



## Prevalence of *Eimeria* Species in Sheep with A Special Reference to Vaccinated Pregnant Ewes for Maternal Immunity for the First Time

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### ABSTRACT

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Sheep coccidiosis is an infection of economic and medical importance and have been observed in almost all alive sheep rearing in the world. Examination of 928 sheep fecal samples from different localities in Kaloubia Governorate for detection of *Eimeria* infection revealed a prevalence rate of 72.5% (n= 673/928). The identified *Eimeria* species oocysts were *Eimeria* candelas (78.3%), *E. granulosa* (63%), *E. ovinoidalis* (41%), *E. parva* (31.5%), *E. pallida* (22.1%), *E. intricata* (6.8%), *E. faurei* (6.2%) and *E. ahasta* (4.9%). The prevalence rate was high in females (76.3%) as compared to males (68.3%). The incidence of infection peaked in winter (83.1%) followed by spring (80.8%), while the lowest rate was in summer (61.0%). High prevalence rate was recorded in ages > 6 months (90.8%), followed by 6-12months (73.6%), while the lowest rate was in sheep over 1 year of age (55.6%). Single infection was recorded in 26.6% of infected sheep. Double infection rate was in 29.7% while mixed infection rate was in 43.7%. With regard to breeds, Rahmany and Osemy breed showed the highest infection rate (80.9% and 73.9%; respectively). On the other hand, Baladi breed showed the lowest infection rate (58.3%). Immunized dam with UV attenuated *Eimeria* oocysts and their progeny showed a significant increase in both IgG and IgM as compared to non-immunized control group. Biochemical analysis Immunized dams and their progeny showed high level of albumin, Beta and Gamma concentration levels as compared to control dams and their progeny. Conclusion, it could be concluded that Egyptian sheep was infected by 8 species of *Eimeria* with predominant of *Eimeria* crandalis and *Eimeria* granulosa. New born lambs from immunized ewes have high serum immunoglobulin especially IgG compared to those from non-immunized ewes. Recommendation, Immunization of pregnant ewes by two doses UV irradiated *Eimeria* oocysts one month before parturition to give protection to their progeny.

**Key word:** *Eimeria* species, UV irradiation, ewes, offspring, immunoglobulin, protein profile, vaccination.

## 1. INTRODUCTION

Sheep coccidiosis is an infection of economic and medical importance and have been observed in almost all alive sheep rearing in the world. (Taylor and Catchpole., 1994). Fifteen *Eimeria* species are considered to have the capability of infecting sheep of all ages and most pathogenic lambs (Platzer et al., 2005, Khan et al., 2011).

Coccidiosis may be clinical or subclinical (Lagares, 2008). Clinical coccidiosis results in higher losses for producers because of medical treatment costs, sever effect on performance of growth and sometimes death of lambs less than 3 months (Reeg et al., 2005; Elmadawy and Elkhaiat, 2014).

The incidence of *Eimeria* species was recorded by El-Akabawy, (1992) in kaloubia, Boshra, (1994) in Kaloubia, Nasr et al., (2008) in Sharkia, Mahran (2009) in Red sea, Abouzeid et al., (2010) in Sinai, Bkheet et al., (2010) in Beheria province, Sultan et al., (2016) in Kafr-El Sheikh, Mahmoud et al., (2018) in Assuit, and Mohamaden et al., (2018) in Suez, where their prevalence was (80.4, 80.67, 76.51, 6.61, 6.7, 70, 16.52, 65 and 57.7%, respectively) in sheep in Egypt.

The conventional control strategy is achieved by careful husbandry combined with in-feed anticoccidial drugs or vaccination with live or attenuated parasites (Blake and Tomley, 2014). It is very urgent to develop safe and effective multivalent vaccine against mixed infection of all the economically important species of *Eimeria* (Chapman et al 2005, del Cacho et al 2012). The use of  $\gamma$  radiation for attenuation of *Eimeria* species oocysts has been previously done in chicken by Jenkins et al.

(1997), X ray radiation of *Eimeria stiedai* in rabbits by Zayan and El-akabawy (2003), X-Ray irradiated *Toxoplasma gondii* in mice by AL-Barwary (2012) and X-rad irradiated *E. ninakohlyakimovae* oocysts in goats by Ruiz et al., (2014). However, few studies were carried out on Eimeriosis in sheep in Egypt as well as no previous studies concerned with immunization of ewes against coccidiosis beside the effect of UV radiation on immunogenicity of *Eimeria* species oocysts infecting ewes and their offspring.

## 2-MATERIAL AND METHODS:

### A. Animals

The present work was conducted on 928 sheep (442 males and 486 females) obtained from different localities in Kalubia Governorate (Shebin–Elkanater, Tokh, Qaliub and Benha), as well as, animals admitted to the Veterinary Teaching Hospital, Faculty of Veterinary medicine, Benha University during the period from June 2016 to the end of May 2017 for detection of *Eimeria* infection.

### B. Collection of fecal samples

Fecal samples were collected directly from the rectum of sheep of different ages (one week - > 3 years), sexes and breeds (Baladi, Rahmani, Oesmi and mixed) labeled in plastic bags for breed, age, sex, date of collection and locality. All the samples were transferred to the laboratory of Parasitology, Faculty of Veterinary Medicine, Benha University, Moshotor for parasitological examination. The collected fecal samples were examined by using concentration flotation techniques according to Pritchard and Kruse (1982). The positive fecal samples for *Eimeria* oocysts were aspirated and sporulated in 2.5%

potassium dichromate according to Solusby (1982) and identified according to Levine (1985).

### C. Immunization of ewes against coccidiosis for maternal immunity.

#### C.1. Animals.

Six pregnant ewes (aged 2 years) were kept in the farm of Faculty of Veterinary medicine, Benha University, under complete hygiene with free access to water and food for two weeks for adaptation with daily examination of their fecal samples to ensure they are free from any parasitic infection.

#### C.2. Attenuation of *Eimeria* species.

*Eimeria* species oocysts (*E. prva*, *E. pallida*, *E. ovinoidalis*, *E. faurei*, *E. crandalis* and *E. granulosa*) were attenuated by exposure to Ultraviolet radiation (Universal UV unit, 110/120 Vac. 50/60 HZ, 253.7 nm, Model No.51418, Gelman Instrument company) for half hour (Ramadan and Nagwa 2007).

Ewes were allocated into 2 groups each of 3 ewes. Each ewe in group 1 was inoculated orally with  $1 \times 10^5$  attenuated *Eimeria* species using stomach tube. Booster dose was given to ewes two weeks later before parturition. Ewes in group 2 were kept as uninfected to be control negative.

#### C.3. Blood samples.

Blood samples were taken from pregnant ewes groups from jugular vein in sterilized test tube without EDTA for separation of serum (Fiege et al.1991) just before first inoculation, second inoculation and at day of parturition for determining IgA, IgG, IgM according to Voller et al. (1976) and biochemical analysis according to Doumas and Biggs, (1972). Blood samples were collected from the newborn lamb on 1<sup>st</sup> day

post parturition after suckling colostrum for determination of the same parameters.

### 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:

Examination of 928 fecal samples from sheep revealed a prevalence rate of 72.5% (n= 673/928). The identified *Eimeria* species oocysts were *Eimeria crandalis* (78.3%), *E. granulosa* (63%), *E. ovinoidalis* (41%), *E. parva* (31.5%), *E. pallida* (22.1%), *E. intricata* (6.8%), *E. faurei* (6.2%) and *E. ahasta* (4.9%) (Photo 1).

The prevalence rate was high in females (76.3%, n=371/486) as compared to males (68.3%, n= 302/442). Further analysis revealed that the highest rate of infection was in winter (83.1%) followed by spring (80.8%). While the lowest rate was in summer (61.2%) (Table 1). The statistical analysis showed a significant decrease in the prevalence rate in autumn and summer and a high significant rate in winter and spring.

A significant difference in the infection rate among ages less than 6 months (90.8%), followed by 6 month-12 years of age (73.6%), while the lowest was rate in sheep over 1 year of age (55.6%) (Table 2). Single infection was recorded in 26.6% of infected sheep, double infection was in 29.7% while mixed infection rate was in 43.7% (Graph. 1).

With regard to Table (3) showed that, Rahmany and Osemy breed showed the highest infection rate (80.9% and 73.9%; respectively). On the other hand, Baladi breed showed the lowest infection rate (58.3%). Statistical analysis showed a significant difference between different

breeds, Rahmany, Osemy and Baladi, while there was no significant difference between Mixed with Baladi and mixed with Osemy

Table (4) indicated that immunized dam with UV attenuated *Eimeria* spp. oocysts showed a significant increase in both IgG and IgM during the whole period of experiment compared to non-immunized control group which showed a significant increase in IgA. Similarly, 24 hours post parturition lambs that suckle colostrum from immunized dams revealed a significant increase in IgG and IgM when compared to control lambs

which showed a significant increase in IgA (Graph. 2).

Electrophoretic analysis of serum from dams and their lambs in each group showed that Immunized dams had high level of albumin, Beta and Gamma concentration levels as compared to control dams that showed increase in  $\alpha_1$  and  $\alpha_2$ . While lambs consequently from immunized dams showed also an increase in Gamma globulin when compared to lambs from control negative group.  $\alpha_1$ , and  $\beta$  globulin were high in Control negative group than immunized group (Table 5, Graph 3, 4, 5, 6).

Table (1). Seasonal prevalence of *Eimeria* species among examined males and female's sheep.

Seasons	The examined males			The examined females			Total		
	No	Positive	%	No	Positive	%	No	Positive	%
Summer	127	61	48 <sup>CB</sup>	110	84	76.4 <sup>abA</sup>	237	145	61.2 <sup>b</sup>
Autumn	63	41	65 <sup>bdA</sup>	169	111	65.7 <sup>bA</sup>	232	152	65.5 <sup>b</sup>
Winter	87	66	75.9 <sup>adB</sup>	132	116	87.9 <sup>aA</sup>	219	182	83.1 <sup>a</sup>
Spring	165	134	81.2 <sup>aA</sup>	75	60	80 <sup>aA</sup>	240	194	80.8 <sup>a</sup>
Total	442	302	68.3 <sup>B</sup>	486	371	76.3 <sup>A</sup>	928	673	72.5

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 (2). The relationship between age of examined sheep and prevalence of *Eimeria* species.

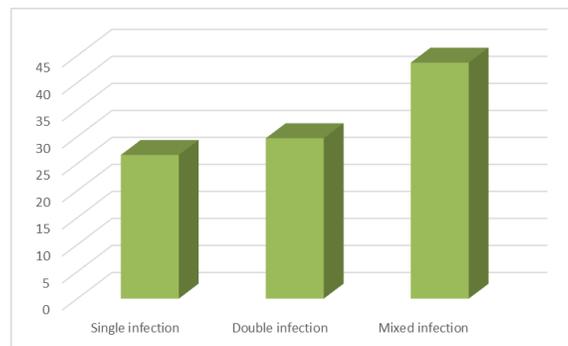
Age	No. of examined sheep	Positive sheep	%
<6 month	260	236	90.8 <sup>a</sup>
6 months - 1 year	364	268	73.6 <sup>b</sup>
> 1 year	304	169	55.6 <sup>c</sup>
Total	928	673	72.5

Table (3): The incidence of *Eimeria* species in different breeds and sexes of examined sheep.

Breed	No. of examined males			No. of examined females			Total		
	No.	Positive	%	No.	Positive	%	No	Positive	%
Baladi	74	39	52.7 <sup>cB</sup>	65	42	64.6 <sup>bA</sup>	139	81	58.3 <sup>c</sup>
Osemy	132	97	73.5 <sup>aA</sup>	152	113	74.3 <sup>bA</sup>	284	210	73.9 <sup>bd</sup>
Rahmany	135	105	77.8 <sup>aA</sup>	189	157	83.1 <sup>aA</sup>	324	262	80.9 <sup>a</sup>
Mixed	101	61	60.4 <sup>bcB</sup>	80	59	73.8 <sup>abA</sup>	181	120	66.3 <sup>cd</sup>
Total	442	302	68.3 <sup>B</sup>	486	371	76.3 <sup>A</sup>	928	673	72.5

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.



Graph (1): Percentage of single and mixed infection with different *Eimeria* sp. Among examined sheep

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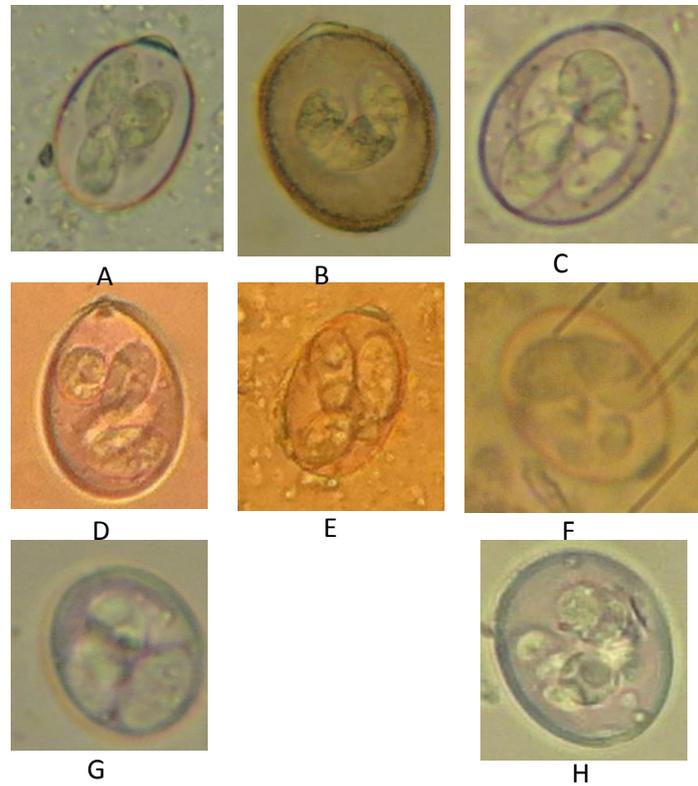
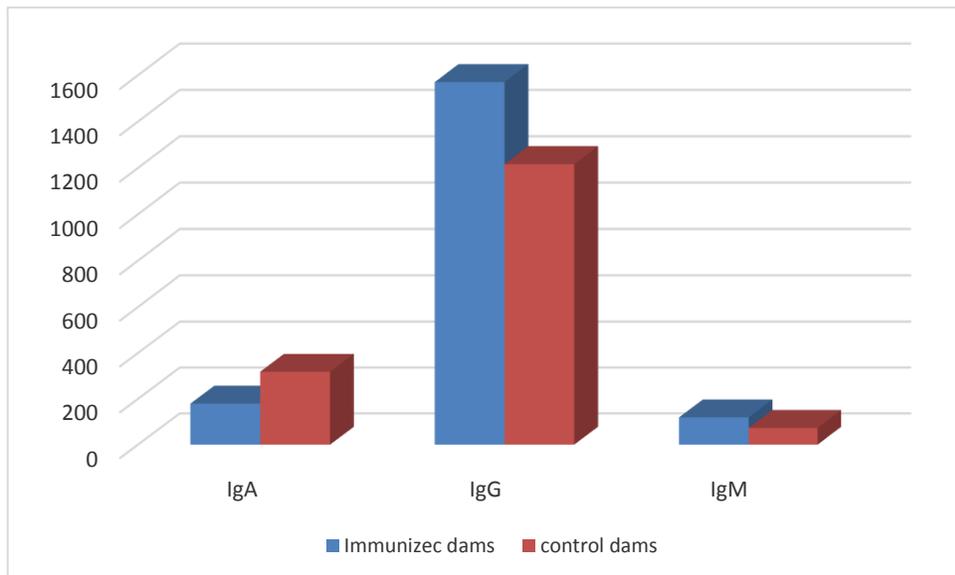


Plate (1): the identified *Eimeria* sp. Among examined sheep.  
 A. *E. ahasta* B. *E. intricata* C. *E. ovinoidalis* D. *E. faurei*  
 E. *E. granulosa* F. *E. crandalis* G. *E. pallida* H. *E. parva*.

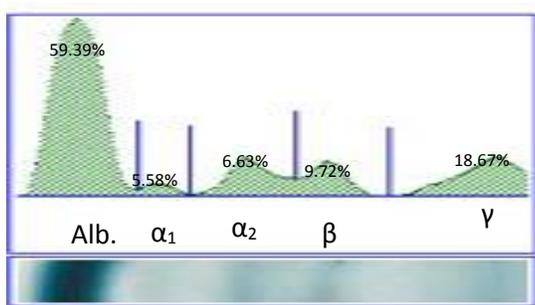


Graph. (2): comparing the levels of different immunoglobulin in one day old lambs delivered from both immunized and non-immunized dams.

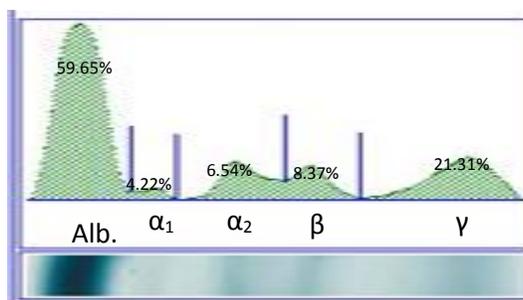
Table (5): Electrophoretic pattern of serum Albumin and different globulins among ewes experimentally inoculated by UV irradiated *Eimeria* oocysts and control negative dams and their progeny.

Fraction	Immunized pregnant dams	Control non-immunized pregnant dams	lambs of Immunized dams	lambs of control dams
Albumin	41.63 ± 0.034 <sup>a</sup>	38.94 ± 0.127 <sup>b</sup>	59.56 ± 0.011 <sup>a</sup>	59.39 ± 0.051 <sup>b</sup>
α <sub>1</sub> globulins	16.74 ± 0.046 <sup>b</sup>	29.09 ± 0.051 <sup>a</sup>	4.22 ± 0.011 <sup>b</sup>	5.58 ± 0.023 <sup>a</sup>
α <sub>2</sub> globulins	5.13 ± 0.063 <sup>b</sup>	25.29 ± 0.051 <sup>a</sup>	6.54 ± 0.017 <sup>a</sup>	6.63 ± 0.023 <sup>a</sup>
β globulins	23.92 ± 0.127 <sup>a</sup>	6.25 ± 0.138 <sup>b</sup>	8.37 ± 0.098 <sup>b</sup>	9.72 ± 0.051 <sup>a</sup>
γ globulins	12.58 ± 0.103 <sup>a</sup>	0.43 ± 0.057 <sup>b</sup>	21.31 ± 0.040 <sup>a</sup>	18.67 ± 0.115 <sup>b</sup>

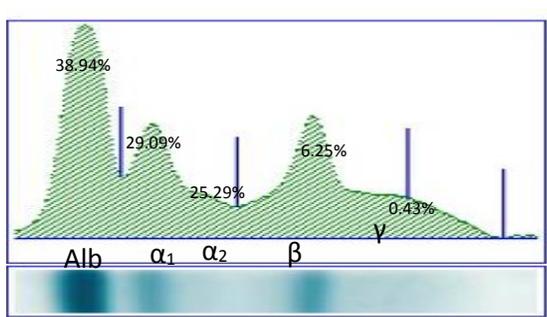
a, b & c: There is no significant difference (P>0.05) between any two means, within the same column have the same superscript letter.



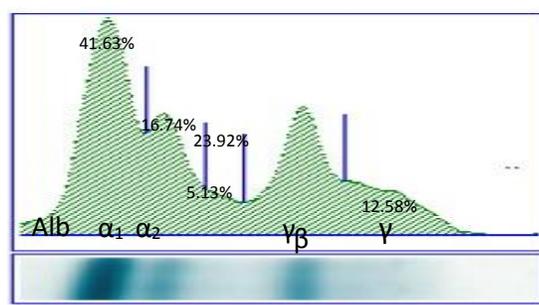
Graph.(3): Electrophoretic profile of lamb serum from control non immunized dams



Graph.(4): Electrophoretic profile of lamb serum from immunized dams



Graph.(5): Electrophoretic profile of serum from control non immunized pregnant dams



Graph.(6): Electrophoretic profile of serum from immunized pregnant dams

#### 4-DISCUSSION:

In the present study, examination of 928 fecal samples from sheep of different sex revealed a prevalence rate of 72.5%. Such result was nearly similar to El-Akabawy (1992), Boshra (1994) in Kaloubia, Nasr et al., (2008) in Sharkia, Bkheet et al. (2010) in Beharia Province and Mahmoud et al. (2018) in Assuit, Egypt where their prevalence was (80.4, 80.67, 76.51, 70, and 65%, respectively). However, these results were higher than those previously reported by Mahran (2009) in Red Sea governorate, Abouzeid et al. (2010) in Sinai governorate, Sultan et al. (2016) in Kafr-Elsheikh and Mohamaden et al., (2018) in Suez, Egypt. Also Sulaiman et al., (2005) in Iraq, Toulah (2007) in Saudi Arabia, Abakar (2010) in Sudan, Majeed et al. (2015) in Kuwait, where their prevalence were (6.61, 6.7, 16.52, 57.7, 60.5, 41, 41.2, and 17.5%, respectively). On the other hand; it was lower than those noted by Bastauerous et al. (2001) in Assuit, Egypt and Ali (2005) in Sudan Where their prevalence were 94.9 and 86% among examined sheep. Such difference may be due to changes in environmental condition.

With regard to the sex, there is a significant difference between sex, in which the prevalence rate was higher in females (76.3%, n=371/486) as compared to males (68.3%, n= 302/442). This result agreed with that noted by El-Akabawy (1992) in Kaloubia who recorded that females were more susceptible to infection (82.3%) than males (78%), and Mohamaden et al., (2018) in Suez, Egypt who recorded that females showed a higher prevalence rate (69.1%) than males (48.4%). On the other hand, this result disagreed with Sulaiman et al., (2005) who observed that

the highest infection rate was in males (58.3%) than females (37.11%), Idris et al. (2012) who observed that male lambs (4.66%) were more susceptible to infection than females (4.15%), and Dabasa et al., (2017) who recorded males more infected (31%) than females (10.4%). While Ali (2005) in Sudan, Nasr et al., (2008) in Sharkia, Yakhchali and Pezaei (2010), Gizachew et al. (2014), Lakew and Seyoum (2016), Yonas and Goa (2017) recorded that the sex of sheep had no significant effect on prevalence of *Eimeria*. Higher incidence in females may be attributed to nature of immune status of females as well as stress caused by pregnancy and lactation.

With respect to the seasonal prevalence of *Eimeria* species among examined sheep, the statistical analysis showed a significant decrease in the prevalence rate of autumn and summer and a higher significant rate in winter and spring. This result agreed with that reported by Ali (2005) in Sudan, who noted that the highest prevalence rate occurred in spring (98%), while the lowest rate was in summer, Nasr et al., (2008) in Sharkia, who noted that the highest prevalence rate was recorded in winter for male and female (78.89% & 90.82%, respectively), followed by spring (71.84%), autumn (66.10%) while the lower infection rate was in summer (52.63%), Mahran (2009) in Red sea governorate, Egypt, who noticed that higher prevalence rate was in winter while the lowest rate was in summer. While this results disagreed with that obtained with El-Akabawy (1992) who reported that, the infection rate was high in autumn (89.3%) followed by winter (82.7%), summer (77.4%) and spring (73.9%), Boshra (1994) Summer had higher prevalence rate (91.04%), followed by

spring (78.57%) and autumn (74.54%) while the lowest prevalence rate was in winter (74.45%), and Mohamaden et al., (2018) in Suez, Egypt, who detected that the highest infection rate was in summer followed by autumn and spring. While the lowest rate was in winter. However, Maingi and Munyua (1994) in Kenya and Kheirandish et al. (2012) in Iran whom reported that there was no significant difference between the seasons

With respect to single infection was recorded in 26.6% (n=179/673) of infected sheep. Double infection rate was in 29.7% (n=200/673) while mixed infection rate was in 43.7% (n=294/673). The present result nearly similar to that recorded by El-Akabawy (1992) in Kaloubia, who investigated that mixed infection with 2-9 different *Eimeria* species was 78.8%, Toulah (2007) in Saudi Arabia, who noticed that mixed infection with multiple infection with three species was 51.22%, Mohamaden et al., (2018) who detected that the mixed infection was 68.3% of the examined sheep in Suez, Egypt. On the other hand, it disagreed with that observed by El-Akabawy (1992) in Kaloubia, who investigated that single infection rate was 1.7% among examined sheep. Boshra (1994) who investigated that Sheep infected with single species was 3.03%, double mixed species was 17.63% while mixed infection with more than two species was 79.33% among examined sheep in different localities in Egypt, Ali (2005) who noted that mixed infection was in 83% of the examined sheep while 17% showed pure infection, Toulah (2007) noticed that mixed infection with two *Eimeria* species was 36.59% while a single species (12.20%) were less common.

Concerning age, the highest infection rate was among ages less than 6 months (90.8%),

followed by 6month-12years of age (73.6%). While the lowest was rate in sheep over 1 year of age (55.6%). This results agreed with that noted by Nasr et al., (2008) in Sharkia, Egypt, who detected that the prevalence rate was higher in young age (38.09%), followed by immature (24.38%), while the lowest prevalence rate was in adult animals (18.30%), Dabasa et al., (2017) found that young age was more susceptible to infection (21.4%) than adult (10.8%), Muhammed et al. (2017) who reported that a higher infection rate was observed in young animals (51.56%) than adult ones (15.53%). On the other hand, it disagreed with that reported by El-Akabawy (1992) in Kaloubia, who investigated that adult sheep 6 – 12 months were highly susceptible to infection (89.2%), followed by those less than 6 months old (79.2%), Abouzeid et al. (2010) in Sinai and Mahmoud et al. (2018) in Assuit, Egypt, who observed that high prevalence rate of *Eimeria* spp. in adult sheep (9.2 and 65.26%) than lambs (8.6 and 63.63%, respectively). While Kheirandish et al. (2012), Gizachew et al. (2014), Rizwan et al. (2017) and Yonas and Goa (2017) observed that there was no significant efficacy of age on the infection rate. Such difference may be attributed to rearing system of sheep or suggesting the development of resistance against *Eimeria* species with a gradual increasing by age.

In regard to breeds, Rahmany and Osemy breed showed the highest infection rate (80.9% and 73.9%; respectively). On the other hand, Baladi breed showed the lowest infection rate (58.3%) The only author discussed the different breed in Egypt was El-Akabawy (1992) in Kaloubia, who reported that a minor difference was noticed among breeds (82%, 80.7%, 79.8% and 74.6% in mixed, Osemy, Rahmany and a falahy breeds respectively). While Biu et al.

(2009), Idris et al. (2012) and Nurzaty Ewani et al. (2014) recorded that no significant difference was observed between breeds in their countries. Lower infection in Baladi breeds may be due to the development of immunity on response to repeated *Eimeria* infection.

The present study showed that immunized dam with UV attenuated *Eimeria* oocysts showed a significant increase in both IgG and IgM during the whole period of experiment compared to non-immunized control group. Similarly, 24 hours post parturition lambs that suckle colostrum from immunized dams revealed a significant increase in IgG and IgM when compared to control lambs which showed a significant increase in IgA. This result agreed with Tizard (1992) who found that all IgG, IgM and IgA of colostrum are derived from serum of dams and transferred to their offspring and Naciri et al. (1994) who reported that hyper immunization of ewes by intramuscular injection with *cryptosporidium parvum* antigen resulted in a significant increase in IgG titer and IgM in serum. Lambs fed with hyper immune ovine colostrum showed high serum IgG antibody titer.

In the present study, immunized dams and their offspring showed high level of albumin, Beta and Gamma globulins concentration levels as compared to control dams and their offspring that showed high level in  $\alpha_1$  and  $\alpha_2$ . This indicated that immunization of pregnant ewes by two doses of UV radiated *Eimeria* oocysts can developed immunity in pregnant ewes and consequently transmitted to their newborn by colostrum which protect them against Eimeriosis in early life. This agree with Akdogan Kaymaz et al., (1999) who observed that there was positive correlation between  $\beta$  globulin and  $\gamma$  globulin in *Eimeria* in dog. Also, there were positive correlation between total protein and albumin

with  $\beta$  globulin, Ghanem et al. (2009) who estimated that globulin showed an elevation in goat as compared to control group Patra et al. (2011) who recorded that an increased in level of total serum globulin especially  $\beta$  and  $\gamma$  globulin in laboratory rats infected with *Eimeria nieschulzi*.

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