–	70.0	3.3	26.7
	Total	236 (43/193)	30.9 (30.2/31.1)	58.9	–	–	–	28.7	1.4	11.0
B		I/N	P							
Sex	Male	13/43	30.2	61.5	–	–	–	23.1	7.7	7.7
	Female	60/193	31.1	58.3	–	–	–	30	–	11.7
Age	<1 year	4/18	22.2	100	–	–	–	–	–	–
	1–2 years	4/78	5.1	75	–	–	–	–	25	–
	>2 years	65/140	46.4	55.4	–	–	–	32.3	–	12.3

N sample size, I infected with *Blastocystis*, M males, F females, P overall prevalence,  $P_M$  prevalence in males,  $P_F$  prevalence in females

<sup>a</sup> Mixed infections

paucity of published information on caprine *Blastocystis* globally, we have provided a more comprehensive account on occurrence of this intestinal protozoa among goats. Since in the present study we did PCR amplification directly on the faecal sample with only seven set of primers, we may actually miss some of the positive samples if they belonged to ST8–13. Hence we prefer to use the term ‘occurrence’ than ‘prevalence’. The occurrence of *Blastocystis* sp. in goats was 30.9 % whereby 236 goats were screened, which was slightly lower compared with other livestock such as pigs (46.8 %; Navarro et al. 2008), ducks (56 %; Abe et al. 2002) and cattle (71 %; Abe et al. 2002). The differences may be due to different management systems in farms,

different adaptation by animals or different way in conducting the studies which based from different countries (Navarro et al. 2008).

Interestingly, the goats from all the farms, with the exception of the Ulu Langat farm, harboured a single *Blastocystis* sp. ST1 infection. Therefore, we initially postulated that locally reared goats only harboured *Blastocystis* sp. ST1 which is commonly found from various animals such as pigs, cattle, dogs, monkeys, chickens, pheasants and geese (Abe et al. 2003b; Yoshikawa et al. 2004b; Noël et al. 2005; Parkar et al. 2007; Stensvold et al. 2009). However, examination of the goats on the Ulu Langat farm revealed the presence of *Blastocystis* sp. ST1, ST3, ST6 and ST7, thus challenging our earlier postulations. Previous studies have indicated that the occurrence of mixed subtypes in animals was uncommon, within the range of 1.8–20 % (Yoshikawa et al. 2004b; Navarro et al. 2008). Nevertheless, in the present study, the occurrence of mixed *Blastocystis* sp. subtypes was 41.1 %, the highest reported thus far among animals. The Ulu Langat farm is surrounded by villages and grass land with some wet areas which may facilitate significant waterborne transmission to humans in the vicinity. In fact, *Blastocystis* sp. ST1, ST3, ST6 and ST7 are known to infect humans in particular the Malaysian populations (Tan et al. 2008, 2009). Therefore, the transmission may be spread from farm personnel or domesticated animals like dogs and cats from the nearby villages. In addition, the drinking water for goats in our study might have been contaminated with *Blastocystis* sp. from goats' droppings.

The age of the host is one of the important factors that can influence parasite transmission among animals. Navarro

**Table 2** Summary of the epidemiological studies of *Blastocystis* sp. in animals

Animals	Prevalence (%)	Reference
Pig	46.8–95	Abe et al. 2002; Navarro et al. 2008
Primates	66.7–85	Abe et al. 2002, 2003b
Cattle	71	Abe et al. 2002
Dog	70.8	Duda et al. 1998
Cat	67.3	Duda et al. 1998
Pheasant	80	Abe et al. 2002
Duck	56	Abe et al. 2002
Amphibian	41.1	Yoshikawa et al. 2004a
Asian elephant	64	Parkar et al. 2010
Giraffe	85.2	Parkar et al. 2010
Quokka	100	Parkar et al. 2010

et al. (2008) reported that piglets were easily infected with *Blastocystis* sp. due to their immature immune system. In contrast, the present study highlighted that 46.4 % of the *Blastocystis* sp. infected goats were from the 2–3 year age group. In addition, the parasite was detected in 8.3 % of the younger animals (<2 years). Hence, our data is incongruent with that reported by Navarro et al. (2008). However, at the present moment, we are unable to provide a satisfactory explanation for the age predilection observed in this study. The adult goats may have adapted in the presence of *Blastocystis* sp. whereby there was no diarrhetic symptoms recorded by the animal handlers.

The gender of the animals was found to be a risk factor of *Blastocystis* infection. Navarro et al. (2008) reported that male pigs (60 %) were at a higher risk to be infected compare to female conspecifics (40 %). In the present study, there was no significant difference in the infection rates between the genders. Hence, we postulated that gender might not be a suitable variable to be taken as a possible risk factor. Nevertheless, further investigations are necessary to accurately describe the relationship between gender and *Blastocystis* infection. It is also important to note that through this cross-sectional study, we could not infer any causal relationships between the risk factors. Thus at the present moment, our postulations are merely based on associations between the presence of infection and the animals' signalment and management in the farms.

The different husbandry practices and feeding regimes adopted in urban and rural farms appear to have a significant influence on the distribution of *Blastocystis* sp. STs. Blastocystosis and other infections may well be controlled or even eliminated due to modern farming practices which place a greater emphasis on hygiene compared to the traditional ones (Navarro et al. 2008). The UPM and Kuala Klawang farms are considered urban intensive farms while Ulu Langat is a rural farm. Therefore, in the Ulu Langat farm, we observed mixed subtypes of *Blastocystis* sp. while others only harboured a single ST infection. The transmission of this parasite was further facilitated by the presence of a dense population of animals in a small area. Navarro et al. (2008) stated that the infection of *Blastocystis* sp. among the male pigs was higher compared to female pigs because male pigs were reared in small area but in large population. In addition, sharing of contaminated food and water may act as an effective mode of transmission. Furthermore, lack of awareness among the staffs or animal handlers may have worsened the scenario.

## Conclusion

The present study is the first to report the occurrence and genetic diversity of *Blastocystis* sp. isolated from goats in

the tropics. Although high prevalence of *Blastocystis* sp. is known to occur among animals and animal handlers, it remains unclear if the isolates recovered in this study have any zoonotic significance.

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