Comparative Analysis of Locally Developed Vaccines and Monensin Against Coccidiosis in Chicks

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A thesis submitted in partial fulfillment of the requirements for the degree of

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IN

PARASITOLOGY

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Declaration

I hereby declare that the work presented in the following dissertation is my own effort, except where otherwise acknowledged, and that the thesis is my own composition. No part of this thesis has been previously presented for any other degree.

Amina Malik

CERTIFICATE

This dissertation "Comparative Analysis of Locally Developed Vaccines and Monensin Against Coccidiosis in Chicks" submitted by **Ms. Amina Malik** is accepted in its present form by the Department of Animal Sciences, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad as satisfying the thesis requirement for the degree of Master of Philosophy in Parasitology.

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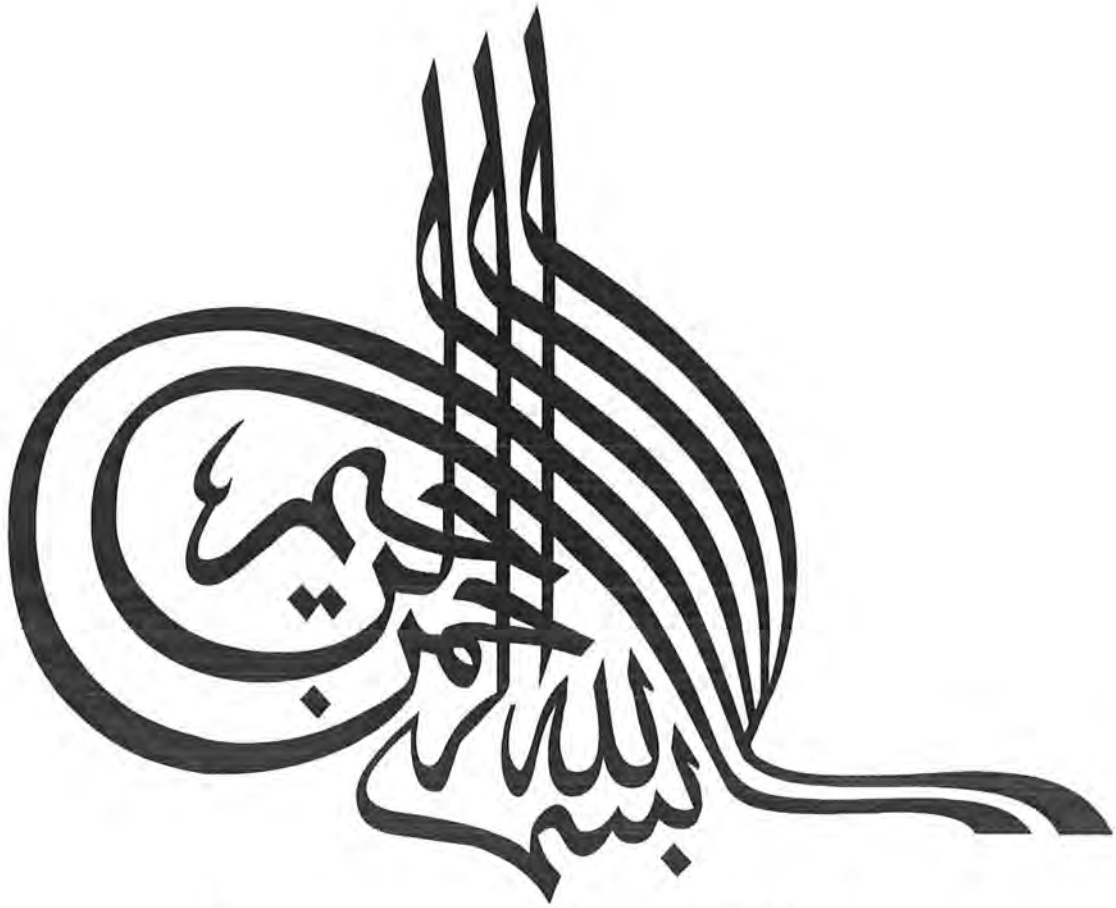

In the name of Allah,
the Most Beneficent,
the Most Merciful

I dedicate this humble effort

To my loving

FATHER AND MOTHER

For their endless sacrifices, support, prayers, advices

without whom none of my success would be possible

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LIST OF ABBREVIATIONS

Names	Abbreviations
Body weight gain	BWG
Feed conversion ratio	FCR
Oocyst per gram	OPG
Mean weight gain	MWG
Aspartate aminotransferase	AST
Alanine aminotransferase	ALT
Alkaline phosphate	ALP

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ABSTRACT

Coccidiosis is a parasitic disease caused by *Eimeria* in chickens, a major cause of economic loss and serious threat to poultry industry. For control of coccidiosis synthetic drugs are widely used either in their feed or water. Excessive use of these drugs resulted in resistance of *Eimeria* strains and rendered it less effective, for this reason vaccines are developed which are highly effective and unique. In present study three types of egg adapted vaccines were prepared (i) Gametocyte (ii) Gametocyte sonicated (iii) Gametocyte inactive by formalin. Twelve hen's eggs (10 days old) were kept at 39 °C and 70% humidity in an incubator and candled for embryo status. At 12th day sporulated *Eimeria* oocyst were inoculated in egg with the help of a sterile syringe. On day 7 post-inoculation, chorio-allantoic fluid was collected to harvest microgametes and macrogametes. Gametocytes were purified by centrifugation (1500 rpm/5 minutes), washed twice with normal saline and stored at 4 °C. Ninety one day old birds were purchased and divided into 6 groups, each containing 5 birds and were kept in triplicates. Group 1 was vaccinated with Gametocytes, Group 2 with sonicated Gametocytes, Group 3 with Gametocytes inactivated by formalin, Group 4 with monensin medicated drug, Group 5 was Infected with *Eimeria spp.* and Group 6 was non Infected. On 14th day of age each bird was inoculated with 1ml of 10,000 sporulated oocysts of *Eimeria*, no infection was given to Group-6. Monensin drug was given to medicated group 4 in feed 100mg/kg on 5th day post infection. 0.2 ml vaccine was given orally to group 1, 2 and 3. Performance parameters such as body weight gain, feed consumed, feed conversion ratio (FCR), oocyst counts (OPG), bloody diarrhea, lesion score, mortality and survival rate, histopathology and biochemical analysis were checked. Weight gain and feed consumption was more in non-infected control group and in gametocyte vaccinated group, approximately similar results were shown by monensin medicated group while in other two vaccinated groups i.e. gametocyte sonicated and formalin inactivated gametocytes showed comparatively less significant results. Oocysts per gram rate was higher in infected non medicated non vaccinated control group while there was mild shedding in gametocyte vaccinated and monensin medicated group. There was no oocyst shedding in non-infected control group. Feed conversion ratio was higher in infected groups. Lower lesion score of zero was shown by infected-medicated and non-infected control group while the other two vaccinated groups (2 and 3) showed lesion score of +2 and +1 respectively. Maximum lesion score

was observed in positive control group of +4. Maximum survival rate of 100% was shown by gametocyte vaccinated group, monensin medicated group and negative control group while poorest survival rate of 50% was shown by infected non medicated and non-vaccinated group. Formalin inactivated gametocyte group 2 and sonicated inactivated gametocyte group 3 showed 80% and 60% survival rates respectively. Serum biochemical parameters such as uric acid, albumin, creatinine ALT, ALP and AST were investigated in all groups. Serum biochemical analysis showed significant decrease in albumin and ALP concentrations and increase level of uric acid, AST, ALT and creatinine in infected non medicated non vaccinated, formaline inactivated gametocytes and sonicated gametocytes vaccinated groups. Less severe histopathological lesions were found in gametocyte vaccinated and Monensin medicated groups, mild to moderate lesions were found in gametocyte sonicated and gametocyte inactivated with formalin. More agglutination was recorded in gametocyte vaccinated group as compared to other two vaccinated groups. While no agglutination was observed in infected group. It is concluded from present work that among three egg adapted vaccines prepared i.e., gametocyte, gametocyte sonicated and gametocyte inactivated with formalin, gametocyte (pure) vaccines were significantly more effective than monensin medicated group (ionophore drug) as well as other two vaccinated groups.

INTRODUCTION

Poultry is among the quickly expanding segments of agricultural sector (Bachaya *et al.*, 2012) and is more quickly increasing in the developing countries. According to a survey organized by food and agriculture organization in 2007 reported that 75% of the poultry exists in the established countries (Mwale & Masika, 2011). Annually agricultural crop production has been rising at an estimate of 8-10%. 37 billion eggs and one billion broilers is nation annual utilization rate in US. Approximate of income flexibility for meat and eggs principally propose that utilization of these products can be expected to widen efficiently. In countryside per person utilization of eggs is less than half of that in inner city (Conroy *et al.*, 2005). Poultry is not only rich source of proteins and other vital component but it also help to improve economy rate of the country. It provides chances for employment and livelihood and contributes 19% of the total poultry meat production worldwide (Chapman, 2009).

In the advanced world the chief form of poultry manufacturing is poultry rearing. Every family in countryside and every 5th family in inner city income is correlated with poultry manufacturing activities in one way or the other, rural production contribute 98.5% to national egg with an annual output of 78,000 metric ton and 99.2% to poultry meat production with an annual output of 72,300 metric tons (Alemu *et al.*, 2012; Numan *et al.*, 2005). World population is increasing drastically so are their needs and it is estimated that up to 2050 it will raise up to 9 billion thus poultry demand is also increasing day by day as it is rich source of nutrients and proteins (Yegani, 2009). From 1995 to 2005 demand of chicken meat and eggs has increased 53% and 39% respectively (Scanes, 2007). The obstructions in this way are parasitic diseases such as coccidiosis (Li *et al.*, 2006).

Coccidiosis is a parasitic disease caused in chickens by *Eimeria spp.* one of major cause of economic loss and is a serious threat to poultry industry. In commercial poultry coccidiosis is more dangerous and cause death in flocks. It is most expensive disease to commercial poultry and cause $127 million loss annually worldwide (Usman *et al.*, 2011 & Masood *et al.*, 2013). Moreover coccidiosis inhibit growth of birds by causing epidemic diseases (Jun *et al.*, 2013).

Eimeria spp. belong to kingdom Protista, phylum Apicomplexa and are monoxenous parasties infect single host and are highly host specific. Life cycle of *Eimeria spp.* consists of two stages endogenous and exogenous stage (Price, 2012). There are about 1700 described species of genus *Eimeria* (Yang *et al.*, 2015). Many effect birds within 3 to 18 weeks and young ones are more susceptible to parasite. It mostly effects intestinal mucosa of birds and other animals. Coccidiosis can be prevented by good ventilation, cleaning and proper decontamination of feeders and drinkers moreover the stocking density and spacing are also among precatory measures (Munteanu & Demirbag, 2009).

There are nine species of *Eimeria* infecting chickens including *Eimeria acervulina*, *Eimeria brunette*, *Eimeria maxima*, *Eimeria mitis*, *Eimeria necatrix*, *Eimeria praecox*, *Eimeria mivatis*, *Eimeria hagani* and *Eimeria tenella* (Jordan *et al.*, 2011). Most pathogenic species of *Eimeria* are *E. tenella* and *E. necatrix*. *E. acervulina*, *E. mitis* and *E. mivati* are less pathogenic while *E. praecox* and *E. hagani* are lesser pathogenic species (Bachaya *et al.*, 2012). Common mean of infection is through mechanical transmission, between houses and farms moreover coccidial infections greatly depends on number of oocyst and immune status of the bird (Amer *et al.*, 2010). As few as 10 oocyst of *E. tenlla* give rise to 500,000 oocyst generation when ingested by chickens (Chapman & Jeffers, 2014). *Eimeria* life cycle is complex and consists of three stages sporogony, shizogony and gametogony. The cycle begins by the ingestion of sporoluated oocyst having four sporozoites (Chapman *et al.*, 2010). Oocysts are released in intestinal lumen where it is aided by bile, trypsin, Co_2 and release sporozoites which attach and invade host epithelium. The merozoites are released by sporozoites and finally these merozoites enter host cell. In host cell these merozoites mature into male and female gametes (Allen *et al.*, 2002). After fertilization oocyst is produced which is then sheded in feces and the cycle continues (Sharman *et al.*, 2010). Oocyst are highly resistance to adverse environmental conditions, composed of double wall made up of proteins and lipids which protects it from mechanical and chemical damage, under favorable conditions it become sporulated which is infective stage. Complete life cycle may vary from eight to nine days (Quiroz-Castañeda *et al.*, 2015). Six to nine days after infection are very crucial where maximum oocyst output is observed (Allen *et al.*, 2002). Higher prevalence of infection during rainy season indicating that

warmth temperature and humid condition favors the oocyts sporulation and subsequent transmission (Amin *et al.*, 2014)

Eimeria cause infection wherever poultry is raised (Ruff, 1999). Coccidial infections may be classified as, (i) Clinical coccidiosis, chronic phase which is characterized by diarrhea, morbidity, mortality and by injurious effects on economic performance (ii) Subclinical coccidiosis is asymptomatic phase i.e., not showing any clear symptoms (iii) Mild coccidiosis, showing no adverse effects (Williams, 2002). The signs and symptoms of cocciodiosis may vary from weight loss, bloody diarrhea, decreased egg production and if left untreated, may lead to death (Shah, 2013). All species of *Eimeria* invade intestinal epithelial cells resulting from mild to severe pathological conditions and cause malabsorption which ultimately leads to economic loss (Lee *et al.*, 2009). Intestinal infection leads to reduced feed efficiency and body weight (Su *et al.*, 2015).

Clinical diagnosis of coccidiosis is carried out by postmortem, examination of intestinal scarping and by microscopy of oocysts. Microscopy is considered as a technical method for species identification and lesion soring i.e. macroscopic examination of visible lesion (Mcdougald, 1998; Dezfoulian *et al.*, 2010). Anticoccidial sensitivity testing (AST) is a method to find out drug resistance against different coccidial isolates, biochemical and molecular techniques such as PCR are also among advanced diagnostic techniques (Gussem, 2006; Morris, 2015). McMaster technique is standard for oocyst counting (Haug *et al.*, 2008). Framers may diagnose coccidiosis by observing bloody diarrhea (Adewole, 2012). Oocyst wall is highly resistant and variety of methods are used to break it, most effective method is to add glass beads (Lee *et al.*, 2015). For the study of tissues, histopathology is performed (Amare *et al.*, 2012).

Currently coccidiosis is controlled by synthetic chemicals (drugs) either in feed or water. Major growth of poultry industry occurred during past 50 years due to use of coccidiostats in feed which has resulted in an increase in excellent poultry products for consumers (Tewari and Maharana, 2011). However these are costly and also develop resistance in *Eimeria Spp* (Chapman, 1997). Continuous emergence of new strains of *Eimeria*, continuous use of same drugs and modification of host immune system to them, drugs are less effective (Dalloul & Lillehoj, 2005). The development of vaccine is highly

effective and unique. For the control of pathogen in commercial poultry live vaccines either attenuated or non-attenuated oocyst are mostly used. According to a survey conducted in USA between 1995-999 it was reported that drugs are used worldwide (Chapman & Jeffers, 2014). Protective immunity is stimulated by live *Eimeria* vaccines (Price, 2012). *Eimeria* infections stimulates antibody and cell mediated immune responses (Laurent *et al.*, 2001). Synthetic coccidiostats and natural coccidiostats both differ in their mode of action against biological activity, metabolism and chemical structure of parasite. Another contrast lies between the way in which they eradicate parasite, natural coccidiostats kill the parasite at its extracellular stages i.e., when it has not entered cells while the synthetic drugs kill the intracellular stages when parasite has entered cells and gone through development (Chapman, 2007).

Monensin (1971) is an antibiotic used against coccidiosis in broiler chickens. Its activity is very broad and act on trophozoites and 1st generation shizonts and is more effective than amprolium (1960), clopidol (1968) and zoalene (1960). Monensin gives protection against all species at 0.01-0.121% concentration in feed (Kant *et al.*, 2013).

The new methods for developing vaccines against coccidiosis has aid their use in hatchery and in economic stability (Chapman *et al.*, 2002). There are three main types of vaccines (i) attenuated (ii) non-attenuated (iii) live. Main aim of any vaccine either attenuated or recombinant, is to protect against infection (Jenkins, 1998). Reproductive ability of *Eimeria* species are limited by live vaccines. Live vaccines can be given in variety of ways, in water, in feed, by sprying or in ovo (Price *et al.*, 2015).

The first commercial vaccine was CocciVac® which was introduced in US in 1952 and was based on several wild type strains of *E.tenella* oocysts. This type of vaccine is developed from fluid of chorio allontic membrane of egg (Sharman *et al.*, 2010). Various efforts are laid for designing an effective vaccine against coccidiosis by attenuating different strains of *Eimeria* either by physical or biochemical means. In vaccinated chickens immune response was detected by modified splenic cell migration inhibition test. Vaccines like immunocox and coxivac are commercially prepared worldwide, immunocox is also available in Pakistan, but it does not show 100% protection and disease occur inspite of vaccination (Akhtar *et al.*, 2003). Acquired immunity against avain coccidiosis is

attained by gut associated lymphoid tissues which contain B and T lymphocytes (lillehoj & lillehoj, 2000). Live vaccines contain either attenuated or virulent strains. Two types of attenuated vaccines are commercially prepared (i) Paracox containing strains of all species of coccidia (ii) livacox containing precocious and egg passed lines. Live attenuated vaccines have low reproductive rate which is its greatest benefit and do not cause clusters at mucosal site of infection which initiates strong immune response and cause minimal tissue damage at the infected site (Allen & Fetterer, 2002; sharman *et al.*, 2010).

The advanced method for the control of coccidiosis is in-ovo injection of live coccidian (Weber *et al.*, 2004). The antibodies which are raised against antigens present on the surface of gametocyte of *Eimeria spp.* can stop growth and development of gametes and halt the transmission of the disease (Hafeez *et al.*, 2007). Recently in-ovo vaccination of broilers birds against infection with *Eimeria tenella* in 18 day old chick embryo, has been demonstrated to be possible (Weber & Evens, 2003).

In egg propagated gametocytes vaccines, sporozoites of *Eimeria* are inoculated into allantoic cavity of 10-days old chick. Life cycle of sporozoites is completed inside egg in eight to nine days at 37°C and 70% humidity. Additionally, the different developmental stages of parasite are observed at suitable timings which may be sampled for further investigations (Jiang *et al.*, 2012).

The present study is planned to develop three types of vaccines (I) Gametocytes (II) Gametocyte inactivated by formalin (III) Gametocytes inactivated by sonication, biochemical analysis of serum of infected birds treated with vaccine and comparative analysis of vaccine with drug (monensin)

Aims and Objectives

The aims and objectives of this study were

1. To develop three types of vaccines i.e., gametocytes, gametocytes inactivated with formalin, gametocytes sonicated against birds infected with *Eimeria spp.*
2. Comparative analysis of vaccine with drug (Monensin) and biochemical analysis of serum of infected birds treated with vaccine.

MATERIALS AND METHODS

2.1 Sample Collection and Storage

The suspicious samples were collected from veterinary research institute Peshawar (VRI) Khyber Pakhtunkhwa and poultry research institute Rawalpindi (PRI) Punjab. Samples were preserved in 16 to 25ml screw cap vials containing 2.5% potassium dichromate ($K_2Cr_2O_7$) in 1:5 and brought to laboratory for further processing. The samples were kept at room temperature for about 7-10 days to facilitate sporulation.

2.2 Sporulation

The *Eimeria* samples were placed in petri dishes and conditioned with 2.5% potassium dichromate, kept at room temperature and aired daily up to 7 to 10 days to facilitate sporulation. Observed under microscope for the confirmation of sporulation (Sharma *et al.*, 2013; Long *et al.*, 1976). Samples were then homogenized in phosphate buffer distilled water having pH 8 for 2-3 min. Trypsin was added to a final concentration of about 1.5% w/v, incubated at 41 °C for about 30 min and was strained through two thickness of muslin cloth. Filtrate was centrifuged at 1500 rpm for 10 minutes and supernatant was discarded. Saturated salt solution 26% was added to pellet, mixed well and centrifuged for 10min at 1500 rpm. Oocysts from the top of salt solution were collected and washed repeatedly for 3-4 times in distilled water, pellet was obtained and kept in 2.5% K2Cr2O7, stored at 4°C for further processing (Eckert & Braun, 1995).

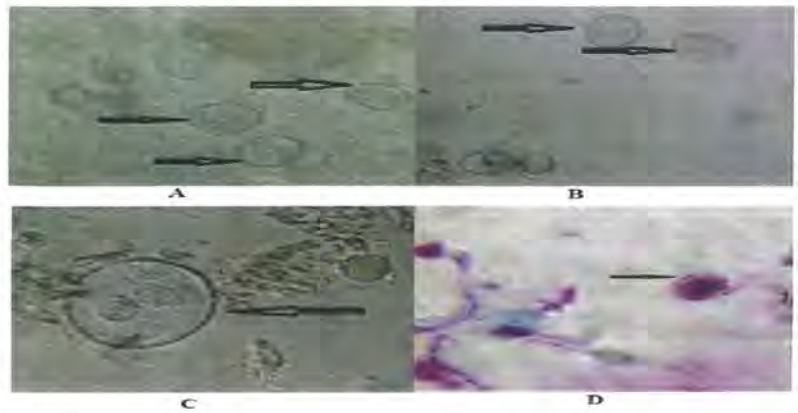

Figure 2.1: Showing (A, B) unsporulated, (C) Sporulated, (D) Giemsa-stained oocyst under light microscope at 40X magnification power.

2.3 Experimental Design

Twelve embryonated chicken eggs (10 days old) were used in this experiment, kept in an incubator at 39°C temperature and 70% humidity. To check the viability of embryo, candling was performed every day. On 12th day, eggs were placed on a plate and area opposite to head was wiped with 70% alcohol and a hole was drilled with the help of a sterile syringe. In the allantoic cavity of each embryo 0.1ml suspension of sporulated oocyst was inoculated along with penicillin by inserting syringe into egg while two embryos were kept as control and were inoculated with PBS. Opening of every egg was sealed by molten wax. Again the eggs were incubated for 7 days. Deaths occurred during 24 hours were not calculated as they were due to bacterial contamination. After inoculation *Eimeria spp.* passed through all developmental stages in chorio allantoic membrane and completes its life cycle. Mature microgametes and macrogametes were observed after 7 days of infection (Jiang *et al.*, 2012).

Chorio allantoic fluid was collected on 7th day after infection to harvest microgametes and macrogametes, for this purpose the eggs were first sterilized with 70% alcohol. The shell over air sac was cracked and removed with the help of sterile forceps and scissor. The chorio allantoic membrane was cracked and then fluid was collected by gently pressing the yolk sac with a sterile syringe. The fluid of each egg was aspirated, collected in separate plastic bottles and centrifuged at 800g for 20 minutes at 4°C temperature to remove RBC's. The gametocytes which entangled with embryo mass in supernatant were separated by treating with 1% trypsin at 39°C for about 2 hours. For the purification of gametocytes the samples were centrifuged at 1500 rpm for 5 minutes and then washed twice with normal saline and stored at 4°C for further use (Jiang *et al.*, 2012)

2.4 Vaccines Formulation

Three types of vaccines were prepared

Vaccine I- Gametocytes

Vaccine II- Gametocyte treated with formalin

Vaccine III- Gametocyte inactivated with sonication.

Gametocytes were sonicated for five minutes by using an ultrasonic homogenizer. The mixture was than centrifuged for 15 min at 250 rpm. Supernatant was collected and was inactivated with 3% formalin (Ayaz *et al.*, 2008).

Day old ninety broiler birds (chicks) were purchased from local hatchery and were brought to animal house (primate faculty) Quaid e Azam University Islamabad. The room was washed with disinfectant before placing birds. Room temperature was maintained at 29 °C, then gradually decreased up to 22 °C in third week and then kept constant till end of the experiment (Christaki *et al.*, 2004). Coccidiostat free feed was provided to birds. Artificial source of light was provided for 24 hrs. Grouping was done on day 5th and total duration of the experiment was 42 days. Total 6 groups were made (group 1, 2, 3, 4, 5 and 6) each containing 5 birds and each group was kept in triplicate

Group 1: Gametocyte

Group 2: Gametocyte sonicated

Group 3: Gametocyte inactivated with formalin

Group 4: Medicated (Monensin)

Group 5: Infected

Group 6: Not infected

Prior to the infection, the fecal litter from all groups and subgroups were sampled and inspected for the presence of any coccidian parasites. No group was found positive for oocysts. Vaccination was done on 6th day. 0.2 ml Gametocyte vaccine was given orally to group 1, 0.2 ml sonicated vaccine to group 2 and 0.2 ml formalin treated vaccine to group 3.Processed oocysts stored at 4 °C were centrifuged 3-4 times in distilled water to remove potassium dichromate. On 15th day post vaccination each bird from group 1 to 5 were inoculated with 1 ml of 10,000 sporulated oocysts. No infection was given to Group-6. Monensin drug at dose of 100 BW/Feed was given orally with the help of a dropper to medicated group 4 on 5th day post infection (Lee *et al.*, 2012).

2.5 Performance Parameters

The effectiveness of biological treatments was evaluated on the basis of following performance parameters: Body weight gain (BWG), feed consumed, feed conversion ratio (FCR), oocyst counts (OPG), bloody diarrhea, lesion score, mortality rate and survival rate, histopathology, indirect hemagglutination test and biochemical analysis.

2.6 Determination of Weight Gain

On 21st day of age (on day of inoculation) the body weight (BW) of all birds present in separate cages presenting different groups was determined and then again weighed on 4-6th week. Each week before feeding, birds were weighed using weighing balance. The weight of birds on 21st day was, therefore, considered as the initial weight. The mean weight gain (MWG) was calculated using the formula:

MWG = (mean final weight of live birds in a cage) - [(mean initial weight of all birds in that cage) + (weight of dead birds)].

(Naidoo *et al.*, 2008: Lee *et al.*, 2013)

2.7 Estimation of feed intake

Amount of feed consumed by each group was determined as, each morning quantity of feed given to each group was weighed from which weight of feed remained the next morning was subtracted.

2.8 Determination of feed conversion ratio

Feed conversion ratio (FCR) was calculated as grams of feed taken by birds to produce one gram of their body weight. FCR was calculated from the 4th week to 6th week of age i.e., 1st to 3rd week post infection. Feed conversion for individual birds cannot be determined because birds were fed as a group. The group feed conversion ratio (FCR) was calculated using the formula: (Naidoo *et al.*, 2008).

$$FCR = \frac{\text{Total feed consumed per group(g)}}{\text{Weight gain of live birds} + \text{Weight gain of dead birds}}$$

2.9 Fecal samples collection and laboratory examination

After 5 days of infection the birds started shedding oocysts. From 6 to 10 days post inoculation fecal samples from each cage were collected and microscopically examined for the presence of oocysts. McMaster's oocyst counting technique was used for counting the coccidian oocysts (Zaman *et al.*, 2012). In a beaker 3 grams of fecal sample was mixed with 42 ml of tap water. Any pallet if present was broken and kept overnight. The suspension was then filtered through two folds muslin cloth, preventing any undissolved debris from passing through it. The two chambers of the McMaster slide were filled with the filtrate and allowed to settle for 2–3 minutes. The McMaster slide was then examined microscopically. Following formula was used for calculating the oocysts count per gram of fecal sample (Naidoo *et al.*, 2008).

$$X/0.15\times 45 \times 10 \times 1/3 = X1000$$

Where X=average oocyst count in 1 chamber; 0.15=volume under ruled area of McMaster slide; 45=total volume of suspension; 10=1/10 dilution factor; 1/3=correction factor.

2.10 Bloody diarrhea

After infection bloody diarrhea was investigated from 4^{th} to 7^{th} day. The degree of bloody diarrhea was given degrees, from 0 (-) to 3 (+++). The zero level is normal level, degree 1 to less than 25%, degree 2 to 26–50%, 3 to 51–75%, and 4 to above 75% hemorrhagic feces in total feces over 24 hours were observed. This percentage was given according to severity of infection where 0 indicate no infection and 75% indicate high infection (Christaki *et al.*, 2004).

2.11 Lesion score

On 7^{th} and 14^{th} day of post inoculation from each group birds were sacrificed humanly. After post mortem intestinal tract of each chicken was examined and lesion score of 0-4 (Johnson & Reid, 1970) was assigned to each group.

Figure 2.2: Showing (A) blood filled caeca, (B) normal caeca of birds at 7th day post infection with *Eimeria spp*.

2.12 Survival percentage

The following formula is used for calculating survival rate of birds (Du & Hu, 2004).

$$\text{Survival \% age} = \frac{\text{Total no of chicks} \_ \text{Total no of dead chicks}}{\text{Total number of chicks}} \text{ X } 100$$

2.13 Mortality percentage

The mortality percentage was calculated using the formula (Naidoo *et al.*, 2008).

$$\text{Mortality \% age} = \frac{\text{Total no of dead chicks in the cage}}{\text{Initial no of birds in cage}} \text{ X } 100$$

2.14 Biochemical analysis

Blood samples were collected from the wing vein of three selected birds of all groups (the infected and uninfected) at 7 day post inoculation with the help of syringe. The blood samples were collected in vacutainer without anticoagulant and centrifuged at 750 × g for 15 min at 4°C. The serum was stored in Eppendorf's tubes at – 20°C until analyzed. Serum samples were used for determination of albumin, uric acid, creatinine, , alanine aminotransferase (ALT) and alkaline phosphatase (ALP) activities by using commercial kits of spectrum, aspartate aminotransferase (AST) by using biomaxima® kit and biochemical analyzer of motenu MTN-658C (Hafeez *et al.*, 2006 : Hashemnia *et al.*, 2014)

2.15 Histopathology

Liver and caecum tissues were taken from all groups on 7th day of infection after post mortem and placed in 10% formalin for 24-48 hours. Tissues were dehydrated by 70, 80, 90, 95, and 100% Ethanol for 60 and 90 minutes respectively. Finally the tissues were dehydrated by xylene twice for 60 minutes each, then embedded in paraffin for about 120 min at 58 °C. Wax blocks were made and placed onto wooden blocks. Tissues were microtomed at 7um thickness. Slides were smeared with a thin layer of albumin using clean brush. The slides were then stained by first keeping in xylene 1 and 11 for 10 minutes each and then passing through different concentrations of ethanol. Finally slides were stained with hematoxylin and eosin (destained by 95% ethanol if needed). Few drops of permount were added on slide and was covered with a cover slip. The slides were than observed under microscope where eosin binds to eosinophilic tissues and hematoxylin binds to basophilic tissues (Kawazoe *et al.*, 2005; Kadhim, 2014).

2.16 Indirect Hemagglutination Test

The serum and RBC's (collected on 5th and 15th day of the post vaccination) were used for detection of antibodies titer and developed immunity in vaccinated birds by indirect hemagglutination test. Erythrocytes of human blood group 'O' were suspended in sterile phosphate buffer saline and washed three times to remove plasma by centrifugation at 1500 rpm for 5 minutes. The erythrocytes (0.5ml) suspended in 10ml phosphate buffer saline (PBS) was mixed with 10ml of 3 different vaccines i.e. gametocytes, formalin inactivated and formalin inactivated sonicated gametocytes vaccines (antigens) in three sterile test tubes and incubated at 37°C for different time durations from 20 to 100 minutes. After incubation, sensitized erythrocytes containing solution were washed twice with PBS by centrifugation. 96 wells of micro titration plate were filled with 100µl of PBS. First row was filled with 100µl of serum sample. 50µl of sensitized RBC's of human blood group "O" were added to each well except last one to which washed RBC's were added as control. Serum and RBC's were gently mixed by tapping. The plates were incubated at 25°C for 25 minutes. Degree of

hemagglutination in each well was recorded (Hayat *et al.,* 1999; Sultana *et al.*, 2014; Zimmerman *et al.*, 1968).

2.17 Statistical analysis and presentation of results

Results were presented as mean ± standard deviation and differences between means were determined using one way analysis of variance (ANOVA). Level of significance between means were considered at $p < 0.05$ using the Tukey test (HSD) by using Minitab16.

RESULTS

All experimental groups expect non-infected (6) showed clinical symptoms like depression, ruffled feathers, haemorrhagic feces, weakness, decreased activity, with death of few birds. These symptoms were mild at 5th day of post infection and become severe at 7th to 9th day of infection, then disappearance of bloody diarrhea starts till the end of the first week post infection. The symptoms were mild in gametocyte vaccinated and medicated groups (1 and 4) as compared to other groups (2, 3 and 5).

3.1 Comparative effect of vaccines and drugs on body weight gain of chicks after infection

Changes in mean body weight gain (g) of coccidian infected broilers birds investigated between 1st and 3rd week post inoculation showed that body weight gain of uninfected non-medicated control group was higher than all vaccinated, medicated and infected non-medicated control groups with mean value of 430.66±4.04 gm. Gametocyte vaccinated group showed maximum weight gain of 417.00±5.29 gm which was approximately similar to medicated group 413.66±7.76 gm showing significant difference (P=0.004). Lowest body weight gain 278.33±5.13 gm was recorded in infected non-medicated control group while formalin inactivated gametocyte and gametocyte inactivated by sonication showed similar results 385.33±7.63 gm and 386.33±7.57 gm respectively (Table 3.1 and Figure 3.1).

Table 3.1: Showing the Comparative effect of vaccines and drug on body weight gain (Mean±SD) of chicks after infection with *Eimeria spp.*

Time (week)	Mean body weight gain (gm) from 1st to 3rd week of post-inoculation					
	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
1st wk	226.30±11.84^{b}	195.30±13.05^{c}	184.00±7.81^{c}	224.00±1.07^{b}	149.60±10.59^{d}	309.00±3.00^{a}
2nd wk	344.60±11.15^{a}	301.00±13^{a}	297.60±14.36^{ab}	339.60±8.38^{a}	231.30±4.04ab	391.30±3.21^{a}
3rd wk	417.00±5.29^{a}	385.33±7.63^{a}	386.33±7.57^{a}	413.60±7.76^{a}	278.30±5.13^{b}	430.60±4.04^{a}

Means having the different superscripts within the same row are significantly different from one another by Tukey test (P=0.004).

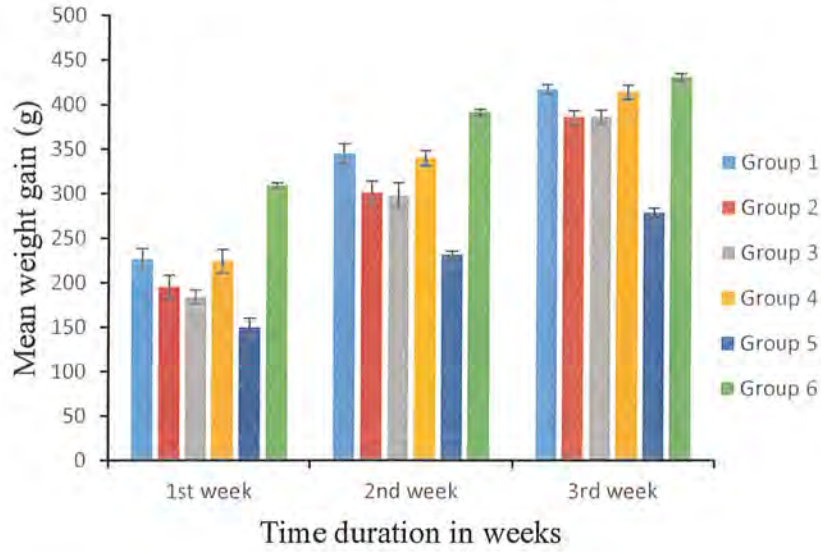

Figure 3.1: Showing body weight gain (Mean±SD) of chicks infected with *Eimeria spp.* between 1st to 3rd week post inoculation.

3.2 Comparative effect of vaccines and drugs on feed consumption of chicks after infection with *Eimeria spp.*

The highest feed consumption of 753.00±5.00 g was shown by negative control group 6. Among vaccinated group maximum feed 707.00±2.60 g was consumed by gametocyte group 1, followed by medicated group 4 (711.00±3.60 g). Infected non-medicated non-vaccinated group showed lowest feed consumption of 681.33±8.02 gm with significant association of P=0.003 while vaccinated group 2 and 3 showed feed consumption of 699.00±1.73 gm and 696.00±5.56 gm respectively (Table and figure 3.2).

Table 3.2: Showing the comparative effects of drug and vaccine on feed consumption ratio of chicks (Mean±SD) infected with *Eimeria spp.*

Time (week)	Feed consumed (g) by chickens during 1-3 weeks post-inoculation.					
	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
1st wk	450.00±10.00^{ab}	431.66±15.27^{bc}	429.66±12.09^{bc}	452.66±9.71^{ab}	409.66±3.51^{c}	470.33±2.08^{a}
2nd wk	589.33±8.14^{ab}	581.00±7.00^{b}	581.66±7.09^{b}	593.00±8.18^{ab}	532.66±7.76^{c}	603.66±3.51^{a}
3rd wk	707.00±2.64^{bc}	699.00±1.73^{bc}	696.00±5.56^{c}	711.00±3.60^{b}	681.33±8.02^{d}	753.00±5.00^{a}

Means having the different superscripts within the same row are significantly different from one another by Tukey test (P=0.003).

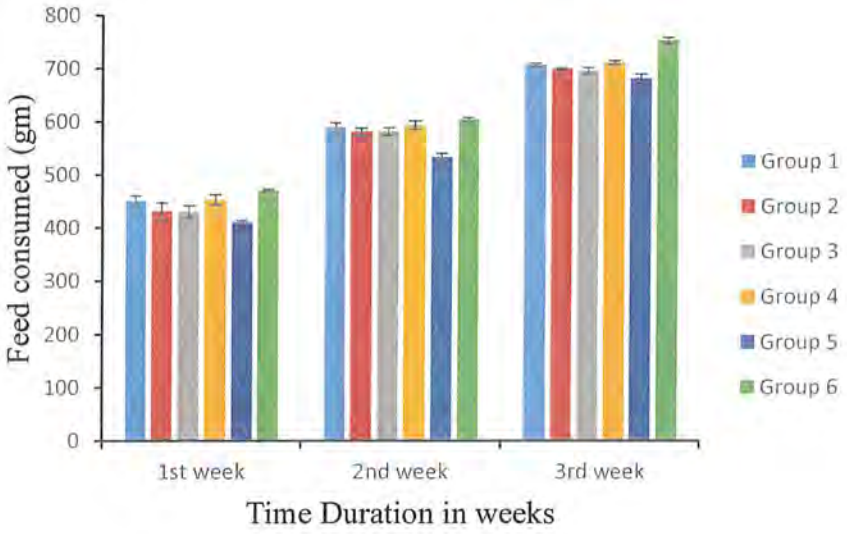

Figure 3.2: Showing mean feed consumed (Mean ± SD) of chicks infected with *Eimeria spp.* between 1st to 3rd week post inoculation.

3.3 Comparative effect of vaccines and drugs on feed conversion ratio of chicks infected with *Eimeria spp.*

The lowest feed conversion ratio was shown by group 1, 4 and 6 with values of 1.69±0.02, 1.71 ±0.04 and 1.74±0.005 respectively, showing significance value of (P=0.002). Due to high level of infection and low feed consumption ratio group 2, 3 and 5 showed maximum feed conversion ratio of 1.81±0.03, 1.79±0.04 and 2.44±0.04 respectively (Table and figure 3.3).

Table 3.3: Showing the comparative effects of drug and vaccine on feed conversion ratio (FCR) of chicks (Mean±SD) infected with *Eimeria spp.*

Time (week)	Feed conversion ratio (FCR) of chickens during 1st to 3rd week post inoculation					
	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
1st wk	1.99±0.14^{b}	2.21±0.12^{b}	2.33±0.14^{b}	2.02±0.15^{b}	2.74±0.18^{a}	1.51±0.01^{c}
2nd wk	1.70±0.07^{cd}	1.92±0.06^{b}	1.95±0.11^{b}	1.74±0.06^{c}	2.29±0.02^{a}	1.53±0.01^{d}
3rd wk	1.69±0.02^{d}	1.81±0.03^{b}	1.79±0.04b^{c}	1.71±0.04^{cd}	2.44±0.04^{a}	1.74±0.005^{bcd}

Means having the different superscripts within the same row are significantly different from one another by Tukey test (P=0.002).

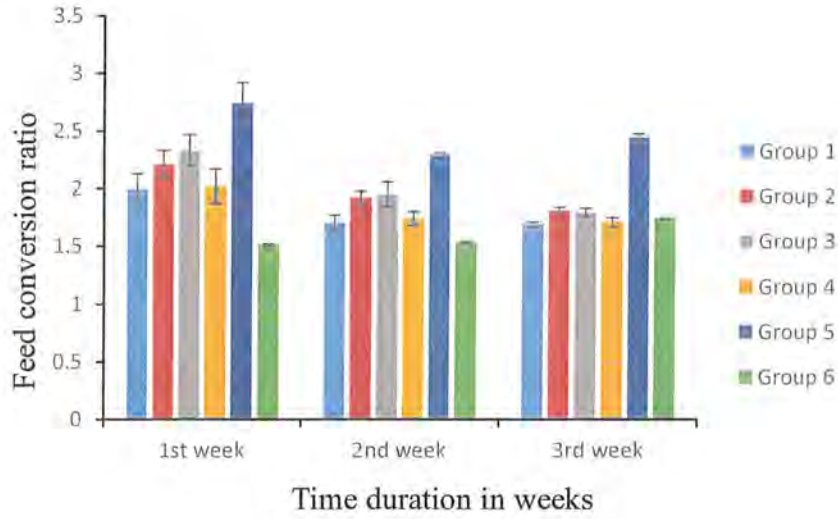

Figure 3.3: Showing feed conversion ratio (Mean±SD) of chicks infected with *Eimeria spp* between 1st to 3rd week post inoculation.

3.4 Comparative effect of vaccines and drugs on oocyst count per gram feces of chicks after infection with *Eimeria spp*

The effect of vaccines and drug on oocyst count per gram of feces of infected birds was calculated during first two weeks of infection. Highest oocyst count was recorded on 7th and 8th day post inoculation in all infected group except negative control which showed no oocyst shedding. Gradual decrease in oocyst shedding was observed on 8th day of

inoculation in all groups. Highest oocyst count (33.65±2.85) was showed by non-medicated, non-vaccinated control group. Monensin treated group showed oocyst count (21.3±2.85) which was similar to gametocyte vaccinated group (22.99±4.14) showing P value of =0.006. Vaccinated group 2 and 3 gametocytes formalin inactivated and sonicated gametocytes showed oocyst count of 25.65±4.44 and 26.64±2.85 respectively (Table and Figure 3.4).

Table 3.4: Showing the comparative effect of vaccines and drug on oocyst shedding in chicks infected with *Eimeria spp.*

Time (Days)	Means ± SD Oocyst per gram of feces (×10³)					
	Group 1	**Group 2**	**Group 3**	**Group 4**	**Group 5**	**Group 6**
6th day	4.00±1.00^{b}	4.33±1.15^{b}	4.33±0.57^{ab}	3.66±0.57^{b}	6.33±0.57^{a}	0.00±0.00^{c}
7th day	5.00±1.00^{b}	5.33±1.15^{b}	5.66±0.57^{b}	4.66±0.57^{b}	8.33±0.57^{a}	0.00±0.00^{c}
8th day	6.00±1.00^{b}	6.66±0.57^{b}	6.66±0.57^{b}	5.66±0.57^{b}	9.33±0.57^{a}	0.00±0.00^{c}
9th day	4.66±0.57^{a}	5.33±0.57^{a}	5.66±0.57^{a}	4.66±0.57^{a}	5.33±0.57^{a}	0.00±0.00^{c}
10th day	3.33±0.57^{ab}	4.00±1.00^{ab}	4.33±0.57^{a}	2.66±0.57^{b}	4.33±0.57^{a}	0.00±0.00^{c}
Total	22.99±4.14	25.65±4.44	26.64±2.85	21.3±2.85	33.65±2.85	00±00

Means having the different superscripts within the same row are significantly different from one another by Tukey test (P=0.006).

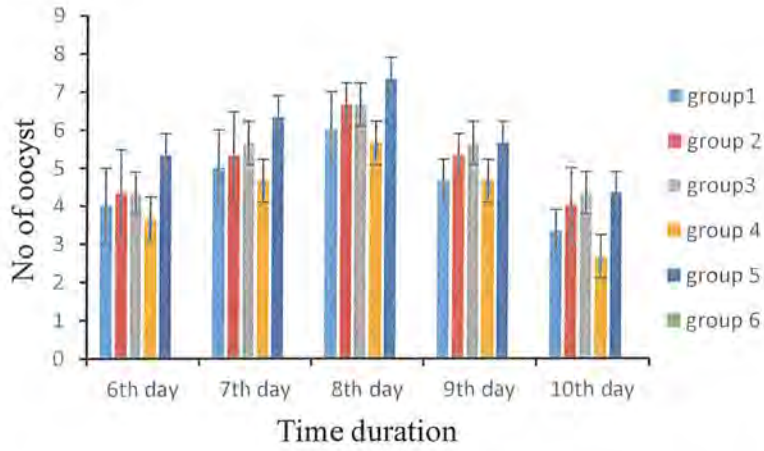

Figure 3.4: Showing oocyst count per gram of feces (Mean±SD) of chicks infected with *Eimeria spp.* between 1st to 3rd week post inoculation.

3.5 Comparative effect of vaccines and drugs on bloody diarrhea, lesion score, mortality and survival rate of chicks infected with *Eimeria spp.*

On 4th day post infection birds in all groups except non-infected control group showed bloody diarrhea. In infected non medicated non-vaccinated group severe diarrhea was observed on 5 to 7th day post infection. In monensin medicated and gametocyte vaccinated group mild diarrhea was observed which disappeared on 5th day post infection. While the other two vaccinated group 2 and 3 showed moderate diarrhea on 4, 5 and 6th day of post infection. On 7 and 8th day of infection bloody diarrhea disappeared in all experimental groups except infected, non-medicated and non-vaccinated group.

Lower lesion score of zero was shown by infected-medicated and non-infected control group while the other two vaccinated groups 2 and 3 showed lesion score of +2 and +1 respectively. Group 1 showed no lesion score. Maximum lesion of +4 score was observed in infected non-medicated and non-vaccinated group.

Maximum mortality of 50% was recorded in infected non-medicated and non-vaccinated group while no mortality occurred in monensin treated, gametocyte vaccinated groups and non-infected control group. Mortality of 20% was observed in formalin inactivated gametocyte group and 40% in sonicated inactivated gametocyte group.

Maximum survival rate of 100% was shown by gametocyte vaccinated, monensin medicated group and negative control group. Vaccinated group 2 i.e., gametocytes inactivated with formalin showed 80% survival rate followed by group 3 i.e., sonicated gametocytes and infected non-medicated group 5 with survival rate of 60 and 50% respectively (Table 3.5).

Table 3.5: Showing the comparative effect of drug and vaccines on bloody diarrhea, lesion score, survival and mortality rate of chicks infected with *Eimeria spp.*

Groups	Bloody diarrhea (Day)					Lesion score	Mortality rate (%)	Survival rate (%)
	3rd	4th	5th	6th	7th			
1	–	+	–	–	–	+0	0	100
2	–	+	++	+	–	+2	20	80
3	–	+	++	+	–	+1	40	60
4	–	+	+	–	–	+0	0	100
5	–	++	+++	++	+	+4	50	50
6	–	–	–	–	–	+0	0	100

+ = less diarrhea, ++ = moderate diarrhea, +++ = heavy diarrhea, — = No diarrhea.

3.6 Indirect Hemagglutination Test

Hemagglutination was observed on 5th and 15th day of post vaccination in all vaccinated groups. More agglutination was recorded in gametocyte vaccinated group than other two vaccinated groups which showed moderate agglutination. Maximum agglutination occurred when samples were kept in an incubator at 37°C for 40 minutes while no agglutination occurred at room temperature. (Figure and Table 3.6 - I and II).

Figure 3.6: Showing degree of hemagglutination in vaccinated chicks.

Table 3.6 (I): Showing Degree of hemagglutination versus time at 37°C in serum of all experimental groups collected on 5th day of vaccination.

Time (minutes)	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
20	–	–	–	–	–	–
40	++	–	–	–	–	–
60	+++	+	+	–	–	–
80	L	++	+	–	–	–
100	L	L	++	–	–	–

+ = low reaction, ++ = moderate reaction, +++ = strong reaction, L = lysis of red blood cells, — = No reaction.

Table 3.6 (II): Showing Degree of hemagglutination versus time at 37°C in serum of all experimental groups collected on 15th day of post vaccination.

Time (minutes)	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
20	–	–	–	–	–	–
40	+++	–	–	–	–	–
60	L	++	+	–	–	–
80	L	+++	+	++	–	–
100	L	L	++	++	+	–

+ = low reaction, ++ = moderate reaction, +++ = strong reaction, L = lysis of red blood cells, — = No reaction.

3.7 Biochemical analysis of different experimental groups

A significant increase in uric acid concentration of 8.70±0.01 mg/dl was observed in infected, non-medicated and non-vaccinated when compared with non-infected control group with uric acid concentration was 6.26±0.07 mg/dl statistically similar to negative control group (6.26±0.07) and gametocyte vaccinated group (6.07±0.04) showing significant association of (P=0.003). Higher uric acid concentration of 7.58±0.41 mg/dl was observed in vaccinated group 3 as compared to vaccinated group 1 and 2 6.07±0.04 and 6.97±0.05 mg/dl respectively (Table and Figure 3.7).

Infected non-medicated non-vaccinated control group showed significant increase in creatinine concentration with value of 1.47±0.08 mg/dl. Concentration of creatinine was

observed to 0.88±0.09 mg/dl in negative control group which was approximately similar to gametocyte vaccinated and monensin medicated group with concentration of 0.86±0.13 and 0.81±0.06 mg/dl respectively showing significant association of (P=0.004). While gametocyte sonicated and gametocyte inactive by formalin showed concentration of 1.02±0.02 and 1.11±0.08 mg/dl respectively (Table and Figure 3.7).

A significant decrease in alkaline phosphatase (ALP) concentration 215.41±0.93 µl was recorded in infected, non-vaccinated and non-medicated group when compared with negative control group i.e., 315.00±20.15. Similarly, vaccinated groups 2 and 3 showed decreased ALP value of 250.4±9.79 and 240.91±17.61 µl respectively (P=0.002). Gametocyte vaccinated group showed significant increase in ALP of 301.06±3.99 µl compared with medicated and negative control groups showing ALP concentration of 290.55±11.79 µl and 315±20.15 µl respectively with P value = 0.003 (Table 3.7 and Figure 3.8).

In negative control group aspartate aminotransferase (AST) concentration was 281.16±3.64 µl which was approximately similar to gametocyte vaccinated group and medicated group showing approximately similar values of 266.92±9.90 µl and 262.83±9.72 µl respectively, thus showing significance association of P=0.003. Significant increase in AST concentration of 314.98±7.48 µl was observed in infected non-medicated, non-vaccinated control group while gradual increase in the concentration of AST occurred in remaining vaccinated groups 2 and 3 298.85±6.57 and 297.99±5.29 µl respectively (Table 3.7 and Figure 3.8).

Normal level of alanine amino transferase (ALT) was observed in negative control group which was 5.54±0.33 µl. Vaccinated group 1 showed 5.28±0.10 and monensin medicated group showed 5.41±0.49µl ALT level thus showing significance of P=0.004. Vaccinated group 2 and 3 showed gradual increase in ALT of 12.48±0.18 and 11.12±0.08 µl respectively. Higher concentration of 13.38±0.30 was shown by infected non-medicated, non-vaccinated group with P=0.004 (Table 3.7 and Figure 3.9).

Negative control group showed albumin level of 1.28±0.01 gm/dl. Albumin concentration decreased significantly to 0.69±0.002 gm/dl in infected non-medicated, non-

vaccinated control group. While in gametocyte vaccinated group and monensin medicated group albumin level was observed as 1.25±0.03 and 1.22±0.03 gm/dl respectively, similar to control group showing significant association of P=0.005. Gradual increase in albumin level occurred in group 2 and 3 with values of 1.04±0.05 and 1.43±0.32 respectively (Table 3.7 and Figure 3.9).

Table 3.7: Showing mean values of biochemical parameters of chicks infected with *Eimeria Spp.*

Parameters	Biochemical values ± S.D					
	Group 1	**Group 2**	**Group 3**	**Group 4**	**Group 5**	**Group 6**
Uric acid	6.07 ± 0.04^{c}	6.97 ± 0.05^{bc}	7.58 ± 0.41^{b}	6.34 ± 0.04^{c}	8.70 ± 0.01^{a}	6.26 ± 0.07^{c}
creatinine	0.86 ± 0.13^{c}	1.02 ± 0.02^{bc}	1.11 ± 0.08^{b}	0.81 ± 0.06^{c}	1.47 ± 0.08^{a}	0.88 ± 0.09^{bc}
ALP	301.06 ± 3.99^{a}	250.4 ± 9.79^{ab}	240.91 ± 17.61^{ab}	290.55 ± 11.79^{ab}	215.41 ± 0.93^{b}	315 ± 20.15^{a}
AST	266.92 ± 9.90^{c}	298.85 ± 6.57^{ab}	297.99 ± 5.29^{ab}	262.83 ± 9.72^{c}	314.98 ± 7.48^{a}	281.16 ± 3.64^{bc}
ALT	5.28 ± 0.10^{d}	12.48 ± 0.18^{b}	11.12 ± 0.08^{c}	5.41 ± 0.49^{d}	13.38 ± 0.30^{a}	5.54 ± 0.33^{d}
Albumin	1.25 ± 0.03^{ab}	1.04 ± 0.05^{bc}	1.43 ± 0.32^{a}	1.22 ± 0.03^{ab}	0.69 ± 0.002^{c}	1.28 ± 0.003^{ab}

Means having the different superscripts within the same row are significantly different from one another by Tukey test.

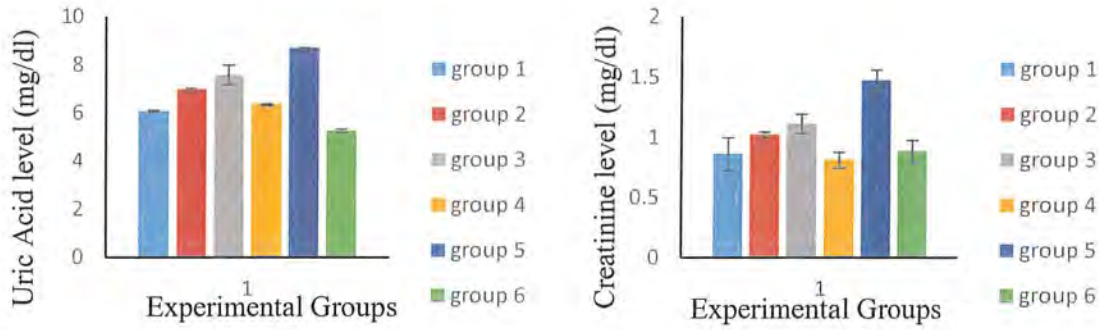

Figure 3.7: Showing uric acid level and creatinine level (mg/dl) of chicks infected with *Eimeria spp.*

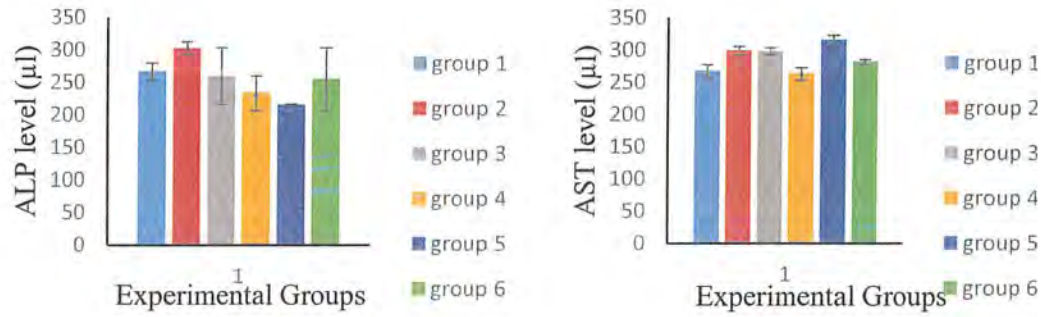

Figure 3.8: Showing ALP and AST level (u/l) of chicks infected with *Eimeria spp.*

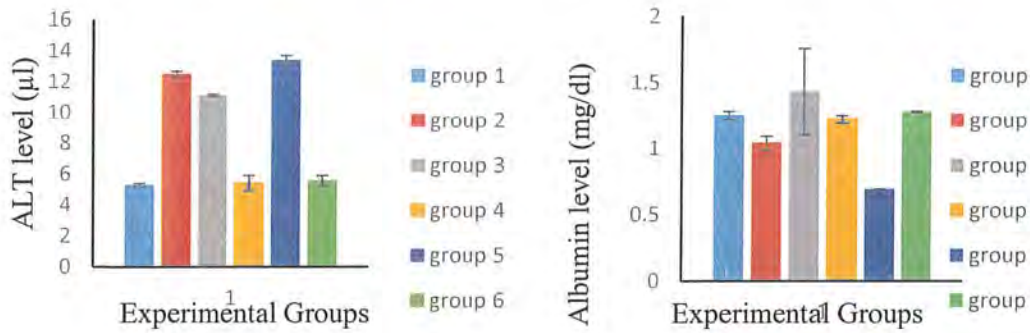

Figure 3.9: Showing ALT and Albumin level (u/l and mg/dl respectively) of chicks infected with *Eimeria spp.*

3.8 Comparative effect of vaccines and drugs on liver and ceaca of chicks infected with *Eimeria spp.*

Histopathological lesions were observed on 7th day post infection. Microscopic examination of permanent histopathological slides showed normal caeca and liver in gametocyte vaccinated, Monensin medicated and negative control group. Histopathological findings of formalin inactivated and gametocyte sonicated vaccine showed presence of schizonts in intercellular spaces and muscosal layer of caeca. In infected non-medicated and non-vaccinated control group showed heavy infection were presence of schizonts, fusion of villi and discrete hemorrhage was observed. *Eimeria spp.* infecting liver parenchyma cells showed histopathological lesions in liver, being severe in positive control group while lesions were less severe in formalin inactivated and gametocyte sonicated groups. No lesions (normal histology) were observed in negative control group, gametocyte vaccinated and monensin medicated groups.

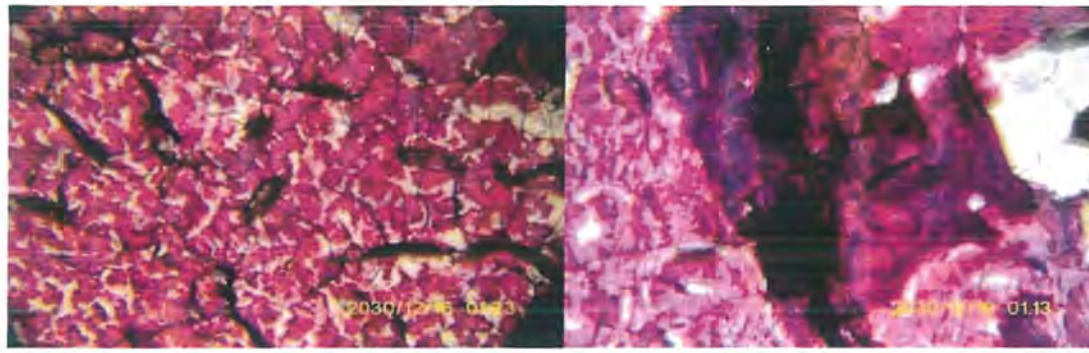

Figure 3.10: Showing comparative histopathological observations of chick's liver of all experimental groups after infection

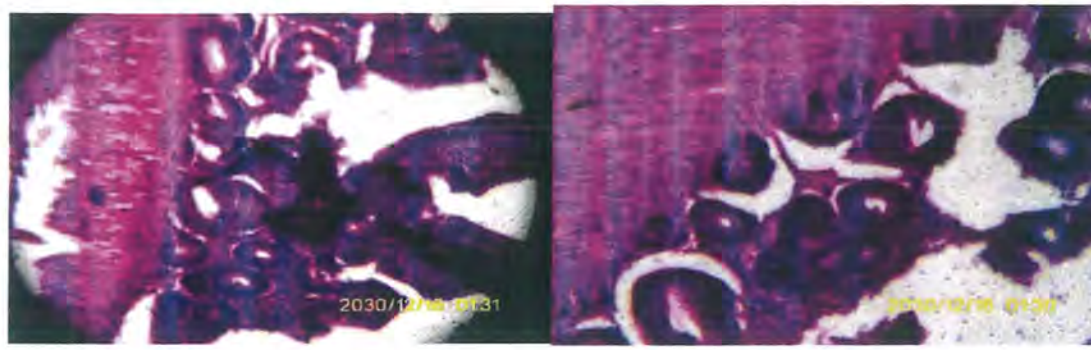

Figure 3.11: Showing comparative histopathological observations of chick's caeca of all experimental groups after infection.

DISCUSSION

Coccidiosis is one of the most important disease of poultry which is caused by intracellular protozoan parasite of genus *Eimeria,* it is highly host specific and resides in intestinal mucosa of birds (chickens). Coccidiosis is a serious threat to poultry industry and to the world's economy that is why variety of drugs have been designed to cure this disease but due to continuous emergence of new strains of *Eimeria*, resistance have been developed in parasite. Use of live vaccines (in-ovo) is an advanced and effective method for treatment of coccidiosis. (Brisibe *et al.*, 2008; Druagan *et al.*, 2014; Abbas *et al.*, 2010).

In this study in-ovo vaccines were used against coccidiosis. Antibodies raised against surface gametocyte antigens of *Eimeria Spp* could inhibit growth, development and fertilization of gametes thereby block transmission of the disease (Hafeez *et al*, 2007).

Increased mean body weight gain was shown by gametocyte vaccinated birds when compared with formalin inactivated gametocyte vaccinated and sonicated gametocyte vaccinated groups. Similar studies were conducted by Weber *et al.*, (2004) and Ding *et al.*, (2004) who reported higher mean body weight gain by vaccinated birds than in non-vaccinated. Same results of higher weight gain was shown by Asim *et al.*, (2001) who reported higher mean weight gain in vaccinated birds and lower body weight gain by medicated birds, while poor body weight gain was reported in non-vaccinated and non-medicated. In contrast Mathis *et al.*, (2014) showed higher mean body weight gain in medicated group than in vaccinated.

Maximum feed consumption was shown by gametocyte vaccinated when compared with formalin inactivated gametocyte vaccinated, sonicated gametocytes and medicated group. Similar results were shown by Lee *et al.*, (2012) who reported higher feed consumption in gametocyte vaccinated group than in others.

Feed conversion ratio in gametocyte vaccinated group was less than formalin inactivated gametocyte vaccinated and sonicated gametocyte vaccinated groups. Del chacho *et al.*, (2012) immunized birds with dendritic cells and reported lower feed conversion ratio (FCR) in immunized birds when compared with non-immunized. William (2002) reported lower FCR in birds treated with ionophore when compared with infected

untreated birds. likewise Danforth, (1988) studied the live oocyst vaccine in the control of avian coccidiosis in field trails reported lower feed conversion ratio in vaccinated medicated birds while higher feed conversion ratio was observed in non-vaccinated and non-medicated groups. Gametocyte vaccinated group showed lower oocyst count than other two vaccinated groups and monensin medicated. Similarly Al –Idreesi *et al.*, (2013) reported decreased oocyst output in vaccinated birds when compared with non-vaccinated birds.

Lower lesion score was observed in birds vaccinated with gametocyte, formalin inactivate, gametocyte sonicated and in monensin medicated birds. Similarly Weber & Evans (2003) reported birds immunized with in-ovo vaccine have less lesion score when compared with non-immunized birds. Likewise Ayaz *et al.*, (2008) reported mild lesion score in gametocyte vaccinated birds followed by gametocyte inactivated with formalin and gametocyte sonicated respectively. In the study of Jenkins *et al.*, (1991) mild lesion score was reported in birds immunized with recombinant gene (GBP-EAMZ250). No mortality occurred in gametocyte vaccinated, monensin medicated and in negative control groups. Similarly Hafeez *et al.*, (2007) also reported no mortality in gametocyte vaccinated birds. While high mortality rate was observed in infected non medicated control group. Williams (2002) reported less mortality in vaccinated birds when compared with medicated birds. Mild bloody diarrhea was observed in gametocyte vaccinated group when compared with other groups. Similar results were shown by Bahrami & Bahrami, (2006) who reported mild bloody diarrhea.

Indirect hemagglutination test was performed to detect antibodies against *Eimeria.* Higher level of agglutination was recorded by gametocyte vaccinated group. Similarly Sultana *et al.*, (2014) reported high level of agglutination in vaccinated groups as compared to non-vaccinated calves. Likewise Anwar *et al.*, (2008) showed maximum agglutination in gametocyte vaccinated group than in livaCox® group. In contrast Lee *et al.*, (2012) reported highest serum anticoccidial titers in birds fed on non-medicated diet when compared with vaccinated chicks.

Severe histopathological lesions were found in non-vaccinated infected group while no noticeable lesions were observed in gametocyte vaccinated group. Similar results

were shown by Kadhim (2014) who reported severe intestinal, caecal and liver lesions in non-vaccinated infected group.

Decreased concentration of alkaline phosphatase (ALP) and albumin was recorded in infected non medicated non vaccinated groups. Similar results were shown by Patra *et al.*, (2010) who reported reduced level of ALP in infected groups which may be due to damage in bone marrow and severe depression in growth. Decreased level of albumin was reported by El-Maksoud *et al.*, (2014) in infected group when compared with non-infected group.

An increased in the concentration of aspartate aminotransferase (AST) and decrease concentration of alanine amino transferase (ALT) were decteded in vaccinated groups when compared with infected non-vaccinated groups. Similar results of increased concentration of AST in vaccinated birds were reported by Mondal *et al.*, (2011). Normal level of ALT was reported by Hashemnia *et al.*, (2014) when compared with control group.

Higher concentration of uric acid and creatinine was recorded in infected non-vaccinated groups. According to Sena *et al.*, (1997); Salwa *et al.*, (1998) and Youssef *et al.*, (2008) significant increase in uric acid level might be due to injury to kidney parenchyma, caused by the harmful effect of *Eimeria* paasite. The higher level of uric acid in broilers infected with *Eimeria* might be due to kidney disfunction (Patra *et al.*, 2010 ; Bowes *et al.*, 1989). Harfoush *et al.*, (2010) reported significant increase in concentration of creatinine from normal level in infected birds.

Conclusion

From this study it is concluded that use of vaccines enhance immunity against coccidiosis and that vaccines are more effective than drug (monensin). More positive results were shown by gametocyte vaccine than other two vaccines i.e., gametocyte sonicated and gametocyte inactivated with formalin. Further expanded and appropriate experimental studies are required to establish their mode of action on parasite.

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