Emirates Journal of Food and Agriculture. 2021. 33(7): 532-543
doi: 10.9755/ejfa.2021.v33.i7.2720
http://www.ejfa.me/

RESEARCH ARTICLE

Biosecurity assessment in relation to the occurrence of some coccidian parasites in poultry farms, with *in vitro* evaluation of *Psidium gujava* as coccidia sporulation inhibitor

Fatma Ali Abouelenien[1]*, Ismail Saad Elshahawy[2], Mohamed Elfatih Hamad[3], Nagham Rafik Elsaidy[1]

[1]Department of Hygiene and Preventive Medicine, Faculty of Vet. Med., Kafrelsheikh University, Egypt, [2]Department of Parasitology, Faculty of Veterinary Medicine, South Valley University, Egypt, [3]Department of Veterinary Medicine, College of Food and Agriculture, United Arab Emirates University, UAE

ABSTRACT

The present study was performed to assess the correlation between biosecurity compliance measures and the prevalence of *Eimeria* and *Cryptosporidium* species in commercial based (CBF) and house hold (HHF) poultry farms, in Delta region Egypt. Additionally, this study aims to evaluate the effect of *Psidium gujava* on sporulation of *Eimeria* oocysts in bird environment (the chicken litter or manure). This study was conducted in 74 poultry farms, from which, 74 litter samples were collected for detection of both species. Additionally, 74 water samples were collected and examined for the presence of Cryptosporium spp. Concurrently a series of experiments were done separately to evaluate the efficacy of *Psidium gujava*. The results showed that the overall prevalence of *Eimeria* spp. was 48.6% with a higher prevalence in CBF (53.3%), while that of Cryptosporium spp. was 54% with the highest prevalence in HHF (62.5%). Application of farms disinfection was insignificantly affected the prevalence of both protozoan parasites. Disposal of farm wastes as land fertilizers significantly decreased the prevalence of both protozoan parasites, in contrast disposing of wastes to fish farms was associated with the highest prevalence of *Eimeria* spp. The highest prevalence of Cryptosporium spp. was recorded in farms that dispose dead birds to domestic rubbish. Rodent control and All in all-out system application were significantly decreased the Cryptosporium spp. prevalence (P=0.012, and 0.025 respectively). Finally, the highest sporulation inhibition percentage (SP% of 86.4) was obtained by using crude watery extract (10%) at a concentration of 2.5ml/ml after 24hr contact time.

Keywords: Biosecurity practices; *Cryptosporidium* spp.; *Eimeria* spp.; Prevalence; *Psidium gujava*

INTRODUCTION

Globally, poultry farming is one of the most intensive and mechanized livestock operations in agriculture. This industry represents an important point in the provision of meat and egg to man and generally plays a dynamic role in the national economy as an income provider (Nnadi and George, 2010).

In Egypt, the poultry industry employs about 2.5 million workers. Furthermore, this sector produces nearly 1.25 billion day-old chicks, 1.1 billion broilers, and 8billion table eggs/year (Byrne, 2019). Poultry production systems in Egypt are quite diverse, ranging from rural very small-scale, extensive poultry production to highly intensive caged systems with over 70,000 birds per house in industrial commercial systems.

The risk for parasitic disease outbreaks among commercial poultry farms can result in significant economic losses for the farmer and the integration. When major disease outbreaks occur in a region, this may also result in great loss of employment.

Of parasitic diseases, Coccidiosis is one of the most dangerous poultry diseases commonly cause economic losses worldwide (Abdisa et al., 2019).

Coccidiosis, is caused by a single-celled protozoan parasite of the genus *Eimeria* and it remains a major constraint to

***Corresponding author:**
Fatma Ali Abouelenien, Department of Hygiene and Preventive Medicine, Faculty of Vet. Med., Kafrelsheikh University, Egypt.
Tel.: +20109462224. **E-mail:** fabou2ga@yahoo. com

Received: 24 February 2021; **Accepted:** 19 June 2021

successful commercial and backyard poultry farming due to its significant-high mortality rates and huge economic losses globally being estimated at more than 3 billion US$ annually in the world (Dalloul and Lillehoj, 2006). Additionally, it accountable for huge morbidity rates which characterized by dysentery, enteritis, bloody diarrhea, emaciation, lower feed conversion rate, delayed sexual maturity, drooping wings, and poor growth (Rehman et al., 2010; Awais et al., 2012).

Several studies have been illustrated the prevalence and economic importance of coccidiosis as a major parasitic disease in both local and exotic breeds of poultry, worldwide (Majaro, 2001; Hadipour et al., 2011; Dakpogan and Salifou, 2013).

Birds are more susceptible to coccidiosis if they are immunosuppressed either by disease or stress. Therefore, it is essential to create a healthy environment. The poultry industry has attempted to limit losses due to parasitic diseases, particularly coccidiosis. However, it is becoming apparent that the extensive use of chemical anti-coccidial drugs in controlling this disease has led to the development of drug-resistant parasites (Al-Mathal, 2010; Ahmed et al., 2018). Additionally, the toxic effect of disinfectants (phenolic, ammonia, methyl bromide and carbon disulfide) and amendments used in controlling coccidiosis, in the surrounding environment, represent a danger to the staff and health of animals and therefore their use has been restricted (Cedric et al., 2018).

Psidium gujava is a curative plant rich in polyphenolic and flavonoid compounds (Patel et al.. 2016). It is utilized in tropical and subtropical nations to treat numerous wellbeing issues. So, it has been accounted for removal of a wide range of biological activities, as, anticough, antibacterial, hemostasis (Ahmed et al., 2018); antidiarrhoeal narcotic (Mohamad et al., 2011), and antioxidant. A lot of previous studies focus on using *Psidium gujava* extracts for sporulation inhibition of *Eimeria* spp. (Cedric et al.. 2018) or using its crude leaf powder as anticoccidial (Ahmed et al., 2018).

Cryptosporidiosis is a zoonotic protozoan disease caused by protozoa of the genus *Cryptosporidium* (Dillingham et al., 2002). Cryptosporidiosis normally causes respiratory problems, gastroenteritis, and diarrhea in chickens and turkeys, while in humans; it causes abdominal pain, nausea, and watery diarrhea lasting 3-4 days. Several studies between 1989 and 2019 declared that *Cryptosporidium* infection has been reported in almost all Egyptian governorates with prevalence rates ranged between 3% and 50% or up to 91% in immunocompromised patients and diarrheic children (Ibrahim et al., 2016, El-Shahawy and Abouelenien, 2019). *Cryptosporidium* oocysts are transmitted between hosts via the fecal-oral route, either directly from contact with faeces of infected birds or indirectly through environmental contamination or from ingestion of contaminated food or water. The association between cryptosporidiosis in birds and their handlers is a topic of interest. Therefore, the prevention of cryptosporidiosis is an important tool for public health (Karanis et al., 2007).

Biosecurity means guarding birds by preventing disease-causing agents from entering the farm and protecting neighbor farms by inhibiting disease from leaving the infected farm. Even if some diseases are unapparent, but causing economic losses in the form of morbidity and decrease in production and others can kill all birds. Developing a good biosecurity plan and adhering to it is the best way to limit the introduction of infectious diseases and parasites into the flock (Ajewole and Akinwumi, 2014; Gomes and Sparks, 2020).

Up to the author's knowledge, no previous studies are investigating the correlation between the biosecurity practices, applied in poultry farms and the prevalence of some parasites, which is of public health importance. So the main aim of the current study is to investigate the correlation between the prevalence of *Eimeria* and *Cryptosporidium* spp. and the compliance with the investigated biosecurity items in poultry farms. The second aim is to evaluate the efficacy of guava leaves extract, guava leaves powder, and crude watery extract of guava leaves on sporulation of *Eimeria* oocyst in bird environment (chicken litter or manure).

MATERIALS AND METHODS

This study was conducted in 74 poultry farms in the Delta region from January 2017 till August 2018 to evaluate the prevalence of *Eimeria* spp. and *Cryptosporidium* spp. between different types of surveyed farms and to assess the correlation between the application of biosecurity measures and the prevalence of these parasites**.**

Questionnaire design

For each type of poultry scale system, a structured questionnaire was designed, both in Arabic and English. The questionnaire aimed to collect data about the presence (P) or absence (A) of each studied biosecurity practice in the surveyed farms (Abouelenien et al., 2020)

Parasitological examination of surveyed poultry farms

Litter sampling and preparation for detection of Eimeria spp.

A total of 74 litter samples were taken during the study period from surveyed farms. Litter materials were collected from several representative areas of the grower chickens house from the top layers of material as a' "handful" of litter (Conway and McKenzie, 2007).

First, the samples were mixed separately and 9 g of it was poured into a 300 ml standing glass jar and 126 ml of water was added to it. The sample was then filtered through a 100 μm pore sieve and poured into a clean test tube with a 15 ml suspension. After centrifugation (1500 rpm for three minutes), the supernatant was discarded, and the saturated salt solution was added to it to give a convex surface at the test tube opening. Each test tube was placed in test-tube stands. Cover-slip was placed on the test tube surface and left to stand for 15 minutes after which they were gently lifted off without brushing against the tubes. This was then placed on microscopic slides sideways in one quick movement to avoid air bubbles on the glass slide and viewed under the microscope. Examinations of the slides were carried out using 10x objective lens. The tested samples were examined for the presence/absence of oocysts and recorded as oocyst-positive or oocyst-negative. Oocyst-positive samples were processed for sporulation by mixing 10 g litter sample with approximately 60 ml 2.5% potassium dichromate (w/v aqueous) and passing the homogenate through a sieve with 1 mm^2 opening. The filtrate (<100 ml) was transferred to a 150 ml Erlenmeyer flask capped with aluminum foil perforated to permit air exchange and then placed on a rotary platform shaker operating at ~100 rpm at room temperature to permit sporulation of viable oocysts. Speciation of coccidia was performed by microscopic features of oocyst morphology (shape, size, and color of the oocysts). The results of the aforementioned identification characteristics used were compared with the identification key (Long and Reid, 1982) for confirmation of species identification.

Detection of Cryptosporidium species in litter and water samples

A total of 148 random litter and water samples (74 each) were simply collected. Approximately 20 g of litter mixed with feces were collected from four corners of each bird pen and mixed to perform one sample then transferred into properly labeled polyethylene plastic bags. Additionally, about 50ml of water from the main water source and drinkers from different corners of the bird pen were also taken and put in labeled glass bottles. All samples were immediately transported to Hygiene and Preventive Medicine Lab., Faculty of Veterinary Medicine, Kafrelsheikh University in the icebox for parasitological examination. All pooled specimens were screened for the presence of *Cryptosporidium* oocysts by Sheather's floatation technique and modified Ziehl-Neelsen staining procedures (Henriksen and Pohlenz, 1981).

Experimental study

A series of experiments were done separately to evaluate the efficacy of *Psidium gujava* (Guava) leaves extract, powder and crude watery extract on the sporulation of *Eimeria* tenella oocyst (field strain) in a bird environment (litter or manure).

Plant materials

Leaves of *Psidium gujava* (Guava) were purchased from the local market of Kafrelsheikh city, authenticated by a botanist of Faculty of Agriculture, Kafrelsheikh University. The leaves were washed in a salt solution to remove possible microbial contaminants and rinsed in clean water then spread to dry under shade at room temperature for one week. The dried leaves were crushed into a coarse powder using an electric grinder, dried again under shade at room temperature and then ground into fine powder. The resultant fine powder was then stored in nylon bags at 4°C until required for use (Ahmed et al., 2018). To obtain the crude watery extract, 10 g of ground leave powder was taken in 100 ml of distilled water in a clean beaker and mixed thoroughly by a stirrer. Then, the beaker was placed on a heater and the content of the beaker was boiled for 15-20 minutes. Cooling at room temperature then filtered and the obtained watery extract then kept in a sterilized airtight bottle at 4°C until used (Jalal Uddin et al., 2016), while guava leaves extract was purchased from PHARO PHARMA Company, Egypt, with the chemical composition of aqueous guava leaves extract (4:1) 0.125 gm (standardized to contains up to 5% total flavones).

Preparation of unsporulated oocysts

Oocysts of *E. tenella* were isolated from naturally infected chicks at the Department of Parasitology, Faculty of Veterinary Medicine, South Valley University. The parasite strain was identified on the basis of parasitological niche of the infected chick, and morphological features described in previous studies (Long and Reid, 1982). The collected oocysts were stored at 4°C in the refrigerator until required.

Test procedures and sporulation inhibition assay

Three treatment trials (crude watery extract (T1), leave powder (T2), leaves extract (T3) were conducted. In each trial, a total of 100 *E. tenella* oocysts were added to the wells of the drawing working plate and mixed with different concentrations of each plant material. The oocyst suspension in these mixtures was incubated for 24 and 48 hr at 25°C with 60-80% humidity and continuous aeration. The control plates contained the diluents (saline solution) and the oocyst suspension. Then, the number of sporulated and non-sporulated oocysts was counted and the percentage of sporulation was estimated by counting the number of sporulated oocysts in a total of 100 oocysts. The oocysts with 4 sporocysts were considered sporulated regardless of the shape and size of the sporocysts (Daugschies et al., 2002).

The sporulation inhibitory percentage was calculated according to the following formula: Sporulation inhibition percentage% (sp.%) = sp.% of control – sp.% extract/sp.% of control ×100 (Daugschies et al., 2002; Cedric et al., 2018)

RESULTS

I- Prevalence of Eimeria spp. and Cryptosporidium spp. in surveyed farms with different rearing systems

Out of 74 poultry farms examined, 36 were found to be infected with *Eimeria* spp., which reflected a total percentage of infection reached 48.6%. Concerning the distribution of infection with *Eimeria* spp. in surveyed farms, the current study declared that 52.3% of the CBF were afflicted, whereas 43.75% of the HHF on the other hand, were found to be positive. Concerning the prevalence of *Cryptosporidium* spp., 40 out of 74 examined farms were found to be infected with an estimated overall prevalence of 54.05%. A higher prevalence (62.5%) was observed in HHF than in CBF (47.6%) as depicted in Table (1). The same table also summarized the relationship between the prevalence rate and the sample type. From this table, it was evident that the manure samples had a higher infection rate (54%) as compared to the water samples (40.5%). Five species of *Eimeria* were identified as mixed infections throughout the current survey from both screened farms Figure (1, g), namely *Eimeria acervulina*, *E. maxima*, *E. necatrix*, *E. mitis* and *E. tenella*. Furthermore, E. tenella and *Eimeria acervulina* occurred most frequently with prevalence of 41.5% and 32%, respectively, whereas the infection rate value of *E. necatrix*, *E. mitis* and E. maxima were found to be 22%, 3% and 1.5% respectively in CFB. On the other hand, among HHF, *Eimeria* necatrix has the highest overall prevalence of 40.2% followed by *E. acervulina* (25.1%), *E. tenella* (15.4%) and *E. maxima* (10.7%), while *E. mitis* represented the lowest prevalence (8.6%). The main morphological features of the recovered *Eimeria* species were illustrated in Figure (1, a-e). Additionally, the mean *Eimeria* oocyst count was 10300 oocysts/g, while the oocyst load of *Cryptosporidium* spp. was very minimal since the smears showed 2 oocysts/slide as shown in Figure (1, f).

II- Correlation between biosecurity measures and prevalence of Eimeria spp. and Cryptosporidium spp.

II.1. Prevalence of Eimeria spp. and Cryptosporidium spp. in relation to the application of Restricted access to the bird and Cleaning and disinfection

Restriction of access to birds inside the farms is a very important step in keeping birds away from the source of infection. Table (2), showed that visitors were not allowed to enter poultry sheds in 58 farms (78.3%), this restriction was found to be associated with the prevalence of *Eimeria* spp. and *Cryptosporidium* spp. Higher prevalence of *Eimeria* spp. (50%) and *Cryptosporidium* spp. (75%) were observed in farms that allow visitors than that restrict visitors. The housing of multiple species was found in 13.5% of surveyed farms, in which the prevalence of *Eimeria* spp. and *Cryptosporidium* spp. was lower than farms that reared only one species. Quarantine of new birds was applied in only 16.2% of surveyed farms and results showed that it has no significant effect on the prevalence of both *Eimeria* spp. and *Cryptosporidium* spp. Most of the farms did not have a fence around them (97.3%), and these farms have a higher prevalence of *Cryptosporidium* spp. (75% vs 0% in farms with fence). Utilization of borrowed equipment/sprayers was insignificantly affecting the prevalence of both *Cryptosporidium* and *Eimeria* spp. in spite of a slight increase the prevalence of *Eimeria* spp. (60%).

Table (3) also revealed that the absence of cleaning and disinfection of farm equipment resulted in a slight increase in the prevalence of *Eimeria* spp. infection but not significantly affect that of *Cryptosporidium* spp. Additionally, disinfection of farm buildings has no significant effect on the prevalence of both protozoan parasites. Table 3 also reveals that 83.8% of farms applied spraying as a method of disinfection versus 5.4% only used fumigation, and this later method caused a significant decrease in the prevalence of both *Cryptosporidium* and *Eimeria* spp. The absence of a foot bath in front of the bird's house caused an increase in the prevalence of *Cryptosporidium* spp.

II-2. Prevalence of Eimeria spp. and Cryptosporidium spp. in relation to the disposal of wastes and dead carcasses in surveyed poultry farms

Methods of disposal of farm wastes (litter/and or manure) were surveyed and results are shown in Table. 4. It was found that farm wastes were disposed to either fish farm (48.6%), land as a fertilizer (21.6%), or to domestic rubbish (37.8%). Disposal of wastes to land as a fertilizer was significantly decreased *Eimeria* spp. and *Cryptosporidium* spp. prevalence (37.5 and 50% respectively), while throwing to canal significantly decreased the prevalence of *Eimeria* spp. (25%). In contrast, highest prevalence of *Eimeria* spp. was associated with the disposal of wastes to fish farms. The highest prevalence of *Cryptosporidium* spp. was due to the disposal of wastes to domestic rubbish.

Bird carcasses were found to be disposed of either through throwing to domestic rubbish (45.9%), burning (27%), throwing to water canals (21.6%), burying (10.8%), or by feeding to pet animals (8.1%). The highest prevalence of *Cryptosporidium* spp. (82.4%) was associated with throwing of dead birds into domestic

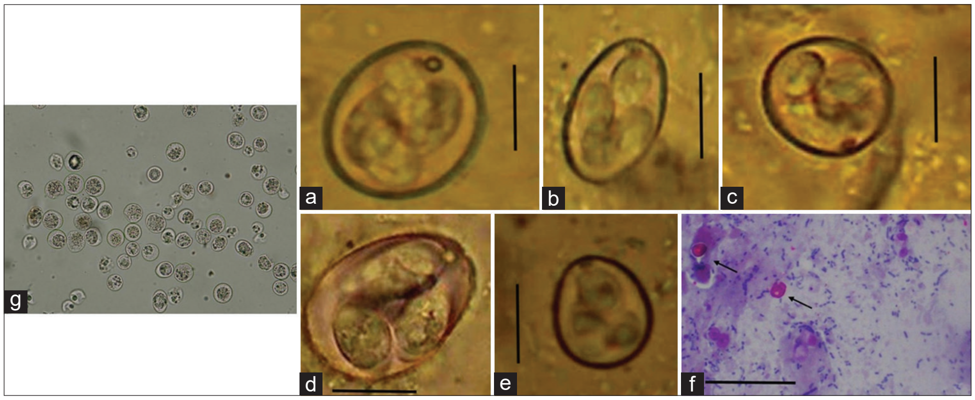

Fig 1. (a-g) Oocysts of *Eimeria* spp. identified in the litter collected from surveyed farms. a: *E. tenella*; b: *E. necatrix*; c: *E. mitis*; d: *E. maxima*; e: *E. acervulina* (Scale bar=20um); f: *Cryptosporidium* species oocysts (arrow) (Scale bar=10um); G: Freshly shed unsporulated oocysts of *Eimeria* spp. in litter samples collected from screened poultry farms.

Table 1: Prevalence of *Eimeria species and Cryptosporidium* species in surveyed farms with different systems

	Commercial based farm		House hold based		P value
Sample type	Water42	Litter/ Manure=42	Water 32	Litter/ Manure=32	
Parasite	N (%)	N (%)	N (%)	N (%)	
Eimeria spp.	-	22 (52.3%)	-	14 (43.75)	0.4617
Cryptosporidium spp.	16 (38.09)	20 (47.61%)	14 (43.75)	20 (62.5)	0.2151

Table 2: Prevalence of *Eimeria species and Cryptosporidium* species in surveyed farms with different bird species

Farm type (Species)								
Type of samples	Broiler (n=46)		Layer (n=8)		Duck (n=6)		Mixed farm (n=14)	
	Water (n=46)	Litter/ manure(n=46)	Water (n=8)	Litter/ manure(n=8)	Water (n=6)	Litter/ manure(n=6)	Water (n=14)	Litter/ manure(n=14)
Eimeria spp.	-	56.52 (n= 26)	-	50 (n= 4)	-	33.33(n=2)	-	28.57 (n=4)
Cryptosporidium spp.	23.91 (n=11)	28.26 (n=13)	50 (n=4)	50 (n=4)	66.66 (n=4)	66.66 (n=4)	-	42.85 (n= 6)

rubbish while the lowest one (25%) was recorded with burying (Table 4).

II-3. Prevalence of Eimeria spp. and Cryptosporidium spp. in relation to managemental and structural Biosecurity practices

Table (5) showed that 94.6% of the surveyed farms had neighboring farms, within 500 m, and this was associated with increased prevalence of *Cryptosporidium* spp. (74. 3%). Another structural biosecurity item, which is the presence of trees around the farms, increases the prevalence of both protozoan parasites. In contrast, the presence of grasses around the farm decreased the prevalence of *Eimeria* and *Cryptosporidium* spp. (45.8 and 66.7% respectively vs 53.8 and 84.6% in case of absence of grasses respectively). The presence of ponds around the surveyed farms did not significantly affect the prevalence of both parasites.

On the other hand, Table.5 summarized the effect of some managemental factors on the level of infection, of these factors keeping different age groups together which were found to increase significantly (P=0.001) the prevalence of *Cryptosporidium* spp. (93.3% vs 59.1% in farms that do not keep different age groups together). Another factor is the presence of isolation pens for diseased birds which did not significantly affect the prevalence of infection. Control of rodents was applied in 45.9% of surveyed farms, which showed a significant decrease in the prevalence of *Cryptosporidium* spp. (58.8% vs 85% in farms which not use rodenticides) with P=0.012. On the other hand, access of wild birds was observed only in 13.5% of the farms under study and it was found that it has no significant effect on infection intensity in these farms.

Most used housing system in the surveyed farms is deep litter system 72 farms out of 74 farms (97.3%), the most used bedding material was wood shaving (41.6%) which significantly (P=0.0129) decreased the prevalence of *Cryptosporidium* spp. Furthermore, the application of All in all-out system of management was significantly associated with decreasing the prevalence of *cryptosporidium* spp. in the examined farms (P=0.025). The presence of rodents

Table 3: Prevalence of *Eimeria species and Cryptosporidium* species in relation to restricted access to the bird; the application cleaning and disinfection in surveyed poultry farms

Restriction to visitors			
Parasite	**A=16**	**P=58**	**P value**
Eimeria spp.	50 (n=8)	48.3 (n=28)	0.902
Cryptosporidium spp.	75 (n=12)	72.4 (n=42)	0.836
Multiple Species rearing			
Parasite	**A=64**	**P=10**	
Eimeria spp.	53.1 (n=34)	20 (n=2)	0.05
Cryptosporidium spp.	75 (n=48)	60 (n=6)	0.320
Isolation and quarantine of new birds			
Parasite	**A=62**	**P=12**	
Eimeria spp.	48.4 (n=30)	50 (n=6)	0.91
Cryptosporidium spp.	71 (n=44)	83.3 (n=10)	0.37
Presence of fence around premises			
Parasite	**A=72**	**P=2**	
Eimeria spp.	47.2 (n=34)	100 (n=2)	0.52
Cryptosporidium spp.	75 (n=54)	0 (n=0)	0.018
Utilization of borrowed equipment/sprayers			
Parasite	**A=64**	**P=10**	
Eimeria spp.	46.9 (n=30)	60 (n=6)	0.43
Cryptosporidium spp.	75 (n=48)	60 (n=6)	0.32
Cleaning and disinfection of farm equipment's			
Parasite	**A=14**	**P= 60**	
Eimeria spp.	57.14(n=8)	46.7 (n=28)	0.4800
Cryptosporidium spp.	71.4(n=10)	73.33(n=44)	0.88
Cleaning and disinfection of poultry houses			
Parasite	**A=8**	**P= 66**	
Eimeria spp.	2(25)	51.5 (n=34)	0.227
Cryptosporidium spp.	4(50)	75.7 (n=50)	0.259
Method of disinfection (spraying)			
Parasite	**A= 4**	**P= 62**	
Eimeria spp.	50 (n=2)	51.61 (n=32)	0 .950
Cryptosporidium spp.	50 (n=2)	77.4 (n=48)	0.214
Method of disinfection (Fumigation)			
Parasite	**A= 62**	**P= 4**	
Eimeria spp.	51.6 (n=32)	50 (n=2)	0.950
Cryptosporidium spp.	77.4(n=48)	50 (n=2)	0.214
Presence of foot bath			
Parasite	**A= 44**	**P=30**	
Eimeria spp.	45.5(n=20)	53.3 (n=16)	0.464
Cryptosporidium spp.	81.8(n=36)	60 (n=18)	0.150

P: means presence of biosecurity practice in surveyed poultry farms (poultry farms that applied the mentioned biosecurity practice)
A: means absence of biosecurity practice in surveyed poultry farms (poultry farms don't apply the mentioned biosecurity practice)

in the farms caused an increase in the prevalence of *Cryptosporidium* spp.

III- Experimental study

The current results demonstrated that crude watery extract (10%) showed the highest sporulation inhibition percentage (86.4%) of *Eimeria* tenella oocyst at a concentration of 2.5ml/ml after 24hr contact time. While mixing of Psidium guava leaves powder with *E. tenella* oocyst in the litter at a concentration of

Table 4: Prevalence of *Eimeria species and Cryptosporidium* species in relation to disposal of wastes and dead carcasses in surveyed poultry farms

Method of disposal of farm wastes (to fish farm)			
Parasite	**P= 36**	**A=38**	
Eimeria spp.	55.6 (n=20)	42 (n=16)	0.247
Cryptosporidium spp.	66.7 (n=24)	78.9 (n=30)	0.234
Method of disposal of farm wastes (land fertilizer)			
Parasite	**P=16**	**A=58**	
Eimeria spp.	37.5 (n=6)	51.7 (n=30)	0.314
Cryptosporidium spp.	50 (n=8)	79.3 (n=46)	0.019
Method of disposal of farm wastes (to domestic rubbish)			
Parasite	**P=28**	**A=46**	
Eimeria spp.	42.8 (n=12)	52.2 (n=24)	0.463
Cryptosporidium spp.	85.7 (n=24)	65.2 (n=30)	0.114
Method of disposal of dead birds (Throwing in canals)			
Parasite	**P=16**	**A=58**	
Eimeria spp.	25 (n=4)	55.2(n=32)	0.033
Cryptosporidium spp.	75 (n=12)	72.4(n=42)	0.837
Method of disposal of dead birds (burying)			
Parasite	**P=8**	**A=66**	
Eimeria spp.	75 (n=6)	45.5 (n=30)	0.114
Cryptosporidium spp.	25 (n=2)	78.8 (n=52)	0.001
Method of disposal of dead birds (fed to pet (dog)			
Parasite	**P=6**	**A=68**	
Eimeria spp.	66.7(n=4)	47 (n=32)	0.357
Cryptosporidium spp.	66.7(n=4)	73.5 (n=50)	0.717
Method of disposal of dead birds (to domestic rubbish)			
Parasite	**P= 34**	**A=40**	
Eimeria spp.	41.2 (n=14)	55(n=22)	0.236
Cryptosporidium spp.	82.4 (n=28)	65 (n=26)	0.094
Method of disposal of dead birds (burning)			
Parasite	**P= 20**	**A=54**	
Eimeria spp.	60 (n=12)	44.4 (n=24)	0.234
Cryptosporidium spp.	80 (n=16)	70.4 (n=38)	0.407

P: means presence of biosecurity practice in surveyed poultry farms (poultry farms that applied the mentioned biosecurity practice)
A: means absence of biosecurity practice in surveyed poultry farms (poultry farms don't apply the mentioned biosecurity practice)

0.25g/g for 24hr, resulted in SP.% of 84.5%. However, Psidium guava extract showed the highest SP% of 72.4% at 10mg/ml after 48hr contact time as described in Table (6).

DISCUSSION

In developing countries, poultry is still the cheapest and most important source of protein. In Egypt vast majority of families in villages rear poultry in their houses (HHF) using an extensive system of housing. Coccidiosis and cryptosporidiosis are known to cause severe losses in poultry farms and have zoonotic importance.

I- Prevalence of Eimeria spp. and Cryptosporidium spp. in surveyed farms with different rearing systems

It was found that the prevalence of *Eimeria* spp. infections were 48.6% in the examined farms and this percentage

Table 5: Prevalence of *Eimeria* species and *Cryptosporidium* species in relation to managemental and structural biosecurity practices in surveyed poultry farms

Presence of neighboring farms (within 500 m)			
Parasite	**P= 70**	**A= 4**	**P value**
Eimeria spp.	48.6 (n=34)	50 (n=2)	0.956
Cryptosporidium spp.	74.3 (n=52)	50 (n=2)	0.287
Presence of trees around the farm			
Parasite	**P= 54**	**A= 20**	
Eimeria spp.	51.9 (n=28)	40 (n=8)	0.365
Cryptosporidium spp.	74.1 (n=40)	70 (n=14)	0.726
Presence of grasses around the farm			
Parasite	**P= 48**	**A= 26**	
Eimeria spp.	45.8 (n=22)	53.8 (n=14)	0.510
Cryptosporidium spp.	66.7 (n=32)	84.6 (n=22)	0.097
Keeping different age group together			
Parasite	**P= 30**	**A= 44**	
Eimeria spp.	46.7(n=14)	50 (n=22)	0.778
Cryptosporidium spp.	93.3(n=28)	59.1 (n=26)	0.001
Isolation pen for diseased birds			
Parasite	**P= 54**	**A= 20**	
Eimeria spp.	24(44.4)	12(60)	0.263
Cryptosporidium spp.	38(70.4)	16(80)	0.566
Rodent control			
Parasite	**P= 34**	**A= 40**	
Eimeria spp.	41.2(n=14)	55 (n=22)	0.236
Cryptosporidium spp.	58.8(n=20)	85(n=34)	0.012
Bedding material used (wood shaving)			
Parasite	**P= 30**	**A= 42**	
Eimeria spp.	60 (n=18)	42.9 (n=18)	>0.151
Cryptosporidium spp.	60 (n=18)	85.7 (n=36)	>0.0129
Bedding material used (Straw)			
Parasite	**P= 24**	**A=48**	
Eimeria spp.	50 (n=12)	50 (n=24)	1
Cryptosporidium spp.	83.3 (n= 20)	70.8 (n=34)	0.248
Bedding material used (Hay)			
Parasite	**P= 18**	**A= 54**	
Eimeria spp.	55.6 (n=10)	48.1 (n=26)	0.586
Cryptosporidium spp.	88.9 (n= 16)	70.4 (n= 38)	0.116
All in all out system			
Parasite	**P= 36**	**A= 38**	
Eimeria spp.	38.8 (n= 14)	57.8 (n= 22)	0.102
Cryptosporidium spp.	61.1 (n= 22)	84.2 (n= 32)	0.025

P: means presence of biosecurity practice in surveyed poultry farms (poultry farms that applied the mentioned biosecurity practice)
A: means absence of biosecurity practice in surveyed poultry farms (poultry farms don't apply the mentioned biosecurity practice)

was laying in the same range as reported by Wondimu et al.. (2019) (29.3 to 63.7%) and was higher than that obtained by Lawal et al.. (2016) who reported 31.8% in their study. This variation might be attributed to different factors such as sampling periods, geographic area and climatic conditions (Lawal et al., 2016). Furthermore, the current investigation also declared that CBF showed higher prevalence than HHF as the intensive system of housing in CBF is associated with higher stocking density than in HHF resulted in more stress on birds. Similarly, Lawal et al.. (2016) reported a higher prevalence of coccidial infection in the intensive system of housing than both the semi intensive and extensive systems in Nigeria, respectively.

Through the present study, five species were detected in the litter of the surveyed farms that we investigated in Kafrelsheikh province. *E. tenella* is the most frequent species in CBF, while *Eimeria* necatrix is the most predominant species in HHF. The establishment of different anticoccidial programs could limit the occurrence of some *Eimeria* species more than others, causing changes in the population of these parasites (Carvalho et al., 2011). Additionally, different types of animal handling practices can also affect the prevalence of *Eimeria* species.

In the current survey, oocyst length, width, and shape were indicative of the *Eimeria* species identification. This finding is in agreement with Al-Quraishy (2009) and Amer et al.. (2010) who reported as the length and width of *E. tenella* in μm (19.5-26 and 16.5-22.8); *E. acervulina* (17.7-20.2 and 13.7-16.3), *E. mitis* (16.4-21.1), *E. necatrix* (12.1-28.9 × 10.8-23.8) and *E. maxima* (21.5-42.5 and 16.5-29.8) to identify *Eimeria* spp. Additionally, the present findings showed that most of the *Eimeria* oocyst shape were ovoid. Similar findings were also reported by Ahmed et al.. (2012) who reported that *E. tenella*, E. acervulina, *E. maxima* and *E. necatrix* oocysts were ovoid shaped.

Globally, poultry are considered as the main transmitter of many pathogens, of which *Cryptosporidium* is one of the most dangerous ones because of it's a wide range of hosts and its zoonotic importance.

In previous studies, the prevalence rates of *Cryptosporidium* spp. varied between different poultry species and different countries. In the current, study the overall prevalence of cryptosporidiosis is 54% tested positive using the modified Ziehl Neelsen staining in both drinking water and manure samples taken from the examined farms, this percentage was higher than that obtained by Helmy et al.. (2017) who found the prevalence of cryptosporidiosis among broilers and layer chickens was 5.7% and 8.3% in Germany. The infection rate values among broilers in Scotland and Greece were 18.7% (Randall, 1982) and 24.3% (Papadopoulou et al., 1988), respectively. In Africa, the prevalence among broiler flocks was 37% in Morocco (Kichou et al., 1996). In Tunisia, 4.5% of individual broiler chickens were tested positive using the Ziehl Neelsen staining of fecal smears (Soltane et al., 2007). In Asia, using histological examination, 36.8% of infection was observed among individual broilers and 33.3% in layers in Japan (Itakura et al., 1984), while in Iran a rate of 23.8% was recorded. In contrast, Bomfim et al.. (2013) reported higher prevalence

Table 6: Effect of Guava crude water extract, Guava leaves powder and guava extract type, concentration and contact time on sporulation inhibition of *Eimeria Tenella* (field strain) in bird environment

Treatment (T)	T1							T2							T3						
	Contact time/hr.	24			48				24			48				24			48		
	Concentration (ml/ml)	S	NS	SP%	S	NS	SP%	Conc. (g/g)	S	NS	SP%	S	NS	SP%	Conc. (mg/ml)	S	NS	SP%	S	NS	SP%
	2	18	82	59.1	32	68	33.3	0.25	4	96	84.5	10	90	58.3	5	18	82	50	46	54	20.7
	2.5	6	94	86.36	10	90	79.1	0.5	10	90	61.5	10	90	58.3	10	30	70	16.7	16	84	72.4
	Control	44	56	-	48	52	-	Control	26	74	-	24	76	-	15	26	74	27.7	24	76	58.6
															20	24	76	33.3	30	70	48.3
															25	24	76	33.3	20	80	56.5
															Control	36	64	-	58	42	-

T1= wells containing fresh *Eimeria* oocysts treated with *Psidium gujava* crude water extract, T2= wells treated with *Psidium gujava* leaves powder, while T3= treated wells with *Psidium gujava* extract

than the current study (86%) in feces of the chickens using PCR in Brazil.

The differences in prevalence rates observed in the current study than the previous ones were mainly due to the sample origin which taken mainly from bird environment manure/litter and drinking water) but most previous study samples were taken from tissue samples or feces of individual birds with the use of different screening methods. On the other hand, differences in the housing systems and hygiene and management practices may also be responsible for low infection rates in birds housed in CBF (47.6%) than higher prevalence (62.5%) in HHF (Wang et al., 2010; Bomfim et al., 2013; Helmy et al., 2017).

II- Correlation between biosecurity measures and prevalence of Eimeria spp. and Cryptosporidium spp.

II.1. Prevalence of Eimeria spp. and Cryptosporidium spp. in relation to the application of Restricted access to the bird and Cleaning and disinfection

The effect of keeping birds isolated from sources of infection with *Cryptosporidium* and/or *Eimeria* spp. was evaluated by measuring the degree of compliance of farms with restriction to visitors, multiple species rearing, isolation and quarantine of new birds, presence of fence around premises, and utilization of borrowed equipment/sprayers on prevalence of Table 2, showed that restriction of visitors is insignificantly affecting the prevalence of *Eimeria* spp. and *Cryptosporidium* spp., however, multiple species rearing is significantly affecting the prevalence of *Eimeria* spp. in the surveyed farm, as higher prevalence was detected in farms reared only one species that is because *Eimeria* infection is strictly host specific (López et al., 2020). The presence of a fence around the farm resulted in a significant decrease in the prevalence of cryptosporidiosis. As the presence of a fence will decrease the entrance of stray dogs, cats and unauthorized persons which contaminate water and feed and indirectly disseminate the infection (Toledo et al., 2017). Utilization of borrowed equipment, cleaning and disinfection of both equipment and farm building were found to be insignificantly affecting the prevalence of both *Eimeria* and *Cryptosporidium* infection in the surveyed farms. This result could be attributed to the oocysts are resistant to most of the used disinfectants commonly used around farms (López et al., 2020). Additionally, Oocysts of *Cryptosporidium* are resistant to chlorine disinfection at the concentrations typically applied during drinking water treatment (Rochelle et al., 2012). Presence of footbaths in farms resulted in a low prevalence of *Cryptosporidium* infection but not significantly affect the prevalence of coccidiosis. As the type of disinfectants mostly used in footbath are hydrogen peroxide and Glutraldehyde products (Gradel et al., 2004; Martinez et al.. 2008). Additionally, most of the used disinfectants are selected on the basis of antibacterial or virucidal properties will often lead to frustrating results if antiparasitic effects are expected (Daugschies et al., 2013).

II-2. Prevalence of Eimeria spp. and Cryptosporidium spp. in relation to disposal of wastes and dead carcasses in surveyed poultry farms

Selling of farm waste (litter and/or manure) to fish farms to be used as fish pond fertilizer was the most used disposal method (48.6% of surveyed farms). In contrast application of manure as land fertilizer was the most used practice in Poland (Kyakuwaire et al., 2019; Drozdz et al., 2020) as it is the cheapest and most environmentally safe method of disposing especially with huge amount worldwide. This method was associated with the highest prevalence of *Eimeria* spp. (55.6%), this may have attributed to the practice of collecting the manure and litter in the farm after its removal and its storage which may extend for one day or more until transferred by trucks to the fish farms. During this period moisture increased and with the help of temperature *Eimeria* spp. oocyst become infective and by the help of wild birds and contaminated clothes or workers hands and tools feed of birds will be contaminated with oocyst and consequently transmit the infection to birds and bird's environment. On the other hand, the highest prevalence of cryptosporidiosis was associated with the disposal of wastes to domestic rubbish (85.7%), which increase the risk of feed and water contamination by bird feces from trash and rubbish by means of wild birds and insects or rodents (Daniels et al., 2003; Toledo et al., 2017). Disposal of waste to land as fertilizer was significantly (P=0.09) decreased the prevalence of *Cryptosporidium* spp. and *Eimeria* spp. This result may have attributed to that farm field is away from the farm and no need to store the waste in the farm before disposal decreasing the chance of water, feed and bird environment contamination with oocyst.

Disposal of dead birds through burning, burying, or feeding to dogs was found to increase the prevalence of *Eimeria* spp. while the prevalence of *Cryptosporidium* spp. was increased in farms that dispose dead birds in canals or domestic rubbish. Burning and burying were the most popular and hygienic methods for disposal of dead carcasses if properly applied but in the current work, it was associated with a high prevalence of *Eimeria* spp. This may have attributed to the improper application of these methods which may involve a long storage period of dead carcasses until burning or open burning process in front of the farm. Additionally, burying occurred in improper places near the farm. Propelling of dead birds in canals or domestic rubbish resulted in contamination of the environment with oocyst which contaminates water (Daniels et al., 2003). Puleston et al.. (2014) recorded an outbreak of cryptosporidiosis with *Cryptosporidium* cuniculus following a water quality incident in North Hampton shire, UK which was confirmed to be caused by a fresh wild rabbit carcass (Oryctolagus cuniculus) immediately below the inlet pipe to a backwash granulated activated carbon tank. It was assumed that the oocysts had been released into the disinfection contact tank from the carcass.

II-3. Prevalence of Eimeria spp. and Cryptosporidium spp. in relation to managemental and structural Biosecurity practices

The presence of neighboring farms within 500 m was positively related to the prevalence of *Cryptosporidium* spp. This nearer distance may have resulted in the circulation of the infection through contaminated drinking water sources. Additionally, the presence of grasses around the farm is associated with decreasing the prevalence of *Cryptosporidium* and *Eimeria* spp., as the presence of grasses around the farm and on the shores of canals may reduce water contamination with oocyst. Keeping different ages together resulted in a significant higher prevalence of cryptosporidiosis (P=0.001), but it was not affecting the prevalence of *Eimeria* spp. This result was in line with Wang et al.. (2010) and Bin-Kabir et al.. (2020) who found no significant difference in *Cryptosporidium* infection between the chicken age group. As young ages are more susceptible to infection with *Cryptosporidium* spp. than adult, so mixed ages increase the contamination of bird environment with a huge number of the oocyst. The presence of an isolation pen to isolate diseased birds was found to decrease the prevalence of both protozoan species. This could have attributed to the separation of the cases from other healthy birds will reduce the amount of excreted oocyst that contaminates bird water and feed.

Using of the rodent control system in the farm was found to significantly reduce (P=0.012) the prevalence of *Cryptosporidium* spp., as rodents are the main source of infection to birds through contamination of feed and water by its excreta (Daniels et al., 2003).

Table (5) showed that there is no significant effect of the type of bedding material on the prevalence of *Eimeria* spp. in spite of a slight increase in its prevalence in the case of wood shaving than straw or hay. This could be attributed to the nature of bedding materials in the surveyed farms was natural bedding material (wood, straw, and hay) which were highly exposed to oocysts as a result of faecal exposure more than artificial one (wired/caged) (Lunden et al., 2000; Prakashbabua et al., 2017). On the other hand, wood shaving used as bedding material was significantly decreased the prevalence of *Cryptosporidium* spp. (P=0.012), in contrast to hay which showed the highest prevalence (88.9%). Wood shaving has more ability to absorb moisture than hay. Also, it increases the ability of oocyst to adhere to it than to be available to the bird due to its high fiber content as compared to hay (Kuczynska et al., 2005).

All in all-out system of management was significantly associated with decreasing the prevalence of *Cryptosporidium* spp. in the examined farms (P=0.025). Similarly, Kochanowski et al.. (2017) found that the use of all-in/all-out production system allows reducing the occurrence of the parasite in farms. This could have resulted from the application of terminal cleaning and disinfection which allow the removal of parasite oocysts which can accumulate and increase the risk of infection.

III- Experimental study

Coccidiosis is one of the most dangerous poultry diseases commonly cause economic losses all over the world (Abdisa et al., 2019). It is worse to mention that *Eimeria* oocysts are highly resistant to most known chemical disinfectants (Daugschies et al., 2002). The tenacity of oocyst increased by the presence of organic matter (Watkinson, 2008) who mentioned that organic matter slightly inhibited the effectiveness of phenols. Most of the previous studies used *Psidium gujava* (Guava) as an antioxidant for increasing bird resistance (Cedric et al., 2018), or as antidiarrheal and antimicrobial (Hassan et al., 2011). In this study *Psidium* *gujava* (Guava) is used to decrease the sporulation percent of coccidial oocyst in litter and or manure of birds under field condition. Currently, the highest inhibition was obtained by crude watery extract (T1) at a concentration of 2.5ml/ml after 24hr contact time (86.4%), Followed by *Psidium gujava* leaves powder (T2) (84.5%) at 0.25g/g, while Psidium guava extract showed highest sp.% of 72.4% at 10mg/ml after 48hr contact time. The highest sporulation inhibition percentage was obtained after 24hr than after 48hr, the same result was obtained by Cedric et al.. (2018) who reported the highest efficacy after 24hr. Additionally, they reported maximum sporulation inhibition activity at 30 mg/ml (88.67%) of Psidium guava extract, which is higher than that obtained in the current study (72.4%) at 10 mg/ml. This difference may have attributed to the presence of litter material which is rich in organic matter and also due to the difference in the use of different *Eimeria* spp. Another study stated a lower sporulation suppression level of 75.9% as compared to the current study by using 39% benzene +22% xylene (1:10 dilution) and 85.5% for 30% cresol soup (1:1 dilution) on *E. tenella* (You, 2014).

Psidium leaves powder (T2) inhibit sporulation by 84.5% at 0.25g/ml, this plant material was used in the previous study of Ahmed et al., (2018) who used it in the chicken diet in the level of 100g and 50g per kg of the diet after artificial infection with *Eimeria* tenella oocyst, they found that the unpalatability of *Psidium gujava* leaves decreased the feed intake of the birds. So the current study declared that the uses of this plant material on bird litter give good results in controlling coccidian transmission. This might be attributed to the presence of flavonoids, phenolic and tannins compounds in *Psidium gujava* extract (Kumar et al., 2021). Although the mechanism by which flavonoids and tannins inhibited the sporulation process is not known (Molan and Farag, 2015), the ability of polyphenolic compounds to inhibit the activities of various endogenous enzymes (Horigome et al.,1988) is well documented. This motivated us to guess that the Gujava extracts, which contain abundant amounts of polyphenolic compounds, may have the capacity to inhibit the enzymes responsible for the sporulation process of the coccidian oocysts.

CONCLUSION

The current research highlights the importance of understanding the correlation between the prevalence of *Eimeria* and *Cryptosporidium* protozoan parasites and the level of application of biosecurity measures. It was found that both protozoan parasites are common among the surveyed poultry farms, with higher *Eimeria* and Cryptosporium spp. prevalence in CBF (53.3%) and in HHF (62.5%), respectively. In reverse to the general understanding of farmworkers and owners, application of farm disinfection was insignificantly affecting the prevalence of both protozoan parasites. Methods used for farm waste and dead carcass disposal were significantly affecting the prevalence of both protozoan parasites. Finally, the highest sporulation inhibition percentage (SP% of 86.4%) was obtained by using of crude watery extract of *Psidium gujava* (10%) at a concentration of 2.5ml/ml after 24hr contact time. This result showed the possibility of decreasing the contamination of bird environment especially the litter with *Eimeria* oocyst which is very helpful in controlling this dangerous protozoan. Additionally, the current method of application rather than adding of *Psidium gujava* to bird feed solves the problems of its use in the previous researches.

CONFLICTS OF INTEREST

Authors doesn´t have conflicts of interest

ACKNOWLEDGMENTS

We would like to thank all the farmers and employees for participating in this study. Their help is greatly appreciated.

Authors' contribution

Fatma Abouelenien: was involved in design, collection of samples, laboratory work, result analysis and manuscript writing.

Ismail Elshahawy: co-supervised the research, provided guide for sample processing, assisted with parasitological sample collection, species identification and corrected the manuscript.

Nagham Elsaidy: was involved in grant writing for the research and correcting the manuscript.

Mohamed Elfatih Hamad: contribute in statistical analysis, writing and English editing.

REFERENCES

Abdisa, T., R. Hasen, T. Tagesu, G. Regea and G. Tadese. 2019. Poultry coccidiosis and its prevention, control. J. Vet. Anim. Res. 2: 103.

Abouelenien, F., M. Eleisway, I. S. Elshahawy, S. Almidany and N. Elsaidy. 2020. Biosecurity practices in commercial and house hold poultry farms in the Delta region, Egypt: I- Correlation between level of biosecurity and prevalence of poultry mites. Thai J. Vet. Med. 50: 315-328.

Ahmed, A., A. M. Olfat, A. N. Aida and S. A. Mohamed. 2012. Studies on coccidia of Egyptian balady breed chickens. Life Sci. J. 9: 568-576.

Ahmed, A. I., M. M. Sulieman, W. A. Istifanus and S. M. Panda. 2018. *In-vivo* anticoccidial activity of crude leaf powder of *Psidium gujava* in broiler chickens. Sch. Acad. J. Pharm. 7: 464-469.

Ajewole, O. C. and A. Akinwumi. 2014. Awareness and practice of biosecurity measures in small scale poultry production in Ekiti State, Nigeria. IOSR-JAVS. 7: 24-29.

Al-Mathal, E. M. 2010. Efficacy of *Commiphora molmol* against hepatic coccidiosis (*Eimeria stiedae*) in the domestic rabbit. J. Food Agric. Environ. 8: 1072-1080.

Al-Quraishy, S., A. S. Abdel-Baki and M. A. Dkhil. 2009. *Eimeria tenella* infection among broiler chicks *Gallus domesticus* in Riyadh city, Saudi Arabia. J. King Saud Univ. Sci. 21: 191-193.

Amer, M. M., M. H. H. Awaad, R. M. El-Khateeb, N. M. Abu-Elezz, A. Sherein-Said, M. M. Ghetas and M. A. Kutkat. 2010. Isolation and Identification of *Eimeria* from Field Coccidiosis in Chickens. J. Am. Sci. 6: 1107-1114.

Awais, M. M., M. Akhtar, Z. Iqbal, F. Muhammad and M. I. Anwar. 2012. Seasonal prevalence of coccidiosis in industrial broiler chickens in Faisalabad, Punjab, Pakistan. Trop. Anim. Health Prod. 44: 323-328.

Bin-Kabir, H., O. Ceylan, C. Ceylan, A. Shehata, H. Bando, M. Essa, X. Xuan, F. Sevinc and K. Kato. 2020. Molecular detection of genotypes and subtypes of *Cryptosporidium* infection in diarrheic calves, lambs, and goat kids from Turkey. Parasitol. Int. 79: 102163.

Bomfim, T. C. B., R. S. Gomes, F. Huber and M. C. M. Couto. 2013. The importance of poultry in environmental dissemination of *Cryptosporidium* spp. Open Vet. Sci. J. 7: 12-17.

Byrne, J. 2019. New Investments in Poultry Feed Mills in Egypt, More Local Corn Planting. Available from: https://www.feednavigator.com/article/2019/03/25/new-investments-in poultry-feed-mills-in-Egypt.

Carvalho, F. S., A. A. Wenceslau, M. Teixeira and G. R. Albuquerque. 2011. Molecular diagnosis of *Eimeria* species affecting naturally infected *Gallus gallus*. Genet. Mol. Res. 10: 996-1005.

Cedric, Y., V. K. Payne, N. A. C. Nadia, N. Kodjio, E. Kollins, L. Megwi, J. Kuiate and M. Mbida. 2018. *In vitro* anticoccidial, antioxidant activities and cytotoxicity of *Psidium gujava* extracts. Res. J. Parasitol. 13: 1-13.

Conway, D. P. and M. E. McKenzie. 2007. Poultry Coccidiosis Diagnostic and Testing Procedures. 3rd ed. Ames, Iowa, Blackwell Publishing.

Dakpogan, H. B. and S. Salifou. 2013. Coccidiosis prevalence and intensity in litter based high stocking density layer rearing system of Benin. J. Anim. Plant Sci. 17: 2522-2526.

Dalloul, R. A. and H. S. Lillehoj. 2006. Recent advances in immunomodulation and vaccination strategies against coccidiosis. Avian Dis. 49: 1-8.

Daniels, M. J., M. R. Hutchings and A. Greig. 2003. The risk of disease transmission to livestock posed by contamination of farm stored feed by wildlife excreta. Epidemiol. Infect. 130: 561-568.

Daugschies, A., B. Bangoura and M. Lendner. 2013. Inactivation of exogenous endoparasite stages by chemical disinfectants: Current state and perspectives. Parasitol. Res.112: 917-932.

Daugschies, A., R. Bose, J. Marx, K. Teich and K. T. Friedhoff. 2002. Development and application of a standardized assay for chemical disinfection of coccidia oocysts. Vet. Parasitol. 103: 299-308.

Dillingham, R. A., A. A. Lima and R. L. Guerrant. 2002. Cryptosporidiosis: Epidemiology and impact. Microbes Infect. 4: 1059-1066.

Drozdz, D., K. Wystalska, K. Malinska, A. Grosser, A. Grobelak and M. Kacprzak. 2020. Management of poultry manure in Poland current state and future perspectives. J. Environ. Manage. 264: 110327.

El-Shahawy, I. S. and F. Abouelenien. 2019. Seroprevalence of *Cryptosporidium* and risks of cryptosporidiosis in residents of Sothern Egypt: A cross-sectional study. Asian Pac. J. Trop. Med. 12: 232.

Gomes, C. C. and N. Sparks. 2020. Exploring the attitudes of backyard poultry keepers to health and biosecurity. Prev. Vet. Med. 174: 104812.

Gradel, K. O., J. C. Jorgensen, J. S. Andersen and J. E. L. Corry. 2004. Monitoring the efficacy of steam and formaldehyde treatment of naturally *Salmonella*-infected layer houses. J. Appl. Microbiol. 96: 613-622.

Hadipour, M. M., A. Olyaie, M. Naderi, F. Azad and O. Nekouie. 2011. Prevalence of *Eimeria* species in scavenging native chickens of Shiraz, Iran. Afr. J. Microbiol. Res. 5: 3296-3299.

Hassan, M. M., A. K. Saha, S. A. Khan, A. Islam, M. Mahabub-Uz-Zaman and S. S. U. Ahmed. 2011. Studies on the antidiarrhoeal, antimicrobial and cytotoxic activities of ethanol-extracted leaves of yellow oleander (*Thevetia peruviana*). Open Vet. J. 1: 28-31.

Helmy, Y. A., J. KruÈcken, E. S. M. Abdelwhab, G. Himmelstjerna and H. M. Hafez. 2017. Molecular diagnosis and characterization of *Cryptosporidium* spp. in turkeys and chickens in Germany reveals evidence for previously undetected parasite species. PLoS One. 12: e0177150.

Henriksen, S. A. and J. F. L. Pohlenz. 1981. Staining of Cryptosporidia by a modified Ziehl-Neelsen technique. Acta Vet. Scand. 22: 594-596.

Horigome, T., R. Kumar and K. Okamotok. 1988. Effects of condensed tannins prepared from leaves of fodder plants on digestive enzymes *in vitro* and in the intestine of rats. Br. J. Nutr. 60: 275-285.

Ibrahim, M. A., A. E. Abdel-Ghany, G. K. Abdel-Latef, S. A. Abdel-Aziz. and S. M. Aboelhadid. 2016. Epidemiology and public health significance of *Cryptosporidium* isolated from cattle, buffaloes, and humans in Egypt. Parasitol. Res. 115: 2439-2448.

Itakura, C., M. Goryo and T. Umemura. 1984. Cryptosporidial infection in chickens. Avian Pathol. 13: 487-499.

Jalal Uddin, A. B. M., M. Rashid, A. I. Khan, M. A. Awal, M. A. Sobhan and A. W. Islam. 2016. Effect of indigenous herbal preparations on coccidiosis of poultry. Res. Agric. Livest. Fish. 3: 145-149.

Karanis, P., C. Kourenti and H. Smith. 2007. Waterborne transmission of protozoan parasites: A worldwide review of outbreaks and lessons learnt. J. Water Health. 5: 1-38.

Kichou, F., F. Saghir and M. El Hamidi. 1996. Natural *Cryptosporidium* sp. infection in broiler chickens in Morocco. Avian Pathol. 25: 103-111.

Kochanowski, M., J. Karamon, J. Dąbrowska, A. Dors, E. Czyżewska-Dors and T. Cencek. 2017. Occurrence of intestinal parasites in pigs in Poland the influence of factors related to the production system. J. Vet. Res. 61: 459-466.

Kumar, N. S., N. M. Sarbon, S. S. Rana, A. D. Chintagunta, S Prathibha, S. K. Ingilala, S. P. Jeevan Kumar, B. Sai Anvesh and V. R. Dirisala. 2021. Extraction of bioactive compounds from *Psidium guajava* leaves and its utilization in preparation of jellies. AMB Express. 11(1): 36.

Kyakuwaire, M., G. Olupot, A. Amoding, P. Nkedi-Kizza and T.A. Basamba. 2019. How safe is chicken litter for land application as an organic fertilizer? A review. Int. J. Environ. Res. Public Health. 16: 3521.

Lawal, J. R., S. M. Jajere, U. I. Ibrahim, Y. A. Geidam, I. A. Gulani, G. Musa and B. U. Ibekwe. 2016. Prevalence of coccidiosis among village and exotic breed of chickens in Maiduguri, Nigeria. Vet. World. 9: 653-659.

Long, P. L. and W. M. Reid. 1982. A Guide for the Diagnosis of Coccidiosis in Chickens. Vol. 404. The University of Georgia, College of Agriculture Experiment Stations, Research Report, pp. 1-17.

López-Osorio, S., J. J. Chaparro-Gutiérrez and L. M. Gómez-Osorio. 2020. Overview of poultry *eimeria* life cycle and host-parasite interactions. Front. Vet. Sci. 7: 384.

Lunden, A., P. Thebo, S. Gunnarsson, P. Hooshmand-Rad, R. Tauson and A. Uggla. 2000. *Eimeria* infections in litter-based, high stocking density systems for loose-housed laying hens in Sweden. Br. Poult. Sci. 41: 440-447.

Majaro, O. M. 2001. New house syndrome: Investigation into the possible role of housefly (*Musca domestica*) in the epidemiology of coccidiosis in chickens. Trop. Vet. 19: 237-242.

Martinez, M., M. Marin, A. C. Torres and M. Lainez. 2008. In Agroalimed (Ed.), Caracterización de las Explotaciones de Pollos de Engorde de la Comunidad Valenciana. Valencia, Spain.

Mohamad, S., N. M. Zin, H. A. Wahab, P. Ibrahim, S. F. Sulaiman, A. S. M. Zahariluddin and S. S. M. Noor. 2011. Antituberculosis potential of some ethnobotanically selected Malaysian plants. J. Ethnopharmacol. 133: 1021-1026.

Molan, A. and A. M. Faraj. 2015. Effect of selenium-rich green tea extract on the course of sporulation of *Eimeria* Oocysts. IOSR J. Dent. Med. Sci. 14: 68-74.

Nnadi, P. A. and S. O. George. 2010. A cross-sectional survey on parasites of chickens in selected villages in the subhumid zones of South-eastern Nigeria. J. Parasitol. Res. 14: 1824.

Papadopoulou, C., E. Xylouri and N. Zisides. 1988. Cryptosporidial infection in broiler chickens in Greece. Avian Dis. 32: 842-843.

Patel, P., R. Sunkara, L. T. Walker and M. Verghese. 2016. Effect of drying techniques on antioxidant capacity of guava fruit. Food Nutr. Sci. 7: 544-554.

Prakashbabua, B., V. Thenmozhi, G. Limona, K. Kunduc, S. Kumar, R. Gargc, E. L. Clark, A. S. R. Raoe, D. G. Raj, M. Raman, P. S. Banerjeec, F. M. Tomley, J. Guitiana and D.P. Blake. 2017. *Eimeria* species occurrence varies between geographic regions and poultry production systems and may influence parasite genetic diversity. Vet. Parasitol. 233: 62-72.

Puleston, R. L., C. M. Mallaghan, D. E. Modha, P. R. Hunter, J. S. Nguyen, C. M. Regan, G. L. Nichols and R. M. Chalmers. 2014. The first recorded outbreak of cryptosporidiosis due to *Cryptosporidium cuniculus* (formerly rabbit genotype), following a water quality incident. J. Water Health. 12: 41-50.

Randall, C. J. 1982. Cryptosporidiosis of the bursa of fabricius and trachea in broilers. Avian Pathol. 11: 95-102.

Rehman, T. U., M. N. Khan, M. S. Sajid, R. Z. Abbas, M. Arshad, Z. Iqbal and A. Iqbal. 2010. Epidemiology of *Eimeria* and associated risk factors in cattle of district Toba Tek Singh, Pakistan. Parasitol. Res. 108: 1171-1177.

Rochelle, P. A., A. M. Johnson, R. De Leon and G. D. Di Giovanni. 2012. Assessing the risk of infectious *Cryptosporidium* in drinking water. J. Am. Water Works Assoc. 104: E325-E336.

Soltane, R., K. Guyot, E Dei-Cas, and A. Ayadi. 2007. Prevalence of *Cryptosporidium* spp. (*Eucoccidiorida*: *Cryptosporiidae*) in seven species of farm animals in Tunisia. Parasite. 14: 335-338.

Toledo, R. D. S., F. D. C. Martins, F. P. Ferreira, J. C. De Almeida, L. Ogawa, H. L. Santos, M. dos Santos, F. Pinheiro, L. Navarro, J. Garcia and R. Freire. 2017. *Cryptosporidium* spp. and *Giardia* spp. In feces and water and the associated exposure factors on dairy farms. PLoS One. 12: e0175311.

Wang, R., F. Jian, Y. Sun, Q. Hu, J. Zhu, F. Wang, C. Ning, Z. Longxian and L. Xiao. 2010. Large scale survey of *Cryptosporidium* spp. in chickens and Pekin ducks (*Anas platyrhynchos*) in Henan, China: prevalence and molecular characterization. Avian Pathol. 39: 447-451.

Watkinson, W. J. 2008. Chemistry of detergents and disinfectants. In: A. Y. Tamime, (Ed.), Cleaning-in Place: Dairy, Food and Beverage Operations. 3rd ed. Blackwell Publishing, Hoboken, New Jersey.

Wondimu, A., M. E. Ermias and B. Y. Yehualashet. 2019. Prevalence of poultry coccidiosis and associated risk factors in intensive farming system of Gondar town, Ethiopia. Vet. Med. Int. 2019: 5748690.

You, M. J. 2014. Suppression of *Eimeria tenella* Sporulation by Disinfectants. Korean J. Parasitol. 52: 435-438.