

## **PREVALENCE AND CHEMOTHERAPY OF COCCIDIAL INFECTIONS IN BROILERS IN MUZAFFARABAD**

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**ABSTRACT:** The present study was performed to know the prevalence of coccidiosis and efficacy of anti-coccidial drugs in the capital city of Muzaffarabad, Azad Kashmir, Pakistan. A total of 573 broiler birds of different age groups were sampled from the study area farms and processed accordingly. Fecal flotation and McMaster egg counting techniques were used for Oocyst identification and counting, respectively. Postmortem of birds was performed to know the lesions and type of coccidiosis present in the birds. Drug trial was also carried out to evaluate the efficacy of anti-coccidial drugs available in the study area market. An overall 19.37% (111/573) prevalence of Coccidiosis was observed in study area. Among different age groups, broilers below three weeks of age were found more susceptible to infection. During summer season higher prevalence was recorded. Different risk factors were found associated with coccidial infection in the study area. Five anticoccidial drugs available in the market have been found effective with different efficacy level. Among the anticoccidial drugs used during the present investigation, Sulphadimerazine 86% had shown better effect. Besides these anticoccidial drugs, a herbal plant locally known as Sumbal was also used and found potent against Coccidiosis. This is a good alternate choice used in field conditions along with synthetic anti-coccidial for the treatment and control of coccidiosis. It was deduced that prevalence of coccidiosis recorded in study area was comparatively lower than prevalence recorded in other parts of the world. Furthermore, Sulphadimerazine 86% have shown comparatively better efficacy than other drugs.

**Key Words:** Prevalence, Sumbal, Anti-coccidial drugs, Sulphadimerazine, Coccidiosis

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### **INTRODUCTION**

Poultry is the second largest and well-known industry of Pakistan with 1.2 billion broiler production annually across the country. Pakistan ranked at 11<sup>th</sup> position on broiler production basis around the globe (Anonymous, 2018). Poultry meat has been considered as cheap source of protein from animal origin now days that fulfil an increasing human meat demand globally (CSA, 2004; Jordal *et al.*, 2002; Tadesse & Teshome, 2018). Poultry (broiler) contributes about 45% to total meat production in Pakistan (ESP, 2017) and in national Gross Domestic Product (GDP) its share is 2% (Zaman *et al.*, 2012). Disease pressure which usually develops because of viral, bacterial and parasitic load influences the welfare and production of poultry all over the world including Pakistan. Coccidiosis is a Protozoan disease that has great impact on commercial broiler production all over the world.

Coccidiosis is caused by species of genus *Eimeria* (*Coccidia*) which belong to apicomplexan group. To date, it has been reported that nine species of *Eimeria* (Morgan *et al.*, 2007) are responsible for causing Coccidiosis in poultry including broiler. Among the nine *Eimeria* Species, (*E. tenella*, *E. maxima*, *E. brunette* and *E. necatrix*) are highly virulent whereas, (*E. acervulina*, *E. mitis* and *E. mivati*) are moderately virulent and *E. praecox* and *E. hagani* are the least virulent (Nematollahi *et al.*, 2008; Jadhav *et al.*, 2011). These Coccidiosis causing species target different parts of digestive system particularly intestinal tract and caecum of their hosts (Chapman, 2014). Coccidiosis not only reduces production of poultry, but also imparts huge economic losses that make it an economically significant disease for the poultry industry with losses estimated more than \$800 million per year in the USA (Sharman *et al.*, 2010). Variable prevalence percentages of Coccidiosis from various parts of the world including Pakistan and adjoining areas of study districts have been reported (Al-

Gawad *et al.*, 2012; Bachaya *et al.*, 2013; Amin *et al.*, 2014; Shamim *et al.*, 2015). A few, epidemiological studies have been conducted in Pakistan and Azad Kashmir (Shamim *et al.*, 2015) on coccidial infection in broilers. Anticoccidial drugs are practicable option used for the managing coccidiosis in poultry with other managemental practices. On the other side, plant based drugs have been used as alternate to chemical drugs in most parts of the world for the control and treatment of parasitic infections (Hussain *et al.*, 2015). Keeping significance of coccidiosis in poultry, present research study was planned to establish the prevalence, speciation and efficacy of different anti-coccidials available in the market of study area.

## MATERIALS AND METHODS

**Study area:** The present study was conducted in the district Muzaffarabad. Muzaffarabad is capital of Azad Kashmir located near the convergence of the Jhelum and Neelum rivers. Muzaffarabad has diverse climate and topography. The climate of Muzaffarabad is mild, and generally warm and temperate but extreme cold in winter. Muzaffarabad is a city with a considerable rainfall. Even in the drier months, there is a lot of rain. The average annual rain fall is 1457 mm.

There are more than 300 broiler farms comprising of one or two sheds. The average capacity of rearing of birds per shed is 1500-3000. All the farms are open type and manually operated with different altitude locations. There is a seasonal farming of broilers at commercial level because of extreme cold weather in the winter season. Broiler production in the study area is reduced to 10-20% in the winter. Poultry sheds are mostly with metal roof and non-cemented floor usually divided in two portions, a small room called brooding room which is used for the rearing of young chicks from day old to the first week of the age and the larger portion used for rearing birds above one week of age.

**Sample scheme:** Before start of sample collection, sample size was determined according to the formula of (Thrushfield, 2007). Formula for sample size calculation was as follows:

$$n = \frac{(1.96)^2 P_{exp}(1-P_{exp})}{(d)^2}$$

Where; n= required sample size,  $P_{exp}$ =Expected prevalence, d= Desired absolute precision.

Simple random sampling procedure was used for the collection of samples from the area of study. Every month farms located at different locations in the study area were visited for the collection of gut and stool samples. The entire samples were properly labeled with necessary information. Data was collected on predesigned Performa (Annexure 1). Birds of different

age groups were sampled. All the collected birds were thoroughly examined before performing post mortem.

**Post mortem:** Post-mortem was performed according to the standard Post-mortem method established for poultry disease diagnosis (Nikam *et al.*, 2012). Briefly, all the internal body parts of the incised birds were examined keenly, particularly intestinal parts e.g. small, large and caeca for recording lesions caused by Coccidiosis causing pathogens. Histopathological lesions were recorded and slides were prepared for differential diagnosis and future reference.

**Faecal Examination:** Drooping/faecal materials were picked up from litter randomly and then carefully examined for existence and identification of oocyst of Coccidia. Faecal flotation and McMaster procedures were used for determining Oocyst per gram of faeces. Oocysts were identified on the basis of their shape and size following the keys of (Soulsby, 1987; Taylor *et al.*, 2012). Briefly, method used by (Obiora *et al.*, 1992) was followed.

**Chemotherapeutic trail:** Standard guidelines were followed for evaluation of chemotherapeutic effectiveness of anti-coccidial drugs available in the market of district Muzaffarabad, Azad Kashmir. Before start of the drug trial, market of Muzaffarabad was surveyed for selection of anticoccidial brands available in the market. Brands selected and purchased for drug trial for present study were as follows; Amprolium 60%, Sulphadimerazine 86%, Sulphaquanaxalone 16%, Toltrazuril 2.5% and Sumbal roots extract.

**Procurement of Chicken for Drug Trail:** Broiler chicks which were one day old were obtained from the hatchery and reared at shed prepared before the procurement of chicks. Broiler birds were then categorized into six groups (A, B, C, D, E and F) randomly. Group A was given Amprolium 60%, group B was given Sulphadimerazine 86%, group C was given Sulphaquanaxalone 16%, group D was given Toltrazuril 2.5%, group E was given Sumbal roots extract and group F was control. Each group was comprised of approximately 30 birds. Birds were offered feed and water *ad libitum* till the end of chemotherapeutic trial. Infection was given to birds at day 23 and there were outbreaks of Coccidiosis on 4<sup>th</sup> day after the infection. Birds were treated with selected anti-Coccidial agents according the manufacturer recommended dose rate.

**Statistical Analysis:** Data were collected and sorted using Statistical Package version 11. Prevalence was calculated according to the formula mentioned in (Thrushfield, 2007). While, Chi-square test was used to check level of significance ( $P>0.05$ ) difference among factors associated with prevalence of coccidiosis.

## RESULTS

This study was consisting of two experiments, one recording of prevalence of coccidiosis and 2nd evaluation of anti-coccidial drugs commonly available in the study area against coccidial infection.

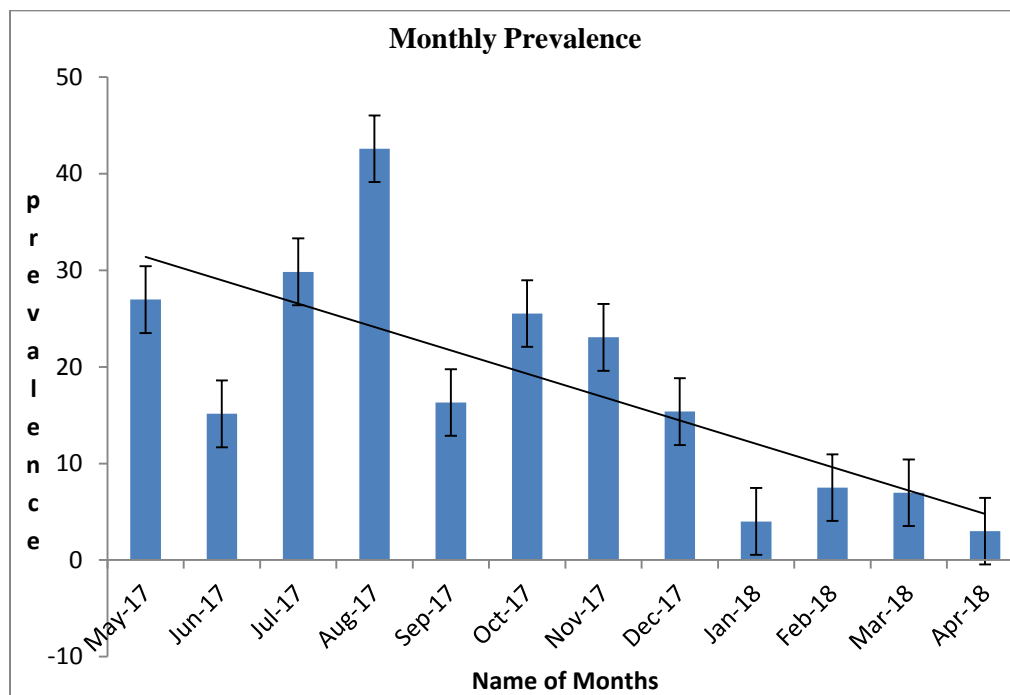
A total of 573 broilers were sampled during the period of May 2017 to April 2018 from different places of district Muzaffarabad. From (573) broilers (111) birds were observed to be positive for coccidiosis. So, overall prevalence of coccidiosis noted during the present study (111/573) was 19.37% (Table 4.1). *Eimeria tenella* and *Eimeria maxima* was identified on the basis of their shape and size. Cases of caecal Coccidiosis were more than intestinal coccidiosis. Month wise significantly ( $P < 0.05$ ) higher prevalence was recorded in the month of August and lowest in the month of January as shown in (Figure 4.1.) similarly, seasonal and broiler bird age wise prevalence was also noted during the present investigation. During the present study, birds from day old to three week old were found significantly ( $P < 0.05$ ) more prone to coccidial infection as compared to the birds above than 3 weeks of age (Figure 4.3).

**Comparative efficacy of anticoccidial drugs:** The present research study was conducted to measure and

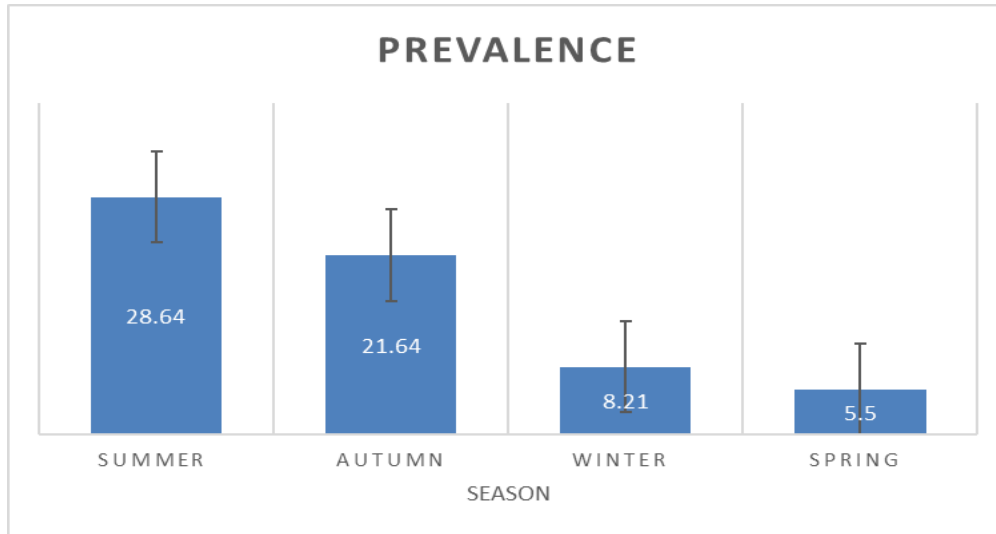
compare the efficacy of Amprolium, Sulphadimerazine 86%, Sulphaquinoxalone 16%, Toltrazuril and herbal plant (Sumbal) roots extracts against *Coccidia* infected broilers in Muzaffarabad. Five anti-coccidial drugs available in the study area market were used to evaluate their efficacy against coccidial infection. Mortality of broiler was also recorded during the drug trial. All the anticoccidial drugs were properly diluted in water and then applied to experimental birds. Significantly ( $P < 0.05$ ) more mortality was seen in control (F) group (43%) during the trial period. However, there was lowest mortality recorded in group B (7%), whereas mortality recorded in group A, C, D and E were 27%, 20%, 30% and 20%, respectively. This rate of mortality was not significantly ( $P < 0.05$ ) different among the groups. Broiler in all groups (A to F) had shown typical signs of Coccidial infection. Anticoccidial efficacy was also noted on the basis of percent reduction in Oocyst in each gram of faces. In this regards non-significant ( $P > 0.05$ ) differences were seen among the anticoccidial agents. Sulphadimerazine have shown much better effect against Coccidial infection as compared to other anticoccidial drugs given in trial. However, an herbal plant (Sumbal) root extract used in this study have also shown positive response to Coccidiosis (Figure 4.4).

**Table 4.1: Over all Prevalence of Coccidiosis.**

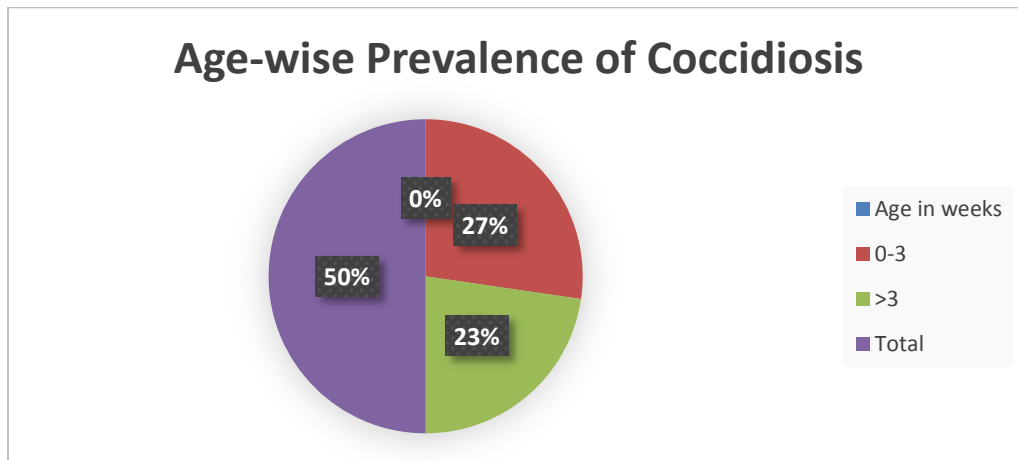
Sr. No	Total Birds Sampled	Birds Positive	Birds Negative	Prevalence %
1	573	111	462	19.37



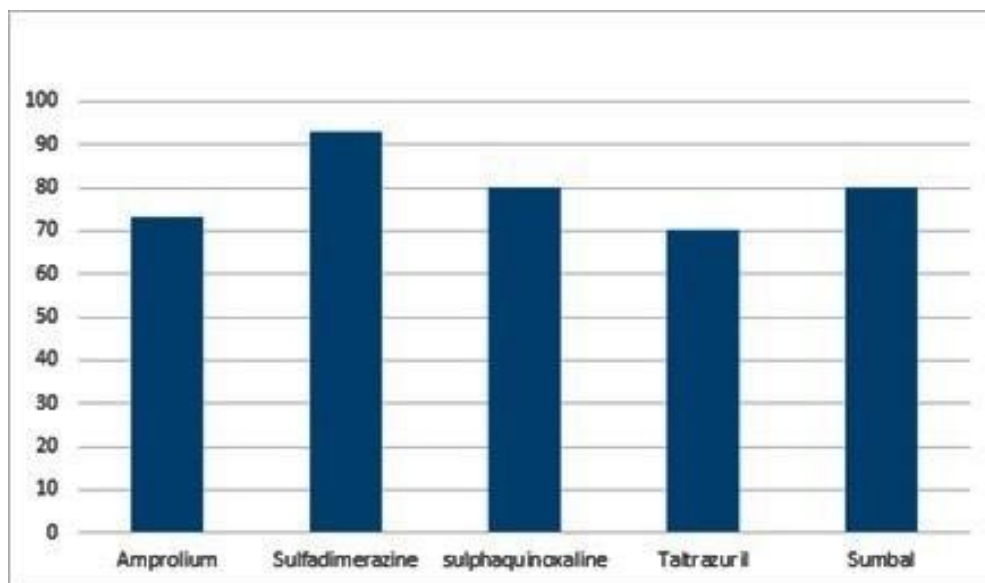
**Figure 4.1. Month wise prevalence of coccidial infection in broiler in study area.**



**Figure 4.2.** Seasonal prevalence of coccidial infection in broiler reared at different location in study area.



**Figure 4.3.** Age wise prevalence of coccidial infection in broiler reared at different location in study area.



**Figure 4.4.** Comparative efficacy of anti-coccidial and Sumbal against natural infection of coccidiosis.

## DISCUSSION

This is the first report regarding prevalence of Coccidiosis from the study area in Pakistan-controlled Azad Kashmir. Planning for control strategies can greatly benefit from studies on disease prevalence. Broilers are known to be a sensitive species of chicken that are prone to many diseases. One of the common illnesses in the broiler sector, coccidiosis has generally been seen as a managerial problem. The overall prevalence in the present study was 19.37%, which is less than the prevalence reported from other regions of the world (26.31%, 46.04%, 43.89%, 25.40%, and 39.58%) but nearly comparable to the prevalence published before (Khan *et al.*, 1990; Jadhav *et al.*, 2011).

Worldwide prevalence of Coccidiosis ranges from 10 % to 90 % in broilers (Morris and Gasser, 2006). The following factors could account for the lower prevalence found in the current study: (a) good litter management (b) anticoccidial agents in feed (c) periodic anticoccidial therapy (d) environmental factors. These are the common elements that were crucial in the global spread of coccidiosis in broilers. One of the management issues is coccidiosis, and damp litter, in particular, encourages the growth and sporulation of the *Coccidia* oocyst that infects broilers.

On the other side, good litter management lessens the likelihood that litter will become moist, which minimizes the risk of coccidiosis. Additionally, the likelihood of a disease breakout is reduced by properly combining anticoccidial medications in feed used for preventive measures. In poultry husbandry, timely vaccination is crucial since coccidiosis impairs immunity (Kabell *et al.*, 2006). Therefore, immunization of birds against other infectious agents is less effective during this immune-compromised stage. Two *Eimeria* species, *Eimeria tenella* and *Eimeria maxima*, were identified in this study and were found to be dominant in the district under investigation (Singh *et al.*, 2015). The prevalence of these two species and other *Eimeria* species in broiler and other chicken species that cause coccidiosis, however, has been observed by a number of researchers (Amer *et al.*, 2013). *Eimeria tenella* is widely found in the caeca of broilers and is thought to be the most pathogenic (Soulsby, 1982). Coccidiosis linked with *Eimeria tenella* is known as caecal coccidiosis. According to (Singh & Meitei, 1982), the dominance of *Eimeria tenella* in nature and its high pathogenicity may explain its increased prevalence.

The terrain and climate of an area, as well as environmental elements, such as rainfall, humidity, and temperature, all have an impact on the likelihood that a disease may spread. Figure 1 illustrates the outline of disease prevalence that varies with temperature, precipitation, and humidity changes. Since these factors are crucial contributors to the occurrence of coccidiosis

outbreaks and favor the growth of coccidial oocysts in any given location (Sharma *et al.*, 2013). In this study, it was shown that coccidiosis had a fluctuating monthly prevalence and persisted throughout the year. Age and season both have an impact on disease incidence. The results of the current study indicated that young age groups were more susceptible to the disease. These findings are similar to (Razmia *et al.*, 2001). This might be due to weakened immune system, and increased stocking density (Sharma *et al.*, 2013).

Between the two age groups, there was a statistically significant difference in prevalence (P 0.05). The present study found that the summer season had the highest prevalence, which may be related to the summer's favorable environmental conditions for coccidial oocyst sporulation, as previously reported (Sharma *et al.*, 2013). However, previous studies showing lowest occurrence in spring time support the present study's findings. As environmental factors such as climate and topography affect illness occurrence in any region, this difference may be caused by the local climate and topography. Furthermore, improved management and extra caring could be the explanations for this difference in seasonal occurrence of coccidiosis in the research location. The difference in coccidiosis prevalence was based on the type of farm floor, as cemented floors are simpler to wash and clean than non-cemented floors. Washing and sanitizing a filthy floor is challenging, and *Eimeria* oocysts that have been retained or trapped in the floor's cracks and crevices begin to sporulate when they find a favorable environment within the farm.

According to the results of present study, it has been noted that among anticoccidial agents, Sulphadimerazine had shown better effects against coccidial infection in broiler reared in the study area. Hunduma & Kebede (2016), shared the finding of their research by reporting that Sulphadimerazine is more effective than Amprolium in the treatment of coccidial infection. Kaboudi *et al.*, (2011) reported that Toltrazuril have better effect than Amprolium. In other investigation, it is also reported that Toltrazuril have shown better results than Amprolium and Sulfaquinoxaline. It has been extensively reported that development of drug resistance (Chapman, 1999) and reasons of variations in findings of present study and studies conducted earlier are due to; 1), different brand and their compositions 2) dose rate 3) type and class of birds 4) methods of treatment 5) parameter 6) duration of experiments 7) infection 8) anti-coccidial drug resistance 9) environmental factor and area 10) drug metabolism difference. These are the major reason of variations in the results. The better response shown by Sulphadimerazine is because of higher percentage of salt in composition and comparatively less used at farms of the study area as compared to other anticoccidial agents. Extensive used of drug may lead to the development of resistance as endorsed by Kaboudi *et*

al., (2016). So farmers must use this anticoccidial for the control and treatment of natural coccidial infection. Sumbal local plant is also evaluated for its anti-coccidial efficacy by hit and trial method against natural coccidial infection and showed anti coccidial effect. This plant was not used earlier for their anti coccidial effect. However, some reports depicting its other medicinal properties are present. Therefore, it must be evaluated by designing separate experimental study for evaluating their efficacy at large scale within laboratory and in field conditions. In addition, to this also finding of the active ingredient in this plant that exhibited anticoccidial properties is imperative. This would be better alternate to synthetic anticoccidial agents.

**Conclusions:** It was concluded that coccidiosis causing *Eimeria* species are present in the study area. Prevalence of coccidiosis recorded comparatively lower than prevalence recorded in other parts of the world. Broiler farmers must be vigilant and aware about different coccidiosis causing pathogens and economic losses associated with the disease particularly in summer season. Caecal coccidiosis is more prevalent in the study area. Anti-coccidial drug i.e. Sulphadimerazine 86% have shown comparatively better efficacy than other tested drugs. However, Sumbal herbal plant used in this trial has also shown anti-coccidial effects.

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