

Study on Poultry Coccidiosis in Tiyo District, Arsi Zone, Ethiopia

Getachew Gari¹, Getachew Tilahun² and Ph. Dorchies³

¹National Animal Health Research Center, P.O. Box: 4, Sebeta, Ethiopia

²Aklilu Lemma Institute of Pathobiology, Addis Ababa University

³Ecole National Veterinary, Toulouse, France

Abstract: The objective of this study was first to investigate the prevalence of poultry coccidiosis and to identify the coccidial species occurring in the study area on local strain and Rhode Island Red breed chicken. The study involved questionnaire survey, fecal examination, necropsy examination and identification of coccidial species based on their morphology, predilection site in the intestine and sporulation time. More than 75% respondents indicated that poultry production and income generated from poultry production in the rural community is the major income source for females and youth and bloody diarrhea predominantly appeared during wet season than chalky, yellow or green diarrhea. Public and private veterinary service centers have no anti coccidial drugs and other medicaments used for poultry diseases. Frequency detection of oocyst in the fecal samples from Rhode Island Red breed and local strain chicken was 80.65% and 61.25% respectively. This finding indicated that coccidial infection in Rhode Island Red breed was significantly higher than in local strain chicken ($p < 0.05$). The lesion score and mean oocyst output per gram feces was also considerably higher in Rhode Island Red breed than in local strain chicken ($p < 0.05$, $p < 0.001$ respectively), which may be the difference due to management system and breed. Clinical coccidiosis occurrence in Rhode Island Red breed and local strain chicken was 22.58% and 12.25% respectively. There was no statistically significant difference in clinical coccidiosis occurrence between the two genotype chickens and system. *Eimeria* species identified in descending order of their occurrence were *E. tenella*, *E. acervulina*, *E. necatrix*, *E. maxima* and *E. mitis*. Mixed infections were the predominant in both production systems. *E. mitis* was diagnosed for the first time in Ethiopia.

Key words: Coccidia, *eimeria*, infection, Local strain chicken, prevalence and Rhode Island Red breed,

Introduction

Coccidiosis remains one of the major disease problems of poultry in spite of advances made in prevention and control through chemotherapy, management and nutrition (Graat *et al.*, 1996). *E. tenella* and *E. necatrix* are the most pathogenic species. *E. acervulina*, *E. maxima* and *E. mivati* are common and slightly to moderately pathogenic; *E. brunetti* is uncommon but pathogenic when it does occur. *E. mitis*, *E. praecox* and *E. hagani* are relatively non-pathogenic species (Soulsby, 1982; Lillehoj and Trout, 1993). The species of coccidia identified in Ethiopia are *E. tenella*, *E. necatrix*, *E. maxima* and *E. acervulina* (Methusela *et al.*, 2002; Ashenafi *et al.*, 2004). Quantitative losses due to coccidiosis in Ethiopia are not well documented, but Kinung'hi *et al.* (2004) has reported that coccidiosis contributes to 8.4% loss in profit in large-scale farms and 11.86% loss in profit in small-scale farms. Losses due to mortality following a severe outbreak may be devastating and incidence rates as high as 80% were observed to occur in the form of an outbreak in Ethiopia (Alamargot, 1987). However, morbidity losses may be even more costly without the producers being aware that their flocks having any disease problem.

Thus, the objective of this study is to conduct prevalence study of poultry coccidiosis in Tiyo Wereda, Arsi Administrative Zone of the Oromia Regional State, Ethiopia. The study area may represent the mid and highland agro-ecology of the zone. Besides the indigenous strain chicken under free-range management system, the study included the Rhode Island Red breed kept under large-scale deep litter management system to observe the importance of coccidiosis in this breed under intensive farm. The species of *Eimeria* occurring in the area were identified and ranked based on their frequency of occurrence.

Materials and Methods

Study Area: Tiyo Wereda is located 175 km Southeast to Addis Ababa at 07°-56'-856N and 39°-08'-260E, 2436 masl and it is one of the Twenty Weredas found in Arsi Zone of Oromia Regional State situated in the North Western part of the Zone. Poultry population in Tiyo Wereda is estimated to be 40648 out of which 2500 are exotic breeds of Rhode Island Red and Bovans breed. The indigenous local strain chickens found in the study area are not well studied and characterized for their genetic identity. However, the genetic potential of these

chickens in egg and meat production is considered as low (Nasser, 1998). At the smallholder farmers level local strain chicken are reared in a traditional backyard system. The birds feed by scavenging around the residence area and occasionally supplemented with food residues and cereals. The housing and nesting place provision are poorly and constructed from the locally available materials. The main source of Rhode Island Red breed is Adama Poultry Breeding and Multiplication Center. The birds are sold to the farmers through the Wereda Agricultural extension program with government subsidy.

Study population: Local strain chickens were bought directly from the farmers randomly in five market centers. Physical appearance, size of the chicken and short interview of owners about the pedigree of their chicken was made to exclude exotic blood or crossbreed chicken. Most chicken bought for the study was approximately in the range of growers and adult age groups. Systematic random sampling method was applied during selection and purchase chicken.

The study was planned to investigate Rhode Island Red breed chicken that were kept under smallholder poultry production system. However, in practical reality these chickens were crossbreed with the local chickens since they were managed together under extensive system. Thus, it was difficult to get the pure breed except F₂ and F₃ generation. Therefore, the samples from Rhode Island Red breed were collected from Adama Poultry Breeding and Multiplication Center to observe the importance of coccidiosis in this breed and to compare the prevalence between breeds and management systems. The farm holds about 13000 chickens under intensive deep litter management and a total of 31 live birds with different age groups and equal sex proportion were randomly collected for the study (Methusela *et al.*, 2002).

Sample size determination was based on the assumption of the possible prevalence rate of the disease recorded in other places and 15% expected prevalence rate was considered from previous researchers. The formula applied to calculate sample size was the formula for simple random sampling method and the study considered 95% level of significance (Thrusfield, 1995).

Study design: The study design consists of cross sectional study to determine the prevalence of coccidiosis in local strain and Rhode Island Red breed chicken and to identify the prevalent *Eimerian* species in the study area. Questionnaire survey was also conducted to collect information from farmers and Adama poultry farm regarding the general condition about poultry production and disease problems in the study area.

Questionnaire survey: The survey was conducted on 100 farmers having direct practice in poultry production from 10 Peasant Associations (PA), 10 farmers in each PA. Pre-tested questionnaire was developed and used to gather information regarding general production system in the study area, assessment of chicken disease occurrence and farmer's treatment practices. Disease assessment in PA's was based on information regarding clinical manifestations of some common diseases encountered and their seasonal occurrence.

Cross sectional study: The study was conducted from September to December 2003 GC. The study methodology involved quantitative and qualitative analysis of fecal examination to investigate oocyst discharge, necropsy examination to grade the intensity of lesions induced (lesion scoring), location of lesion in the intestine and identification of the *Eimerian* species were performed. Measurement the size and shape of sporulated oocyst was done to identify the species of *Eimeria* encountered in the study area.

Fecal examination and eimeria species identification: The local strain chicken purchased was kept overnight in the laboratory for at least 18 hours. The aim was to collect fecal sample from each individual bird and to record ante mortem clinical condition of each bird. The fecal sample of each bird was blended by mortar and pistol and oocyst per gram feces was calculated based on the technique described by MAFF (1979). Positive samples were further examined for species identification based on oocyst size, sporulation time, shape and color of the oocysts.

The procedure applied to harvest oocysts was by floatation technique using saturated Sodium Chloride salt solution (Conway and McKenzie, 1991). The average length and width were measured from at least 10 oocysts to determine the size. Oocyst shape and color was also recorded for each recovered positive cases. Calibration of the microscope objective lens was done based on the procedures described by Conway and McKenzie (1991).

The sporulation time of the oocysts was determined at 29-30°C using the technique described by Conway and McKenzie (1991). The sporulation time was considered when 90% of the oocysts were sporulated.

Gross lesion examination and lesion scores: During 18-24 hours stay of chicken in the laboratory ante mortem clinical condition of each bird was recorded. The chicken was euthenized by cervical dislocation using the technique described by Zander (1978). The gastrointestinal tract was grossly examined carefully. The intestinal portions were divided into 4 sections, the upper part (duodenum and jejunum), the middle part (ileum), lower part (distal ileum and rectum) and cecal

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Table 1: Clinical and sub-clinical coccidial infection cases

No	Breed	Samples examined	Sub-Clinical	Total positive clinical	(%) of cases	(%) of clinical	95% C I positive cases	clinical (%)
1	Local	160	20	78	98	12.5 ^a	61.25 ^a	7.3-17.7
2	RIR	31	7	18	25	22.58 ^a	80.65 ^b	7.0-38.2
	Total	191	27	96	123	14.14	70.95	

Mean values within a column followed by different lower case superscript are significantly different ($p < 0.05$).

Table 2: Frequency detection of fecal oocysts in local strain chickens and Rhode Island Red breed

No	Division	Category	Manag system	Samp. examined	Positive samp	Clinical cases	Preva rate%	C I of preva. rate%	Mean OPG \pm SE	C I of OPG
1	Breed	LSC	F-r	160	98	20	61.25 ^a	53.62-68.88	1809 \pm 251 ^a	1314-2304
		RIR	D-l	31	25	7	80.65 ^b	65.91-95.38	5215 \pm 106 ^b	3161-7271
2	Sex	Male	-	90	59	15	65.56 ^c	55.54-75.56	2017 \pm 99 ^c	1423-2610
		Female	-	101	64	12	63.37 ^c	53.81-72.93	2669 \pm 457 ^c	1763-3575

Mean values within a column followed by different lower case superscript (a-d) are significantly different ($P < 0.05$). Manag - Management, F - r = Free range, Samp - Sample, D - l = Deep litter, Preva - Prevalence, RIR - Rhode Island Red, OPG - Oocyst per gram, LSC - Local Strain Chicken.

pouches. Intestinal gross lesions in any part of the sections were graded from 0 to 4 based on lesion score key (Conway and McKenzie, 1991). The lesion score zero represents absence of lesion and lesion score four is for very severe intestinal /cecal mucosa lesion and fatal cases. The location of the lesion was recorded; intestinal contents from the respective sections were taken and duplicate mucosal scrapping smears made from each section of the intestine.

Statistical analysis: The association of oocyst count per gram feces between the two genotype chicken and sex was computed by chi-square test. The mean Oocyst Per Gram count, prevalence and confidence interval in both genotype chickens were calculated using Stata-7 (Stata-7, 1984-2000). The p-value for statistically analyzed data was considered significant different at $P < 0.05$ probability level (Methusela *et al.*, 2002).

Results

Questionnaire survey: Poultry management in the rural area is free ranging and chicken feed by scavenging around the house with occasional cereal and food residuals supplement. More than 75% respondents indicated that poultry production and income generated from poultry production in the rural community is the major income source for females and children. Disease problems in traditionally managed chicken are very important and more than 90% interviewees responded as medium to high rank for disease problems. Although farmers have their own local names and ways of identifying poultry diseases, the most frequent disease they complain about (>75%) was diarrhea. The other observation was the seasonal occurrence of diarrhea and more than 85% farmers have clearly described the color of feces. More than 75% of the respondents described that bloody diarrhea predominantly appeared during wet season than chalky, yellow or green diarrhea. This observation might be more indicative of the occurrence of coccidiosis. Moreover, the public and private veterinary service centers have no anti coccidial

drugs and other medicaments used for poultry diseases. More than 80% farmers have experience of brooding and rearing chicks during dry season.

Potential risk factors for coccidiosis: In free-ranging local chickens, non-selective picking behavior during feeding can expose chickens to infection. Age group, high moisture conditions that favorably influence oocyst sporulation and development to the infective stage were the potential risk factors. However, in Rhode Island Red breeds that were kept under intensive deep litter system, the potential risks observed from farm assessment through questionnaire were age groups, production systems, flock size, moisture level in the poultry house and level of biosecurity.

Clinical coccidiosis: In the current study, chicken that showed depression, ruffled feather, diarrhea and/or blood mixed droppings were recorded as clinical cases. The results of clinical and sub clinical infection are shown in Table 1. The number of clinical cases in Rhode Island Red breed and local strain chickens was not significantly different. Clinical coccidiosis detection between male and female was also not significantly different in both genotype chickens.

Quantitative and qualitative fecal examination: The prevalence of coccidial infection and Oocyst Per Gram count in local strain and Rhode Island Red chicken is shown on Table 2. The frequency occurrence of the coccidial infection in the Rhode Island Red breed was significantly higher than the local strains ($\text{Chi}^2 = 4.26$; $p < 0.05$). The Oocyst Per Gram count in Rhode Island Red breed was also significantly higher than the local strain chicken ($\text{Chi}^2 = 109.085$; $p < 0.001$). However, the frequency occurrence of coccidial infection and Oocyst Per Gram count between male and female was not statistically significant. The association of clinical case occurrence with Oocyst Per Gram counts was highly significant in both genotype chickens ($\text{Chi}^2 = 106.82$; $p < 0.001$).

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Table 3: Coccidial species investigated in the study area and their identification characteristics

Species identification		<i>E. tenella</i>	<i>E. necatrix</i>	<i>E. acervulina</i>	<i>E. maxima</i>	<i>E. mitis</i>
No	characters					
1	Av. Oocyst length (µm)	22.77	22	18.64	30.26	16.26
2	Av. Oocyst width (µm)	19.37	18.25	15.46	21.78	15.61
3	Shape index	1.18	1.20	1.21	1.39	1.042
4	Frequency occurrence	29	16	23	13	9
5	Oocyst shape	Ovoid	Oblong ovoid	Ovoid	Ovoid	Sub-spherical
6	Sporulation (Hrs) at 29-30°C	20	20	18	30	18
7	Site of occurrence in the intestine	Cecum	Mid intestine (Jejunum and ileum)	Upper intestine (Duodenum)	Mid intestine (Jejunum and ileum)	Upper intestine (Duodenum Jejunum)
8	Pathological lesions induced in host intestine	Hemorrhage & bleeding thickening of cecal wall	Ballooning, Bleeding, white plaques & thickening of int. wall	White round colonies, streaks & mucoide exudates	Ballooning, petechial. hemorrhage, mucoid exudates & thickening of int. wall	no, apparent lesion

Coccidial species identified in this study were *E. tenella*, *E. acervulina*, *E. necatrix*, *E. maxima* and *E. mitis* in descending order of their occurrence. Mixed infections were the predominant case, which accounts for 45 mixed infections. The major proportion of mixed infections amounting to more than 55.6% was consisted of *E. acervulina* as a member of the mixed infection. *E. necatrix* with *E. acervulina* and *E. maxima* with *E. acervulina* have occurred in the highest mixed infection frequency. *E. mitis* was the first diagnosed species of coccidia in this study in Ethiopia and recovered from the samples collected from Adama poultry center. It was the smallest in size as compared to others and sub spherical shape. The size and color of most coccidial species are overlapping to identify based on their morphological feature except for *E. maxima*, which has the largest size and brown red color. The average length and width of each species and their identification characteristics are shown in (Table 3). Most coccidial species were restricted to specific predilection sites in the intestine. However, *E. acervulina* and *E. maxima* were found to occupy the most proportion of the small intestine than other species.

Oocyst sporulation time for *E. acervulina* and *E. mitis* were shorter (18 hours) as compared to other species. *E. tenella* and *E. necatrix* had similar sporulation time of about 20 hours and *E. maxima* took almost 36 hours (Table 3) for sporulation at 29-30°C.

Gross lesions: Post mortem examination is the best method for diagnosis and species identification. The site of their occurrence and characteristic lesions produced by specific *Eimeria* species were used for identification of the species (Table 3). *E. tenella* induced hemorrhage, thickening of the mucosa, clotted blood and cecal cores in the cecum depending on the magnitude of infection and duration of the infection. In some cases concurrent infections with bacterial enteritis obscured the coccidial lesions in duodenum and cecum. *E. necatrix* and *E. maxima* usually shared similar intestinal lesions. Lesions in *E. necatrix* were more severe with bleeding and whitish plaques seen in

the middle intestine on both sides from yolk sac diverticulum, which was not the case in *E. maxima*. *E. acervulina* usually occurred in the duodenal loop characterized by mucoid exudates in intestinal content; white spots were usually evident from the serosal side and eroded mucosal membranes.

The total number of chicken in which intestinal lesions were scored above zero was 98 (61%) in local strain and 25 (81%) in Rhode Island Red breed. The frequency detection of lesions in Rhode Island Red was significantly higher than it appeared in local strain chickens ($p < 0.05$). The detection of intestinal lesions had significant association with the occurrence of clinical coccidiosis ($\text{Chi}^2 = 17.38$; $p < 0.001$). Moreover, lesion score showed significant association with Oocyst Per Gram counts in both genotype chickens ($\text{Chi}^2 = 89.72$, $p < 0.01$). Lesion score between male and female was not significantly different. Microscopic examination of intestinal mucosa scrapping was also made in addition to gross lesion grading to observe different developmental stages of coccidia in the mucosa.

Discussion

Generally, in traditional poultry production system, the input required is minimal and is considered as secondary to other agricultural activities by the smallholder farmers. Housewives and children are usually responsible to undertake poultry production around the homestead. Since these social groups usually stay longer around the home, they can easily look after the chicken. The income obtained from poultry production may also be most accessible source of income during need of cash for women and youths. Thus, from this point of view, poultry production may address the social and economical problems of gender issues and improve the income source and long-term economic potential of women in the rural community. Indigenous knowledge of farmers on poultry coccidiosis might not be very specific. Nevertheless, they have keen awareness regarding the risk factors for the occurrence of the disease and they describe the disease based on the clinical signs. The current observation showed that

public and private veterinary services overlook the health impact on poultry production and no drugs and biological preparations for poultry were available in their stock. Therefore, farmers apply their own indigenous practices to treat and control chicken diseases, which may not be usually effective. The magnitude of the disease problem based on the farmer's response was highly amenable to appropriate technical interventions to increase the production and productivity of this resource. The prevalence of coccidial infections in the local strain chickens in this study was higher than in previous findings (Ashenafi *et al.*, 2004). The climatic conditions, agro-ecological set-up and lack of adequate information on the subject may be attributed to the variation in the maintenance of the disease. The relatively wet climate and cooler temperatures in the high and mid altitudes of the study area may be more favorable for the occurrence of coccidiosis. The finding may consolidate the importance of coccidiosis in the indigenous chicken. The frequency occurrence of coccidial infection in Rhode Island Red breed was significantly higher ($p < 0.05$) than the local strain and this could be due to management system and breed factor. The higher Oocyst Per Gram count in Rhode Island Red breed can be related with the higher frequency occurrence of coccidiosis in the deep litter system due to relatively higher oocyst accumulation in the deep litter. The amount of oocyst discharged from infected chicken depends on the dose of oocysts ingested and the immunological status acquired from pre-exposure (Bumstead *et al.*, 1991; Williams, 2001). Thus, the chances of the chickens to pick-up large numbers of sporulated oocyst can be more likely in the Rhode Island Red kept in deep litter management than the local strain chickens. The results of this study are consistent with the finding in large and small-scale deep litter rearing systems (Methusela *et al.*, 2002). However, occurrence of coccidiosis was not significantly affected by sex. This indicates that there was no significant natural resistance variation in relation to the sex (Pinard-Van Der Laan *et al.*, 1998).

The most economically important species of coccidia *E. tenella*, *E. acervulina*, *E. necatrix* and *E. maxima* were found in this study, which is incongruent with the previous researchers reports (Mathusela *et al.*, 2002; Ashenafi *et al.*, 2004). Nevertheless, *E. mitis* was the first diagnosed species of coccidia in this study in Ethiopia based on morphological and other characteristics from the samples collected from Adama Poultry Center. Further confirmation of the identity of this species in the free ranging chicken may be required in the future. The distribution of coccidial species in free-ranging chicken and under intensive management was not significantly different. However, *E. acervulina* was the most dominant species occurring in the intensive deep litter system, which is in agreement with the findings of Methusela *et al.* (2002). The higher biotic potential of this species may

favor its dominance occurrence in a confined production system. Due to this fact Reid (1978) has stated that the significance of this species as a pathogen has increased steadily within the recent years in large poultry establishments. Mixed infections were frequently encountered accounting for 45 mixed infection.

Lesion score showed significant association with clinical coccidiosis and Oocyst Per Gram detection frequency ($p < 0.001$, $p < 0.01$ respectively). This may be explained that chicken that had lesions may more likely manifest clinical disease and also shade large amount of oocysts in droppings (Methusela *et al.*, 2002). Thus, the lesion score in Rhode Island Red breed was significantly higher than local chicken ($p < 0.05$) and that may be the mirror reflection with the frequency detection of fecal oocyst in the deep litter management system. Lesion score in local strain chicken was usually confused or overlapping with the lesions produced by gastrointestinal parasites and enteric infectious diseases. Thus, the lesion score parameter may be affected and not reliable to indicate the level of infection and the pathogenicity of coccidial species involved when superimposed with other infections in the local strain chickens. *E. acervulina* and *E. maxima* occupied much greater area beyond the typical restricted sites of their species occurrence. This tendency may be explained that the merozoites are being carried by the peristaltic and anti peristaltic waves of the intestine beyond already infected areas in heavy infection cases (Williams, 2001). The occurrence of sub-clinical coccidiosis was significantly higher than clinical coccidiosis in both genotype chicken and management systems. The birds might have developed immunity to the trickle infections acquired from the environment and maintain the state of balance to the infection in the form of sub-clinical disease. The effect of sub-clinical coccidiosis on the production performance of chicken and its economic significance should be further studied.

Coccidial infection in deep litter management was significantly higher than in the free-ranging system. This showed that management system plays a great role in the epidemiology of coccidial infection. However, the observed high prevalence rate of coccidiosis in the indigenous chicken showed that it is one of the economically important diseases, which deserve appropriate prevention and control measures.

Acknowledgement

The authors would like to acknowledge the financial support of the Office of the School of Graduate Studies and Research programs Addis Ababa University. We would like to express our thanks to Agricultural Development Bureau of Oromia Regional State and Debre Zeit Poultry Research Division staff of EARO for their support during research work. Our special thanks go to Dr. Hailu Wondimu and the staff of Asella Regional

Veterinary Laboratory for their assistance during the research work. We are highly thankful to Adama Poultry Breeding and Multiplication Center for their cooperative spirit to supply us with Rhode Island Red breed chicken.

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