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Coccidiosis in Japanese quails (*Coturnix coturnix japonica*) in Kalioubia governorate: prevalence and treatment trials

Mohamed Y. Ramadan, Reham S. Elmadawy, Tolba M Ibrahim.

Parasitology Department, Faculty of Veterinary Medicine, Benha University.

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ABSTRACT

This study was undertaken to identify *Eimeria* species in Japanese quail (*Coturnix coturnix japonica*) from different localities in Kalioubia governorate. In addition, an experimental study has been done on quail to study the efficacy of some natural anticoccidial products (propolis and neem; *Azadirachta indica*) compared to amprol (Amprolium hydrochloride plus ethopobate) as a chemical anti-coccidial drug on the oocyst output, pathogenicity, weight gain, mortality, severity of diarrhea, intestinal lesion scores and oocysts sporulation. Out of 900 examined quails, 575 (63.8%) were infected by *Eimeria*. Quail farms in Abozabl showed the highest infection (73.3%), while the lowest infection (58.6%) was recorded in quail farm in Kafrshokr. The detected species were *E. bateri, E.uzura, E. tsunodai, , Eimeria bahli* and *Eimeria colini*. On the basis of the study results, it was concluded that that amprol, propolis and neem had comparable significant effectivity in reduction of oocyst counts, vanishing coccidiosis symptoms, declining the mortality cases, waning the inflammatory intestinal lesion. Moreover, propolisoutperform the other groups in increasing the body gain of coccidiosis infected quails and showed a significant declining of the percentage of sporulation of *Eimeria* oocysts obtained from treated birds

1. INTRODUCTION

The production of quail is a branch of poultry modern industry like animal production systems. Japanese quails breeding is widespread worldwide which has a brilliant part in aviculture due to the increase of the consumption of meat and exotic eggs and it is considered as a substitute to chicken production. Japanese quails in recent decades become an important for scientific research as it is used widely in studies concerning nutrition, genetics, embryology, toxicology, pathology, and physiology (Cardozo *et al.,* 2010, Jatoi*et al.,* 2013). In common with the condition met in breeding of other poultry spp., parasitosis is a preventive factor in high production rearing commercial farms and in rustic breeding systems, where significant economic losses can be noted. The most pathogenic parasites infecting poultry is coccidiosis caused by *Eimeria* spp. and it is characterized by severe enteropathy, which endorses lowering reproduction, anorexia, decreased posture in adults, and increasing the level of mortality (Norton and Pierce 1971, Ruff *et al*., 1984, Teixeira *et al*., 2004). The study aimed to characterize *Eimeria* spp. infecting Japanese quail farms in Kalioubia governorate. Moreover, and due to the hazard effect of the extensive use of chemical anticoccidial drugs which led to the development of drug-resistant parasites and the side effects of some anticoccidial drugs which have serious consequences for future control (Williams, 2006). Therefore, a second aim of this study is to evaluate the efficacy of some natural anticoccidial products as propolis and neem (*Azadirachta indica*) compared to amprol (Amprolium hydrochloride) as chemical anticoccidial drug.

2. MATERIAL AND METHODS

2.1 Examination of Japanese quail for coccidiosis

2.1.1. Birds

A total of 900 Japanese quails from different farms in Kalioubia governorate (Abo Zabel, Kafr Shoker, Shbeen Elqanater and Elqanater Elkhyreh) were examined for *Eimeria* infection.

2.1.2. Fecal samples

Fecal dropping was collected from Japanese quails. Samples were placed into plastic bags and later processed at parasitology laboratory, Faculty of Veterinary Medicine, Benha university.

2.1.3. Preparation of oocysts

Oocysts were sporulated in 2.5% aqueous potassium dichromate (K2Cr2O7) in petri dishes at room temperature. The oocysts after sporulation were recovered by centrifugation using sugar saturated solution as described by Duszynski & Wilber (1997) and used subsequently.

* Corresponding author: rehamelmadawy@gmail.com

2.1.4. Morphometric characters of the oocysts

Eimeria species were described based on morphological characters as well as morphometric characteristics (dimensions) of oocysts were described according to the key of Norton and Pierce (1971) using a binocular microscope and an eye piece micrometer.

2.2. Efficacy of chemical and natural drugs on quail coccidiosis

2.2.1. Drug preparation

The chemical drug used was amprol powder; Amprolium hydrochloride plus ethopobate (20%). The used propolis was a dry dark-colored natural material which was purchased as powder from the market, and it was diluted with water to be used at 20 % concertation. (Asy, 2011). The leaves of neem *(Azadirachta indica) used in this study were* collected from the neem trees in Kalioubia governorate, then the Fresh leaves of neem were grinded with water using an electric blender, sieved and the resultant extract (neem juice) was collected and kept in dark bottle till use to be added in the quail waterer (Patrick et al.,2010).

2.2.2. Experimental design

A total of 250 quails (One day old Japanese quails) were purchased from a local flock for experimental study. The quails were kept in an isolated room and were fed on clean balanced ration free from antibiotics and anticoccidial drugs with free access of clean source of water all period of experiment. The quails were allocated into 5 groups: each of 50 birds. Each quail in four groups was inoculated with 10^3 mixed spp. of *Eimeria* oocysts (*E. bateri*, *E.uzura*, E. *tsunodai*, *Eimeria bahli* and *Eimeria colini*). In group 5 birds were kept as control negative (CN).

2.2.3. Drug application

Application of the drugs occurs on 6th day post infection (DPI). All the used drugs were provided in drinking water of quail. The quails in group 1 were treated by amprol (Amprolium hydrochloride 20%). Group 2, the quails were treated with propolis (20%). Group 3 (neem group) were treated with aqueous extract of neem (20%). Quails in group 4 were kept as a control positive (CP) without treatment.

2.2.4. Oocysts counting

Fecal sample were collected daily for 30 days from each group of quails and the number of oocysts per gram of feces (OPG) was determined using a technique described by (Menezes and Lopes, 1995). The sporulation % of the oocysts was also studied till 10th DpI to examine the efficacy of the drugs on oocyst sporulation after treatment.

2.2.5. Clinical symptoms

The feed intake body weight of birds and mortalities were daily recorded and clinical signs after infection were observed.

2.2.6. Necropsy

The dead bird in each group was necropsied. where, parts of the small intestine (jejunum and ileum) and caeca examined for gross lesion according to Johnson and Reid (1970).

2.3. Statistical analysis

It was done by two ways ANOVA using statistical package for the social sciences (SPSS) ver25.

3. RESULTS

The present study showed that the overall *Eimeria* infection in quail farms in Kaluobia governorate was 63.8%. Quail farms in Abozabl showed the highest infection (73.3%), while the lowest infection (58.6%) was recorded in in Kafrshokr farm. The detected *Eimeria* spp. were *E. bateri*, *E.uzura,* E. *tsunodai*, *E.bahli* and *E. colini* (Table1, 2, Fig. 1).

Upon studying the effect of propolis, neem and amprol on oocysts counts, the results revealed that oocysts shedding started on 6th DpI (prepatent period) was decreased gradually ($p< 0.05$) from 6th DpI(250 OpG), till complete stoppage of oocyst excretion on 12nd DPI in amprol treated group. Moreover, declining of oocysts excretion occurred and totally stopped on 16th and 20th DpI for propolis and neem treated groups; respectively. While a continuation of oocysts shedding till 30th DpI in CP group was recorded (Table 3).

In different treated group quail suffered from symptoms of coccidiosis including: ruffled feathers, depression, decrease appetite, bloody diarrhea, Enlarged two ceca and bloody cecal core on the 4th DpI with a percentage of 6% each for amprol, neem and 8% for propolis groups, but this symptoms subsided gradually from 8th DpI till the end of the experiment as compared to CP group ($p< 0.05$) which suffered from upgraded pathogenicity from 4th DpI (6%-66%) till the end of the experiment (Table 4, Fig.2). The total numbers of the dead birds at the end of the experiment were 4%, 6% and 6% for amprol, propolis and neem groups; respectively, without any significant difference($p> 0.05$) throughout the experiment, while the percentage of dead birds in CP group was (32%) among quails compared to the other groups ($p< 0.05$) (Table 5, Fig. 2). In amprol and propolis groups, only one bird (2%) in each group showed cecal enlargement, ballooning and bloody cecal core on 7th DPI, Though, quails in neem group showed the same cecal lesion in two birds on 7th and 10th DPI, but no significant difference was recorded ($p> 0.05$) except with CP, where 28% of the quails showed lesions (Table 6).A weekly comparison showed a pronounced increase in the body weight of quails in all groups from 1st to 4th week, however propolis group achieved the heaviest body gain ($p< 0.05$) compared to amprol, neem and CP groups (Table 7).

Regarding the effect of the used drugs on the percentage of sporulation of *Eimeria* oocysts, the results exhibited that propolis showed the highest reduction percent of sporulation (29%) compared to the other ($p< 0.05$, Table8).

Table 1: Incidence of Eimeria spp. in quail farms in different localities in Kalioubia governorate

Locality	No. examined	No. infected	%	Detected *Eimeria* spp.
Abo zabel	225	165	73.3	*E. bateri, E.uzura, E. tsunodai, E. bahli and E. colini*
Kafr shoker	225	132	58.6	*E. bateri, E.uzura, E. tsunodai,*
Shbeen elqanater	225	141	62.6	*E. bateri, E.uzura, E. tsunodai,*
Elqanater elkayrea	225	137	60.8	*E. bateri, E.uzura, E. tsunodai, , E. bahli and E. colini*
Total	900	575	63.8	

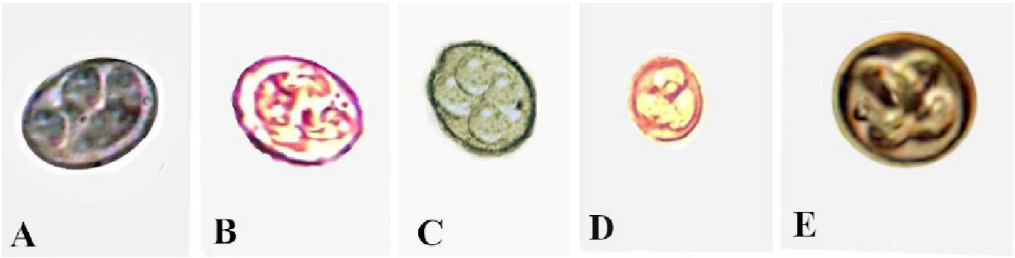

Fig (1): Eimeria species infecting Japenes quail (Coturnix coturnix japonica). A. *Eimeria bateri*, B. *Eimeria uzura*, C. *Eimeria tsunodai*, D.*Eimeria bahli*, E. *Eimeria colini.*

Table (2) Morphology of the Eimeria oocysts

Characters	*E. bateri*	*E. uzura*	*E. tsundai*	*E. bahli*	*E. colini*
Oocyst's measurement (um)	21.2X15	18.2X13.4	20X18	16.7X17.5	24.2X20.6
Micropyle	-	-	+	+	-
Number of Polar granule (s)	1	2- 4	1	-	1
Oocystic residium	-	-	-	-	-
sporocysts	pear shape with stieda bodies and sporocystic residium	fusiform with stieda bodies and sporocystic residium	ovoid with stieda bodies and sporocystic residium	oval with stieda bodies and sporocystic residium	Curved fusiform with stieda bodi and sporocystic residium

Table 3: Average number of oocysts per gram (OPG) excreted by quail treated by amprol, propolis and neem compared to Control positive (CP) group. (50 quails in each group)

DpI	No. of oocysts			
	Amprol group	Propolis group	Neem group	Cp
1th-5th	$0a^{A}$	$0a^{A}$	$0a^{A}$	$0g^{A}$
6th	$0a^{A}$	0aA	$0a^{A}$	$0g^{A}$
7th	$0a^{A}$	0aA	$0a^{A}$	$0g^{A}$
8th	$0a^{A}$	0aA	$0a^{A}$	$0g^{A}$
9th	$0a^{A}$	0aA	$0a^{A}$	$0g^{A}$
10th	250^{aB}	600^{aB}	550^{aB}	1850^{defA}
11th	150^{aB}	550^{aB}	600^{aB}	3800^{abcA}
12th	150^{aB}	600^{aB}	500^{aB}	$4000a^{bcA}$
13th	100^{aB}	550^{aB}	450^{aB}	$4400a^{bA}$
14th	50^{aB}	250^{aB}	400^{aB}	4500^{aA}
15th	50^{aB}	300^{aB}	300^{aB}	$3650a^{bcA}$
16th	0^{aB}	200^{aB}	300^{aB}	3000^{cdA}
17th	0^{aB}	100^{aB}	400^{aB}	$4000a^{bcA}$
18th	0^{aB}	100^{aB}	200^{aB}	3750^{abA}
19th	0^{aB}	50^{aB}	100^{aB}	3000^{cdA}
20-30th	0^{aB}	0^{aB}	200^{aB}	3250^{bcA}

a, b & c: There is no significant difference (P>0.05) between any two means, within the same column have the same superscript letter.A, B & C: There is no significant difference (P>0.05) between any two means for the same attribute, within the same row have the same superscript letter. LSDG (Group): 529, DpI : day post infection , CP: Control positive

Table 4: Percentage of coccidiosis symptoms showed in quails treated by amprol, proplis and neem groups (50 quails in each group).

DPI	Amprol	Proplis	Neem	CP
4th	3 (6%) ^{aA}	4(8%) ^{aA}	3 (6%) ^{aA}	3(6%) ^{fA}
6th	2 (4%) ^{aA}	2 (4%) ^{aA}	2(4%) ^{aA}	6(12%) ^{fA}
8th	2 (4%) ^{aB}	2 (4%) ^{aB}	2(4%) ^{aB}	8 (16%) ^{efA}
10th	0.00^{aB}	0.00^{aB}	0.00^{aB}	11 (22 %) ^{efA}
12th	0.00^{aB}	0.00^{aB}	0.00^{aB}	18 (36%) ^{cdA}
14th	0.00^{aB}	0.00^{aB}	0.00^{aB}	23(46%) ^{bcA}
16th	0.00^{aB}	0.00^{aB}	0.00^{aB}	30 (60%) ^{abA}
18th	0.00^{aB}	0.00^{aB}	0.00^{aB}	33 (66%) ^{aA}
20th	0.00^{aB}	0.00^{aB}	0.00^{aB}	15 (30 %) ^{cdeA}

a, b & c: There is no significant difference (P>0.05) between any two means, within the same column have the same superscript letter. A, B & C: There is no significant difference (P>0.05) between any two means for the same attribute, within the same row have the same superscript letter., LSD (group): 9.72, DpI : day post infection , CP: Control positive

4. DISCUSSION

Coccidian infections particularly with *Eimeria* species are one of most hazardous diseases facing production industry. *Eimerian* species are highly pathogenic causing great losses. The available information on coccidian species infecting quails in Egypt is very scarce.In this present study the prevalence of *Eimeria* infection among farmed Japanese quails (*Coturnix coturnix japonica*) examined was 63.8%. In this respect, Elmadawy (2001) in Kalioubia, Egypt observed the infection of 80% of the quail, Abedel-Aal and El-Sayed (2003) in Sharkia, Egypt who recorded that 76.6% of the examined quails were *Eimeria* infected, Abd-El-Maged (2005) in Egypt who recorded an infection rate of 31.5% and Basiouny *et al* (2017) found the infection in 23.21% of the examined farm quails in Sharkia, Egypt. Moreover, Bashtar *et al*. (2010), Mohammad (2012) in Iraq, Arafat and abbas (2018) in Saudi Arabia noted *Eimeria* infection in 80%, 49.4% and 31.78% 52% of Japanese quails. Five species of *Eimeria* were detected in the study, these are *E. bateri*, E. uzura, *E. tsunodai*, *E. bahli* and *E. colini*. These results coincided with that of El-Morsy *et al.,* (2016) in Mansoura and Basiouny *et al*. (2017) in Sharkia, Egypt. Though, Abedel-Aal and El-Sayed (2003) in Sharkia, Egypt found more than six species of *Eimeria* namely; *E. bateri, E. bahli, E. oreortygis, E. uzura, E. tsuondai, E. colini*, and un identified *Eimeria* sp. from common quails (*C.coturnix*), while Abd-El-Maged (2005) in Sharkia, Egypt recorded two species of *Eimeria*; *E. bateri* and un identified *Eimeria* sp. in farm's quails.

Table 5: Mortality rate of quails in each of the treated group by amprol, Propolis and neem compared to CP group. (50 quails in each group).

DpI	No. of dead birds (%)			
	Amprol group	Propolis group	Neem group	CP
3rd	0^{aA}	0^{aA}	0^{aA}	0^{dA}
4th	0^{aA}	0^{aA}	0^{aA}	0^{dA}
5th	0^{aB}	1 (2%) ^{aA}	0^{aB}	1 (2%) ^{cdA}
6th	1 (2%) ^{aA}	1 (2%) ^{aA}	1 (2%) ^{aA}	1 (2%) ^{cdA}
7th	1 (2%) ^{aB}	1 (2%) ^{aB}	1 (2%) ^{aB}	2 (4%) ^{bcA}
8th	0^{aC}	0^{aC}	1 (2%) ^{aB}	5 (10 %) ^{aA}
9th	0^{aB}	0^{aB}	0^{aB}	3 (6%) ^{bA}
10th	0^{aB}	0^{aB}	0^{aB}	2 (4%) ^{bcA}
11th	0^{aB}	0^{aB}	0^{aB}	1 (2%) ^{cdA}
12th	0^{aB}	0^{aB}	0^{aB}	1 (2%) ^{cdA}
13rd-30th	0^{aB}	0^{aB}	0^{aB}	0^{dA}
Total	2 (4%) ^{aB}	3 (6%) ^{aB}	3 (6%) ^{aB}	16 (32%) ^{bC}

a, b & c: There is no significant difference (P>0.05) between any two means, within the same column have the same superscript letter. A, B & C: There is no significant difference (P>0.05) between any two means for the same attribute, within the same row have the same superscript letter. LSD(Group): 1.11, DpI : day post infection , CP: Control positive

The results in the present work clarified that coccidiosis in experimentally infected quails caused mild to severe pathogenicity. This verify the opinion of many authors, since Norton & Pierce (1971) recorded that experimentally infected young Japanese quails with *E. bateri* and showed mild loss of weight, anorexia and softening of feces were observed. Tsunoda & Muraki (1971) also reported a minimum pathogenicity in experimentally infected Japanese quails which suffered from diarrhea and anemia. Nevertheless, Ruff & Fagan (1984) showed mortality, low weight gain and poor reproductive performance. The severity of the symptoms recorded in this study may be attribute to the young age of the experimentally infected quail (Teixeira *et al.,* 2004). The macroscopic lesion of recorded due to *Eimeria* was mainly restricted to the two ceca including congestive and hemorrhagic lesion, this observation coincided with that of Mohamed (2012) and the cecal ballooning which was the main gross lesion reported by Umar *et al.* (2014).

Table 6: Percentage of cecal lesion among different groups of quails treated by amprol, propolis and neem compared to CP group. (50 quails in each group).

DpI	No. of Birds having caecal lesion (%)			
	Amprol group	Propolis group	Neem group	CP
7th	1 (2 %) ^{aA}	1 (2 %) ^{aA}	1 (2 %) ^{aA}	1 (2 %) ^{cdA}
10th	0	0	1 (2 %) ^{aB}	6 (12%) ^{aA}
13th	0	0	0	3 (6%) ^{bA}
16th	0	0	0	3(6%) ^{bA}
20th	0	0	0	1 (2%) ^{cdA}
21st- 30th	0	0	0	0
Total	1 (2 %) ^{aA}	1 (2 %) ^{aA}	2 (4%) ^{aA}	14 (28%) ^{bC}

a, b & c: There is no significant difference (P>0.05) between any two means, within the same column have the same superscript letter. A, B & C: There is no significant difference (P>0.05) between any two means for the same attribute, within the same row have the same superscript letter. LSD(Group): 1.31, DpI : day post infection , CP: Control positive.

Fig 2: A-H The clinical symtoms and PM lesion due to of quail coccidiosis. A-C Control positive group of quails showing symptoms of ruffled feathers, loss of appetite ,retardation of growth, walls recumbent position, emaciation and death, D-G. Caecal congestion, bloody tinged intestinal contents with enlargement due to Eimeria bateri, H. Ballooned caecum in quail due to Eimeria tsunodai.

The current study demonstrated the beneficial effect of the usage of amprol, propolis and neem extract when used individually against quail coccidiosis, where they have been reported cessation of oocyst outputs, disappearance of clinical signs, lesions and improvement of body weight among treated quail when compared to the positive control group. This is possibly due to the inhibition of the intestinal mucosal inflammation which is indicative of the increased nutrient absorption through the intestinal wall and enhanced conversion ratio of feed.

Table 7: Average body weight (gm) among quails treated by amprol, propolis and neem compared to CP group. (50 quails in each group).

	Weight (gm) of quails				
Week	Amprol group	Propolis group	Neem group	C P	CN
1st	42^{dA}	47^{dA}	40^{dA}	45^{dA}	46^{dA}
2nd	100^{cAB}	115^{cA}	110^{cA}	90^{cB}	103^{cA}
3rd	165^{bA}	180^{bA}	180^{bA}	140^{bB}	164^{bA}
4th	195^{aB}	220^{aA}	200^{aB}	175^{aC}	223^{aA}

a, b & c: There is no significant difference (P>0.05) between any two means, within the same column have the same superscript letter. A, B & C: There is no significant difference (P>0.05) between any two means for the same attribute, within the same row have the same superscript letter. SD(Group):15.08, DpI: Day post infection CP: Control positive, CN: control negative.

Mainly in this study, amprol as a chemical showed the best effect on quail coccidiosis in term of reduction oocyst count and reduction of sporulation%, followed by Propolis and Neem, this is because amprol is a long term and widely used anticoccidial agent which was previously proved to effectively reduces the level of fecal *Eimeria* oocysts in cattle and poultry (Gibbons *et al*; 2016 , Avais *et al;* 2016) as it antagonize thiamine (vitamin B1), where rapidly dividing coccidia have a high requirement for thiamine (Richard and Gerhold, 2014).

Table 8: Efficacy of different drugs on sporulation % of Eimeria in quails treated by amprol, propolis and neem compared to CP group. (50 quails in each group).

	% of sporulation			
DPI	Amprol group	Propolis group	Neem group	Control Positive
6th	10 $\%^{cB}$	$16\%^{cB}$	$12\%^{dB}$	$60\%^{bA}$
7th	$20\%^{bcC}$	$28\%^{bBC}$	$36\%^{cB}$	$65\%^{bA}$
8th	$24\%^{bD}$	$39\%^{aC}$	$55\%^{aB}$	$79\%^{aA}$
10th	$42\%^{aB}$	$32\%^{abC}$	$44\%^{bcB}$	$78\%^{aA}$
Average	$27.2\%^{aA}$	$29\%^{aA}$	$39.8\%^{aB}$	$72.4\%^{aC}$

a, b & c: There is no significant difference (P>0.05) between any two means, within the same column have the same superscript letter.A, B & C: There is no significant difference (P>0.05) between any two means for the same attribute, within the same row have the same superscript letter. LSD(Group): 9.38, DpI : day post infection , CP: Control positive

Although limited research discussed the effects of other used natural drugs (propolis and neem) on quail coccidiosis. The neem effect on coccidiosis was previously recorded by (Toulah et al. 2010), Zyan *et al.* (2017), Eze *et al*. (2019) and Dogo *et al*. (2020) against chicken coccidiosis. So, far the precise mechanism of action of neem against coccidia is unknown, but the report of the National Research Council (1992) proposed that *Azadirachta indica* aqueous leaf extract when taken orally enhances the cellular immunity response and increasing antibody level. Thus, most pathogens can be eliminated easily before they cause ill effects associated with this disease. Likewise, for animal coccidiosis, many investigators indicated that Propolis helps to improve symptoms of coccidiosis including appetite and body gain (El- Akabawy *et al.,* 2004, Abdel-Maged *et al.,* 2013, Ahmed *et al*., 2014). Moreover, it decreased OPG and stopped diarrhea in goats (Ghanem *et al.,* 2009). The anticoccidial efficacy of propolis against quail coccidiosis might be attributed to preventing the damage of the intestinal epithelium caused by the multiplication of *Eimeria* stages which impaired absorption, utilization and assimilation of some elements such as iron and copper (Omar 1995). In this study, an evidence to support a lower oocyst sporulation rate was obtained after treatment application in different groups, where the average number of sporulated oocysts were the uppermost among CP (72.4%) followed by Neem (39.8%), propolis (29%) and amprol (27.2%) groups. This can indicate the potent effect of the used drugs not only on the endogenous stage of *Eimeria* but also on the sporulation of oocysts which help in controlling the disease.

4. CONCLUSION

Coccidiosis is a very ubiquitous disease in Japanese quail. The prevalence of infection was 63.8%. Five species of Eimeria were detected, Those are *E. bateri*, *E.uzura*, *E. tsunodai,Eimeria bahli* and *Eimeria colini.* The present study of the therapeutic trials using a chemical drug (amprol) and two natural drugs (propolis and neem), it was concluded that that amprol, propolis and neem had comparable significant effectivity in reduction of oocyst counts, vanishing coccidiosis symptoms, declining the mortality cases, waning the inflammatory intestinal lesion. Moreover, propolisoutperform the other groups in increasing the body gain of coccidiosis infected quails and showed a significant declining of the percentage of sporulation of *Eimeria* oocysts obtained from treated birds. The hazard effect of the used chemical drug (amprol) makes the therapeutic potential of these natural compounds and/or extracts (propolis and neem) in coccidiosis treatment is considered promising to avoid the peril outcome of the chemical. Further studies are needed to the reason of lowering oocyst sporulation rate by natural drugs and if this related to change in the gametogenesis process or in the structure of the oocysts or due to any other cause.

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