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TREATMENT AND PROPHYLAXIS - ORIGINAL PAPER

Oral inoculation of ultraviolet-irradiated *Eimeria* species oocysts protects chickens against coccidiosis

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Abstract

Prevention of coccidiosis is one of the best ways of controlling disease. Therefore, the present study was carried out to evaluate the protective effect of ultraviolet (UV)-irradiated sporulated oocysts of *Eimeria* species against coccidiosis in layer chickens. One hundred forty-four one-day-old layer chicks were randomly divided into 4 groups ($n = 36$), including non-immunized/non-challenged negative control group (NC group), non-immunized/challenged control group (NIC group), non-irradiated sporulated oocyst/challenged group (CA group), and UV-irradiated sporulated oocyst/challenged (UV group). At the age of 4 days, chickens in groups UV and CA were both orally inoculated with 1.0×10^4 UV-irradiated and non-irradiated sporulated oocysts of *Eimeria* species, respectively. Chickens in groups NIC and NC were served as positive and negative controls, respectively. Chickens in all groups were orally challenged with 7.5×10^4 sporulated oocysts of *Eimeria* species except the NC group at the age of 21 days. The results revealed that chicks receiving UV-irradiated sporulated oocysts had no signs of illness with minimal or no changes in the cecal integrity and a significantly lower oocyst shedding (OPG) than in the NIC group. Additionally, the cytokine gene expression profiles were evaluated. Expression levels of IL-2, IL-12, and IFN-γ were significantly higher in the spleen of chicks in the UV and CA groups than in the NC group post-challenge. As expected, treatment with irradiated oocysts resulted in a significant reduction in oocyst shedding and maintenance of cecal mucosal integrity. Furthermore, the body weight was higher in chickens inoculated with UV-irradiated oocysts than their non-irradiated counterparts. In conclusion, our results demonstrate that inoculation with UV-irradiated sporulated oocysts of *Eimeria* species can produce a substantial reduction in infection symptoms.

Keywords *Eimeria* species · Chicks · UV irradiation · Cytokines

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Introduction

The global poultry industry is currently witnessing several challenges, among which is the development of effective vaccines to combat bacterial, viral, and parasitic pathogens that are posing a constant threat to this sector. In the context of enteric infections, coccidiosis is regarded as one of the most devastating diseases that cause substantial economic losses in poultry production. Coccidiosis is a parasitic disease caused by a protozoan parasite belonging to the genus *Eimeria* (El-Ashram and Suo 2017; El-Ashram et al. 2015; Huang et al. 2018). In chickens, *Eimeria* infection is primarily associated with hemorrhagic gastroenteritis, malabsorption, and bloody diarrhea. Furthermore, the mucosal damage caused by *Eimeria* spp. and the subsequent extravasation of protein-rich fluids into the intestinal lumen constitute a major risk factor for acquiring *Clostridium perfringens* infection (Peek and Landman 2011a). Evidence indicates that young chicks are more susceptible to coccidiosis than older ones (Peek and Landman 2011a).

Therefore, prevention of coccidiosis requires induction of immune responses in the early life of chicks. Even though the undeniable success of anticoccidial drugs and other synthetic chemicals in prevention or control of coccidiosis, the alarming rise of antimicrobial resistance and emergence of drug-resistant strains necessitates the need for more efficacious alternative strategies to control avian eimeriosis. Precocious strains have been proven to be effective against *Eimeria* infection (e.g., Paracox 8 and Paracox 5) (Shivaramaiah et al. 2013). However, the production of such a vaccine is very laborious and time-consuming. Additionally, the production of attenuated and wild-type *Eimeria* oocysts requires a large number of chickens. Therefore, a novel anticoccidial vaccine, which is not cost-effective and easy to prepare, is needed (Li et al. 2004; Shirley et al. 2005). Ultraviolet irradiation is a physical, non-thermal method that has been extensively used for inactivating or killing microorganisms, including protozoa, viruses, and bacteria (Cho et al. 2007; Erickson and Ortega 2006; Fino and Kniel 2008; Yaun et al. 2004) by damaging the nucleic acid of organisms, with a loss in the ability to replicate and cause disease. Numerous studies have proven the effectiveness of UV irradiation to inactivate several parasites, such as various stages of *Schistosoma mansoni* (miracidia, sporocysts, and cercariae), *Haemonchus contortus* larvae (Aboelhadid et al. 2013), *Isospora turdi* oocysts, *Toxoplasma gondii* oocysts and tachyzoites (Kannan et al. 2014; Ware et al. 2010; Zhao et al. 2013), and *Cryptosporidium* oocysts (Abbaszadegan et al. 1997; Clancy et al. 1998; Dyksen et al. 1998; Huffman et al. 2000). Moreover, a study in mice demonstrated that immunization with UV-irradiated *Eimeria papillata* sporulated oocysts resulted in a reduction in oocyst output following experimental infection (Al-Quraishy et al. 2011). Similarly, a low-energy electron irradiation (LEEI) was used to protect chickens against cecal coccidiosis (Thabet et al. 2019). Given the remarkable potential of UV to inactivate a wide range of protozoan parasites, the current study was undertaken to evaluate the protective efficacy of UV-irradiated *Eimeria* species oocysts against *Eimeria* infection in chickens.

Materials and methods

Preparation of *Eimeria* oocysts

Mixed species of *Eimeria tenella* (80%), *Eimeria necatrix* (10%), *Eimeria maxima* (5%), and *Eimeria acervulina* (5%) oocysts were used. *Eimeria* spp. were derived from the field isolate and maintained by passaging in coccidia-free chicks ((El-Ashram and Suo 2017; El-Ashram et al. 2015; Huang et al. 2018). Sporulating and sporulated oocysts were counted by the McMaster method.

Exposure of *Eimeria* oocysts to UV irradiation

Sporulating and sporulated *Eimeria* oocysts were added to a Petri dish that contained 25 mL of saline solution (0.9% sodium chloride) and then exposed to UV irradiation at a wavelength of 254 nm (ETS Vilber-Louramat, Marne LaVallee, Cedex, France), as previously described (Allam and Aboel Hadid 2009). After 1 h of exposure, the saline solution was replaced with 2.5% potassium dichromate for the induction of sporulation. The sporulation of the oocysts was observed after 48 h of the incubation. The control-unexposed, oocysts were incubated in 2.5% potassium dichromate for sporulation.

Investigation of the viability of the irradiated sporulating oocysts

The viability of the irradiated sporulating oocysts was examined by oral inoculation of 3.0×10^4 oocysts into chicks ($n = 5$). After 7 days of infection, fecal samples were tested for the presence of the *Eimeria* oocysts.

Chicks and experimental design and challenge

One-day-old Tetra male layer chicks (purchased from Cairo Company Limited for chickens, Egypt) were housed in wire cages on dry, clean wood shavings in the laboratory of the Department of Poultry diseases, Faculty of Veterinary Medicine, Beni-Suef University. Chicks were fed un-

medicated diet commercial starter layer diet, which contains about 20% crude protein and 2900 kcal ME/kg diet ad libitum and monitored for weight gain and general health problems. Then, the chicks were randomly divided into 4 groups (n = 36), including non-immunized/non-challenged negative control group (NC group), non-immunized/challenged control group (NIC group), non-irradiated sporulated oocyst/challenged group (CA group), and UV-irradiated sporulated oocyst/challenged (UV group). At the age of 4 days, chickens in groups UV and CA were both orally inoculated with 1.0×10^4 UV-irradiated and non-irradiated sporulated oocysts of *Eimeria* species, respectively. Chickens in groups NIC and NC served as positive and negative controls, respectively. Chickens in all groups were orally challenged with 7.5×10^4 sporulated oocysts of *Eimeria* species except the NC group at the age of 21 days (Table 1).

Evaluation the protective capacity of irradiated oocyts (clinical and parasitological examination)

Clinical examination of the inoculated chickens was conducted on a daily basis for the presence of mortality and bloody diarrhea. Chicken droppings were collected for parasitological examination and oocyst count at day 7 post-immunization and post-challenge, respectively. Furthermore, lesion score and body weight gain were determined at day 7 post-immunization and post-challenge, respectively (Johnson and Reid 1970).

Evaluation of cytokines gene expression by quantitative real-time PCR

Randomly selected spleen samples were aseptically obtained from 3 chicks per group at 7 days post-immunization and from 6 chicks per group on day 7 post-challenge and preserved at − 20 °C until further use. Total RNA was extracted from the tissue samples using the RNeasy mini kit (QiagenRNeasy Mini Kit, catalog no. 74104, Germany) according to the kit manufacturer's instructions and (El-Ashram et al. 2016). Purified RNA was eluted in 50-μl RNase-free water and stored at − 70 °C. The primer and probe sequences of IFN-γ, IL-2, IL-12, and 28S rRNA are shown in Table 2. TaqMan probes were labeled with the fluorescent reporter dye FAM (6-carboxyfluorescein) at the 5′ end and with the fluorescent quencher dye TAMRA (6-carboxytetramethylrhodamine) at the 3′ end. RT-PCR was performed using the QuantiTect probe RT-PCR kit catalog no. 204443 (Applied Bio-systems, Qiagen, Germany). Amplification and detection of specific products were performed using the Stratagene MX3005P Real-Time PCR System (Applied Bio-systems). Relative expression levels of target genes were calculated using −ΔΔCt method (Abouhajer et al. 2018; El-Ashram et al. 2017b; El-Ashram et al., 2018).

Histopathological examination

Histopathological examination of cecal and splenic tissues was done according to a previously described method (Bancroft et al. 2012; Ding et al. 2011). Small pieces of the cecal tissue were collected in 10% buffered formalin for histopathology. The fixed tissues were washed in running tap water over-night, dehydrated, and infiltrated by paraffin wax. Serial paraffin sections (5-μm thickness) were obtained, and the sections were deparaffinized in three, consecutive washings in xylol for 5 min, and rehydrated with five, successive washings with alcohol in descending order of 100, 95, 80, 70, and 50% in deionized water. The histological sections were then subjected to conventional hematoxylin and eosin (H&E) staining procedure (El-Ashram et al. 2017a. Morphometric analysis of the cecal mucosa and the germinal centers of spleen were performed using Image J analysis software program (NIH, Bethesda, MD). The endogenous stages

Table 1 Experimental design

Day Group	Immunization dose (4-day-old chicks)	Follow-up and sample collection (11-day-old chicks)	Challenge dose (21-day-old chicks)	Follow-up and sample collection (at day 7 post-challenge)
Negative control (non-immunized/ non-challenged, NC)	–	–	–	All chicks were sacrificed humanely
Positive control (non-immunized/ challenged, NIC)	PBS*	–	Orally infected with 7.5×10^4 sporulated oocyts	All chicks were sacrificed humanely
Immunized with UV-untreated sporulated oocysts/challenged (CA)	Immunized with 1.0×10^4 untreated sporulated oocysts	Cervical dislocation and postmortem (PM) investigation ($n = 3$)	Orally infected with 7.5×10^4 sporulated oocyts	All chicks were sacrificed humanely
Immunized with UV-treated sporulated oocysts/challenged (UV)	Immunized with 1.0×10^4 UV-treated sporulated oocysts	Cervical dislocation and PM investigation ($n = 3$)	Orally infected with 7.5×10^4 sporulated oocyts	All chicks were sacrificed humanely

Table 2 Real-time PCR primer sequences and GenBank accession numbers of the targeted genes

Target genes		Primer and TaqMan probe* sequences (5¯–3¯)	Gen bank accession numbers	References
28SrRNA	F	GGCGAAGCCAGAGGAAACT	X59733	Balu et al. (2011)
	R	GACGACCGATTTGCACGTC		
	P	(FAM)-AGGACCGCTACGGACCTCCACCA-(TAMRA)		
IL- 2	F	TTGGAAAATATCAAGAACAAGATTCATC	AJ009800	Kaiser et al. (2000)
	R	TCCCAGGTAACACTGCAGAGTTT		
	P	(FAM)-ACTGAGACCCAGGAGTGCACCCAGC-(TAMRA)		
IFN- γ	F	GTGAAGAAGGTGAAAGATATCATGGA	Y07922	Kaiser et al. (2000)
	R	GCTTTGCGCTGGATTCTCA		
	P	(FAM)-TGGCCAAGCTCCCGATGAACGA-(TAMRA)		
IL-12p35	F	TGGCCGCTGCAAACG	NM213588.1	Balu et al. (2011)
	R	ACCTCTTCAAGGGTGCACTCA		
	P	(FAM)-CCAGCGTCCTCTGCTTCTGCACCTT-(TAMRA)		

* TaqMan probes were labeled with the fluorescent reporter dye FAM at the 5′ end and with the fluorescent quencher dye TAMRA at the 3′ end

of *Eimeria* species were blindly recorded in microscopic fields of cecal tissue at a magnification of × 40. The microscopic scoring of cecal lesions was done according to a previously published method (Gibson-Corley et al. 2013).

Statistical analysis

Data were analyzed using ANOVA and a Tukey multiple range test to determine differences between groups. Results are expressed as means ± SD. Probability values of less than 0.05 ($P < 0.05$) were considered significant.

Results

UV irradiation effect on unsporulated and sporulated *Eimeria* oocysts

The exposure of sporulating oocysts to UV irradiation blocked sporulation. Furthermore, the sporulated oocysts were exposed to a UV irradiation system for 1 h and subsequently used for chicken inoculation.

Protective capacity of UV-irradiated *Eimeria* sporulated oocysts in layer chicks (clinical and parasitological findings)

At day 7 post-inoculation, clinical examinations revealed that chicks immunized with irradiated oocysts were apparently healthy and had no bloody diarrhea. However, chicks inoculated with non-irradiated oocysts suffered from severe bloody diarrhea. With respect to the oocyst counts, the UV group had lower oocyst counts ($2.9 \times 10^3 \pm 1.0 \times 10^2$) compared with the CA group ($2.55 \times 10^4 \pm 5.0 \times 10^2$) (Fig. 1 post-immunization), while the control groups (NC and NIC) had no disease symptoms and did not shed oocysts. Meanwhile, post-challenge findings revealed that there was no mortality in any of the groups after challenge with 7.5×10^4 sporulated oocysts. The challenged chickens had variable clinical signs, such as reduced feed intake, dullness, and bloody diarrhea. Severe bloody diarrhea was observed in the challenged non-treated group (NIC), while chickens that received the irradiated and non-irradiated oocysts showed mild bloody feces. In addition, 10% of the immunized chicks showed bloody contents in the cecum. The fecal oocyst counts in the UV group were significantly lower than in groups CA and NIC. The oocyst count of the CA group was significantly lower than

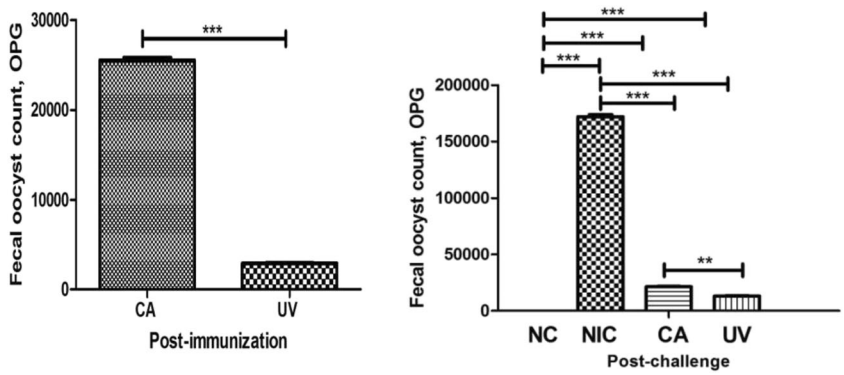

Fig. 1 Fecal oocyst counts in CA and UV groups at day 7 post-immunization and in all groups (including the NC, NIC, CA, and UV) at day 7 post-challenge (***$p < 0.001$ and **$p < 0.01$)

in the NIC group (Fig. 1 post-challenge). Moreover, there were differences in lesion score values in treated groups (CA and UV) which was decreased significantly in the later groups compared with the NIC group (Fig. 2). Additionally, the body weight gain was significantly higher in groups (CA and UV) than in NIC group ($P < 0.05$) (Fig. 3).

Gene expression profiling of pro-inflammatory cytokines following challenge with *Eimeria* species

The expression of pro-inflammatory mediators, including IL-2, IL-12, and INF-γ, which play crucial roles in the initiation of inflammation during *Emieria* infection, was measured. At day 7 post-inoculation, the expression of the cytokines was significantly upregulated in the immunized groups (CA and UV) compared with the control groups. The transcription levels of all cytokines were significantly higher in the CA group than in UV group (Fig. 4 post-immunization), indicating that pre-treatment with irradiated oocysts induces a local protective immunity against *Eimeria* infection without causing tissue damage. Furthermore, at day 7 post-challenge, the expression levels of IL-2, IL-12, and INF-γ in CA and UV groups were significantly higher than those in NC and NIC groups. Importantly, treatment with irradiated oocysts resulted in significantly higher levels of these cytokines compared with the group treated with non-irradiated oocysts (Fig. 4 post-challenge).

Histopathological findings

Gross examination revealed a bilateral cecal core in the group CA chicks, while there were no cecal lesions with the absence of fibronecrotic cecal core in other groups at day 7 post-inoculation. Cecal mucosa of NC chicks appeared with normal

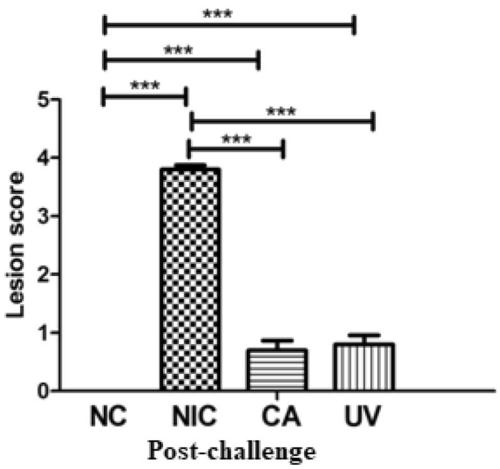

Fig. 2 Lesion scores in all groups, including the NC, NIC, CA, and UV groups at day 7 post-challenge (***$p < 0.001$)

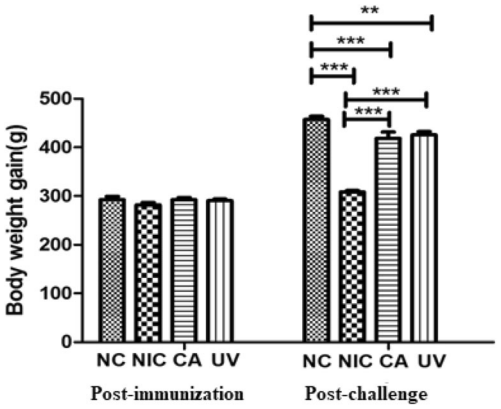

Fig. 3 Body weight gains at day 7 post-immunization; there is no significant difference between different groups. At day 7 post-challenge, the immunized groups (CA and UV) showed significance difference with NIC groups (***$p < 0.001$ and **$p < 0.01$)

crypts at day 11 of age. Submucosa was occupied by lymphoreticular tissue, with less-developed cecal glands (Fig. 5a, b). In the UV group, cecal mucosa appeared more or less normal with marked hyperplasia and few degenerative changes, and the cecal crypts appeared more developed. Furthermore, cecal submucosa had more developed cecal

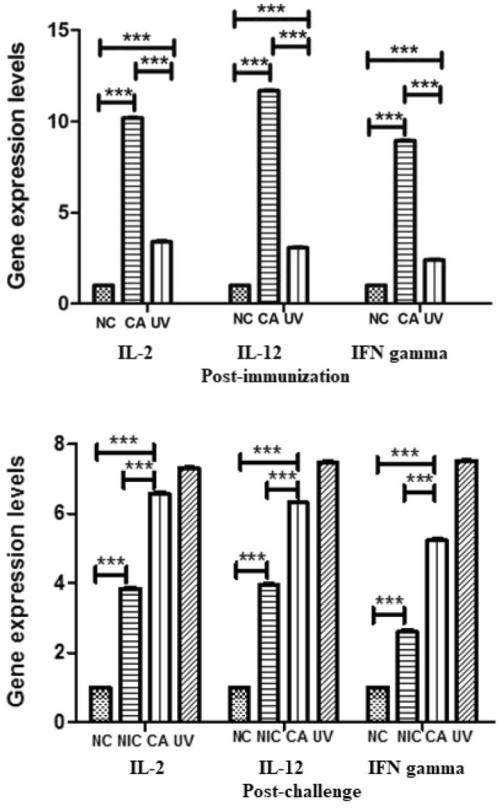

Fig. 4 Gene expression levels of IL-2, IL-12, and IFN gamma at day 7 post-immunization showed that CA group significantly higher levels than other groups. At day 7 post-challenge, UV group had the higher levels of cytokines than other groups (***$p < 0.001$)

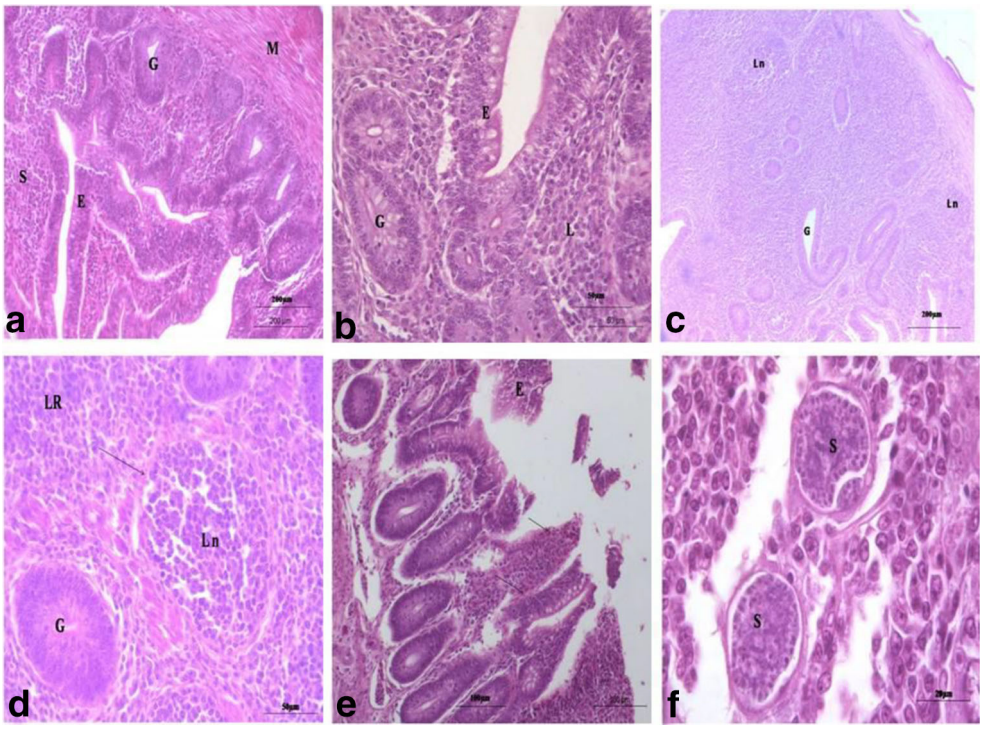

Fig. 5 Histopathological pictures of the cecum in the negative control group (NC) and non-immunized infected control group (NIC). **a**. Cecum in the NC group shows a normal cecal mucosa (E), cecal gland (G), submucosa (S), and muscular layer (M) (H&E staining × 200). **b**. A higher magnification of cecum in the negative control group displays a normal cecal mucosa with crypt (E), well-developed cecal gland (G), and proliferating lymphoreticular tissue (L) (H&E staining × 100). **c**. Cecum in the NC group at day 7 post-challenge shows a normal cecal mucosa, cecal gland (G), and submucosa with well-organized lymphatic nodules (LN) (H&E staining × 200). **d**. Cecal submucosa in the NC group has a normal well-developed cecal gland (G), proliferating lymphoreticular tissue (LR), and well-organized lymphatic nodule (LN) (H&E staining × 100). **e**. A panoramic picture in the cecum of the NIC group at day 7 post-challenge shows sloughed mucosa and hyperplasia in the crypt and cecal gland epithelium. Note congestion and bleeding (arrow) in the cecal submucosa. **f**. Cecal tissue of the NIC group has different parasitic stages (H&E staining × 1000)

glands and well-demarcated cecal tonsils (Fig. 6a). In addition, endogenous stages of *Eimeria* species, which appeared in the cecal tissues, were 5.27 ± 1.67 in the examined field (Fig. 6b), while cecal mucosa of the CA group showed a marked degeneration and mild desquamation with hemorrhage. Cecal crypts were more developed with hyperplastic epithelium. Submucosa had well-developed cecal tonsils and more prominent cecal glands (Fig. 7a). Moreover, different endogenous stages of *Eimeria* species, mainly gametocytes, were observed in the cecal tissue (8.45 ± 2.25 per field Table 3) (Fig. 7b). However, at day 21 of age, cecal submucosa in the NC group had well-demarcated cecal tonsils and highly active cecal glands (Fig. 5c, d). In the NIC group, cecal lumen appeared to contain cecal core, which formed mainly from desquamated epithelial cells, blood cells, mucous, and different enougenous stages of *Eimeria* species. Cecal mucosa showed a severe degeneration and sloughing; however, epithelial cells of intestinal crypts showed a marked stratification. Cecal submucosa displayed hemorrhage and proliferation of submucosal glands, which showed a variable degree of necrosis accompanied with various endogeneous stages of *Eimeria* within epithelial cells or even in the cecal lumen (Fig. 5e, f). In the UV group, cecum appeared to be normal with hyperplasia in crypts and well-developed cecal glands accompanied with hyperplasia in cecal tonsils with very few endogenous stages of *Eimeria* species sequestrated by lymphocytes (Figs. 6c and 2d). However, chicks inoculated with non-irradiated oocysts showed that cecal tissue appeared to have a minimal degeneration and hyperplasia in the cecal epithelia, cecal glands, and cecal tonsils with endogenous stages of *Eimeria* species (Fig. 7c, d).

Histomorphometry of the immune organs and scoring of parasitic stages were reported in Table 3. The parasitic stages in the UV group had the lowest number of endogenous stages of *Eimeria* species post-challenge than in the other groups (2.64 ± 0.690 per field).

Discussion

Eimeria infection in chickens occurs through the ingestion of sporulated oocysts. Upon entry into the digestive tract, the mechanical and biochemical action breaks down the wall of the oocysts, resulting in the release of sporozoites into the intestinal lumen. This process is referred to as excystation

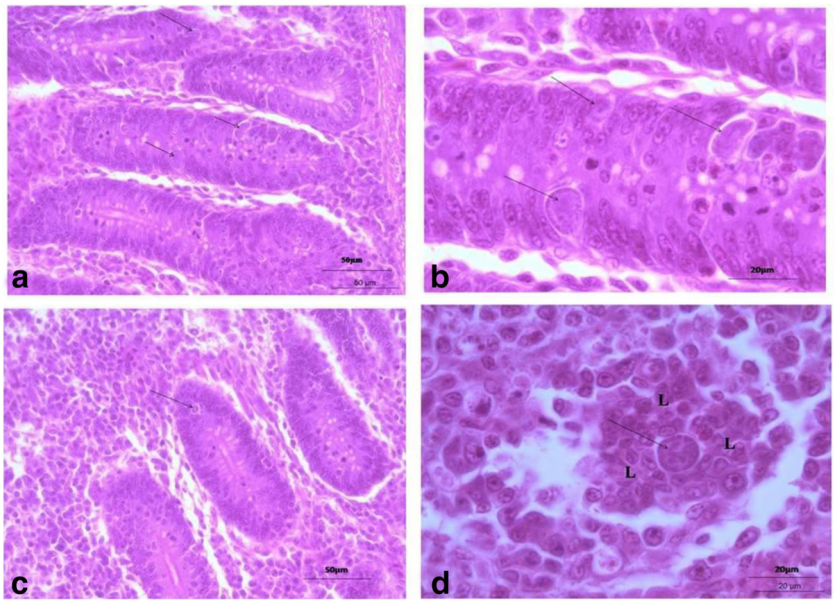

Fig. 6 Histopathological pictures of the cecum in the UV group at day 7 post-immunization and post-challenge. **a**. Cecum in the UV group at day 7 post-immunization shows cecal tissue with proliferative mucosal epithelia and cecal tonsils (H&E staining × 100). **b**. Cecal gland with *Eimeria* stages (H&E staining × 1000). **c**. Intact cecal mucosa in the UV group at day 7 post-challenge displays crypt hyperplasia, proliferating cecal tonsils, and cecal glands with few *Eimeria* stages (arrow). **d**. A higher magnification in the cecal tissue in the UV group at day 7 post-challenge illustrates *Eimeria* stage sequestered with lymphocytes (H&E staining × 1000)

(Thompson et al. 1953). Subsequently, the liberated sporozoites penetrate the epithelial cells of the intestine (Conway and McKenzie 2008), eventually leading to gastrointestinal damage. To date, three methods have been used for the production of live attenuated coccidiosis vaccines, including a selection of genetically stable precocious lines, passage through embryonated chicken eggs, and use of irradiation (Williams 1998; Fetterer et al. 2014a, 2014b). Despite the high cost of production, selection for precociousness is the most commonly used method for production of commercial coccidiosis vaccines (Fetterer et al. 2014a; Peek and Landman 2011b). Given the fact that an ideal anticoccidial vaccine must be cost-effective, thus, an affordable and more efficacious vaccine is needed to protect chickens against *Eimeria* infection.

In the present study, we investigated the ability of UV irradiation to reduce the reproductive potential of *Eimeria* oocysts and the potential of UV-irradiated sporulated oocysts to protect chickens against *Eimeria* infection. Previous studies have shown that exposure of organisms to UV radiation causes distortion of the DNA helical structure (Allam and Aboel Hadid 2009; Kniel et al. 2007; Oguma et al. 2001). For instance, exposure to UV light, at wavelength 200 to 280 nm, causes cross-linking between neighboring pyrimidine nucleoside bases in the complementary nucleic acid strand

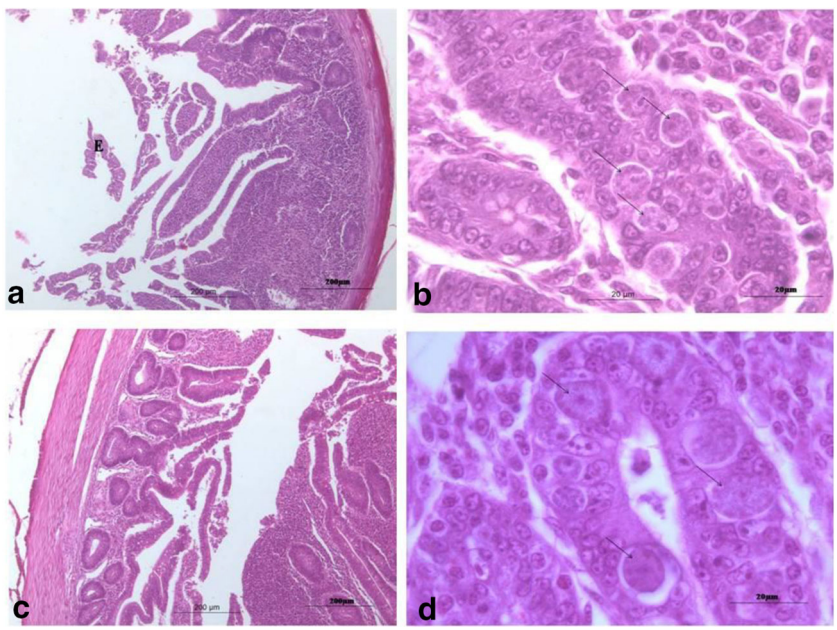

Fig. 7 Histopathological pictures in chicks immunized by non-irradiated sporulated oocysts (CA) at day 7 post-immunization and post-challenge. **a**. Cecal mucosa in the CA group at day 7 post-immunization shows mucosal damage (E) and bleeding (H&E staining × 100). **b**. Numerous coccidian parasites in various stages within the cecal tissue (H&E staining × 1000). **c**. Sloughed cecal mucosa with crypts hyperplasia, proliferating cecal tonsils and cecal glands at day 7 post-challenge (H&E staining × 100). **d**. A higher magnification of the cecal tissue at day 7 post-challenge shows numerous coccidian parasites in various stages within the cecal epithelial tissue (H&E staining × 1000)

Table 3 Histomorphometry of the immune organs and scoring the number of coccidian parasites in various stages and pathological lesions

Group	No of *Eimeria* stages/field		Scoring of pathological lesions			Changes in immune organs		
	At day 7 PI	At day 7 PC	Degenerated cells/field	Sloughed cells/field	Congestion	Thickening of the cecal tonsils (μm)	Size of germinal centers of spleen (μm)	Ratio of germinal center/splenic tissue
NC	0	0	0	0	0	851.23 ± 9.620^{b}	$49{,}498 \pm 1108.9^{a}$	0.021^{a}
NIC	0	17.27 ± 2.97^{a}	4	3	3	68.44 ± 103.31^{b}	$32{,}058 \pm 1864.6^{b}$	0.014^{b}
CA	8.45 ± 2.25^{b}	4.82 ± 1.33^{b}	2	1	2	903.13 ± 77.14^{a}	$58{,}806 \pm 2629.5^{a}$	0.017^{b}
UV	5.27 ± 1.67^{c}	2.64 ± 0.690^{c}	1	1	0	1021.1 ± 34.24^{a}	$40{,}399 \pm 1287.7^{b}$	0.025^{a}
p value	< 0.0001	< 0.0001				< 0.0001	< 0.0001	< 0.0001

[a, b, c,] Means in the same column with different superscripts are significantly different ($p < 0.05$)

PI, post-immunization; *PC*, post-challenge; *NC*, negative control (non-immunized/non-challenged); *NIC*, positive control (non-immunized/challenged); *CA*, immunized by UV-untreated oocysts/challenged; *UV*, immunized by UV-treated sporulated oocysts/challenged

(DNA or RNA), consequently leading to a reduction in the pathogen ability to replicate (*Eimeria acervulina*) (Kniel et al. 2007). The commonly used UV radiation wavelength is 240–290 nm due to its harmful effects on microorganisms (Cadet et al. 2005). Therefore, based on previous work by the authors (2009b), we used wavelength 254 nm.

In general, our results revealed that immunization with UV-irradiated oocysts protected chickens against *Eimeria* infection as evidenced by the absence of clinical signs or symptoms of active *Eimeria* infection. There was no bloody diarrhea, and a significant reduction in oocyst shedding was observed. However, immunization with non-irradiated oocysts resulted in hemorrhagic diarrhea, cecal lesions, dense lymphoeosinophilic infiltration, and large number of different developmental stages of *Eimeria* in the intestinal tissue. These findings are in agreement with an earlier report indicating that early administration of gamma-irradiated *Eimeria* oocyst vaccine protects chickens against coccidiosis (Fetterer et al. 2014b). Immunization of goats with live attenuated oocysts by gamma radiation resulted in ameliorated clinical coccidiosis in goat kids (Ruiz et al. 2014). In addition, UV exposure can completely inhibit *Toxoplasma* tachyzoite replication in mice (Li et al. 2017; Yang et al. 2010). Recently, a low-energy electron irradiation (LEEI) has been used as new promising technology for the production of attenuated *E. tenella* oocyst vaccine (Thabet et al. 2019).

Eimeria infection is associated with a local inflammatory reaction manifested by increased vascular permeability (Rose and Long 2009) and increased production of pro-inflammatory cytokines and chemokines, including tumor necrosis factor-α (TNF-α), IL-1β, IL-6, and CXCLi2 (formerly IL-8) as well as T cell helper-type 1 (Th1) cytokines (Hong et al. 2006a, b). These soluble mediators together with the nitric oxide produced by the innate immune cells contribute to the initiation of inflammation, which in turn provide protective immunity against *Eimeria* infection (Allen 1997a, b; Hong et al. 2006b). Excessive production of these molecules, however, can result in inflammation of the intestinal mucosa.

Oocyst counts in the UV and CA groups at day 7 post-challenge were significantly lower than those in the NIC group. Decreasing oocyst production may be due to increased production of IL-8, IFN-γ, IL-15, TGF-4, and IL-1, which increase host protection against coccidiosis and reduce the intracellular development of *Eimeria* resulting in the reduction of fecal oocyst shedding (Lillehoj et al. 2004; Zhang et al. 2012a). In addition, IFN-γ acts to slow down sporozoite replication in chicken macrophages and fibroblasts by an upregulation of the pro-inflammatory cytokine IL-ip and the CC chemokine K203 (Dimier et al. 1998; Laurent et al. 2001). These mediators in turn enhance cytotoxic and phagocytic activity of macrophages, epithelial cells, and neutrophils. Recombinant chicken IFN-alpha pre-treatment of macrophages inhibits intracellular replication of *E. tenella* (Hériveau et al. 2000) and decreases fecal oocyst shedding (Yun et al. 2000a; c), besides the increased level of IgA+ plasma cells and IgA rich cecal contents have been shown to inhibit sporozoite invasion and development in cell cultures (Davis et al. 1978).

The results revealed that body weight gain was significantly ($P < 0.05$) higher in the UV and CA groups than in the NIC group. These results may be due to an increased level of IFN-γ and lymphotactin post-immunization (Zhang et al. 2012b). Furthermore, a recombinant chicken IFN-γ administered to chickens prior to infection with *E. acervulina* resulted in an increased weight gain relative to controls (Lowenthal et al. 1997). In contrast, body weight gain decreased in the NIC group after challenge with *Eimeria* species oocysts because *Eimeria*-infected chicks must divert energy from growth to fight the infection, thus disrupting body weight gain (Klasing et al. 1987; Wang et al. 2017). The reduced body weight gain of the CA group compared with the UV group may be due to the introduction of a virulent dose, even a low dose (more than 10^3) of *Eimeria* spp. oocyst during the early stage of the live vaccine production or non-irradiated oocysts from environment may cause growth performance impairment (Chapman et al. 2002; Lee et al. 2011; Lehman et al. 2009).

Chicks in the CA and UV groups showed significantly higher levels of the pro-inflammatory mediators, including IL-2, IL-12, and INF-γ for both pre-challenge and post-challenge samples. Additionally, the CA group had higher levels of the pro-inflammatory mediators than in the UV group. Furthermore, in the post-challenge, the CA and UV groups showed significantly higher levels of IL-2, IL-12, and INF-γ than in the NC and NIC groups. Importantly, treatment with irradiated oocysts resulted in significantly higher levels of these cytokines compared with the group treated with non-irradiated oocysts. Interleukin-2 enhances Thl-type responses and acts as a potent growth factor for a variety of cell types, including T cell differentiation, B cell development, and NK cell activation (2009a; Lin et al. 2015), while IL-12 also plays a critical role during *Eimeria* infection by promoting the early production of IFN-γ (Lillehoj and Lillehoj 2000; Lin et al. 2015). Additionally, IFN-γ expression is a common marker of cellular immunity against avian coccidiosis, which is mediated by CD4+ and CD8+ effector lymphocytes (Choi et al. 1999; Lin et al. 2015). High production of IL-12 and IFN-γ had a protective effect (Min and Lillehoj 2002; Zhang et al. 2012b). In the same vein, the results of this study revealed significantly high levels of IFN-γ, IL-2, and IL-12 gene expression at day 7 post-immunization and challenge in the UV-irradiated and non-irradiated oocysts compared with controls. These findings indicate that the high levels of IL-2, IL-12, and IFN-γ have a critical role in protection against coccidiosis (Dalloul and Lillehoj 2005). However, high pro-inflammatory cytokines can induce damage to host tissue (Gazzinelli and Denkers 2006; Zhang et al. 2012b). These results are in close parallel with the results of Hanieh et al. (2012), who reported a significant enhancement of IL-2 mRNA transcripts after the primary as well as the secondary infections with *E. acervulina*, in the spleen and intestine (Lillehoj et al. 2004; Yun et al. 2000b). The histopathological findings in the cecal tissues at day 11 post-inoculation proved that UV irradiation resulted in the reduction of the number of endogenous parasitic stages compared with the CA group. Furthermore, the inoculation of chicks with irradiated oocysts protects them against secondary infection by *Eimeria* species. The most pronounced characteristic of the UV group is the increased lymphocyte population in the cecal tonsils. Several investigations have indicated that lymphocytes in cecal tonsil may be involved in the intestinal immune response to *Eimeria*. In addition, lymphoid nodules were found at the base of cecal tonsils, accumulating as dense aggregates of lymphocytes containing irregularly scattered lymphoid tissues and germinal centers (del Cacho et al. 1993). This study was limited by the absence of a comparison group (i.e., inoculation of chicks with irradiated oocysts without challenge); however, the efficacy of UV-irradiated oocyst vaccine was mainly evaluated by clinical signs, mortality rate, OPG, and lesion scores, which preclude the need for this group.

Conclusion

In this study, UV irradiation at wavelength 254 nm resulted in a significant reduction of the reproductive potential of mix species of *Eimeria* oocysts. Furthermore, irradiated oocysts induced a strong protective immune response against challenge with wild-type *Eimeria* species (i.e., non-irradiated oocysts). However, further studies are needed to study the attenuation process in the context of irradiated oocyst progeny.

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Compliance with ethical standards This research was performed in compliance with the recommendations and guidelines stated by the animal care committee of the Faculty of Veterinary Medicine, Beni-Suef University (BSU 2017).

Conflict of interest The authors declare that they have no conflict of interest.

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