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Research Article
Identifying Intra-Specific Variability in the Virulence of *Eimeria tenella* Using SCAR Markers

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Abstract

Background and Objective: Coccidia are major parasitic pathogens of poultry, with infection characterized by intestinal lesions, blood loss, body weight loss, a poor feed conversion ratio, increased susceptibility to other microorganisms and mortality. The present study was undertaken to identify intra-specific variability in the virulence of *Eimeria tenella* strains using conventional polymerase chain reaction (PCR) and sequence characterized amplified region (SCAR) markers. **Materials and Methods:** A single PCR-based assay was performed to detect and identify *E. tenella* using SCAR markers for four strains that represent four Egyptian governorates (Alexandria, Beheira, Gharbia and Kafr El-Sheikh). The Coccivac B® vaccine was used as a source of *E. tenella* (wild type). **Results:** Sequencing and a phylogenetic tree of all *E. tenella* strains showed 100% identity, except for the Gharbia governorate strain, which exhibited 97.86% identity with the other governorate strains. However, these results did not correlate with an evaluation of strain virulence using 25×10^3 oocysts per chick, as the Alexandria strain was found to be the most virulent (60% mortality and a significant reduction in weight gain). The Beheira strain was the second most virulent strain (33.33% mortality), followed by the Gharbia and Kafr El-Sheikh strains (no mortality). **Conclusion:** These results indicate that the nucleotide variations identified between the Gharbia strain and other strains may occur infrequently or that the portion of the genome under study is not involved in the pathogenicity of *E. tenella*. Furthermore, the SCAR markers used in this study may be species specific (*E. tenella*) and may not reveal intra-specific variations.

Key words: Coccidia, Egypt, *Eimeria tenella*, pathogenicity, poultry, SCAR markers

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Coccidiosis, caused by protozoa of the genus *Eimeria*, is one of the most important parasitic diseases in chickens and is characterized by intestinal lesions, blood loss, body weight loss, a poor feed conversion ratio (FCR), increased susceptibility to other microorganisms and mortality[1]. Seven species of *Eimeria* that infect chickens cause hemorrhagic (*E. tenella*, *E. necatrix* and *E. brunette*) or malabsorptive (*E. acervulina*, *E. maxima*, *E. mitis* and *E. praecox*) coccidiosis, with absolute host and tissue specificity[2]. Moreover, during the course of natural infection, chickens may be infected by several species of *Eimeria* at the same time[3]. *Eimeria tenella* is considered to be the most economically damaging, ubiquitous and pathogenic *Eimeria* species in chickens[4]. Diagnosis of coccidiosis is based on comparison of clinical signs, intestinal pathology in the host, the morphology of different parasite stages in fecal material or the intestine and the pre-patent period[5]. Recently, *Eimeria* species have been identified by several polymerase chain reaction (PCR)-based assays targeting different regions of the genome, including the 5S rRNA, small subunit rRNA[6], sporozoite antigen gene EASZ240/160[7], internal transcribed spacer-1 (ITS-1)[8] and ITS-2[9]. Control of this disease through anticoccidial drugs[3] presents some disadvantages, such as drug resistance[10] and public concern due to chemical residues in foodstuffs[11]. Alternative means for controlling coccidiosis diseases include vaccination of birds using live wild or attenuated vaccines[12], though this method is hindered by live parasite complexity and production constraints. In addition, antigenic diversity is observed among some isolates, such as *E. tenella*, *E. maxima* and *E. acervulina*, from different geographical regions worldwide[13], which in turn results in variability in pathogenicity and the host-pathogen relationship[14]. Although it is known that *Eimeria* species and/or strains can vary in pathogenicity, practical methods to identify *Eimeria* isolates according to their virulence are lacking yet invaluable[15]. Indeed, no antigenic marker to date has been shown to strictly correlate with *Eimeria* pathogenicity. Shirley[16] and Morris and Gasser[17] stressed that identification of the genetic variation in *Eimeria* species is vital for understanding the pathogenicity and epidemiology of chicken coccidiosis. In addition, assessment of the virulence and pathogenicity of various field *Eimeria* isolates is very important for formulating effective control strategies[5]. Therefore, the aim of the present study was to verify the virulence of field Egyptian *E. tenella* strains using conventional PCR and sequence characterized amplified region (SCAR) markers in comparison with *in vivo* studies.

MATERIALS AND METHODS

Parasites: Four *E. tenella* strains were isolated from ceca of naturally infected broiler flocks from four Egyptian governorates (Alexandria, Beheira, Gharbia and Kafr El-Sheikh)[18]. Coccivac B® (a wild-type vaccine used as a source of *E. tenella*) is the commercial vaccine used in Egypt. Each isolate was separately induced to sporulate (via forced aeration with continuous mixing using a magnetic stirrer) with 2.5% potassium dichromate for 2-3 days. The materials used for the processing, collection, isolation and storage of oocysts were sterilized by autoclaving or treatment with boiling water. Pure strains of *E. tenella* were obtained by single oocyst isolation from four field isolates (Alexandria, Beheira, Gharbia and Kafr El-Sheikh) by the agar plate method according to Su *et al.*[8]. Seven-day-old broiler chicks (Cobb) were kept free from coccidiosis by collecting them individually at 1 day of age and housing them in purpose-built metal cages (30×22×22 cm) (1 chick/cage) under these conditions until the end of the experiment[8]. Chicks were fasted for 2 h before inoculation. Food (commercial broiler starter feed, 23% protein, free from anticoccidial drugs and antibiotics) and water were supplied ad libitum. Each inoculated chick/isolate was examined separately for oocysts in the feces by the flotation technique beginning from the 5th day post-infection (P.I). The chicks produced oocysts. Feces and cecal content were collected on waterproof paper after euthanasia on the 10th day P.I. for purification and sporulation. Sporulated oocysts of each strain were propagated in two-week-old coccidium-free broiler (Cobb) chicks in wire-floored battery cages. A dose of 6×10^3 sp. oocysts/chick was inoculated directly into the crop to obtain a sufficient amount for DNA extraction and further *in vivo* evaluation of the virulence of *E. tenella* strains.

DNA extraction from pure *Eimeria* oocysts: Sporulated oocysts (15×10^6) were crushed via the glass bead grinding method described by Hnida and Duszynski[19] using glass beads 0.75-1 mm in diameter that were sterilized with Clorel® (sodium hypochlorite, less than 5%and sodium hydroxide, less than 5%) at 4°C for 10 min. DNA was extracted using the phenol/chloroform extraction method followed by ethanol precipitation[20].

PCR amplification: The forward and reverse SCAR markers for *E. tenella* were 5′-CCGCCCAAACCAGGTGTCACG -3′ and 5′-CCGCCCAAACATGCAAGATGGC-3′, respectively. These primers were used for amplification of the target DNA fragment of *E. tenella* with an expected amplicon size of

539 bp[21]. PCR reactions were performed in a 50 µL reaction volume containing 5 µL genomic DNA, 5 µL 10x buffer, 1 µL of dNTPs mix, 1 µL of each primer (10 pmol), 1 µL of Taq polymerase (5 units μL^{-1}) and 36 µL of ddH_2O. The reaction mixture was placed in a thermal cycler (Techne, Tc-3000, California, USA)and the amplification program was as follows: step 1 (1 cycle), 95°C for 5 min; step 2 (35 cycles), 94°C for 1 min, 64°C for 1 min and 72°C for 1 min; step 3, 72°C for 10 min. The PCR products were stored at -20°C until needed.

Electrophoresis of amplified PCR products: Eight microliters of each PCR product was mixed with 2 µL of gel loading buffer and electrophoresed through a 2% agarose gel with ethidium bromide at 140 V for 45 min. A 100-bp DNA ladder was used as a size marker and bands were visualized on a UV transilluminator and photographed using a gel documentation system (gel Doc. Alpha-chem. Umager, USA). DNA sequencing, data analysis and phylogenetic tree construction: Sequencing was performed for all five samples (Alexandria, Beheira, Gharbia and Kafr El-Sheikh strains and Coccivac B®). The PCR products were sent to Macrogen Company (South Korea) for sequencing in two directions using an ABI 3730XL DNA sequencer (Applied Biosystems, USA). Sequence comparisons were performed using the BLAST program from the National Center for Biotechnology Information website http://www.ncbi.nlm.nih.gov/. Sequences were aligned using CLUSTALW version 2.1[22]. A neighbor-joining tree was constructed using MEGA version-6 [23].

***In vivo* evaluation of the virulence of *E. tenella* strains:** One hundred and eighty commercial broiler chicks (Cobb) were used in two experiments. The first experiment measured the virulence of the Alexandria and Beheira strains and the second experiment measured the virulence of the Gharbia and Kafr El-Sheikh strains. Each experiment included a non-infected control group. All birds were maintained coccidium-free from 1 day of age without the use of anticoccidial drugs and were housed in cleaned and sanitized wire-floored battery cages with continuous illumination. Food and water were available ad libitum. All experimental groups were ensured to be free from coccidia by fecal examination through flotation in saturated NaCl on three successive days before infection. On the 14th day, inoculation was performed directly into the crop with 25×10^3 sporulated oocysts of *E. tenella* prepared in 1 mL of distilled water.

Parameters: Body weight gain and feed intake were determined weekly; the FCR was calculated, where FCR = total feed consumption by the birds in a cage divided by the weight gain of surviving birds+weight gain of dead birds in the cage. Lesion scores on the 6th day P.I.[24], the oocyst index[25], coccidiosis-induced mortality and daily oocyst counts from the 7th-13th day P.I. were calculated using McMaster's technique[26].

Statistical analysis: Analysis of variance (one-way ANOVA) for collected data was performed using Statistical Analysis System[27].

RESULTS

PCR amplification of *E. tenella* strains and DNA sequencing: Amplification of the target DNA fragment of *E. tenella* using the SCAR marker sequence produced the expected amplicon size of 539 bp, confirming that the five samples were *E. tenella* (Fig. 1). Single-nucleotide polymorphisms (SNPs) showed nucleotide sequence variation among the strains, especially the Gharbia strain (10 nucleotides) (Table 1).

Similarity coefficient and dendrogram: The similarity coefficient among the Alexandria, Beheira, Kafr El-Sheikh and Coccivac B® strains was 100% but it was 97.86% for the Gharbia strain. Identical results were observed for the Gharbia strain when comparing the sequences of the studied *E. tenella* strains with similar sequences in GenBank; the sequence identity of our isolates with two accessions ranged from 96-99% in the forward or reverse direction. However, the Gharbia strain showed only 94% identity with gb|FJ515794.1| and 93% identity with gb|AY571634.1| (Table 2). The

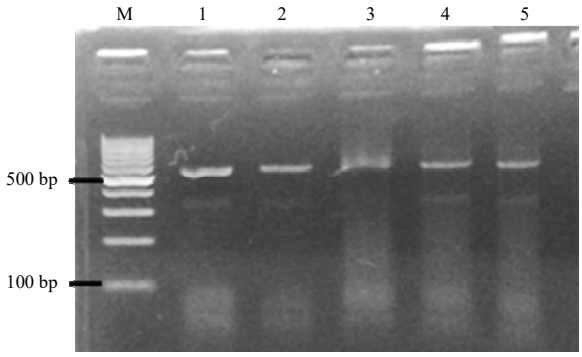

Fig. 1: Ethidium bromide stained agarose gel (2%) of PCR products representing amplification of *Eimeria tenella* genomic DNA with band size 539-bp. M: DNA molecular weight marker 100 bp, 1-5: *Eimeria tenella* strains

Table 1: *Eimeria tenella* strains and nucleotide sequence variation by single nucleotide polymorphisms (SNPs)

	Nucleotide No.									
Eimeria tenella strain	213	249	293	313	374	389	409	415	418	422
Alexandria	G	G	-	C	C	T	T	C	C	C
Beheira	G	G	-	C	C	T	T	C	C	C
Kafr Elsheikh	G	G	-	C	C	T	T	C	C	C
Coccivac B®	G	G	-	C	C	T	T	C	C	C
Gharbia	A	C	C	G	T	G	G	G	T	G

Table 2: Similarity coefficient among five *Eimeria tenella* strains

E. tenella strain	Alexandria	Beheira	Kafr Elsheikh	Coccivac B®	Gharbia
Alex	-	-	-	-	-
Beheira	100.00	-	-	-	-
Kafr Elsheikh	100.00	100.00	-	-	-
Coccivac B®	100.00	100.00	100.00	-	-
Gharbia	97.86	97.86	97.86	97.86	-

Table 3: Dynamic of changes in performance and parasitological parameters vs negative control group in Exp.1 (evaluation of the virulence of Alexandria and Beheira strains of *Eimeria tenella*) (results are expressed as Mean±SE)

Parameters	Age	A	B	C
Weight gain (g±S.E)	0-7 days P.I.	349.95±17.82^{a}	223.30±15.92^{b}	264.11±15.32^{b}
Feed Intake (g±S.E)		417.00±21.07^{a}	428.33±36.12^{a}	395.67±1.45^{a}
FCR		1.19±0.005^{b}	1.94±0.196^{a}	1.51±0.081^{b}
Weight gain (g±S.E)	7-14 days P.I.	408.44±0.79^{b}	327.90±17.33^{b}	369.94±16.28^{b}
Feed Intake (g S.E)		548.67±32.52^{a}	710.00±36.79^{a}	636.67±29.04^{a}
FCR		1.34±0.075^{b}	2.15±0.34^{a}	1.72±0.04^{ab}
Lesion score	6th day P.I.	0.00±0.00^{b}	4.00±0.00^{a}	4.00±0.00^{a}
Oocyst index		0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}
Mortality (%)		0	60	33.33

A: Non-infected group, B: infected group with Alexandria strain, C: infected group with Beheira strain. P.I.: post infection. Groups within the same row with the same superscription letters show no significant differences ($p \leq 0.05$)

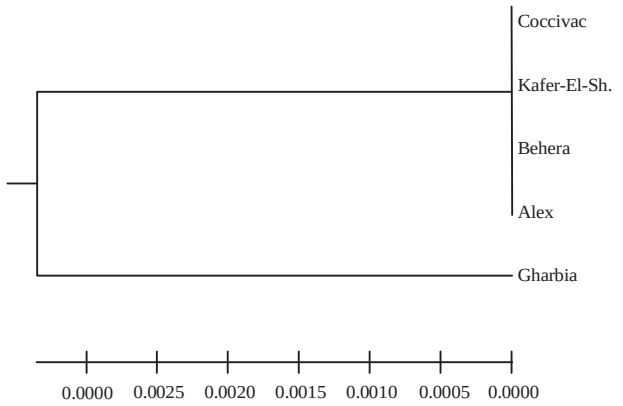

Fig. 2: Dendrogram (phylogenetic tree) based on sequence of *Eimeria tenella* strains using SCAR markers

dendrogram (phylogenetic tree) based on the sequences of the five *E. tenella* strains using SCAR markers produced two main clusters. The first cluster included Coccivac B®, Alexandria, Beheira and Kafr El-Sheikh and the second cluster included the Gharbia strain (Fig. 2). Consequently, the first cluster was listed under GenBank accession number gb|KT985454| with the name the Coccivac B® vaccine strain; the Gharbia strain was listed under accession number gb|KT985455|.

Virulence of four Egyptian strains of *E. tenella*: The Alexandria and Beheira isolates of *E. tenella* (Exp. 1) caused a statistically significant decrease in weight gain during the 1st and 2nd week P.I. compared to uninfected controls (Table 3). The Gharbia and Kafr El-Sheikh isolates (Exp. 2) had the same effect but only in the 1st week P.I., followed by compensatory growth during the 2nd week P.I. (Table 5). However, feed intake was not significantly reduced in the groups infected with any of the isolates in Exp. 1 and 2 (Table 3-5).

Regarding FCRs, chicks infected with the Alexandria isolate of *E. tenella* had markedly poorer (higher) FCRs at 1 and 2 weeks after infection than uninfected chicks and FCR was significantly higher than that of chicks infected with the Beheira strain only during the 1st week P.I. In contrast, infection with the Beheira isolate resulted in a higher FCR but the difference was not significant compared to uninfected chicks at two weeks P.I. (Table 3).

Table 4: Oocyst count $\times 10^3$ g^{-1} feces from 7th-13th day P.I. on Exp.1 (evaluation of the virulence of Alexandria and Beheira strains of *Eimeria tenella*)

Group	7th day P.I.	8th day P.I.	9th day P.I.	10th day P.I.	11th day P.I.	12th day P.I.	13th day P.I.
A	0.00±0.00^b	0.00±0.00^b	0.00±0.00^b	0.00±0.00^b	0.00±0.00^b	0.00±0.00^b	0.00±0.00^a
B	549.54±0.35^a	213.79±0.49^a	100.00±0.43^a	21.38±0.15^b	31.62±0.58^a	16.98±0.81^{ab}	19.49±0.76^a
C	1288.25±0.62^a	117.49±0.57^a	151.36±0.69^a	8.13±0.17^c	38.90±0.39^a	25.12±0.18^a	7.94±0.32^a

A: Non-infected group, B: infected group with Alexandria strain, C: infected group with Beheira strain. Groups within the same column with the same superscription letters show no significant differences ($p \leq 0.05$)

Table 5: Dynamic of changes in performance and parasitological parameters vs negative control group in Exp.2 (evaluation of the virulence of Gharbia and Kafr El-Sheikh strains of *Eimeria tenella*) (results are expressed as Mean±SE)

Parameters	Age	D	E	F
Weight gain (g±S.E)	0-7 days P.I.	452.67±10.35^a	362.67±17.7^b	378.67±13.84^b
Feed Intake (g S.E)		378.33±26.17^b	458.33±1.67^{ab}	463.33±1.2^{ab}
FCR		0.84±0.07^b	1.27±0.07^a	1.23±0.04^a
Weight gain (g±S.E)	7-14 days P.I.	586.65±78.53^a	503.00±16.37^a	552.67±17.89^a
Feed intake (g S.E)		933.33±35.43^a	896.33±20.33^a	974.00±30.82^a
FCR		1.61±0.11^a	1.79±0.09^a	1.76±0.02^a
Lesion score	6th day P.I.	0.00±0.00^b	2.33±0.33^a	2.50±0.5^a
Oocyst index		0.00±0.00^b	1.00±0.00^{ab}	3.00±2.00^a
Mortality (%)		0	0	0

D: Negative control group; E: Infected with Gharbia strain, F: infected with Kafr El-Sheikh strain. Groups within the same row with the same superscription letters show no significant differences ($p \leq 0.05$)

Table 6: Oocyst count $\times 10^3$ g^{-1} feces from 7th-13th day P.I. on Exp. 2 (evaluation of the virulence of Gharbia and Kafr El-Sheikh strains of *Eimeria tenella*)

Group	7th day P.I.	8th day P.I.	9th day P.I.	10th day P.I.	11th day P.I.	12th day P.I.	13th day P.I.
D	0.00±0.00^b	0.00±0.00^b	0.00±0.00^b	0.00±0.00^b	0.00±0.00^b	0.00±0.00^a	0.00±0.00^a
E	831.80±0.14^a	128.80±0.75^a	61.66±0.58^a	50.12±0.68^a	2.63±0.67^b	1.82±0.26^a	5.62±0.98^a
F	363.10±0.49^a	74.13±0.09^a	45.71±0.56^a	99.31±0.39^a	10.23±0.83^{ab}	0.00±0.00^a	1.58±0.31^a

D: Non-infected group, E: infected group with Gharbia strain, C: infected group with Kafr El-sheikh strain. Groups within the same column with the same superscription letters show no significant differences ($p \leq 0.05$)

Groups infected with either the Gharbia or Kafr El-Sheikh *E. tenella* isolates had markedly poor FCRs during the 1st week P.I. relative to uninfected chicks. In the 2nd week P.I., differences between the FCRs of infected and uninfected chicks were much smaller and non-significant (Table 5).

Both the Alexandria and Beheira isolates resulted in non-statistically significant differences in lesion scores (4±00)and the oocyst index (number of oocysts in cecal droppings on the 6th day P.I.) was zero. Nonetheless, the mortality rates were 60 and 33.33%, respectively (Table 3). The Gharbia and Kafr El-Sheikh isolates produced moderate lesions, with no significant differences between them. The oocyst index was higher with the Kafr El-Sheikh strain than the Gharbia strain but with no significant difference. No mortality was recorded for any treatment with either strain (Table 5).

Differences in mean daily oocyst production for the Alexandria or Beheira isolates were not significant, except on day 10 P.I. (Table 4). The same was true for the Gharbia and Kafr El-Sheikh strains; no statistically significant difference in oocyst count was observed between the infected and uninfected groups, except on the 7th, 8th and 10th days P.I. (Table 6).

DISCUSSION

Cantacessi *et al.*[28] and Schwarz *et al.*[29] stated that cloning and sequencing methods provide new methods of examining genetic diversity among *Eimeria* spp. infecting chickens'. In addition, Brisse *et al.*[30] first demonstrated the feasibility of utilizing SCAR markers for discriminating genetic sub-classifications of a unicellular parasite and this study was performed to prove the success or failure of PCR-based assays and sequencing using SCAR markers to identify the virulence of *E. tenella* strains. In the current study, the data obtained by conventional PCR showed that all five strains under study were in fact *E. tenella*. Furthermore, matching the sequences of these isolates (forward or reverse direction) with those in GenBank revealed a high percentage of identity, ranging from 93-99%. The similarity coefficient among the isolates was 100%, even when the vaccinal strain was included. The exception was the Gharbia strain, which differed from all other isolates in the selected sequences by 2.14%. This difference in the Gharbia strain was explained by SNPs that occurred at 10 positions (nucleotides 213-422). Shirley and Bumstead[31] also identified only a 2% difference between *E. tenella* isolates using the random amplification of polymorphic DNA

(RAPD) technique. However, Procunier *et al*.[32] considered that this very low level of variability highlights the advantages of RAPD for identifying differences between strains. Therefore, we first addressed whether this percent difference for the Gharbia isolate affects its virulence compared to other isolates. We next sought to determine whether the isolates showing 100% identity with the vaccinal strain (Alexandria, Beheira and Kafr El-Sheikh) are in fact the vaccinal strain or if this amplified fragment of DNA is simply shared (species-specific). El-Nahas *et al*.[33] reported a 70.6% similarity coefficient for five isolates of *E. tenella* with nearly the same geographical distribution as in our study (Marsa Matruh, Alexandria, Beheira, Kafr El-Sheikh and Gharbia) by RAPD PCR. This genetic variation coincides with variations in their pathogenicity[33]. Awad *et al*.[34] reported 78-96% sequence similarity for ITS-1 between five *E. tenella* isolates and Coccivac B®. However, these similarity values were not related to the protection conferred by the vaccine against the five field isolates. Therefore, a battery trial was performed to assess the virulence of these regional E. *tenella* strains (Alexandria, Beheira, Gharbia and Kafr El-Sheikh) using a dose of 25×10^3 sporulated oocysts/chick. Conway *et al*.[35] stated that "…body weight gain normally is not affected or is only slightly reduced in mild infection, while in severe infections weight gains may be as much as 40-60% lower than non-infected control birds". However, reductions in weight gain at 7 days P.I. by the Alexandria and Beheira isolates were 36.2 and 24.5%, respectively and those caused by the Gharbia and Kafr El-Sheikh strains were 19.9 and 16.3%, respectively, relative to the non-infected control group. This finding may indicate that the dose did not cause a severe infection, even though the dose did result in mortality rates of 60 and 33.3% for the Alexandria and Beheira strains, respectively. This idea was explained by Shumard and Callendar[36], who reported a poor correlation between mortality rates and weight gain in young birds. A different range of mortality was also obtained for various *E. tenella* strains by Abu-Akkada and Awad[37], with results indicating that mortality is strongly influenced by the pathogenicity and virulence of each isolate. Although feed intake was not significantly different among the groups, the birds infected with the Alexandria, Gharbia and Kafr El-Sheikh isolates had poorer FCRs. This growth depression may have been due to the cecal lesions caused by *E. tenella* and the subsequent malabsorption of nutrients, anorexia and listlessness[38]. Nonetheless, the lesion score was assessed on the 6th day P.I., a time when mortality had occurred with the Alexandria and Beheira isolates and the lesion score and oocyst index were assessed in dead birds. Therefore, the lesion score was 4.00 ± 0.00 and the oocyst index zero for both isolates because no oocyst production occurred on the 6th day P.I. (Schizont rupture). The Gharbia and Kafr El-Sheikh isolates resulted in lower lesion scores of 2.33 and 2.5, respectively (as assessed in euthanized birds). It was obvious that the lesion score was related to weight gain, as described previously by Long *et al*.[39], who observed a significant weight depression in susceptible broiler birds with lesion scores of 3 or 4 at 6 days P.I. with *E. tenella*. However, Conway *et al*.[35] reported that lesion scoring does not fully reflect the degree of disease severity in induced infection. Williams[40] reported no relationship between the virulence and fecundity of strains of *Eimeria*, which explained the lack of significant differences in oocyst counts between chicks of different groups. Based on these data, it was obvious that the Gharbia and Kafr El-Sheikh strains had lower virulence, even though they were isolated from cases of clinical coccidiosis with bloody diarrhea, high mortality, a cecal core and a sausage-like appearance of the ceca. The findings may explain why the occurrence and severity of avian coccidiosis are influenced by many other factors, such as starvation, immunosuppression, age and genetic background of the birds. In addition, Warren[41] showed that dietary vitamins have an effect on the degree of *E. tenella* pathogenicity. Furthermore, the studied strains were obtained from single oocysts and may have inherently had lower virulence or may have been attenuated with coccidia vaccines such as Livacox®. Egyptian farms, especially broiler farms, do not use these vaccines due to poor biosecurity. Therefore, the ranking of *E. tenella* isolates according to virulence from high to low is as follows: Alexandria, Beheira, Gharbia and Kafr El-Sheikh. The Alexandria strain appeared to be the most virulent, the Kafr El-Sheikh strain had the least virulence and the Beheira isolate was between these two. Additionally, Coccivac B® is known to be pathogenic; sequence identity between it and the other isolates was 100% according to amplification and sequencing of the selected part of the genome. Moreover, sequence identity for the Gharbia isolate with all other studied isolates was 97.86%, though it was found to be similar to the Kafr El-Sheikh isolate in virulence. This may indicate that the nucleotide variations identified between the Gharbia isolate and other isolates may be silent or that the portion of the genome under study contains repetitive DNA and is not involved in the pathogenicity. Overall, further study is required to investigate the effect of this variation in the protection conferred by the Coccivac B® vaccine against these strains.

SIGNIFICANCE STATEMENT

This study found that nucleotide variations between the Gharbia strain and other strains (Alexandria, Beheira and Kafr El-Sheikh) may be silent or that this part of the genome

contains repetitive DNA that is not involved in their pathogenicity. In addition, intra-specific variability in the virulence of *Eimeria tenella* strains using conventional PCR and SCAR markers may be beneficial for the diagnosis and control of coccidia infections. This study will help future researchers to uncover new and critical information with relevance to improving diagnosis and control measures for protozoal infections, with development of a new treatment strategy for coccidia.

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