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Protection against *Eimeria stiedae* in Rabbits by using sonicated sporulated oocyst vaccine

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Abstract
This study was designed to evaluate a sonicated sporulated oocyst vaccine against *E. stiedae* in rabbits, in Parasitology department/ Faculty of Veterinary Medicine/ University of Baghdad, from the beginning of January to the end of December 2015. Forty rabbits were divided randomly into 4 equal groups. Different doses of sonicated sporulated oocysts of *E. stiedae* were given to group I and II, subcutaneously, and challenged by 1000 sporulated oocysts of *E. stiedae* on day 20 post vaccination. Group III had been injected the challenge dose, while group IV were administered water only. All rabbits were subjected for oocyst shedding rate and blood sampling to estimate total and differential leukocyte count, serum antibody titers and hepatic enzymes. Results showed significant differences in oocyst shedding rate between vaccinated and non-vaccinated rabbits, commenced at day 17 post challenge. The Ab titers in vaccinated rabbits were markedly higher than that of the infected rabbits, despite no significant ($P>0.05$) differences between vaccinated groups. Non-vaccinated rabbits showed significantly higher WBC count, neutrophil and eosinophil with lower lymphocyte percentage than other groups on day 14 PC onwards. Disturbed serum ALP, ALT and AST levels were obvious in G3.

Keywords: *Eimeria stiedae*, Rabbits, oocyst, vaccine

1. Introduction
Rabbits coccidiosis is a contagious aggressive disease caused by *Eimeria spp,* most often affect youngsters that eat food contaminated by feces from infected or carrier dam or environment, since the disease is normally inhabit the intestine of most bunnies [1, 2]. Mostly, *Eimeria spp* affect the intestine favoring a different part for each species [2]. Symptoms are species specific and may include weakness, severe blood or mucus diarrhea, abdominal pain, and if weight loss exceeds 20%, death occurs within 24 hours [2]. Rapid death may occur preceded weakness and lethargy without diarrhea. Out of the eleven different *Eimeria* species, only *Eimeria stiedea* affects the liver [2].
Eimeria stiedea causes devastating hepatic coccidiosis in rabbits resulting in highest morbidity and mortality rate [3]. It causes thickening of the bile ducts causing stunting and a pot belly with less often fatal. In contrast to chicken coccidia, scarce knowledge is currently valuable concerning rabbit coccidiosis, although Eimeria stiedai was observed by Leeuwenhoek as early as 1674 [4]. In 1879, Leuckart named them Coccidium oviforme and distinguished liver and intestinal coccidian [4].
Beside sanitation, control measures overcoming coccidiosis could be achieved by anticoccidial drugs and vaccination. Regarding medicaments, an emerging drug resistance [5], a relatively long withdrawal period and changing the meat taste due to such residues act as problematic public health issue [6, 7]. Far from these disadvantages, vaccine is the most plausible solution economically and for the consumers' preference. Degree of efficiency of the coccidia vaccine is crucial to be good alternative for the anticoccidial drugs. Different types of anticoccidial vaccines are available for use in fields including attenuated, non-attenuated and killed vaccines with different efficiencies in controlling coccidiosis [8, 9]. This study aimed to evaluate a sonicated sporulated oocyst vaccine against *E. stiedae* in rabbits and its use in prophylaxis against coccidiosis in rabbits.

2. Materials and Methods

2.1 Source of the rabbit and parasite

The study conducted in Animal house and Parasitology department of Veterinary Medicine College/ University of Baghdad, from the beginning of January to the end of December 2015. Twenty four rabbits were purchased from Abo Ghraib local market, Baghdad city. Fecal samples from these rabbits were examined daily for the presence of *E. stiedae* oocysts. Two of the rabbits gave positive result for this investigation. Accordingly, these rabbits were killed, and the contents of bile ducts were obtained in perti dish, and examined for the oocysts. The isolation and identification of *E. stiedae* oocysts was done at Parasitology Lab, College of Veterinary Medicine-University of Baghdad. The oocysts were preserved in 2.5% potassium dichromate solution for sporulation.

2.2 Preparation of Sonicated Oocysts

The sporulated oocysts were washed 4 times with physiological saline solution (pH 7.2) and then concentrated to 4000 oocysts/ml by centrifuging at 2700 rpm for 5 minutes. These oocysts were subjected to ultra-sonication using an Ultrasonic Homogenizer (Soni prep-150/Germany) for 2×30 seconds in jacketed vessel with cool water [10]. The homogenate was centrifuged at 3000 rpm for 30 minutes. Then supernatant above the pellet was collected and sterilized by millipore filters (0.45) micron [11].

2.3 Experimental Design

Rabbits, aged 4-8 weeks, weighing between 500 and 1000 gm, were purchased from Institute of Vaccine and Sera, Ministry of Health, Baghdad. They were undergone bacteriological and parasitological examination by direct fecal examination and blood smear testing. Out of 87 rabbits examined, only 40 showed no parasitic infection. These rabbits were divided randomly into 4 equal groups. The first group (G1) was subcutaneously injected with 1 mg/ml sonicated Ag (sporulated oocysts of *Eimeria stiedae*). Half dose of the previously mentioned Ag was administered to the second group (G2) by the same manner. The third group (G3) was injected subcutaneously with 1 ml PBS containing 1000 oocysts/ml and considered as a positive control group. The fourth group (G4) was injected subcutaneously with 1 ml PBS and considered as a negative control group.

During the experimental period, rabbits were kept in metal individual cages with grids in the bottom at 15-20℃ in the experimental animal house of College of Veterinary Medicine-University of Baghdad. The trays where the feces is collected were daily cleaned using water and disinfectant throughout the experiment. The animals were fed with standard commercial pellet and purified water was supplied ad libitum. Challenge with 1000 oocyst/rabbit (orally) was performed on the 20th day after vaccination.

2.4 Oocyst Count

Oocysts shedding were calculated six times; on day 17, 20, 23, 26, 29 and 32 PC. Fecal samples were collected from each rabbit in each group. The samples were collected from trays after the homogenization of the feces with a stick, placed in plastic containers, labeled with a group number and date and transported to the laboratory for estimation the number of the oocyst per gram of feces (OPG) using concentration McMaster method [12]. This technique can detect as low as 20 oocysts per gram of feces, and the procedure is more flexible when many samples were handled simultaneously.

Fig 1: sporulated *Eimeria stiedae* oocyst(100x)

2.5 Antibody Titer

Blood sera were obtained individually from all rabbits on Day 14, 20, 28, 42, 56 and 70 post immunization. Sera were subjected to passive haemagglutination (PHA) test [13] to evaluate the humoral immune response in all rabbits against *E. stiedae*.

2.6 Hematological Parameters

Using 5 ml sterile disposable syringe, blood was obtained by heart puncture and 0.5 ml of the blood was drained into EDTA vacutainer tubes for total and differential WBC, while the rest of blood was put into plain vacutainer tube and allowed to stand in refrigerator for 15 minutes and centrifuged at 3500 rpm for 5 minutes for serum collection. Serum was used to detect the activities liver enzymes, mainly ALP, AST and ALT by colorimetric methods [14, 15] and according to the instruction of Kit- manufacturer (Biosystems/ Spain).

2.7 Histopathology

Pieces of liver (≈1cm^3) were fixed in 10% buffer formalin for 48 hours for routine histopathological examination [16].

2.8 Statistical Analysis

Data were analyzed via statistical package for social science (SPSS version 16). Two-way ANOVA test was used to find least significant differences among average oocyst count, antibody titer and weight. P-value of less than 0.05 was considered significant.

3. Results and Discussion

3.1 Clinical Signs

Clinical signs of the disease appeared only on the rabbits of the G3. These signs included anorexia, depression, diarrhea, rough body coat, distended abdomen, and sometimes icterus. Three rabbits from this group died at day 29, 30 and 32 PC. Rabbits in G1, G2 and G4 devoid these signs with no mortality until the end of the experiment.

3.2 Oocyst Count

There were significant differences (P<0.05) in oocyst shedding between G1, G2 (the vaccinated groups) and G3 (non vaccinated group) at day 17 PC, the first oocyst shedding in feces, till day 32 PC. Within groups, there were significant increments in oocyst shedding when time progressed. This difference was more obvious in G3 where oocyst output at least 9 times higher than G1 and G2 rabbits (Table 1). These results agreed with many previous studies [10, 17]. Akhtar and Bahrami reported a decline in oocysts shedding following vaccination with sonicated oocysts. Active immunization depends on the introduction of suitable antigen to the host. To

induce effective immune response, this antigen should have many properties among which are proteineous nature and immunogenicity [18, 19]. Based upon the results of reduced oocyst shedding in immunized rabbits, it is reasonable to postulate that these proteineous aggregates contain antigenic epitopes that stimulate effective immune response which partially protects rabbits from the infection.

Table 1: Oocyst shedding rate

Day	Groups			
	G1	G2	G3	G4
0	*	*	*	*
17	310.7± 39.6^{Aa}	432.4± 56.8^{Aa}	2296.1±186.3^{Ab}	*
20	935.8±112.4^{Ba}	922.7±71.4^{Ba}	2588.3±156.5^{Ab}	*
23	2829.6±191.7^{Ca}	3115.2±182.6^{Ca}	14337.1±318.4^{Bb}	*
26	5137.7±216.1^{Da}	4933.1±229.5^{Da}	44658.1±1934.6^{Cb}	*
29	4812.5±236.4^{Da}	4522.9±218.6^{Da}	42872.6± 813.5^{Cb}	*
32	2633.5±221.4^{Ca}	3026.9±262.3^{Ca}	29665.8± 1017.4^{Db}	*
56	116.2± 6.4^{Ea}	142.7±13.6^{Aa}	32392.4±776.2^{Eb}	*

Data expressed as mean±SE
Different superscript refers to significant differences at $P<0.05$
* Not detected

After 14 days of vaccination, the average antibody titer increased significantly in both vaccinated groups and peaked at day 28 of vaccination. The antibody titer was dose-related and maintained at day 70 (Table 2). There is almost general agreement that the immunity against intracellular microorganisms is of cell mediated immunity (CMI) type [18]. Immunity against *Eimeria* is not an exception from that dogma, and CMI is thought to be the main adaptive immune response in this context [2]. However, estimation of such response is not easy, and measuring antibody titer can give a moderate indication about immune response in cases involving the evaluation of different vaccines. Furthermore, humoral immune response in coccidial infection is not absolutely worthless, and antibody of IgA is supposed to have a role in the resistance of such infection. As there was obvious protection in the vaccinated group manifested by reduced oocyst shedding, it can be assumed that relatively high percentage of IgA is present in the serum of vaccinated rabbits although it could not possible to estimate each antibody isotype separately.

Table 2: Antibody titers in different groups

Days	Groups			
	G1	G2	G3	G4
	9.18± 0.62 ^{Da}	7.63± 0.91^{Da}	8.22± 0.45^{Da}	7.92± 0.68 ^{Da}
14	532.7± 35.6 ^{Aa}	428.3± 42.7^{Ab}	68.1± 4.9^{Ac}	7.81± 0.55 ^{Dd}
28	1320.1±57.9^{Ba}	811.6±84.6^{Bb}	176.6±10.2^{Bc}	8.41± 0.89 ^{Dd}
42	835.6±77.8^{Ca}	634.2±93.5^{Bb}	92.4±8.1^{Cc}	7.66± 0.71 ^{Dd}
56	592.6±68.8^{Aa}	446.2±50.8^{Aa}	44.3±7.2^{Db}	6.76± 0.85 ^{Dc}
70	483.9±51.7^{Aa}	305.1±43.8^{Cb}	32.6±7.2^{Dc}	7.25± 0.49 ^{Dd}

Data expressed as mean±SE
Different superscript refers to significant differences at $P<0.05$

The relatively low levels of antibody titers in G3 following challenge may be attributed to the subcutaneous administration of the challenge dose, a quite different from the natural oral route. The proteineous nature of the antigen used in the vaccine ensures the stimulation of T-cell dependent immune response. Because the antigen is protein, it has to be recognized by T-helper cells which activates B cells, and the antibodies produced by plasma cells will be of different isotypes including IgA. Furthermore, these antibodies are of higher affinity to antigen compared to T-cell independent immune response which occurs when the antigen is of polysaccharide nature [18]. There was no peculiar amount of antigens that provoke typical immune response. Significant increased antibody titers in G1 compared with G2 (Table 2) illustrate the effect of the dose of antigen used in this study. This result is coincided by the higher amount of lymphocytes occurred in G1 blood view (Table 3). Furthermore the higher dose does not elicit any adverse reaction such as hypersensitivity or tolerance state [19].

3.3 Total and differential Leukocyte Count

Overall, total and differential WBC counts in all groups were within normal limits for the entire experimental period, except for G3 where significant neutrophil increments and inverse lymphocyte decrements were recorded on day 28 onward (Table 3). On the other hand, significant differences were recorded between G1 AND G2, G4, except on day 56 PC where G2 had significantly higher WBC count than both G1 and G4 (Table 3).

Leukocytes are the main reservoir of both the innate and adaptive immune response. An infection accompanied with massive inflammation may result in WBC reduction due to depletion of the primary effector cells, neutrophils mainly, responsible in pathogens clearance in an acute inflammatory process [20]. Activation and regulation functions of innate and adaptive immune cells are controlled by neutrophils, playing a crucial role in the pathogenesis of infections caused by intracellular pathogens and chronic inflammation [21].

Results of Lymphocytes support the notion of relatively low percentage of stem cells directed by GM-CSF in commitment with lymphoid lineage. On the other hand, the main player that stimulates the lymphoid transformation of stem cell is interleukin (IL)-7 [22]. This cytokine is secreted by stromal cells in the bone marrow and thymus. Keratinocytes, dendritic cells and hepatocytes are also produce considerable quantities of IL-7 in cases of infections especially with viral agents [23]. Transforming growth factor-β (TGF-β) and IL-7 down-regulate the expression of the other. Moreover, the former cytokine down-regulates IL-7 mRNA and protein secretion from bone marrow stromal cells [24]. Knowing the fact that infection with *Eimeria* increases 5 to 8 folds in the expression of TGF-β in IEL and 2.5 folds in spleen cells [25], it will be no longer surprising the reduction in lymphoid lineage of stem cells, and eventually lower percentage of lymphocytes. It is likely that this relative reduction in lymphocyte percentage may be considered as one mechanism by which the parasite can overcome the immune response. As it is well known, lymphocyte is the cornerstone of both humoral and CML response. Reduction in these cells can undoubtly diminishes the adaptive immune response. However, vaccinated group have normal lymphocyte percentage as compared with G4.

Table 3: Total and differential leucocytic count

Day	Parameter	G1	G2	G3	G4
0	Total WBC	5822.4±95.1^{Aa}	5916.2±109.2^{Aa}	5792.7±118.5^{a}	5893.5± 99.2^{Aa}
	Neutrophil	30.7±1.09^{Aa}	30.1±0.89^{Aa}	29.9±0.89^{Aa}	29.5±1.1^{Aa}
	Lymphocyte	62.7±1.22^{Aa}	61.8±1.17^{Aa}	63.2±1.12^{Aa}	63.5±1.34^{Aa}
	Monocyte	9.91±0.87^{Aa}	10.5±1.02^{Aa}	11.6±0.83^{Aa}	10.3±0.99^{Aa}
	Eosinophil	0.361±0.085^{Aa}	0.304±0.097^{Aa}	0.328±0.105A^{a}	0.293±0.071^{Aa}
14	Total WBC	6114.6±81.3^{Ba}	6201.2±122.8^{Bab}	6302.7±188.9^{Bb}	5795.1±113.8^{Aa}
	Neutrophil	31.4±1.26^{Aa}	29.7±1.19^{Aa}	30.8±0.92^{Aa}	28.9±1.1^{Aa}
	Lymphocyte	64.1±1.51^{Aa}	63.7±1.34^{Aa}	64.9±1.62^{Aa}	62.7±1.23^{Aa}
	Monocyte	10.4±0.69^{Aa}	11.2±1.14^{Aa}	9.8±1.18^{Aa}	10.9±0.81^{Aa}
	Eosinophil	0.497±0.119^{Aa}	0.423±0.077^{Aa}	0.539±0.122^{Aa}	0.325±0.101^{Aa}
28	Total WBC	5926.2±126.3^{ABa}	6197.9±99.51^{Ba}	6213.4±115.2^{Bb}	5811.7±82.5^{Ac}
	Neutrophil	28.6±1.12^{Aa}	30.1±0.88^{Aa}	33.7±1.04^{Bb}	29.4±0.83^{Aa}
	Lymphocyte	69.6±1.17^{Aa}	62.3±0.95^{Ba}	49.6±1.88^{Cb}	61.9±1.34^{Ba}
	Monocyte	11.6±0.83^{Aa}	10.7±0.97^{Aa}	11.2 ±1.07^{Aa}	11.1±0.88^{Aa}
	Eosinophil	0.981±0.291^{Ba}	0.899±0.256^{Ba}	1.883 ±0.41^{Bb}	0.288±0.073^{Ac}
42	Total WBC	6019.7±133.7^{ABa}	6152.8±161.4^{ABa}	8293.1±253.2^{Cb}	5912.1±115.3^{Ac}
	Neutrophil	29.2±0.85^{Aa}	31.4±1.24^{Aa}	34.6±1.22^{Bb}	29.1±0.99^{Aa}
	Lymphocyte	68.5±1.29^{Aa}	62.9±0.64^{Ba}	51.2±2.11^{Cb}	60.8±1.31^{Ba}
	Monocyte	10.9±1.23^{Aa}	11.7±0.91^{Aa}	11.5±1.31^{Aa}	11.2±1.15^{Aa}
	Eosinophil	0.376±0.813^{Aa}	0.283±0.161^{Aa}	2.556±0.411^{Cb}	0.327±0.087^{Aa}
56	Total WBC	5922.8±98.1^{Aba}	6083.2±117.6^{ABb}	7735.9±319.4^{Dc}	5732.4±86.8^{Aa}
	Neutrophil	29.8±1.13^{Aa}	28.9±1.17^{Aa}	30.1±0.95^{Ab}	29.3±0.88^{Aa}
	Lymphocyte	66.2±0.98^{Aa}	63.7±1.41^{Aa}	47.4±2.31^{BCb}	63.4±1.29^{Aa}
	Monocyte	11.1±0.83^{Aa}	11.1±0.47^{Aa}	14.6±0.56^{Ab}	11.16±0.42^{Aa}
	Eosinophil	0.514±0.263^{Aa}	0.413±0.111^{Aa}	2.513±0.331^{Cb}	0.495±0.129^{Aa}
70	Total WBC	6141.1±141.9^{ABa}	5992.5±143.7^{ABa}	7249.6±192.1^{Db}	5699.3±102.3^{Ac}
	Neutrophil	30.1±0.93^{Aa}	28.8±0.85^{Aa}	29.9±1.23^{Ab}	29.4±0.93^{Aa}
	Lymphocyte	67.3±1.68^{Aa}	62.1±0.91^{Bac}	46.7±2.14^{Cb}	61.9±1.15^{Bc}
	Monocyte	10.6±0.24^{Aa}	10.6±0.27^{Aa}	12.5±0.36^{Ab}	10.7±0.21^{Aa}
	Eosinophil	0.413±0.104^{Aa}	0.428±0.13^{Aa}	2.19±0.471 ^{Cb}	0.366±0.131^{Aa}

Data expressed as mean±SE

Different superscript refers to significant differences at $P<0.05$

Monocytes showed no significant differences among the four groups except on day 56 PC onward, where G3 had significantly higher monocyte percentage (Table 3). This result partially supports the aforementioned results regarding the neutrophil percentage and the role of GM-CSF. Regarding eosinophils, day 14 PC witnessed mild non-significant increase in G1, G2 and G3, and significant sustained peak was observed only in G3 at day 28 PC onward.

The GM-CSF and IL-3 control the early stages of eosinophils differentiation, while maturation in the later stage of is controlled by IL-5 produced by mast cells and activated T cells [26]. The primary function of these cells is a defense against parasitic helminths, organisms that failed to be phagocytosed. The mechanism by which oesinophils act is binding and responding to carbohydrate ligands present on the parasitic surface, e.g. the Lewis-related and cell-adhesion molecules. After becoming activated, they degranulate on to or around their targets causing detrimental effects [27].

Eimeria spp as protozoan parasites do not induce eosinophilia, because they are intracellular parasite and do not have the compound characterized the helminth nematode where eosinophils might bind. The mild elevation in eosinophils percentage in vaccinated groups on day 28 PC and in G3 during almost the entire experimental period can be attributed to the activity of GM-CSF cytokine as this cytokine stimulate the formation of granulocytes (including eosinophils) from the bone marrow stem cells. Results of total and differential leukocytes count support the efficiency of the sonicated sporulated *E. stiedae* vaccine in protection of rabbits against coccidial infection.

3.4 Liver Enzymes

ALT, AST and ALP

Significant increments in ALT, AST and ALP were observed in G1, G2 and G3 at day 14 PC compared with G4 and pre challenge values (Table 4). These values tend to normal at day 42 in G1 and G2, while the rise is still obvious in G3 till the end of the experiment.

The main target for *E. stiedea* is the liver and bile ducts. The activity of most of these enzymes increases in case of liver injury. Therefore, measuring the activity of these liver enzymes is of great important in screening the parasite pathogenesis and the evaluation of the efficiency of the vaccine [28]. Liver activity of the ALT is about 3000 times that of the serum's. Thus, in cases of hepatocellular injury, release of ALT as well as AST from damaged hepatocytes increases their activities in the serum [29]. Upon increased hepatocellular death, the serum AST/ALT ratio, known as De Ritis quotient, tends to be doubled and is predictive of prolonged complications including fibrosis [30]. De Ritis below 0.7 indicates inflammatory type liver injury, while De Ritis higher than 0.7 indicates liver necrosis [31]. Rise in ALP activity more than that of ALT refers to obstructive jaundice [32].

The present study results revealed that a mild degree of liver injury has been occurred in G1, G2 and G3 commenced at day 14 PC, returned to normal values in G1 and G2 at day 42, while significant increments continued in G3 till the end of experiment (Table 4). Screening of AST and ALT values could give a conclusion that most, if not all, De Ritis quotients are > 0.7 which indicates necrosis type liver injury resulted from damage to the liver cells. Exaggerated elevation

of ALT, AST and ALP values especially in G3 indicates the obstructed jaundice which is caused by enlarged bile duct accompanied by lumen narrowing and eventually partial obstruction of the ducts.

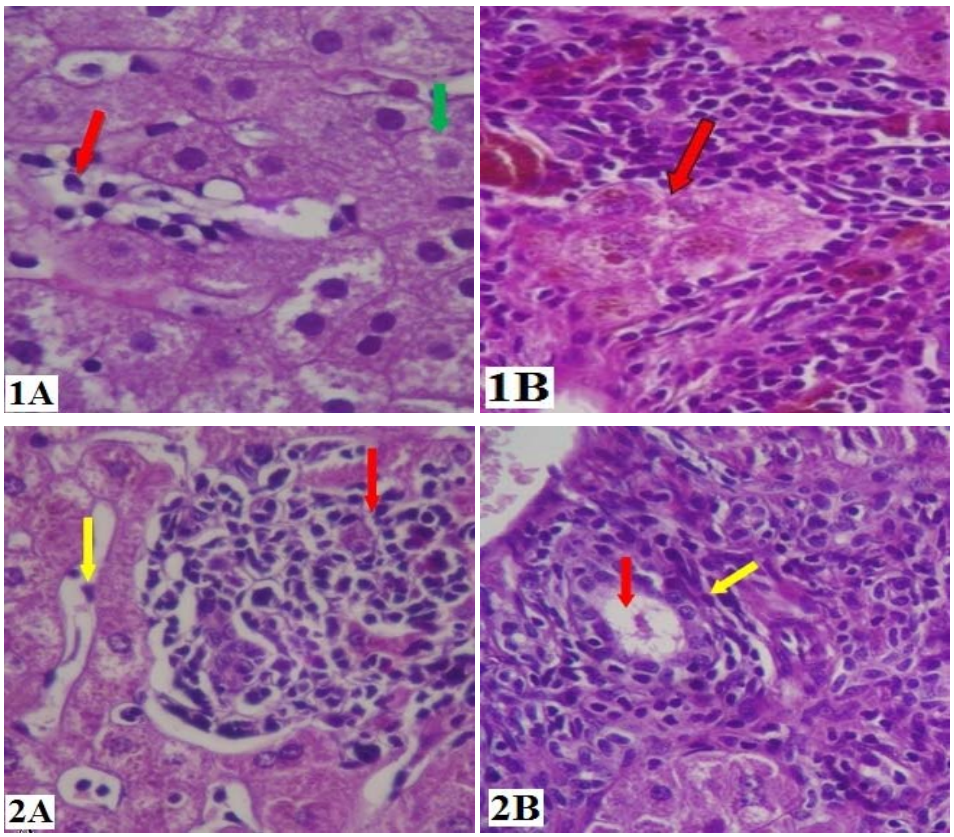
Table 4: Average ALT, AST and ALP concentrations (U/L) in different groups

Day	Enzymes	G1	G2	G3	G4
0	ALT	6.81±0.32^{Aa}	7.27±0.56^{Aa}	7.05±0.49^{Aa}	6.32±0.37^{Aa}
	AST	13.37±0.91^{Aa}	12.67±0.47^{Aa}	13.08±0.85^{Aa}	12.89±0.93^{Aa}
	ALP	14.57±0.79^{Aa}	15.66±1.06^{Aa}	13.97±0.93^{Aa}	15.12±0.61^{Aa}
14	ALT	7.99±0.43^{Ba}	8.18±0.36^{Ba}	9.15±0.61^{Ba}	6.51±0.42^{Ab}
	AST	16.52±1.21^{Ba}	16.71±0.62^{Ba}	18.39±1.13^{Ba}	13.15±0.82^{Ab}
	ALP	21.55±1.97^{Ca}	23.21±2.77^{Ba}	26.59±3.03^{Ba}	14.19±0.81^{Ab}
28	ALT	8.21±0.62^{Ba}	7.92±0.59^{Ba}	14.55±1.21^{Cb}	7.09±0.41^{Aa}
	AST	15.22±1.27^{ABa}	14.62±0.96^{Cab}	27.55±2.63^{CDc}	12.73±1.13^{Ab}
	ALP	19.28±2.12^{BCa}	18.96±2.47^{ABa}	31.64±2.93^{Bc}	15.39±0.90^{Ab}
42	ALT	7.84±0.71^{Aab}	8.52±0.81^{Ab}	13.82±0.85^{Cc}	6.99±0.42^{Aa}
	AST	13.22±1.15^{Aa}	13.73±0.85^{ACa}	29.33±2.81^{Db}	12.85±1.22^{Aa}
	ALP	16.22±1.97^{ABa}	17.11±1.85^{ABa}	28.61±1.98^{Bc}	14.99±0.87^{Aa}
56	ALT	6.77±0.33^{Aa}	7.39±0.48^{Aba}	15.64 ±0.69^{Cb}	7.63±0.49^{Aa}
	AST	12.76±0.89^{Aa}	12.98±1.17^{ACa}	23.18±2.16^{Cb}	13.19±0.96^{Aa}
	ALP	16.94±1.06^{ABab}	15.85±1.13^{Aa}	29.36±2.11^{Bc}	15.88±0.92^{Ab}
70	ALT	6.35±0.41^{Aa}	7.28±0.53^{Aba}	13.02 ± 0.57^{Cb}	6.84±0.51^{Aa}
	AST	13.29±1.01^{Aa}	14.32±1.09^{Aca}	26.11±3.18^{CDb}	12.27±1.03^{Aa}
	ALP	15.18±0.89^{Aa}	14.94±0.91^{Aa}	27.99±1.81^{Bb}	14.63±1.01^{Aa}

Data expressed as mean±SE
Different superscript refers to significant differences at $P<0.05$

3.5 Histopathology of the liver

Eimeria stiedai migrates from the duodenum to liver via the portal vein as well as the lymphatic system and develops in the epithelium of bile ducts [33, 34]. Histopathological changes of G1 liver rabbits showed hypertrophy of hepatocytes and moderate kupffer cells infiltration (Fig 1-A1), degenerated schizonts within liver section (Fig 1-B1). On the other hand, G2 rabbits showed granulomatous-like lesion in liver parenchyma with slight kupffer cell proliferation (Fig 1-A2), multifocal MNCs infiltration mainly around proliferated bile ductules (Fig 1-B2). The G3 rabbits showed sloughing with necrotic tissue debris in the lumen of bile ductules (Fig 1-A3) and oocysts and developmental schizont stages in the bile duct (Fig 1-B3). An extra intestinal localization of sporozoites [35-38] seems to be an essential part of *E. stiedai* cycles. These observations coincided with the present findings. Moreover, Fitzgerald (1970) found sporozoites of *E. stiedai* within lymphoid cells. He supposed that the sporozoites may be carried to the liver via blood, while [39] assumed that the transport of sporozoites settled in the liver throughout whole host organ.



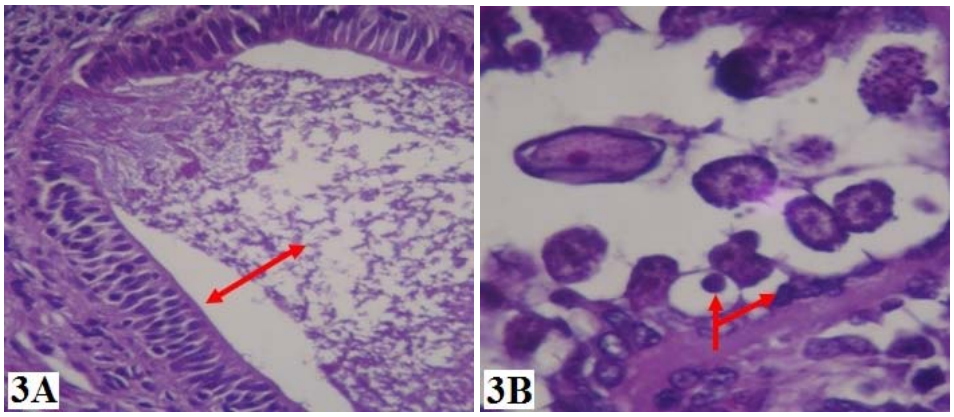


Fig 1: Histopathological changes of liver (H&E, 40X). G1 rabbits showed A1; Hypertrophy of hepatocytes (green arrow) and moderate kupffer cells infiltration (red arrow). B1; Degenerated schizonts within liver section (arrow). G2 rabbits showed A2: granulomatous-like lesion in liver parenchyma (red arrow) with slight kupffer cell proliferation (yellow arrow). B: multifocal MNCs infiltration (yellow arrow) mainly around proliferated bile ductules (red arrow) (40X). G3 rabbits showed A3: sloughing with necrotic tissue debris in the lumen of bile ductules (red arrow). B3: oocysts and developmental schizont stages in the bile duct (red arrow).

6. Conclusion

The present study concluded that vaccination of rabbits with sonicated sporulated oocyst of *E. stiedae* is beneficial in protection against coccidiosis, despite the dose. The higher dose of the vaccine (1mg/ml) stimulated higher humoral immune response without obvious side effects. Thus this dose is more preferable than the smaller dose (0.5 mg/ml). No signs of hypersensitivity or tolerance appeared on the animals vaccinated with either dose which indicate the proper quantities of these doses.

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8. Reference

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