

## Case Control Study of Avian Coccidiosis in Commercial Poultry

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**Received:** August 05, 2020

**Published:** February 17, 2021

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### Abstract

Commercial poultry sector is one of the most important segments of global trade and production. Coccidiosis, a worldwide disease of economic importance, significantly influences the production potential of this sector. To decrease the prevalence and consequent economic losses, identification of factors, which influence the frequency and persistence of illness, is pre requisite. A retrospective case control study was conducted for a period of one year in order to identify the risk factors associated with coccidiosis infection. Total of 70 broiler and 15 layer flocks of varying age and population sizes were selected as cases. For each case flock, one apparently healthy flock of similar breed, age and population size was also selected as control. Data of these 85 case control pair flocks regarding disease incidence and associated factors, recorded from January through December 2018, were analyzed for measures of association. Monsoon season, no vaccination, broiler breed, winter season, post monsoon season, spring season, open shed, wet litter;  $\leq 4$  weeks age, coccidiostat excluded feed and crowding appeared as putative risk factors exhibiting significant ( $P < 0.05$ ) association with coccidiosis infection by odds ratios of 2.88, 3.35, 3.66, 5.70, 6.77, 7.12, 8.14, 8.50, 9.28, 9.33 and 11.40 respectively. Non-significant ( $P > 0.05$ ) association of coccidiosis infection was recorded with layer breed and scarce flushing with odds ratios of 0.73 and 0.94 respectively. Incidence and persistence of coccidiosis infection in commercial poultry population is significantly influenced by management and seasonal factors.

**Keywords:** Avian Coccidiosis; Risk Factors; Case-Control Flocks; Odds Ratio; Broiler and Layer Breeds; Management Factors; Seasonal Factors

## Abbreviations

WHO: World Health Organization; CI: Confidence Interval

## Introduction

Among animal protein sources, commercial poultry is considered as most important white meat type with respect to global trade and production [15]. South Asian countries including Pakistan share 24.8% of the total world production. In Pakistan, the contribution of poultry industry in total meat production is 26.8% which is 1.26% of GDP. It is cheapest animal protein source of the country in terms of meat and eggs [10]. According to recommendations of world health organization (WHO), 27g of animal protein is minimal individual daily requirement [18]. Pakistan is producing 38% less than this minimal individual requirement. The deficiency is being intensified due to uninhibited population growth. The average annual net yield per bird in Pakistan is 51 which are 80% less than the average annual per bird yield of developed countries [15]. Among major disease problems hampering production potential of commercial poultry, coccidiosis is the most commonly reported disease of economic importance worldwide. Seven species of a protozoan parasite of Eimeria genus are reported to be responsible for avian coccidiosis. Among these seven species *E. maxima*, *E. necatrix*, *E. tenella* and *E. brunette* are more pathogenic compared to *E. mitis*, *E. acervulina* and *E. praecox* [11]. Up to 70% of production loss has been reported to be associated with this disease [12]. Significant morbidity, poor growth, enteritis and bloody diarrhoea are characteristic features of disease [16]. Young chickens of 3 - 8 weeks age are mostly affected. The disease is transmitted by ingestion of oocysts from contaminated faecal material [14]. Specie level identification of parasite is made on the basis of oocysts morphology, sporulation time, affected intestinal zones, schizont size and pattern of grass lesions appearance [13].

Besides providing protection from hard floor, bedding material may act as important source of disease transmission. Increased moisture content due to urination or spillage with decreased evaporation rate produce damp bedding material which facilitates coccidian oocysts sporulation [2]. Commercial poultry farms are at high risk of coccidiosis due to intensive farming and massive stocking densities. Information on different epidemiological aspects of avian coccidiosis, influencing disease occurrence and spread, is quite scanty. Present case-control study is therefore designed for identification of risk factors associated with this disease.

## Materials and Methods

### Case definition

Based on criteria described in published literature, following definition for clinical outbreak of coccidiosis was used for selection of a case flock.

An affected broiler or layer flock with characteristic clinical signs of inappetence, bloody diarrhoea, weight loss with necropsy findings of whitish round plaques in duodenum, bleeding and disruption of caecal glands, small pin point haemorrhages leading to erosions in ileum and presence of oocysts in droppings [8-17].

### Study area and population

Being high density poultry farm region, District Chakwal was selected as study area. According to data of Livestock and Dairy Development Department, Chakwal, Punjab, all commercial poultry farms of this District including 658 broiler and 184 layer farms with capacity of 4221800 and 1324700 birds respectively were selected as study population.

### Sample size

Sample size was estimated using epi-tools, epidemiological software. The values of expected exposure in controls, assumed odds ratio for  $\leq 4$  weeks age as putative risk factor, confidence level and desired study power were specified as 0.12, 0.03, 0.95 and 0.8 respectively. The desired sample size per group was 85 flocks.

### Case-control flocks

A total of 70 broiler and 15 layer flocks of different age groups and population sizes affected by coccidiosis were selected as cases. For each case flock, an apparently healthy flock of similar breed, age and population size which did not exhibit characteristic clinical and necropsy findings mentioned in case definition, was selected as control. In this way 85 case control pair flocks of similar breed (broiler or layer), age and population sizes were selected from the study population.

### Data collection

The data of each case-control flock regarding disease (coccidiosis) incidence, breed, age, season, flushing, use of coccidiostat in feed, vaccination, crowding, litter moisture status, shed type (open or controlled), faecal specimens and intestinal contents of affected live and dead birds collected and analyzed, were recorded in per-forma.

### Laboratory analysis

The specimens were analyzed by floatation technique. Two grams of faecal sample was mixed with flotation solution (saturated salt solution) by using stirring rod, one drop of methylene blue was added to enhance visibility of oocysts. Resulted solution was sieved through strainer in test tube to remove extra particles. Test tube was filled up to the top with sieved solution and cover slip was placed over it. This solution was then allowed to settle for 30 minutes so that oocysts can float over the top and adhere with cover slip. Cover slip was then placed on a glass slide and examined under microscope with resolving power of 10X, 40X and then 100X for oocysts presence and morphology [5].

### Statistical analysis

The data were analyzed for measures of association using 2 by 2 table analysis module of epi-info version 7.2.3.1 software, developed for pair matched case control study. Odds ratios and their 95% confidence limits were estimated in order to identify the factors associated with coccidiosis infection. Mc Nemar's Chi-Square

test was applied to measure the significance. The P-values of < 0.05 were considered as statistically significant [3].

### Results

Significant ( $P < 0.05$ ) association of disease was recorded with broiler breed,  $\leq 4$  weeks age, no vaccination, use of coccidio-stat excluded feed, crowding, wet litter, open shed, winter, spring, monsoon and post-monsoon seasons. Largest odds ratio (11.40) with 95% confidence limits of 4.5697 - 28.4398 was recorded for crowding followed by coccidiostat excluded feed (9.33; 4.0215 - 21.6615),  $\leq 4$  weeks age (9.28; 4.2580-20.2501), wet litter (8.50; 3.6479 - 19.8062), open shed (8.14; 3.7142 - 17.8521), spring season (7.12; 3.3994 - 14.9335), postmonsoon season (6.77; 3.3662 - 13.6470), winter season (5.70; 2.9110-11.1612), broiler breed (3.66; 2.0717 - 6.4896), no vaccination (3.35; 1.9507 - 5.7631) and monsoon season (2.88; 1.6902 - 4.9376). Non significant ( $P \geq 0.05$ ) association of disease was recorded for layer breed and no use of flusher with odds ratios and 95% confidence limits of 0.73; 0.5193-1.0283 and 0.94; 0.4867-1.8326 respectively. Detailed results are presented in table.

Serial #	Variable	Cut off Points	Study Groups		OR	95% CI		$\chi^2$ Value	P-Value
			Cases n = 85	Controls n = 85		Lower	Upper		
	Layer Breed	Yes	7	57	0.73	0.5193	1.0283	3.2667	0.7070
	Flushing	Scarce	67	17	0.9444	0.4867	1.8326	0.0286	0.08657
	Monsoon Season (June-Sept)	Yes	67	52	2.88	1.6902	4.9376	16.5143	0.0000
	Vaccination	No	68	57	3.35	1.9507	5.7631	21.6216	0.0000
	Broiler Breed	Yes	70	55	3.66	2.0717	6.4896	22.8571	0.0000
	Winter Season (Dec-Feb)	Yes	75	57	5.70	2.9110	11.1612	32.9701	0.0000
	Post monsoon Season (Oct-Nov)	Yes	76	61	6.77	3.3662	13.6470	38.6286	0.0000
	Spring Season (March-May)	Yes	77	57	7.12	3.3994	14.9335	36.9385	0.0000
	Open Shed	Yes	78	57	8.14	3.7142	17.8521	39.0625	0.0000
	wet Litter	Yes	79	51	8.50	3.6479	19.8062	35.5263	0.0000
	Age	$\leq 4$ weeks	78	65	9.28	4.2580	20.2501	46.7222	0.0000
	Coccidiostat in Feed	No	79	56	9.33	4.0215	21.6615	40.3226	0.0000
	Crowding	Yes	80	57	11.40	4.5697	28.4398	43.6129	0.0000

**Table:** Risk factors of avian coccidiosis in commercial poultry population (Broilers and layers).

Significant at  $P < 0.05$  OR (odds ratio); CI (confidence interval); analyzed using Epi info version 7.2.3.1 software.

## Discussion

Seasons significantly influenced the disease incidence. Spring, winter, post-monsoon and monsoon seasons appeared as putative risk factors. Poultry feeding and management is influenced by seasonal factors i.e. rainfall, humidity and temperature. Humid and cold weather stimulates the birds for increased feeding with decreased water intake. During this season the birds crowd together for warmth. This situation is reversed during hot season when feed consumption is decreased with increased water intake. This seasonal variation and consequently induced response of birds significantly influence the disease outbreak occurrence and propagation [7]. Among seasonal factors, rain fall, temperature and humidity are reported to be associated with development of coccidiosis [6]. Ambient temperature (25°C to 30°C) and high humidity produce favorable environment for sporulation and survival of coccidian oocysts [5]. Spring being growth season in Pakistan produced favorable environment for development and dissemination of disease thereby promoting the oocysts sporulation and survival [19]. The disease association with spring, post-monsoon and monsoon seasons may be due to favorable environmental factors including temperature and humidity whereas its association with winter season may be due to increased stress as extreme weather conditions seriously influence the ability of bird's immune system to combat the invading pathogens by imposing stress [1]. Results of present study with respect to seasonal factors are in accordance with previous reports [2-19]. Our findings are not in agreement with some other previous reports [4-20]. The contradiction may be due to different seasonal patterns, geo-climatic conditions and study designs.

Use of aflatoxin contaminated feed, production stress, intensive farming and dose of coccidiostat feed additives, crowding, floor type, decreased ventilation and poor management are major predisposing factors of coccidiosis [9-19]. In present study, crowding, wet litter, open shed and absence of coccidiostat feed additives exhibited significant association with occurrence and dissemination of coccidiosis infection. Findings of present study are in agreement with previous reports [2-5] with respect to management related risk factors.

The disease exhibited significant association with Broiler breed. Previous reporters also reported higher prevalence of coccidiosis in broiler breed. They attributed this higher prevalence to intensive rearing, poor management, contaminated feeds, high stocking den-

sity and poor ventilation [5]. Management system, age, and breed are among putative risk factors of coccidiosis [21].

Younger chicken of  $\leq 4$  weeks age exhibited significant association with coccidiosis infection. Our findings with respect to significant association of disease with younger age of chicken are supported by previous findings of [5] whereas differ from some other studies of [20]. The difference is may be due to different study designs as they divided the younger chickens of 1 to 4 weeks age in two groups i.e. 0 - 15 days and 16 - 30 days. Higher prevalence of coccidiosis in growing chickens i.e. birds of 5 - 7 weeks age have also been reported in previous findings [20]. They attributed this higher prevalence to increased oocysts concentration in litter and decreased immunity during this age as according to their report, chickens at younger (1 - 4 weeks) age have sufficient maternal immunity for protection which decreases at growing (5 - 7 weeks) age. Higher disease incidence in younger chicken recorded during present study may be due to decline in maternal immunity induced by seasonal and managerial stress factors as these factors also exhibited significant association with this infection.

## Conclusion

Poor management including wet litter and crowding, together with environmental and seasonal exposure intensify the vulnerability of susceptible commercial poultry population leading to development and spread of coccidial infection. Monitoring of feeding, water intake, temperature, humidity and crowding with varying age and seasons will improve management and reduce seasonal and environmental exposure.

## Acknowledgements

The principal author acknowledges the cooperation of Live-stock and Dairy Development Department, Govt of Punjab, Chakwal, Pakistan, for collection of necessary data and laboratory specimens and Dr. Altaf Mahmood for designing present study.

## Conflict of Interest

There is no conflict of interest among concerned parties.

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