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RESEARCH ARTICLE

Evaluation of Sonicated Oocyst Vaccine in Protection Against Hepatic Coccidiosis in Rabbits

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Abstract

Background: Hepatic coccidiosis, caused by Eimeria stiedae, is considered to be a devastating disease for rabbit colonies resulting in high morbidity and mortality. Many anticoccidial drugs became less effective against this microorganism, and there is urgent need for appropriate alternative for these compounds.

Aims: this study aimed to evaluated the protective efficacy of sonicated E. stiedae sporulated oocyst against the experimental infection with this parasite.

Materials and Methods: A total of 18 rabbits were divided into three equal groups: the first (G1) and second group (G2) were vaccinated s/c with 1 ml and 0.5 ml respectively of sonicated E. stiedae sporulated oocyst, while the third group (G3) represented positive control group. Challenge with 1000 oocyst/animal (orally) was performed on the 20th day after vaccination. Oocyst per gram of feces (OPG) was counted on day 17, 20, 23, 26, 29 and 32 post challenge (PC). Blood samples were obtained from each rabbit on 14, 20, 28, 42, 56 and 70 after immunization. Passive haemagglutination (PHA) test was used to estimate antibody titer. All rabbits were weighted on 1, 14, 28, 42, 56 and 70 day post vaccination.

Results: There were highly significant differences in oocyst shedding between the vaccinated groups (G1 and G2) and non vaccinated group (G2) from the first day of oocyst shedding till the day 32 PC**,** while there were no significant differences between G1 and G2 although G2 had higher oocyst shedding rate**.** The same significant differences were recorded regarding Ab titers after 14 days of vaccination until the end of the study. Calculated weight gain revealed that G1 and G2 had very close weight gains (196 gm, 180 g and 199 gm respectively) compared to only 57 gm for G3.

Conclusion: This vaccine can give partial protection against infection with E. steidae in rabbits. Such vaccine can be subjected to further study to improve its efficiency in order to be a suitable alternative for anticoccidial drugs.

Introduction

Hepatic coccidiosis, caused by Eimeria stiedea, is considered to be a devastating disease for rabbit colonies resulting in high morbidity and mortality (Kvicerova et al., 2008). Beside sanitation and other managemental arrangements, there are two main types of control measures to overcome this disease: anticoccidial drugs and vaccination. For decades, anticoccidial drugs have been used effectively as a control measure for coccidia. Unfortunately, many of these compounds became no longer effective against this organism due to emerging of drug resistance (Chapman,

1998). The other problem associated with the anticoccidial drugs is a public health problem. The withdrawal period for many of these drugs about one week (Kant et al., 2013) which is relatively long period. Farmers have to balance between the risk of coccidiosis in their flocks and the presence of drug residues in the carcasses of slaughtered animals with subsequent effects on the consumers' health. In fact, drug residues in the consumed meat does not only effect public health, but also may change the consumers' preference as a result of the change in the taste of meat containing such residues (Peek and Landman, 2011).

Lacking all these disadvantages, vaccine seems to be the most plausible solution both from an economical and consumers' preference standpoints. A plethora of studies have been conducted on vaccination against coccidian. However, the vast majority of these studies were concerning avian coccidiosis (Price and Barta, 2010; Price, 2012; Jenkins et al., 2013; Milbradt et al., 2014) with only limited studies regarding hepatic coccidiosis in rabbits (Abul Megeed et al., 2005; Akpo et al., 2012). The current study aimed to evaluated a type of killed vaccine (sonicated oocysts of E. stiedae) in protection the rabbits against this parasite.

Materilas and Methods

Source of the Parasite

A total of 24 rabbits were purchased from the local market and farms in 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/ Collage of Veterinary Medicine / Baghdad University.

Preperation of Sonicated Oocysts

The sporulated oocysts in potassium dichromate solution were washed 4 times with physiological saline solution (pH 7.2) and then concentrated to 4000 oocysts/ ml. 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 **(**Akhtar et al., 1998). The homogenate was centrifuged at 3000 rpm for 30 minutes. Then supernatant above the pellet was collected and sterilized by millipore filters (0.45micron) according to Fue and Lee (1976) with some modification.

Experimental Design

Eighteen rabbits aged between 4 and 8 weeks and weighing between 500 and 1000 gm, were purchased from Abo-Greeb farm western of Baghdad. They were undergone parasitological examination by direct fecal examination and blood smear testing. No parasitic infection was recorded in any animal. These rabbits were divided randomly into three groups as follows

I- The first group (6 rabbits) was injected subcutaneously with 1 mg/ml sonicated Ag (sporulated oocysts of Eimeria stiedae).

II- The second group (6 rabbits) was injected subcutaneously with 0.5 mg/ml sonicated Ag (sporulated oocysts of Eimeria stiedae).

III- The third group (6 rabbits) was injected subcutaneously with 1 ml PBS and considered as a positive control group.

During the experimental period, animals were kept in metal individual cages with grids in the bottom in the experimental animal house of Veterinary Collage/Baghdad University. The trays where the feces is collected were daily cleaned throughout the experiment. Temperature of the breeding house was controlled at 15-20 ^{o}C. The animals were fed with commercial pellet food, and water was supplied ad libitum. The trays where the feces is collected were daily cleaned using water and disinfectant. Challenge with 1000 oocyst/animal (orally) was performed on the 20th day after vaccination.

Oocyst Count

Oocysts shedding was 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 (Roepstorff and Nansen, 1998.). 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.

Antibody Titer

Blood samples were obtained from each rabbit on 14, 20, 28, 42, 56 and 70 after immunization. Passive haemagglutination (PHA) test (Herbert, 1978) was applied to evaluated the humoral immune response of vaccinated rabbits against E. stiedae.

Growth Performance

All rabbits were weighted on 1, 14, 28, 42, 56 and 70 day after vaccination.

Statistical Analysis

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

Results and Discussion

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. One rabbit from this group died at day 29 PC. Rabbits in G1, G2 and G4 devoid these signs with no mortality until the end of the experiment.

Oocyst Count

The first oocyst output in feces was observed on day 17 PC (prepatent period was 17 days). Overall, there were highly significant differences in oocyst shedding between the vaccinated groups (G1 and G2) and non vaccinated group (G2) from the first day of oocyst shedding till the day 32 PC (table 1). Furthermore, there were no significant differences between G1 and G2 although G2 had higher oocyst shedding rate. The oocyst shedding pattern simulated that of natural infection i.e starting with relatively low numbers (240±167.8, 400±167.8 and 2053±666.8 in G1, G2 and G3 respectively; reaching its peak at day 26 PI (4533±502.6, 4933±896.1 and 41547±4832.6 respectively) and then declining at day 32 PI (2987±333.5, 3227±711.6 and 22827±2463.5 respectively). Although vaccinated group still shed oocyst, there was a reduction in oocyst out put 8.051 times in G1 and 7.141 times in G2 as compared to non-vaccinated group.

Table 1: Oocyst output in different groups

Group / Day	Mean± SE		
	G1	G2	G3
17	240± 68.508[a]	400± 68.508[a]	2053± 272.209[b]
20	827± 167.385[a]	960± 71.554[a]	2693± 191.183[b]
23	2667± 228.775[a]	3253± 200.975[a]	12288± 488.983[b]
26	4533± 205.177[a]	5093± 242.359[a]	41664± 2412.02[b]
29	3787±225.014[a]	4027± 272.209[a]	39392± 795.829[b]
32	2987±143.924[a]	3227± 290.41[a]	23008± 1211.5[b]

Note: Different small litters indicate significant differences

Antibody Titer

Table 2 shows antibody titers in different groups. After 14 days of vaccination, average antibody titer in G1 reached 469±42.66 and differed significantly from both G2 (341±53.97) and G3 (29±6.532). In either vaccinated groups (G1 and G2), antibody titers keep rising to their maximum values after 56 of vaccination (491±125.406 and 427±53.97 respectively) during which the significant differences between the two groups disappeared. After that, a little decline in these titers was observed (469±42.667 and 341±53.97 respectively), and the significant difference between the two groups returned back. On the other hand, no much variations in antibody titers from the initial value was recorded in G3 except the mild elevation from 29±6.532 to 107±13.492 after 8 days of challenge dose.

Table 2: Antibody titers in different groups

Groups	Mean±SE

	G1	G2	G3
14	469± 42.66 [a]	341± 53.97[b]	29± 6.532[c]
28	1109± 205.51[a]	768± 114.487[a]	107± 13.492[b]
42	768± 114.487[a]	512±114.487[a]	51± 7.838[b]
56	491± 125.486[a]	427± 53.97[a]	32± 8.764[b]
70	469± 42.667[a]	341± 53.97[b]	27± 9.992[c]

Note: Different small litters indicate significant differences

Growth Performance

From the first day until day 56 of the experiment, there were no significant differences among the four groups regarding rabbits' weight, although G3 (positive control) showed relatively low weights compared to the other three groups. At day 70, average weight of G1, G2 and G4 (1739±31.1 g, 1755±38.5 g and 1724±27.2 g respectively) differed significantly from that of G3 (1606±63.1 g)(table 3). Calculated weight gain revealed that G1, G2 and G4 had very close weight gains (196 g, 180 g and 199 g respectively) compared to only 57 g for G3.

Table 3: Average weight (gram) of rabbits in different groups

Groups / Days	**Mean ± SE**		
	G1	G2	G3
1	1543 ±33.4[a]	1575±35.5[a]	1549±23.7[a]
14	1580±31.2[a]	1590±35.1[a]	1574 ±26.6[a]
28	1607±31.1[a]	1615±36.5[a]	1568±26.4[a]
42	1646±32.9[a]	1654±35.1[a]	1584 ±25.3[a]
56	1693±30.9[a]	1713±39.6[a]	1596 ±26.2[a]
70	1739±31.1[a]	1755±38.5[a]	1606 ±27.2[b]

Discussion

Hepatic coccidiosis in rabbits is one of the most important disease that threaten rabbit production industry. The clinical signs appeared on the infected rabbits ere almost recorded in all previous studies on hepatic coccidiosis in rabbits (Pakandl, 2009; Abu-Akkada et al., 2010; Al-Naimi et al., 2012). Some of these signs such as depression and anorexia are general for all infectious diseases, however, distended abdomen and icterus are an indication for liver infection which is also not restricted for hepatic coccisdiosis. Hence, it is difficult to diagnose this disease depending entirely on the clinical signs.

The current study revealed a significant reduction in oocyst shedding in the vaccinated rabbits. These results are in accordance with many previous studies (Akhtar et al., 1998; Bahrami et al., 2006) which revealed reduction in oocysts shedding after vaccination of animals with sonicated oocysts.

Active immunization depends on the introduction of suitable antigen to the host. This antigen should have many properties among which proteineous nature and immunogenicity (Levinson, 2012). Beside abolishing the infectivity of the sporulated oocysts, sonication of protein results in the formation of aggregates with high stability against resolubilization upon exposure to heat, detergents and reducing agents (Stefani and Dobson, 2003). Based on the results of previous and current studies regarding reducing oocyst shedding in immunized animals, it is reasonable to postulate that these proteineous aggregates contain antigenic epitopes that stimulate effective immune response partially protects rabbits from the infection.

There is almost general agreement that the immunity against intracellular microorganisms is of CMI type (Levinson, 2012). Immunity against Eimeria is not an exception from that dogma, and CMI is thought to be the main adaptive immune response in this context (Pakendl, 2009). 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 represented at least 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.

The relatively low levels of antibody titers in G3 even after the challenge dose may be related to the route of administration because the challenge dose was given orally while the vaccine was given subcutaneously.

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 (Parija, 2012).

There is no peculiar amount of antigens that provoke typical immune response. Very low doses of antigen do not stimulate immune response, either because of too few lymphocytes on contact or because a non response state is elicited. Conversely, high doses of antigen increase the risk of hypersensitivity reaction or even can elicit tolerance state (Parija, 2012). It seems that there is little difference between the two doses (1 ml and 0.5 ml) used in this study regarding the protection against challenge dose. Furthermore the higher dose does not elicit any adverse reaction such as hypersensitivity.

Weight loss in rabbits infected with hepatic coccidiosis has been recorded in many literatures (fox et al., 2002; Varga, 2014). Of course diarrhea has detrimental effect on weight loss, but through observations of infected rabbits, it was anorexia rather than diarrhea which had the main cause of diminished weight gain. Furthermore, the role of the liver and bile duct is well documented in digestion and metabolism of absorbed nutrients. Damage caused by E. stiedae for this organ can further augment weight loss.

From these results it can be concluded that such type of vaccine can give partial protection against infection with E. steidae in rabbits. Taking into account the cost disadvantages of anticoccidial drugs, such vaccine can be subjected to further study to improve its efficiency in order to be suitable alternative for anticoccidial drugs.

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