



Immunization of Lambs Against Coccidiosis by Using Ultraviolet Irradiated *Eimeria* Oocysts for the First Time

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ABSTRACT

The study was conducted to study the effect of Ultra-violet radiated *Eimeria* oocysts on immune pattern of lambs. For this purpose, *Eimeria* sp. (*E. crandalis*, *E. granulosa*, *E. ovinoidalis*, *E. parva*, *E. pallida* and *E. faurei*) were collected from infected sheep and propagated in a lamb for increasing the number of oocysts. The collected *Eimeria* sp. from the lamb, were sporulated in potassium dichromate 2.5% and divided into 3 parts; the 1st part was exposed to UV radiation for half hour and the 2nd part for one hour and the third left without irradiation. Nine lambs were classified into 3 equal groups; G1 was inoculated by half hour UV irradiated *Eimeria* oocysts, G2 was inoculated by one-hour UV irradiated *Eimeria* oocysts and G3 was inoculated by non-irradiated *Eimeria* oocysts. Fecal samples were collected from all lamb groups from 5th to 21th days, identified and count. Challenge by non-attenuated *Eimeria* was carried out at 21th day post inoculation. The obtained results revealed that immunization of lambs by using one-hour UV irradiated *Eimeria* oocysts showed low number of oocysts in feces before and after challenge compared to other groups. The same group also showed high level of γ globulin as well as increasing levels of IgG. It was recommended that immunization of lambs by one-hour UV irradiated *Eimeria* oocysts could protect lambs against Coccidiosis.

Keyword: *Eimeria* species, Lambs, UV irradiation, immunoglobulin, immunization.

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1. INTRODUCTION

Sheep coccidiosis is of economic and medical importance and the infection of sheep have been observed in almost all sheep rearing in the world (El Madway and Elkhait, 2014). All ages of sheep are susceptible to *Eimeria* infection, but lambs are more severely affected

by clinical coccidiosis and disease outbreak (Khan et al., 2011). The coccidiosis may be clinical or subclinical. Both the clinical and the subclinical form of the disease compromise animal health, possibly leading to their death (Lagares, 2008).

Anticoccidial control relies primarily on routine chemoprophylaxis using ionophore and/or chemical drugs, but resistance develops rapidly and is now widespread (Chapman and Jeffers, 2014). Alternative control strategies are urgent to be developed due to the rise of drug resistance, the increasing legislation restrictions on the use of coccidias, the high costs of new drug development and the potential reversion of pathogenicity, as well as the limited cross-protection among *Eimeria* species (del Cacho et al., 2012; Ding et al., 2012; Blake and Tomely, 2014; Clarke et al., 2014; Chapman, 2014). However, it is very urgent to develop safe and effective multivalent vaccine against mixed infection of all the economically important species of *Eimeria* (del Cacho et al., 2012, Chapman et al., 2005).

The use of γ radiation for attenuation of *Eimeria* species oocysts has been previously done in chicken by Gilbert et al., (1998), X ray in rabbits by Zayan and El-Akabawy (2003), X ray in goats by Ruiz et al. (2014). However, no previous studies concerned with attenuation of sheep *Eimeria* species were used in immunization of sheep against coccidiosis despite of its sever pathogenic effect. So, the present work is a trail to study the effect of UV radiated *Eimeria* oocysts on immune status in lambs for the first time.

2-MATERIAL AND METHODS

A. Collection and Propagation of *Eimeria* species.

The *Eimeria* sp. (*E. crandalis*, *E. granulosa*, *E. ovinoidalis*, *E. parva*, *E. pallida* and *E. faurei*) used in this study were isolated from naturally infected sheep in Qaloubia governorate, Egypt. These species were

propagated and maintained by oral inoculation in lamb (2 month of age). Fecal samples were examined on 5th to 20th day post inoculation (DPI) for oocysts detection. *Eimeria* species oocysts were collected, isolated, counted and sporulated in 2.5% potassium dichromate at room temperature (Ruiz et al., 2014). The sporulated oocysts were identified according to Solusby (1982).

B. Attenuation of *Eimeria* oocysts

Three suspensions of sporulated oocysts (each containing 2×10^6 oocyst/ ml) were washed several times by distilled water to remove excess of potassium dichromate. The first suspension was exposed to Ultraviolet radiation (Universal UV unit, 110/120 Vac. 50/60 HZ, 253.7 nm, Model No.51418, Gelman Instrument company) for half hour. The second suspension was exposed to ultraviolet irradiation for one hour (Ramadan and Nagwa 2007). The third suspension was kept without attenuation.

C. Evaluation of the efficacy of UV irradiation on infectivity and immunogenicity of *Eimeria* oocysts in lambs

C.1. Animals

A total of 9 Baladi breed lambs aged 2-3 months were purchased from local sheep farm. Lambs were maintained under parasite free condition in a separate room with concrete floors. The rooms were cleaned daily with water and 10% ammonia solution. The fecal matter of each lamb was daily examined to ensure they were free from any parasitic infestation. The lambs were treated using sulphadiazine injection for 4 days and albendazole 5%.

C.2. Experimental design

Lambs were allocated into 3 equal groups. Each lamb in 1st group (G1) was inoculated with 1×10^5 mixed *Eimeria* oocysts (*E. parva*, *E. pallida*, *E. ovinoidalis*, *E. faurei*, *E. crandalis* and *E. granulosa*) by using a stomach tube which was previously exposed to UV irradiation for 30 minutes. The second group (G2) was inoculated by 1×10^5 UV irradiated oocysts for 60 minutes. Lamb in the third group (G3) were inoculated by 1×10^5 non-attenuated oocysts. Furthermore, the lambs in all groups were challenge at 21th day by 1×10^5 non-attenuated *Eimeria* oocysts.

Fecal samples were collected daily from each animal in all lamb groups for detection, identification and counting of *Eimeria* oocysts in feces (Ruzi et al., 2014). Also blood samples were collected weekly from each lamb group starting from zero day to end of the experiment according to Sultana et al., (2014). Serum was separated for determination of IgG, IgM, IgA according to Voller et al. (1976) and biochemical analysis according to Doumas and Biggs, (1972).

D. Statistical analysis data was analyzed by Two-way ANOVA using SPSS (ver. 20). Difference between groups were considered significant at $P < 0.05$.

3. RESULTS

Clinical symptoms were noticed on lamb groups as follow: lambs in group 1 which was inoculated by UV irradiated oocysts for 30 minutes suffered from moderate diarrhea, recumbency, fever and off food at 12th to 14th day post first inoculation, while lambs in group 2 that inoculated by UV irradiated oocysts for 60 minutes showed only mild diarrhea at 13th

day post inoculation. On the other hand; lambs in group 3 which was inoculated by non-attenuated *Eimeria* strains showed more potent symptoms than the other two groups where the animals suffered from severe diarrhea, recumbency, fever, off food and mortality at 10th to 17th day post first inoculation (Table 1). All lambs' groups showed no clinical signs of coccidiosis after challenge.

Experimentally infected lambs with sporulated oocyst displayed a pre-patent period of 10 day. The control positive group showed a typical time course of oocyst shedding. However, the maximum oocysts per gram (OPG) of control positive and 1st group where was a significantly higher on 16th DPI (5.4×10^4 , 5.1×10^4 ; respectively) as compared to 2nd group on 14th DPI (2.1×10^4). Similarly, OPG were gradually declined from 17th DPI and continued afterward (Graph.1). There was a significant difference between different groups and between days in the same group.

After challenge, there was an overall reduction in OPG in all lamb groups. A significant difference occurred between groups in which, the maximum OPG (1800) was on 36th DPI in group 3. Group 2 the maximum shedding (600) OPG was on 36th DPI, while in group 1 the maximum shedding was on 34rd, 36th (900) (Graph.2).

The concentration of IgA in G1 was a significantly higher than the other two groups from 14th to 49th DPI. G3 showed the lowest IgA level as compared with the other groups (Graph. 3).

On 21st DPI the level of IgG in G1, G2 was a significantly much higher than G3. The level in G1 continued with a nearly steady high

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level till the end of the experiment, but in G2 it declined firstly, then elevated again on 49th DPI. In G3 the concentration of IgG was increased till 28th DPI than G1 and G2 but it gradually declined till the end of the experiment (Graph. 4).

ELISA test displayed that the level of IgM in G1 had a significant lower level than the other two groups. It reached its peak on 35th DPI, then gradually declined to the end of the experiment. In G2, the level of IgM reached its top concentration on 7th DPI, but it declined gradually, then elevated gradually again till the end of the experiment to reach a much higher level than G1 and G3 on 49th DPI. G3 showed a higher elevation of IgM than G1 and G2 on 28th to 35th DPI but its concentration decreased

gradually till the end of the experiment (Graph.5).

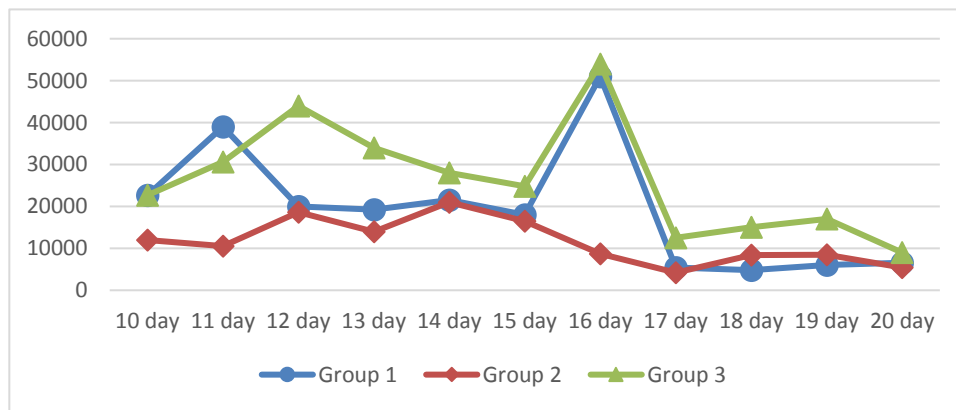
Electrophoretic analysis of serum of different groups of lambs showed a significant difference in which; albumin concentration decreased in both G1 and G2 compared to G3 in which albumin concentration increased on 21 days. α_1 globulin of G3 increased than zero day while in G1&G2 decreased than the value on day zero. α_2 globulin increased only in G1 than G2 & G3 while β globulin increased in all groups than the value on day zero. γ globulin in G2 was a highly significant than the other two groups and from the value on day zero (control day) as shown in (Table 2 & Graph. 6, 7, 8, 9, 10, 11).

Table 1. Clinical manifestations in lambs experimentally infected by irradiated and non-irradiated *Eimeria* oocysts.

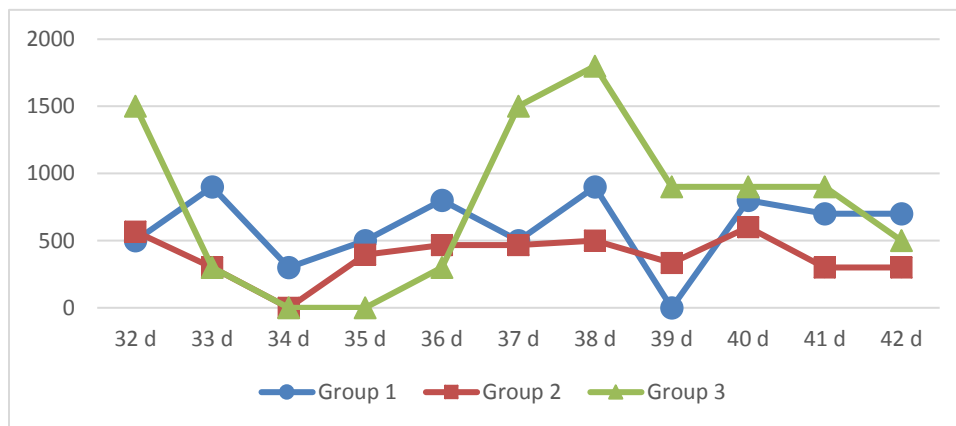
Symptoms	Days of appearance of clinical symptoms		
	Group 1 (30-minute UV)	Group 2 (60-minute UV)	Group 3 (Control positive)
Diarrhea	12 th , 13 th , & 14 th DPI	13 th (soft) DPI	10 th - 17 th DPI
Recumbency	12 th , 13 th , & 14 th DPI	Negative	10 th - 17 th DPI
Fever	12 th , 13 th & 14 th DPI	Negative	10 th - 17 th DPI
off food	12 th , 13 th & 14 th DPI	Negative	10 th - 17 th DPI

Table (2). Electrophoretic pattern of serum Albumin and different globulins among lambs experimentally inoculated by UV irradiated and non- irradiated *Eimeria* oocysts.

Fraction	Group 1 (30-minute UV)		Group 2 (60-minute UV)		Group 3 (Control positive)	
	At zero day	At 21 st day	At zero day	At 21 st day	At zero day	At 21 st day
Albumin	46.21	38.05±0.086 ^b	37.64	29.39±0.202 ^c	35.49	40.01±0.046 ^a
α ₁ globulins	16.12	14.21±0.051 ^b	28.92	12.64±0.207 ^c	17.28	20.52±0.277 ^a
α ₂ globulins	1.55	10.32±0.069 ^a	25.36	8.27±0.075 ^b	5.55	4.29±0.046 ^c
β globulins	31.44	35.06±0.069 ^a	7.96	28.77±0.3 ^b	41.59	35.09±0.051 ^a
γ globulins	4.68	2.35±0.098 ^b	0.12	20.93±0.381 ^a	0.09	0.10±0.011 ^c

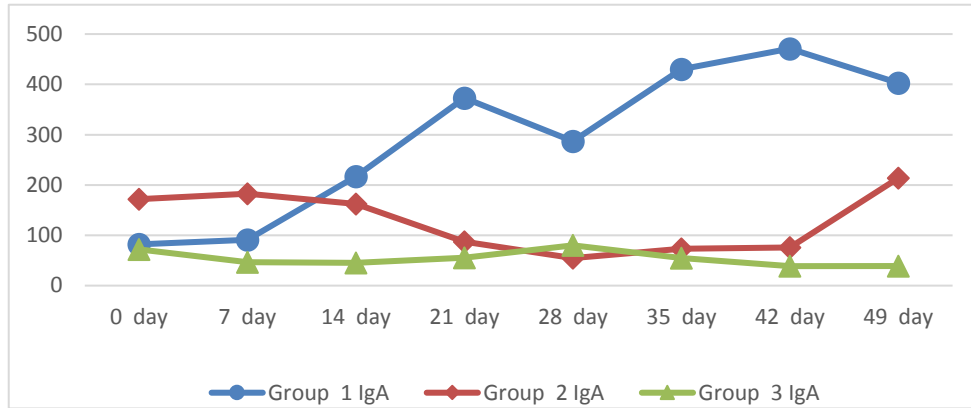


Graph (1): Effect of UV irradiation on oocysts shedding.

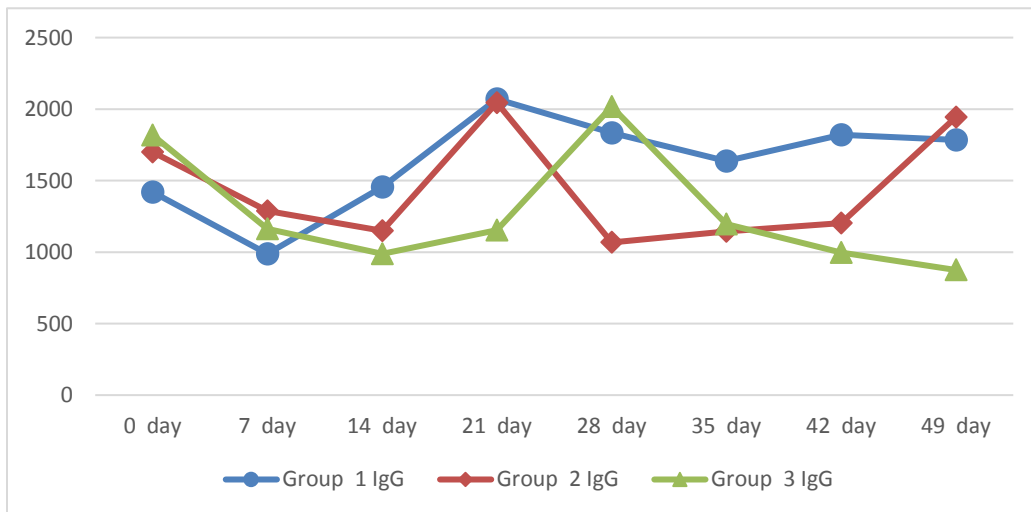


Graph (2): Number of *Eimeria* oocysts in experimentally infected lambs post challenge.

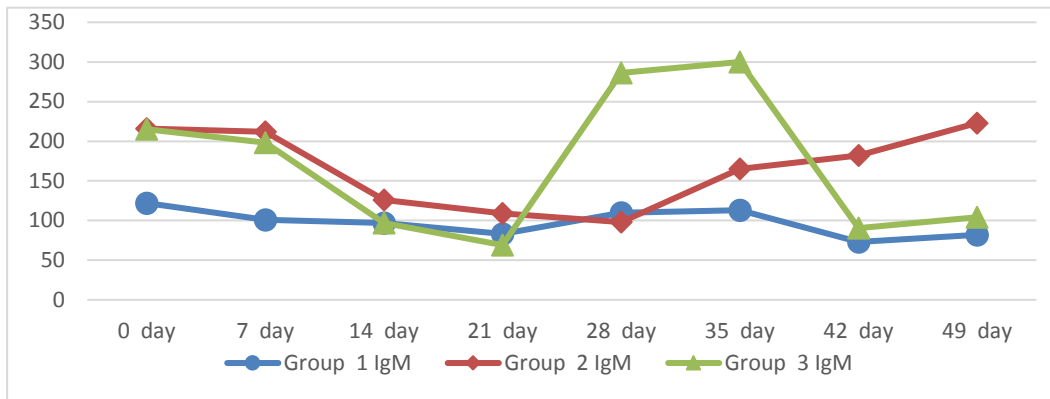
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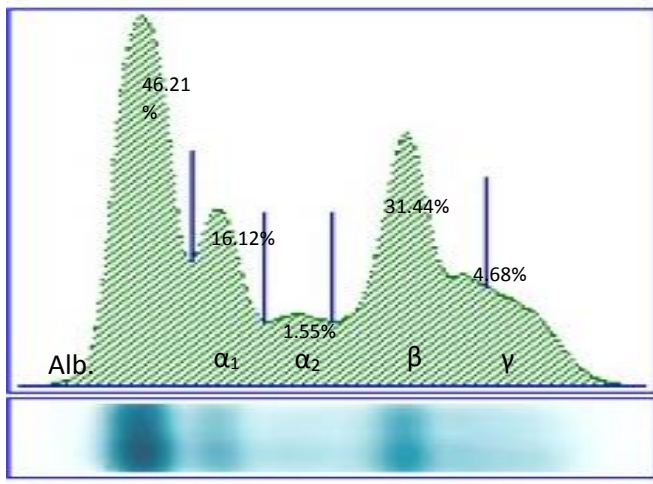
Graph (3): Immunoglobulin A (IgA) level in lamb experimentally infected by UV irradiated and non-irradiated of *Eimeria* oocysts.



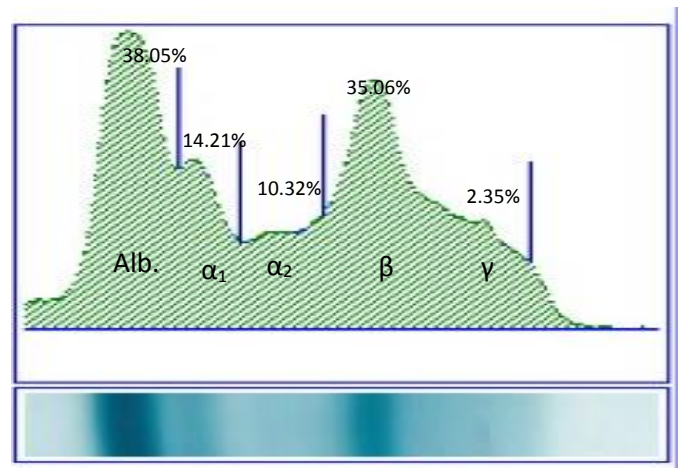
Graph (4): The level of IgG among lambs experimentally inoculated by UV irradiated and non-irradiated *Eimeria* oocysts.



Graph (5): The level of IgM among lambs experimentally inoculated by UV irradiated and non-irradiated *Eimeria* oocyst.

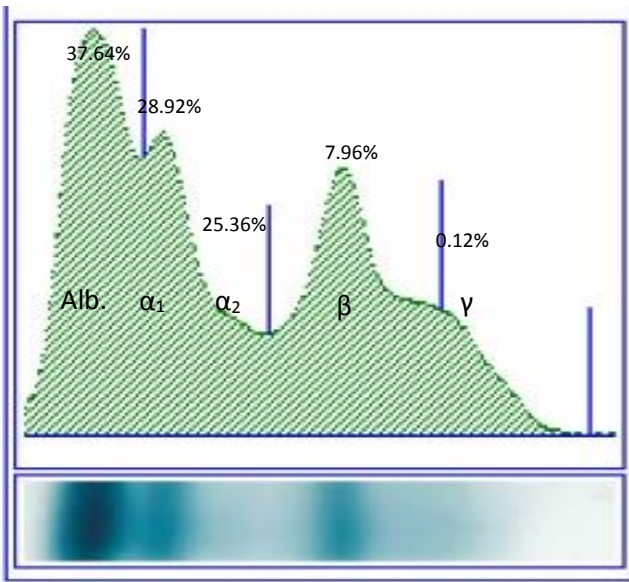


Graph (6): Serum profile of electroporesis of G1 (infected with half hour UV irradiated oocysts) on zero day of the experiment

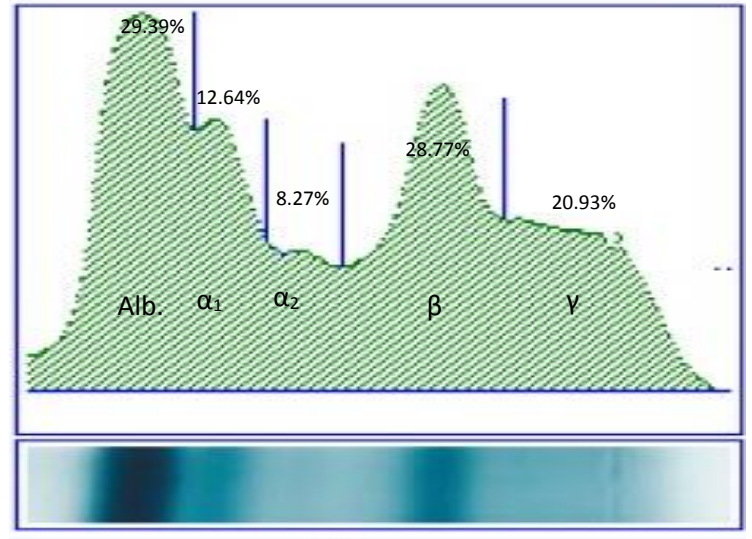


Graph (7): Serum profile of electroporesis of G1 (infected with half hour UV irradiated oocysts) on 21st day of the experiment

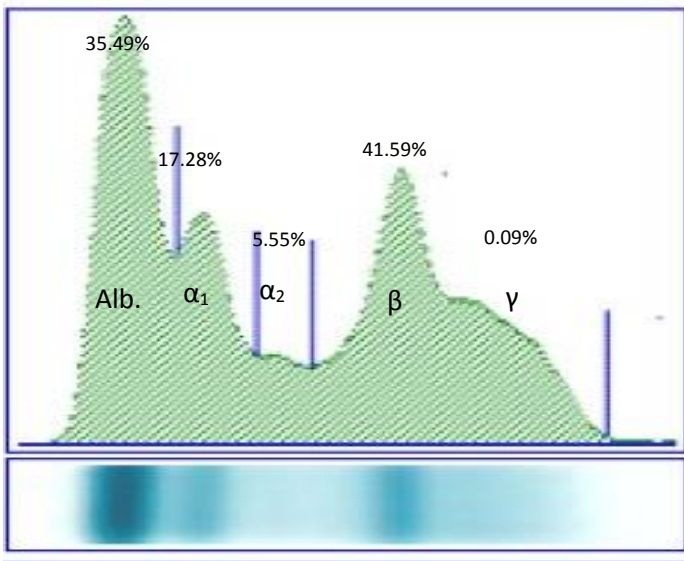
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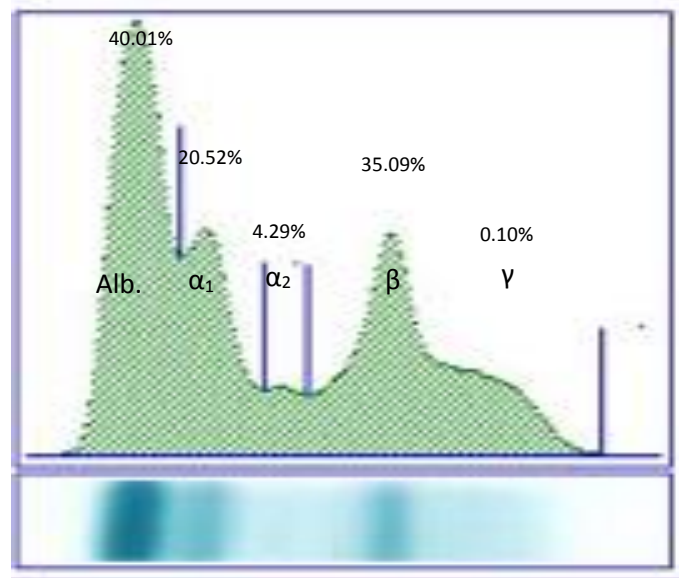
Graph (8): Serum profile of electrophoresis of G2 (infected with one hour UV irradiated oocysts) at zero day of the experiment



Graph (9): Serum profile of electrophoresis of G2 (infected with one hour UV irradiated oocysts) at zero day of the experiment



Graph (10): Serum profile of electrophoresis of G3 (Control positive group) on zero day of the experiment



Graph (11): Serum profile of electrophoresis of G3 (Control positive group) on 21st day of the experiment

4-DISCUSSION

The effect of UV irradiated *Eimeria* oocysts on immune status in lambs was carried out for the first time. Clinical signs appear more potent in lambs that inoculated by non-attenuated *Eimeria*. Lambs that inoculated by UV irradiated oocysts for 30 minutes suffered from diarrhea, recumbency, fever and off food. While lambs which were inoculated by UV irradiated oocysts for 60 minutes showed only diarrhea. However, lambs which were inoculated by non-attenuated *Eimeria* strains; clinical symptoms appeared more potent than the other two groups where the animals suffered from diarrhea, recumbency, fever, off food on 10th to 17th DPI except one lamb; diarrhea persist until death on day 35 DPI. Such signs were previously noted by Ruiz et al., (2013) who showed that the signs of coccidiosis were diarrhea, dehydration, anorexia and recumbency in non-attenuated *Eimeria* oocyst in goat. Regarding to oocysts count per gram of feces, the results displayed a pre-patent period of 10 day. The control positive group showed a typical course of oocyst shedding. However, the maximum oocysts per gram (OPG) of control positive and 1st group were was a significantly higher on 16th DPI (5.4×10^4 , 5.1×10^4 ; respectively) as compared to 2nd group on 14th DPI (2.1×10^4). The lowest oocysts shedding was recorded in G 2 (4.2×10^3) as compared to the other two groups. The recorded low number of oocysts in one hour attenuated group may be due to failure of sporozoites to invade the columnar epithelial cells of intestine and reproduce inside it. In this respect Chatterjee et al. (1996) mentioned that irradiated sporozoites of *Plasmodium berghei* inhibited sporozoites invasion during challenge

infection. Also, Dubey et al. (1998) found that sporulated oocysts of *Toxoplasma gondii* and *Cryptosporidium* irradiated at 0.4 k Gy of ¹³⁷Cs were able to encyst and sporozoites were infective but not capable of inducing a viable infection in mice also Ramamurthy et al. (2006) who recorded that a dose of 528 Gy of γ irradiated was sufficient to arrest replication but not host cell penetration by tachyzoites.

After challenge, the maximum OPG (1800) was on 36th DPI in G3. In G2 the maximum shedding was on 30th (567 OPG), 36th (500OPG), 34th and 35th (467 OPG) DPI. While in G1 the maximum shedding was on 31rd, 36th (900 OPG), 34th and 38th (800 OPG) DPI. Such result indicated that UV irradiation of *Eimeria* oocysts succeeded in decreasing the infectivity and reproducibility of *Eimeria* species in immunized lambs with superiority of those exposed to one hour of irradiation. This results agreed with Zayan and El-Akabawy (2003) noticed that OPG was 40 fold (1×10^5) fewer in rabbit group inoculated by 1×10^4 X ray irradiated *Eimeria stiedai* than group (40×10^5) inoculated by 1×10^4 non irradiated oocysts before challenge, while no datable oocysts were observed after challenge in both groups, and Ruiz et al., (2014) who showed that Immunized group with X-ray irradiated *E. ninakohlyakimovae* oocysts in goat showed significantly reduced shedding of oocysts by 95.3% when compared to oocysts output induced by non-attenuated oocysts. While the course of re-challenge infection there was a significant reduction of oocysts shedding in animals immunized either with X rad-attenuated

oocysts (87.7%) or non-attenuated oocysts (60.5%).

With respect to the effect of UV irradiation on Immunoglobulin, it was recorded that, the concentration of IgA in G1 was higher than the other two groups (G2, G3) from 14th to 49th DPI. G3 showed the lowest IgA level as compared with the other groups (G1 and G2). G1 showed higher titer level on 42nd DPI and the lowest level on zero-day DPI while; Group 2 showed higher titer level on 49th DPI and the lowest level on 28th DPI. However, group 3 revealed high titer on 28th DPI while the lowest level was on 42nd and 43th DPI. This indicated that immunization of lambs by UV attenuated *Eimeria* species succeeded in rising IgA which usually secreted in mucous that secreted by goblet cell in intestine and attack *Eimeria* sporozoites and merozoites. This result agreed with Zorgi et al., (2011) who showed that high levels of IgA antibodies in the immunized mice serum with irradiated *Toxoplasma gondii*.

On 21st DPI The level of IgG in G1, G2 was much higher than G3. The level in G1 continued with a nearly steady high level till the end of the experiment, but in G2 it declined gradually, then elevated again on 49th DPI. In G3 the concentration of IgG was at a higher level on 28th DPI than G1 and G2 but it gradually declined till the end of the experiment revealed that there was a significant difference ($P>0.05$) between different groups and between different days in the same group. These results agreed with Sher and Coffman (1992) who found that more IgG being produced during subsequent exposure to the stimulating antigen responses which were generally stronger and more rapid at this time. This indicated that immunization of lambs by UV attenuated

Eimeria species succeeded in rising IgG which usually present in serum and protect animals against reinfection with superiority of lambs that inoculated by one-hour UV attenuated *Eimeria*.

ELISA test displayed that the level of IgM in group 1 had a lower level than the other two groups. In group 2, the level of IgM elevated gradually till reached its top concentration on 49th DPI to reach a much higher level than G1 and G3 on 49th DPI. Group 3 showed a higher elevation of IgM than G1 and G2 on 28th to 35th DPI but its concentration decreased gradually till the end of the experiment. The statistical analysis showed there was a significant difference ($P>0.05$) between different groups. This result agreed with AL-Barwary (2012) recorded that the IgM level was higher at day 15 post injection and then started to decline in irradiated groups and non-irradiated groups and a second dose of *Toxoplasma* tissue cysts caused an increase in the IgM level at day 3 post challenged and remained in the peak at 15th to 30th days post challenge.

It was noted that IgG predominate over the other two immunoglobulins (IgA and IgM) throughout the experiment in response to infection. This indicated that immunization of lambs by UV attenuated *Eimeria* species succeeded in rising IgG which usually present in serum and protect animals against reinfection.

Regarding the effect of UV attenuated *Eimeria* on protein profile, serum of lambs experimentally inoculated by UV irradiated and non-irradiated *Eimeria* oocysts showed that albumin concentration decreased in both G1 and G2 as compared to G3 in which albumin concentration increased on day 21 than the value on day zero. These results agreed with Abdel-Salam and Mahran (2004) who reported

that the values of albumin was significantly decrease in goats infected with coccidiosis and El-Dessouky and El-Masry (2005) who found a high significant decrease of albumin in infected calves with *Cryptosporidium parvum* as compared with control group. The presence of infection is assumed to cause nutrient mal-absorption lead to intestinal protein loss and consequently reduced total protein and albumin Bangoura et al. (2007). Also, the albumin level of G3 were surprisingly high this due to hemoconcentration rather than increased availability of serum protein. Bangoura et al. (2007) who discussed this observation in which sever *Eimeria zurenii* oocysts infection massive water loss induces hemoconcentration which obscures serum protein loss. α_1 globulin of G3 increased than zero day while in G1 & G2 decreased than the value on day zero. α_2 globulin increased only in G1 than G2 & G3. This due to increase level of acute inflammatory cells due to infection by *Eimeria* species while β globulin increased in all groups than the value on day zero.

Gamma globulin of G2 had a high significant value than the other two groups this is due to the presence of attenuated sporozoites have the ability to penetrate but not replicate inside intestinal cells stimulate the host's immune system leading to increased synthesis of γ globulin (Holst and Svensson, 1994; Vijay pandaey et al., 2010).

Conclusion: Immunization of lambs by UV irradiated *Eimeria* oocysts resulted in reduction of oocysts number after challenge also lead to elevation of different immunoglobulin specially IgG in immunized lambs.

Recommendation: Immunization of lambs with attenuated strains of *Eimeria* exposed to UV light for one hour and more researches in immunization of lambs against coccidia should be performed.

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