Tropical Biomedicine 37(1): 210–217 (2020)

Epitope variances demonstrated by *Blastocystis* sp. ST3 symptomatic and asymptomatic isolates

Sheela, D.S.[1], Chandramathi, S.[2] and Suresh, K.[1*]
[1]Department of Parasitology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia
[2]Department of Microbiology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia
[*]Corresponding author email: suresh@um.edu.my
Received 15 November 2019; received in revised form 18 November 2019; accepted 20 November 2019

Abstract. *Blastocystis* sp. is an enteric protozoan parasite of humans and many animals. *Blastocystis* sp. subtype 3 (ST3) proves to be the highest frequency case in most populations around the world and it is further distinguished into symptomatic and asymptomatic isolates based on the clinical symptoms exhibited by infected individuals. Phenotypic and genotypic studies implicate the distinctiveness of this parasite which may describe its pathogenesis. However, the antigenic distinctiveness which describes the antibody mediated cell lysis of this parasite has not been explored. This study was aimed to identify the cross-reactivity and cytotoxicity effect between three isolates of symptomatic and asymptomatic *Blastocystis* sp. ST3 respectively. Antigen specificity and diversity of this parasite was performed by co-culturing sera (10-fold dilution) obtained from mice immunised with *Blastocystis* sp. symptomatic and asymptomatic antigens and the respective *Blastocystis* sp. ST3 live cells through complement dependant cell cytotoxicity (CDC) assay. The results obtained has shown that, the sera (at 10-fold diluted concentration) from symptomatic and asymptomatic solubilised antigen immunised mice were able to specifically lyse the respective live parasites with an average percentage of 82% and 86% respectively. There were almost 50% cross-reactivity observed between the three isolates of *Blastocystis* sp. ST3 from symptomatic and asymptomatic group proving high antigen diversity or rather low antigen specificity within the same group. However, there was only 17% cross-reactivity observed between the mice sera and parasitic cells of different groups (symptomatic vs asymptomatic isolates) suggesting high specificity between these two groups. We, for the first time have proven that through CDC analysis there were epitopes dissimilarities between *Blastocystis* sp. ST3 symptomatic and asymptomatic isolates which may allow the parasite to set up diverse immune modulations such as imbalanced Th1/Th2 responses in an infected host.

INTRODUCTION

Blastocystis sp. is an intestinal parasite found in humans and many animals (Chen *et al.*,1999; Gould *et al.*, 2013; Ragavan *et al.*, 2014) and isolated through parasitological stool surveys (Tan *et al.*, 2008). Clinically, *Blastocystis* sp. infection in human can be categorised into asymptomatic or no symptoms and symptomatic which comes with associated indications such as abdominal pain, diarrhoea, constipation, cramps, nausea and fatigue (Coyle *et al.*, 2012; Sekar *et al.*, 2013). Various studies have implicated *Blastocystis* sp. ST3 as highly prevalent (Tan *et al.*, 2008; Ragavan *et al.*, 2015; Deng *et al.*, 2019) along with that, *in-vitro* studies which proved its potentiality in triggering a higher immune response upon stimulation (Tan *et al.*, 2008; Kumarasamy *et al.*, 2013).

The antigenic specificity of *Blastocystis* sp. ST3 isolates was demonstrated through the characterization of the adaptive immune response by performing antigenicity and immunogenicity assessments (Sheela Devi and Suresh, 2019). It was observed that, the symptomatic and asymptomatic isolates of this parasite demonstrated distinct adaptive immune responses which may indicate their

unique pathogenic role. However, there was difference of immune response observed among the isolates of the same group (symptomatic versus symptomatic isolates and/or asymptomatic versus asymptomatic isolates). A study conducted by Mirza *et al.* (2011) had proven that, there were antigenic heterogeneity of protein profiles obtained from patients infected with symptomatic *Blastocystis* sp. isolates. It was concluded that, each of the isolates may display a different pathogenic role. Nevertheless, they were no antigen diversity assessment performed among the isolates of symptomatic and asymptomatic *Blastocystis* sp. ST3 to identify the degree of the antigenic uniqueness among them.

Generally, antigen specificity defines the ability of an immune system to respond differently towards various antigens whereas antigen cross-reactivity evaluates the degree where various antigens were recognised as similar by the immune system. The molecular determinants play a major role in dictating the specificity and cross-reactivity across all antigens by segregating the populations based on its unique variations (Frank, 2002). The antibodies cross-reactivity between symptomatic and asymptomatic *Blastocystis* sp. ST3 has not been characterised so far. It was reported that, parasite surfaces share molecules which immensely overlap with antibody binding sites which are also known as epitopes (Frank, 2002). Previous studies have also reported antibody cross-reactivity among *Blastocystis* sp. isolates of human and animal origins (Tan *et al.*, 1997; Tan *et al.*, 2001). Therefore, it becomes imperative to investigate the degree of specificity and cross-reactivity between *Blastocystis* sp. ST3 symptomatic and asymptomatic isolates to confirm the differences of adaptive immune response demonstrated by these isolates. In this study the antigen diversity of these parasite was determined through the analysis of complement mediated cytotoxicity (CDC) assay. Past studies have proven CDC assay as an efficient method in differentiating and identifying cross-reactivity mediated by using polyclonal antibodies (Konishi *et al.*, 2007; Kitai *et al.*, 2010). Therefore, the antigenic heterogeneity of this parasite was further substantiated by performing CDC assay. CDC assay, is a mechanism where antibodies lyse the targeted cells by activating a cascade of complement-related reactions. The antigenic diversities were determined based on the degree of cell lysis obtained among these isolates.

MATERIALS & METHODS

Source of *Blastocystis* sp.

Blastocystis sp. parasites were obtained from random stool sample collection in a survey carried out at a particular rural area in, Malaysia. Isolates respectively from symptomatic and asymptomatic individuals were continuously cultured in Jones' medium. Individuals with symptoms showed flatulence, abdominal pain, diarrhoea and constipation. This information was obtained using a questionnaire. Samples with *Blastocystis* sp. were selected through direct faecal screening and cyst concentration technique to select the samples with only *Blastocystis* sp. as the sole symptom causative agent. ST3 identification of the isolates was determined through Polymerase Chain Reaction (PCR) technique (Sheela Devi and Suresh, 2019).

Axenization of *Blastocystis* sp. and isolation of solubilised antigen

Harvested cysts were washed in sterile saline, cultured in Jones' medium supplemented with 10% heat-inactivated horse serum and incubated at 37°C. After 2 days, parasites from all isolates were assessed using direct microscopy to confirm the presence of the parasite (vacuolar form). The xenic *Blastocystis* sp. cultures were axenised. The clear layer containing the parasites were harvested and re-suspended in basal Jones' medium (without supplementation). The axenic cells were sonicated and the homogenates were incubated at 4°C overnight. The homogenates were the centrifuged at 13,000×g for 15 min. The supernatant (solubilised *Blastocystis* sp. antigen), was filter sterilized, and the protein concentration was determined by Bradford assay (Sheela Devi and Suresh, 2019).

Animal Selection, Housing and Ethical Clearance

Female Balb/c mice (n=24) with the age ranged from 4-6 weeks old were selected. The Balb/c mice were purchased from Animal Laboratory, University Putra Malaysia, Serdang, Malaysia and were kept in an individually ventilated cage (IVC) cage at Animal Satellite Laboratory, Department of Parasitology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia during this study. Prior to the experiment, the mice were acclimatised to the new environment for one week by housing in groups of four in IVC cages. The animal protocols were approved by Institutional Animal Care and Use Committee, University of Malaya (approved ethical number: 2014-04-01/PARA/R/SKG) as described by Sheela Devi and Suresh (2019).

Mice Immunization and Polyclonal Antibody production

Mice (n=24) were randomly divided into groups of three. The mice were immunised intraperitoneally (IP) by using a 27-G needle. The priming dose of 100 µl (40 µg/ml) aliquots of *Blastocystis* sp. of ST3 AS (1-3) and S (1-3) solubilised antigens respectively were emulsified in equal volume of Freund's complete adjuvant (CFA) to 100 µl (ratio of 1:1). The subsequent three injections with incomplete Freund's adjuvant (IFA) of 100 µl emulsion with the booster dose of 100 µl (20 µg/ml) aliquots of *Blastocystis* sp. of ST3 AS (1-3) and S (1-3) solubilised antigen. 200 µl of *Blastocystsis* sp. of is ST3 AS (1-3) and S (1-3) solubilised antigen (20 µg/ml) with PBS was injected as the final boost but without the adjuvant, at day 59. Mice which served as negative control were injected with PBS instead of solubilised antigen. The method was previously described by Sheela Devi and Suresh K (2019).

Sera and *Blastocystis* sp. ST3 Cells Cytotoxicity Analysis

For the CDC assay, ST3 symptomatic (S1-3) and asymptomatic (AS1-3) *Blastocystis* sp. cell suspension containing 5×10^4 cells in 50 µl of sera-free Jones Medium was mixed with an equal volume of test sera diluted in Jones Medium at (1:10/10-fold). This mixture was incubated on ice for 30 min. 11 µL of human complement (Sigma, USA) was added to make a final concentration of 10% and was incubated at 37°C for 4 hours. After 4 hours the cells were subjected for CCK-8 analysis to quantitate the viable cells following protocol by Konishi *et al*., 2007.

Calculations

The percentage of specific cell lysis was calculated according to the manufacturer's instructions by using the following formula:

$$\% \text{ Viable Cells: } \frac{\text{Average OD450 of wells containing antigen stimulated cells}}{\text{Average OD450 containing medium}} \times 100$$

Cell Cytotoxicity and Cross-reactivity Study Design

The cell lysis was observed between the cells and sera of ST3 *Blastocystis* sp. following the experimental matrix design in Table 4. The following terms were used to describe the cell cytotoxicity reactions.

1. Same isolates described for the reaction between (S1 cells with S1 Sera) and (AS1 cells with AS1 sera)
2. Same group described for the reaction between (S1 cells with S2 and S3 sera) and (AS1 cells with AS2 and AS3 sera)
3. Different group described for the reaction between (S1 cells with AS1, AS2 and AS3 sera).

Table 1. The matrix experimental design of cell cytotoxicity and cross-reactivity analysis between *Blastocystis* sp. ST3 symptomatic and asymptomatic cells and sera obtained from immunised mice

Cell	AS1	AS2	AS3	S1	S2	S3
Sera						
AS1	√	√	√	√	√	√
AS2	√	√	√	√	√	√
AS3	√	√	√	√	√	√
S1	√	√	√	√	√	√
S2	√	√	√	√	√	√
S3	√	√	√	√	√	√

Table 2. Percentage Specific Cell Lysis Among *Blastocystis* sp. ST3 Symptomatic and Asymptomatic Isolates

Symptomatic Isolates	S1	S2	S3	Average A & B 84%
% Lysis (A)	96.0±0.1	79.4±0.9	71.9±0.1	
Asymptomatic Isolates	AS1	AS2	AS3	
% Lysis (B)	84.0±0.3	87.2±1.9	85.7±0.6	

Data is given as mean ± SD (n=2). The comparison was carried out with between *Blastocystis* sp. ST3 symptomatic and asymptomatic group. There was no significant difference observed.

Table 3. Cross-reactivity among *Blastocystis* sp. ST3 symptomatic group

Blastocystis sp. Cells	Sera (1:10)	Lysis (%)
S1	S2	49.0±2.3
	S3	29.3±1.4
S2	S1	37±2.1
	S3	55.5±1.5
S3	S1	39.3±1.2
	S2	53.1±0.2

Data is given as mean ± SD (n=2).

Table 4. Cross-reactivity among *Blastocystis* sp. ST3 asymptomatic group

Blastocystis sp. Cells	Sera (1:10)	Lysis (%)
AS1	AS2	49.7±0.7
	AS3	59.5±0.6
AS2	AS1	48.6±0.54
	AS3	37.4±0.1
AS3	AS1	41.3±1
	AS2	48.7±0.1

Data is given as mean ± SD (n=2).

Statistical analysis

Data for cell lysis and was analysed by one-way analysis of variance (ANOVA) using SPSS software 22. The graphs were plotted using Graph Pad Prism 8.

RESULTS

1.1 Cell Lysis of Same Isolates

The *Blastocystis* sp. ST3 cells symptomatic (S1-3) and asymptomatic (AS1-3) isolates were co-cultured with sera obtained from mice injected with symptomatic (S1-3) and asymptomatic (AS1-3) isolates diluted at 1:10. The percentage cell lysis is reflected in Table 2. It was observed that *Blastocystis* sp. symptomatic (S1-3) and asymptomatic (AS1-3) isolates induced significantly higher cell lysis when they were co-cultured with the sera of the same isolates. The average percentage cell lysis for symptomatic group was 82% whereby those of the asymptomatic group was 86%. Therefore, the average of 83% was used as benchmark for specific cell lysis for the subsequent comparisons.

1.2 Percentage Cell Lysis of Same Group (Symptomatic)

The *Blastocystis* sp. ST3 cells symptomatic (S1-3) cells were co-cultured with sera obtained from mice injected with symptomatic (S1-3) isolates diluted at 1:10. The percentage lysis among the isolates is shown in Table 3. It was observed that *Blastocystis* sp. symptomatic (S1-3) cells induced average cell lysis of 44% when they were co-cultured with the sera of the same group (S1-3).

1.3 Percentage Cell Lysis of Same Group (Asymptomatic)

The ST3 *Blastocystis* sp. cells asymptomatic (AS1-3) cells were co-cultured with sera obtained from mice injected with asymptomatic (AS1-3) isolates diluted at 1:10. The percentage lysis among the isolates is shown in Table 4. It was observed that *Blastocystis* sp. asymptomatic (AS1-3) cells induced average cell lysis of 48% when they were co-cultured with the sera of the same group (AS1-3).

Table 5. Cross-reactivity between *Blastocystis* sp. ST3 symptomatic and asymptomatic group

Blastocystis sp. Cells	Sera (1:10)	Lysis (%)	*Blastocystis* sp. Cells	Sera (1:10)	Lysis (%)
S1	AS1	16.6±0.2		S1	25.4±0.6
	AS2	20.2±0.4	AS1	S2	13.5±0.6
	AS3	13.4±0.4		S3	11.5±0.2
S2	AS1	5.7±0.3		S1	18.4±0.3
	AS2	16.8±0.04	AS2	S2	21.9±1.1
	AS3	4.2±0.1		S3	29.0±0.2
S3	AS1	9.9±0.04		S1	19.7±0.1
	AS2	11.4±0.6	AS3	S2	22.7±1.3
	AS3	13.7±0.3		S3	36.7±0.2

Data is given as mean ± SD (n=2).

1.4 Percentage Cell Lysis of Different Group (Symptomatic with Asymptomatic) The ST3 *Blastocystis* sp. cells symptomatic (S1-3) and asymptomatic (AS1-3) cells were co-cultured with sera obtained from mice injected with asymptomatic (AS1-3) and symptomatic (S1-3) isolates diluted at 1:10. The percentage lysis among the isolates is shown in Table 5. It was observed that *Blastocystis* sp. asymptomatic and symptomatic groups induced average cell lysis of 17% cross-reactivity upon co-culture.

DISCUSSION

The sera of the mice injected with symptomatic and asymptomatic *Blastocystis* sp. antigen produced polyclonal antibodies. In order to identify the degree of polyclonal antibodies specificity and efficacy, the antibodies and cells were interacted at 10-fold sera dilution (10^1). We have proven in our previous study (Sheela Devi and Suresh, 2019), that there was a presence of dominant IgG antibodies in the sera sample of the injected mice. Therefore, 10-fold sera dilution containing polyclonal antibodies were used in this study to observe the cell lysis. In theory, the specific antibodies which were present in the mice sera, will initiate the binding of the respective antigens expressed on the surface of the *Blastocystis* sp. cells *in vitro* with antibodies. This will eventually form an antigen-antibody complex which may induce the complement activation and lyse the *Blastocystis* sp. cells. CCK-8 cell cytotoxicity kit was used to measure the NADH+ content of the live *Blastocystis* sp. cells which denotes the metabolic activity of the live cells.

In this study the positive and negative cut off points were set based on the specific sera and cell lysis of the respective isolates. The positive cut off value was set based on the average value of specific percentage cell lysis by symptomatic and asymptomatic group which was 84%. In contrast, the negative cut off point were set based on the specific cell lysis between cells and sera obtained from PBS injected mice. It was shown that PBS immunised mice did not exert specific cell lysis in this assay. Therefore, negative (PBS control cell lysis) and specific *Blastocystis* sp. cell lysis at more than (>84%) and lower than (<1%) were used as a high and low cut off point for all the cell lyses.

The specificity of an antibody is defined by its ability to recognize one specific antigen. As a general example, an antibody that recognizes the *Blastocystis* sp. antigen will not be able to recognize helminth (parasite), on the contrary, an antibody that recognizes helminth will not able to recognize the *Blastocystis* sp. (Tan KS *et al.*, 2001). This phenomenon is defined as antibody specificity where each and every B lymphocyte cell is able to produce antibody of one kind. In this study, the specificity of the *Blastocystis* sp. from the symptomatic and asymptomatic groups were evaluated

Blastocystis sp. ST3 Live Cells			% Cell Lysis: Serum (1:10) Symptomatic 1	2	3	Asymptomatic 1	2	3	PBS Control	IgG (OD492)
	Symptomatic	1	92	37	39	25	18	20	0.68	2.63
		2	49	79	53	14	22	23	0.16	1.83
		3	29	55	72	12	29	37	0.99	2.37
	Asymptomatic	1	17	6	10	84	49	41	0.37	1.48
		2	20	17	11	49	87	49	1.76	1.13
		3	13	4	14	60	37	86	0.44	1.10

Low <1%
Medium <84%
High >84%

Figure 1. Summary percentage of ST3 *Blastocystis* sp. cell lysis at 1:10 sera dilution.

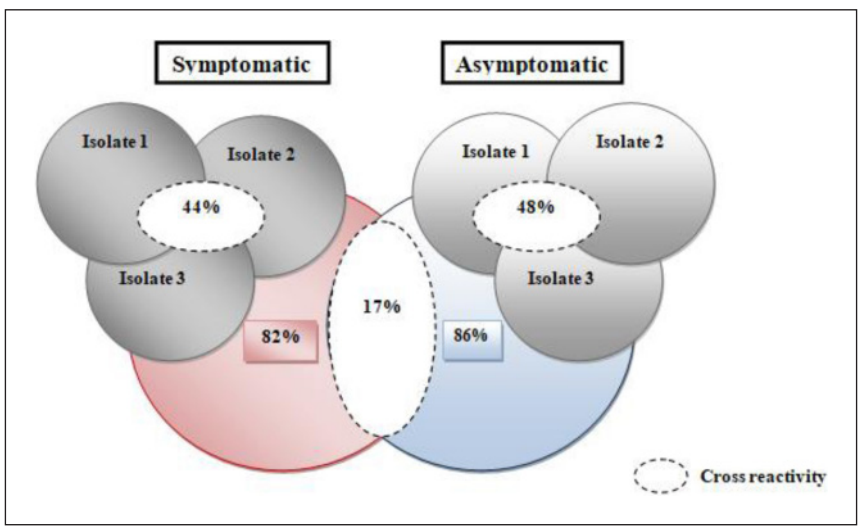

Figure 2. Summary percentage of cell-cytotoxicity and cross-reactivity induced by *Blastocystis* sp. ST3 symptomatic and asymptomatic isolates.

and it was shown to induce high specific cell lysis. The polyclonal antibodies produced against each isolate of *Blastocystis* sp. ST3 symptomatic and asymptomatic group was able to recognise the cell surface membrane of its specific cell.

The specific cell lysis at 1:10 sera concentration was observed in both symptomatic and asymptomatic group with an average percentage value of 82% and 86% respectively with no significant difference between the two isolates. These results were compared with the IgG antibody OD reading obtained at 1:10 dilution. However, the IgG OD readings have shown a contrast results which has shown higher IgG content in symptomatic isolates where average readings of 2.3 and 1.2 OD were observed in sera obtained from mice injected with symptomatic and asymptomatic isolates respectively with a significant difference of $P<0.01$. This scenario could be due to the higher influence of IgG1/IgG2a antibody isotype contents which was proven in our previous study (Sheela Devi and Suresh, 2019). Studies suggested that, IgG1 isotype performs a higher classical complement mediated lysis effector function in comparison to IgG2 isotype (Beenhouwer *et al*., 2007; Wang *et al*., 2017). In our previous study it was proven that, asymptomatic isolates were Th2 dominant with higher IgG1 isotype and symptomatic were Th1 dominant with higher IgG2a isotype secretions. Therefore, the differences between cell lysis and sera IgG content could be due to higher IgG1 isotype in comparison to sera obtained from mice injected with symptomatic isolates.

The diversity of the antibodies raised against of *Blastocystis* sp. ST3 symptomatic and asymptomatic isolates were further evaluated through the cross-reactivity assay in this study. The antibodies were cross-reacted (1) among symptomatic isolates (2) among asymptomatic isolates (3) between symptomatic and asymptomatic isolates. The antibodies which were cross-reacted among symptomatic isolates at sera dilution of 1:10 showed an average of 44% cell lysis. On the other hand, the cell lysis among asymptomatic isolates showed an average of 48% cell lysis. This showed that symptomatic and asymptomatic isolates shared almost half the epitopes among its own group which showed a medium cell lysis at lesser than 84%. In contrast, the cross-reactivity between symptomatic and asymptomatic group showed average cell lysis of 17%. The cell lysis between symptomatic and asymptomatic group were exerted higher than the negative cut off value more than 1% but significantly lower than positive cut off value which is higher than 84%. These data demonstrated a minimal cross-reactivity between symptomatic and asymptomatic isolates. Therefore, CDC cross reactivity analysis has further proven the existence of diversity between the solubilised antigen of symptomatic and asymptomatic isolates which supports the distinct adaptive immune response elicited by these isolates.

CONCLUSION

In conclusion this study has demonstrated that, *Blastocystis* sp. ST3 symptomatic and asymptomatic isolates were able to induce complement mediated antibody cell lysis as depicted in Figure 2 above. Sera obtained from mice injected with 20 µg/ml symptomatic and asymptomatic solubilised antigens were able to exert specific lysis implicating high antibody-cell binding specificity. In other words, this observation evidenced the high antigen specificity. There were almost 50% cross-reactivity observed between *Blastocystis* sp. ST3 isolates originated from the same group proving high antigen diversity (low specificity) among symptomatic or asymptomatic isolates. Only 17% cross-reactivity observed between the sera and cells of different group (symptomatic and asymptomatic isolates). Altogether, this study clearly implicates that large level of epitopes dissimilarities between these two groups of *Blastocystis* sp. ST3 (symptomatic vs asymptomatic isolates) may allow the parasite to set up diverse adaptive immune modulation tactics (imbalanced Th1/Th2)

responses as proven in our previous study (Sheela Devi and Suresh, 2019), in order to maintain its survival in the host.

REFERENCES

Beenhouwer, D.O., Yoo, E.M., Lai, C.W., Rocha, M.A. & Morrison, S.L. (2007). Human immunoglobulin G2 (IgG2) and IgG4, but not IgG1 or IgG3, protect mice against Cryptococcus neoformans infection. *Infection and Immunity* **75**: 1424-35.

Chen, X.Q., Singh, M., Ho, L.C., Tan, S.W. & Yap, E.H. (1999). Characterization of protein profiles and cross-reactivity of *Blastocystis* antigens by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblot analysis. *Parasitological Research* **85**: 343-6.

Coyle, C.M., Varughese, J., Weiss, L.M. & Tanowitz, H.B. (2012). *Blastocystis*: to treat or not to treat. *Clinal Infectious Diseases* **54**: 105-10.

Deng, L., Chai, Y., Zhou, Z., Liu, H., Zhong, Z., Hu, Y. & Peng, G. (2019). Epidemiology of *Blastocystis* sp. infection in China: a systematic review. *Parasite* **26**: 41.

Frank, S.A. (2002). Immunology and Evolution of Infectious Disease. Princeton (NJ): Princeton University Press; Chapter 4, Specificity and Cross-Reactivity.

Gould, R. & Boorom, K. (2013). *Blastocystis* surface antigen is stable in chemically preserved stool samples for at least 1 year. *Parasitological Research* **112**: 2469-71.

Kitai, Y., Kondo, T. & Konishi, E. (2010). Complement-dependent cytotoxicity assay for differentiating West Nile virus from Japanese encephalitis virus infections in horses. *Clinical and Vaccine Immunology* **17**: 875-878.

Konishi, E., Kitai, Y. & Kondo, T. (2007). Utilization of complement-dependent cytotoxicity to measure low levels of antibodies: application to non-structural protein 1 in a model of Japanese encephalitis virus. *Clinical and Vaccine Immunology* **15**: 88-94.

Kumarasamy, V., Kuppusamy, U.R., Samudi, C. & Kumar, S. (2013). *Blastocystis* sp. subtype 3 triggers higher proliferation of human colorectal cancer cells, HCT116. *Parasitological Research* **112**: 3551-5.

Mirza, H., Wu, Z., Kidwai, F. & Tan, K.S.W. (2011). A Metronidazole-Resistant Isolate of *Blastocystis* spp. is Susceptible to Nitric Oxide and Downregulates Intestinal Epithelial Inducible Nitric Oxide Synthase by a Novel Parasite Survival Mechanism. Adams JH, ed. *Infection and Immunity* **79**: 5019-5026.

Ragavan, N.D., Govind, S.K., Chye, T.T. & Mahadeva, S. (2014). Phenotypic variation in *Blastocystis* sp. ST3. *Parasites & Vectors* **7**: 404.

Sekar, U. & Shanthi, M. (2013). *Blastocystis*: Consensus of treatment and controversies. *Tropical Parasitology* **3**: 35-9.

Sheela Devi, S. & Suresh, K. (2019). Immunogenic and Antigenic Heterogeneity of *Blastocystis* sp. Subytpe 3 from Symptomatic and Asymptomatic Individuals. *Tropical Biomedicine Journal* **36**: 1.

Tan, K.S. (2008). New insights on classification, identification, and clinical relevance of *Blastocystis* spp. *Clinical Microbiology Reviews* **21**: 639-665.

Tan, K.S., Ibrahim, M., Ng, G.C., Nasirudeen, A.M., Ho, L.C., Yap, E.H. & Singh, M. (2001). Exposure of *Blastocystis* species to a cytotoxic monoclonal antibody. *Parasitological Research* **87**: 534-8.

Tan, S.W., Singh, M., Ho, L.C., Howe, J., Moe, K.T., Chen, X.Q., Ng, G.C. & Yap, E.H. (1997). Survival of *Blastocystis hominis* clones after exposure to a cytotoxic monoclonal antibody. *Int J Parasitol* **27**: 947-54.

Wang, X., Mathieu, M. & Brezski, R.J. (2017). IgG Fc engineering to modulate antibody effector functions. *Protein & Cell* **9**: 63-73.