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Prevalence and Associated Risk Factors of *Eimeria* spp. Infection in Goats at Northern and Southern Egypt

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ABSTRACT

This study was conducted to explore the prevalence of Eimeria spp. infection in goats in northern and southern Egypt in Behera, Menofia, Assiut, Sohag governorates through morphological identification. and to elucidate the associated risk factors for infection with Eimeria in goats. The overall prevalence was (40.63%). Four species of Eimeria viz., E. caprovina (14.84%) followed by E. arloingi (9.36%), then E. hirci (8.89%) the lowest infection rate was E. caprina (7.53%) were identified. Young kids were more susceptible (65.32%) compared to adults (15.76). Infection was during the winter cold season (74.92%) than Autumn (18.12%), and Summer (15.11%). On the other hand; locality did not significantly affect the infection rate. Goats reared on free-range grazing system in opened areas was more susceptible to Eimeria spp. infection (45.08%) compared to those housed in pens. (36.16%).

Keywords: *Eimeria* spp., Prevalence, Goats, Risk factors, Egypt.

INTRODUCTION

Goats are one of the most important domestic animals worldwide especially in tropical production systems, and they are a major source of cash income and food protein for rural farmers, because of low input requirements such as small initial capital, fewer resources, and maintenance costs [1]. They also produce milk and meat in usable quantities using marginal lands, poor pastures, and/or crop residues. Furthermore, only short periods are needed to rebuild flocks after the disaster [2]. Goats can be infected by numerous internal parasites including the protozoan *Eimeria* [3].

Caprine coccidiosis caused by protozoa of the genus *Eimeria* is one of the foremost parasitic diseases influencing the goat industry in many parts of the planet [4]. Thirteen *Eimeria* spp. are known to infect goats, of which nine species are commonly identified supported oocyst morphology and predilection site [5-6]. Infection of goats with *Eimeria* spp. occurs through the ingestion of sporulated oocysts; within the bowel sporulated oocysts release sporozoites that infect intestinal epithelial cells [7].

The most common signs of infection are diarrhea with or without mucus or blood, dehydration, emaciation, weakness, anorexia, and death. Some goats show constipation and die acutely without diarrhea [8]. The disease may occur under stress factors such as weaning, dietary changes, inclement weather, or travel and regrouping [9].

Diagnosis of *Eimeria* spp. infection is based on history, age, postmortem lesions, and fecal examination for oocysts, the latter

may be present in very large numbers in both healthy and diseased animals so that postmortem or oocyst differentiation is advisable [6]. Coccidiosis is considered to be one of the most economically important diseases of intensively-reared goats worldwide [10]. Therefore, the objective of this study was to determine the prevalence and associated risk factors of *Eimeria* spp. infection in northern and southern Egypt, including animal's sex, age, seasonal variations, locality, as well as breeding system.

MATERIALS AND METHODS

Ethical considerations

The study protocol was carefully reviewed and approved by the local guidance of Research, Publication, and Ethics of the Faculty of Veterinary Medicine, Sohag University, Egypt, which complies with all relevant Egyptian legislations.

Study area and animal data

One thousand, two hundred and sixty (1260) rectal fecal samples were collected from apparently healthy goats (406 adults, 854 kids, 663 females, and 597 males) from different localities in northern and southern Egypt such as; Behera, Menofia, Assiut, and Sohag governorate as shown in (Figure 1) during the period from November 2019 to September 2020, each sample was collected in a clean plastic cup, then labeled with age, sex, locality, season, breeding system, and delivered directly to Parasitology lab, Faculty of Veterinary Medicine, Assiut and Sohag University.

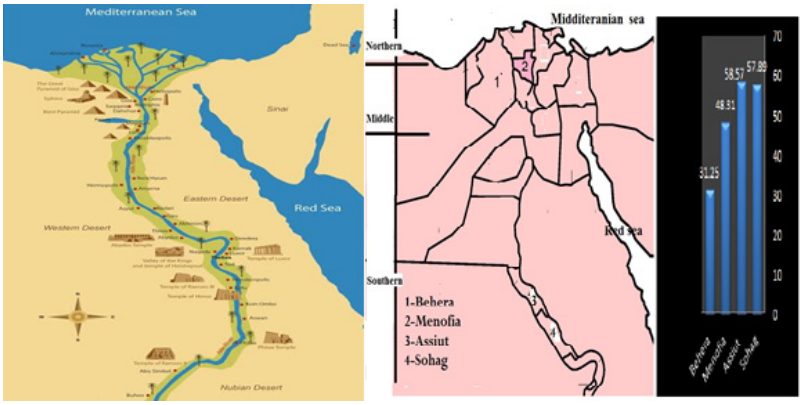

Figure 1. Map of Egypt showing percent of infection in different localities at northern and southern regions

Microscopic examination

Simple gravity sedimentation technique by Urquhart: A 10 gm of fecal pellets were thoroughly mixed with 50 mL tap water in a 250 mL beaker by using a depressor blade, an extra 50 mL tap water was added to the suspension which was strained through two layers of wet gauze into a sedimentation flask, and the material was let to sediment for 1 hour, the supernatant was decanted carefully and a sample was left to sediment for 1 hour, then repeated washing was done until a clear supernatant was obtained. a small portion of the sediment was removed by using a long capillary pipette, and placed on a glass slide, and covered with a cover slide, examined by (10x) objective lens of the microscope, and confirmation was made by (40x) [11].

Flotation method by Kaufmann: A 5 gm-10 gm of fecal pellets were placed in a shell vial cup and mixed 50 mL of tap water with a spatula, the mixture was then filtered through a wire mesh screen with an aperture of 500 µm-800 µm to get rid of large debris, and the fluid was collected in a bowl. The debris left on the screen was discarded, the suspension was transferred to a conical vial and filled with tap water to the highest, then allowed for settling for 30 min, the supernatant was discarded carefully. From the remaining sediment (of approximately 10 mL), a sample of 2 mL was poured into a centrifuge tube. Saturated NaCl was added until a convex meniscus stood above the top of the tube, a thick 19 mm × 19 mm square cover glass was placed on the tube, ensuring that no bubble was trapped under that coverslip. The tube was centrifuged at 2000 rpm for 2 min-3 min., the cover glass was picked off and the sample was placed on a slide and examined under a microscope by using a magnification of 40x-100x [12].

Sporulation of coccidian oocysts by Soulsby: The collected oocysts were taken in clean Petri-dishes (5 cm in diameter) containing 2.5% solution of potassium dichromate. The covers of the Petri-dishes were lined with moist filter papers before tightly covering the plates, and incubated at 27°C. The Petri dishes were daily exposed to air for oxygenation of oocysts. The Petri-dishes were examined daily to follow up on the process of sporulation, and the time consumed for sporulated oocysts was

accurately estimated. Then the contents of Petri-dishes were centrifuged at 1500 rpm for 3 minutes, and the supernatant fluid was decanted. The sediment was suspended in distilled water and the centrifugation was repeated several times until the supernatant fluid becomes clear, sediment was examined microscopically for the morphology and measurements of the sporulated oocysts [13].

Molecular detection of the Eimeria oocysts

Fecal samples: Only four positive samples that represented the observed sporulated oocysts of *Eimeria* species under the study were chosen and preserved at -20°C for DNA extraction by using the Genomic DNA Purification kit (Applied biotechnology, USA) following manufacturer's instructions. The positive samples were incubated for 20 min at 65°C in 200 µL of Nuclei Lysis Solution, and 40 µL of a 20 mg/mL Proteinase K solution, the fecal samples were homogenized utilizing the ceramic beads to obtain the optimal extraction of DNA, and the tubes were centrifuged at 10,000 × g (gravity)to eliminate debris. Then the supernatant was collected, and 100 µL of isopropanol was added to each sample. The mixture was run through the filter columns at 13,000 × g (gravity) for 1 min. DNA bound to filter was washed and eluted following manufacturer instructions. The extracted DNA concentration and purity were measured using a NanoDrop® spectrophotometer (Nano Drop Technologies, Inc. Wilmington, DE, USA) and stored at -20°C until use.

Molecular detection of the Eimeria oocysts from fecal samples based on ITS-1 rDNA gene

The ITS-1 DNA from each sample was amplified using conventional PCR. The cycling conditions and primer sequences of the forward and reverse primers were as follows: Forward 5′-GCAAAAGTCGTAACACGGTTTCC-3′, Reverse 5′-CTGCAATTCACAATGCGTATCG-3′ [12]. The cycling conditions were: initial denaturation at 94°C for 5 min followed by 35 cycles of 94°C for 45 s, 55°C for 1 min and 72°C for 1 min, this was followed by a final extension at 72°C for 7 min. The amplification products from ITS-1 rDNA were separated on 1.6% agarose gel containing 0.4 µg/mL of ethidium bromide at 90 V for 40 min-60 min and then imaged.

Statistical analysis

SPSS 22.00 software (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY:IBM Corp.) was used for statistical analysis. The prevalence results were expressed as a percentage of infected to all examined animals. The oocyst measurements were expressed as a mean ± standard error (M ± SE). The Chi-square test was done to express the significance of results at 95% confidence level at ($p \leq 0.05$) between different variables categories.

RESULTS

The present study revealed different species of *Eimeria* spp. in goats, the overall prevalence of *Eimeria* spp. infection was 40.63% (512/1260) of examined goats, furthermore, the study elucidated the different associated risk factors of *Eimeria* spp. infection to goats under an environmental condition in Egypt, including the age, sex, season, breeding system, and localities, and that was verified in (Table 1).

Table 1. Prevalence of *Eimeria* spp. infection in examined fecal samples of goats in Egypt and associated risk factors

Variables	No. exam.	No. infect	%	Chi-square (χ^2)	p-value
Overall infection	1260	512	40.63		
Age groups					
Suckling Kids (<6 months)	398	260	65.32	2.235	0.0035
Weaned kids (6-12 months)	456	188	41.22		
Adults (>1 year)	406	64	15.76		
Sex of animal					
Male	597	288	48.24	2.591	0.107
Female	663	224	33.78		
Season					
Winter	339	254	74.92	2.177	0.0014
Spring	321	158	49.22		
Summer	298	45	15.11		
Autumn	302	55	18.21		
Locality					

Sohag	339	154	45.42	1.977	0.7314
Assiut	291	95	32.64		
Menofia	328	115	35.06		
Behera	302	148	49.01		
Breeding system					
Free range grazing system	721	325	45.08	2.977	0.0214
housed closed system	539	195	36.17		
***Eimeria* spp.**					
E.arloingi	1260	118	9.36	1.909	0.0608
E.caprovina	1260	187	14.84		
E.hirci	1260	112	8.89		
E.caprina	1260	95	7.53		

Four different species. were detected in a study such as; *E. arloingi* (9.36%), *E. acaprovina* (14.84%), *E. hirci* (8.89%), and *E. caprina* (7.53%). The identification was based on morphological characters and measurements including the length and width of oocyst, sporocyst, sporozoites and micropyle, and micropylar cap, residual bodies, and steel body of the sporulated oocyst, By PCR assay, four different species amplified bands at electrophoresis (Figure 2). Young suckling goat kids revealed the highest infection rate (65.32%) followed by weaned kids (41.22%) and the lowest rate was observed in adults (15.76%) with a p-value (0.0035). Even though the male goats (48.24%) revealed a higher infection rate than the female (33.78%), the p-value (0.107) showed that the sex of the animal is not a significant factor that affects the infection rate at a 95% confidence level. the infection rate in the winter cold season was significantly the highest (74.92%) at p-value (0.0014), followed by spring (49.22%), then autumn (18.12%), and the lowest infection rate was in summer (15.11%) at 95% confidence level, and so, it is considered a potential risk factor for *Eimeria* spp. infection. Behera governorate showed the highest rate of infection (49.01%) in goats, Sohag (45.42%), Menofia (35.06), and Assiut governorate (32.64%). However, the locality of infection is not statistically considered a potential risk factor since the p-value was 0.7314. It is revealed that; goats reared on free-range grazing systems in opened areas were susceptible to *Eimeria* spp. infection (45.08%) more than goats breeds or reared in the closed housed system (36.16%) with a p-value (0.0214) as shown (Table 1 and Figure 3). Among the species observed in the present study, *E. caprovina* (14.84%), showed the highest infectivity, followed by *E. arloingi* (9.36%), then *E. hirci* (8.89%), and *E. caprina* (7.53%) at 95% confidence interval at p value of (0.0608). Hence, no specific *Eimeria* species was restricted to a specific locality or country (at 95% confidence level) as shown in (Figures 4-7).

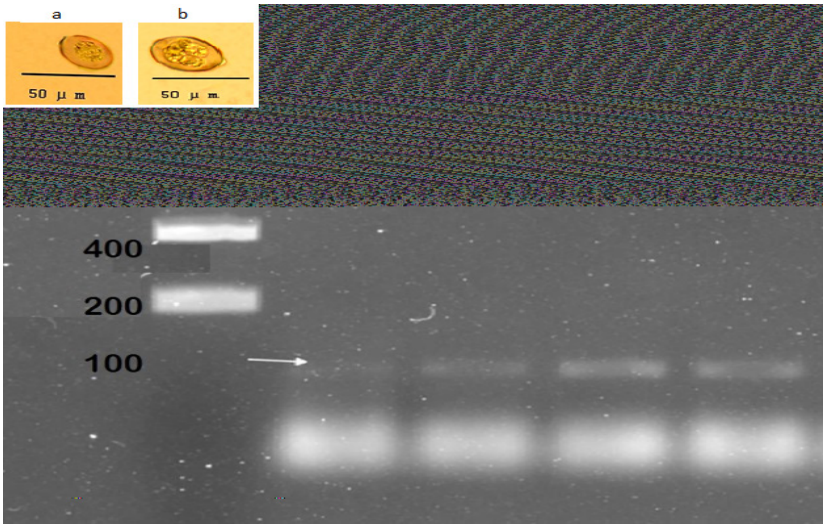

Figure 2. PCR agarose gel electrophoresis of sporulated *Eimeria* oocyst amplified DNA by ITS-1 Primer, DNA ladder is located on the left side of the gel, the size of fragments is represented in base pairs; a-d: represent positive fecal samples a: *Eimeria arloingi*, b:*E.caprovina*. c: *E. hirci*. d: *E. caprina*, all species give bands at 100 bp.

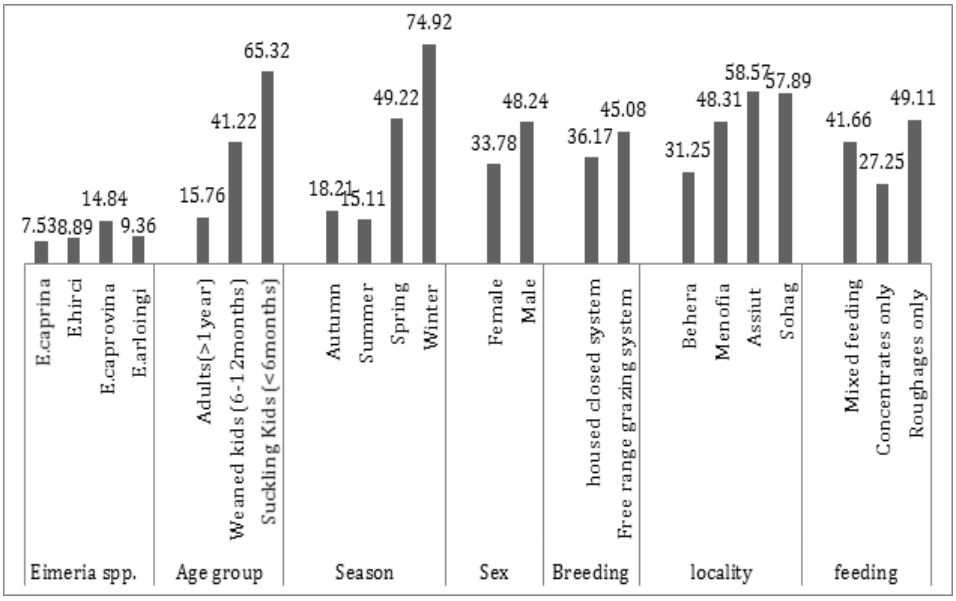

Figure 3. Prevalence of *Eimeria* spp. infection in examined fecal samples of goats in Egypt and associated risk factors

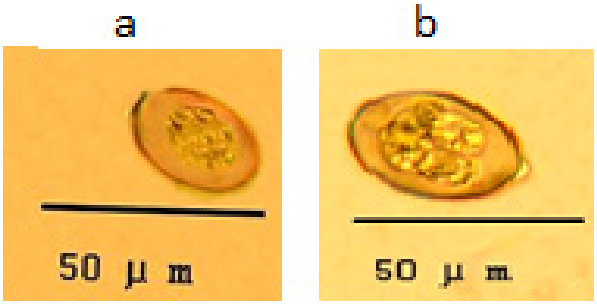

Figure 4. (a): *Eimeria arloingi* Sporulated oocyst; (b): Unsporulated oocyst

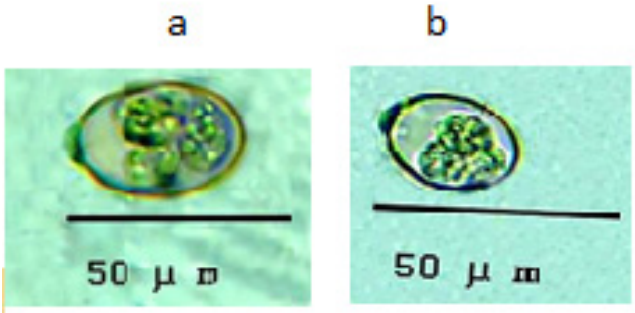

Figure 5. (a): *Eimeria caprovina* Sporulated oocyst; (b): Unsporulated oocyst

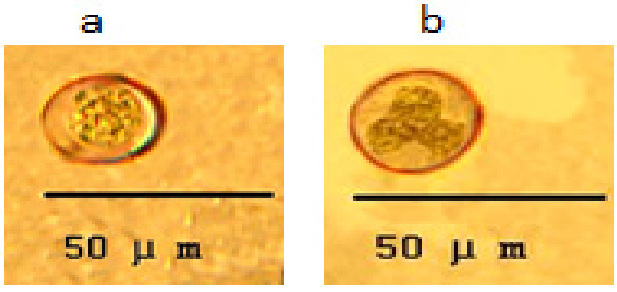

Figure 6. (a): *Eimeria hirci* Unsporulated oocyst; (b): Sporulated oocyst

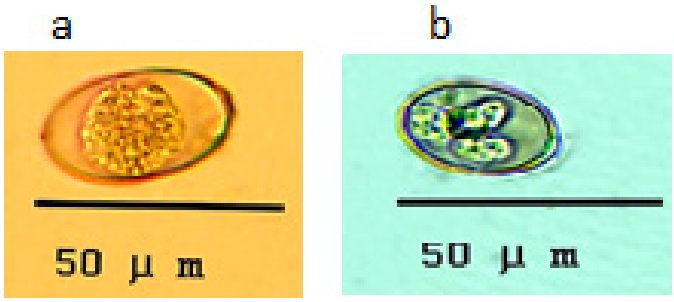

Figure 7. (a): *Eimeria caprina* Unsporulated oocyst; (b): Sporulated oocyst

DISCUSSION

The overall prevalence of infection in the present study was (40.63%) in surveyed goats. However, at Upper Egypt, El Shahawy found 65.07% positivity, while Hassan at Giza Governorate (Egypt) observed 76.89% [1,14]. In Iran, Radfar observed 89.27% positivity, while in Ethiopia, Terefe observed 100% positivity for the presence of *Eimeria* spp. oocysts in the faeces of goats [2,9].

Results of the current study revealed that the age of the animals is a contributing factor for the infection of goats with *Eimeria* spp. This may be due to the low immune status in young kids, and the absence of humoral or cellular immune response that can counter-attack the sporozoites into epithelial cells of the small intestine of the host. Similar results were observed in Kenya, in Pakistan, and in Egypt, in Egypt, found that the adult goats were more susceptible than kids with a prevalence rate of 72% and 45% respectively1, [15-17]. Animal of both sexes was equally susceptible for coccidiosis in goats as previously reported in Egypt, Saudi Arabia, and in Northeastern Brazil [10,17,18]. However, in India, stated that the female goats were more susceptible than males for infection with *Eimeria* spp. Season of the year is a potential risk factor that significantly affects the prevalence of *Eimeria* sp. infection in goats, and cold winter and spring seasons, the infectivity rate is highest [19]. This may be due to the availability of the suitable temperature and humidity, and oxygenation that is needed for oocyst sporulation. On the contrary; the dry hot season's summer and autumn revealed the lowest percent of infection with *Eimeria* sp. in goats [14,19]. However, Smith and Sherman mentioned that hot and humid weather is particularly conducive to sporocyst development and outbreaks of clinical coccidiosis were common during summer [20]. Ashraf and Nepote observed that in Maryland (United States) peak infection level was observed during winter [21]. Recently, Osman reported a higher infection rate in winter in New Valley Governorate, Egypt [22].

Goats reared by the free-range system of rearing were more at risk of infection with *Eimeria* sp. infection than housed goats in a closed system. To conclude, there's a significant relationship between age, seasonal variations, breeding system, for the occurrence of *Eimeria* sp. infection, while the species of the parasites, sex of the host, locality, etc had no influence.

Different species of *Eimeria* were known to infect goats (Levine, 1985), Four species of *Eimeria* were identified namely; *Eimeria arloingi*, *E. caprina*, *E. caprovina, E. hirci*, and *E. caprovina* which were not previously reported in goats in Assiut Governorate, Kahan and Greiner detected them in Florida, USA, while found seven species in Upper Egypt viz., *Eimeria alijevi*, *E. arloingi, E. caprovina*, *E. ninakohlyakimovae*, *E. hirci*, *E. jolchijevi* and *E. aspheronica* [4,14].

E. ninakohlyakimovae, *E. hirci*, *E. caprina*, *E. christenseni*, *E. jolchijevi*, *E. apsheronica* and *E. arloingi* in goats in suez canal governorate [23]. Five species *E. arloingi*, *E. parva*, *E. ninakohlyakimovae*, *E. christenseni*, and *E. faorei* in Iran were identified [9]. Six species; *Eimeria jolchijevi, Eimeria arloingi, Eimeria alijevi, Eimeria caprina, Eimeria hirci, and Eimeria christenseni* were studied in China [24]. In Egypt, identified seven species, *E. ninakohlyakimovae, E. hirci, E. caprina, E. christenseni, E. jolchijevi, E. apsheronica*, and *E. arloingi* [18], and in Mexico, determined eight species; *E. caprovina, E. christenseni, E. hirci, E. arloingi, E. caprina, E. alijevi, E. ninakohlyakimovae,* and *E. jolchijevi* [25].

CONCLUSION

The overall prevalence of *Eimeria* spp. infection in examined goats in Egypt was (40.63%). There were four *Eimeria* spp. detected viz., *Eimeria arloingi, E. caprina, E. caprovina,* and *E. hirci*. Young kids were more susceptible

than adults while the infection was higher in cold winter and spring seasons. Goats that were reared on a free-range system were highly susceptible compared to animals sheltered under housing animals. Animal sex, locality of infection, species of *Eimeria* did not significantly affect the infection in goats.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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