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Intestinal growth and pathology of *Giardia duodenalis* assemblage subtype A_I, A_{II}, B and E in the gerbil model

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Running title: Variable growth and pathogenicity among *Giardia* assemblages A, B and E.

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SUMMARY

Introduction: This study investigated the significance of the genetic differences between assemblages A, B and E on intestinal growth and virulence.

Methods: Intestinal growth and virulence were studied in 2 laboratory (A_I: WB and B: GS/M-83-H7) and 6 field isolates of assemblage subtype A_I, A_{II}, B and E_{III}. Intestinal trophozoite burdens, body weight and faecal consistency were monitored until day 29 post-infection (pi), morphological (mucosal architecture and inflammation) and functional (disaccharidase and alkaline phosphatase enzyme activity) damage to the small intestine were evaluated on day 7 and 18 pi.

Results: The assemblage subtypes A_I and B were more infectious and produced higher trophozoite loads for a longer period compared to the subtypes A_{II} and E_{III}. The body weight of infected gerbils was significantly reduced compared to uninfected controls, but did not differ between the assemblage subtypes. Consistent softening of the faeces was only observed with assemblage B. Assemblage B next to assemblage subtype A_I elicited relatively higher pathogenicity, characterized by more extensive damage to mucosal architecture, decreased brush-border enzyme function and infiltration of inflammatory cells. Assemblage E_{III} and A_{II} isolates showed relatively low virulence.

Conclusion: The *Giardia* assemblage subtypes exhibit different levels of growth and virulence in the gerbil model.

Keywords: assemblage subtypes A_I, A_{II}, B and E; growth dynamics; virulence; gerbil model.

INTRODUCTION

Giardiasis is quite common among people (Plutzer, J. *et al.* 2010), domestic and farm animals (Ballweber, L. R. *et al.* 2010; Geurden, T. *et al.* 2010) and despite intense research, the disease mechanisms, host response and factors determining the variability in clinical outcome are still poorly understood. Specific virulence factors linked to *Giardia* pathogenicity have not been identified yet (Roxstrom-Lindquist, K. *et al.* 2006). The clinical manifestations of giardiasis may vary from asymptomatic carrier-state to long-lasting diarrhoea and malabsorption (Robertson, L. J. *et al.* 2010). Studies on epithelial cell lines (Chavez, B. *et al.* 1995), laboratory animals (Belosevic, M. *et al.* 1989; Cevallos, A. *et al.* 1995), man (Oberhuber, G. *et al.* 1997; Hanevik, K. *et al.* 2007) and livestock (Ruest, N. *et al.* 1997; Koudela, B. and Vitovec, J. 1998) demonstrated a variable degree of damage to the small intestinal surface, ranging from no abnormalities to microvillus brush-border injury, villus atrophy and crypt hyperplasia, which explain the typical symptoms of malabsorption and maldigestion (Buret, A. *et al.* 1991; Buret, A. *et al.* 1992).

Currently, isolates of *G. duodenalis* are assigned to 7 genotypes: assemblages A and B infect humans and a broad range of animals, while assemblages C, D, E, F and G are restricted to domestic animals, livestock and wild animals (Read, C. M. *et al.* 2004; Caccio, S. M. and Ryan, U. 2008). This host restriction may suggest that assemblages C to G are phenotypically distinct from assemblages A and B, although a clear correlation between genetic and biological markers has not yet been established (Roxstrom-Lindquist, K. *et al.* 2006). T-cell stimulating antigens (Astiazaran-Garcia, H. *et al.* 2009), variant-specific surface proteins (VSP) (Franzen, O. *et al.* 2009; Jerlstrom-Hultqvist, J. *et al.* 2010) and other surface antigens (Aggarwal, A. and Nash, T. E. 1987; Franzen, O. *et al.* 2009) are variably expressed on the WB (assemblage A_I), GS (assemblage B) and P15 (assemblage E_{III}) strains. Hence, it is of interest to study how these differences may relate to biologic properties, such as infectivity and pathogenicity. Some authors report a relation of assemblage A with symptomatic infections and

assemblage B with asymptomatic cases in man (Haque, R. *et al.* 2005; Sahagun, J. *et al.* 2008), while others just found the opposite (Geurden, T. *et al.* 2009; Singh, A. *et al.* 2009) or no relation at all (Kohli, A. *et al.* 2008; Ajjampur, S. S. *et al.* 2009).

Variation in infectivity, pathogenicity and immune response has extensively been demonstrated in laboratory animal models (Aggarwal, A. and Nash, T. E. 1987; Majewska, A. C. and Gustowska, L. 1996; Williamson, A. L. *et al.* 2000), even within isolates of the same host species (Visvesvara, G. S. *et al.* 1988; Cevallos, A. *et al.* 1995) and among clones of a same isolate (Udezulu, I. A. *et al.* 1992). Differences in virulence have been defined in terms of infectivity rates (Visvesvara, G. S. *et al.* 1988), minimal infective dose (Visvesvara, G. S. *et al.* 1988), maximal parasite load (Udezulu, I. A. *et al.* 1992; Williamson, A. L. *et al.* 2000) or small intestinal injury (Cevallos, A. *et al.* 1995; Majewska, A. C. and Gustowska, L. 1996). However, the pathogenic significance of the various genotypes has yet to be established since information about the infecting genotype is mostly not available. In addition, available literature on pathology involves *G. muris* infections in mice (Buret, A. *et al.* 1990) or is based on a small number of *G. duodenalis* isolates (Buret, A. *et al.* 1991; Cevallos, A. *et al.* 1995), trophozoites of a reference strain with a long history of laboratory culturing (Aggarwal, A. and Nash, T. E. 1987; Belosevic, M. *et al.* 1989) or heterogeneous *Giardia* populations (trophozoites or pooled cyst samples) without prior cloning (Visvesvara, G. S. *et al.* 1988; Majewska, A. C. and Gustowska, L. 1996). Furthermore, diverse endpoints are used, resulting in a great variability and difficult interpretation of published data.

Taking advantage of the fact that *in vitro* trophozoite culture and gerbil infections have recently been established for assemblages A, B and E strains (Benere, E. *et al.* 2010) enabled us to investigate the possible correlation between the assemblage subtype and *in vivo* phenotype for intestinal growth and overall virulence in the gerbil model.

MATERIALS AND METHODS

Parasite cultures: Eight strains of *G. duodenalis* were selected according to genotype (assemblages A, B and E), host species (human [h] or cattle [c]) and history (lab or field strain). The WB (ATCC 30957; assemblage A_I) and GS/M-83-H7 (ATCC 50581; assemblage B) strains were obtained from the Swiss Tropical and Public Health Institute, Basel, Switzerland (R. Brun) and from the Institute of Parasitology, Berne, Switzerland (N. Mueller). All field isolates, four of assemblage A (Ah1, Ah2, Ac1, Ac2) and two of assemblage E (Ec1, Ec2), were cloned and characterized by sequence analysis of the glutamate dehydrogenase (gdh), β-giardin (bg), triosephosphate isomerase (tpi) gene (Benere, E. *et al.* 2010). Assemblage A and E trophozoites were routinely maintained in TYI-S-33 medium at pH 6.8, supplemented with 10% heat-inactivated bovine serum and 1 µL/mL fresh bovine bile (Benere, E. *et al.* 2007). Assemblage B trophozoites were grown in TYI-S-33 medium supplemented with 0.5 g/L powder bovine bile and 10% heat-inactivated fresh human serum (Benere, E. *et al.* 2010). Detachment of trophozoites for preparation of inocula was achieved by chilling the cultures on ice for 20 min. The number of trophozoites was determined using 0.4% trypan blue (w/v) (Sigma, Bornem, Belgium) and 0.04% formol in a KOVA Glasstic® counting slide (Bayer, Brussels, Belgium).

Animal model: Four to five week-old male SPF gerbils (34.7g ± 0.6) were purchased from Janvier (St Isle, France). After one week acclimatisation, they were randomly allocated to experimental groups of four animals each. Before experimental infection, previous contacts with *Giardia* were excluded by microscopic examination of faeces collected over three consecutive days. The experimental infections were established using fresh trophozoites from axenic *in vitro* cultures. The gerbils were fasted overnight before oral inoculation with 10^6 trophozoites in 500 µL phosphate buffered saline (PBS). All animal experiments were approved by the ethical committee of the University of Antwerp.

Experimental design: The growth dynamics of the *G. duodenalis* isolates were determined by monitoring the number of trophozoites in the small intestine on day 4, 7, 11, 14, 18, 21, 25 and 29 post infection (pi). Clinical signs of diarrhoea and evolution of body weight were also monitored. Faecal consistency was scored using an ordinal scale with 0 = normal, 1 = slight softening, and 2 = severe softening. Faecal cyst excretion was not monitored. On each time point, four gerbils were euthanized with 100% CO_2 for collection of the entire small intestine and enumeration of the number of trophozoites. Briefly, the intestine was opened longitudinally and suspended in PBS on ice for 35 min to detach trophozoites. After removal of the intestine, the test tube was centrifuged at 800 g for 10 min at 4°C, to separate the trophozoites, which were then counted using a KOVA Glasstic® chamber. If no trophozoites were observed, the entire suspension was transferred to a 6-well plate and further examined using an inverted microscope, achieving a limit of detection of about 10^4 trophozoites/gerbil. To represent the growth dynamics of each strain as a single value, the area under the infection curve from day 0 to 18 (AUC) was calculated using GraphPad Prism software (San Diego California, USA).

Sample collection: Duodenal and jejunal segments were collected for histopathological analysis from animals sacrificed on day 7 and 18 pi. Beginning at the gastro-duodenal junction, the first 5 mm was discarded, the next 5 mm was collected for histopathological examination and the next 15 mm for the evaluation of brush-border enzyme activities. Similar tissue samples from the middle jejunum section were taken. The samples for evaluation of enzyme activities were immediately frozen in liquid nitrogen and stored at -80°C until further processing.

Histology: The small intestinal segments were fixed for 2 h in 4% paraformaldehyde (0.1M, pH 7.4) at room temperature and rinsed 4 times in PBS (0.01 M, pH 7.4) for 10 min. Tissues were dehydrated through a graded series of ethanol and at random embedded in paraffin wax. Sections (4 µm) were made, rehydrated, mounted and routinely stained with haematoxylin – eosin. Villus height (µm) and

crypt depth (µm) were measured in thirty perpendicular oriented villus-crypt units, divided over minimum ten high power fields (HPF). The epithelial cell height (µm) of three scattered enterocytes was determined in each villus. All measurements were performed by the same person at 100x using morphometric analysis software (Axiovision 4.8, Zeiss, Germany). Inflammation was assessed using a semi-quantitative estimation of the amount of inflammatory cells present in the mucosa of five randomly chosen HPF's: Score 0 = comparable to uninfected control (UC), Score 1 = a slightly increased number of inflammatory cells in the villi; Score 2 = moderate increased number of inflammatory cells in both villi and crypt; Score 3 = severe inflammation with higher numbers of inflammatory cells compared to score 2. For each strain, data are presented as the weighted means of the scores obtained for each individual gerbil.

Brush-border enzyme activity: The intestinal segments collected on day 7 pi were homogenized in ice-cold distilled water using a tissue grinder, frozen in liquid nitrogen and kept at -80°C until analysis. The activities of sucrase, maltase and lactase were determined by measuring the D-glucose liberated from the respective substrates using the glucose oxidase-peroxidase system (DAHLQVIST, A. 1964). Alkaline phosphatase was measured by the rate of splitting the paranitrophenylphosphate substrate into the yellow paranitrophenol (Millington, P. F. and Tovell, P. W. 1969). Enzyme-specific activities (SA) are expressed as µmol substrate hydrolysed per min and related to 1 g of protein, estimated according to the Bradford method (Bradford, M. M. 1976). The results are expressed as mg protein per g fresh organ weight. All reagents were from Sigma Chemical Co. (St Louis, Mo, USA) or from Merck (Darmstad, Germany).

Statistics: All results were compared to the UC and expressed as group means ± standard error of the mean (SEM). For the statistical evaluations, SPSS® 17.0 (SPSS Inc, Chicago Illinois, USA) was used. Comparison between the *Giardia* isolates was performed using a one-way analysis of variance (ANOVA) with LSD *post hoc* for histopathology and enzyme activity data, and AUC values generated

from intestinal trophozoite burdens and weight gain. The categorical inflammation data were analysed using a Chi-square test. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Infection dynamics (Figure 1): All isolates showed a similar basic pattern of infection but with variable levels of trophozoite burdens. All gerbils inoculated with GS/M-83-H7, WB and A_I field isolates were positive on day 4 pi, while only 50 - 75% were positive for the E_{III} field isolates and none for the A_{II} field isolate. Trophozoites steadily increased in number and remained high until day 14 pi, except for assemblage A_{II} which already started to decrease on day 11 pi. All isolates showed low trophozoite loads at day 18 pi (mean 1 x 10^6/gerbil). Minimal residual burdens were still present on day 29 pi (mean 4.3 x 10^4/gerbil), except for the assemblage E_{III} field isolates that cleared around day 25 pi. All assemblage subtypes displayed significant different growth dynamics ($p<0.05$), with the exception of assemblage subtype A_{II} and E_{III}, which showed comparable growth ($p>0.05$). The A_I lab (WB) strain demonstrated the highest trophozoite burdens and A_{II} and E_{III} subtypes the lowest.

Clinical manifestations: The overall weight gain per assemblage subtype was significantly lower in infected gerbils (AUC= 491.7 ± 16.7), compared to the UC (AUC= 769.4 ± 59.2), but was not significantly different among the assemblage subtypes ($p<0.05$). Gerbils infected with the A_I lab (WB), A_I field and E_{III} field isolates showed occasional slight softening of the faeces, while infection with the GS/M-83-H7 strain resulted in more consistent softened faeces from day 7 till day 16 pi.

Mucosal architecture (Figure 2): On day 7 pi, the duodenal villus height did not differ from the UC while the crypt depth was significantly increased by about 38% to 47% ($p<0.05$), except for the assemblage subtype A_{II} field isolate. Jejunal villus height became reduced by 23% to 29% after

infection with A_I, A_{II} and E_{III} field isolates, but the degree of villus shortening remained comparable between the subtypes mentioned. Jejunal crypt depth was increased by 35% and 16% upon infection with the assemblage B (GS/M-83-H7) lab strain and A_I field isolates, respectively ($p<0.05$). Epithelial cell height in the duodenum did not differ from the UC, except for the assemblage B lab strain, while a reduction of 15% to 23% was observed in the jejunum for all strains without significant differences between the different subtypes. By day 18 pi, almost full recovery was noted. Villus height had returned to UC values in both duodenum and jejunum. However, crypt hyperplasia was still present in the duodenum and jejunum of gerbils infected with the WB (A_I lab) strain (+59%, +70%) and in the jejunum of GS/M-83-H7 (+22%) and A_I field (+23%) isolate infected animals ($p<0.05$). Epithelial cell height had returned to UC values in the jejunum, but was still markedly decreased in the duodenum of gerbils infected with the GS/M-83-H7 (-35%) and A_I field (-14%) isolates ($p<0.05$).

Inflammation (Table 1): A slight to moderate inflammatory infiltration developed by day 7 pi and tended to increase by day 18 pi. In addition, the inflammatory infiltration was generally more severe in the duodenum than in the jejunum. Comparing the changes among the assemblage subtypes, a significant difference ($p=0.006$) was only found in the duodenum at day 18 pi. Light microscopic analysis revealed strong infiltration of inflammatory cells in both villi and crypts after infection with the GS/M-83-H7, A_I lab and field isolates (Figure 3). In contrast, only a slight to moderate inflammation was observed with the A_{II} or E_{III} field isolates. The inflammatory cell population consisted predominantly of plasma cells, lymphocytes and macrophages.

Brush-border enzyme activity (Figure 4): Since the disaccharidases (sucrase, maltase and lactase) showed similar SA profiles, only the sucrase SA is presented in figure 4. All assemblage subtypes significantly decreased the duodenal disaccharidases (32% to 84%) and alkaline phosphatase SA (26% to 82%). In the jejunum, only the assemblage B (GS/M-83-H7) lab and the A_I field strain significantly reduced SA of all disaccharidases (11% to 61%) while the alkaline phosphatase SA was decreased

(22% to 54%) for all assemblages except subtype A_{II}. The B lab strain showed the highest reduction in all four brush-border enzyme SA. In contrast, subtype A_{II} caused the lowest reduction in all four brush-border enzyme SA in the duodenum and in alkaline phosphatase SA in the jejunum.

DISCUSSION

Although a large genetic and clinical variability has been identified among *G. duodenalis* isolates (Roxstrom-Lindquist, K. *et al.* 2006), it is still unclear whether the various genotypes are associated with different disease outcome or intrinsic virulence. This study specifically aimed to explore the significance of the differences between assemblage subtypes A_{I}, A_{II}, B and E_{III} on intestinal growth and virulence characteristics in the gerbil model.

Our study indicates that the assemblage subtype can indeed be associated with particular intestinal growth characteristics since all displayed significantly different growth dynamics, with the exception of A_{II} and E_{III} field isolates. The assemblage subtypes A_{I} (lab and field isolates) and B were more infectious and produced higher trophozoite loads for a longer period of time compared to the subtypes A_{II} and E_{III}. The A_{I} lab strain demonstrated the highest trophozoite burden, significantly different from all other isolates including their assemblage-matching A_{I} field strains. This may be linked to the long term *in vitro* cultivation and successive passages that may have altered its growth characteristics. For example, the colonization capacity of the P0-1 lab strain (assemblage A_{I}) was also found to be higher and more regular compared to an assemblage-matching field isolate (Sotiriadou, I. and Karanis, P. 2004). Independent of the isolate, the acute phase of infection with high trophozoite burdens occurred between day 4 and day 11 pi with appreciable reduction by day 18 to 29 pi, an infection pattern which is similar to what previously has been described for the WB and GS/M-83-H7 strain (Aggarwal, A. and Nash, T. E. 1987; Belosevic, M. *et al.* 1989).

Morphological analysis of the small intestine on day 7 and 18 pi, respectively representing the acute and recovery phase of infection, revealed crypt hyperplasia, villus atrophy and reduced epithelial cell height. Unlike the increased crypt depth, decrease in villus and epithelial cell height was not consistently observed and not uniformly distributed over the small intestine, a phenomenon also observed in *Giardia* infected patients (Farthing, M. J. 1997). Our results suggest a correlation of mucosal damage with the assemblage subtype, with considerable alterations after infection with assemblage subtype A_I and B lab strain, moderate damage after A_I field strains and only a low extent of damage after assemblage subtype A_{II} and E_{III} infections. Likewise, assemblage-linked reduction of brush-border enzyme activities were greatest in assemblage B infected animals, followed by assemblage subtype A_I (lab and field isolates), E_{III} and A_{II}. The morphological and functional changes of the WB strain were within the same range to those found by other researchers (Belosevic, M. *et al.* 1989; Leitch, G. J. *et al.* 1993). However, the morphological damage in GS/M-83-H7 infected gerbils was not observed in BALB/c mice (Byrd, L. G. *et al.* 1994).

The activities of intestinal brush-border enzymes (e.g. alkaline phosphatase and disaccharidases) are markers of epithelial digestive function (Mahmood, S. *et al.* 2005) and were measured to assess the relevance of the observed villus and crypt modifications. Severe reductions in brush-border enzyme activities were found, even in the absence of obvious villus or epithelial cell damage. These findings are consistent with previous reports (Buret, A. *et al.* 1990; Cevallos, A. *et al.* 1995) and suggest that brush-border enzyme deficiencies in giardiasis are correlated with a diffuse loss of epithelial brush-border microvillus surface area rather than with alteration in villus structure. Also consistent to previous reports (Buret, A. *et al.* 1991; Astiazaran-Garcia, H. *et al.* 2000), the clinical manifestations of intestinal pathology remained fairly minimal: the net weight gain of infected gerbils was decreased compared to the uninfected controls but did not differ between the isolates, and softening of faecal pellets was noted for some assemblages. In symptomatic patients, assemblage B has been associated with severe acute and persistent diarrhoea and assemblage A with mild intermittent diarrhoea

(Homan, W. L. and Mank, T. G. 2001). In our study, assemblage B infected gerbils also presented the highest faecal score and diarrhoea has indeed been described in gerbils infected with human or animal strains of *G. duodenalis* (Buret, A. *et al.* 1991; Majewska, A. C. and Gustowska, L. 1996). Unfortunately, information on the genotype was not available making it impossible to align our findings. It also became clear that the severity of the clinical features (body weight, soft faeces) may not be related to the extent of mucosal abnormalities since assemblage A_I induces a high degree of mucosal and functional damage without causing obvious signs of diarrhoea.

Immediately related to the fact that *G. duodenalis* preferentially colonizes the duodenum (Buret, A. *et al.* 1991), all isolates induced a significant larger reduction (10% to 28%) in the activity of the 4 brush-border enzymes in the duodenum compared to the jejunum. The mucosal abnormalities and brush-border enzyme malfunctions were markedly present during the acute phase of infection (day 7 pi) with clear signs of recovery by day 18 pi. A direct correlation between the period of highest parasite loads and morphological/functional damage can therefore be suggested, which has already been hypothesised in previous literature reports (Belosevic, M. *et al.* 1989; Araujo, N. S. *et al.* 2008). Consistent with this, the mucosal architecture in mice infected with the human BRIS/83/HEPU/106 isolate was quickly restored, whereas the avian BRIS/95/HEPU/2041 strain induced a long term infection with villous atrophy still evident at 26 days pi (Williamson, A. L. *et al.* 2000).

Although *Giardia* infections in man are generally associated with little or no mucosal inflammation (Oberhuber, G. *et al.* 1997; Roxstrom-Lindquist, K. *et al.* 2006), a remarkable infiltration of inflammatory cells in the lamina propria was observed with all assemblage subtypes in this study. The experimental infections were established with sterile trophozoite cultures and one can therefore be sure that the observed inflammation must be linked to *G. duodenalis*. This is further supported by observations of mucosal inflammation in naturally infected human and cattle (Ruest, N. *et al.* 1997; Hanevik, K. *et al.* 2007; Mokrzycka, M. *et al.* 2010), goats experimentally infected with isolates from

pigs (Koudela, B. and Vitovec, J. 1998) or gerbils infected with isolates from human or rodents (Majewska, A. C. and Gustowska, L. 1996). The inflammatory cell population of predominantly mononuclear cells is consistent with other reports (Oberhuber, G. *et al.* 1997). The degree of infiltration varied depending on the isolate, the time point in the infection and the intestinal region. The highest infiltration of inflammatory cells was found in the duodenum with assemblage B and A_I (lab and field) being most severe while assemblage A_{II} or E_{III} only showed slight to moderate inflammation. Quite remarkably, all infections demonstrated high inflammation scores at day 18 pi when the parasite load had already declined. Similarly, the mucosal inflammation in gerbils infected with a rodent isolate (CP-117) was higher on day 21 pi than on day 7 pi (Majewska, A. C. and Gustowska, L. 1996) and lymphocyte counts in *Giardia*-infected patients returned to normal some time after treatment and eradication of the parasite (Wright, S. G. and Tomkins, A. M. 1977).

In summary, the assemblage subtypes A_I and B are more infectious, produce higher trophozoite loads for a longer period and elicit relatively greater pathogenicity, characterized by higher intestinal inflammation and more extensive damage to mucosal architecture and function. The assemblage subtypes A_{II} and E_{III} are clearly less virulent, although it must be recognized that not all factors contributing to virulence have been addressed in this study. The lack of genetic information in most published reports on pathogenicity has been a major shortcoming, but should become a standard requirement in future studies on *Giardia* in view of the present study results. The inclusion of even more isolates would be beneficial to corroborate our major findings.

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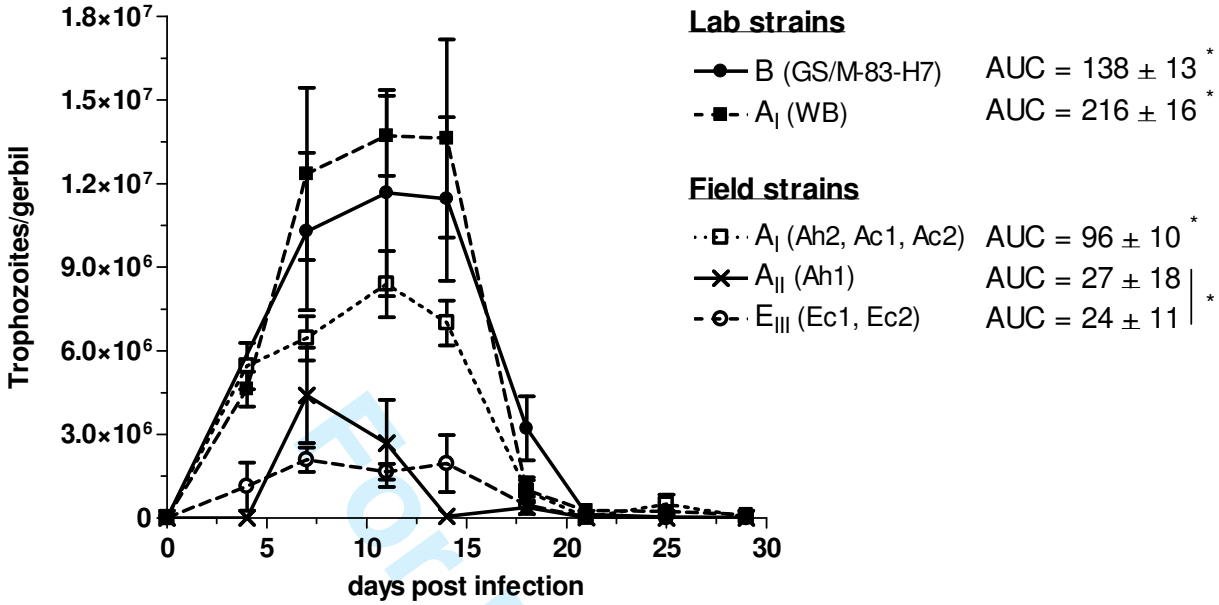
Table 1: Weighted means of the inflammation scores[#] obtained in gerbils infected with lab and field strains of assemblages A, B and E. *(each data point represents the mean of 4 gerbils)*

Strain	Day 7 pi		Day 18 pi	
	Duodenum	Jejunum	Duodenum	Jejunum
UC	0	0	0	0
Lab strains				
B: GS/M-83-H7	1.7	1.0	3.0*	1.7
A_I: WB	1.3	0.3	2.8*	2.3
Field strains				
A_I: Ah2, Ac1, Ac2	1.5	1.5	2.8*	1.7
A_{II}: Ah1	0.8	0.5	1.3*	1.5
E_{III}: Ec1, Ec2	1.1	0.6	1.9*	1.0

[#]Score 0 = comparable to uninfected control (UC), Score 1 = a slightly increased number of inflammatory cells in the villi; Score 2 = moderate increased number of inflammatory cells in both villi and crypt; Score 3 = severe inflammation with higher numbers of inflammatory cells compared to score 2.

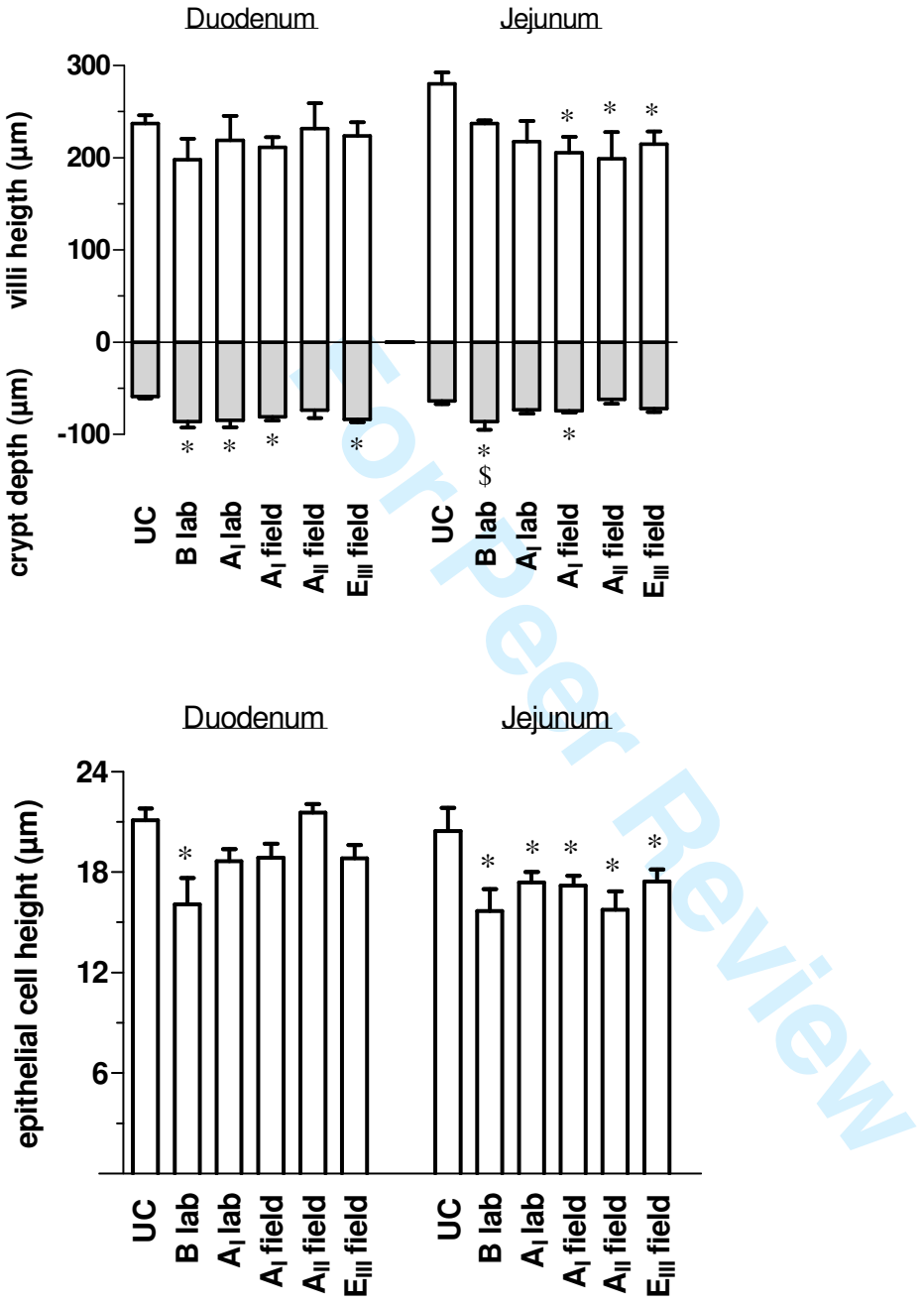
* Significant difference between the assemblage subtypes B/A_I and the assemblage subtypes A_{II}/E_{III} (P = 0.006).

Figure 1: The growth dynamics of lab and field strains of assemblage A, B and E. *(each data point represents the group means ± SEM of 4 gerbils)*



*: significant different from the other strains, $p<0.05$

Figure 2: Analysis of crypt depth and villus and epithelial cell height in the small intestine of gerbils 7 days pi with lab and field strains of assemblage A, B and E. *(each data point represents the group means ± SEM of 4 gerbils)*



*: significantly different from the UC, p<0.05

$: significantly different from the other strains (except from the A_I lab strain), p<0.05

Figure 3: Light microscopic analysis of the small intestine of the uninfected control (UC) (a) and the WB lab strain of assemblage subtype A_I showing a moderate (score 2) (b) to severe (score 3) (c) infiltration of inflammatory cells in both villi and crypts 18 days pi. (200x magnification)

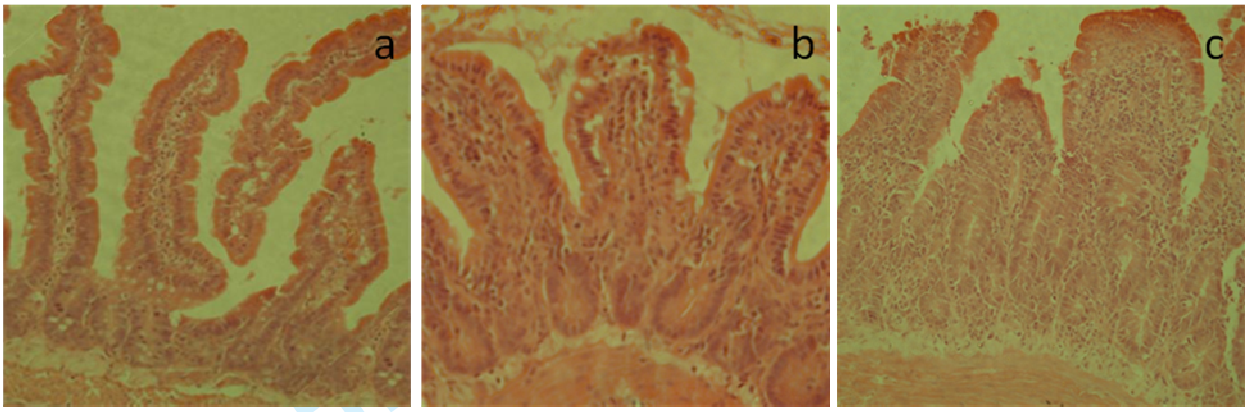
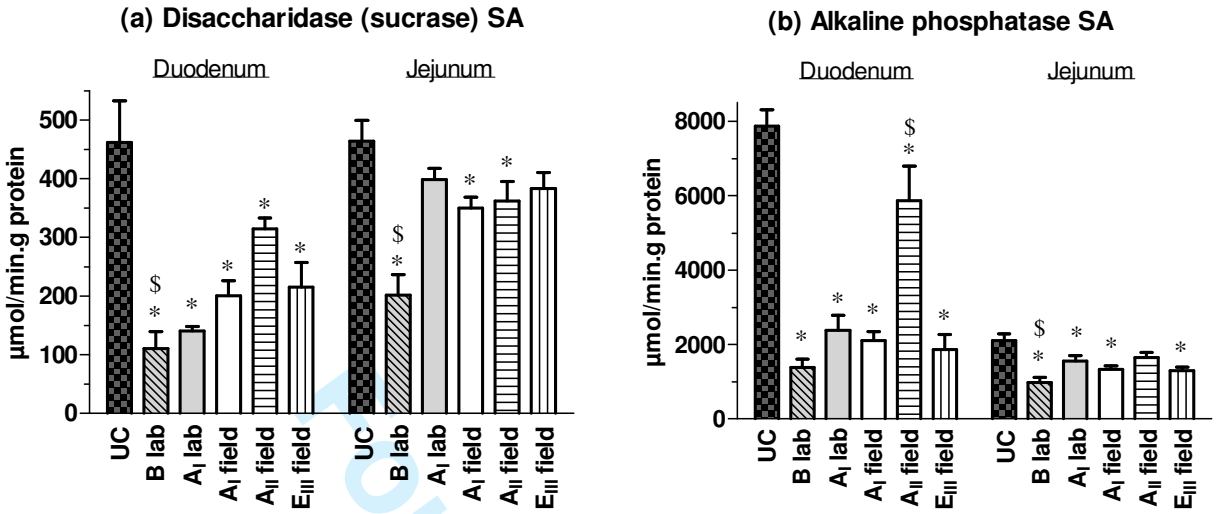


Figure 4: Brush-border enzyme activity in the small intestine of gerbils 7 days pi with lab and field strains of assemblage A, B and E: (a) Disaccharidase (sucrase) and (b) alkaline phosphatase SA. *(Each data point represents the group means ± SEM of 4 gerbils)*



*: significant different from the UC, $p<0.05$

$: significant different from the other strains (except from the A_I lab strain for duodenum sucrase and A_I, E_{III} field strains for jejunal alkaline phosphatase), $p<0.05$