EVALUATION OF THERAPEUTIC AND PROPHYLACTIC
POTENTIALS OF TOLTRAZURIL AGAINST CAECAL
COCCIDIOSIS OF CHICKENS IN BANGLADESH

A Thesis

Submitted to
Bangladesh Agricultural University, Mymensingh
In partial Fulfillment of the Requirements for the Degree of

Master of Science
in
Pathology

By

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November, 2011
Dedicated to My Beloved Parents
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The Author

November, 2011
ABSTRACT

Coccidiosis is a fatal disease of poultry. In order to combat caecal coccidiosis of poultry this study was designed to evaluate the therapeutic and prophylactic efficacy of Toltrazuril at different doses as well as its combination with other therapeutic and immunomodulatory agents. Forty five, day old chicks, were randomized and divided into nine groups. Group A served as noninfected, non-medicated control. The chicks of Group B, C, D, E, F, G, H and I were challenged orally with 1x10^4 sporulated oocysts of *E. tenella* on the 10th and 15th day of their age to induce infection. Chicks of Group B were infected and non-medicated while chicks of Group C, D, and E were treated with Toltrazuril at a dose rate of 3.5, 7, and 12 mg/kg body weight respectively on day 22 and 23 of age; the early stage of clinical coccidiosis. Chicks of Group F were treated on day 22 and 23 with Toltrazuril and Vitamin K at a dose rate of 7mg/kg and 2.5 mg/kg body weight respectively. Similarly chicks of Group G were treated with Toltrazuril 7 mg/kg and Sulfaclozine 150 mg/kg body weight and the chicks of Group H were treated with Toltrazuril 7 mg/kg and Oyster Mushroom powder 100 mg/kg body weight. In chicks of Group I, Toltrazuril was given as prophylactic at a dose rate of 3.5 mg/kg body weight from day 10 up to day 24 of experiment. The efficacy of Toltrazuril was evaluated based on oocysts counts per gram (OPG) of faeces, weight gain, morbidity, mortality and necropsy findings. The maximum reduction of OPG counts in therapeutic trial on day 24 were detected in Group F (0.09±0.03 thousand, p<0.05) and Group G (1.04±0.04 thousand, p<0.01). Concerning the mean body weight recorded as compared to healthy control on day 24, Group F (850.75±2.40 g, p<0.01) chickens showed highest performance in terms of therapeutic trial, followed by Group H (809.90±1.91 g, p<0.01) and Group G (797.22±1.20 g, p<0.01) chickens. Chickens belonging to Group B showed lowest performance in terms of body weight (643.97±1.649g) and OPG of faeces count (64.44±1.38 thousand) (p<0.01). In prophylactic trial chickens of Group I showed the highest performance in terms of body weight (942.60±1.44g) and OPG of faeces count (0.03±0.01 thousand) (p<0.01). Results showed Toltrazuril as an effective prophylactic agent against caecal coccidiosis of chicken and supplementation of Vitamin K in feed of infected flock reduced morbidity and mortality.
# CONTENTS

<table>
<thead>
<tr>
<th>CHAPTER</th>
<th>TITLE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACKNOWLEDGEMENT</td>
<td></td>
<td>iii-iv</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td></td>
<td>v</td>
</tr>
<tr>
<td>LIST OF CONTENTS</td>
<td></td>
<td>vi-vii</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td></td>
<td>viii</td>
</tr>
<tr>
<td>LIST OF FIGURE</td>
<td></td>
<td>ix</td>
</tr>
<tr>
<td>LIST OF ABBREVIATION AND SYMBOLS</td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>CHAPTER I</td>
<td>INTRODUCTION</td>
<td>1-2</td>
</tr>
<tr>
<td>CHAPTER II</td>
<td>REVIEW OF LITERATURE</td>
<td>3-13</td>
</tr>
<tr>
<td>2.1.</td>
<td>Reviews on importance and prevalence in Bangladesh</td>
<td>3-4</td>
</tr>
<tr>
<td>2.2.</td>
<td>Reviews on therapeutic and prophylactic agent</td>
<td>4-12</td>
</tr>
<tr>
<td>2.2.1.</td>
<td>Reviews on drugs and drugs resistance</td>
<td>4-11</td>
</tr>
<tr>
<td>2.2.2.</td>
<td>Reviews on mushroom</td>
<td>11</td>
</tr>
<tr>
<td>2.2.3.</td>
<td>Reviews on Vitamin K</td>
<td>11-12</td>
</tr>
<tr>
<td>2.3.</td>
<td>Reviews on pathogenecity of <em>E. Tenella</em></td>
<td>12-13</td>
</tr>
<tr>
<td>CHAPTER III</td>
<td>MATERIALS AND METHODS</td>
<td>14-27</td>
</tr>
<tr>
<td>3.1.</td>
<td>Preparation of experimental house</td>
<td>14</td>
</tr>
<tr>
<td>3.2.</td>
<td>Chicks</td>
<td>154</td>
</tr>
<tr>
<td>3.3.</td>
<td>Feed</td>
<td>14-15</td>
</tr>
<tr>
<td>3.4.</td>
<td>Collection of therapeutic agents</td>
<td>16</td>
</tr>
<tr>
<td>3.5.</td>
<td>Management of the experimental chicks</td>
<td>16-17</td>
</tr>
<tr>
<td>3.5.1.</td>
<td>Brooding</td>
<td>16</td>
</tr>
<tr>
<td>3.5.2.</td>
<td>Feeding and drinking</td>
<td>16</td>
</tr>
<tr>
<td>3.5.3.</td>
<td>Parasite</td>
<td>17</td>
</tr>
<tr>
<td>3.6.</td>
<td>Production of fresh oocysts for experimental infection</td>
<td>17</td>
</tr>
<tr>
<td>3.6.1.</td>
<td>Cleaning of oocysts</td>
<td>17</td>
</tr>
<tr>
<td>3.7.</td>
<td>Experimental design</td>
<td>17-18</td>
</tr>
<tr>
<td>3.7.1.</td>
<td>Groupings</td>
<td>17-18</td>
</tr>
<tr>
<td>3.7.2.</td>
<td>Preparation of oocyst dose</td>
<td>18</td>
</tr>
<tr>
<td>3.7.3.</td>
<td>Preparation of therapeutic and prophylactic doses</td>
<td>21</td>
</tr>
</tbody>
</table>
CONTENTS (Contd.)

<table>
<thead>
<tr>
<th>CHAPTER</th>
<th>TITLE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.7.4.</td>
<td>Recording of daily weight</td>
<td>21</td>
</tr>
<tr>
<td>3.7.5.</td>
<td>Recording of recovery and mortality rate</td>
<td>22</td>
</tr>
<tr>
<td>3.7.6.</td>
<td>Counting of faecal oocyst</td>
<td>22</td>
</tr>
<tr>
<td>3.8.</td>
<td>Post-mortem examination of broilers</td>
<td>22</td>
</tr>
<tr>
<td>3.9.</td>
<td>Histopathological examination</td>
<td>22-27</td>
</tr>
<tr>
<td>3.9.1.</td>
<td>Collection of samples</td>
<td>22</td>
</tr>
<tr>
<td>3.9.2.</td>
<td>Preparation 10% buffered formalin</td>
<td>22-23</td>
</tr>
<tr>
<td>3.9.3.</td>
<td>Chemicals required</td>
<td>23</td>
</tr>
<tr>
<td>3.9.4.</td>
<td>Histopathological examination procedure</td>
<td>23</td>
</tr>
<tr>
<td>3.9.5.</td>
<td>Processing of tissues</td>
<td>23-24</td>
</tr>
<tr>
<td>3.9.6.</td>
<td>Staining procedure</td>
<td>24</td>
</tr>
<tr>
<td>3.9.6.1.</td>
<td>Preparation of Harris hematoxylin solution</td>
<td>24</td>
</tr>
<tr>
<td>3.9.6.2.</td>
<td>Preparation of eosin solution (1% stock alcoholic eosin)</td>
<td>24-25</td>
</tr>
<tr>
<td>3.9.6.3.</td>
<td>Routine hematoxylin &amp; eosin staining procedure</td>
<td>26-27</td>
</tr>
<tr>
<td>3.9.6.4.</td>
<td>Histopathological studies &amp; photomicrograph</td>
<td>27</td>
</tr>
<tr>
<td>3.10.</td>
<td>Statistical analysis</td>
<td>27</td>
</tr>
<tr>
<td>CHAPTER IV</td>
<td>RESULTS</td>
<td>28-41</td>
</tr>
<tr>
<td>4.1.</td>
<td>Mean body weight record</td>
<td>28</td>
</tr>
<tr>
<td>4.2.</td>
<td>OPG counts</td>
<td>28</td>
</tr>
<tr>
<td>4.3.</td>
<td>Mortality and recovery rate record</td>
<td>28-29</td>
</tr>
<tr>
<td>4.4.</td>
<td>Post-mortem findings after treatment</td>
<td>29</td>
</tr>
<tr>
<td>4.5.</td>
<td>Histopathology</td>
<td>35-36</td>
</tr>
<tr>
<td>CHAPTER V</td>
<td>DISCUSSION</td>
<td>42-45</td>
</tr>
<tr>
<td>CHAPTER VI</td>
<td>SUMMARY AND CONCLUSION</td>
<td>46-47</td>
</tr>
<tr>
<td>REFERENCES</td>
<td></td>
<td>48-63</td>
</tr>
<tr>
<td>TABLE</td>
<td>TITLE</td>
<td>PAGE</td>
</tr>
<tr>
<td>--------</td>
<td>----------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>Table 1</td>
<td>Composition of the starter feed as provided by the manufacturer</td>
<td>15</td>
</tr>
<tr>
<td>Table 2</td>
<td>Composition of the grower feed as provided by the manufacturer</td>
<td>15</td>
</tr>
<tr>
<td>Table 3</td>
<td>Therapeutic agents used to study therapeutic and prophylactic potentials of caecal coccidiosis</td>
<td>16</td>
</tr>
<tr>
<td>Table 4</td>
<td>Doses for the therapeutic and prophylactic trial</td>
<td>21</td>
</tr>
<tr>
<td>Table 5</td>
<td>Preparation of 10% buffered formalin</td>
<td>22</td>
</tr>
<tr>
<td>Table 6</td>
<td>Time required for dehydrating tissues</td>
<td>23</td>
</tr>
<tr>
<td>Table 7</td>
<td>Preparation of Harris hematoxylin solution</td>
<td>24</td>
</tr>
<tr>
<td>Table 8</td>
<td>Preparation of eosin solution (1% stock alcoholic eosin)</td>
<td>24</td>
</tr>
<tr>
<td>Table 9</td>
<td>Therapeutic and prophylactic efficacy trial of Toltrazuril in broiler chicken in term of body weight gain</td>
<td>30</td>
</tr>
<tr>
<td>Table 10</td>
<td>Therapeutic and prophylactic efficacy trial of Toltrazuril in broiler chicken in term of OPG count</td>
<td>31</td>
</tr>
</tbody>
</table>
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>FIGURE</th>
<th>TITLE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fig. 1</td>
<td>Different therapeutic agents used for the management of induced caecal coccidiosis in chickens</td>
<td>19</td>
</tr>
<tr>
<td>Fig. 2</td>
<td>Vaccination of chicks with BCRDV on day 2</td>
<td>19</td>
</tr>
<tr>
<td>Fig. 3</td>
<td>Sporulated oocyst of <em>Eimeria tenella</em> (arrow) derived after culture.</td>
<td>20</td>
</tr>
<tr>
<td>Fig. 4</td>
<td>Feeding in the experimental Group B on day 11</td>
<td>20</td>
</tr>
<tr>
<td>Fig. 5</td>
<td>Post mortem examination of the chickens of Group A did not reveal any major lesion</td>
<td>32</td>
</tr>
<tr>
<td>Fig. 6</td>
<td>Distended caeca filled with blood tinged contents as seen in Group B</td>
<td>32</td>
</tr>
<tr>
<td>Fig. 7</td>
<td>Profuse haemorrhage was seen after opening up the caecal tube of Group B chicken that died of induced coccidiosis</td>
<td>33</td>
</tr>
<tr>
<td>Fig. 8</td>
<td>Haemorrhagic lesion was seen in the mucosa after washing up the caeca of Group D chicken</td>
<td>33</td>
</tr>
<tr>
<td>Fig. 9</td>
<td>Caeca after washing of Group F showing no lesion on day 24</td>
<td>34</td>
</tr>
<tr>
<td>Fig. 10</td>
<td>Caecal section of the chicken of Group A (healthy control) did not reveal any significant microscopic lesion (H &amp; E, 100X)</td>
<td>37</td>
</tr>
<tr>
<td>Fig. 11</td>
<td>Caecal section of the chicken of Group B (infected untreated) showing desquamation of epithelia, merozoites/schizonts in mucosa, and infiltration of reactive cells (H &amp; E, 100X)</td>
<td>37</td>
</tr>
<tr>
<td>Fig. 12</td>
<td>Caecal section of chicken of Group C treated with 3.5 mg Toltrazuril /kg body weight showing erosion and desquamation of crypt epithelia, infiltration of reactive cells in the lamina propria with merozoites/schizonts in the crypt of villi (H &amp; E, 400X)</td>
<td>38</td>
</tr>
<tr>
<td>Fig. 13</td>
<td>Caecal section of chicken of Group D treated with 7 mg Toltrazuril /kg body weight showing arrested coccidial development with necrosis, haemorrhages, erosion, and desquamation of epithelial cells (H &amp; E, 100X)</td>
<td>38</td>
</tr>
<tr>
<td>Fig. 14</td>
<td>Caecal section of chicken of Group E treated with 12 mg Toltrazuril /kg body weight showing haemorrhages in caecal mucosa and submucosa, infiltration of inflammatory cells (H &amp; E, 100X)</td>
<td>39</td>
</tr>
<tr>
<td>Fig. 15</td>
<td>Caecal section of chicken of Group F treated with 7 mg Toltrazuril and 2.5 mg Vitamin K/kg body weight showing diffuse infiltration of reactive cells specially eosinophils and mononuclear cells in the mucosa and submucosa, degeneration of caecal gland but no significant haemorrhage present (H &amp; E, 100X)</td>
<td>39</td>
</tr>
<tr>
<td>Fig. 16</td>
<td>Caecal section of chicken of Group G treated with 7 mg Toltrazuril and 150 mg Sulfaclozine/kg body weight showing slight desquamation of caecal epithelia, necrosis, and degeneration of mucosa and caecal gland (H &amp; E, 100X)</td>
<td>40</td>
</tr>
<tr>
<td>Fig. 17</td>
<td>Caecal section of chicken of Group H treated with 7 mg Toltrazuril and 100 mg Oyster mushroom/kg body weight showing desquamation of epithelial lining, infiltration of reactive cells in mucosa and submucosa (H &amp; E, 100X)</td>
<td>40</td>
</tr>
<tr>
<td>Fig. 18</td>
<td>Caecal section of chicken of Group I (used as prophylactic trial) treated with 3.5 mg Toltrazuril/kg body weight showing no significant lesion related to coccidiosis (H &amp; E, 100X)</td>
<td>41</td>
</tr>
</tbody>
</table>
## LIST OF ABBREVIATION AND SYMBOLS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAU</td>
<td>Bangladesh Agricultural University</td>
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<tr>
<td>BCRDV</td>
<td>Baby chick Ranikhet disease vaccine</td>
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<tr>
<td>cm</td>
<td>Centimeter</td>
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<tr>
<td>Co.</td>
<td>Company</td>
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<td>dl</td>
<td>Deciliter</td>
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<td>et al.</td>
<td>Associate</td>
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<td>etc.</td>
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</tr>
<tr>
<td>Fig.</td>
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</tr>
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<td>Kcal</td>
<td>Kilocalorie</td>
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<td>kg</td>
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<td>g</td>
<td>Gram</td>
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<td>Ltd.</td>
<td>Limited</td>
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<tr>
<td>Max.</td>
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<td>Min.</td>
<td>Minimum</td>
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<td>No.</td>
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<td>OPG</td>
<td>Oocyst per gram</td>
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<td>rpm</td>
<td>Rotation per minute</td>
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<tr>
<td>SE</td>
<td>Standard error</td>
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<tr>
<td>µm</td>
<td>Micrometer</td>
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<tr>
<td>°C</td>
<td>Degree Celsius</td>
</tr>
<tr>
<td>±</td>
<td>Plus minus</td>
</tr>
</tbody>
</table>
CHAPTER I
INTRODUCTION
CHAPTER I
INTRODUCTION

Poultry farming in Bangladesh has grown as an emerging and prospective industry and many landless farmers are found to involve with poultry rearing (Huque, 2001). A total of 5 million people are engaged in this sector (Saleque, 2006). At present chicken contributes 51% of total meat production in Bangladesh (Raha, 2007). There are about 110,800 small and large scale poultry farms in this country (Anon, 2006) and per capita annual consumption of meat is 5.99 kg against the universal standard 80 kg per head (Raha, 2007).

There are several constraints of poultry industries in Bangladesh including outbreak of infectious diseases causing economic loss and discouraging poultry rearing (Das et al., 2005). Among the different diseases, parasitic infection brings a great threat to poultry industry. Coccidiosis is a common and fatal disease in poultry. Intestinal coccidiosis, caused by various species of *Eimeria*, is an economically important (estimated to be 2 billion dollars a year) disease of poultry (Zhang and Zeng, 2005). *Eimeria spp.* are belonging to the phylum Apicomplexa causing coccidiosis of farm animals and birds. *Eimeria tenella* is the most important species, as it causes caecal coccidiosis in chickens (Shirley, 1986). *Eimeria tenella* primarily invades and resides in the linings of caeca of exposed chickens (Vervelde and Vermeulen, 1995 and Yun *et al.*, 2000).

In Bangladesh a number of drugs are available for the treatment and prevention of coccidiosis. Anticoccidial drugs remain important for a long time, although resistance development could limit their use (Stephen *et al.*, 1997). Moreover, the price of synthetic anticoccidials is too much high and efficacy is not so satisfactory. Anticoccidial therapeutic agents must fulfill some main criteria including a high level of efficacy against all developmental stages of pathogenic *Eimeria species* infecting poultry and at the same time, they shouldn't interfere with the immune response of the host during and after treatment of coccidial infections at therapeutic dosages (El-Banna *et al.*, 2005).
Toltrazuril is a symmetrical triazinetrione compound and 2.5% oral solution has been shown to be effective against all species of *Eimeria* infecting chickens (Mehlhorn *et al*., 1988). It is active against all intracellular developmental stages including those of schizogony and gametogony (Mehlhorn *et al*., 1984). Toltrazuril has chemoprophylactic (Gjerde and Helle, 1991) and therapeutic effects (Chapman, 1987; Mehlhorn *et al*., 1988; Mathis *et al*., 2004; Ghanem *et al*., 2008) against coccidiosis and does not interfere with the development of immunity (Grief, 2000). Chemoprophylaxis with Toltrazuril enhances immunity against poultry coccidiosis (Grief, 2000). It has been proved that therapeutic medication with Toltrazuril protects the birds from clinical coccidiosis (Ramadan *et al*., 1997).

There are other different therapeutic and prophylactic agents for coccidiosis in different species. Among these vitamin-K added to a deficient diet reduces mortality due to *Eimeria tenella* or *E. necatrix* (Ryley *et al*., 1978), sulfaclozine may reduce the deleterious effects of coccidiosis in broilers (Sanei *et al*., 2009) and dietary supplementation with oyster mushroom powder enhances anti-inflammatory activity which is mediated through the inhibition of NF-κB and AP-1 signaling (Jedinak *et al*., 2011).

To evaluate therapeutic and prophylactic potentials of Toltrazuril against poultry coccidiosis this study was undertaken with the following objectives:

i. To study the therapeutic potentials of Toltrazuril against caecal coccidiosis in chickens.

ii. To study the prophylactic effect of Toltrazuril against caecal coccidiosis in chickens.

iii. To compare the therapeutic and prophylactic potentials of Toltrazuril against caecal coccidiosis in chickens in terms of body weight gain, oocyst counts per gram (OPG) of faeces, morbidity and mortality pattern and postmortem lesions.
CHAPTER II

REVIEW OF LITERATURE

It is true that coccidiosis is one of the most serious problems in poultry industry throughout the world including Bangladesh. A few relevant published information on the experiment have been reviewed in the following paragraphs. The reviews have been arranged in the chronological order.

2.1. Reviews on importance and prevalence in Bangladesh

Coccidiosis in poultry appeared as an important disease as it is caused higher mortality and morbidity in farm practices.

Kutubuddin (1973) made a study on mortality due to coccidiosis of chicken in BAU poultry farm and recorded 14.66% mortality of the birds due to coccidiosis. Sarker (1976) collected 300 dead birds from Bangladesh Agricultural University (BAU) Poultry farm and based on post mortem examination about 12% infectivity with coccidiosis was recorded.

Mondal and Qadir (1978) reported subclinical coccidiosis (54.14%) of chicks in (BAU) poultry farms and among the infected birds 23.75% were found to infect with *E. tenella*, the most predominant species in Bangladesh. Karim (1988) studied chicken coccidiosis in Bangladesh, and reported 24.8% of mortality of chicken due to coccidiosis in October, 1986.

Karim and Tress (1990) identified coccidiosis as one of the major diseases of poultry in Bangladesh. The authors also identified five species of *Eimeria* named *E. tenella, E. necatrix, E. acervulina, E. maxima and E. brunetti*, that cause coccidiosis of chicken in Bangladesh. Karim and Begum (1994) reported the incidence of *E. tenella* as a major species of chicken coccidia caused great economic loss to the poultry industry in Bangladesh. The authors also reported two additional species: *E. precox* and *E. mitis* in chicken with high morbidity and mortality.
Karim et al (1994) recorded higher prevalence of chicken coccidiosis during winter or cold months, and found *E. tenella* (70%), *E. maxima* (40%), *E. brunetti* (30%) and *E. necatrix* (20%) by lesion scoring of birds. The authors reported that *E. acervulina* was the commonest species and found in 80% birds examined.

Bhattacharjee et al (1996) made a study on prevalence of chicken coccidiosis in Bangladesh and reported the prevalence is 9.4% cases. Islam et al. (1996) investigated the prevalence of chicken coccidiosis in dead birds. The authors found that 39.2% of the birds (out of 337) were affected with coccidiosis.

Talha (1999) studied the prevalence of coccidiosis in different poultry farms of Bangladesh. The author reported 45% mortality rate due to chicken coccidiosis in Bangladesh. Karim (2001) reported the second most pathogenic species is *E. necatrix* but least common in Bangladesh.

Giasuddin et al. (2002) did a statistics on mortality due to coccidiosis in Bangladesh and reported 4-5% mortality rate in chicken due to coccidiosis in Bangladesh. Giasuddin et al. (2003) made a study on the prevalence of coccidiosis in chicken in Bangladesh and reported 9.17% prevalence.

Saleque (2003) collected data (1999-2002) from Central Disease Investigation Laboratory and BRAC laboratory, and reported gradual higher prevalence of coccidiosis in poultry farms over the last 3 years.

2.2. Reviews on therapeutic and prophylactic agents

2.2.1 Reviews on drugs and drug resistance

Warren et al. (1966) examined the response of field strains of coccidia to several coccidiostats during 1964 and 1965. The authors reported strains of all species of *Eimeria*, and more strains of coccidia had a tendency to be resistant to coccidiostats that were used. The coccidiostats used included 0.0125% amprolium, 0.009% coccidiostat 'D' (a mixture of 8 part sulphaquinoxaline and 1 part diaveridine), 0.01% nitrofurazone, 0.0145% coccidiostat 'P' (a mixture of 16 percent amprolium, 12 parts sulphaquinoxaline and 1 part ethopabate), 0.0125 percent sulphaquinoxaline and 0.125 percent zoalene.
Joyner (1970) in a study reported resistant response to coccidiostatic drugs in different strains of *Eimeria* spp. The author also reported that the drug resistance may be induced to most coccidiostat in common use and failure of prophylaxis may occur in the presence of resistant strains.

Oikawa *et al.* (1975) reported that 97% of the strain of *E. acervullina* was resistant to decoquinate and 93% resistant to clopidol; a considerable percentage of the strain of *E. tenella* and *E. necatrix* were resistant to amprolium, clopidol and decoquinate, and only a small percentage of these strains were resistant to sulfadimethoxime.

Chapman (1978) studied on the development of resistance in the Houghton strain of *E. tenella* to anticoccidial drugs like amprolium, clopidol and methyl benzoquate. The author reported that amprolium and clopidol developed resistance more rapidly in experiments.

Gill and Bajwa (1979) studied on drug resistance of different field isolates of *Eimeria* spp. The authors reported 71.5% resistant to sulphaquinoxaline, 58.8% to bifuran, 34.1% to amprolium, 6.3% to clopidol and 5.6% to nicarbazin against chicken coccidiosis.

Jeffers and Bentley (1980) reported that polyether anticoccidials as an ineffective coccidiostat against *Eimeria* spp and developed resistance against coccidia readily. Krylov and Zaions (1981) reported that 97.5% field isolates of *E. tenella* exhibited resistance to nitrofuran derivatives, 75% to 3, 5-dinitro denzamide derivatives, 47.5% to sulphanylantides and 10% to thiamin derivatives.

Mathis and McDougald (1982) studied the efficacy of clopidol, amprolium, nequinate, zoalene and sulfaquinoxalines, and found that *E. tenella, E. acervulina* and *E. maxima* had high frequency of resistance against these drugs. On the other hand, very few isolates were resistant to nicarbazin, robenidine or halofuginone.

Chapman (1982) collected field isolates of *E. acervulina* from broiler and breeder farms throughout UK and reported sulphaquinoxaline and suophaquinoxaline plus pyrimethamine were only partially effective coccidiostat against the field isolates.


Chapman (1982) studied over a period of one year in UK and collected isolates from broiler farms where monensin had been used continuously for 28 and 38 crops (broiler isolates) and from breeder farms where the drug had never been used (breeder isolate). The author reported that the broiler isolates were partial resistant to monensin.

Chapman (1983) reported that several *Eimeria* spp. were resistant to aprinocid which had been used for 5 to 7 successive flocks @ 60 ppm in diet. Few isolates were found sensitive where aprinocid had never been used.

Chapman (1984) studied with Houghton strain of *E. tenella* and found that after 16 passages the parasite become resistant (partial) to monenisn at the concentration of 100 ppm. At higher concentrations of the drug, the parasite was not resistant. Chapman (1986) studied the resistance of monensin against chicken coccidiosis and reported that the field isolates of *E. tenella* were partly resistant to monensin.

Sanda (1986) made a therapeutic trial to study the efficacy of monensin against chicken coccidiosis. The author supplied monensin in feed for 21 days in *E. tenella* infected chicks and the birds were recovered well.

Rommel (1987) observing the resistant strains of *Eimeria*, and stated that it was necessary to seek an alternative to the use of anticoecidials because of the development of resistant coccidial parasite and need to reduce drug residues in tissues.

Salisch (1987) used maduramicin at 6 ppm and chicken coccidiosis was controlled. Maduramicin treatment resulted in significantly lower lesion scores and fewer parasites (oocysts) were seen in litter samples.

Munoz and Rodriguez (1988) used maduramincin, monensin, salinomycin, narasin and lasalocid in the treatment of *Eimeria spp*. The authors observed highest Anticoccidial Index (AI) activity in maduramicin treated group at different dose rates (AI = 164.59, 169.37 and 170.24 at 5 ppm, 6 ppm and 7 ppm respectively).

Varga *et al.* (1988) studied anticoccidial activity of maduramicin, monensin, narasin, salinomycin and lasalocid. The authors used maduramicin ammonium at the recommended dietary level of Smg/kg body weight in battery and floor pen trials. In both
the trials, the authors found the efficacy of maduramicin superior to other ionophorous anticoccidials against *E. tenella* and *E. acervulina* infection.

**Reather and Paeffgen (1989)** studied the sensitivity to monensin (100 ppm); narasin (70 ppm), salinomycin (60 ppm), maduramicin (5 ppm) and lasalocid (90 ppm) of coccidiosis infected broiler chickens in nine European countries. The authors reported that five of 26 isolates were sensitive to all ionophorous drugs tested, while the rest of the isolates (81%) were partially or entirely resistant to one or several polyether. *E. tenella* and *E. brunetti* isolates were highly resistant to ionophores.

**Harder et al. (1989)** tested that the anticoccidial properties of toltrazuril in *Eimeria falciformis*-infected mice and were potentiated by the simultaneous application of pyrimethamine, trimethoprim, or sulfadimidine. Their results suggest that toltrazuril primarily affects the respiratory chain and secondarily, two enzymes involved in pyrimidine synthesis.

**Salisch and Shaksholik (1989)** studied the efficacy of ionophorous drugs against *E. tenella* and *E. acervulina*. The authors reported that salinomicin and maduramicin were most effective against *E. acervulina* and *E. tenella* respectively. On the other hand, maduramicin was least effective against *E. acervulina* and monensin was slightly effective against *E. tenella*.

**Salisch and Friederichs (1991)** studied the efficacy of maduramicin and salinomycin against *E. brunetti*. The authors reported that maduramicin (5 ppm in feed) gave better result by suppressing the oocysts production completely.

**AL-Taee et al. (1993)** studied the efficacy of maduramicin and monensin against *E. tenella* infection in chicken. The authors reported that maduramicin is more effective than monensin.

**Zhang et al. (1995)** studied the efficacy of maduramicin against *E. tenella* infection in chicken. The authors reported that the recommended dose of maduramicin was 2.5-4 ppm for prevention and 4-5 ppm for treatment against *E. tenella* infection.
Grief et al. (1996) reported that 55% strains from 15 *E. acervulina* and five *E. brunetti* strains were resistant to several anticoccidial drugs like maduramicin (5 ppm), monensin (100 ppm), salinomysin (6fl ppm), nicarbazin (125 ppm), halofuginone (3 ppm), diclazuril (1 ppm) and toltrazuril (25 Ppm).

Stephan et al. (1997) studied ten *Eimeria* field isolates from North Germany in battery tests for sensitivity to selected anticoccidials. Chapman (1997) studied that anticoccidial drugs are widely used for the control of coccidiosis in the fowl which has inevitably led to the development of drug resistance.

Greif (2000) studied immunoglobin level and activity of immune sera after toltrazuril treatment; toltrazuril may be involved in the enhancement of immunity. Chapman (2000) studied that immunological response is recognized as the only major practical alternative to chemotherapy for the control of coccidiosis.

Lakkundi et al. (2002) studied the effect of toltrazuril and amprolium on body weight and feed efficiency of broiler chicken experimentally infected with *Einreria tenella*. The authors reported that toltrazuril (25 ppm) was very effective irrespective of degree of infection, whereas the efficacy of amprolium (240 ppm) was better in moderate than in heavy infection.

Lakkundi et al. (2002) evaluated the effect of toltrazuril and amprolium on body weight and feed efficacy in broiler chickens experimentally infected with caecal coccidiosis. The authors found that Toltrazuril treated birds did not excrete oocysts, but had better body weight gain and feed efficiency than uninfected birds. The caecal lesion score was mild in this group. In the amprolium treated birds, the OPG was less than in infected controls and mean body weight was lower than toltrazuril treated birds. However, cumulative feed efficiency was similar in both treatments. The lesion score was moderate in amprolium treated birds. The results indicated that toltrazuril was very effective irrespective of degree of infection, whereas the efficacy of amprolium was better in moderate than in heavy infection.

Lakkundi et al. (2002) evaluated histopathological anticoccidial activity of toltrazuril and amprolium in experimentally induced caecal coccidiosis in broiler chicken (n=280).
was reported that toltrazuril prevented the establishment of caecal coccidiosis by degeneration and disintegration of the first generation of schizonts. However, in the amprolium-treated birds, few intact first and second generation schizonts and also micro and macrogametocytes were noticed on microscopic examination. Occasionally, oocysts were also found. It was concluded that toltrazuril has a coccidiocidal effect, whereas amprolium is coccidiostatic in nature based on the histopathological studies.

**Jin-GuangMing et al. (2003)** selected one hundred and thirty 21-day-old healthy chickens to study the resistance of *two Eimeria tenella* isolates, Nanjing isolate and Fengyang isolate, to diclazuril, maduramicin, clopidol, robenidine and amprolium. The authors reported that two isolates were light resistant to amprolium, sensitive or light resistant to diclazuril, middle resistant to clopidol, middle resistant or complete resistant to maduramicin and robenidine.

**Ghazala-Nawaz et al. (2003)** tested the efficacy of 2.5% toltrazuril against experimentally induced coccidiosis in broiler chicks. It was shown that treatment with 2.5% toltrazuril at 7 mg/kg in drinking water for 2 consecutive days, for 8 h per day was effective for controlling experimentally induced acute coccidiosis and immediately stopped mortality.

**Grilli et al. (2003)** conducted a study to investigate the effect of a therapeutic medication with toltrazuril on the control of coccidiosis and broiler performance in the absence of coccidiostat-medicated feed. They reported that the toltrazuril single treatment controlled coccidiosis and oocyst shedding was decreased at 35 days of age. The results indicated that in case of early coccidiosis challenge, the treatment should be anticipated between 10-14 days of age to avoid death of chicken due to damage at earlier onset.

**Mathis et al. (2003)** conducted a 42-day broiler floor pen study conducted comparing the anticoccidial efficacy of toltrazuril (Baycox) as a stand alone treatment and as an additional treatment to in-feed anticoccidial programs. The authors reported that Toltrazuril most completely eliminated all coccidial lesions and dramatically reduced oocyst shedding and Toltrazuril can thus be used for supplemental control with in-feed anticoccidials or as a primary anticoccidial with nonmedicated feed.
Dhillon et al. (2004) tested the efficacy of toltrazuril against 6 different levels of *Eimeria tenella* infection in 2-week-old chicks was evaluated. The authors reported that the treatment resulted to lower mortality and reduced oocyst production. Moreover, therapy resulted to complete elimination of the clinical signs at lower levels of infection. The drug interfered in the development of immunity only at the lower levels of infection.

Mathis et al. (2004) conducted a 56-day floor pen study to determine the appropriate time to administer toltrazuril (BaycoxReg.) for control of coccidiosis in broiler chickens. They reported that the final performance for the salinomycin (SAL/SAL) was significantly less compared to all toltrazuril and nicarb/salinomycin (NIC/SAL) birds. All toltrazuril treatments at days 2-3 provided good coccidiosis control with accompanying performance. The absence of clinical coccidiosis relapse during the last third of the growout along with moderate oocyst counts and low lesions was indicative of unimpaired coccidiosis immunity.

Sheng-chao et al. (2005) studied efficacy evaluation of combinations among selected anticoccidials against *Eimeria tenella* in chicken. Results showed that the combination of polyether ionoporous antibiotics and synthetic chemical coccidials acted later in the life cycle of *E. tenella* had the synergistic efficacy against coccidiosis and the good promotion of weight gain in chickens.

Claeskens et al. (2007) assessed Toltrazuril (BaycoxReg.) stand-alone treatment and Paracox-5 (live attenuated vaccine) in a field trial for broiler coccidiosis control. They found that within treatment groups, the feed conversion ratio and daily weight gain were significantly influenced by the month of hatch. It was concluded that a single treatment with toltrazuril is a valuable alternative to vaccination for coccidiosis control in a rotation programme.

Sanei et al. (2009) studied the effect of sulfaclozine 30% (Esb3) on experimental coccidiosis in broiler cockerels. They reported that sulfaclozine may reduce the deleterious effects of coccidiosis in broilers. Sentepe and Eraslan (2010) studied the Pharmacokinetic of sulfaclozine in broiler chickens.

2.2.2. Reviews on mushroom
Manzi et al. (1999) made a comparative study on various components of nutritional interest, such as water, protein, total amino acids, ash and minerals, in mushrooms of different species (Pleurotus ostreatus, Pleurotus eryngii, Pleurotus pulmunarius and Lentinula edodes).

Wasser (2002) made a study on medicinal mushrooms. The author reported that these mushroom represent an unlimited source of polysaccharides with antitumor and immunostimulating properties.

Borchers et al. (2008) studied the immunobiology of mushroom. They reported that mushrooms have beneficial effects on immune function with subsequent implications for inhibition of tumor growth.

Jedinak et al., 2011 done a study on anti-inflammatory activity of edible oyster mushroom. They reported that oyster mushroom (Pleurotus ostreatus) has significant anti-inflammatory effect which is mediated through the inhibition of NF-κB and AP-1 signaling.

2.2.3. Reviews on Vitamin K

Dam (1942) studied the chemistry and physiology of Vitamin K. Stephens et al. (1960) performed research on sources and levels of vitamin k in relation to cecal coccidiosis. They reported that Vitamin K supplementation reduces the mortality of chicken due to caecal coccidiosis.

Harms et al. (1960) made a comparison of various sources and levels of vitamin k activity using chicks with cecal coccidiosis. Warren (1968) studied the Vitamin requirements of the Coccidia of the chicken. He reported that for normal development of the parasites, Eimeria acervulina and E. tenella in the chicken, dietary thiamine, riboflavin, biotin, nicotinic acid and folic acid are required. E. acervulina required thiamine for second schizogony and sporulation; riboflavin for first schizogony and possibly gametogony, biotin for the development of the sporozoites/trophozoites and for gametogony, and nicotinic acid for schizogony and gametogony. E. tenella required
thiamine and riboflavin for gametogony; biotin for first schizogony; and nicotinic acid for second schizogony.

Ryley et al. (1978) studied the use of vitamin K deficient diets in the screening and evaluation of anticoccidial drugs. They found that Vitamin K (as menaphthone sodium bisulphite) added to a deficient diet reduced mortality due to Eimeria tenella or E. necatrix, had a slight effect on haematocrit, but had no obvious effect on weight gain or faecal blood; 0·1 ppm gave a maximal response. The effect of vitamin K on mortality was not absolute; the magnitude of the effect depended on the size of the challenge dose of oocysts.

2.3. Reviews on pathogenecity of E. tenella

Tyzzer (1929) reported that E. tenella was the most pathogenic species of all the avian coccidia. Waxlen (1941) found that death occurred due to haemorrhage associated with second generation schizonts of E. tenella.

Goodrich (1944) observed that oocysts of E. tetella survived for 24 to 25 months and retained its pathogenecity at room temperature in 2.5% potassium dichromate solution. Edgar (1955) reported that the prepatent period of several species of coccidia were one day less than that was reported previously. The author also stated that sporulation time of E. tenella was only 19 hours at 29°C, 21 hours at 26°C and 24 hours at 20°C.

Doran and Vetterling (1969) reported 23-59% mortality in 3 week old chicks infected with 10^5 oocysts of E. tenella. Long (1973) studied the pathogenecity of E. tenella and reported that there was haemorrhage, sloughing of caecal mucosa, thinning of caecal wall and eventual fibrosis and thickening of the walls.

Dikovskaya (1974) studied the pathogenecity of E. tenella in different areas of USSR and recorded 12.5-80% mortality of chicks by this parasite. Mondal and Qadir (1978) identified 23.75% infection in chicken due to E. tenella which is the commonest one.

Seeripto (1984) observed that infection of village chickens with 5 x 10^4 oocysts of E. tenella cause only a slight decrease in body weight, but the layers and broilers showed clinical signs of coccidiosis and had a weight loss of 19.4% and 14.2% respectively.
Mortality was recorded in village chickens when the inoculated dose of oocysts was increased from $5 \times 10^4$ to $15 \times 10^4$, but the rate was much lower than in broilers.

**Nayak and Rai (1985)** studied the pathogenicity of several species of *Eimeria* in broiler chickens. The authors reared day old broiler chicks in presterilized experimental cages in similar environmental conditions, food and water. At the age of 6 weeks the birds were infected orally with $2 \times 10^4$ oocysts of *E. tenella*, *E. necatrix* and *E. acervulinx* in mixed culture. The authors reported that the total erythrocyte count, haemoglobin and packed cell volume (PCV) were significantly lower in infected birds.

**Shirley and Millard (1986)** studied the pathogenicity of *E. tenella* in chickens. The authors gave treatment of several infected birds, but unfortunately the authors found no difference in body weight gain of treated and untreated birds.

**Jungmann and Mielke (1989)** studied on *E. tenella* irradiated vaccine and reported that the immunized chickens challenged with *E. tenella* developed no clinical sign. On the other hand, the controls (*E. tenella* infected, not immunized) had severe haemorrhagic enteritis with 88% mortality.
CHAPTER III

MATERIALS AND METHODS
CHAPTER III

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The experiment was conducted at the Department of Pathology, Faculty of Veterinary Science, Bangladesh Agricultural University (BAU), Mymensingh to study the therapeutic and prophylactic potentials of Toltrazuril against caecal coccidiosis of chicken.

3.1. Preparation of experimental house

The experimental poultry house was properly cleaned, washed and then dried up. The room was fumigated with formaldehyde and with ammonia before introduction of chicks. The feeder, waterer and cages were cleaned with water and then fumigated with ammonia.

3.2. Chicks

Forty five, Cobb 500 day old chicks were included in this study. The chicks were collected from Kazi Hatchery, Gazipur. Vaccination of chicks with BCRDV was performed on day 2 and 19 (Fig.2).

3.3. Feed

The broiler chicks were provided with standard broiler starter and grower ration (Champion Starter and Champion Grower, Quality Feeds Ltd.) according to the age (Table 1 and 2). The starter feed was given from day 1 to day 10 and the grower feed was given from day 11 up to the end of the experiment. The feed was stored in a dry and coccidia free area.
Table 1: Composition of the starter feed as provided by the manufacturer

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Feed composition</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Humidity (max.)</td>
<td>11</td>
</tr>
<tr>
<td>2.</td>
<td>Protein (min.)</td>
<td>24.5</td>
</tr>
<tr>
<td>3.</td>
<td>Fat (min.)</td>
<td>5</td>
</tr>
<tr>
<td>4.</td>
<td>Carbohydrate (max.)</td>
<td>4</td>
</tr>
<tr>
<td>5.</td>
<td>Lysin (min.)</td>
<td>1.4</td>
</tr>
<tr>
<td>6.</td>
<td>Methionine (min.)</td>
<td>0.65</td>
</tr>
<tr>
<td>7.</td>
<td>Calcium (min.)</td>
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</tr>
<tr>
<td>8.</td>
<td>Phosphorus (min.)</td>
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<tr>
<td></td>
<td><strong>Total metabolic energy</strong></td>
<td><strong>3150 kcal/kg</strong></td>
</tr>
</tbody>
</table>

Table 2: Composition of the grower feed as provided by the manufacturer

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Feed composition</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Humidity (max.)</td>
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</tr>
<tr>
<td>2.</td>
<td>Protein (min.)</td>
<td>24</td>
</tr>
<tr>
<td>3.</td>
<td>Fat (min.)</td>
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</tr>
<tr>
<td>4.</td>
<td>Carbohydrate (max.)</td>
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</tr>
<tr>
<td>5.</td>
<td>Lysin (min.)</td>
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<tr>
<td>6.</td>
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<tr>
<td>8.</td>
<td>Phosphorus (min.)</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td><strong>Total metabolic energy</strong></td>
<td><strong>3175 kcal/kg</strong></td>
</tr>
</tbody>
</table>
3.4. Collection of therapeutic agents

The therapeutic agents for the experiment (Fig.1) are presented in Table 3.

Table 3: Therapeutic agents used to study therapeutic and prophylactic potentials of caecal coccidiosis

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Therapeutic agent</th>
<th>Trade name</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Toltrazuril</td>
<td>Cox-Zero®</td>
<td>Local market</td>
</tr>
<tr>
<td>2.</td>
<td>Vitamin-K</td>
<td>Rena-K®</td>
<td>Local market</td>
</tr>
<tr>
<td>3.</td>
<td>Sulfaclozine</td>
<td>Nava Cox®</td>
<td>Local market</td>
</tr>
<tr>
<td>4.</td>
<td>Oyster mushroom</td>
<td>-</td>
<td>Horticulture Center, Kewatkhali, Mymensingh, Bangladesh.</td>
</tr>
<tr>
<td></td>
<td>powder</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3.5. Management of the experimental chicks

The following procedures were adapted during the experimental period.

3.5.1. Brooding

The experimental chicks were brooded in two brooders provided with temperature of 35°C ± 1°C at 1st week of age and then gradually decreased the temperature at the rate of 2.5°C per week up to 24 days of age. Temperature was corrected by adjusting the height of the heat source, i.e. the electric bulb used for heating. The birds were reared in coccidia free condition as the utensils were sprayed with ammonia earlier. Strict biosecurity was maintained.

3.5.2. Feeding and drinking

The chicks were given feed twice a day in the morning and in the afternoon, and water *ad libitum*. During the 1st week feed was supplied on large brown paper and water in waterer. After 1st week the feeders were provided. Feeders and waterers were cleaned daily. After 10 days when the chicks were placed in cages then water was given in beakers.
3.5.3. Parasite

A single oocyst derived strain of *E. tenella* as a field isolate was used in this experiment which was collected from a chicken affected with caecal coccidiosis.

3.6. Production of fresh oocysts for experimental infection

For the production of fresh oocysts, each seven days old chicks (n=6) was infected with $10^4$ sporulated oocysts. Faeces were collected from 10-11 days post infection (dpi) and were kept in 2% potassium dichromate solution, while the concentration of oocyst was found in higher concentration than oocysts (Fig.3) were collected and used in this study.

3.6.1. Cleaning of oocysts

The oocysts were cleaned from the faeces by adopting the method described by Ryley (1973) with required modifications. After collection in 2% potassium dichromate solution the faeces was homogenized using a food blender. The homogenized mixture was then centrifuged at 700 rpm for 5 minutes and the supernatant was discarded. The sediment was resuspended by using saturated salt solution (specific gravity 1.200) and centrifuged at 500 rpm for 5 minutes. With the help of a plastic Pasteur pipette the oocyst rich scum was collected from the supernatant. The oocysts suspension was then passed through a mash (90 µm apperture) sieve collected from local market. The suspension was diluted with water to at least ten times of its original volume. After centrifuging the suspension at 1100 rpm for 5 minutes the supernatant was discarded. The oocysts rich sediment was resuspended in 2% potassium dichromate and aerated at room temperature for 48 hours. The sporulation percentage was determined by counting a total of 100 oocysts (both sporulated and unsporsulated). The oocysts were stored at 4°C temperature for subsequent use.

3.7. Experimental design

3.7.1. Groupings

The chicks (n = 45) at the age of day 10 were divided into 9 equal groups (Group A, B, C, D, E, F, G, H and I). The chicks were reared in separate cages. The Group A was
maintained as a control group without induction of infection and treatment and Group B was maintained as infected but untreated (Fig.4). The next 6 groups (Group C to H) were used for therapeutic trial (Table 4) and Group I was used for prophylactic trial (Table 4) exposed to coccidial infection.

3.7.2. Preparation of oocyst dose

Oocyst suspension was centrifuged at 500 rpm for 5 minutes in test tubes and the supernatant was discarded. The sediment after resuspending in distilled water was centrifuged again. This process was repeated until the potassium dichromate was cleaned off. The final sediment was resuspended in distilled water and the number of oocysts per milliliter was counted by McMaster counting technique. Finally the number of oocysts as infective inocula \((10^4\) sporulated oocysts) was adjusted to a volume of 0.5 to 1 ml with water and each prepared dose was administered orally to every chick of Group B, C, D, E, F, G, H and I on day 10 and 15.
Fig. 1: Different therapeutic agents used for the management of induced caecal coccidiosis in chickens.

Fig. 2: Vaccination of chicks with BCRDV on day 2.
Fig. 3: Sporulated oocyst of *Eimeria tenella* (arrow) derived after culture.

Fig. 4: Feeding in the experimental Group B on day 11.
### 3.7.3. Preparation of therapeutic and prophylactic doses

After appearance of clinical signs and symptoms the chicks of different groups were treated as follows:

Table 4: Doses for the therapeutic and prophylactic trial

<table>
<thead>
<tr>
<th>Grouping</th>
<th>Days of treatment (from- to)</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A (Control group)</td>
<td>-</td>
<td>Without treatment</td>
</tr>
<tr>
<td>Group B (Infected untreated)</td>
<td>-</td>
<td>Without treatment</td>
</tr>
<tr>
<td>Group C</td>
<td>22-23</td>
<td>3.5 mg Tolrazuril/kg body weight</td>
</tr>
<tr>
<td>Group D</td>
<td>22-23</td>
<td>7 mg Tolrazuril/ kg body weight</td>
</tr>
<tr>
<td>Group E</td>
<td>22-23</td>
<td>12 mg Tolrazuril/ kg body weight</td>
</tr>
<tr>
<td>Group F</td>
<td>22-23</td>
<td>7 mg Tolrazuril and 2.5 mg Vitamin K/ kg body weight</td>
</tr>
<tr>
<td>Group G</td>
<td>22-23</td>
<td>7 mg Tolrazuril and 150 mg Sulfaclozine/ kg body weight</td>
</tr>
<tr>
<td>Group H</td>
<td>22-23</td>
<td>7 mg Tolrazuril and 100 mg Oyster mushroom/ kg body weight</td>
</tr>
<tr>
<td>Group I (used in prophylactic trial)</td>
<td>10-24</td>
<td>3.5 mg Tolrazuril/ kg body weight</td>
</tr>
</tbody>
</table>

### 3.7.4. Recording of daily weight

Chicks were weighed individually from the day of starting (day 1) till the end of the experiment (day 24) every day at morning. The average mean individual weight was calculated from these values.
3.7.5. Recording of recovery and mortality rate

In response to the treatment the recovery and mortality rate were recorded up to the end of the experiment.

3.7.6. Counting of caecal oocyst

The faeces were collected every day after post infection immediately after defecation. The faeces of all groups were homogenized in a food blender separately and made up to 0.5 liter by adding water. After thorough mixing by gentle stirring, 1 ml of suspension was diluted in the saturated salt solution to make a desired dilution (x 10) and mixed by gentle shaking. A portion of the suspension was withdrawn with the help of an ordinary plastic transfer pipette and the two chambers of McMaster egg counting slide was filled. After 3-5 minutes the oocysts in the two chambers, were counted by using low power objective (x 10). The number of oocysts per bird was calculated by dividing the figure by 0.3 and multiplied by the dilution factor and then divided by the number of chicks in each subgroup.

3.8. Post-mortem examination of broilers

At the end of experiment (day 24), post-mortem examination of the broilers were performed. Caeca, intestine, liver and spleen were examination. The gross post-mortem findings of these organs were recorded. The caeca were collected for histopathology.

3.9. Histopathological examination

3.9.1. Collection of samples

Caeca from the experimental chickens were collected in 10% neutral buffered formalin and used for histopathological study.

3.9.2. Preparation 10% buffered formalin

Table 5: Preparation of 10% buffered formalin

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>37-40% formalin</td>
<td>100 ml</td>
</tr>
<tr>
<td>Distilled water</td>
<td>900 ml</td>
</tr>
<tr>
<td>Sodium Phosphate (monobasic)</td>
<td>4 gm</td>
</tr>
<tr>
<td>Sodium Phosphate (dibasic)</td>
<td>6.5 gm</td>
</tr>
</tbody>
</table>
The above ingredients were mixed thoroughly, preserved in an air tight container split in plastic jar @ 250ml/jar

3.9.3. Chemicals required

(i) Alcohol (50%, 70%, 80%, 95% & absolute)
(ii) Chloroform
(iii) Paraffin
(iv) Xylene
(v) Distilled water
(vi) Hematoxylin
(vii) Acid alcohol
(viii) Ammonium water
(ix) Eosin

3.9.4. Histopathological examination procedure

Fixed tissue sections (caeca) were processed for paraffin-embedding, sectioning (Luna, 1968) and staining with Hematoxylin & Eosin stain.

3.9.5. Processing of tissues

The formalin fixed tissues were properly trimmed. The tissues were washed overnight under running tap water to remove formalin. The tissues were dehydrated in ascending grades of alcohol.

Table 6: Time required for dehydrating tissues

<table>
<thead>
<tr>
<th>Alcohol Percentage</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>50% alcohol</td>
<td>1 hour</td>
</tr>
<tr>
<td>70% alcohol</td>
<td>1 hour</td>
</tr>
<tr>
<td>80% alcohol</td>
<td>1 hour</td>
</tr>
<tr>
<td>95% alcohol</td>
<td>1 hour</td>
</tr>
<tr>
<td>100% alcohol</td>
<td>1 hour X 2</td>
</tr>
</tbody>
</table>

The tissues were cleared in two changes in chloroform, 1.5 hour in each. The tissues were embedded with molten paraffin wax at 56°C for two changes, 1.5 hour in each. Paraffin block containing tissue pieces were made using templates. The tissues were sectioned with a microtome at 5 µm thickness, allowed to spread on warm water bath (40°C) containing a small amount of gelatin & taken on
oil and grease free glass slides. The slides were air dried and kept in cool place until staining.

3.9.6. Staining procedure

3.9.6.1. Preparation of Harris hematoxylin solution

Table 7: Preparation of Harris hematoxylin solution

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematoxylin crystals</td>
<td>5 gm</td>
</tr>
<tr>
<td>Alcohol (100%)</td>
<td>50 ml</td>
</tr>
<tr>
<td>Ammonium or potassium alum</td>
<td>100 ml</td>
</tr>
<tr>
<td>Distilled water</td>
<td>100 ml</td>
</tr>
<tr>
<td>Mercuric oxide (red)</td>
<td>2.5 gm</td>
</tr>
</tbody>
</table>

The hematoxylin was dissolved in the alcohol and the alum in the water by the aid of heat. Two solutions were thoroughly mixed and boiled as rapidly as possible. After removing from heat, mercuric oxide was added slowly and reheated to simmer until it become dark purple. The solution was then removed from heat immediately and plunged the vessel into basin of cold water until become cool. The solution was kept in the dark. Immediately before use the solution was filtered and 2-4 ml of glacial acetic acid was added per 100ml of solution to increase the precision of the nuclear stain.

3.9.6.2. Preparation of eosin solution (1% stock alcoholic eosin)

Table 8: Preparation of eosin solution (1% stock alcoholic eosin)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eosin Y, water soluble</td>
<td>1 gm</td>
</tr>
<tr>
<td>Distilled water</td>
<td>20 ml</td>
</tr>
<tr>
<td>Dissolved and 95% alcohol</td>
<td>80 ml</td>
</tr>
</tbody>
</table>

Working eosin solution

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eosin stock solution</td>
<td>1 part</td>
</tr>
<tr>
<td>Alcohol, 80%</td>
<td>3 parts</td>
</tr>
</tbody>
</table>
Flow Chart for Histopathology:

Fixation in neutral buffered formalin

Trimming (0.5-1cm thick)

Overnight washing (8-12 hour)

Dehydration in ascending grades of alcohol
(50%, 1 hour; 70%, 1 hour; 80%, 1 hour; 95%, 1 hour; 100%: 2 changes, 1 hour in each)

Clearing in chloroform/ Xylene
(2 changes, 1 hour and 30 minutes in each)

Embedding in paraffin (3 hour)

Blocking with paraffin

Sectioning on a Microtome

Staining with Hematoxylin & Eosin

Examination under light microscope
3.9.6.3. Routine hematoxylin & eosin staining procedure

The tissue sections were deparaffinized in 3 changes of Xyline (3 minutes in each). Rehydrations of the sectioned tissues were done through descending grades of alcohol.

95% alcohol for 2 minutes

↓

80% alcohol for 2 minutes

↓

70% alcohol for 2 minutes

↓

Distilled water for 5 minutes

↓

Staining with Harris Hematoxylin for 15 minutes

↓

Washing in running tap water for 15 minutes

↓

Differentiated in acid alcohol: 2 to 4 dip (1 part HCl in 99 parts 70% alcohol)

↓

The tissue sections were then washed in tap water for 5 minutes.

↓

Blue in ammonia water (2-3 quick dips)

↓

The sections were then stained with eosin for 1 minute.
Differentiation & dehydration in 95% alcohol: 3 quick dips.

The stained sections were then cleaned by 3 changes in Xylene, 5 minutes in each. Finally the sections were mounted with coverslip using DPX.

3.9.6.4. **Histopathological studies & photomicrograph**

The tissues were examined & photomicrography was taken at the Pathology Laboratory, BAU, Mymensingh.

3.10. **Statistical analysis**

Group mean values of weight gain and oocysts output of the chicks were compared by a students 't' test with N, + N2-2 degrees of freedom (Bailey, 1981).
CHAPTER IV

RESULTS
CHAPTER IV

RESULTS

4.1. Mean body weight record
The effect of oral administration of Toltrazuril at different doses were evaluated in chickens alone or in combination with Vitamin-K, Sulfaclozine, and Oyster mushroom against chicken coccidiosis (Table 9). The mean initial weight of chicks for all groups were almost similar, which was 37 g and above as recorded on day 1. The pre-infection body weight values at day 10 recorded for all groups were almost similar which was more than 217 g. Significant (P<0.01) increase of body weight was recorded on day 21 following artificial infection in which the highest increase (780.21±1.29 g) was seen in Group A chicken (healthy control). After treatment (on day 24), there were significant (P<0.01) increase in body weight of all groups used for therapeutic trial with the maximum mean weight gain in Group F (850.75±2.40 g) which was the nearest value to Group A (healthy control) and in Group I (used for prophylactic trial). The chickens of Group I gained highest (P<0.01) increase in mean body weight (942.60±1.44 g) on day 24 of the experiment.

4.2. OPG Counts
In Group A, OPG counts remained "0" throughout the experimental period (healthy control). The initial mean OPG of chickens for other therapeutic trial groups were almost similar, which was 49 thousands (P<0.01) and above recorded on day 21. After treatment there were sharp decline of OPG counts in all groups. But the decline was very significant (P<0.05) in Group F which was 0.09±0.03 thousand. In Group I (used in prophylactic trial), the pre-treatment value on day 10 and post-treatment on day 24 were 0 and 0.03±0.01 thousand (P<0.01) respectively (Table 10).

4.3. Mortality and recovery rate record
The clinical signs of caecal coccidiosis appeared on day 20 and became severe on day 21. A total of 3 chicks died during the experimental period from Group B (untreated affected group) and found 60% mortality. Only one bird died in Group C treated with Toltrazuril 3.5 mg/kg. In Group I there was no visible morbidity and mortality.
After treatment, there was most significant recovery from coccidiosis in Group F with 100% recovery rate. Among Groups B and C, the chicks did not recover as the Group B was untreated and Group C was treated with low dose of Toltrazuril. The chicks of Group D, E, G and H had 20, 60, 60 and 80% recovery rate respectively.

4.4. Post-mortem findings after treatment

Group A
Appeared healthy all over the study, lesion was not observed at any part of gastrointestinal tract (Fig.5).

Group B
The caeca were distended and filled with blood tinged contents, wall showed patchy haemorrhage (Fig. 6 and 7).

Group C, D, E, G and H
There were little haemorrhage found on caecal wall with no blood tinged contents (Fig.8). All other organs were apparently uninfected and normal.

Group F
No significant change was observed in caeca and all other part of intestine (Fig.9).

Group I
In Group I the gastrointestinal tract appeared healthy. The caeca did not reveal any change.
Experimental infection was given at 10 and 15 days of age and therapeutic measure were taken on day 22 and 23 of this study. Prophylactic trial was set on day 10 of the experiment.

Table 9: Therapeutic and prophylactic efficacy trial of Toltrazuril in broiler chicken in term of body weight gain

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>On day 1 (Pre-infection)</td>
</tr>
<tr>
<td>Group A (Control)</td>
<td>37.94±0.15 NS</td>
</tr>
<tr>
<td>Group B (infected untreated)</td>
<td>37.87±0.38 NS</td>
</tr>
<tr>
<td>Group C (3.5 mg Toltrazuril/ kg body weight)</td>
<td>37.72±0.56 NS</td>
</tr>
<tr>
<td>Group D (7 mg Toltrazuril/ kg body weight)</td>
<td>37.91±0.47 NS</td>
</tr>
<tr>
<td>Group E (12 mg Toltrazuril/ kg body weight)</td>
<td>38.26±0.68 NS</td>
</tr>
<tr>
<td>Group F (7 mg Toltrazuril and 2.5 mg Vitamin K/ kg body weight)</td>
<td>37.85±0.62 NS</td>
</tr>
<tr>
<td>Group G (7 mg Toltrazuril and 150 mg Sulfachlortetin/ kg body weight)</td>
<td>38.00±0.52 NS</td>
</tr>
<tr>
<td>Group H (7 mg Toltrazuril and 100 mg Oyster mushroom/ kg body weight)</td>
<td>37.75±0.57 NS</td>
</tr>
<tr>
<td>Group I (used in prophylactic trial)</td>
<td>37.95±0.61 NS</td>
</tr>
</tbody>
</table>

The above values represent the mean ± standard error (SE) of the body weight of 5 chickens

*=Significant at 5 percent level (P<0.05)

**=Significant at 1 percent level (P<0.01)
<table>
<thead>
<tr>
<th>Groups</th>
<th>OPG count (thousand)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-treatment values</td>
<td>Post-treatment values</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(on day 21)</td>
<td>(on day 24)</td>
<td></td>
</tr>
<tr>
<td>Group A (Control)</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td></td>
</tr>
<tr>
<td>Group B (infected untreated)</td>
<td>50.55±2.368**</td>
<td>64.44±1.38**</td>
<td></td>
</tr>
<tr>
<td>Group C (3.5 mg Toltrazuril/ kg body weight)</td>
<td>52.71±1.796**</td>
<td>14.43±1.36**</td>
<td></td>
</tr>
<tr>
<td>Group D (7 mg Toltrazuril/ kg body weight)</td>
<td>51.65±2.112**</td>
<td>1.58±0.38**</td>
<td></td>
</tr>
<tr>
<td>Group E (12 mg Toltrazuril/ kg body weight)</td>
<td>49.46±1.33**</td>
<td>1.37±0.27**</td>
<td></td>
</tr>
<tr>
<td>Group F (7 mg Toltrazuril and 2.5 mg Vitamin K/ kg body weight)</td>
<td>50.44±1.62**</td>
<td>0.09±0.03*</td>
<td></td>
</tr>
<tr>
<td>Group G (7 mg Toltrazuril and 150 mg Sulfaclozine/ kg body weight)</td>
<td>46.60±1.86**</td>
<td>1.07±0.04**</td>
<td></td>
</tr>
<tr>
<td>Group H (7 mg Toltrazuril and 100 mg Oyster mushroom/ kg body weight)</td>
<td>49.05±0.97**</td>
<td>1.21±0.10**</td>
<td></td>
</tr>
<tr>
<td>Group I (used in prophylactic trial)</td>
<td>0.00±0.00NS</td>
<td>0.03±0.01**</td>
<td></td>
</tr>
</tbody>
</table>

The above values represent the mean ± standard error (SE) of the body weight of 5 chickens

*=Significant at 5 percent level (P<0.05)

**=Significant at 1 percent level (P<0.01)
Fig. 5: Post mortem examination of the chickens of Group A did not reveal any major lesion.

Fig. 6: Distended caeca filled with blood tinged contents as seen in Group B.
Fig. 7: Profuse haemorrhage was seen after opening up the caecal tube of Group B chicken that died of induced coccidiosis.

Fig. 8: Haemorrhagic lesion was seen in the mucosa after washing up the caeca of Group D chicken.
Fig. 9: Caeca after washing of Group F showing no lesion on day 24.
4.5. Histopathology

At the end of the experiment caeca from the birds of the different groups were collected for the histopathological study.

**Group A**

In Group A (control healthy) the caeca showed almost normal structure. Microscopic lesion was not seen related with caecal coccidiosis (Fig.10).

**Group B**

The microscopic lesions of the chicks sacrificed on day 24 of this study consisted of slight thickening of the villi due to infiltration of mononuclear cells and eosinophils in lamina propria (Fig.11). The glands of the crypt region contain mucous exudates in the lumen. Merozoites/schizonts were found in contact with villi and crypt epithelium. Inflammation preceded further additional haemorrhage in the lamina propria and submucosa (Fig.11). Desquamation of crypt epithelia was found (Fig.11).

**Group C**

There were erosion and desquamation of crypt epithelia, infiltration of reactive cells in the lamina propria (Fig.12). Merozoites/schizonts were also found in the crypt of villi (Fig.12).

**Group D**

The caeca collected from the chicken of Group D showed arrested coccidial development with necrosis, haemorrhages, erosion, and desquamation of epithelial cells (Fig.13).

**Group E**

There were haemorrhages in caecal mucosa and submucosa, infiltration of inflammatory cells (Fig.14) indicating diffuse acute caecitis.

**Group F**

Microscopic lesions of caeca of the chicken of Group F showed diffuse infiltration of reactive cells specially eosinophils and mononuclear cells in the mucosa and submucosa but haemorrhage was not seen (Fig.15). There was also degeneration of caecal gland.

**Group G**

Slight desquamation of caecal epithelia, necrosis, and degeneration of mucosa and caecal gland were found (Fig.16).
**Group H**
Desquamation of epithelial lining, infiltration of reactive cells in mucosa and submucosa were found (Fig.17).

**Group I**
In this group the caeca showed almost normal structure as Group A in which the histopathology of caeca did not reveal any significant lesion related to coccidiosis (Fig.18).
Fig. 10: Caecal section of the chicken of Group A (healthy control) did not reveal any significant microscopic lesion (H & E, 100X).

Fig. 11: Caecal section of the chicken of Group B (infected untreated) showing desquamation of epithelia, merozoites/schizonts in mucosa, and infiltration of reactive cells (H & E, 100X).
Fig. 12: Caecal section of chicken of Group C treated with 3.5 mg Toltrazuril /kg body weight showing erosion and desquamation of crypt epithelia, infiltration of reactive cells in the lamina propria with merozoites/schizonts in the crypt of villi (H & E, 400X).

Fig. 13: Caecal section of chicken of Group D treated with 7 mg Toltrazuril /kg body weight showing arrested coccidial development with necrosis, haemorrhages, erosion, and desquamation of epithelial cells (H & E, 100X).
Fig.14: Caecal section of chicken of Group E treated with 12 mg Toltrazuril/kg body weight showing haemorrhages in caecal mucosa and submucosa, infiltration of inflammatory cells (H & E, 100X).

Fig.15: Caecal section of chicken of Group F treated with 7 mg Toltrazuril and 2.5 mg Vitamin K/kg body weight showing diffuse infiltration of reactive cells specially eosinophils and mononuclear cells in the mucosa and submucosa, degeneration of caecal gland but no significant haemorrhage present (H & E, 100X).
Fig. 16: Caecal section of chicken of Group G treated with 7 mg Toltrazuril and 150 mg Sulfaclozine/kg body weight showing slight desquamation of caecal epithelia, necrosis, and degeneration of mucosa and caecal gland (H & E, 100X).

Fig. 17: Caecal section of chicken of Group H treated with 7 mg Toltrazuril and 100 mg Oyster mushroom/kg body weight showing desquamation of epithelial lining, infiltration of reactive cells in mucosa and submucosa (H & E, 100X).
Fig. 18: Caecal section of chicken of Group I (used as prophylactic trial) treated with 3.5 mg Toltrazuril/kg body weight showing no significant lesion related to coccidiosis (H & E, 100X).
CHAPTER V

DISCUSSION

To have maximum body weight gain in a shortest possible time by spending minimum feed is the key to success in poultry industry. One of the major constraints having direct negative impact on body weight gain is coccidiosis in chicken. Karim and Trees (1990) identified five species of *Eimeria* (*E. tenella, E. necatrix, E. acervullina, E. maxima and E. brunette*) in chicken in Bangladesh. The occurrence of the relatively less pathogenic species *E. precox* and *E. mitis* in chicken in Bangladesh was identified by Karim and Begum (1994). Thus all the 7 pathogenic species of chicken *Eimeria* are present in Bangladesh.

About $10^4$ oocysts of *E. tenella* once directly introduced into the crop of chicks normally produce a moderate to severe infection with a very low rate of mortality (Karim, 1988). Faecal oocyst count and body weight gain were used as the criteria for the detection of infection and severity of infection. However, faecal oocysts count may not always correspond with the weight depression, since often a large number of oocysts may be produced without apparent effect on weight gain (Karim, 1988). Since the uninfected control chicks had a steady and uninterrupted gain in weight, and did not pass any oocysts, it can be presumed that the effect on body weight gain was due to experimental infection. In addition, since the premises, waterer, feeder, brooder etc. were disinfected with ammonia that kills *Eimeria* oocysts (Xie *et al.* 1983), the possibility of any additional infection can be excluded.
From the body weight records, the maximum mean weight gained was seen in chicks of non infected healthy chicks (Group A) and was 982.79 g and the minimum mean weight was achieved (643.97±1.649 g) by the infected untreated chickens of Group B. The mean weight of other groups remained under this limit. Moreover, it was also noticed that multiplication of the oocysts administered to test groups was also higher than usual. The members of Group I (used as prophylactic trial) weighed 942.60 g and the group was placed in 2nd position. Among the groups of therapeutic trial the chickens of Group F gained the maximum mean weight which was 850.75 g. The findings of the present study were correlated with the observation of Lakkundi et al. (2002), who evaluated the effect of Toltrazuril and amprolium on body weight and feed efficacy in broiler chickens experimentally infected with caecal coccidiosis. They found Toltrazuril treated birds had better body weight gain and feed efficiency than uninfected birds.

The picture of OPG counts in Group I showed that the birds became immune to some extent after administration of low dose of Toltrazuril (3.5 mg/kg) for 15 days as prophylactic trial showed range of OPG counts between 0 and 0.03 thousand. This depicts prophylactic efficacy of the Toltrazuril. However, due to high challenge infection, the immunity remained fluctuating. The therapeutic trial in different groups from day 22 to 23 showed mark reduction of mean OPG counts. The maximum reduction was found in Group F (treated with Toltrazuril 7 mg/kg and Vitamin K 2.5 mg/kg) which was only 0.09 thousand. Similarly, the Group H (treated with Toltrazuril 7 mg/kg and Sulfaclozine 150 mg/kg) had the second position of mean OPG counts having mean quantity of 1.21 thousand. The present findings substantiated with the findings of Grief (2000), who claimed that during evaluation studies, Toltrazuril acted against all intracellular schizonts and being correlated with a higher reduction in oocyst excretion, lesion scoring and increased weight gains.

From the records of mortality and recovery rate from coccidiosis, it showed that among the groups of the therapeutic and prophylactic trial the Group F gave the maximum recovery and lowest mortality rate which were 100 and 0% respectively. Among the other groups, the Group E, G and H had 60, 80 and 60% recovery rate.
Only one bird died in Group C treated with Toltrazuril at a dose rate of 3.5 mg/kg body weight. This study could be compared with Dhillon et al. (2004), who tested the efficacy of Toltrazuril against 6 different levels of *Eimeria tenella* infection in 2-week-old chicks and found that the treatment resulted to lower mortality and reduced oocyst production. Moreover, therapy resulted to complete elimination of the clinical signs at lower levels of infection.

In histopathological study, it was found that the Group A revealed very normal structure of caecal section as it was used as control group. The group of prophylactic trial (Group I) also showed normal structure as Group A indicating the prophylactic efficacy of Toltrazuril. The histopathology of the samples of Group F showed diffuse infiltration of reactive cells specially eosinophils and mononuclear cells in the mucosa and submucosa but no significant haemorrhage indicated the anti-haemorrhagic impact of Vitamin K. In other treated groups (Group C, D, E, G, and H), it was observed that there were some abnormalities of caeca like infiltration of inflammatory cells in the mucosa and submucosa, desquamation of crypt epithelia, presence of merozoites/schizonts in the caecal crypt and villi with only exception in Group E in which haemorrhage was present prominently in mucosa and submucosa but other lesions were so prominent. In caecum of Group B, there were slight thickening of the villi due to infiltration of mononuclear cells and eosinophils in lamina propria, mucous exudates in the lumen of the glands of the crypt region, merozoites/schizonts in contact with villi and crypt epithelium. There were also inflammatory infiltrations in additional to haemorrhages in the lamina propria and submucosa and desquamation of crypt epithelia indicating the microscopic lesions due to caecal coccidiosis. This study could be compared with Lakkundi et al. (2002) who evaluated histopathological anticoccidial activity of Toltrazuril and amprolium in experimentally induced caecal coccidiosis in broiler chicken (n=280). It was reported that Toltrazuril prevented the establishment of caecal coccidiosis by degeneration and disintegration of the first generation of schizonts. It may be concluded that Toltrazuril had a coccidiocidal effect (Lakkundi et al. 2002).
Toltrazuril alone as prophylactic use or use of Toltrazuril in combination with Vit-K or Sulfaclozine or mushroom can protect chicken from caecal coccidiosis. However, it needs to study while chickens treated with Toltrazuril, what concentration of Toltrazuril remain active in the flesh and what public health risk is leying with the residual value of Toltrazuril in meat and body tissue.
CHAPTER V

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DISCUSSION

To have maximum body weight gain in a shortest possible time by spending minimum feed is the key to success in poultry industry. One of the major constraints having direct negative impact on body weight gain is coccidiosis in chicken. Karim and Trees (1990) identified five species of *Eimeria* (*E. tenella, E. necatrix, E. acervullina, E. maxima and E. brunette*) in chicken in Bangladesh. The occurrence of the relatively less pathogenic species *E. precox* and *E. mitis* in chicken in Bangladesh was identified by Karim and Begum (1994). Thus all the 7 pathogenic species of chicken *Eimeria* are present in Bangladesh.

About $10^4$ oocysts of *E. tenella* once directly introduced into the crop of chicks normally produce a moderate to severe infection with a very low rate of mortality (Karim, 1988). Faecal oocyst count and body weight gain were used as the criteria for the detection of infection and severity of infection. However, faecal oocysts count may not always correspond with the weight depression, since often a large number of oocysts may be produced without apparent effect on weight gain (Karim, 1988). Since the uninfected control chicks had a steady and uninterrupted gain in weight, and did not pass any oocysts, it can be presumed that the effect on body weight gain was due to experimental infection. In addition, since the premises, waterer, feeder, brooder etc. were disinfected with ammonia that kills *Eimeria* oocysts (Xie *et. al.* 1983), the possibility of any additional infection can be excluded.
From the body weight records, the maximum mean weight gained was seen in chicks of non infected healthy chicks (Group A) and was 982.79 g and the minimum mean weight was achieved (643.97±1.649 g) by the infected untreated chickens of Group B. The mean weight of other groups remained under this limit. Moreover, it was also noticed that multiplication of the oocysts administered to test groups was also higher than usual. The members of Group I (used as prophylactic trial) weighed 942.60 g and the group was placed in 2nd position. Among the groups of therapeutic trial the chickens of Group F gained the maximum mean weight which was 850.75 g. The findings of the present study were correlated with the observation of Lakkundi et al. (2002), who evaluated the effect of Toltrazuril and amprolium on body weight and feed efficacy in broiler chickens experimentally infected with caecal coccidiosis. They found Toltrazuril treated birds had better body weight gain and feed efficiency than uninfected birds.

The picture of OPG counts in Group I showed that the birds became immune to some extent after administration of low dose of Toltrazuril (3.5 mg/kg) for 15 days as prophylactic trial showed range of OPG counts between 0 and 0.03 thousand. This depicts prophylactic efficacy of the Toltrazuril. However, due to high challenge infection, the immunity remained fluctuating. The therapeutic trial in different groups from day 22 to 23 showed mark reduction of mean OPG counts. The maximum reduction was found in Group F (treated with Toltrazuril 7 mg/kg and Vitamin K 2.5 mg/kg) which was only 0.09 thousand. Similarly, the Group H (treated with Toltrazuril 7 mg/kg and Sulfaclozine 150 mg/kg) had the second position of mean OPG counts having mean quantity of 1.21 thousand. The present findings substantiated with the findings of Grief (2000), who claimed that during evaluation studies, Toltrazuril acted against all intracellular schizonts and being correlated with a higher reduction in oocyst excretion, lesion scoring and increased weight gains.
From the records of mortality and recovery rate from coccidiosis, it showed that among the groups of the therapeutic and prophylactic trial the Group F gave the maximum recovery and lowest mortality rate which were 100 and 0% respectively. Among the other groups, the Group E, G and H had 60, 80 and 60% recovery rate. Only one bird died in Group C treated with Toltrazuril at a dose rate of 3.5 mg/kg body weight. This study could be compared with Dhillon et al. (2004), who tested the efficacy of Toltrazuril against 6 different levels of *Eimeria tenella* infection in 2-week-old chicks and found that the treatment resulted to lower mortality and reduced oocyst production. Moreover, therapy resulted to complete elimination of the clinical signs at lower levels of infection.

In histopathological study, it was found that the Group A revealed very normal structure of caecal section as it was used as control group. The group of prophylactic trial (Group I) also showed normal structure as Group A indicating the prophylactic efficacy of Toltrazuril. The histopathology of the samples of Group F showed diffuse infiltration of reactive cells specially eosinophils and mononuclear cells in the mucosa and submucosa but no significant haemorrhage indicated the anti-haemorrhagic impact of Vitamin K. In other treated groups (Group C, D, E, G, and H), it was observed that there were some abnormalities of caeca like infiltration of inflammatory cells in the mucosa and submucosa, desquamation of crypt epithelia, presence of merozoites/schizonts in the caecal crypt and villi with only exception in Group E in which haemorrhage was present prominently in mucosa and submucosa but other lesions were so prominent. In caecum of Group B, there were slight thickening of the villi due to infiltration of mononuclear cells and eosinophils in lamina propria, mucous exudates in the lumen of the glands of the crypt region, merozoites/schizonts in contact with villi and crypt epithelium. There were also inflammatory infiltrations in additional to haemorrhages in the lamina propria and submucosa and desquamation of crypt epithelia indicating the microscopic lesions due to caecal coccidiosis. This study could be compared with Lakkundi et al. (2002) who evaluated histopathological anticoccidial activity of Toltrazuril and amprolium in experimentally induced caecal coccidiosis in broiler chicken (n=280). It was reported that Toltrazuril prevented the establishment of caecal coccidiosis by degeneration and disintegration of the first generation of schizonts. It may be concluded that Toltrazuril had a coccidiocidal effect (Lakkundi et al. 2002).
Toltrazuril alone as prophylactic use or use of Toltrazuril in combination with Vit-K or Sulfaclozine or mushroom can protect chicken from caecal coccidiosis. However, it needs to study while chickens treated with Toltrazuril, what concentration of Toltrazuril remain active in the flesh and what public health risk is leying with the residual value of Toltrazuril in meat and body tissue.
CHAPTER VI

SUMMARY AND CONCLUSION
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The present research was undertaken to evaluate the therapeutic and prophylactic potentials of Toltrazuril against chicken coccidiosis in Bangladesh. For therapeutic trial 40 broiler chicks were divided into eight groups and five in each were used. The infection was induced by administrating orally about 10,000 sporulated oocyst of *Eimeria tenella* to chicks of 10 and 15 days of age. Group A was left as noninfected, non-medicated control. Chicks of group B were infected and non-medicated while chicks of Group C, D, and E were treated with Toltrazuril at rate of 3.5, 7, and 12 mg/kg body weight on day 22 and 23 of age when the chicks were severely infected. In chicks of Group F, Toltrazuril was given at rate of 7mg/kg body with a combination with Vitamin K at the rate of 2.5 mg/kg body weight on same days. Similarly chicks of Group G were treated with Toltrazuril 7 mg/kg and Sulfaclozine 150 mg/kg body weight and the chicks of group H were treated with Toltrazuril 7 mg/kg and Oyster Mushroom powder 100 mg/kg body weight. For prophylactic trial the chickens of Group I were treated with 3.5 mg Toltrazuril/kg body weight before and during exposure to coccidial infection.

After treatment, the Group F among the groups of therapeutic trial gave the best result on the basis of body weight, OPG counts, and histopathology. The Group I (used as prophylactic trial) gave output almost similar to Group A (healthy control).

From the present research it could be concluded that combination of 7 mg Toltrazuril and 2.5 mg Vitamin K/kg body weight would be best solution against caecal coccidiosis than other doses of Toltrazuril and combination used in the study. This study also suggested that 3.5 mg Toltrazuril/kg body weight could be of value as prophylactic measure against caecal coccidiosis.
In this study *E. tenella* oocysts were used. Further study is needed to determine the efficacy of Toltrazuril against other species of *Eimeria* and these researches would provide a clear idea on the efficacy of Toltrazuril against chicken coccidiosis. Clinical caecal coccidiosis in chicken can best be treated with Toltrazuril and Vit-K supplement or use of Toltrazuril alone as prophylactic dose. Use of mushroom with Toltrazuril reduced morbidity and pathologic lesions following exposure to *E. tenella*. Analysis is needed to find out the cost benefit effectiveness of use of the mushroom.
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