Journal of the Egyptian Society of Parasitology, Vol. 51, No. 3, December 2021
J. Egypt. Soc. Parasitol. (JESP), 51(3), 2021: 641 – 646
(Online: 2090-2549)

EFFICACY OF FECAL CALPROTECTIN AS A MARKER FOR THE PATHOGENICITY OF *BLASTOCYSTIS HOMINIS*

By

AMIRA RAAFAT, IMAN R. ABDEL-SHAFI, MOUSA A. M. ISMAIL*, And NOHA M. AMIN

Department of Medical Parasitology, Faculty of Medicine, Cairo University, Cairo, Egypt (*Correspondence: dr.mousa.abdelgawad@gmail.com)

Abstract

Fecal calprotectin (f-CP) is a marker of inflammation in intestine, calprotectin is released by whilte blood cells, macrophages, and epithelial cells.

This study used the f-CP level as a factor to determine the pathogenicity of *Blastocystis hominis*. The f-CP levels were measured in *Blastocystis hominis* patients as compared to negative *B. hominis* ones with gastrointestinal symptoms related to healthy controls.

A total of 235 stool samples were divided into three groups. G1: 88 stool samples of patients with gastrointestinal complaints positive only for *blastocysts hominis.* G2: 75 stool samples of patients with gastrointestinal complains and *B. hominis* free. G3: 72 stool samples of healthy volunteers, with neither gastrointestinal symptoms nor microbiological infection.

The levels of f-CP were determined by specific ELISA showed that the f-CP concentration was highest in G1 & G2 as compared separately with G3, with significant differences ($P < 0.001$), but without significant difference between G1 & G2 ($p = 0.453$).

Keywords: *Blastocystis hominis,* Fecal calprotectin, Gastrointestinal symptoms.

Introduction

Blastocystis hominis is a protozoan that infects the human gastrointestinal tract. The pre-valence of *Blastocystis* varied from 3 % to 50% in different countries worldwide. Nevertheless, the parasite is a neglected parasite of the gastrointestinal tract (Al and Hökelek, 2007). However, the presence of *Blastocystis hominis* in patients without other pathogens causing gastrointestinal symptoms should be considered as a pathogen that causes symptoms. *Blastocystis hominis* patients commonly experience diarrhea and abdominal pain vomiting or fatigue (Ning *et al,* 2020). Despite findings that support *Blastocystis hominis* as a possible pathogen, some doubts remain regarding its pathogenicity (Fouad *et al,* 2011). However, it's associated with a broad gastrointestinal symptoms were reported (Abou El Naga and Negm, 2001). By proper treating of *B. hominis* infected patients the symptoms disappeared by cure of intestine was inflammation of mucosa (Ning *et al,* 2020).

Fecal calprotectin (f-CP) binds calcium and zinc was reported in 30 to 60% of neutrophil cytosol (Chen *et al,* 2012). F-CP concentrations were measured in bacteria, viruses, parasites, colorectal cancer, and inflammatory bowel diseases of the gastrointestine, which could be detected in patients' feces (Aykur *et al,* 2019). Besides, gut diseases, neutrophils migrate into lumen, releasing calprotectin damaging mucosal epithelial cells (Bustinduy *et al,* 2013).

This study aimed to evaluate fecal calprotectin inflammation levels of *Blastocystis hominis* in patients with gastrointestinal troubles as compared to patients with gastrointestinal troubles but without *B. hominis*, also, to evaluate f-CP of *B. hominis* potential pathogenicity.

Materials and Methods

The participants (235) were divided in three groups. G1: 88 patients with gastrointestinal symptoms and only infected with Blastocystis *hominis*. G2: 75 patients with gastrointestinal symptoms and *B. hominis* free. G3: 72 health cross-matched volunteers. Medical sheets were filled out on each one.

Patients' classification was based on the morning stool examined of stained smears (El Saftawy *et al,* 2019). Enhancement of growth of *Blastocystis* was done by Jones' medium using 10% horse serum (Irikov *et al*, 2009).

Fecal calprotectin was ELISA evaluated (Drg: Hybridxl Calprotectin Kit) in feces >

200µg/g was positive (Radin *et al,* 2014).

Statistical analysis: Data were computerized and analyzed by the statistical package for the Social Sciences (SPSS) version 28 (IBM Corp., Armonk, NY, USA). Comparisons between quantitative variables were done by using non-parametric Kruskal-Wallis & Mann-Whitney tests (Chan, 2003). Exact test was used instead when expected frequency was < 5 P-values less than 0.05 were considered as statistically significant.

Ethical Statement: Ethical approval was obtained from the ethical committee of Faculty of Medicine, Cairo University and its consecutive adjustments and analogous ethical standards. All procedures related to the human samples were covenant with the ethical standards settled by the National Research Committee and the 2000 Helsinki Declaration.

Results

The participants were <20 years old, >20-40 years old and > 40 years old, with the f-CP level was high in G1& G22 than control, highest level was among age group 20-40 of G1, but with significant differences ($p < 0.01$), But, no significant difference between ages in same group ($p > 0.05$). The gastrointestinal symptoms in G1 were diarrhea, abdominal pain and weight loss (45.5%, 18.2% &18.2%), followed by vomiting and fever (17% & 13.6%) respectively. Symptoms in G2 were diarrhea, abdominal pain and fever (45.3%, 18.7% & 17.3%), followed by vomiting and weight loss (16% & 16%) respectively. The f-CP concentration was highest in G1. F-CP levels of G1 &G 2 when compared separately with control showed significant ($p < 0.001$), but without difference between G1 & G2 ($p = 0.453$). F-CP patients with cli-nical data were not significant except for abdominal pain and fever.

The results were shown in tables (1, 2, 3, 4 & 5) as well as figures (1 & 2)

Table 1: Sexes distribution among groups

Sex	G1		G2		control		P value
	Count	%	Count	%	Count	%	
Male	42	47.7%	23	30.7%	20	27.8%	0.016
Female	46	52.3%	52	69.3%	52	72.2%	

Table 2: Age (years) distribution among groups.

Ages year	G1				G2				control				P value
	Mean	Median	Min.	Max.	Mean	Median	Min.	Max.	Mean	Median	Min.	Max.	
	31.11±18.85	32.5	5.0	60.0	30.53±18.42	30.0	5.0	60.0	27.75±18.71	25.0	5.0	60.0	0.354

Table 3: F-CP levels in different age groups.

Ages year	G1				G2				Control				P value
	Mean	Median	Min.	Max.	Mean	Median	Min.	Max.	Mean	Median	Min.	Max.	
<20	193.50±190.57	100.0	10.0	800.0	129.35±107.30	90.0	10.0	300.0	21.09±12.30	22.5	5.0	40.0	< 0.001
>20-40	331.39±321.22	200.0	10.0	800.0	124.12±80.24	90.0	50.0	300.0	19.17±11.28	17.5	5.0	40.0	< 0.001
>40	298.47±315.88	120.0	20.0	1000.0	131.11±105.44	90.0	30.0	300.0	16.36±12.55	10.0	5.0	40.0	< 0.001

Table 4: F-CP distribution among groups

Fecal calprotectin	G1				G2				Control				P value
	Mean	Median	Mini.	Max.	Mean	Median	Mini.	Max.	Mean	Median	Mini.	Max.	
	262.26±275.82	100.0	10.0	1000.0	128.80±99.86	90.0	10.0	300.0	19.17±12.13	17.50	5.0	40.0	< 0.001

Table 5: Clinical data and calprotectin values of patients in G1 & G2

Symptoms	G1				G2				
	fecal calprotectin (ng/mg)				fecal calprotectin (ng/mg)				P value
	Mean	Median	Mini.	Max.	Mean	Median	Mini.	Max.	
Diarrhea	256.23±283.39	100.0	10.0	900.0	123.82±97.20	90.0	10.0	300.0	0.369
Vomiting	251.80±271.12	150.0	20.0	800.0	172.50±101.19	150.0	50.0	300.0	0.792
Fever	340.83±305.09	325.0	20.0	1000.0	114.62±98.03	60.0	30.0	300.0	0.048
Abdominal pain	285.0±292.35	150.0	50.0	800.0	120.0±112.11	50.0	10.0	300.0	0.031
Weight loss	159.56±155.42	95.0	10.0	450.0	141.67±109.86	125.0	20.0	300.0	0.873

Discussion

Generally, the *B. hominis* is one of the common zoonotic intestinal parasite (Windsor *et al,* 2005), of worldwide distribution (Stensvold, 2013). *Blastocystis* consists of several species, living in the gastrointestinal tracts of species as diverse as humans, farm animals, birds, rodents, reptiles, amphibians, fish, and even cockroaches (Yoshikawa *et al,* 2007). Fecal-oral transmission is the most accepted pathway, and recent studies have shown that transmission involves only the

cyst form of the parasite (Yoshikawa *et al,* 2004). The extent to which human-human, human-animal, and animal-human transmission occurred was still unknown, but genomic studies gave evidence for all three routes, though experimental studies have yet to provide conclusive proof for the existence of either (Yoshikawa *et al,* 2000). Infections with this parasite are spread worldwide and it is frequently the most commonly isolated protozoan in parasitological surveys (Chandramathi *et al,* 2010). In developing countries, *B. hominis* was more predominant (30 to 50%) as compared to (1.5 to 10 %) in developed countries (Li *et al,* 2007).

In the present study, the main clinical manifestations in a descending order were diarrhea, abdominal pain, weight loss, vomiting and fever among *B. hominis* patients as compared to less severity of diarrhea, abdominal pain, fever, vomiting, and weight loss among *B. hominis*-free patients. Ok *et al.* (1999) reported that the pathogenicity of *B. hominis* occurred in patients without or with gastrointestinal symptoms. Yakoob *et al*. (2004) reported that *B. hominis* caused irritable bowel syndrome. Boorom *et al.* (2008) found that *B. hominis* was the commonest protozoa in patients presenting with gastrointestinal symptoms. Laodim *et al.* (2012) reported that abdominal pain was the most frequent symptom in *B. hominis* patients followed by diarrhea. Also, Ibrahim *et al.* (2020) reported that *Bastocystis* patients complained of altered bowel habits with the predominance of diarrhea.

In the present study, there was no significant difference between male and females blastocystosis patients. Dagci *et al*. (2014) stated that blastocystosis in males and females was more or less in equal percentage. Yunus *et al.* (2015) reported that blastocystosis was 0.56% in females and 0.53% in males, but without a risk factor. Also, Ibrahim *et al.* (2020) reported infection of 39% males and 61% females, without significant difference as to ages or sexes.

In the present study, among the three different groups, the f-CP level was high in blastocystosis patients and blastocystosis-free patients than control. The highest level was in age group 20-40 in blastocystosis patients. The f-CP levels were significant between blastocystosis patients and blastocystosis-free patients than control in all age groups ($p< 0.01$), without significant difference among ages of same group ($p > 0.05$). But, f-CP levels were no significant among symptomatic and healthy ones ($p < 0.01$). This agreed with Aykur *et al.* (2020) who didn't find significant difference among age groups of same group ($p > 0.05$).

In the present study, the f-CP levels were highest among *B. hominis* patients with gastrointestinal symptoms than patients with gastrointestinal symptoms, without significant difference ($p =0.453$). Yahya *et al.* (2017) found f-CP was (3.22%) positive in stools of *Blastocystis hominis* patients compared to (41.75 %) *Entamoeba histolytica* patients, followed by (21.27%) in *Giardia lamblia* patients ($P<0.05$). Lam *et al.* (2014) found a significant positive correlation between gastrointestinal pathogens and f-CP levels ($P<0.0001$). Also, Rady *et al.* (2019) reported fecal calprotectin significant association ($p<0.05$) with *Cryptosporidium parvum* (52.63%), and *G. lamblia* (45.45%). No doubt, Calprotectin a 36-kDa zinc and calcium-binding protein of about 60% as dissolved in cytoplasm of neutrophils, which was measured in several body fluids, such as the urine, plasma, and cerebrospinal fluid, and tissue samples and feces (McMahon and Chhabra, 2018). Also, the non-invasive usage of f-CP was a stool marker to monitor inflammation (Rutgeerts *et al,* 2012, Caimmi *et al*, 2018), and for mucosal inflammatory activity in IBD (Wei, 2016).

Kostakis *et al.* (2013) reported that the f-CP levels of patients with gastrointestinal disorders were higher than healthy controls. Aykur *et al.* (2020) found that the most common gastrointestinal symptoms in groups of patients with gastrointestinal symptoms were diarrhea and abdominal pain. They in contrast found statistically significant difference in f-CP levels in the groups presented with gastrointestinal symptoms such as diarrhea, abdominal pain and weight loss,

when compared with healthy controls ($p < 0.0001$).

In the present study, *B. hominis* infected patients showed the highest value of f-CP indicating an inflammation of the mucosa of the gastrointestinal tract, supporting the potential pathogenicity of *Blastocystis hominis*. Lam *et al.* (2014) reported a substantial correlation between the occurrence of intestinal pathogens and f-CP levels ($P= 0.0001$), and that f-CP levels were higher in patients with positive intestinal pathogens than those without. Also, Abu El-Fetouh *et al.* (2021) found that colonic mucosa of experimentally infected Albino rats a week after *B. hominis* infection showed focal atrophy with unusual inflammatory cells, with aggregates of eosinophils and parasites present in the colon.

Conclusion

Using fecal calprotectin clarified the pathogenicity of *Blastocystis hominis* as a reliable indicator of inflammation in patients' intestines. The f-CP can be a good marker for pathogenicity of *Blastocystis hominis*.

Authors' contributions: All authors equally contributed in this work.

Conflicts of interest: Authors declared that they have neither conflict of interests nor received fund.

Acknowledgement

The authors would like to thanks all the participants whether patients or healthy volunteers who shared in this applied study.

References

Abu El-Fetouh, NI, Abdelmegeed, ES, Attia, RA, El-Dosoky, I, Azab, MS, 2021: Genotyping of *Blastocystis hominis* symptomatic isolates and kinetics of associated local CD3 & CD20 cell infiltrate. Parasitologists U. J. 19:115-22.

Abou El Naga, IF, Negm, AY, 2001: Morphology, histochemistry and infectivity of *Blastocystis hominis* cyst. J. Egypt. Soc. Parasitol, 31, 3: 627-635.

Al, FD, Hökelek, M, 2007: Is *Blastocystis hominis* an opportunist agent? Türk. Parazitol. 31, 1:28-36.

Aykur, M, Armagan, G, Vardar, R, Dagci, H, 2020: Fecal calprotectin as a factor that supports the pathogenicity of *Dientamoeba fragilis.* Microbial Pathogen.139:103868.

Aykur, M, Kurt, C, Erdogan, D, Avcı, C, Vardar, R, *et al,* 2019: Investigation of *Dientamoeba fragilis* prevalence and evaluation of sociodemographic and clinical features in patients with gastro-intestinal symptoms. Acta Parasitol. 64, 1:162-70.

Boorom, KF, Smith, H, Nimri, L, Viscogliosi, E, Spanakos, G, *et al,* 2008: Oh my aching gut: Irritable bowel syndrome, *Blastocystis,* and asymptomatic infection. Parasit. Vectors 1, 1:40-8.

Bustinduy, AL, Sousa-Figueiredo, JC, Adriko, M, Betson, M, Fenwick, A, *et al,* 2013: Fecal occult blood and fecal calprotectin as point-of-care markers of intestinal morbidity in Ugandan Children with *Schistosoma mansoni* infection. PLoS Negl. Trop. Dis. 7, 11:e2542.

Caimmi, C, Bertoldo, E, Venturini, A, Caramaschi, P, Frulloni, L, *et al,* 2018: Relationship between increased fecal calprotectin levels and interstitial lung, disease in systemic sclerosis. J. Rheumatol. 46, 3:274-8.

Chan, YH, 2003: Biostatistics 103: Qualitative data, tests of independence. Singapore. Med. J. 44, 10:498-503.

Chandramathi, S, Suresh, K, Kuppusamy, U R, 2010: Elevated levels of urinary hyaluronidase in humans infected with intestinal parasites. Ann. Trop. Med. Parasitol. 104, 5:449-52.

Chen, CC, Huang, JL, Chang, CJ, Kong, M, S, 2012: Fecal calprotectin as a correlative marker in clinical severity of infectious diarrhea and usefulness in evaluating bacterial or viral pathogens in children. J. Pediatr. Gastroenterol. Nutr. 55, 5:541-7.

Dagci, H, Kurt, O, Demirel, M, Mandiracioglu, A, Aydemir, S, *et al,* 2014: Epidemiological and diagnostic features of *Blastocystis* infection in symptomatic patients in Izmir Province, Turkey. Iran. J. Parasitol. 9:519-29.

El Saftawy, EA, Amin, NM, Hamed, DH, Elkazazz, A, Adel, S, 2019: Hidden impact of different *Blastocystis* genotypes on C3 & IgE serum levels: A matter of debate in asthmatic Egyptian children. Indi. J. Parasit. Dis. 43, 3:443-51.

Fouad, SA, Basyoni, MMA, Fahmy, RA, Kobaisi, MH, 2011: The pathogenic role of different *Blastocystis hominis* genotypes isolated from patients with irritable bowel syndrome. Arab J. Gastroenterol.12, 4:194-200.

Ibrahim, SS, Ismail, MAM, Shaker, MA, Khalil, DM, Raafat, A, 2020: *Bastocystis hominis* in diabetic patients and non-diabetic with irritable bowel syndrome in Beni-Suef City, Egypt. J. Egypt. Soc. Parasitol. 50, 3:683-8.

Irikov, OA, Antokhin, AI, Romanov, YA, 2009: Study of the dynamics of *Blastocystis hominis* reproduction in vitro. Bull. Exp. Biol. Med. 148, 1:99-102.

Kostakis, ID, Cholidou, KG, Vaiopoulos, AG, Vlachos, IS, *et al,* 2013: Fecal calprotectin in pediatric inflammatory bowel disease: A systematic review. Dig. Dis. Sci. 58: 309–319.

Lam, YA, Warouw, SM, Wahani, AM, Manoppo, JI, Salendu, PM, 2014: Correlation between gut pathogens and fecal calprotectin levels in young children with acute diarrhoea. Paediatr. Indones. 54:193-7.

Laodim, P, Intapan, PM, Sawanyawisuth, K, Laummaunwai, P, Malewong, W, 2012: A hospital based study of epidemiological and clinical data on *Blastocystis hominis* infection. Foodbor. Pathol. Dis. 9, 12:1077-82.

Li, LH, Zhang, XP, Lv, S, 2007: Cross-sectional surveys and subtype classification of human *Blastocystis* isolates from four epidemiological settings in China. Parasitol. Res. 102:83-90.

McMahon, CW, Chhabra, R, 2018: The role of fecal calprotectin in investigating digestive disorders, J. Lab. Precis. Med. 3: 19.

Ning, CQ, Hu, Z, Chen, J, Ai, L, Tian, G, 2020: Epidemiology of *Blastocystis* infection from 1990 to 2019 in China. Infect. Dis. Poverty 9, 1:168-72.

Ok, UZ, Girginkardesler, N, Balcioglu, C, Ertan, P, Pirildar, T, *et al,* 1999: Effect of trimethoprim sulfamethaxazole in *Blastocystis hominis* infection. Am. J. Gastroenterol. 94, 11:245-7.

Radin, JN, Gaddy, JA, Loh, JT, Piazuelo, M B, Kehl-Fie, TE, *et al,* 2014: The host protein calprotectin modulates the *Helicobacter pylori* cag-type iv secretion system via zinc availability PLoS Pathog. 10, 10:e1004450.

Rady, HI, Elkazazz, A, Saftawy, EA, Abdelrazek, NM, 2019: Parasites and *Helicobacter pylori* in Egyptian Children with or without diabetes with gastrointestinal manifestations and high calprotectin level. J. Egypt. Soc. Parasitol. 49, 1: 243-8.

Rutgeerts, P, Van Assche, G, Sandborn, WJ, Wolf, DC, Geboes, K, *et al,* 2012: Adalimumab induces and maintains mucosal healing in patients with Crohn's disease: Data from the extend trial. Gastroenterology 142, 5:1102-11.

Stensvold, JR, 2013: *Blastocystis*: Genetic diversity and molecular methods for diagnosis and epidemiology. Trop. Parasitol. 3, 1:26-34.

Wei, SC, 2016: Could fecal calprotectin enter mainstream use for diagnosing & monitoring inflammatory bowel disease? Intest. Res. 14:293-4.

Windsor, JJ, Macfarlane, L, 2005: Irritable bowel syndrome: the need to exclude *Dientamoeba fragilis*. Am. J. Trop. Med. Hyg. 72:501-42.

Yahya, JS, Chateen, AA, Adil, AR, 2017: Fecal calprotectin among patients infected with some protozoan infections. Int. J. Curr. Microbiol. App. Sci. 6, 6:3258-74.

Yakoob, J, Jafri, W, Jafri, N, Islam, M, Asim Beg, M, 2004: In vitro susceptibility of *Blastocystis hominis* isolated from patients with irritable bowel syndrome. Brit. J. Biomed. Sci. 61, 2:75-7.

Yoshikawa, H, Abe, N, Iwasawa, M, *et al,* 2000: Genomic analysis of *Blastocystis hominis* strains isolated from two long-term health care facilities. J. Clin. Microbiol. 38, 4:1324-30.

Yoshikawa, H, Wu, Z, Howe, J, Hashimoto, T, Geok-Choo, N, *et al,* 2007: Ultrastructural and phylogenetic studies on *Blastocystis* isolates from cockroaches. J. Eukaryotic Microbiol. 54, 1:33-7.

Yoshikawa, H, Yoshida, K, Nakajima, A, Yamanari, K, Iwatani, S, *et al,* 2004: Fecal-oral transmission of the cyst form of *Blastocystis hominis* in rats. Parasitol. Res. 94, 6:391-6.

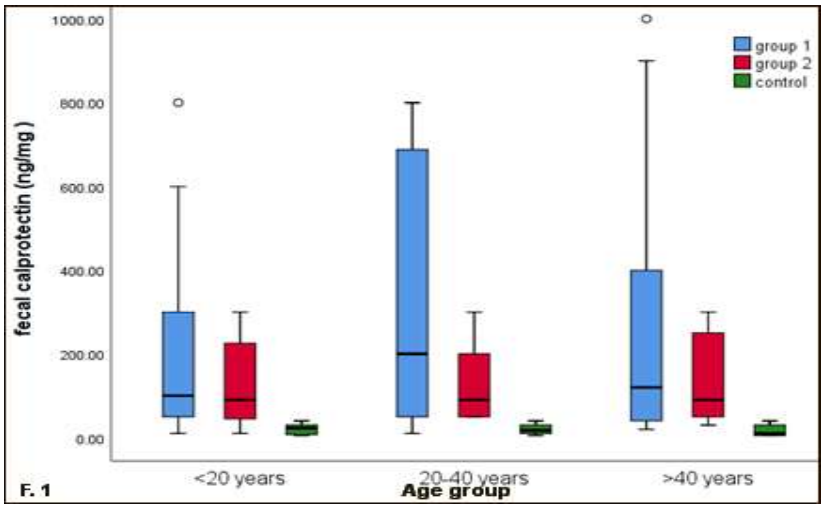
Yunus, E, Yilmaz, H, Cengiz, ZT, Ekici, A, 2015: Clinical significance & prevalence of *Blastocystis hominis* in Van, Turkey. Saudi Med. J. 36, 9:1118.

Explanation of figures

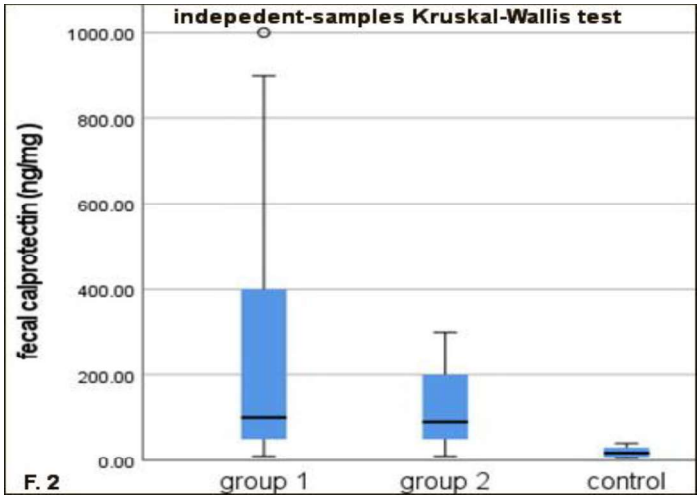
Fig. 1: Evaluation of fecal calprotectin levels by age groups.

Fig. 2: Relationship of fecal calprotectin values among groups.

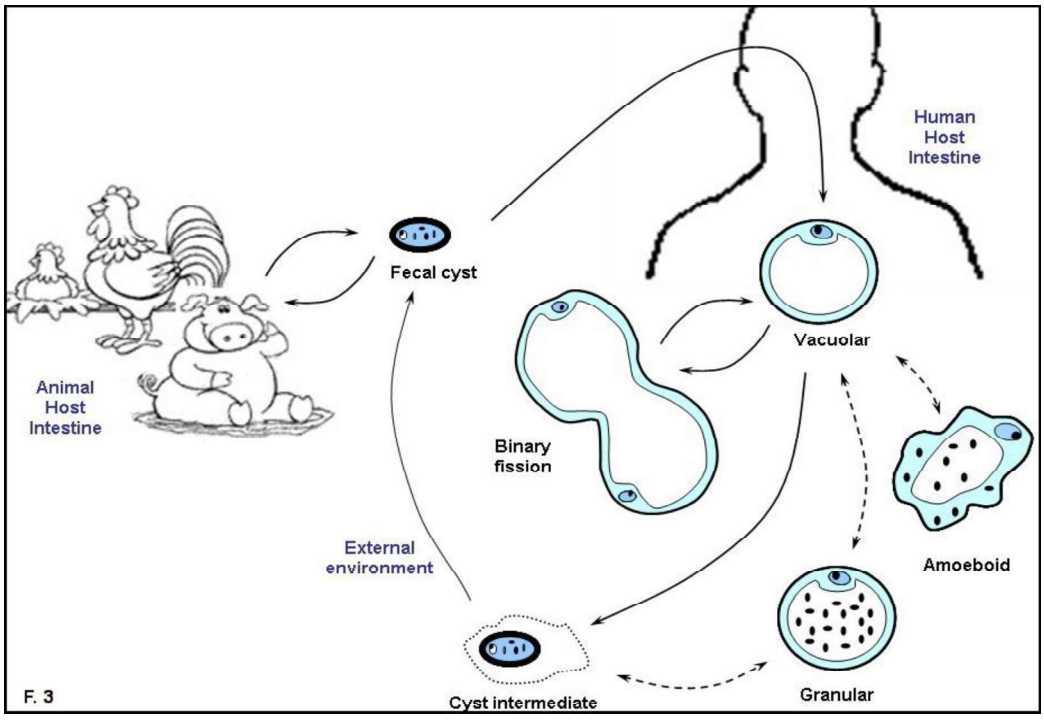
Fig. 3: *B. hominis* life cycle after Tan KS, 2004: Blastocystis in humans and animals: new insights using modern methodologies. Vet. Parasitol. 126, 1/2:121-44


1000.00
800.00
600.00
400.00
200.00
0.00
fecal calprotectin (ng/mg)
group 1
group 2
control
<20 years
20-40 years
>40 years
Age group

F. 1


independent-samples Kruskal-Wallis test
1000.00
800.00
600.00
400.00
200.00
0.00
fecal calprotectin (ng/mg)
group 1
group 2
control

F. 2


Human
Host
Intestine
Fecal cyst
Vacuolar
Animal
Host
Intestine
Binary
fission
Amoeboid
External
environment
Cyst intermediate
Granular

F. 3