Global Veterinaria 8 (3): 285-291, 2012
ISSN 1992-6197

Coccidiosis Prevailing in Parent Stocks: A Comparative Study Between Growers and Adult Layers in Kombolcha Poultry Breeding and Multiplication Center, Ethiopia

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Abstract: A cross sectional study was conducted in 828 dead grower chickens and parent stocks in Kombolcha poultry farm, Ethiopia, between October 2009 and March 2010. The objectives of this study were to evaluate the prevalence of coccidiosis, to identify the prevalent species of *Eimeria* and to assess the predisposing factors. The study involved post mortem examination of dead birds, mucosal scraping examination, examination of gross and histopathological changes and identification of *Eimeria* species. Overall prevalence 25.24% (95 % CI: 22.28 - 28.20) of clinical coccidiosis was assessed. Prevalence rates of 22.3% (95 % CI: 19.20 - 25.65) and 35.3% (95 % CI: 28.47- 42.05) was recorded in White leghorn (WLH) grower chickens and in parent stocks of WLH and Rhode Island Red (RIR) breeds, respectively. The prevalence of clinical coccidiosis was significantly higher in adults than the growers ($P < 0.05$). However, no statistically significant difference was observed between sexes and breeds. *E. tenella*, *E. brunetti*, *E. necatrix*, *E. acervulina* and *E. maxima* were identified. *E. maxima* were found for the first time in the farm. Coccidiosis prevalence has decreased among growers, but is reciprocal in the adults alarming the need for control and prevention measures in the parent stocks.

Key words: Adult Layers · Coccidiosis · Eimeria · Ethiopia · Growers · Prevalence

INTRODUCTION

Coccidiosis remains one of the most expensive and common diseases of poultry in-spite of advances made in prevention and control through chemotherapy, management, nutrition [1] and genetics [2,3]. In all parts of the world where confinement rearing is practiced, coccidiosis represents a major disease problem demanding the attention of poultry producers, feed manufactures and poultry disease experts [4]. In Ethiopia despite the immense research works done by several outstanding researchers in the area of poultry coccidiosis in different parts of the country [5-11], the disease is still continued being a major problem demanding much research and investigation. Regarding the disease poultry coccidiosis in Amhara region in Kombolcha Poultry Breeding and Multiplication centre (KPBMC), information is scant except Lobago *et al.* [12] who have done his research half decades ago on Rhode Island Red (RIR) grower chickens of age 1- 60 days. However, no research has been done in White Leghorn grower chickens of 1- 60 days age and parent stocks, yet. Therefore, the objectives of this research were to evaluate the prevalence of coccidiosis in White Leghorn grower chickens from 1- 60 days age, to identify the prevalent species of *Eimeria*, to determine the prevalence of poultry coccidiosis in the adult (> 230 days old) parent stocks of Rhode Island Red (RIR) and White Leghorn exotic breeds and to assess some of the predisposing factors associated with occurrence of poultry coccidiosis.

MATERIALS AND METHODS

Study Area: The study was conducted in Kombolcha poultry breeding and multiplication centre (KPBMC), Kombolcha, south Wollo, north eastern Ethiopia of the Amhara national regional state. It is located 380 km north of Addis Ababa and 500 km west of Bahir Dar at an

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altitude of 1864 meter above sea level the centre is situated at 11° 07' N latitude and 39^0 44' E longitudes. The size of the farm is 7.5 hectare. The area has experienced a bimodal rain fall distribution with a three year an annual average of 1038 mm, annual mean temperature of 18°C and relative humidity from 23.9% to 79% [13].

Study Birds: The study was performed in two different bird age groups in which the first study group was carried out in layer type White Leghorn (WLH) breeds of grower chickens age from 1-60 days, which were kept for an extension service program. The second study group was comprised of adult breeders (layers) of Rhode Island Red (RIR) and White Leghorn breeds to evaluate the significance of coccidiosis in these breeds and to compare the prevalence between ages (young versus adult). The farm hold 19,212 WLH grower chickens of five different batches' hatched with a week age difference (interval) reared in 6 houses and 5,158 adult breeders (layers) of RIR and WLH bovans breeds, kept under intensive deep litter management system.

Housing and Poultry Management: The Kombolcha poultry breeding and multiplication centre (KPBMC) is a deep litter large scale state owned intensive poultry farm with a land holding capacity of 7.5 hectare. Currently, the farm has 13 functional poultry houses from which 6 were used for rearing of 19,212 grower chickens and the remaining 7 houses were for raising of parent stocks, during the study period. Four of the rearing houses have an area of 307 m^2 and the rest two houses have 207 m^2 each. Each of the four parent stock houses has an area of 350 m^2 and the remaining three houses have an area of 307 m^2.

The farm has flock sizes ranging from 2180 - 4190 per rearing house and 1170 - 2000 per raising (parent stocks) houses. Feed is given in troughs suspended at a breast height for chickens above 3 wks of age, but for the very early age chicks the trough is put on the ground. Watering is given through pipe using cup connected with hollow plastic tubes and suspended above the ground. They are closed and opened manually but operate automatically when pressed by chicken's mouth [12].

The farm's flock health management was basically based on prevention and comprised of vaccination, medication, bio - security and sanitation (cleaning and disinfection). Vaccination against New castle disease was given in three rounds at l-7, 18-21 and 60th days of age. Moreover, Gumboro (infectious bursal disease) vaccination was also given at 21st and 28th days of age. Anticoccidial drugs were also given to the growers starting from the second weeks of age for 7 to 14 days during the growing periods for prevention purposes.

Study Design and Sample Size Determination: The study design consists of cross sectional study to determine the prevalence of coccidiosis in dead birds of grower chickens of WLH breeds and adult breeders (layers) of RIR and WLH bovans breeds and to identify the prevalent species of Eimeria in the study area. The sample size was determined based on the assumption of the possible or expected prevalence rate of the disease recorded in the study area which was 38.34% [12]. The formula applied to calculate sample size was the formula for simple random sampling method and the study has considered 95% CI and 5% absolute precision [14]. Therefore, a minimum sample size of 363 birds was considered for this study.

Post Mortem Examination: Post mortem examination was conducted on these randomly selected 638 dead grower chickens and 190 adult parent stocks following the procedure described by Conway and McKenzie [15] and the gastro intestinal tract was thoroughly examined for gross pathological changes as described by Gari *et al.* [11] and Lobago *et al.* [12].

Mucosal Scraping Examination: Mucosal scrapings to demonstrate the parasite developmental stages of coccidia along with lesions by microscopic examination were done according to Lobago *et al.* [12].

Histopathological Examination: Tissue samples of intestines about 1-3 cm length were collected and submitted to the pathology section of the Faculty of Veterinary Medicine, University of Gondar and processed for histopathological examination according to Luna [16].

Identification of Species of Coccidian: *Eimeria* species were identified morphologically and histopathologically based on the identification characteristics given by Conway and McKenzie [15].

Data Analysis: The data were analyzed using SPSS version-17 statistical software. Pearson's Chi square test has been used to measure statistical significance of results. In order to consider a result to be statistically significant 95% CI and P value < 0.05 has been taken.

RESULTS

Overall Prevalence of Clinical Coccidiosis in Growers and Adult Layers: Out of the total 828 birds examined, overall prevalence 25.24% of clinical coccidiosis in both growers WLH and adult layers of WLH and RIR breeds was recorded (Table 1). The difference between the growers and adults was statistically significant $p < 0.05$. The prevalence was higher in adults (35.3%) as compared to growers (22.3%).

Prevalence of Coccidiosis and *Eimeria Species* Identification in Growers of WLH Breeds: Out of the 638 sampled dead birds clinical coccidiosis was found in 142 (22.3%) birds. Of these, clinical coccidiosis positive cases of 16.5%, 51.4%, 67.3% and 41.0 % were found from 21-30 days, 31-40 days, 41-50 days and 51-60 days of age, respectively. There were no cases in the first twenty days of age. The highest number of clinical coccidiosis cases (67.3%) were, recorded at age 41-50 days. There was a statistically significant difference ($P<0.05$) in the prevalence of coccidiosis at different age of birds (Table 2).

In the current study, five Eimeria species, namely *E. acervulina*, *E. necatrix*, *E. brunetti, E.tenella* and *E.maxima* were identified in single infections. Mixed infections were the predominant cases recorded due to *E. tenella* together with any of the four species (*E. brunetti*, *E. necatrix*, *E. acervulina* and rarely with *E. maxima*). The distribution and occurrence of Eimeria species at different age groups as either single or mixed infections were significantly different and in all species, the peak of occurrence or prevalence was noted at age 41-50 days. Regarding the temporal distribution of the Eimeria species identified, either as single or mixed infections at different age of birds, *E. tenella* and *E. brunetti*, occurred most frequently with prevalence of 37.86% and 29.22%, respectively. Whereas the rest three species identified, *E. acervalina* (15.22%) *E. necatrix* (12.35%) and *E.maxima* (5.35%) were low in prevalence. *E. maxima* were diagnosed for the first time in the current study in this study area. It was the largest in size, ovoid in shape and has golden brown colour observed during microscopic examinations of mucosal scraping smears taken from the middle small intestine. Moreover, histopathological examination results revealed large gametocytes.

Prevalence in RIR and WLH Parent Stocks (Layers): In a trial to find whether there is a difference in the prevalence of clinical coccidiosis in adult layers between RIR and WLH breeds, it was found that out of the 190 examined dead birds, clinical coccidiosis was found in 67 (35.3%) birds. Of this total prevalence, the prevalence of clinical coccidiosis in RIR and WLH was found to be 26(36.1%) and 41(34.7%), respectively. Moreover, the prevalence in male and female birds of both genotypes was 32.3% and 36.8%, respectively (Table 3). The occurrence of clinical coccidiosis in the RIR and WLH bovans breeds was not significant. However, the prevalence was a bit higher in Rhode Island Red breed than White Leghorn breed (Table 3). Likewise, the prevalence between male and female was also not significant (Table 3).

Table 1: Overall prevalence of clinical coccidiosis in growers and adult layers in Kombolcha poultry farm

Age group	No. examined	No. of positive cases	Prevalence (%)	95% CI
Growers	638	142	22.3 %	0.1920 - 0.2565
Adults	190	67	35.3%	0.2847- 0.4205
Total	828	209	25.24 %	0.2228 - 0.2820

Table 2: Mortality rates due to Coccidiosis of White Leghorn grower chickens in Kombolcha Poultry farm between 2009/2010

Age group	No. examined	No. of positive cases	Prevalence (%)	95% CI
1-10 days	224	0	0 %	0 - 0.0169
11-20 days	102	0	0%	0 - 0.0363
21-30 days	79	13	16.5 %	0.0988 - 0.2615
31-40 days	37	19	51.4 %	0.3589 - 0.6655
41-50 days	113	76	67.3 %	0.5816 - 0.7522
51-60 days	83	34	41.0 %	0.3101 - 0.5171
Total	638	142	22.3 %	0.1920 - 0.2565

$\chi^2 = 261.995^a$, $df = 5$; p value = 0.000

Table 3: Breed and sex prevalence of clinical coccidiosis in adult layers (parent stocks)

Risk factors		No of sampled dead birds	No of positive cases (%)	95 % CI	χ^2 (P- value)
Breed	RIR	72	26 (36.1)	0.2501- 0.4720	0.037[a] (P=0.848)
	WLH	118	41 (34.7)	0.2616 - 0.4334	
Total	190	67 (35.3%)	0.2847- 0.4205		
Sex	Female	125	46 (36.8%)	0.2835 - 0.4525	0.378[a] (P= 0.539)
	Male	65	21 (32.3%)	0.2094 - 0.4368	
Total		190	67 (35.3)	0.2847- 0.4205	

Cannibalism, high stocking density, inadequate cleaning of utensils, absence of isolation pen (mixing of sick and healthy birds in a house), defective feeders and waterers which allow the birds to enter in to the utensils and defecate in them, defective or mistaken inclusion of anti coccidial drugs in feed/water and the deep litter system (quality of the litter/infrequent change and turning of the litter) were identified as the main predisposing factors responsible for the prevalence of coccidiosis (25.24 %) in the present study.

DISCUSSION

The results of the present study illustrate that the disease coccidiosis is still prevalent in KPBMC with prevalence rates of 22.3% (142/638) and 35.3% (67/190) in dead sampled White leghorn (WLH) grower chickens of age 1-60 days and in parent stocks of WLH and Rhode Island Red (RIR) breeds, respectively. Mean while, the prevalence of clinical coccidiosis in adult parent stocks is significantly higher than the young (1-60 days) age WLH breeds $P < 0.05$ ($P = 0.000$). This finding is not inline with findings of Methusela *et al.* [7] and FAO/ILRI [17]. This discrepancy may come from the managerial problems in which less emphasis was given to the parent stocks where there is high stocking density, poor quality of litter, inadequate cleaning, poor ventilation system, high burden of predators like mice and rats, lack of prophylactic strategy against the disease and presence of different ages of birds in a single house. Plus to these factors extensive use of certain anticoccidial drugs for prevention purposes, starting from the second week of age of the growing period for two weeks duration, which might not allow the development of immunity at adult stage [2, 3, 18], may be the other contributor. Inappropriate anti-coccidial prophylactic dose application in the farm has also been incriminated for drug resistance and increased prevalence of the disease in the current study. This finding, on the other hand, may be supported by other authors and researchers justifications regarding age and susceptibility to coccidiosis [2, 3, 19].

In the current study coccidiosis prevalence was 22.3% in dead WLH chickens 1-60 days of age. It was also observed that there was a statistically significant difference ($P<0.05$) in the prevalence of coccidiosis among the six different age groups examined. When compared with the previous study done in this study area by Lobago *et al.* [12] in dead RIR chickens 1-60 days of age, the prevalence of clinical coccidiosis in the present study has decreased by approximately two-fifth of the previous prevalence i.e. 16.04% in the study site. This high reduction of prevalence of coccidiosis observed in the current study may be ascribed mainly to the application of preventive measures which basically rely on the use of anticoccidial drugs that were given at early ages starting from the second weeks of age for duration of 7 to 14 days of the growing periods. This may be also due to breed difference as the previous study had been done on RIR breeds. The other reason may be due to the slight improvement of the management system and bio security measures when compared to the setup in the previous study [12]. There was an improvement in the stocking density with an observed range of 8.43 birds / m^2 to 16.71 birds / m^2 which normally was expected to be between 10-15 birds / m^2 [20]. However, the prevalence of clinical coccidiosis 22.3% in the present study was attributed to the absence of isolation pen, occurrence of cannibalism (vent picking) and some managerial errors such as defective feeders and waterers that increase the risk of litter contamination and oocyst build-up and higher litters moisture.

The results of the present study is in consistent with the findings of Gari *et al.* [11] and Tehetena [21], who assessed prevalence rates of 22.58% in deep litter system and 23.1% in small and large scale production systems in Ethiopia, respectively. Although the disease coccidiosis appeared to occur for the first time since 28th day of age, the highest frequency occurred in the age group between 41-50 days (6-7 weeks). This finding is in congruent with the findings of other authors and researchers regarding the frequency occurrence of clinical coccidiosis with respect to the age of birds [9, 12, 22]. This was because most coccidian infections occur at the age of 3-4 weeks

but clinical diseases develop one or more weeks later. As a result the clinical diseases appear to reach climax at 5-7 weeks of age and as the age exceeded 7 weeks, most birds will develop immunity against the diseases. However, this period may be prolonged by mistaken /defective use of anticoccidial drugs; hence a slight change was observed in the frequency of occurrence of clinical coccidiosis in that the peak of the disease was observed at the age of 41-50 days (6-7 weeks) unlike the findings of Lobago *et al.* [12].

In the present study, five Eimeria species were identified, namely, *E. acervulina*, *E. necatrix*, *E. brunetti*, *E. tenella* and *E. maxima*. There was a significant variation in distribution or frequency of occurrence of these identified species with respect to age ($p < 0.05$). In all species the maximum prevalence was observed at the age of 41 to 51 days (6-7 weeks), which is true with *E. tenella* infection which generally affects chicks below 10 weeks of age with maximum prevalence in 4 to 8 weeks old chicks, but in chicks below 2 weeks it is rarely seen [23], whilst older birds are generally immune as a result of prior infection [24] and the infections with *E. acervulina* and *E. maxima* are seen at 3 - 6 weeks of age and then *E. necatrix* at 8 - 18 weeks of age, whereas *E. brunetti* is seen both early and late [25]. However, in the previous work, four Eimeria species namely, *E. acervulina*, *E. necatrix*, *E. brunetti* and *E. tenella* [12], were reported in this study area and the current finding adds the fifth species *E. maxima* which was diagnosed for the first time as one of the prevalent Eimeria species in the study site. *E. maxima* were found to be the first diagnosed species of Eimeria in this study area. This can probably be either due to the prophylaxis use of anticoccidial drugs in feed or water, which were observed during the study period or presumably due to emergence of drug resistance in which the most frequently given drug being amprolium, which is usually effective for control of *E. tenella* and *E. brunette* [12], or may be due to breed difference. It is likely that resistance has developed to more recent anticoccidial drugs [26-29] and very few drugs are equally efficacious against all *Eimeria species* [25], the occurrence and incidence of disease is also, to a great extent affected by the type of chicks reared and breeds sensitivities to infection [22]. The practice of feeding premixed coccidiostats to poultry in large poultry-raising and production establishments has reduced the significance of *E. tenella* and *E. necatrix* and emphasized the importance of other species, such as *E. maxima* [30]. Moreover, many coccidiostatic drugs have been directed against *E. tenella*, with the result that other species are increasingly incriminated as a cause of poultry coccidiosis [31]. Nevertheless, this finding is in agreement with the previous reports [9, 11, 32] who investigated *E. maxima* in Ethiopia.

In an attempt to study the prevalence of clinical coccidiosis in 190 dead sampled adult parent stock RIR and WLH breeds, prevalence rate of 67 (35.3%) was found. This result was in agreement with the results obtained from the previous studies in Ethiopia that reported prevalence rates of 50.8% [5] and 48.2% [8] in deep litter systems. It had also almost equal rate of prevalence with that of Dereje [33] who assessed 36.78% in deep litter system. However, there were no observed significant difference in the occurrence of coccidiosis between the two breeds $P < 0.05$ ($P=0.848$) although a slightly higher prevalence rate of 36.1% was noted in RIR breed than the WLH (34.7%) breeds. However, this insignificant difference observed between these two breeds may be due to the effects of environmental factors in-spite of the genotypic factor in which both genotypes were exotic breeds. This finding may be supported by Saif [34] who stated that chickens selected for their zoo-technical performance are particularly sensitive to coccidia infection. Indeed, these exotic birds were selected for their capacities in producing eggs and meat; and because of this selection, these animal are much more susceptible to diseases than the traditional backyard poultry [35]. Previously, it was reported that a significant difference was observed in natural resistance to coccidiosis between indigenous and RIR exotic breeds in which coccidial infection in RIR breeds was significantly higher than in local strain chicken [11]. This report indicated that the effects of agro-ecological factors outweigh over the genotypic variations in the susceptibility or sensitivity of breeds to coccidial infections in which the RIR exotic breeds were more susceptible/sensitive than the native local breeds under the hostile environment. Thus, no significant natural resistance variation was observed between RIR and WLH breeds to coccidiosis under natural infection. Like wise, the occurrence of clinical coccidiosis was not significantly affected by sex this finding was in concordance with Gari *et al.* [11] who reported that no significant natural resistance variation in relation to the sex to coccidiosis infections [36]. Thus, sex has not effect in the occurrence of clinical coccidiosis.

In conclusion, poultry coccidiosis remains a major burden to poultry producers and veterinary health professionals in the farm by changing its mode of occurrence from time to time as to the variation in the

conditions of the management system. Hence, demanding a lot of interventions and research to develop long-lasting and sustainable prevention and control strategies so as to get rid of the disease.

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