Immunization of V-line rabbits against intestinal coccidiosis by using attenuated Eimeria oocysts.

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A B S T R A C T

The present work aimed to study the immunization of V-line rabbits against coccidiosis by using attenuated Eimeria oocysts by different methods (UV irradiation , formalin and freezing-thawing) on Eimeria oocysts and its effect on immune pattern of V-line rabbits. For this aim, Eimeria species (E. exigua,E. flavescens, Emedia, E magna, E. irresidua, E.nagpurensis, E. coecicola and E. intestinalis)were collected from infected and dead rabbits. The collected Eimeria species were sporulated in potassium dichromate 2.5% and divided into four parts ; 1stpart exposed to UV for an hour ,2nd part exposed to six freeze-thaw cycles, 3rd part was diluted with 2.5 ml of formalin 37% and 4th part left without treatment. Fifteen V-line rabbits were classified into 4 groups;G1 was treated by formalin treated Eimeria oocysts,G2 was inoculated by UV irradiated Eimeria oocysts, G3 was inoculated by freezing-thawing oocysts and G4 was inoculated by non-treated Eimeria oocysts. Challenge by non-attenuated Eimeria was carried in 14th day post inoculation. Fecal samples were collected from all groups from 6th to 35thdays, identified and count. Blood samples were collected at days (zero,7th ,14th ,21st,28th and 35th) for determination of cellular and humeral immunity. The obtained results recorded that immunization of rabbits by UV irradiated Eimeria oocysts showed low number of oocysts in feces before and after challenge compared to other groups. The same group showed high level of IgG .It recommended that immunization of rabbits by UV irradiated Eimeria oocysts could protect rabbits against coccidiosis.

Key words: *immunization, Eimeria species, oocyst, rabbits*
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1. INTRODUCTION

Coccidiosis of rabbits remains one of the most causes of digestive disorders in fattening rabbits (Vancraeynest et al., 2008). The intestinal coccidial species inducing weight reduction, diarrhea and mortality due to villi atrophy which lead to malabsorption of nutrients, electrolyte misbalance, anemia, hypoproteinemia and dehydration, especially in young rabbits (Pakandl, 2009).

The conventional method of controlling the disease include administration of Anti-coccidial drugs which is successful only in case of rabbits that have been infected for less than five or six days, diarrhea and mortality may still be seen for a few days after the initiation of treatment. Relapse is regularly observed after one or two weeks. Immunity is species specific as exposure to one species does not protect against the other species .Because of the development of drug resistance against commonly available coccidiostat/ coccidiocidal, there is a growing demand for alternative methods for prophylactic and treatment, Chapman(1998).

Immunization against coccidiosis for production of live attenuated vaccine was previously carried out in rabbits by using precocious lines of Eimeria magna and Eimeria media in Benin, (Akpo et al.2012), In Egypt using gamma irradiated Eimeria oocysts in rabbits (Zayan and El-Akabawy 2003). There were another methods

used for protection against coccidiosis as using of butyric acid glycerides (BAGs 4 g/kg diet) or BAGs mixed with clopidol (125 g/kg)in diet of broiler chickens infected with 1×10^4 sporulated oocysts of *E. maxima*,(Ali et al.2014).

Few studies were carried out on immunization of eimeriosis in rabbits in Egypt, so the present work is a trial to study the effect of *Eimeria* oocysts treated by different methods (UV irradiation ,formalin and freezing-thawing) on oocysts shedding as well as immune status in rabbits.

2. MARERIALS AND METHODS

2.1. Collection of Eimeria species.

Eimeria species (*E. exigua, E. flavescens, E media, E magna, E. irresidua, E.nagpurensis, E. coecicola and E. intestinalis*) were collected from infected and dead rabbits in Qaloubia government, Egypt, sporulated in potassium dichromate 2.5% at room temperature according to Ruiz et al., (2014). The collected *Eimeria* spp. was allocated in 4 suspensions each one (40ml) contained $1x10^5$ oocysts and used for the following preparation:

2.2. Attenuation of oocysts by using formalin, Noaman N. A. (2011).

The 1st oocyst suspension was diluted with 2.5 ml of formalin 37%, then before using it, the sediment washed by centrifugation at 1500 rpm for 10 min, discard supernatant and re-suspended by clean distill water to clean it from formalin.

2.3. Attenuation of oocysts by using ultraviolet irradiation (UV), Ramadan et al. (2018).

The 2nd oocyst suspension was exposed to UV radiation for 1 hour using universal UV units 100-240 V .50 ⁄ 60 HZ .Company Model.No.06 13930. 6x8w, 312nm, BXT-26-M, made in EEC.

2.4. Attenuation of oocysts by using freezing and thawing method. Kniel et al. (2007).

The 3rd oocyst suspension was exposed to six freeze-thaw cycles (-18ºC for 30 min and immersion in a 41ºC water bath for 10 min).

2.5. Animals and experimental grouping

Fifteen V- line healthy rabbits aged 28 days were purchased and weighted (690-800 gm) from the rabbits farm of Faculty of Agriculture, Benha University, Egypt. The rabbits were under observation (for one week) with daily examination of their feces to ensure that they are free from any parasitic diseases.

At zero day of the experiment, all rabbits, age 35 days, were weighted separately before the beginning of experiment then allocated into four groups where the first three groups composed of four rabbits. Rabbits in group one (G1) were inoculated with 1ml of suspension contain $1x10^5$ attenuated sporulated oocysts) by 2.5% formalin by a stomach tube. Group 2(G2) were inoculated by1ml of $1x10^5$ UV irradiated oocysts). Group3 (G3) was inoculated by 1ml of $1x10^5$ attenuated by freezing and thawing). G4 (contain three rabbits) each rabbit was inoculated with 1ml of $1x10^5$ non attenuated sporulated Eimeria oocysts. A challenge dose of intestinal *Eimeria* oocysts ($1x10^5$) were administrated to each rabbit in the all groups 14 day post infection.

Clinical symptoms and body weight of all rabbit in each group were reported. Fecal samples were collected daily from each groups, separately in clean cups starting from zero day till the end of the experiment (day35) for identification and counting of Eimeria oocysts Ruzi et al.,(2014) . Blood samples were collected from the optic vein of each rabbit and divided in two samples, first one was free from EDTA for serum separation for determination of IgG, IgM and IgA Matos et al . (2017), whereas the second sample was treated with EDTA for determination of cellular immunity according to Mohamed (2002).

2.6. Statistical analysis

All data were analyzed statically by SPSS by two-way ANOVA using SPSS version 20. Difference between groups were considered significant at $P<0.05$, Steel *et al.* (1997).

4. RESULTS

Animal in the control G4, suffered from severe

off food in the 9th and 11th day post infection and suffered from sever to moderate diarrhea in the period from 9th to 13th day post -infection. Off food was sever in G1 and G3 at 9th day PI, and then decline to moderate in day 11th PI and at 13th PI. While in day 15th became light. Rabbits in G2 showed light off food in day 11th and 13th, while in day 15th eating was normal. diarrhea was moderate in G1and G2 at 7th and 9th day PI ,then it became light at day 13th PI. Dullness was ranged from sever to moderate in G1, G2 andG4 from 7th till 13th PI .mortality was recorded in G3 where only one rabbit was died on 13th DPI. So rabbits in G2 were the best one, and then followed by those in G1 and G3 compared to G4, (Table1).

With respect to relationship between weight gain and methods of attenuation, our result denoted that G2 showed the highest weight gain followed by G4 then G3 and finally G1, (Table 2)

The shedding of oocysts started at 6th day after initial infection .There is a significant decrease in shedded Eimeria oocysts in G1, G2 and G3 compared to G4 at 6th day. The highest no. of shedded Eimeria oocysts was recorded at 11th day in G1 followed by G4, while the lowest number was recorded in G2 and G3. There was significance difference between G1, G2 and G3 compared with G4 in day 6th and 15th. At day 19th day the figure showed that G2 had the lowest No. of shedded oocyst in feces followed by G3 on the other hand G1 showed the highest number of Eimeria oocyst in feces, (Table 3)

At 21st day, there was an overall reduction in oocyst per gram (OPG) in all rabbit groups. At day 23rd there is a significant decrease in Eimeria oocysts shedded in feces in group 3 and group 2. From 29th till the end of the experiment, number of oocysts released in feces was constant in G1, G2 and G3 compared to G4, (Table 4).

There was significance difference between G1, G2 and G3 compared to G2 at day 14th and 21st PI and betweenG1 and G3 compared to G4. There was significant difference in G1at day 21st, 28th and 35th DPI while in G2 at 28th and 35th PI, (Table 5).

IgG from 14 till the end of experiment showed a significant difference when compared with zero day .G2 was the only group had significance difference compared with other days. Table (5).

Level of IgM in G4 was higher than other groups at 7th and 14th DPI. At 21st and 28th PI, G2 recorded the highest level of IgM compared to other groups while G3 showed the highest level of IgM at 35th post challenge. At 21st and 28th day PI G1 recorded the lowest level of IgM, (Table 5)

Total leukocytic count showed that there was no significant difference between all groups from 21st day to the end of the experiment.G1 recorded the highest level at 7th and 14th day PI compared to other groups, (Table 6)

Lymphocyte showed that there was no significant difference between all groups at 21st day and 28th day. The highest level recorded in G3 and G4 at 7th and 14th day respectively compared to other groups,(Table 6)

Neutrophils recorded the highest level on 7th and 35th DPI in G2 while G1 showed the highest level of Neutrophil in 14th post challenge. G4 showed the lowest level of Neutrophil on 14th, 28th and 35th post challenge. G3 showed the lowest level of Neutrophil on 7th and 21st post challenge, (Table 6)

There was no significant difference between all groups except eosinophil mild increased in G1 on 28th DPI and on 21st DPI in G3, (Table 6)

No significance in monocyte all over the experiment except on 7th DPI which recorded significant difference in G2 (17) compared to G1, G3 and G4 (22,23 and 20)respectively. (Table 6)

Table (1). Clinical symptoms in V-line rabbits experimentally infected with different attenuated *Eimeria* species.

Days (PI)	symptoms	G1	G2	G3	G4
7	Off food	++	+	+	++
	Diarrhea	++	+	++	++
	Dullness	+++	++	+++	+++
	Mortality	Negative	Negative	negative	Negative
9	Off food	+++	++	+++	+++
	Diarrhea	++	+	++	+++
	Dullness	+++	+	+++	+++
	Mortality	negative	Negative	negative	Negative
11	Off food	++	+	++	+++
	Diarrhea	+	+	+	++
	Dullness	++	+	++	+++
	Mortality	Negative	Negative	negative	Negative
13	Off food	++	+	++	++
	Diarrhea	+	+	+	++
	Dullness	++	+	++	++
	Mortality	Negative	Negative	positive	Negative
15	Off food	+	Negative	+	+
	Diarrhea	Negative	Negative	negative	+
	Dullness	+	Negative	+	+
	Mortality	Negative	Negative	negative	Negative

+:light ++:moderate +++:sever PI: post infection

G1: Formalin group, G2: UV group, G3: freezing and thawing,G4:control positive group

Table (2): Effect of different methods of attenuation of *Eimeria* species on body weight of V-line rabbits.

Group	Initial body weight \ animal (g)	Final body weight \animal (g)	Change in weight gain/animal (g)	
			(g)	(%)
G1	755	1.400	645	85.13
G2	690	1.850	1260	182.61
G3	800	1.650	850	106.25
G4	710	1.650	940	132.39

G1: Formalin group, G2: UV group, G3: freezing and thawing,G4:control positive group

Table (3): Effect of different methods of *Eimeria* attenuation on infectivity of Eimeria species on experimentally infective V. line rabbits.

Days after inoculation	Groups G1	G2	G3	G4
0-5	0^{eA}	0^{eA}	0^{eA}	0^{fA}
6	$2.49x10^{5cB}$	$1.80x10^{5cB}$	$9.00x10^{4cC}$	$8.18x10^{5bA}$
9	$2.97x10^{5bA}$	$1.45x10^{5cB}$	$1.43x10^{5bcB}$	$1.98x10^{5dAB}$
11	$1.87x10^{6aA}$	$2.70x10^{5abcC}$	$8.37x10^{5aB}$	$1.18x10^{6aA}$
13	$5.61x10^{5bA}$	$4.05x10^{5abA}$	$6.52x10^{5aA}$	$5.10x10^{5bA}$
15	$3.93x10^{5bB}$	$4.35x10^{5aB}$	$4.71x10^{5aAB}$	$7.45x10^{5bA}$
17	$4.55x10^{5bA}$	$2.2x10^{5bcB}$	$2.22x10^{5bB}$	$3.60x10^{5cA}$
19	$6.00x10^{4dA}$	$1.50x10^{4dB}$	$3.80x10^{4dA}$	$4.00x10^{4eA}$

G1: Formalin group, G2: UV group,G3:freezing and thawing,G4:control positive group
a, b & c: There is no significant difference (P>0.05) between any two means, within the same column have the same superscript letter.
A, B & C: There is no significant difference (P>0.05) between any two means for the same attribute, within the same row have the same superscript letter.

(4): Effect of different methods of *Eimeria* attenuation on infectivity of Eimeria species on experimentally infective V- line rabbits post challenge.

Days after inoculation	Groups G1	G2	G3	G4
21	$6.00x10^{4aAB}$	$4.05x10^{4aB}$	$5.20x10^{4aB}$	$1.12x10^{5aA}$
23	$7.80x10^{4aAB}$	$4.05x10^{4aB}$	$4.50x10^{4aB}$	$1.23x10^{5aA}$
25	$1.95x10^{4bB}$	$9.00x10^{3bC}$	$8.40x10^{4aA}$	$1.40x10^{5aA}$
27	$3.00x10^{3cC}$	$1.50x10^{3cD}$	$1.50x10^{4bB}$	$6.40x10^{4aA}$
29	$1.50x10^{3cB}$	$1.50x10^{3cB}$	$1.50x10^{3cB}$	$4.50x10^{3bA}$
31	$1.50x10^{3cA}$	$1.50x10^{3cA}$	$1.50x10^{3cA}$	$1.50x10^{3cA}$
33	$1.50x10^{3cB}$	$1.50x10^{3cB}$	$1.50x10^{3cB}$	$3.00x10^{3bcA}$
35	$1.50x10^{3cB}$	$1.50x10^{3cB}$	$1.50x10^{3cB}$	$3.00x10^{3bcA}$

G1: Formalin group, G2: UV group,G3: freezing and thawing,G4:control positive group
a, b & c: There is no significant difference (P>0.05) between any two means, within the same column have the same superscript letter. A, B & C: There is no significant difference (P>0.05) between any two means for the same attribute, within the same row have the same superscript letter

Table (5): Immunoglobulins (IgA, IgG, IgM) level in V-line rabbits experimentally infected by attenuated and non-attenuated *Eimeria* oocysts.

Day	parameter	G1	G2	G3	G4
0	IgA	209^{dB}	228^{bB}	209^{bB}	290^{abA}
	IgG	1012^{cB}	1066^{cAB}	1038^{cB}	1117^{cA}
	IgM	187^{cA}	203^{bA}	194^{cA}	207^{cA}
7	IgA	252^{cdA}	276^{abA}	253^{abA}	250^{bA}
	IgG	1086^{bcB}	1200^{bA}	1076^{cB}	998^{dC}
	IgM	201^{bcA}	204^{bA}	201^{cA}	210^{cA}
14	IgA	238^{cdB}	280^{abAB}	276^{aAB}	286^{abA}
	IgG	1112^{bB}	1152^{bAB}	1118^{cB}	1205^{bA}
	IgM	255^{aB}	211^{bC}	204^{cC}	296^{aA}
21	IgA	286^{bcAB}	275^{abAB}	260^{abB}	312^{aA}
	IgG	1238^{aA}	1210^{bA}	1250^{bA}	1198^{bcA}
	IgM	214^{abcC}	300^{aA}	256^{bB}	246^{bcBC}
28	IgA	309^{bA}	296^{aA}	286^{aA}	295^{abA}
	IgG	1280^{aAB}	1215^{bB}	1320^{abA}	1319^{aA}
	IgM	215^{abcB}	321^{aA}	300^{aA}	304^{aA}
35	IgA	392^{aA}	298^{aB}	218^{bC}	294^{abB}
	IgG	1300^{aB}	1410^{aA}	1398^{aA}	1372^{aA}
	IgM	248^{abB}	244^{bB}	315^{aA}	291^{abA}

G1: Formalin group, G2: UV group,G3:freezing and thawing,G4:control positive group

a, b & c: There is no significant difference (P>0.05) between any two means, within the same column have the same superscript letter. A, B & C: There is no significant difference (P>0.05) between any two means for the same attribute, within the same row have the same superscript letter.

Table 6: Total and differential leukocytic count

Day	Parameter	G1	G2	G3	G4
0	Total WBC	7.90^{abA}	6.50^{abBC}	7.50^{aAB}	5.50^{cC}
	Lymphocytes	40^{cBC}	45^{cAB}	47^{cA}	35^{cC}
	Neutrophils	41^{aA}	40^{abA}	32^{bcB}	44^{aA}
	Eosinophils	2^{abA}	2^{aA}	2^{aA}	2^{abA}
	Monocytes	16^{bB}	14^{bB}	20^{bA}	14^{bB}
7	Total WBC	8.30^{aA}	5.80^{bB}	5.50^{cB}	7.50^{aA}
	Lymphocytes	37^{cB}	36^{dB}	48^{bcA}	40^{cB}
	Neutrophils	38^{aA}	43^{aA}	26^{cC}	37^{abA}
	Eosinophils	2^{abA}	2^{aA}	2^{aA}	2^{abA}
	monocytes	22^{aA}	17^{aC}	23^{aA}	$^{B}20^{aB}$
14	Total WBC	7.90^{abA}	6.80^{abB}	6.10^{bcB}	6.80^{abB}
	Lymphocytes	49^{bC}	61^{aAB}	56^{aB}	65^{aA}
	Neutrophils	44^{aA}	32^{bBC}	37^{abB}	26^{cC}
	Eosinophils	2^{abA}	1^{abB}	2^{aA}	2^{abA}
	Monocytes	5^{cd}	5^{cdA}	5^{cA}	$^{A}7^{cA}$
21	Total WBC	6.90^{bcA}	7.60^{aA}	7.00^{abA}	7.00^{abA}
	Lymphocytes	53^{abA}	55^{abA}	55^{abA}	53^{bA}
	Neutrophils	43^{aAB}	38^{abA}	$^{B}37^{abB}$	44^{aA}
	Eosinophils	2^{abB}	0^{bD}	3^{aA}	1^{bC}
	Monocytes	3^{dB}	3^{dB}	6^{cA}	2^{dB}
28	Total WBC	6.00^{bcA}	5.60^{bA}	6.00^{bcA}	6.00^{cdA}
	Lymphocytes	52^{abA}	54^{abA}	53^{abcA}	55^{bA}
	Neutrophils	40^{aA}	40^{abA}	37^{abA}	35^{bA}
	Eosinophils	3^{aA}	2^{aB}	2^{aB}	3^{aA}
	Monocytes	5^{cdA}	4^{cdB}	7^{cA}	$^{B}7^{cA}$
35	Total WBC	5.60^{dB}	6.00^{bAB}	5.00^{cB}	6.90^{abA}
	Lymphocytes	57^{aA}	51^{bcAB}	50^{abcB}	57^{bA}
	Neutrophils	37^{aAB}	42^{aA}	41^{aA}	34^{bB}
	Eosinophils	1^{bB}	2^{A}	2^{aA}	1^{bB}
	Monocyte	6^{cA}	6^{cA}	7^{cA}	7^{cA}

G1: Formalin group, G2: UV group, G3:freezing and thawing,G4:control positive group
a, b & c: There is no significant difference (P>0.05) between any two means, within the same column have the same superscript letter. A, B & C: There is no significant difference (P>0.05) between any two means for the same attribute, within the same row have the same superscript letter.

4. DISCUSSION

The present study aimed to investigate the effect of immunization of rabbits by different attenuated oocysts on infectivity, immune-genicity, clinical symptoms and body weight of V-line rabbits. Immunization of rabbits against eimeriosis by different attenuated sporulated *Eimeria* oocysts on experimentally infected rabbits with this attenuated oocysts showed a pre-patent period 5-6 days. Our result close ratio is consistent with that of Akpo et al.(2012) who found that whole

oocyst output was measured 3-8 days after inoculation .

The current result showed that the degree of clinical symptoms was decreased in the attenuated groups than that of the control group, this result was in agreement with that of Lee et al.(2009) who recorded that *Cryptosporidium parvum* oocysts where subjected to gamma irradiation at various doses (1, 5, 10, and 25 kGy) showed reduction of the infectivity and repair of the infectivity after irradiation and Ramadan et al. (2018)who reported that immunization of lambs by using one hour UV irradiated *Eimeria* oocysts showed no signs of recumbency, fever and off food ,only lambs showed soft feces at 13th PI and, while our result were disagreed with Ramamoorthy et al.(2006) who evaluated that vaccination of female mice with two intraperitoneal inoculation of 1x10^{6} of 528 Gy of gamma irradiated *Neospora caninum* tachyzoites and challenged intraperitoneally with 1x10^{7} *N. caninum* tachyzoites. All vaccinated mice remained healthy and showed no obvious signs of neosporosis. The present result revealed that UV attenuated group showed the highest weight gain this result agreed with Zayan and El-Akababwy (2003) who estimated that the highest body weight gain was recorded in G2(inoculated with 1x10^{4} gamma irradiated *E.siedai* at dose of 14K) and G4(control non-immunized and non-challenged). With respect to oocysts shedding there was a significance difference in G1.G2 and G3 compared to G4 in day 6 and 15 PI .from 29 till the end of the experiment the number of oocysts were decline in G1.G2 and G3. The same result was in agreement with that of Chatterjee et al., (1996) who proved that gamma irradiated sporozoites of *Plasmodium berghei* inhibited sporozoites invasion during challenge infection, (Dubey et al.1996) ,Omata et al. (2001) ,Jenkins et al.(2004), Ramamoorthy et al. (2006) and Ramadan et al. (2018). It was cleared that, there was no a significant difference in level IgA between all groups through experiment except at day 35th, G1 followed by G2 recorded the highest level compared to other groups while G1 recorded the lowest level at 14th DPI compared to control group while G3 recorded the lowest level at 21st and 35th DPI. This result was agreed with Pakandl et al .(2008) who demonstrated that rabbits infected with 2000 oocysts of *Eimeria flavescens* and *E. intestinalis* showed that the mean level IgA antibodies was slightly enhanced at 14 and 21 days post inoculation. Generally, no difference in the antibody was found between the samples taken at 0 and 7 days post-infection and Lillehoj and Trout (1993) who reported that IgA is probably the most important isotype involved in coccidial infections, IgA was detected in birds infected with Eimeria tenella at 7 days post infection. Smith et al. (1995) who said that levels of IgA in mice infected by *E. nieschulzi* started to increase after 12 post-primary infection (DPI), reached peak levels between days 20 and 30 DPI and were slightly increased by challenge infection. Pakandl et al .(2008) demonstrated that rabbits infected with 2x10^{3} oocysts of E.flavescens and *E. intestinalis* showed antibody responses against them and the mean level IgA antibodies was slightly enhanced the same period .On the other hand our result was disagreed with Omata et al. (2001) who found that immunized rabbits with *E.Stiedai* antigen developed a high level of IgA antibody against this antigen, Guo et al .(2004) who estimated that the levels of IgA and IgM in bird infected with sporulated oocysts of *E.tenella* who reported specific IgA increased on 14th DPI and slightly decreased on 21st DPI, Zorgi et al. (2011) who showed that high level of IgA antibodies in the immunized mice serum with gamma irradiated *Toxoplasma gondii*.

Our result revealed that G2 from 14 till the end of experiment showed a significant difference when compared with zero day .G2 was the only group had a significant difference compared with other days. This results was consistent with Lillehoj and Ruff (1987) who reported that parasite reactive serum IgG antibodies reach maximum levels 8-14 days after oral infection with different Eimeria sp.(*E.tenella, E.acervulina or E.maxima*), Sher and Coffman(1992) who recorded that more IgG being produced during subsequent exposure to the stimulating antigen responses are generally stronger and more rapid. This indicated that immunization by attenuation of Eimeria spp. by different methods succeeded in rising IgG which usually present in serum and protect rabbits against reinfection. Our data indicated that G2 recorded the highest level of IgM at 21st and 28th DPI compared to other groups, while G3 showed the highest level at 35th DPI (day post infection). This results coincided

with Sher and Coffman(1992) who recorded that IgM produced in largest amounts during response against protozoal disease ,Smith et al. (1995) and Ramadan et al. (2018), noticed that specific serum IgM levels were increased during primary infection but returned to background levels at the end of the patent period and were not affected by challenge infection.

The present data recorded that, in leukocytic count there was no a significant difference between all groups from 21st day to the end of the experiment.G1 recorded the highest level at 7th and 14th day PI compared to other groups. Leukocytic count change could be considered as a part of clinical intestinal coccidiosis complex. Inflammatory process locally recruits leukocytes, so they are lost through damaged intestinal mucosa in the next phase, which is the probable reason of their count decrease. Count increase in later phase should be attributed to haemo-concetration as a result of fluid loss, Kulišić et al.(2006).

Our data indicated that Lymphocyte showed that there was no significance difference between all groups at 21st day and 28th day. The highest level recorded in G3 and G4 at 7th and 14th day, respectively, compared to other groups.

The current results revealed that mild increase in level of neutrophil count at 7th DPI (day post infection) in G2 and there was mild increase in G1 at14th post challenge. G4 showed the lowest level of Neutrophil at 14th, 28th and 35th day post challenge. Neutrophils mainly, responsible in pathogens clearance in an acute inflammatory process (Newburger and Dale, 2013), 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 (Mantovani et al., 2011).

Eosinophils in our result which recorded that there was no significant difference between all groups except Eosinophil mild increased in G1 on 28th DPI and on 21st DPI in G3. This result agreed with Al-Taee and AlZubaidi, (2017) who said that there was mild elevation in eosinophils percentage in vaccinated groups on day 28 PI and in G3 during almost the entire experimental period.

Monocytes showed no significance difference all over the experiment in all groups except G2 which recorded significance difference on 7th DPI compared with other groups our results agreed with Kulisic et al.(2006) who found that There was no statistically significant difference in WBC count between uninfected (Control) and infected rabbits group A($2x10^5$ oocysts)and group B ($4x10^5$ oocysts), as well as between the groups of infected rabbits neither on day 0, nor at any time during the experiment, Al-Taee and Alzubaidi,(2017) who said that Overall, total and differential WBC counts in all groups were within normal limits for the entire experimental period, except for G3(injected subcutaneously with 1 ml PBS containing 1000 oocysts/ml) where significant neutrophil increments and inverse lymphocyte decrements were recorded on day 28 onward. On the other hand, significant differences were recorded between G1 (was subcutaneously injected with 1 mg/ml sonicated Ag (sporulated oocysts of Eimeria stiedae) and G2(Half dose of the previously mentioned Ag), G4(negative control group) , except on day 56 PC where G2 had significantly higher WBC count than both G1 and G4. Monocytes showed no significant differences among the four groups except on day 56 PC onward while there was no change in eosinophils count in coccidious rabbits during experiment. 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. Reduction in these cells can undoubtly diminishes the adaptive immune response.

5-Conclusion

The present study concluded that vaccination of rabbits with UV attenuated sporulated intestinal Eimeria oocyst is more beneficial in protection against coccidiosis which achieved the highest body weight gain , the lowest clinical signs and the lowest oocysts shedding compared to other methods of attenuation(formalin attenuated oocyst and freezed-thawed oocysts)and stimulated higher humoral immune response without obvious side effects.

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