PREVALENCE OF *Eimeria* spp. IN THE FAECAL SAMPLES OF GOAT (*Capra hircus* Linnaeus, 1758) AND THE EFFECT OF OAT PLANT EXTRACT (*Avena sativa* Linnaeus, 1753) IN THEIR SPORULATION

Submitted by

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In partial fulfillment of the requirements for the award of the degree of Master of Science in Zoology with special paper Parasitology

Submitted to

Central Department of Zoology

Institute of Science and Technology

Tribhuvan University

Kritipur, Kathmandu

Nepal

DECLARATION

I hereby declare that the work presented in this thesis entitled "Prevalence of *Eimeria* spp. in the faecal samples of goats (*Capra hircus* Linnaeus, 1758) and the effect of oat plant extract (*Avena sativa* Linnaeus, 1753) in their sporulation" has been done by myself, and has not been submitted elsewhere for the award of any degree. All sources of information have been specifically acknowledged by reference to the author(s) or institution(s).

Date..14th March, 2023

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RECOMMENDATION

This is to recommend that the thesis entitled "Prevalence of *Eimeria* spp. in the faecal samples of goats (*Capra hircus* Linnaeus, 1758) and the effect of oat plant extract (*Avena sativa* Linnaeus, 1753) in their sporulation" has been carried out by Nisha Kiran Shrestha for the partial fulfillment of Master's Degree of Science in Zoology with the special paper Parasitology. This is her original work and has been carried out under our supervision. To the best of our knowledge, this thesis work has not been submitted for any other degree in any institution.

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LETTER OF APPROVAL

On the recommendation of supervisor "Prof. Dr. Mahendra Maharjan" this thesis submitted by Nisha Kiran Shrestha entitled "Prevalence of *Eimeria* spp. in the faecal samples of goats (*Capra hircus* Linnaeus, 1758) and the effect of oat plant extract (*Avena sativa* Linnaeus, 1753) in their sporulation" is approved for the examination and submitted to the Tribhuvan University in partial fulfillment of the requirements for Master's Degree of Science in Zoology with special paper Parasitology.

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CERTIFICATE OF ACCEPTANCE

This thesis work submitted by Nisha Kiran Shrestha entitled "Prevalence of *Eimeria* spp. in the faecal samples of goats (*Capra hircus* Linnaeus, 1758) and the effect of oat plant extract (*Avena sativa* Linnaeus, 1753) in their sporulation" has been accepted as partial fulfillment for the requirements of Master's Degree of Science in Zoology with special paper Parasitology.

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LIST OF ABBREVIATIONS

NAST	Nepal Academy of Science and Technology
ACR	Anticoccidial resistance
OPG	Oocyst per gram
E.	*Eimeria*
µg/ml	Micro gram per milliliter
hrs	Hours
DMSO	Dimethyl sulfoxide
Conc.	Concentrated

ABSTRACT

Coccidiosis is an infectious disease caused by the parasite of genus *Eimeria* that develops in large intestine of small ruminants such as goat. It is distributed worldwide with high infection rate. This study was conducted from February to August, 2022 to evaluate the effect of oat plant (*Avena sativa*) extracts in sporulation of *Eimeria* species in faecal sample of goat at Duwakot, Changunarayan Municipality, Bhaktapur, Nepal. Altogether, 310 faecal sample of goat and plant *Avena sativa* were collected. Faecal sample was collected and preserved in 2.5% potassium dichromate solution and the plant was collected and dried at room temperature in the laboratory of Nepal Academy of Science and Technology (NAST). The faecal samples were examined microscopically by direct wet mount technique, sedimentation and floatation technique. The intensity of faecal sample with heavy infection of *Eimeria* by using oocyst per gram (OPG) count with the help of Mc Master chamber technique. The plant extracts were made by grinded, dissolved it with solvents, filtered and evaporated.

Out of 310 samples, 183 (59%) samples were found to be positive. From which 138 light infection (+), 20 samples revealed the mild infection (++) and 25 samples revealed heavy infection (+++).Then, heavily infected samples were treated with plant extract dose on different concentration (0 μg/ml, 0 μg/ml with 10% DMS0, 0.5 μg/ml, 1 μg/ml, 5 μg/ml, 10 μg/ml, 15 μg/ml, 20 μg/ml and 25 μg/ml) for 0, 24, 48 hours. The rate of sporulation was maximum at the concentration of plant extract to 20 μg/ml in 24 hours and 10 to 20 μg/ml in 48 hours. This study found that there was significant difference between rate of sporulation and dosage concentration of plant extract ($p<0.5$). This study revealed that the utilization of plant extract may have anti-coccidial effect depending on its dose and time of incubation.

Keywords: *Eimeria*, Coccidiosis, OPG count

CHAPTER-1

INTRODUCTION

Coccidiosis is a serious and infectious disease caused by the parasite of genus *Eimeria* which develop in large intestine of small ruminants (Mohamaden, 2018). *Eimeria* is an apicomplexan protozoan parasite that develops in the small and large intestine in young ruminant animals such as goats causing more pathogenic effects in digestive system (Catlier et al. 2012). It has various phases of development as oocyst, sporocyst, sporozoites, micropyls. Oocyst is ovoid shaped thick walled which disintegrates into sporozoite when entered into the host (Bwan and Htun, 2021).

A preliminary faecal examination can be performed by using the modified McMaster method (Hutchinson, 2009) to determine the number of oocysts per gram (OPG) of faeces and to identify which goats were infected with *Eimeria* . For the identification of *Eimeria* species, it was based on morphometric characteristics of the oocysts and in some cases, it may based on the presence or absence of a micropyle and oocyst shape (Levine and Ivens, 1967; Saravia et al., 2021).

Eimeria spp. is distributed worldwide, and the infection rates can reach more than 90% in some areas of the world (Cavalcante et al., 2012; Mohamaden et al., 2018; Juszczak et al., 2019). In the context of developing country such as Nepal, there is high prevalence rate of coccidian parasites due to lack of sanitation and hygiene as well as lack of proper medication.

Globally, more than 20 species of *Eimeria* were reported in goat by various researcher and found that it affect digestive system causing diarrhea and many problems which results lower productivity (Etsay, 2020). In the context of developing countries like Nepal, there is high burden of *Eimeria* due to lack of Knowledge and sanitation in farmers and beared the problems as decrease in productivity. In Nepal, different morphologic forms and the species of *Eimeria* were reported which are as follows: *E. ninakohlyakimovae* , *E. alijevi*, *E. capralis*, *E. masseyensis*, *E. hirci*, *E. tunisiensis*, *E. charlestoni*, *E. jolchejevi*, *E. arloingi*, *E. caprina*, *E. aspheronica*, *E. jolchejevi*, *E. christenseni*, *E. hirci* and *E. caprovina* (Ghimire et al., 2022) .

According to reports from Asian countries as Sri Lanka, Malaysia, China, and India, the most frequent species of *Eimeria* in goats are *E. ninakohlyakimovae, E. christenseni, E. arloingi, E. parva, E. caprina,* and *E.* alijevi (Bawm, 2020). In the developed countries such as USA, various common *Eimeria* species were found which are *Eimeria christenseni, E. kochali, E. caprina, E. ninakohlyakimorae, E. jolchiievi and E. caprovina*. Among them, rate of prevalence of *Eimeria christenseni* (52%) is higher than other species (Young et al, 2011). Similarly, in Egypt seven species of *Eimeria* were found and identified in goat. identified as *E. ninakohlyakimovae, E. hirci, E. caprina, E. christenseni, E. jolchijevi, E. apsheronica,* and *E. arloingi.* In goats, *E. arloingi* (37.04%), *E. ninakohlyakimovae* (30.86%) and *E. hirci* (24.69%) (Mohamaden et al., 2018).

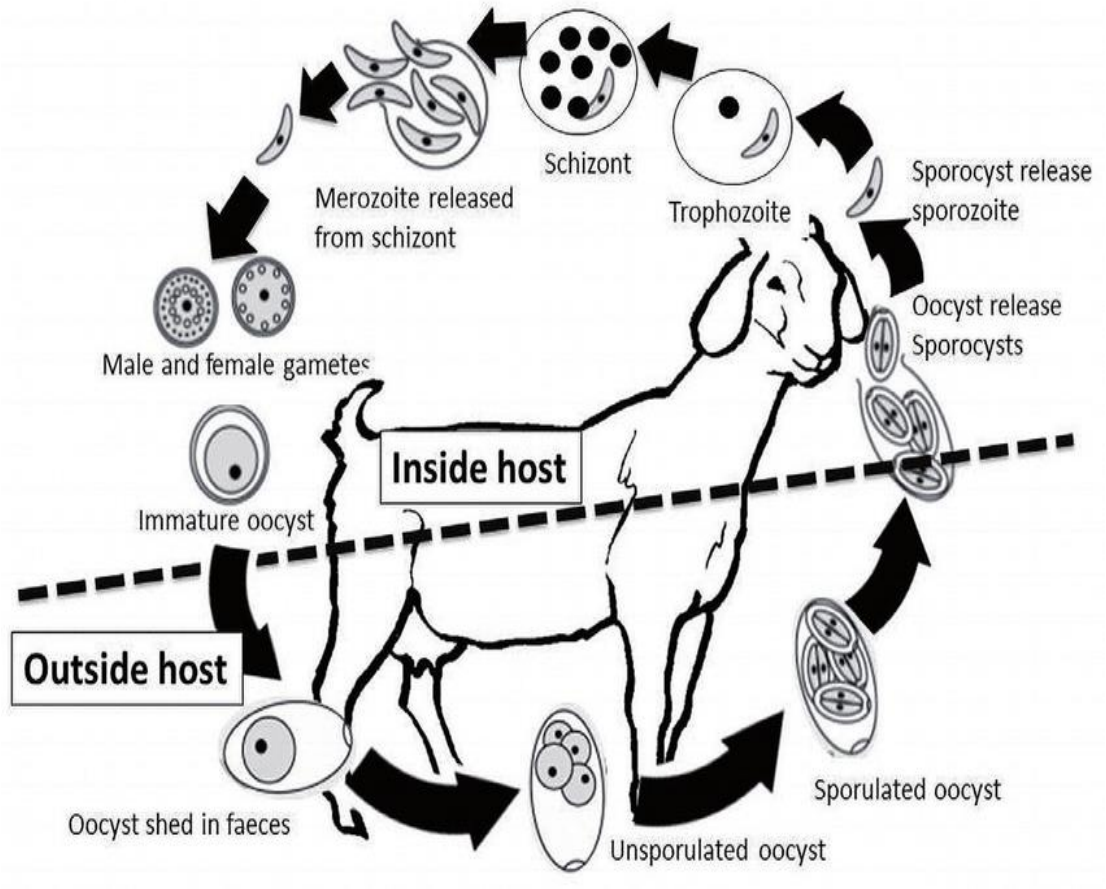

Figure 1: Lifecycle of *Eimeria* (Source: Bwan and Htun, 2021)

Eimeria requires only one host as ruminants to complete their life cycle including two stages as schizogony/merogony and gamogony (Figure 1). The life cycle involves an extracellular oocyst maturation stage (sporogony) as well as a parasitic intracellular stage inside the host with a sexual reproduction followed by an asexual reproduction.

After 2–7 days, unsporulated oocysts move through the feces, infecting the environment. Four sporoblasts are formed from the initial single cell, and each develops into a single sporocyst with two sporozoites. The sporulated oocysts can survive in unfavorable environments. The oocyst is typically ovoid-shaped and has thick walls. The oocyst's walls disintegrate after the host consumes it, releasing sporozoites from the sporocysts invade an epithelial cell, the sporozoites enter the small intestine and grow into the first schizont (Han et al., 2022). A second generation of schizonts may be started by the motile merozoites that the schizonts release, or they may grow into gamont, gametes, and non-sporulated oocysts that are released with the feces. The second generation of merozoites often infiltrate epithelial cells during the large intestine's schizogony are brought on by gametogony and second-generation schizogony. Typically, the *Eimeria* species' prepatent period for goats is around 19 days (Bwan and Htun, 2021).

It is mostly enteropathogenic to ruminants which develop in small and large intestine and then invade into intestinal epithelial cells that lead to loss of nutrients, loss of electrolytes, malabsorption and enteritis. The main symptoms of this disease are bloody diarrhea, dehydration, fever, loss of appetite, loss of weight, poor and retarted growth and death may occur in severe cases (Kaur, 2017). Thus, these parasitic infections are also responsible for causing heavy losses due to reduced production, morbidity and mortality in animals (Lutu, 1983) especially in developing countries like Nepal.

To avoid production losses and decreased productivity, effective husbandry methods, maintaining hygiene, and medications are crucial in lowering the spread of infectious oocysts (Iqbal et al., 2013). For the treatment and prevention of coccidiosis in ruminants, a number of anticoccidial medications are available. These include both ionophores and synthetic pharmaceuticals including sulfonamides, amprolium, decoquinate, and the triazines, diclazuril and toltrazuril (monensin, lasalocid). Amprolium, sulfonamides, and triazines are used therapeutically to treat young animals exhibiting signs of illness or as a preventative measure by being added to feed (Daugschies and Najdrowski, 2005). Nevertheless, continuous usage introduction of commercial coccidiostat has led to parasite's resilience. Due to this and the price alternatives to commercial medications, and methods of coccidiosis is studied and practiced (Gonzaga, 2021).

Anticoccidial resistance (ACR) develops in the case of consumption of commercial drugs in the intensive long-term use of anticoccidial drugs (Oden et al., 2018). To overcome from the toxic side effect of commericial drug, a unique approach to treating coccidiosis is to utilize phytogenic feed additives instead of infeed medications. This method has many advantages, including safety, lack of a grace period, natural origin, little to no side effects, and cost effectiveness (Qaid, 2021). Various plant extracts are utilized to prevent the risk of development of resistivity by *Eimeria* (Han et al., 2022).

Various natural products used by scientist to study the anticoccidial activity of *Eimeria* spp. The plant extract such as of *Coleus aromaticus* is a medicinal plant which is rich in various compound including thymol, carvacrol, and saponins are known to exhibit anticoccidial activity (Felici et al., 2020). These compounds present in the *C. aromaticus* leaf extract may exhibit anticoccidial effects. Saponins prevent the growth of protozoans by interacting with cell membrane cholesterol that may result coccidial effect. The phenolic content which includes carvacrol (Sidiropoulou et al., 2020) may affect the oocyst by interacting on the oocysts' in a cytoplasmic membrane (Gonzaga et al., 2021).

The genus *Avena sativa* is the member of subfamily Pooideae in the family Poaceae. It includes a group of diploid, tetraploid, and hexaploid species in which all but one are annual and self-pollinated. This plant is a rich source of protein, contains a number of important minerals, lipids, β-glucan, a mixed-linkage polysaccharide, phytoconstituents like avenanthramides, an indole alkaloid-gramine, flavonoids, flavonolignans, triterpenoid saponins, sterols, and tocols (Singh, 2013). These compounds may act on the oocyst of *Eimeria* spp. and may exhibit the coccidial effect (Lyrene, 1975).

Various experiments were performed in different plants that have killed the oocyst of *Eimeria* and can save the young infected goats. The fruit extract of *Ruta pinnata* have important anticoccidial activity against oocysts and sporozoites of *Eimeria* by testing the viability of the *E. ninakohlyakimovae* sporozoites and their ability to infect bovine colonic epithelial cells after incubation (Lopez, 2018). Likewise, *Avena sativa* is also highly proteinous and rich in various compound which need to be studied in order to determine the anticoccidial activity against oocyst and sporozoite of *Eimeria*.

1.2 Research Objectives

The current study was performed to fullfill

1.2.1 General Objective

1. To determine the rate of prevalence and burden of *Eimeria* spp.in the faeces of goat and to evaluate the effect of plant (*Avena sativa*) extracts in their sporulation.

1.2.2 Specific Objectives

1. To determine the prevalence of *Eimeria* spp. in the faecal samples of goats.
2. To determine the intensity and burden of *Eimeria* spp. in the faecal samples of goats.
3. To determine the optimum dose and time of sporulation of *Eimeria* spp. *in vitro* by oat plant extract.

1.3 Significance of Study

Nepal is a developing country. Many people depend on agriculture and animal husbandry as a source of income to earn their livelihood. Many ruminants such as goats were died due to the excessive burden of coccidian parasites as *Eimeria* spp which may be due to lack of information to the people about the parasitic pathogenicity of livestock. The goat farming is also in poor and unhygienic manner and hence is heavily infected with different parasites that affect on various system as digestive system of ruminants and may lead to death. Likewise, the infection caused by *Eimeria* in goats can cause significant economic loss leading to the poor health and reduced growth (Nwosu *et al.*, 2007). In market, various synthetic drugs are found in which the parasite develop resistivity against that drug. So, alternative approach to treating coccidiosis is to utilize phytogenic feed additives instead of infeed medications that has many advantages, including safety, lack of a grace period, natural origin, little to no side effects, and cost effectiveness (Qaid, 2021).

Many studies have been carried out in different part of the world reveal that some plant extract shows anticoccidal effect (Lopez, 2018) but this type of study seems to be lacking in Nepal. So, this study must be carried to prevent the side effect of drugs.

Avena sativa is highly nutritious plant which may have anticoccidal effect against *Eimeria*. The alternative medicine such as plant extract have various compound that shows anticoccidial effect of parasites and finally save goat from this parasitic burden as well as coccidiosis. For that the dosage and time of action of plant extract plays great role. This study has detailed study about the role of *Avena sativa* concentration will effect rate of sporulation of *Eimeria* species *in vitro* in goat. This study will help to fulfill the knowledge gap.

CHAPTER-2

LITERATURE REVIEW

Eimeria infection are common in goats worldwide and clinical coccidiosis occur after heavy infection (Young, 2011). *Eimeria* causes coccidian parasitic diseases affecting the profitability of ruminant production systems (Keeton and Navarre, 2018). Young ruminant animals as goat are particularly affected by *Eimeria* species, often in the period around weaning. Infection may result in diarrhoea, reduced growth and occasional deaths (Daugschies and Najdrowski, 2005; Ruiz et al., 2006; Chartier and Paraud, 2012). The plant extract i.e. *R. pinnata* have important anticoccidial activity against oocysts and sporozoites of *Eimeria* (Lopez, 2018)

Ghimire and Bhattarai (2019) asserted that goats are heavily infected with *Eimeria.* All around the nation, goats were at a significant risk of sickness and mortality. They reported that 400 goats' feces in a goat market in Kathmandu contained *Eimeria* followed by *Strongyle*, *Trichuris*, *Strongyloides*, *Moniezia*, *Entamoeba*, *Fasciola*, *Balantidium*, *Cryptosporidium*, *Capillaria*. The species and prevalence rate of the 15 various morphologic types of *Eimeria spp.* that were found in Nepal are as follows: *E. ninakohlyakimovae* (83.0%), *E. alijevi* (75.2%), *E. capralis* (75.2%), *E. masseyensis* (67.2%), *E. hirci* larger form (63.2%), *E. tunisiensis* (47.3%), *E. charlestoni* (33.0%), *E. jolchejevi* larger form *E. arloingi* (32.4%), *E. capralis* (32.4%) (Ghimire et al., 2022).

Similarly, Dixit et al (2016) discovered that in India, goat juveniles in Jabalpur had a 98.05% total prevalence of gastrointestinal parasites, with *Eimeria* spp. followed by *Strongyle*, *Strongyloides* spp, *Amphistome* spp. and *Trichuris* spp. having the greatest percentages. While Singh et al., (2014) also looked at 960 goat feces samples to determine the prevalence of gastrointestinal parasitic infections in goats in Madhya Pradesh, India, they discovered 906 positive samples with coccidian parasites such as *Eimeria* species being the most common (82.4%), followed by Strongyles (69.27%), Amphistomes (22.71%), *Strongyloides spp*. (9.17%), *Trichuris* spp. Likewise, more than 20 species of *Eimeria spp*. have been found in goats in different parts of the world, which lowers productivity (Etsay, 2020).

The most significant parasite illnesses affecting the profitability of ruminant production systems are those caused by *Eimeria* species (Keeton and Navarre, 2018). Animals that are young are especially affected. Infection can cause diarrhea, stunted growth, and occasionally fatalities (Daugschies and Najdrowski, 2005; Ruiz et al., 2006; Chartier and Paraud, 2012). Traditional methods for controlling ruminant coccidiosis include appropriate management and preventive or metaphylactic use of anticoccidials (Daugschies and Najdrowski, 2015).

Coccidiosis affects both the weight and the amount of milk produced by goats. Tropical areas also increase the prevalence of these parasite diseases because of the warm, humid climate conditions that promote coccidia sporulation. By taking drugs as directed, the illness can be prevented. Chemical components in anticoccidial products target the parasite across different phases of its life cycle. In contrast to amprolium, monensin, and lasalocide, sulfonamides are active in the early phases of the life cycle. On the other hand, because it impacts the complete coccidia cycle, toltrazuril has both therapeutic and preventative benefits (Taylor, 2009). The parasite might eventually become resistant to these drugs. widespread and ongoing use of the same (Gonzaga, 2021). In order to stop *Eimeria* from developing a resistance to medications that have been commercially produced, numerous studies on plant extracts were conducted.

By assessing the *E. ninakohlyakimovae* sporozoites' survival and their capacity to infect bovine colonic epithelial cells after incubation, *R. pinnata* fruit extract exhibits significant anticoccidial action against oocysts and sporozoites of *Eimeria* (Lopez, 2018).

The apparent lack of action of the commercial anticoccidials studied is likely due to bioconversion of antiparasitic medications in the host (Lanusse et al., 1995). Toltrazuril and two of its primary metabolites were tested at various concentrations for their anticoccidial action *in vitro* in order to explore this possibility. Toltrazuril is treated by substantial metabolism in the host, resulting in toltrazuril sulphoxide and eventually toltrazuril sulphone (ponazuril) (Lim et al., 2010), which exhibits anticoccidial action against goat *Eimeria* infections (Gibbons et al., 2016).

In Korea, Hur et al., 2005 reveal that the potential direct anticoccidial impact of feeding condensed tannin-containing plants on the formation of *Eimeria* oocysts, twelve native Korean goats that had been accidentally exposed to mixed species of

Eimeria and showed that feeding fresh pine needles and oak leaves to goats along with lucerne chaff (40 g condensed tannins dry matter showed immediate anticoccidial effects as seen by a dramatic drop in oocyst generation. Oocysts per gram of feces (OPG) from goats fed pine needles and lucerne chaff were measured two days after eating.

This phytomedicine can be extract for anticoccidal effects in other paraites. In Brazil, the plant extract was performed experiment for hatching of *Hemonchus. contortus* eggs was prevented by the aqueous extract of dried and fresh plant material as well as the alkaloid-rich portion of the dried leaves of *S. brasiliensis*. Alkaloid fraction's key plant substance was pyrrolizidine alkaloid integerrimine (PA). However, the dried plant's aqueous extract showed more effectiveness compared to their non-polar or alkaloid-enriched fractions, indicating that, whereas PAs contributed to the ovicidal effect. Other plant components may possibly have a role in the plant's ovicidal impact. Aside from that, the aqueous extract inhibiting larvae movement with an aqueous extract from dried plant was more effective than with an aqueous extract from fresh plant (Soares, et al, 2018).

Similarly, Gonzaga et al., 2019 performed their research in Philippines to revealed the anticoccidial activity of the ethanol extract *Coleus aromaticus* of leaves. The efficacies of three concentrations i.e. 600, 800, and 1000 mg extract/ kg bodyweight of *C. aromaticus* extract were tested for anticoccidial activity and evaluated by comparing with a commercial coccidiostat, toltrazuril. The efficacy rate of administering 600 mg/kg extract (92.29%) was comparable to that of toltrazuril (96.65%) at 21 days post-treatment. They showed that three extract concentrations were significant but was lower than toltrazuril. Hence, from this research the extract has the potential in reducing the oocyst per gram (OPG) counts of naturally-infected goats. In the context of Nepal, the literature about the activity of plant extract in coccidal effect of apicomplexan parasites have not found yet.

CHAPTER-3

MATERIALS AND METHODS

3.1 Materials Required

1. Sample collecting vials
2. Gloves
3. Mask
4. Spatula
5. Mortar and Pestle
6. Tea strainer
7. Beakers
8. Measuring cylinder
9. Centrifuge tubes
10. Centrifuge machine
11. Test-tube stand
12. Droppers
13. Glass slides
14. Toothpicks
15. Coverslips
16. Cotton bud
17. Petri dish
18. Refrigerator
19. Light Microscope (Optika Microscopes Italy, B-383PLi)
20. Incubator
21. Rotator (IKA RV 10)
22. Laminar flow
23. Burner
24. Micropipettes
25. McMaster chamber
26. Grinder
27. Conical flask (500 and 1000ml)

3.2 Chemicals required

1. 2.5% potassium dichromate ($K_2Cr_2O_7$) solution
2. Distilled water
3. 0.9% NaCl/ Saline solution
4. Stain (Lugol`s Iodine)
5. 45% Concentrated sodium chloride (conc. NaCl)
6. Dimethyl Sulphoxide (DMSO)
7. Solvents (Hexane, Chloroform, Ethyl acetate and Methanol)

3.3 Methods

This study determined the role of plant (*Avena sativa*) extract in sporulation of *Eimeria* species *in vitro*. The plant and faecal samples was collected purposively from farm and goats respectively.

3.3.1 Plant collection and storage

The plant *Avena sativa* was collected approximately 10kg from the farm located at Duwakot-2, Changunaryan Municipality. The collected plant was brought at Animal Research Laboratory of Nepal Acadamy of Science and Technology (NAST) for preparation of oat plant extract. It was dried and chopped and stored in room temperature.

3.3.2 Faecal sample collection from goat and preservation

Approximately 310 faecal samples was collected in the container with 2.5% potassium dichromate in the month of Fenruary 2022 and brought at Animal Research Laboratory of NAST for Laboratory diagnosis. The faecal samples were preserved in the refrigerator at 4^{o}C.

3.3.3 Laboratory examination

The laboratory procedures were divided into five phases:

3.3.3.1 Preparation of plant extract

The plant *Avena sativa* (approximately 10kg) was dried and chopped. From it only 200 gram dried plant was grinded and dissolved in solvents. The plant was dissolved in a solvent (Hexane, Chloroform, Ethyl Acetate and Methanol respectively) for 7 to 10 days. Then, it was filtered and the solvent was evaporated with the help of rotator. Again, the residue was dissolved with the help of solvent for 2 to 3 days. After 3 days, it was filtered and evaporated the solvent with the help of rotator (Soares, 2019).

3.3.3.2 Identification of parasite (i.e. *Eimeria*)

About 5 gm of the sample was taken in the mortar, and 10 ml saline (0.9% NaCl) was added on it. The mixture was filtered with the help of tea strainer. The filtrate was examined as per literature (Ghimire et al., 2021).

i. Direct wet mount technique

To detect the trophozoites, cysts, oocysts, eggs, and larval stages of the endoparasites, the filtrate of stool sample was directly observed at 2.5% potassium dichromate, 0.9% saline solution, and Lugol's Iodine (Ghimire and Bhattarai, 2019).

ii. Saturated salt floatation technique

About one ml of filtrate and 13 ml of NaCl was kept in the 15 ml centrifuge tubes. The mixture was centrifuged for 5 min, and the centrifuge tubes with mixtures were kept in the test tube stands. The concentrated solution of NaCl was added entirely to the brim forming convex surface at the top at the tube. The tube was covered by the coverslip so that the solution touched the coverslip. After 15–20 min, coverslips were removed and kept on glass slides. The slides were examined under the microscope (Ghimire and Bhattarai, 2019).

iii. Sedimentation technique

One ml of filtrate and 13 ml of 0.9% NaCl were mixed in a 15 ml centrifuge tube. The mixture was centrifuged for 5 minutes. The supernatant liquid was discarded, and the settled solution was used for the experiment. Two drops of the solution was kept on a glass slide containing Lugol's iodine, and parasitic stages were examined on the microscope (Ghimire and Bhattarai, 2019).

3.4 Preparation of solution of plant extract

The plant extracts were made in different concentration (i.e. (0 µg/ml, 0* µg/ml with 10% DMSO, 0.5 µg/ml, 1 µg/ml, 5 µg/ml, 10 µg/ml, 15 µg/ml, 20 µg/ml and 25 µg/ml) by adding media (2.5% Potassium dichromate solution) in it. To determine the volume of extract following formula was used:

$$V_1C_1=V_2C_2$$

Where,

V_1= Volume of oat plant extract

V_2= Volume of media

C_1= Concentration of oat plant extract

C_2= Concentration of media

3.5 Assessment of *Eimeria* burden in fresh sample

The parasitic infection burden was determined by studying the intensity of the parasites. It was performed after the identification of oocyst of parasites by the microscope and examined under 10X and 40X.

Table 1: Burden of *Eimeria oocyst* in field (X 400)

Intensity/ Burden of oocyst of *Eimeria*	**No. of oocyst /field (X 400)**
Light infection (+)	1-3
Mild infection (++)	4-10
Heavy infection (+++)	>10

(Source: Soulsby, 2012)

Parasite severity or burden was measured by quantifying the number of eggs of nematodes and oocysts of *Eimeria* released per gram of faeces (OPG) by applying the McMaster technique (Adhikari & Ghimire, 2021; Soulsby, 2012). Two Cell McMaster Counting Slide (Hawksley and Sons Ltd.) following the manufacturer's recommendations was used. Briefly, three grams *Eimeria* positive stool samples were weighed and filtered through a tea strainer into a 50 ml beaker using 43 ml of floatation fluid made up of 45% NaCl. Then, 0.15 ml of the filtrate was placed into each depth of the McMaster slide using a pipette, and the slide was examined using a 100× total magnification under a compound microscope. All oocysts or eggs in both chambers were counted, and their sum was multiplied by 100. Finally, the resulting product will be divided by 2 to calculate the oocyst per gram (OPG) (Adhikari & Ghimire, 2021).

$$\text{OPG} = \frac{\text{Oocyst of chamber (Left + Right)}}{2} \text{X100}$$

3.6 Preservation of stool sample

The stool sample was preserved in 2.5% of potassium dichromate solution and was kept in refrigerator.

3.7 Culture experiment

i. Dose and kinetic responses of plant extract on sporulation

The sterile culture plates were taken and labeled. 1 gm stool was weighted. The stool, 2ml of 2.5% potassium dichromate and 1 ml of plant extract dose on different concentration (0 µg/ml, 0* µg/ml with 10% DMSO, 0.5 µg/ml, 1 µg/ml, 5 µg/ml, 10 µg/ml, 15 µg/ml, 20 µg/ml and 25 µg/ml) were added. The faecal sample with 1 ml

potassium dichromate was used as positive sample. The culture plates were incubated at 28 °C for 0 hour, 24 hours and 48 hours respectively.

The protocols of this experiment were:

No treatment: 0 µg/ml → 2.5% $K_2Cr_2O_7$ → incubated at 28 °C (0, 24, 48 hours) → OPG count

Control: 0* µg/ml →2.5% $K_2Cr_2O_7$ + 10% DMSO → incubated at 28 °C (0, 24, 48 hours) → OPG count

0.5 µg/ml →2.5% $K_2Cr_2O_7$ + 10% DMSO+ 0.5 µg/ml (plant extract) → incubated at 28 °C (0, 24, 48 hours) → OPG count

1 µg/ml →2.5% $K_2Cr_2O_7$+ 10% DMSO+ 1µg/ml (plant extract) → incubated at 28 °C (0, 24, 48 hours) → OPG count

5 µg/ml →2.5% $K_2Cr_2O_7$+ 10% DMSO+ 5 µg/ml (plant extract) → incubated at 28 °C (0, 24, 48 hours) → OPG count

10 µg/ml →2.5% $K_2Cr_2O_7$+ 10% DMSO+ 10 µg/ml (plant extract) → incubated at 28 °C (0, 24, 48 hours) → OPG count

15 µg/ml →2.5% $K_2Cr_2O_7$+ 10% DMSO+ 15 µg/ml (plant extract) → incubated at 28 °C (0, 24, 48 hours) → OPG count

20 µg/ml →2.5% $K_2Cr_2O_7$+ 10% DMSO+ 20 µg/ml (plant extract) → incubated at 28 °C (0, 24, 48 hours) → OPG count

25 µg/ml →2.5% $K_2Cr_2O_7$ + 10% DMSO+ 25 µg/ml (plant extract) → incubated at 28 °C (0, 24, 48 hours) → OPG count

3.8 Statistical Analysis

Data were analyzed by using Microsoft Excel 2010. Chi-square tests were used to calculate p-values. These values were used to analyse the statistical significance of various variables like concentration or dose of plant extract, kinetics of sporulation, time, rate of mortality and others. The p-value less than 0.05 ($p<0.05$) were considered as statistical significance. Data were present in the tables and graphs.

CHAPTER-4

RESULTS

4.1 Prevalence of *Eimeria* species in faecal sample of goat

A total of 310 faecal samples of goat were examined microscopically for the identification of *Eimeria spp.* by performing direct smear method, sedimentation and floatation method. This study revealed that the rate of prevalence of *Eimeria* infection was 59% in goat (Figure 2).

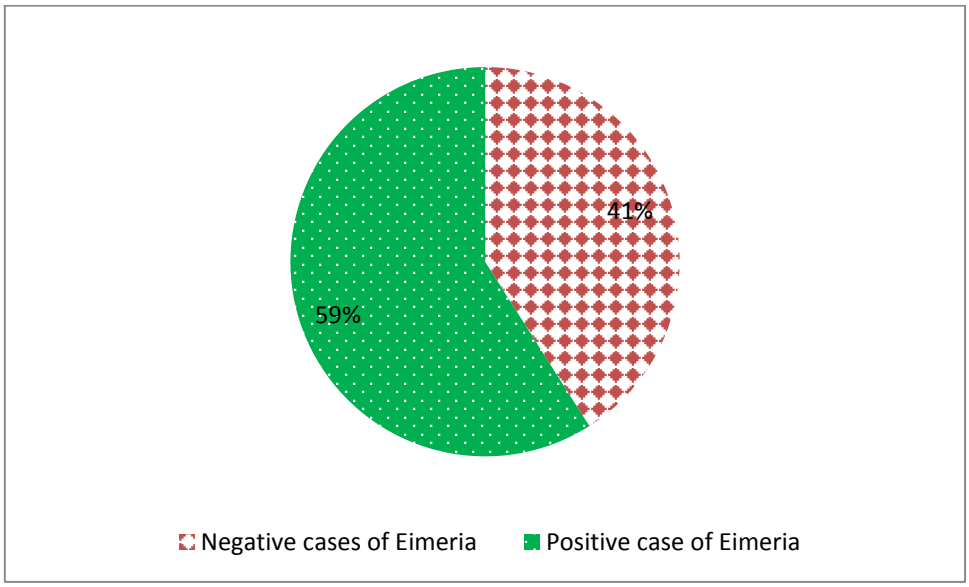

Figure 2: Prevalence of *Eimeria* species infection in goat.

4.2 Intensity of *Eimeria* species in goat

Out of 310 samples, 183 sample found to be *Eimeria* infection. Out of 183 samples, 138 samples revealed light infection (+), 20 samples revealed the mild infection (++) and 25 samples revealed heavy infection (+++).

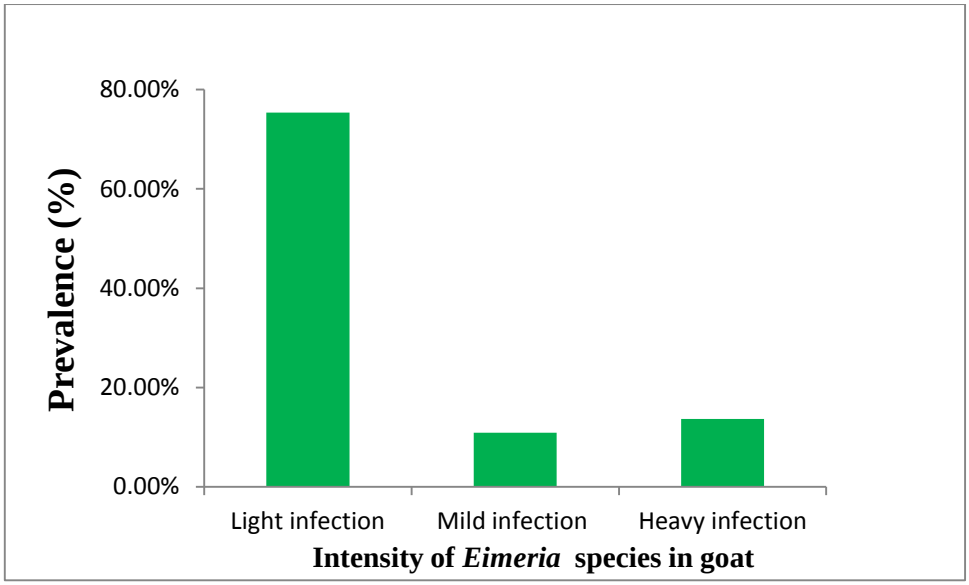

Figure 3: Intensity of *Eimeria* species in goat

Table 2: Burden of *Eimeria* species by OPG count in the faecal sample of goat (N=25)

	Mean	Median	Standard Deviation	Minimum	Maximum
OPG (N=25)	4087.5	3875	405.75	1600	7300

The intensity of *Eimeria* species was determined in heavy infected sample (i.e. 25) by using oocyst per gram (OPG). Its value ranged from 1600 to 7300 in heavy infected sample. This study revealed that the average OPG per gram was found to be 4087.5. The standard deviation was found to be 405.75 and median was found to be 3875.

4.3 Kinetics of sporulation of *Eimeria* spp. after 0, 24 and 48 hours of incubation

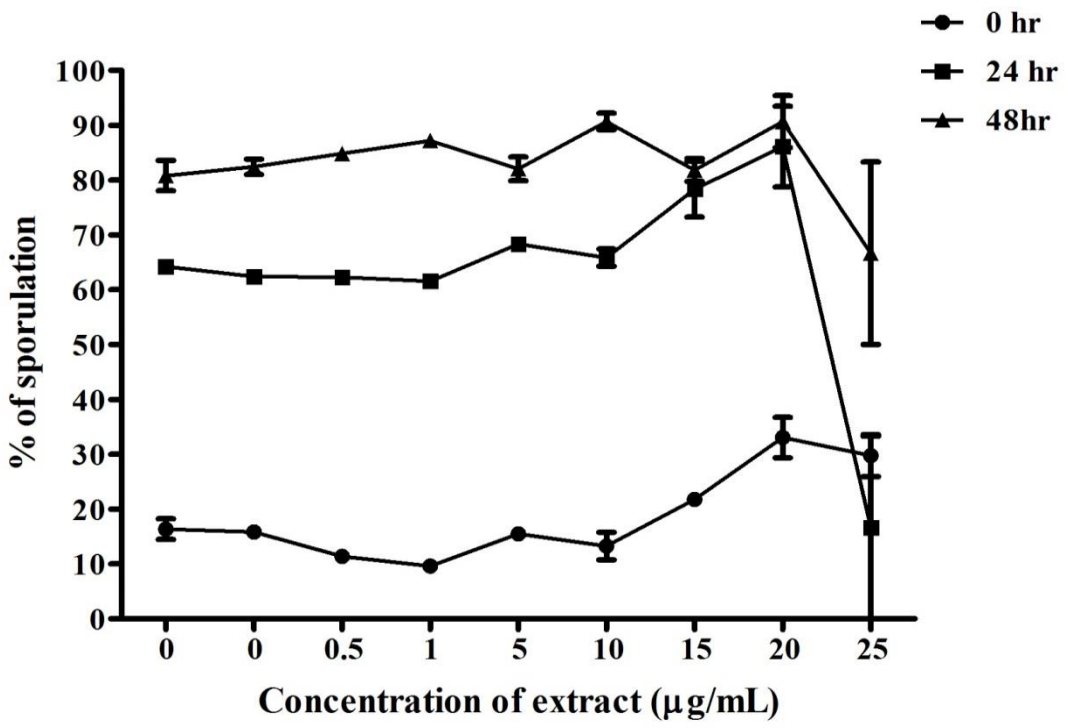

Figure 4: The kinetics of sporulation of *Eimeria* after 0, 24 and 48 hours of incubation

Note: Each point represents % of sporulation after 3 replicates (Mean ± SEM) at different concentration of extract (µg/ml) with respect to time.

This experiment demonstrated that the kinetics of sporulation after 0 hour of incubation were decreased from 0.1 µg/ml and then slightly increased from 1 concentration of plant extract and from 5 µg/ml concentration of plant extract, the kinetics of sporulation gradually increased. It also showed that the rate of sporulation is increased nearly 2 times in increasing dose of plant extract from 0 µg/ml to 25 µg/ml (Figure 4).

After incubation for 24 hours, the rate of sporulation became nearly constant when the extract was used at the dose of 0 to 0.1 µg/ml. However, it was increased up to more than 80% on increasing the concentration from 5 to 20 µg/ml. At 25 µg/ml, the percentage of sporulation decreased sharply. For example, from the dose of 20 to 25 µg/ml, the reduction was about 4 times on sporulation. Here, the rate of sporulation was maximum at the concentration of plant extract at 20 µg/ml.

Following incubation for 48 hours, the rate of sporulation of *Eimeria* was slightly on increasing trend upto 1 µg/ml and then fluctuation upto 25 µg/ml. The rate of

sporulation from 5 µg/ml to 20 µg/ml become fluctuated but at 25 µg/ml, it reduced to nearly, 23%.Here, the rate of mortality of *Eimeria* affected the rate of sporulation. In 48 hours incubation, the rate of sporulation was maximum at the concentration of plant extract at 10 to 20 µg/ml (Figure 4).

This experiment demonstrated that the time period plays greater role in the kinetics of sporulation of *Eimeria* spp. Here, the kinetics of sporulation ranges from 8.8 to 30% in 0 hour incubation. This means the rate of sporulation was increased 3.4 times with increasing concentration 0 to 25 µg/ml. This showed that the rate of sporulation increased 3.4 times from 0 µg/ml to 25 µg/ml concentration of plant extract. Likewise, in 24 hours, the rates of sporulation were slightly increasing when increasing drug concentration (concentration of Plant extract) from 0 to 20 µg/ml whereas it was decreased in 25 µg/ml. Similarly, in 48 hours, the rate of sporulation of *Eimeria* was on increasing trend upto 5 µg/ml and then fluctuation upto 15 µg/ml and then in increasing trend. This revealed that, there was significant difference ($p<0.05$) between rate of sporulation and dosage concentration of plant extract (Figure 4).

CHAPTER-5

DISCUSSIONS

Coccidiosis is an omnipresent parasitic intestinal disease that results in the productive performance of different hosts such as broiler chicken, sheep, goat, cattle and other ruminants. In ruminants, as cattle and buffaloes the rate of prevalence of coccidian parasite was recorded as 29.39% and 35.46%, respectively and the overall rate of prevalence of coccidian parasite was recorded as 32.17% at India (Gupta et al., 2016). According to Etsay et al., (2020), the overall rate of prevalence for the coccidian parasite was 86.19% of which, 87.31%, in sheep and 85.03% in goats. Similarly, Terfa et al., (2023) studied the rate of gastrointestional parasite burden in cattle and found that overall coccidian protozoan parasite was found to be 36%. Likewise, Mohamaden et al., (2018) revealed that the rate of prevalence of coccidian parasites were found to be 60% in goats and 57.70% in sheep from subclinical coccidiosis.

Eimeria is a coccidian parasite that affects the small intestine of ruminants as goats. In this study, out of 310 faecal samples of goats were taken out of which 189 sample i.e. only 59% of sample was found to be positive in *Eimeria* spp. This means the rate of prevalence was found to be 59%. According to Singh et al., 2020 revealed the prevalence of *Eimeria* species was (90.96%).Among them, the rate of prevalence in kid goats were 100% and adult goats were 84.61% at Mathura, Uttar Pradesh, India. Likewise, the combined prevalence rate was found to be 78.7% (Diao et al., 2022). Similarly, in Egypt, Mohamaden (2018) found 60% rate of prevalence of *Eimeria* spp. in goat. Etsay et al., 2020 revealed that the prevalence rate of *Eimeria* spp. was 85.03% in Ethopia. These rate of prevalence were higher in compared to this study which may due to the proper management and proper medication of livestock and Hanseen (2020) found that rate of prevalence is 83.6% and , only nine species *(E. arloingi, E. alijevi, E. ninakohlyakimovae, E. hirci, E. christenseni, E. aspheronica, E. jolchijevi, E. caprina and E. caprovina)* were identified in goats. In Iran, the overall prevalence was 23.23% in which the young male sheep had the highest prevalence (37.61%). They found the highest percentage belonged to the *E. intricata* (39%), followed by *E. faurei* (16%), *E. ovina* (16%), *E. parva* (12%), *E. pallida* (7%), *E. ahsata* (6%) and *E. ovinoidalis* (4%) (Yakhchali and Rezaei, 2010). In USA, to

Young et al., 2011 showed the common *Eimeria* species found are *Eimeria christenseni, E. kochali, E. caprina, E. ninakohlyakimorae , E. jolchiievi and E. caprovina* were found.

Among them, rate of prevalence of *Eimeria christenseni* (52%) is higher than other species but in Nepal, E. ninakohlyakimovae (83.0%). In Nepal, Ghimire et al., 2019 found that the rate of prevalence of *Eimeria* spp. in goat was 80.75%. Likewise, in 2022 he found that the rate of prevalence of *Eimeria* spp. was 92.4% and identified 15 different morphologic forms of *Eimeria*. But in this current study, the morphological forms of *Eimeria* were not studied which is the limitation. Although, different morphologic forms had been detected, they were not studied at species level. In summary, the current study is significant in the context of knowledge regarding prevalence of *Eimeria* spp. in the goats. It also indicates the immediate action of therapeutic preventive strategies in current goat population at the local level.

This research was also carried out the intensity and burden of parasites based on the numbers of oocysts under light microscope. The intensity was measured by observing oocysts found that 13.67% of the *Eimeria* positive faecal sample were heavily infected. From these faecal samples, McMaster technique generated 1600 to 7300 OPG. It indicates that goats of the current population were heavily infected with *Eimeria* species although symptoms and pathologies were not detailed in this study. Similarly, the study carried out by Yakhchali and Rezaei (2010) found that the highest intensity of *Eimeria* species was revealed to be 63.58% in faecal sample of sheep in Iran. The heavy infected goat may suffer diarrhea and hindered growth that lowered the productivity. The pathogenicity of different *Eimeria* species is depends upon the location of replication of the parasite in the host and may influence the clinical outcome of the disease. In general, the severity of coccidiosis is determined by the proliferation capacity of the pathogenic *Eimeria* species, which is defined as the number of merozoites produced by merogonia, and cells destroyed by each sporulated oocyst ingested. So, primoinfective dose (number of viable oocysts ingested) and the magnitude of reinfection may influence the development and course of the disease (Ruiz et al., 2013). The main significance of this intensity is to determine the impact of intensity and severity health condition on goat. This impact may cause the loss to farmer and provide the idea of control measures in future.

To control coccidiosis, use of drugs such as ionophores and decoquinate are predominantly used around the world (Gibbon et al., 2016). They performed research and demonstrated that amprolium and ponazuril were effective in decreasing faecal coccidia oocyst counts in this group of goats. Similarly, Young et al., (2011) determined the efficacy of amprolium for the treatment of pathogenic *Eimeria* species in Boer goat kids. However, there are no drugs approved for treatment of clinical cases of coccidiosis in this species. The side effects were seen in the continuous consumption of these allopathic medicines such as the parasite may develop resistance and may not work in future. To overcome the issue of drug resistivity and expensiveness drugs derived from natural products may have a huge alternative. This is particularly due to environmental friendly, cheaper and easily available plant and plant products. There are many reports of using the extract of various plants such as garlic, oregano and other plant extract in various animals such as sheep, chicken and other ruminants. According to Sidropoulou et al., 2020, the combined use of essential oil of oregano and garlic may have anticoccidial effect *in vitro*. There may be presence of chemical compounds that may play role in anticoccidial effect. Likewise, this phytomedicine can be extracted for anticoccidal effects in other paraites. In Brazil, the plant extract was performed experiment for hatching of *Hemonchus. contortus* eggs was prevented by the aqueous extract of dried and fresh plant material as well as the alkaloid-rich portion of the dried leaves of *S. brasiliensis* (Soares, et al., 2018). The fruit extract of *R. pinnata* has an important anticoccidial activity against oocysts and sporozoites of *Eimeria* by testing the viability of the *E. ninakohlyakimovae* sporozoites and their ability to infect bovine colonic epithelial cells after incubation (Lopez, 2018). The benefit of the phytomedicine was that it did not have any side effect.

In this study, the extracts of *Avena sativa* have been used for the assessment of rates of sporulation at their different doses. Similarly, *Avena sativa* is a plant which is consumed by ruminants. This plant is rich in minerals, lipids, β-glucan, a mixed-linkage polysaccharide, phytoconstituents like avenanthramides, an indole alkaloid-gramine, flavonoids, flavonolignans, triterpenoid saponins, sterols, and tocols (Singh, 2013). The presence of antioxidant may also enhance the rate of sporulation in increasing dosage amount (Bawn and Htun, 2021). So, the extract of *Avena sativa* was used in various concentration at 0, 24, 48 hours. This showed the effect in sporulation

rate and also enhanced the anticoccidial effect. The composition of plant compound may determine the anticoccidial effect. This study revealed that the rate of sporulation depends upon the temperature and time of incubation but also there are other environmental factors that affect rate of sporulation directly or indirectly. Sporulation may be due to the minerals, phytoconstituents and antioxidant property.

This experiment indicated that in natural condition, the oocyst sporulate, however, us of extracts at 20 μg/ml enhanced the rate of sporulation. However, its dose at 25 μg/ml reduced the sporulation at 0, 24, 48 hours of incubation. This suggests that optimum dose of extract is required for the oocyst sporulation *in vitro*.

This experiment counted all the types of *Eimeria* spp. that might be the reason why the variable results of sporulation were obtained. Different doses and incubation periods have different incubation periods in different oocysts. The oocyst structures solely determine the penetrating or invading characters of drugs *in vitro* (Ghimire et al., 2019).That is why stimulation by extract might be different for sporulation rates of different oocysts and cumulatively, they have impact on sporulation.

Although sporulation effects *in vitro* may not be followed/ synchronized by *in vitro* or in situ experiments, the current study gives an idea how the extract affects sporulation. *Avena sativa* is a predominant popular for goats in Nepal and grows usually winter temperature becomes 3°C to 21°C (minimum to maximum). If *in vivo*, follows the *in vitro* results, the plant fed by the goats at the optimum dose might have an impact on sporulation in the faecal samples. It consequently reduces the patent period and if hosts are available, sporulated oocysts can be easily transmitted. If the oocysts do not get proper hosts, then, they may die due to disintegration or other mechanisms.

This study also determined that the use of plant extract also plays greater role in sporulation of oocyst. Not only that the environment also plays role in sporulation and dispersion of faecal matter of goat and other ruminants. The main motto of this experiment to determine the effectiveness of plant extract against *Eimeria*. This study may motivate the farmer to utilize this phytomedicine to reduce the side effect of medicine and to kill the parasites.

CHAPTER-6

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Hence, this study revealed that the rate of sporulation depends upon activity of plant extract (*Avena sativa*). The highest rate of sporulation was seen in 20 µg/ml concentration of *Avena sativa* after 0, 24 and 48 hours of incubation at 28 °C. This showed that the extract of *Avena sativa* have sporulating effect and can be studied further. According to this study, the time of incubation is directly related to the rate of sporulation of *Eimeria*.

6.2 Recommendations

1. Further molecular study of *Eimeria* spp. should be done to evaluate sporulating effects of plant extract in different species.
2. The medicine which are sold in market have side-effects. In order to control the side effect, different medicinal and plant extract must be studied in order to discover phytomedicine to minimize the side-effect of synthetic medicine.

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ANNEX 1

Appendix 1: Letter of approval from Changunaran Municipality Ward No. 2

फोन नं. : ०१-६६१९३६६

चाँगुनारायण नगरपालिका
Changunarayan Municipality
नगर कार्यपालिकाको कार्यालय
Office of the Municipal Executive
२ नं. वडा कार्यालय, दुवाकोट, भक्तपुर (2 No. Ward Office, Duwakot, Bhaktapur)
बागमती प्रदेश, नेपाल (Bagmati Province, Nepal)

पत्र संख्या : २०७९/०८०
चलानी नं. : ४४९

मिति : २०७९-०५-२०

विषयः अनुमती दिईएको सम्बन्धमा।

श्री निशा किरण श्रेष्ठ,
चाँगुनारायण नगरपालिका-२ दुवाकोट ।

प्रस्तुत विषयमा प्राणी शास्त्र केन्द्रिय विभागको चलानी नं.९४८ मिति २०७९/०५/१९को पत्र प्राप्त भई व्यहोरा अवगत भयो । सोहि पत्रको व्यहोरा अनुसार चौथो सेमेष्टर Parasitology extrace -Avena sativa concentration in the rate of sporulation of Eimeria species in Faecal sample of goat शिर्षकमा अध्ययन तथा अनुसन्धान गरिरहेकोले निजको शोध कार्यको लागी यस चाँगुनारायण नगरपालिका-२ दुवाकोटमा अनुमती दिईएको व्यहोरा जानकारी गरिन्छ ।

२९/०५/२०

सोम प्रसाद प्रधान
वडाअध्यक्ष

सोम प्रसाद प्रधान
वडा अध्यक्ष

ANNEX 2

Appendix 2: Key characteristics of included studies on *Eimeria* spp. found in goat in different countries

Year	Site/ country	*Eimeria* species	*Eimeria* Species in %	Total Sample (N)	Positive sample (n)	Reference
2011	U.S.A	*Eimeria christenseni* *E. kochali* *E. caprina* *E. ninakohlyakimorae* *E. jolchiievi* *E. caprovina*	52% 23% 9% 6% 5% 5%		40	Young et al.
2011	China	*Eimeria christenseni* *E. hirci* *E. arloingi* *E. caprina* *E. jolchiievi*	26.9% 20.7% 83.3% 51.7% 68.4%	584	568	Zhao et al.,

		E. alijevi	81.6%			
2018	Egypt	*E. ninakohlyakimovae* *E. hirci* *E. caprina* *E. christenseni* *E. jolchijevi* *E. apsheronica* *E. arloingi*	30.86% 24.69% 17.28% 16.05% 12.35% 16.04% 37.04%	135	25 20 14 13 10 13 30	Mohamadena et al.,
2018	India	*E. arloingl* *E. ninakohlyakimovae* *E. christenseni* *E. hirci* *E. alijevi* *E. arloingi*		60	58	Kaur et al.,
2020	Myanmar	*E. christenseni* *E. arloingi*	13.9% 25.4%	280	39 71	Bamn et al.

		E. hirci	20.7%		58	
2020	Ethopia	*E. arloingi* *E. ninakohlyakimovae* *E. christenseni* Mixed Overall	30% 13.33% 8.89% 47.78% 56.5%	384	27 12 8 43 56.5%	Etsay et al.,
2020	Egypt	*E. alijevi* *E. ninakohlyakimovae* *E. hirci* *E. arloingi* *E. jolchijevi* *E. christenseni* *E. aspheronica* *E. caprina* *E. caprovina*	34.4% 31.1% 31.1% 47.5% 21.3% 31.1% 24.6% 19.7% 16.4%	125	21 19 19 29 13 19 15 12 10	Hassanen et al.,
2022	Nepal	*E. ninakohlyakimovae*	83%		823	Ghimire et al.,

		E. alijevi *E. capralis* *E. masseyensis* *E. hirci* *E. tunisiensis* *E. charlestoni* *E. arloingi* *E. jolchejevi* *E. Caprina* *E. aspheronica* *E. jolchejevi* *E. christenseni* *E. hirci*	75.2% 75.2% 67.2% 47.3% 33% 32.4% 32.4% 19.3% 16.5% 13.5% 9.4% 9.4% 8.1%		745 666 626 469 327 321 321 191 164 134 93 93 80	
2023	Nepal	*Eimeria* species are not identified	59%	310		Present study

ANNEX 3

Appendix 3: Two-way ANOVA

Two-way ANOVA

Source of Variation	% of total variation	P value
Interaction	8.76	P<0.0001
Incubation Periods	78.55	P<0.0001
Concentration of Extracts	6.85	P<0.0001

Source of Variation	P value summary	Significant?
Interaction	***	Yes
Incubation Periods	***	Yes
Concentration of Extracts	***	Yes

Source of Variation	Df	Sum-of-squares	Mean square	F
Interaction	16	6561	410.1	5.064
Incubation Periods	2	58830	29410	363.2
Concentration of Extracts	8	5132	641.4	7.921
Residual	54	4373	80.98	

Number of missing values 0

Bonferroni posttests

0 hr vs 24 hr

Concentration of Extracts	0 hr	24 hr	Difference	95% CI of diff.
0	16.33	64.25	47.92	23.86 to 71.98
0	15.84	62.39	46.55	22.49 to 70.61
0.5	11.41	62.29	50.87	26.81 to 74.93
1	9.603	61.54	51.93	27.87 to 75.99
5	15.49	68.33	52.84	28.78 to 76.90
10	13.28	65.87	52.59	28.53 to 76.65
15	21.77	78.33	56.57	32.51 to 80.63
20	33.06	86.11	53.05	28.99 to 77.11
25	29.76	16.67	-13.09	-37.15 to 10.97

Concentration of Extracts	Difference	t	P value	Summary
0	47.92	6.522	P<0.001	***
0	46.55	6.335	P<0.001	***
0.5	50.87	6.924	P<0.001	***
1	51.93	7.068	P<0.001	***
5	52.84	7.191	P<0.001	***
10	52.59	7.157	P<0.001	***
15	56.57	7.699	P<0.001	***
20	53.05	7.220	P<0.001	***
25	-13.09	1.782	P > 0.05	ns

0 hr vs 48hr

Concentration of Extracts	0 hr	48hr	Difference	95% CI of diff.
0	16.33	80.80	64.47	40.41 to 88.53
0	15.84	82.43	66.59	42.53 to 90.65
0.5	11.41	84.84	73.43	49.37 to 97.49
1	9.603	87.21	77.61	53.55 to 101.7
5	15.49	82.04	66.54	42.48 to 90.60
10	13.28	90.69	77.42	53.36 to 101.5
15	21.77	81.84	60.08	36.02 to 84.14
20	33.06	90.67	57.61	33.55 to 81.67
25	29.76	66.67	36.91	12.85 to 60.97

Concentration of Extracts	Difference	t	P value	Summary
0	64.47	8.775	P<0.001	***
0	66.59	9.062	P<0.001	***
0.5	73.43	9.993	P<0.001	***
1	77.61	10.56	P<0.001	***
5	66.54	9.056	P<0.001	***
10	77.42	10.54	P<0.001	***
15	60.08	8.176	P<0.001	***

20	57.61	7.841	P<0.001	***
25	36.91	5.023	P<0.001	***

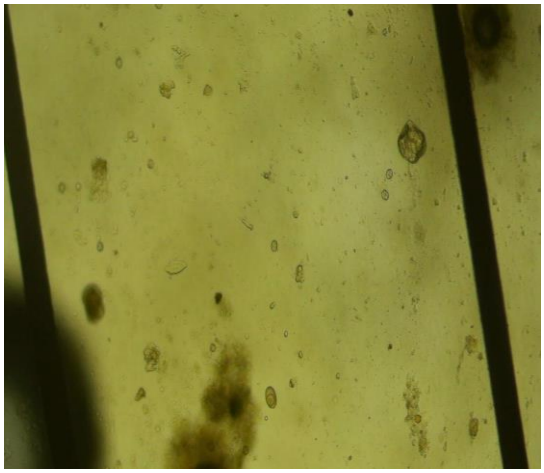
24 hr vs 48hr

Concentration of Extracts	24 hr	48hr	Difference	95% CI of diff.
0	64.25	80.80	16.55	-7.507 to 40.61
0	62.39	82.43	20.04	-4.020 to 44.10
0.5	62.29	84.84	22.55	-1.505 to 46.61
1	61.54	87.21	25.68	1.616 to 49.73
5	68.33	82.04	13.70	-10.36 to 37.76
10	65.87	90.69	24.83	0.7679 to 48.89
15	78.33	81.84	3.510	-20.55 to 27.57
20	86.11	90.67	4.562	-19.50 to 28.62
25	16.67	66.67	50.00	25.94 to 74.06

Concentration of Extracts	Difference	t	P value	Summary
0	16.55	2.253	P > 0.05	ns
0	20.04	2.727	P > 0.05	ns
0.5	22.55	3.069	P < 0.05	*
1	25.68	3.494	P<0.01	**
5	13.70	1.865	P > 0.05	ns
10	24.83	3.379	P < 0.05	*
15	3.510	0.4777	P > 0.05	ns
20	4.562	0.6208	P > 0.05	ns
25	50.00	6.805	P<0.001	***

ANNEX 4

Appendix 4: Photographs



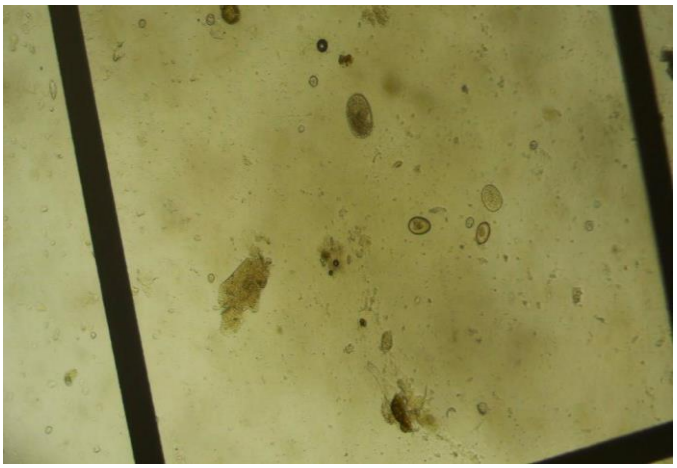


Photo 1 and 2: Egg of sporulated and unsporulated *Eimeria* spp. in chamber by McMaster chamber in 10X magnification

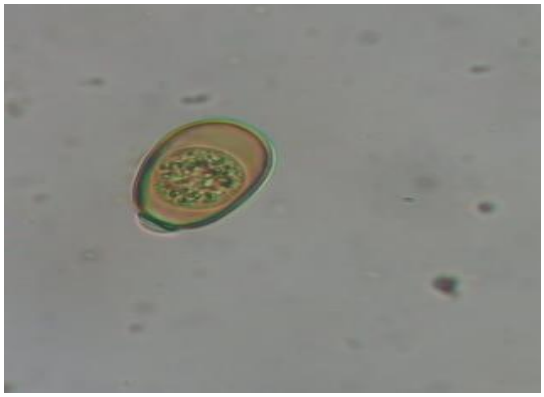


Photo 3: Egg of *Eimeria* spp. during floatation (in 40X magnification)

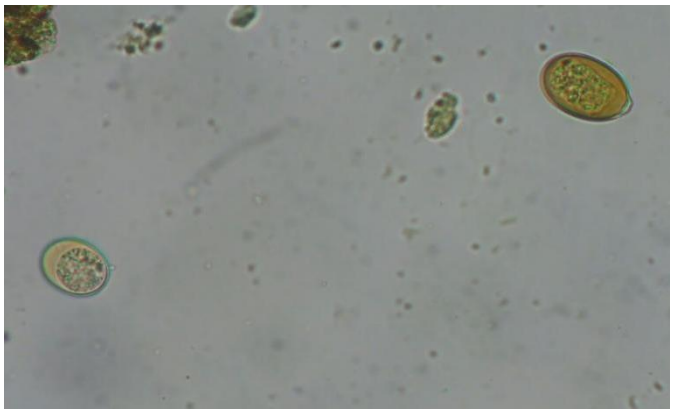


Photo 4: Egg of different morphological forms of *Eimeria*

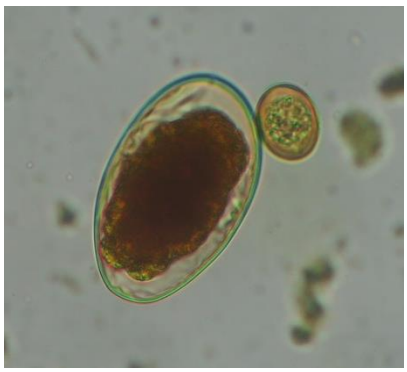


Photo 5: Egg of *Eimeria* spp. and *Strongyle* spp. (in 40X magnification)

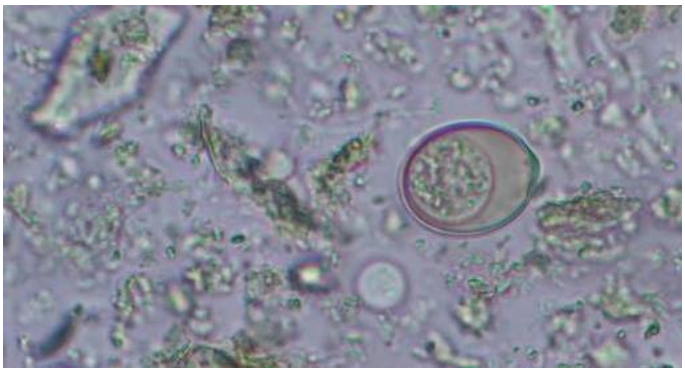


Photo 6: Egg of unsporulated *Eimeria* spp (0.025mm* 0.02mm*400)

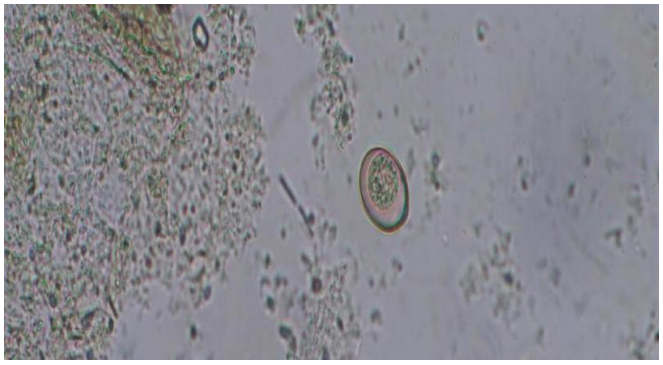


Photo 7: Egg of unsporulated *Eimeria* spp (0.023mm* 0.018mm*400)

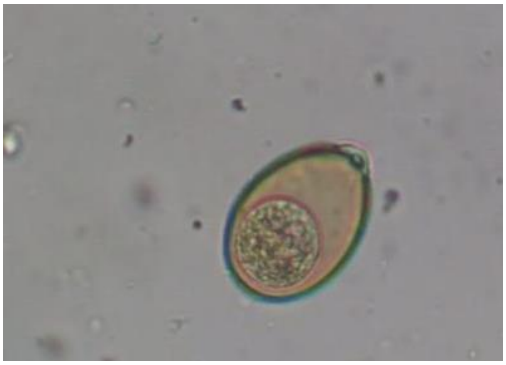


Photo 8: Egg of unsporulated *Eimeria* spp (0.037mm* 0.024mm*400)

Photo 9 and 10: Collection of oat plant (*Avena sativa)* and faecal sample of goat respectively

Photo 11: Fitting apparatus (including sample) with rotator

Photo 12: Centrifuge during sedimentation

Photo 13: Preparing slide of faecal sample

Photo 14: Centrifuge during sedimentation and floatation technique