



BIOCHEMICAL AND ELECTRON MICROSCOPIC CHANGES INDUCED BY GIARDIA EXPERIMENTALLY INFECTED LAMBS

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ABSTRACT

The present study was conducted to evaluate the effect of *Giardia* on the biochemical serum constituents of experimentally infected lambs, in addition to studying the observed alterations accompanying *Giardia* infection in the intestinal mucosa using scanning electron microscopy. Twenty lambs were allotted into two equal groups, Group A (non infected) was kept as control negative and Group B (infected) was orally inoculated by 10⁶ *Giardia* cysts. The biochemical changes were assessed in both groups on 7th, 14th, 21th and 30th days post inoculation (dpi). The study disclosed that *Giardia* induced a significant drop in the levels of serum electrolytes (chloride, sodium, potassium and calcium), blood glucose, different enzymes (lipase, amylase and alkaline phosphatase). Furthermore, the levels of urea, liver enzymes (alanine aminotransferase and aspartate aminotransferase), inflammatory marker (C-reactive protein) and oxidative stress marker (malondialdehyde) were elevated, but nitric oxide was declined by 30th dpi. The scanning electron microscopy of the intestinal mucosa of the infected lambs revealed a notable alteration which was fully explained. All the presented results interpret the pathophysiological effect of *Giardia* which adversely affects the health status of lambs.

Keywords: *Giardia*, Biochemical parameters, Oxidative stress, Electron microscopy.

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1. INTRODUCTION

Giardia sp. is the most common and important intestinal parasite and it is a frequent common cause of diarrheal diseases through the world. It localized and multiplies in the small intestines of human and domestic animals [1, 2]. In ruminant, *Giardia* causes impairment in feed conversion efficiency which adversely affects the health status and causes enormous economic losses to livestock industry, greatly affects reproductive performance and causes significant production losses [3, 4]. Giardiasis outbreak in sheep mostly showed no clinical sign or mortality, but *Giardia*-infected lambs had malabsorption, decreased weight gain, and reduced feed efficiency [5]. The pathophysiological changes associated with *Giardia* infection include: malabsorption of electrolytes and nutrients, intestinal microvillus atrophy, loss of epithelial barrier function due to the produced ulceration and infarction, increased permeability and narrowing of the lumen [6, 7]. The Changes in the sero-biochemical constituents and the hypothesis of decreased activity of the defense system for protecting tissues from the free radical damage associated with *Giardia* infection in lambs need further investigation. This was explained in the current study by detecting the alteration in the sero-biochemical parameters, electrolytes and digestive enzymes and by assessing

the level of MDA and Nitric oxide in experimentally infected lambs compared with non-infected ones. Besides, identifying the alteration induced by *Giardia* on the intestinal epithelium using scanning electron microscopy.

2. MATERIALS AND METHODS

Cysts. *Giardia* cysts were collected from naturally infected sheep. They were identified after lugol's iodine and Giemsa staining. All the collected cysts were liquefied in distilled water and stored at 4°C. Concentration of fecal specimens was practiced by zinc sulfate floatation technique, followed by twice washing of the concentrated cysts by centrifugation at 1000 rpm for 10 minutes. Lastly, the supernatant was decanted and the sediment was resuspended in a saline solution. Haemocytometer was used to quantify the density of the cysts inoculum. The final inoculum were adjusted to 10⁴ Cysts/ lamb.

Animal groups. Lambs used in this study were of both sexes and aged 6-8 months. They were kept in well-ventilated bin cages and provided with standard food and water. Twenty lambs were equally allocated into two groups; group A (non-infected group) and Group B (infected group). The lambs were kept in acclimatized conditions for two weeks. During this period, the feces of the lambs were examined daily by direct wet smear and floatation techniques to exclude the existence of any parasites before the experiment.

Ethical approval. Lambs in this work were permitted by the local committee of the Faculty of Veterinary Medicine, Benha University and according to the guidelines of the National Institute of Health in Egypt.

***Giardia* cyst inoculation.** Each lamb in group B was inoculated orally by a single feeding of *Giardia* cysts (10⁴ Cysts/ lamb).

Cysts detection. Because of the intermittent shedding of *Giardia* cyst is, the lambs feces were examined 3 times daily for ten days till appearance of *Giardia* cysts and establishment of the infection among all lambs.

Serum collection. Ten ml of blood was collected from the jugular vein of each lamb in group A and B into non heparinized tubes. The blood samples were left to stand for 30- 60 minutes at room temperature for clotting. The clotted blood were centrifuged at 3,000 rpm for 10 min for serum separation, all the sera were stored at -20°C till further analysis [8].

Biochemical assessments. Estimation of biochemical changes was done by analyzing the serum samples of the two groups for chloride, sodium, potassium, calcium, creatinine (mg/dl), urea, alanine aminotransferase (ALT), aspartate aminotransferase (AST), C- reactive protein(CRP), glucose, amylase, lipase, alkaline phosphatase (ALP), malondialdehyde (MDA) and nitric oxide (NO) using a high performance liquid chromatography method (HPLC, Sykam 1125 pump system, Germany) [9-11]. The changes in the biochemical parameters were assessed on 7th, 14th, 21th, 30th dpi by comparing the mean values ± SE of lambs in A and B groups.

Electron Microscopy

Two infected lambs were sacrificed at the end the experiment and small portions of their duodenum, proximal part of the jejunum and the lower digestive tract were obtained to identify the changes associated with *Giardia* trophozoites and cysts. The specimens were kept in glutaraldehyde (2.5%) at 4°C for scanning electron microscope (SEM). They were then rinsed in distilled water and dehydrated in acetone alcohol series (25%, 50%, 75%, and 100% for 10 min each). The critical point drying was done using CO₂ to complete the drying process. Finally, the samples were coated by gold using a spraying tool and they were visualized with SEM [12].

Statistics

The statistical analysis was applied using SPSS soft ware (Ver. 16). The results were appraised using One-way Anova and post hoc multi comparisons test at a significance level $P < 0.05$. Descriptive statistical values as median, mean, standard deviation, maximum and minimum were also calculated for comparing of all variables [13].

3. RESULTS

Clinical Symptoms

The prepatent period of *Giardia* ranged between 8-10 days in group B (infected group). The cysts shedding continued all through the period of the experimental study (Fig. 1. a, b). The infected lambs suffered from decreased weight gain, loss of feed efficacy and diarrhea. The onset of diarrhea was on 14th dpi and continued intermittently throughout the study period.

Biochemical Assessment

The mean \pm SE of the serum constituents values of each lamb are displayed in table.1. On 30th dpi the biochemical analysis of the sera revealed a statistically significant decrease ($P < 0.05$) in the levels of serum electrolytes as chloride, sodium, potassium and calcium (60.50 ± 0.87 , 123.3 ± 1.58 , 4.02 ± 0.20 and 2.29 ± 0.11 ; respectively) among infected lambs compared to non infected ones. The beginning of declining of sodium and potassium was on 14th dpi. Whereas, *Giardia* infection did not induce a significant effect ($P > 0.05$) on the level of creatinine. Nevertheless, the infection significantly raised the level of urea to 209.7 ± 0.62 by 30th day only. Concerning liver enzymes, a substantial increase ($P < 0.05$) in the level of ALT was noticed from 21th dpi to 30th dpi (18.45 ± 1.01 and 20.95 ± 1.32 respectively), while the level of AST was only elevated on 30th dpi (98.04 ± 4.12). The maximum mean concentration of serum CRP (10.70 ± 1.06) was noticed on 30th dpi. Serum glucose significantly decreased to 70.51 ± 0.28 on 30th dpi, Serum amylase declined efficiently on 14th dpi (50.76 ± 3.96) and continue declining afterward. A noticeable decrease in the levels of serum lipase and alkaline phosphatase was recorded on 21th dpi (9.98 ± 1.14 and 107.32 ± 0.76 ; respectively) and the declining persisted till 30th dpi (10.11 ± 0.53 and 96.25 ± 0.80 ; respectively). Regarding the oxidative stress markers, MDA level significantly activated on both 21th dpi and 30th dpi (3.42 ± 0.27 and 3.99 ± 0.20), but NO declined to 40.10 ± 0.67 by 30th dpi.

Electron Microscopy

In the infected lambs, scanning electron microscopy of the duodenum and the proximal part of the jejunum revealed varying degrees of destruction in the intestinal villi. Large number of pear shaped trophozoites measuring 12-18 X 7- 10 μ m were seen clasped in situ by their convex sucking discs and bulging above the brush border of the microvilli. Some intestinal villi showed absence of the microvillus border with their basic epithelium. The connective tissue of the intestinal mucosa and submucosa showed fibrosis. (Fig. 1. d,e). Correspondingly, oval shaped *Giardia* cysts measuring 9- 12 X 8-10 μ m were noticed located in the lower digestive tract, without evident changes in the intestinal structure. (Fig 1. c).

4. DISCUSSION

In the current study, a recorded significant decrease in the electrolyte level (CL, Na, K and Ca) was encountered among infected lambs as compared with the non-infected ones. This aforesaid results came in agreement with other reports [1, 14, 15]. Previous studies demonstrated that the reduced absorption of Na^+ is due to the loss of the absorptive power of the intestinal epithelium [16, 17]. In contrast, Ayaz, et al. [18] reported that K and Na levels didn't significantly alter in animals infected with many endoparasites. Moreover, [19, 20]

proved that *Giardia* may change the secretory response of chloride in human and mice. This may explain the low chloride level reported in this study among *Giardia* positive lambs.

The recorded change in the electrolyte balance could be explained by the intestinal inflammation produced due to the activity of *Giardia* trophozoites. Such inflammation and the consequential impairment in electrolytes secretion and absorption often lead to acid, base and electrolyte imbalance which may cause diverse complications in body [21]. This may also interpret the recorded cases of diarrhea which were noticed during the experiment.

The non-significant effect of *Giardia* on creatinine level was formerly revealed by Rosa, et al. [22]. Though, *Giardia* showed a significant decreasing effect on urea level as previously encountered by Ragbetli, et al. [1]. In our opinion, this elevation may be created as a result of the increase of nitrogen metabolism in *Giardia* infection. The malnutrition effect of *Giardia* among infected lambs was associated with a low ALP level. Since, the hepatobiliary integrity is measured by the serum ALP [23] so the low ALP level accuses the indirect negative effect of *Giardia* on liver tissues and bile duct. Accordingly, this effect on the liver tissues could explain the recorded elevation of the liver enzymes (ALT and AST) among infected lambs in the study.

Mostly, the low levels of amylase and lipase among infected lambs was in accordance with the result of Buret [6]. This outcome was explained by the drawback effect of *Giardia* trophozoites on the pancreatic function which inhibits pancreatic lipase and amylase activities [24].

The impairment of serum glucose level was also observed among infected lambs. This observation may reflect the malabsorption and maldigestion effects accompanying *Giardia* infection [25].

The significant elevation of CRP among infected than non infected lambs may be accredited to the damage produced by the attachment of *Giardia* trophozoites to the mucosa of the intestine which continuously stimulated the hepatocytes for elevating the synthesis of CRP as innate defense mechanism. Mainly, CRP cooperates with the raised mucosal phagocytic activities along with IgA which acts as a humoral defense mechanism against *Giardia* [26, 27].

The high level of MDA among infected lambs is an indication of the oxidative stress produced in the infected tissues [28] due to *Giardia*. The decreased activity of the defense mechanism for protecting the tissues from the damage produced by *Giardia* might be one of the reasons of elevation the level of MDA among infected lambs [29].

As regard, the significant decrease in the level of serum NO among infected lambs was consistent with another report [1]. Nitric oxide formation has been encountered in many parasitic infections including *Giardia* [30, 31]. It is considered as only part of an immunopathological series against infection [32]. The low NO level may be credited to the release of arginine deiminase and flavohemoglobins by *Giardia* trophozoites which consumes all the local arginine (precursor of nitric acid formation) [33].

The intestinal changes scanned by the electron microscopy came in agreement with other reports on *Giardia* [7, 34]. Such changes ensured that the colonization of the intestine with *Giardia* trophozoites caused diffuse destruction in the microvillus brush border which in turn resulted in maldigestion and malabsorption effects. Accordingly, a combination of electrolyte hyper secretion and malabsorption appears to be accountable for accumulation of fluid in the intestine, which aggravate diarrhoea [35, 36]. All the above mentioned alterations mainly had a drawback effect on the biochemical serum constituents specially electrolytes [21].

5. CONCLUSION

The study advanced our standing of the pathophysiological effect of *Giardia* on lambs. It induced a significant declining effect on serum electrolytes (CL, Na, K, and Ca), digestive enzymes (Lipase, amylase and ALP) and glucose. Likewise, it significantly elevated the level of urea, Liver enzymes (ALT, AST), liberated inflammatory

markers (CRP). Moreover, *Giardia* induced a significant activation and declining effect on MDA and NO levels; respectively. The Electron microscopy revealed the alteration induced in the intestinal mucosa due to the attachment of *Giardia* trophozoites. These changes interpret the pathogenicity of *Giardia* which adversely affects the health status of lambs.

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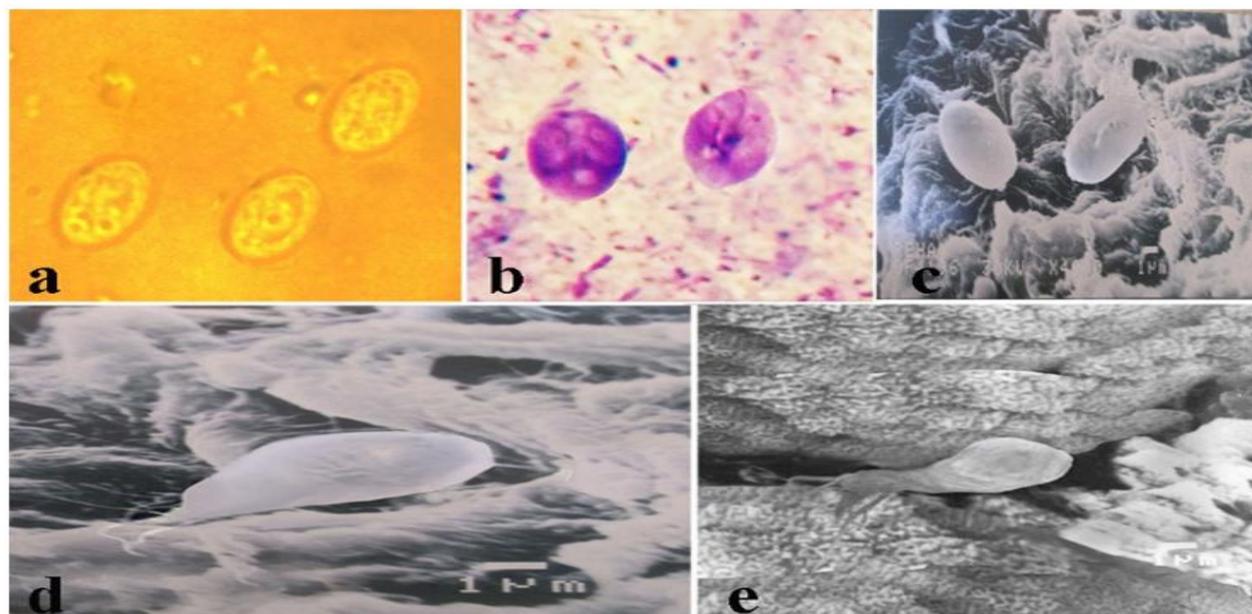


Fig-1. *Giardia* cyst: a. Stained by lugol's iodine, b. Stained with Giemsa stain. Scanning electron microscopy of the intestinal mucosa showing: c. Oval shape *Giardia* cyst, D. Dorsal view of the pear shape trophozoite of *Giardia*. E. *Giardia* trophozoites attached by their adhesive disc to the intestinal mucosa.

Table-1. Comparing the mean ± SE of the serum biochemical values of *Giardia* infected and non infected lambs

Serum parameter	Group A (Non infeced)	Group B (Infected)			
		7 th dpi	14 th dpi	21 th dpi	30 th dpi
Mean ± SE					
Chloride (mEq/L)	94.67 ± 0.18 ^a	94.80 ± 1.04 ^a	90.76 ± 3.21 ^a	70.87 ± 0.39 ^b	60.50 ± 0.87 ^c
Sodium (mEq/L)	158.7 ± 0.40 ^a	158.22 ± 0.45 ^a	140.87 ± 1.81 ^b	133.65 ± 2.11 ^c	123.3 ± 1.58 ^d
Potassium (mEq/L)	5.48 ± 0.15 ^a	5.2 ± 0.64 ^a	3.34 ± 0.20 ^b	4.11 ± 0.05 ^b	4.02 ± 0.20 ^b
Calcium (mg/ dl)	3.100 ± 0.29 ^a	3.51 ± 0.18 ^a	3.02 ± 0.01 ^a	2.10 ± 0.25 ^b	2.29 ± 0.11 ^b
Creatinine (mg/ dl)	1.41 ± 0.24 ^a	1.21 ± 0.01 ^a	1.17 ± 0.99 ^a	1.15 ± 0.14 ^a	1.37 ± 0.22 ^a
Urea (mg/ dl)	174.5 ± 1.40 ^b	176 ± 0.55 ^b	175.20 ± 0.46 ^b	177.53 ± 1.46 ^b	209.7 ± 0.62 ^a
ALT (U/L)	11.05 ± 0.10 ^b	12.34 ± 0.25 ^b	12.54 ± 0.88 ^b	18.45 ± 1.01 ^a	20.95 ± 1.32 ^a
AST (U/L)	62.48 ± 1.03 ^b	69.21 ± 6.49 ^b	70.56 ± 0.32 ^b	70.86 ± 3.53 ^b	98.04 ± 4.12 ^a
CRP (mg/dl)	5.74 ± 0.88 ^c	6.21 ± 0.26 ^c	6.59 ± 0.47 ^{b c}	8.54 ± 0.13 ^b	10.70 ± 1.06 ^a
Glucose(mg/dL)	112.09 ± 0.83 ^a	111.34 ± 0.21 ^a	109.95 ± 1.20 ^a	112.09 ± 0.53 ^a	70.51 ± 0.28 ^b
Amylase (U/L)	82.66 ± 1.13 ^a	80.27 ± 0.23 ^a	50.76 ± 3.96 ^b	46.69 ± 1.78 ^b	36.09 ± 1.48 ^c
Lipase (U/L)	21.49 ± 0.27 ^a	21.33 ± 1.47 ^a	20.41 ± 0.20 ^a	9.98 ± 1.14 ^b	10.11 ± 0.53 ^b
ALP (IU/L)	193.7 ± 0.98 ^a	192.98 ± 0.80 ^a	194 ± 1.15 ^a	107.32 ± 0.76 ^b	96.25 ± 0.80 ^b
MDA (mml/L)	2.78 ± 0.45 ^b	2.70 ± 0.35 ^b	2.72 ± 0.33 ^b	3.42 ± 0.27 ^a	3.99 ± 0.20 ^a
NO (umol/L)	46.11 ± 0.11 ^a	45.04 ± 0.61 ^{ab}	45.020 ± 0.17 ^{ab}	43.20 ± 1.27 ^b	40.10 ± 0.67 ^c

Source: ^{a,b,c} letters of different subscript are considered significant at (p < 0.05)

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