

**STUDY ON PREVALENCE OF HELMINTH
INFESTATION AND ANTHELMINTIC RESISTANCE IN
CALVES**

Thesis

Submitted to the

BIHAR ANIMAL SCIENCES UNIVERSITY, PATNA



In partial fulfillment of the requirements for the degree of

MASTER OF VETERINARY SCIENCE

In

VETERINARY MEDICINE

By

Dr. Pinky Rani

(VM0019/2019-20)

BIHAR VETERINARY COLLEGE

BIHAR ANIMAL SCIENCES UNIVERSITY PATNA-800014

2022

DEPARTMENT OF VETERINARY MEDICINE
BIHAR VETERINARY COLLEGE, PATNA - 14
(Bihar Animal Sciences University, Patna, Bihar)

CERTIFICATE – I

This is to certify that the thesis entitled “**Study on prevalence of helminth infestation and anthelmintic resistance in calves**” submitted in partial fulfilment of requirement for the award of the degree of **Master of Veterinary Science in the discipline of Veterinary Medicine** of faculty of Post-Graduate Studies, Bihar Animal Sciences University, Patna, Bihar is the bona fide research carried out by **Dr Pinky Rani, Registration no. – VM0019/2019 – 20**, ~~son~~daughter of Shri **Nemu Lal Ram** under my supervision and that no part of this thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of this investigation have been fully acknowledged.

Endorsed

Place: Patna

Date:

Dr. Bipin Kumar
(Major Advisor)
Assistant Professor cum Junior Scientist
Department of Veterinary Medicine,
Bihar Veterinary College, Patna-14

DEPARTMENT OF VETERINARY MEDICINE
BIHAR VETERINARY COLLEGE, PATNA - 14
(Bihar Animal Sciences University, Patna, Bihar)

CERTIFICATE – II

This is to certify that the thesis entitled “**Study on prevalence of helminth infestation and anthelmintic resistance in calves**” submitted by **Dr. Pinky rani**, Registration No.–**VM0019/2019-20**, søn/daughter of Shri **Nemu lal Ram** to Bihar Animal Sciences University, Patna, Bihar in partial fulfillment of the requirement for the degree of **Master of Veterinary Science in the discipline of Veterinary Medicine** has been approved by the advisory Committee after an oral examination of the student in collaboration with an External examiner.

Dr. Bipin kumar
Major Advisor

(External Examiner)

Members of the Advisory committee

- 1. Dr. Arvind Kumar Das**
Asst. Professor
Deptt. Of Veterinary Medicine
- 2. Dr. Rajesh Kumar**
Asst. Professor
Deptt. Of Veterinary Surgery and Radiology
- 3. Dr. Nirbhay Kumar**
Asst. Professor
Deptt. Of V.P.T
- 4. Dr. Pankaj Kumar (Dean Nominee)**
Asst. Professor
Deptt. Of V. M. C.

Dr. Arvind Kumar Das,
HOD, Deptt. Of Veterinary Medicine, BVC, Patna

DRI-cum- DEAN, Post Graduate Studies,
Bihar Animal Sciences University, Patna-14

ACKNOWLEDGEMENT

*First and foremost I want to express my heartiest gratitude to Almighty God who has been continuous source of inspiration and strength that help me way in accomplishment of this endeavor. Words alone are insufficient to convey my sincere feelings of indebtedness and fathomless gratitude towards my major advisor, **Dr. Bipin Kumar**, Major Advisor, Assistant Professor, Department of Veterinary Medicine, Bihar Veterinary College, Patna for his learned guidance repleted with stimulating counsel, close supervision, constant encouragement, and healthy criticism during the course of investigation. His painstaking supervision of the manuscript warrants special mention, without which this research undertaking would not have completed.*

*Heartiest gratefulness to express my profound sense of gratitude and sincere regards to members of my advisory committee **Dr. Arvind Kumar das**, Assistant Professor and Head, Department of Veterinary medicine, **Dr. Rajesh Kumar** Assistant Professor, Department of Veterinary Surgery and Radiology, **Dr. Nirbhay Kumar**, Assistant Professor, Department of Veterinary pharmacology, Bihar Veterinary College, Patna for their valuable suggestions and sincere guidance which made this work possible in stipulated time.*

*I am highly obliged to **Dr. Pankaj Kumar** Dean PGS nominee, Assistant Professor, Department of Veterinary microbiology, Bihar Veterinary College, Patna for valuable suggestions and innumerable cooperation during the entire research work,*

*I am highly obliged to **Dr. Ajit Kumar**, Assistant Professor Department of Veterinary Parasitology, Bihar Veterinary College, Patna for providing necessary facilities, valuable suggestions and sincere guidance.*

My sincere thanks are also to all Assistant Professors-cum Jr. Scientists of Bihar Veterinary College, Patna for their co-operative behaviour, valuable suggestions and moral support during the research work,

*My special thanks are due to **Dr. Anil Kumar**, Assistant Professor – cum junior scientist Department of Veterinary Clinical Complex, **Dr. Sonam Bhatt.**, Assistant Professor – cum junior*

scientist Department of Veterinary Medicine, **Dr. R.K. Sinha**, Assistant Professor – cum junior scientist Department of Veterinary Medicine, **Dr. Vivek Kumar Singh**, Assistant Professor – cum junior scientist Department of Veterinary Clinical Complex,

I am particularly obliged and thankful to **Dr. Dr. J. K. Prasad**, Dean, Bihar Veterinary College, Patna-14 for providing necessary facilities during the research period.

A deep sense of gratitude is expressed to Bihar Animal Sciences University (BASU), Patna, Bihar, for providing facilities to conduct this investigation.

A deep sense of gratitude is expressed to **Honorable Vice Chancellor, Registrar, DRJ cum Dean PGS, Director Research, D.S.W, Hostel Warden, Hostel Superintendent and All University Officers** of Bihar Animal Sciences University, Patna, Bihar, for providing facilities to conduct this investigation.

My thanks are also extended to all the respected seniors **Dr. Rajeev Kumar, Dr. Sudhir Kumar, Dr. Menka Kumari**, many colleagues like **Dr. Rupesh Kumar, Dr. Sweta Kumari, Dr. Abhishek Deep, Dr. Bhartendu Vimal**, most loving junior, **Dr. Mukesh Kumar, Dr. Prabhakar Pandey** and **all other friends** who helped me directly or indirectly during my research work with a company of whom helped me to overcome the stressful moment of investigation and physically help from time to time during the course of study.

I am also thankful to the Librarian and the staff-members of the library of the Bihar Veterinary College, Patna-14 for rendering their cooperation.

Thanks are also to the non-teaching staff members **Mr. Manichand, Mr. Rajesh Singh, Mr. Ratnesh Kumar, Mrs. Khushi Kumari, Mr. Manish Kumar, Mr. Rajeev Ranjan, Mr. Vicky, Mr. Jagat Ram, Mr. Neeraj Kumar and Sikha** department of Veterinary Medicine and other department for their kind help during the research work.

I am flooded with deep emotions and blow of my head, expressing profound sense of gratitude to all members of my family especially my lovely mother **Mrs. Urmila Devi** and my father **Mr. Nemu Lal Ram**, elder brothers **Mr. Manish Kumar** and **Dr. Amrit Kumar**. And elder sister **Mrs. Rekha Kumari** for their affectionate care, moral support, constant encouragement blessings, love, gracious sacrifice and inspiration to pursue higher education. Lastly, thank you to my husband, **Mr. Nalin Pratap Rana**, who encouraged me through countless hours of working on research, I could not have done this project without you all.

I thank God for giving me patience and strength to overcome the difficulties which crossed my way in accomplishment of this endeavor.

Last but not the least I would thank every person connected to my life here in Patna or anywhere else who couldn't find a separate mention in this acknowledgement.

All may not have been mentioned but none has been forgotten.

Place: - Bihar Veterinary College, Patna

Date: -

(Dr. Pinky Rani)

TABLE OF CONTENTS

Chapter	Title	Page
1.	Introduction	01-03
2.	Review of Literature	04-12
3.	Materials and Methods	13-25
4.	Results and Discussion	26-42
5.	Summary and Conclusion	43-44
6.	Bibliography	45-53
7.	Brief Resume Of Student	54

LIST OF ABBREVIATIONS AND SYMBOLS

ABBREVIATIONS	FULL FORM
<i>et al</i> :	<i>All others</i>
% :	Percentage
@ :	At the rate
i.e. :	That is
<i>viz.</i> :	Namely
<i>b.wt</i> :	Body weight
IM :	Intramuscular
IV :	Intravenous
T1 :	Treatment Group 1
T2 :	Treatment Group 2
T3 :	Treatment Group 3
% :	Percentage
conc. :	Concentration
C :	Control
cc :	Cubic centimeter
mg :	Milligram
ml :	Milliliter
<i>GIN</i> :	Gastrointestinal nematode
<i>D0</i> :	Pre-treatment
<i>D14</i> :	Post-treatment
<i>IVM</i> :	Ivermectin
<i>ABZ</i> :	Albendazole
<i>FBZ</i> :	Fenbendazole

<i>FEC</i>	:	Faecal egg count
<i>FECR</i>	:	Faecal egg count reduction
<i>FECRT</i>	:	Faecal egg count reduction test
<i>AR</i>	:	Anthelmintic resistance
<i>TT</i>	:	Targeted treatment
<i>CI</i>	:	Confidence interval

LIST OF TABLES

Sl. No.	Table no.	Description	Page no.
1.	1.	EPG of control group at day zero and at day 14th	28
2.	2.	EPG of groupT2 at day zero and at day 14th	28
3.	3.	EPG of groupT3 at day zero and at day 14th	29
4.	4.	EPG of groupT4 at day zero and at day 14th	30
5.	5.	Egg hatch assay for fenbendazole	32
6.	6.	Egg hatch assay for ivermectin	33
7.	7.	Egg hatch assay for levamisole	33
8.	8.	Percentage reduction in EPG of control group	35
9.	9.	Percentage reduction in EPG of T1 group after treatment (given fenbendazole)	36
10.	10.	Comparison between observed and expected values of % reduction in EPG of control group and T1 group	37
11.	11.	Value of chi square for fenbendazole	37
12.	12.	Percentage reduction in EPG of T2 group after treatment (given ivermectin)	38
13.	13.	Comparison between observed and expected values of % reduction in EPG of control group and T2 group	39
14.	14.	Value of chi square for ivermectin	39
15.	15.	Percentage reduction in EPG of T3 group after treatment (given Levamisole)	40
16.	16.	Comparison between observed and expected values of % reduction in EPG of control group and T3 group	41
17.	17.	Value of chi square for Levamisole	42
18.	18.	Difference in values of chi square for three drugs	42

LIST OF FIGURES

Sl. No.	Fig. no.	Description	Page no.
1	1	Faecal smear examination. (a) Smear making (b) <i>H. Contortus</i> (c) <i>Monezia</i>	16,17
2	2	Sample collection from ILFC, Patna	18
3	3	Sample collection from private farms, Patna	18
4	4	Charging McMaster slide	20
5	5	Examining McMaster slide under microscope	21
6	6	Eggs of Helminth observed during EPG (a) <i>Cooperia</i> (b) <i>Trichuris</i>	21
7	7	Putting Clayton lane tube in centrifuge machine.	24
8	8	Graphical representation of comparative EPG among the groups	32
9	9	Hatched egg seen after 48 hrs of incubation	34
10	10	Graphical representation of % reduction in EPG after 14 days in control group	35
11	11	Graphical representation of % reduction in EPG after 14 days in T1 group	36
12	12	Graphical representation of % reduction in EPG after 14 days in T2 group	38
13	13	Graphical representation of % reduction in EPG after 14 days in T3 group	41

DEPARTMENT OF VETERINARY MEDICINE
BIHAR VETERINARY COLLEGE, PATNA - 14
(Bihar Animal Sciences University, Patna, Bihar)

Title of thesis **“Study on prevalence of helminth infestation and anthelmintic resistance in calves”**

Name of student: **Dr. Pinky Rani**

Adm. No.:VM0019/2019-20

Major discipline: **Veterinary Medicine** Minor discipline: **Veterinary Surgery & Radiology**

Date of thesis submission: 27.01.2022

Total pages of thesis: 55

Major Advisor: **Dr. Bipin Kumar**

ABSTRACT

The main objective of research was to find out the prevalence of helminth infestation and the status of anthelmintic resistance in the calves in Patna and its surrounding areas. For the research, we targeted the calves of 0 to 6 months of age.

Collected faecal samples of calves of targeted category on zero Day was examined under the microscope and saw the worm infection was in the collected faecal samples. The infected samples were separated from the infection-free samples. Faecal samples with helminth infestation was selected for further research.

The following three parameters to be positive for parasitic egg when processed further in study to determine the level of anthelmintic resistance in selected faecal samples-

- (i) EGG PER GRAM (EPG)
- (ii) FECRT
- (iii) EGG HATCH ASSAY (EHA)

All the helminth infested calves were divided into four groups, each consists of 10 animals. The first group was not given any medicine for treatment and kept as positive control. The second group of calves (T1) were given fenbendazol. The third group (T2) was given ivermectin and the fourth group (T3) was given levamisole.

All the helminth infested calves were put on for EPG (egg per gram) count in the samples collected on day zero. After treatment, on day 14th, again the status of eggs of helminth was observed in the faecal samples of all the calves by EPG.

In FECRT results, the percentage value of FECR is less than 90%. In research FECR values were found for fenbendazole, ivermectin and levamisole to be 58.33%, 65% and 84.21% respectively which were in different percentages for all the drugs used, showed that the helminth has acquired resistance to the drugs used in the treatment groups T1, T2 and T3.

EHA was used to measure the amount of anthelmintic drug resistance, at three different **concentrations (concentration1, concentration2 and concentration3)** of these three medicines were used whose quantity was 1µg/ml, 5µg/ml and 10 µg/ml respectively. Helminths were found to be developed resistance to **concentration1** of all three drugs. Resistance to even **concentration2** of fenbendazole and levamisole were found. The resistance against the **concentration3** of these drugs was not developed in all the three anthelmintic drugs and these medicines were still effective at higher concentration. It was also found that the resistance against the **concentration2** of levamisole did not develop in helminth.

Therefore, the results can be proved to be very helpful in dealing with helminth infestation and anthelmintic resistant in future.

INTRODUCTION

Animal husbandry is an important part of agriculture which plays an important role in the economy of the country. Livestock production is a foremost earning source for the agricultural sustainability of poor farmers in rural areas, especially when crop production is not a profitable source of income (Khajuria *et al.*, 2013; Ahmed *et al.*, 2020).

In recent years, a rapid increase in both the prevalence and magnitude of anthelmintic resistance, has increased tremendously (Kaplan *et al.*, 2012). Helminth infestation in animals have adverse effects on its health. Gastrointestinal nematode is a major problem in animals. Due to helminth infestation, stockbreeders have to suffer with a huge financial loss. Parasitism has always caused major problems in achieving maximum output from livestock production systems (Mehmood *et al.*, 2017; Ijaz *et al.*, 2018; Nasir *et al.*, 2018; Zafar *et al.*, 2019; Ahmad *et al.*, 2019; Batool *et al.*, 2019; LI *et al.*, 2019a, 2019b; Khater *et al.*, 2020; LI *et al.*, 2020). These parasites affect the cattle's health which leads to decreased production and Average Daily Gain (ADG), resulting in estimated economic losses at over \$2.5 billion a year for cattle producers (Williams and Loyacano, 2001). The tropical environmental condition of India is much favorable to survival and spread of helminths. Animals get infected with parasites by consumption of contaminated soil and grasses. These worms have to be removed so, animals should be provided with suitable anthelmintic drugs in regular interval of time to keep animals free from various types of helminths. Doing so makes animal grow fast and produces better milk and meat. Parasitic infections leads to loss of blood in animals, reduced body weight, reduced milk production, low fertility rates and increased mortality. Due to helminth infestation in stomach of milch animals performance decreases and milk production falls and meat animals weight does not increase, due to which the livestock industry suffers huge economical losses. Severe infection inflicts a substantial impact on milk, meat and wool production, reduces weight gain by 23-63%, and in 25% cases death occurs before weaning (Singh *et al.*, 2015).

Anthelmintic resistance has evolved as a serious problem for the control of many species of important livestock helminths. The problem of gastrointestinal helminths infection are an important cause of disease and creates significant threat to health and production losses in the live stocks industries throughout the world (Fikru *et al.*, 2006) Gastrointestinal (GI) parasites cause impaired digestion and affect the absorption of minerals, particularly calcium and phosphorus (Speedy 1992).

In normal doses (fenbendazole at the dose rate of 10mg/kg b.wt, ivermectin at the dose rate of 0.2mg/kg b.wt, levamisole at the dose rate of 7.5mg/kg b.wt), the anthelmintic drug is not effective on the worm because in animals the efficacy to that anthelmintic is reduced.

“Drug Resistance” is defined as the ability of worm in a population to survive drug treatment that are generally effective against the same species and stage of infection at the same dose (Sangster, 1999). Repeated treatment with frequent use of anthelmintic reduces the number of susceptible parasites, but increases the proportion of resistant parasites in the herd of animals. Parasites become resistance to that drug by giving a low dose of anthelmintic drug below recommended dose. Resistance also occurs by using the same anthelmintic for long time.

Parasites are prolific breeders, therefore, the development of resistance increases quickly and thus anthelmintic resistance spreads fast to animals. Anthelmintic resistance appears in the newborn and young animals with greater intensity than in the adult animals. It is important to detect anthelmintic resistance early in the course of its development, so that appropriate controlling strategies can be devised and implemented to reduce the spread of anthelmintic resistance in worms.

Anthelmintic resistance in animals will become a major worldwide barrier to livestock production if ignored and anthelmintic drug will be completely ineffective. Intensive use of anthelmintic drugs in the livestock industry has led widespread resistance among all the existing anthelmintic. Various methods and tests have been described to explain which drugs are still effective against a particular parasite population on a farm and it would enable the choice of effective anthelmintic drug in the field.

Since, there is very little or no information is available on the efficacy of the broad-spectrum anthelmintic against worm infestation in calves in Bihar. Therefore this study has been undertaken to determine the prevalence of worm infestation and anthelmintic resistance in dairy calves with following objectives-

1. To study the prevalence of helminth infestation in different age groups of calves in and around Patna.
2. To study the efficacy of different anthelmintic drugs in calves.
3. To study the resistance against different anthelmintic drugs in calves.

Purpose of the study

- There is very little or no information is available on the efficacy of the broad spectrum anthelmintic against worm infestation in calves in Bihar.
- The aim of this study is to determine the prevalence of worm infestation and anthelmintic resistance in dairy calves.

Definitions of Terms

Helminths. Macroscopic parasitic worms that are characterized into three broad groups: Cestodes, Trematodes and Nematodes (Castro, 1996).

Anthelmintic - Any medications that is designed to kill parasitic worms, both internally and externally (Merriam-Webster, n.d.).

Anthelmintic resistance (AR) - defined as the ability of worm in a population to survive drug treatment that are generally effective against the same species and stage of infection at the same dose.

Fecal Egg Count (FEC)- Quantifiable interpretation of the parasitic load of an individual based on a ratio of fecal material and flotation solution under a McMaster slide to determine the amount of Eggs Per Gram (EPG) are in the sample (Nielsen, 2015).

Fecal Egg Count Reduction Test (FECRT)- An equation used to determine the rate of parasitic resistance to anthelmintic on market by conducting a FEC on day zero and again on day 14th then comparing the two with a mathematical formula. If FECRT's are reduced by 90% after the day 14th administration, the anthelmintic given in considered to be resistance to parasites in the herd. (Gasbarre, 2014).

Egg hatch assay (EHA)- The assay is less time consuming, easier and results are obtained faster when compared with other detection tests such as faecal egg count reduction test (FECRT) and larval development assay (LDA)

REVIEW OF LITERATURE

- Waller *et al.* (1987) demonstrated that nematodes of domestic animals have shown resistance to common anthelmintic especially in warm and humid parts of the world. This has been suspected to be due to frequent dosing and poor therapeutic strategies.
- Shalaby *et al.* (2013) observed that the frequent use of the same group of anthelmintic, use of anthelmintic in suboptimal doses, prophylactic mass treatment of domestic animals and frequent and continuous use of a single drug have contributed to the widespread development of anthelmintic resistance.
- Hepworth *et al.* (2015) recommended to keep animals on dry lot for 12 to 24 hours after deworming to ensure that eggs and larvae that survive the anthelmintic are not deposited on safe pasture then he presupposed that 24 hours after treatment there should not be viable helminth eggs; therefore, the hatching of viable eggs seven days after treatment in their study is an indication that the worms survived.
- Howell *et al.* (2008) demonstrated that Ivermectin being the most widely used because of its effect against ectoparasites has been grossly overused; hence it is highly susceptible to resistance development.
- Macial *et al.* (1996) described that anthelmintic resistance(AHR) in GIN can however arise due to risk factors associated to the use of anthelmintic, such as under-dosage, overuse, and use of the same chemical compound without rotation.
- Leathwick and Luo (2017) suggested that avoiding routes of administration with low bioavailability has been suggested. The better efficacy was found with orally applied than injectable or pour-on drugs. He also evaluated the change in gene frequency at different dosing rates and concluded that AHR was increased at a lower and more variable dose.

- Sutherland and Leathwick (2011) suggested that oral application was recommended most often because injectable drugs may result in a temporary suppression of the nematodes egg production, which complicates the interpretation of the results.
- Barnes *et al.* (1995) suggested that efficacy of the drugs might be improved by combining multiple drugs rather than administering a unique drug.
- Leathwick (2012) suggested that it will be beneficial if we practice the use of drugs rationally despite a certain degree of lateral resistance between drugs of the same chemical group.
- Campbell and Benz (1984) reviewed that the efficacy of ivermectin in cattle by confirmed high efficacy for the majority of GIN found in cattle. Their study suggested that a low effectiveness of ivermectin for most nematodes genera.
- Kaplan and Vidya Shankar (2012) demonstrated that this effect might be explained by a possible overuse of ivermectin over the years. Resistance of ivermectin was found to be relatively common and widespread.
- Lespine *et al.* (2012) described that under-dosing may cause increased AHR. Their results indicate that efficacy of levamisole increased proportionally with increasing dosing, indicating a correct administration of anthelmintic drugs in most studies.
- Gasbarre *et al.* (2009a) demonstrated that an interval of 7 days may be insufficient for a complete efficacy of ivermectin.
- Sutherland *et al.* (2009) described that the resistance to the BZ appears to involve all the major species infecting cattle, which could reflect the extended period of use since their introduction in the 1960s.
- Demeler, J. *et al.* (2010) and Taylor, M.A. *et al.* (2002) described that the accurate determination of resistance status in GIN infecting cattle is more difficult than in small

ruminants. This is partly because egg output in feces tends to be lower, which makes accurate calculation of drug efficacy problematic.

- Gasbarre, L.C. *et al.* (2009) suggested that the availability of highly effective anthelmintic drugs has allowed changes in the manner in which livestock are farmed with increased stocking rates and reductions in the use of alternative control practices.
- Borgsteede *et al.* (1992) stated that using the EHT for detection of either BZ or levamisole resistance in trichostrongylid nematodes of cattle. However, to date no standardized EHT protocol for which data on the repeatability of data generated within laboratories and reproducibility of result between laboratories has been published.
- Edmonds *et al.* (2010) demonstrated that over the past decade there have been increasing numbers of reports of anthelmintic resistance in gastrointestinal nematodes of cattle worldwide, and most of these concern resistance to the ivermectin.
- Suarez and Cristel (2010) demonstrated that resistance to ivermectin, benzimidazole and to both ivermectin and benzimidazole was present on 60% (15/25), 32% (8/25) and 28% (7/25) of farms respectively.
- Levamisole resistance was not detected. Across all 25 farms the mean reduction in FEC for ivermectin, benzimidazole and levamisole were 81.0, 93.3 and 97.6% respectively.
- Soutello *et al.* (2007) stated that in Sao Paulo state, Brazil, 23 of 25 cattle farms had < 90% reduction in FEC following treatment with ivermectin, and on 19 of these farms reductions in FEC were < 50%.
- Waghorm *et al.* (2006) stated that all laboratories participating in EHA used the standard methods developed by the National Committee for Clinical Laboratory standards. Equivalent methods and standards have so far not been established for in vitro testing of anthelmintic resistance. In the face of increasing anthelmintic resistance, such standardized methods are required in bovine nematodes.

- Sargison *et al.* (2009) stated that route of administration is also important; pour-on formulation in cattle may yield reduced efficacy that is not consistent with a diagnosis of resistance and thus should not be used in a FECRT for the purpose of establishing the presence or absence of resistance on a farm.
- Kochapackdee *et al.* (1995) stated that the percentage of FECR after treatment was calculated as follows:- $\%FECR = [1 - (\text{arithmetic mean FEC post treatment} \div \text{arithmetic mean FEC pre treatment})] \times 100\%$. Resistance was considered present if both the $\%FECR$ was less than 95% and the lower limit of the 95% confidence interval (CI) less than 90%, doubtful if only one criteria was met and absent (susceptible) in all other cases (Coles *et al.*, 1992)
- Baiak *et al.*, (2018) noted that Mode of application interfered ($P < 0.05$) with the efficacy of anthelmintic drugs in cattle. Efficacy of orally applied drugs (90%) was superior to that of injectable (78%) and pour-on drugs (77%). Injectable drugs did not differ ($P > 0.05$) from pour-on drugs.
- Lawal-Adebowale *et al.*, (2012) implicated that the plant could be a good alternative to the commonly used synthetic anthelmintic. The plant is also readily available and it is found as a shrub in most rural backyard gardens in developing countries.
- Certain climate conditions may favor the development of nematodes. Simultaneously, the immune system response to infections resulting from the increased presence of nematodes may be reduced due to stress associated to unfavorable climate conditions such that AHR to drugs will augment (Amarante, 2014).
- Vercruyse *et al.*, (2013) noted that negative impact of GIN on livestock farms is further exacerbated by the escalating spread of anthelmintic resistance (AR).
- Charlier *et al.*, (2018) mentioned that the diagnosis of gastrointestinal helminth infection is mainly based on the detection of worm eggs through faecal egg counts (FEC).

- Charlier *et al.*, (2014) suggested that one of the options to make GIN control practices more sustainable is to lower drug application frequency by targeting treatment (TT) to the whole group of animals when infection is high while preserving a pool of unexposed parasites in refugia as free-living stages.
- Kohler *et al.*, (2001) described that each class of anthelmintic has a unique mode of action against parasites. Imidazothiazoles (IM), such as levamisole, are acetylcholine agonists that act on the nervous system of the parasite.
- Mansour *et al.*, (2002) described that acetylcholine agonists drugs cause muscle contraction and paralysis in the helminth, resulting in the eventual expulsion of the parasite from the body.
- Monteiro *et al.*, (2011) observed that broad-spectrum anthelmintic are more commonly used in ruminants because they are capable of eliminating large numbers of parasites, besides being of easy administration and safe to the hosts.
- Burke *et al.*, (2005) evaluated that the highest resistance has been observed with ivermectin and albendazole or fenbendazole and low to moderate resistance has been observed with levamisole.
- Anon *et al.*, (2007) conducted a survey that anthelmintic resistance has been reported in both the commercial and resource-poor farming sectors in at least 13 countries, and, the situation is considered the worst in the world, with high levels of *Haemonchus contortus* resistance to all classes of anthelmintic.
- Jackson *et al.*, (2001) noted that there are anthelmintic still available, but multiple drug-resistant helminth strains have quickly developed, and producers and animal health professionals must now seek alternative methods of treatment and prevention.
- Sangster *et al.*, (2018) demonstrated that resistance has already developed to two new active ingredients, monepantel and derquantel. This was despite spiroindole- derquantel-being marketed as a combination product to slow the development of resistance.

- Sutherland *et al.*, (2011) surveyed that in recent years AR has become evident that this is also an emerging problem for cattle.
- Torres-Acosta *et al.*, (2012) described that resistance against drugs belonging to the same anthelmintic drug class is called side resistance, whereas cross and multidrug resistance refer to resistance against two or multiple drugs belonging to different anthelmintic drug classes.
- Vijayasarithi *et al.*, (2016) noted that the main factors for the selection for anthelmintic resistance are high-treatment frequency, under dosing and the use of the same anthelmintic class over several years.
- Cernanska *et al.*, (2008) evaluated that drugs individually or in combination, together with the risk of under dosing and continued use of one class of anthelmintic, irrespective of efficacy status are frequently encountered factors enhancing development of anthelmintic resistance.
- Borges *et al.*, (2015) & Da Cruz *et al.*, (2004) noted that many livestock studies report an inefficient management system, as well as, a lack of knowledge about the correct use and dosage of drugs and not respecting the interval time between dosage administrations.
- Lacey *et al.*, (1994) described that BZ resistance in livestock has been widely distributed throughout the world since the development of the drugs in the early 1960s.
- The origin of BZ resistance in livestock has been speculated about. The animal migration and gene flow among nematodes as well as spontaneous mutations and the presence of rare alleles in the population, could be responsible for the spread of resistance among animal nematodes.
(Silvestre *et al.*, 2002; Humbert *et al.*, 2001; Roos *et al.*, 1990)
- Coles *et al.*, (19992) noted that the egg count using the McMaster chamber, and several variations on the original technique, is the most frequently used technique to conduct the

FECRT. The FECRT is an in vitro test that provides an estimate of anthelmintic efficacy by comparing worm egg counts from animals before and after treatment.

- Haryuningtyas *et al.*, (2001) demonstrated that in vitro research stated that goats on farms in Lithuania stated that 61.9% were resistant to Ivermectin, 33.4%, and Levamisole, also 100% to Benzimidazole from 13 goat farms. Anthelmintic resistance had occurred if their use was continuous without prior examination and antihelmintic with incorrect dosage.
- Verma *et al.*, (2018) noted that the frequent usage of anthelmintic such as Albendazole and Ivermectin cause resistance by mutation gene of helminths. The Albendazole has caused β -tubulin isotype 2 mutations, deletion, and also altered metabolism and/or uptake of helminths and The Ivermectin has been reported to cause Mutations in GluCl and/or GABA-R genes of helminths.
- Borges *et al.*, (2013) evaluated that anthelmintic resistance has an impact on treatment costs and productivity. The results stated that a decrease in body weight between 9.05-9.4 kg had occurred in cattle that had anthelmintic resistance compared to cows that were not resistant.
- Faecal Egg Count Reduction Test (FECRT) is one of the methods to measure of anthelmintic resistance of gastrointestinal helminth that recommended by The World Association for the Advancement of Veterinary Parasitology (WAAVP).
- Kudo *et al.*, (2014) [14] reported that faecal egg count (FEC) reduction of Ivermectin at 14 days post-treatment ranged from 16 to 87% in 2009 and from 24 to 96% in 2010 of northern Japan in 2009 and 2010.
- Blackhall *et al.*, (1998) reported that AR caused by a change in the gene frequency of that population resulting from drug selection.
- Kaplan *et al.*, (2002) suggested that lacking a better method, FECRT remains the practical gold standard for detecting resistance in the field.

- Craven *et al.*, (1999) demonstrated that correlations between in vitro and in vivo studies for anthelmintic resistance were found to be poor and they were not able to use the outcome of one test to predict the result of another.
- Rajagopal *et al.*, (2017) described that benzimidazoles are extensively used for nematode control in livestock leading to the development of drug resistance which has now evolved as a major problem worldwide. The first report of benzimidazole resistance in India was by Varshney and Singh (1976).
- Calvete *et al.*, (2012) EHA based on discriminating doses will also be useful in situations when there are no sufficient nematode eggs available for assaying all the drug concentrations.
- Gasbarre *et al.*, (2014) noted that the control of parasites in dairy cattle relies most commonly on commercially available anthelmintic drugs; however, resistance to anthelmintic drugs by GINs has increased over the last few years.
- Devi *et al.*, (2014) noted that frequent and prolonged use with indiscriminating administration and improper formulations of synthetic drugs has regrettably led to an upsurge in resistance in the parasites against these salts.
- Sanyal *et al.*, (2003) demonstrated that resistant parasites become more pathogenic, prolific and acquire increased adaptability and survivability in all their free-living phases in the host.
- Qadir *et al.*, (2010) noted that apart from resistance against anthelmintics, toxic residues, the high cost of production and the unavailability and inaccessibility of these synthetic drugs, especially in remote rural areas, have spurred investigations to explore alternative methods.
- Among the alternatives, botanicals have been very effective against a wide range of parasites. (Abbas *et al.*, 2017a, 2017b, 2018, 2019; Hussain *et al.*, 2017; Idris *et al.*, 2017; Zaman *et al.*, 2017; Khater *et al.*, 2018; Fayaz *et al.*, 2019).

- Calvet *et al.* (2012) demonstrated that egg hatch assay (EHA) based on discriminating doses will also be useful in situations where there are no sufficient nematode eggs available for assaying all the drug concentrations. Hanna *et al.* (2010) shown that the machinery for egg formation can be disrupted very severely by triclabendazole treatment. Prichard, (2008) showed that the benzimidazoles have many benefits, including the following-

- (i) Selectivity and relatively low mammalian toxicity.
- (ii) Broad spectrum of activity
- (iii) High efficacy
- (iv) Ease of administration and
- (v) Low cost.

Suarez and Cristel, (2007) show that resistance to ivermectin, benzimidazole and to both ivermectin and benzimidazole was present on 60% (15/25) 32% (8/25), and 28% (7/25) of farms respectively.

MATERIALS AND METHODS

Place

- The present study was conducted in Department of Veterinary Medicine, Bihar Veterinary College, Bihar Animal Sciences University, Patna

Sources of Calves

- The study were carried out on calves of ILFC and few private farms in and around Patna
- (Animals free from anthelmintic for at least 8 weeks) were selected for the study
- All samples of such animals were brought to Department of Veterinary Medicine for the study

Therapeutic study

1. In vitro study was carried out in Department of Veterinary Medicine and in vivo ILFC, BASU Patna and few private farms in and around Patna

(Therapeutic trials of anthelmintic were done on all the positive animals found positive in private farms.)

Experimental Design

- The experimental calves were randomly divided into four group viz. **T1**, **T2**, **T3** and **C**. Each consisting of 10 animals. The calves in group **C** were kept as control and were not treated with any medicine. The group **T1** was treated with fenbendazole at the dose rate of 10mg/kg b.wt. The calves in group **T2** were administered ivermectin at the dose rate of 0.2mg/kg b.wt. The calves in group **T3** were administered with levamisole at the dose rate of 7.5mg/kg b.wt at day zero and fecal smear were seen as if it is still passing helminthic eggs/larva or not.

AGE GROUP

0-6 months age groups of calves screened for the helminth infestation.

All animals randomly divided into three groups.

Efficacy of Medicine:

The efficacy of each drug was assessed in term of reduction in the number of ova following treatment. For this, the faecal samples were collected on day zero and subsequently on 14th day post treatment using egg per gram counting technique as described before. The efficacy of all three drugs were evaluated on the basis of negative faecal sample (i.e. declining rate of epg) on post supplementation days.

Treatment groups

C : Control group of calves without treatment.

T₁ : Treatment group of calves has given Fenbendazole.

T₂ : Treatment group of calves has given Ivermectin.

T₃ : Treatment group of calves has given Levamisole.

Experimental design of anthelmintic trials in calves

Group	No. of calves	Anthelmintic used	Days of Observation	
			0 th day	14 th
T1	10	Fenbendazole@10mg/kg b. wt	Faecal sample examination.	
T2	10	Ivermectin @ 0.2mg/kg b. wt		
T3	10	Levamisole @ 7.5mg/kg b. wt		
C	10	Control (untreated)		

STATISCAL ANALYIS: Statistical analysis were done with the method of snedecor and cochrn (1991) analysis.

To study the prevalence of helminth infestation in different age groups of calves

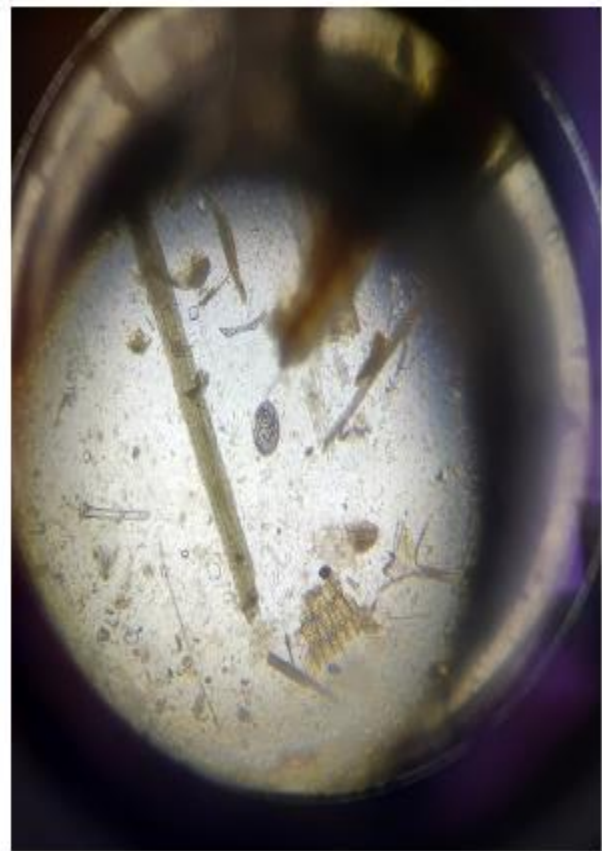
The fecal samples from calves were collected from different parts of Patna such as; Raja Bajar, Beur, Danapur, Sipara, Digha, Gardanibag, ILFC, BVC Patna, Kankarbagh. Thereafter the fecal samples were screened for helminth infestation. The qualitative estimation of presence of egg of helminth has been done by fecal smear examination. A total of 54 samples were collected majority of them (40 calves) were found +ve for helminth infestation. The +ve animals were selected for further study.

Direct Smear Method:

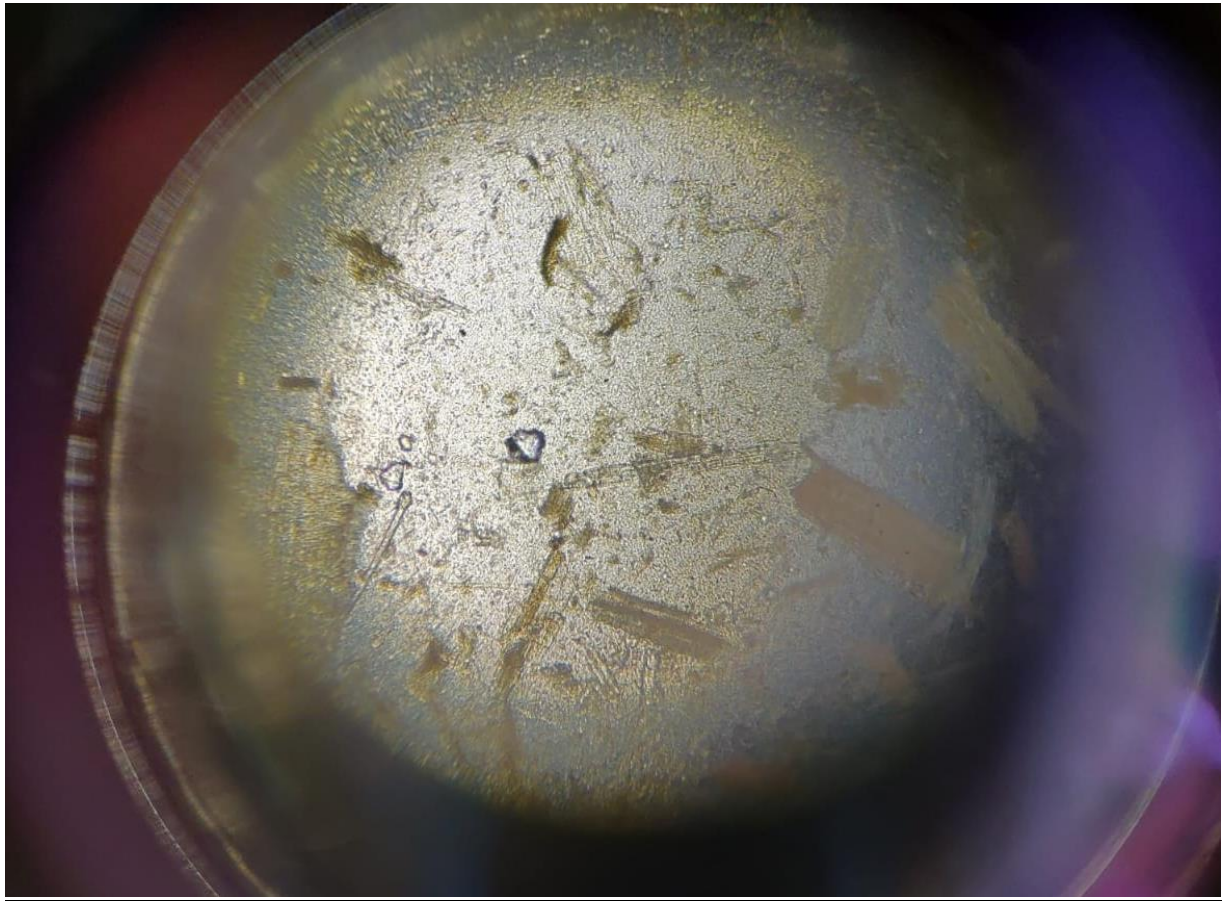
A small quantity of fresh feces was placed on a slide, mixed with a small droplet of water or normal saline, with the help of needle evenly spread over the slide, a coverslip placed on the fluid and examined under 10x followed by 40x.



(a)



(b)



(c)

Fig. 1. Faecal smear examination. (a) Smear making (b) *H. Contortus* (c) *Moneizia*

FAECAL SAMPLE COLLECTION

- Faecal samples collected fresh from the rectum of the animals pre-treatment and post-treatment in air-tight bags. All faecal samples were brought to the Department of Veterinary Medicine laboratory for examination and identification of gastrointestinal helminths and EPG by Mac Master Technique Post-treatment samples collected on 14th day of treatment. EPG has been calculated pre-treatment and post treatment.



Fig. 2. Sample collection from ILFC, Patna



Fig. 3. Sample collection from private farms, Patna

DIAGNOSTIC APPROACHES

The presence of helminthic eggs has been determined through egg per gram (EPG)

We use the EPG method to look for egg-load of helminths in infected faecal samples. The McMaster Technique was used for estimation of EPG. EPG gives us an accurate account of eggs. It also tells us the level of helminths infestation in calves.

The efficacy of anthelmintic drug has been determined through:-

FECAL EGG COUNT REDUCTION TEST (FECRT)

Through this test, we try to know how effective a given medicine is. In FECRT, we calculate the percent reduction in egg-count in infected fecal samples of calves with the help of EPG. In this method, the value of EPG of the faecal samples of infected calves is obtained at zero day. Thereafter treating the calves with the desired drug such as fenbendazole, ivermectin and levamisole, Again on day 14th fecal sample were collected and EPG count were done as per above procedure.

Anthelmintic Resistance has been determined through:-

EGG HATCH ASSAY (EHA)

EHA is conventional tests, was done in vitro to assess the efficacy/resistance of anthelmintic drugs in question. The assay is less time consuming, easier and results are obtained faster when compared with other detection tests such as faecal egg count reduction test (FECRT) and larval development assay (LDA).

EGG PER GRAM (EPG)

The process of the EPG technique is mainly used to see egg-load in faecal samples. In this process, first we took 2 grams of infected faecal sample and mixed it in 28 ml of sheather's solution. (sheather's solution- 454 gm of table sugar + 355 hot water) After this, with the help of a sieve, we filter the solution in another vessel. Using disposable pipette or syringe, we take 1 cc

of solution and carefully fill it in the Mc Master slide. Then, we observed McMaster slide under 10x microscope and counted the egg in each chamber of the Mc Master slide.

We did this process for 40 infected samples which were divided into 4 groups. This revealed the egg-load in all the infected samples.

BY McMASTER TECHNIQUE

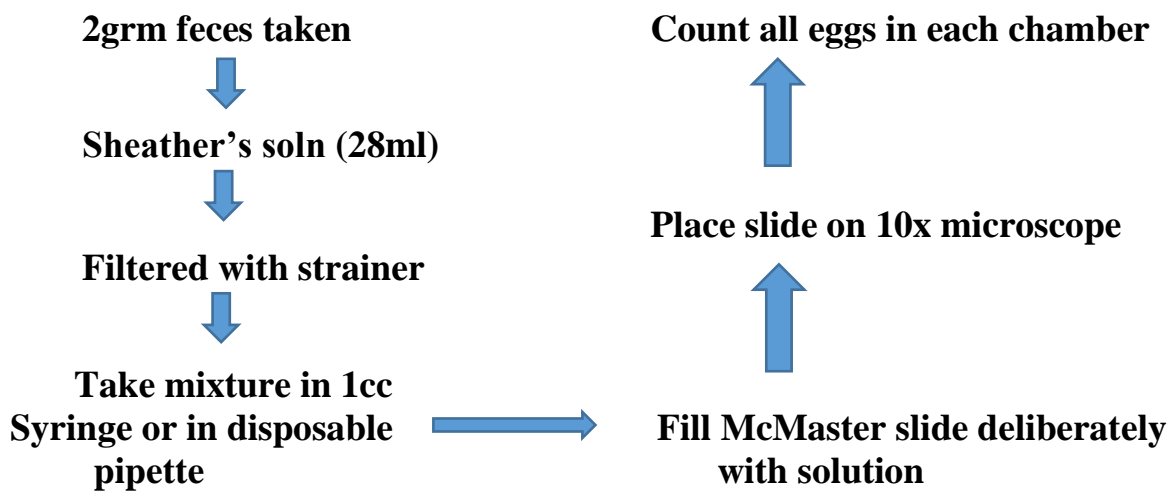


Fig. 4. Charging McMaster slide



Fig. 5. Examining McMaster slide under microscope



(a)



(b)

Fig. 6. Eggs of Helminth observed during EPG (a) *Cooperia* (b) *Trichuris*

The percent in faecal Egg count Reduction (FECRT)

Fecal Egg Count Reduction Test (FECRT) is one of the methods to measure of anthelmintic resistance of gastrointestinal helminth that recommended by The World Association for the Advancement of Veterinary Parasitology (WAAVP). Kholik *et al.*, (2019). To perform a FEC Reduction test (FECRT) a fecal egg count should be performed on Day 'zero' and 14th. The FECRT helps to determine the efficacy of the anthelmintic that was used.

FECRT was calculated by the formula as given below:

$$\text{Percent reduction in EPG} = \frac{\text{Pre-treatment mean EPG} - \text{Post treatment mean EPG}}{\text{Pre-treatment mean EPG}} \times 100$$

The standard interpretation of the FECRT results in livestock was on the basis of following criteria: FECRT > 98%, highly effective; FECRT 90- 98%, effective; FECRT 80-89%, moderately effective; and FECRT < 80% insufficiently active or resistance

EGG HATCH ASSAY

EHA is made up of 2 parts. In the first procedure, we removed the egg of helminths from the faecal sample. We used those samples in this process in which the EPG values were high. First, we took 10 grams of infected faecal sample and dissolved it in 200 ml distilled water. We filtered the solution into 8 Clayton Lane tube. We put all these eight Clayton Lane tubes into the centrifuge machine and spin at 1000rpm for 2 minutes. This is followed by removing the tubes from the centrifuge machine and suck off the supernatant from its upper part. After this, NaCl solution is added to the remaining solution in the tubes till the meniscus is formed. We put a cover slip over the meniscus in tubes and left it for 4 to 5 hours. Further, we removed the cover slip and washed these in the centrifuge tube with the help of pipette. We put the centrifuge tube in the centrifuge machine and rotate it at 1000 rpm for 2 minutes. After removing the supernatant from the centrifuge tube, distilled water is added to it. Then with the help of pipette, 1ml of this solution of centrifuge tube is poured into the petri dish. And observed the eggs present per milliliter with the microscope.

EXTRACTION/COLLECTION OF EGGS FROM THE FECES

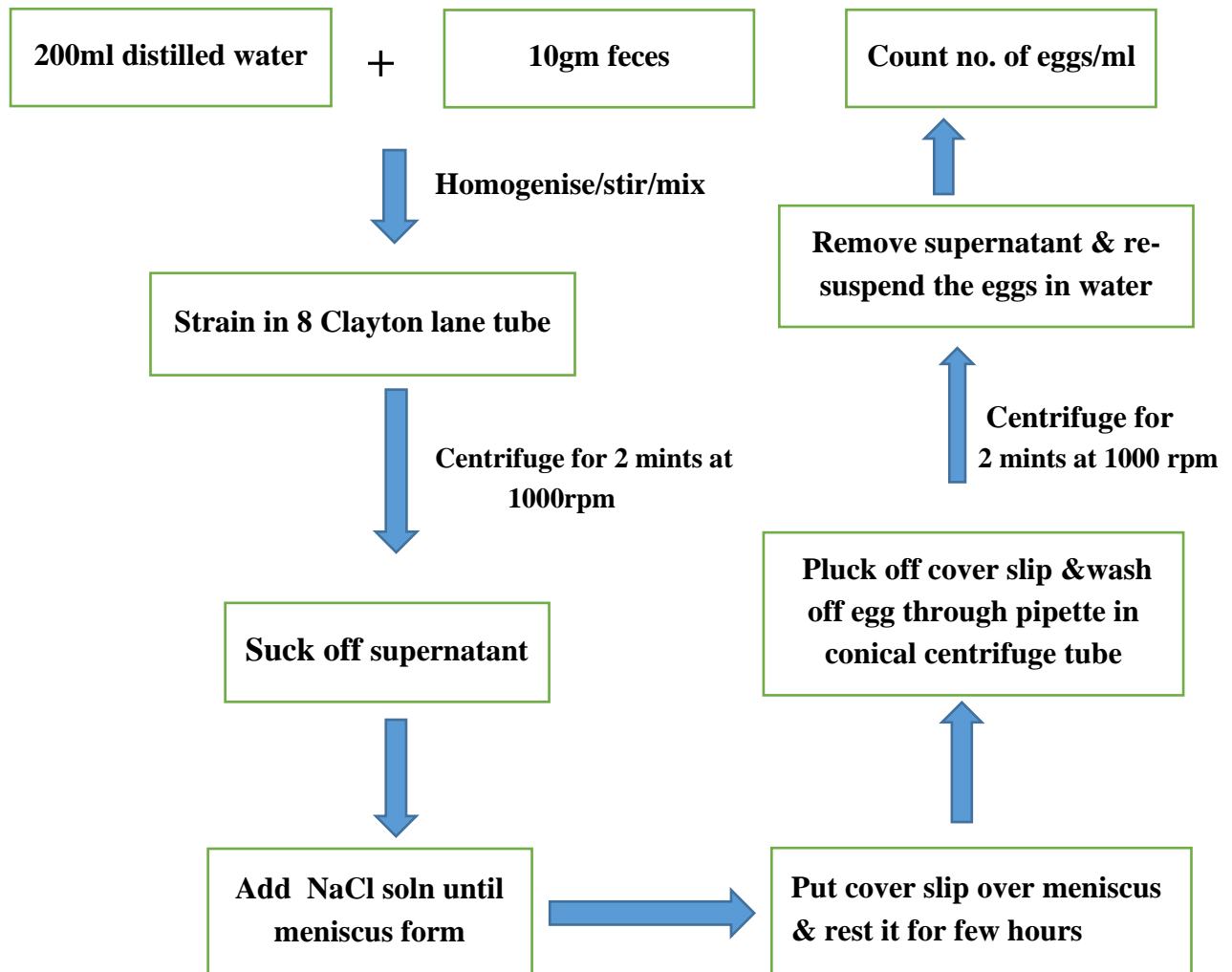
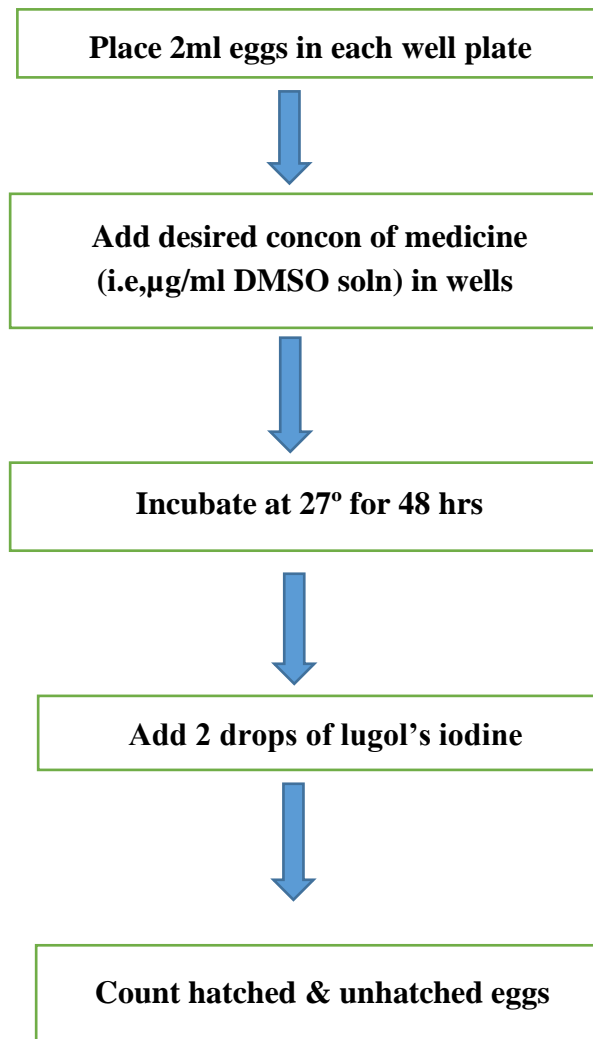




Fig. 7. Putting Clayton lane tube in centrifuge machine.

In the second procedure of EHA, We put 2ml of the solution of eggs into the each Well-plates. (We made three concentrations of desired drugs i.e. 01 $\mu\text{g/ml}$, 05 $\mu\text{g/ml}$ and 10 $\mu\text{g/ml}$ in DMSO solution). Then in each well of the three well-plates, we poured 1ml each of the solutions containing 1 $\mu\text{g/ml}$, 5 $\mu\text{g/ml}$ and 10 $\mu\text{g/ml}$ concentrations of fenbendazole to the three well-plates. Now all the three well-plates are incubated at 27 °C temperature for 48 hrs. We added two drops of Lugol's iodine solution to all the three well plates. We emptied the three well-plates separately in three petri dish and observed them under the microscope. This gives us the number of hatched and unhatched eggs. This whole process was repeated for ivermectin and levamisole.

PROCEDURE FOR TEST





RESULTS AND DISCUSSION

4

RESULTS AND DISCUSSIONThe prevalence of helminths infestation in different age groups of calves

During this study period, 54 samples were collected from in and around Patna. Forty (40) were found to be infected with one or more species of gastro intestinal parasites i.e; *Monezia*, *H.Contortus*, *Cooperia*, *Trichuris*. The prevalence of the disease was recorded as per the formula described below-s

$$\text{Prevalence (\%)} = \frac{\text{No. of faecal samples found positive}}{\text{Total no. of faecal samples examined}} \times 100$$

The overall prevalence of helminth infestation in this study was found to be 74.07%. The prevalence rate in helminth parasitic infection in calves were found 74.04%. Almost severe finding has been reported by many workers across the country. As per finding of Kashyap *et. al.*, (1997) 40.3% prevalence of gastrointestinal helminth was found in Malwa region of Madhya Pradesh and Gupta *et. al.*, (2012) also recorded 68.93% prevalence in cattle and buffalo in same locality. The variation in finding with earlier report might be due to the difference in the number of faecal sample examining, age of the animal, geo-planetic condition of the area and period of study. High temperature and humidity favors the survival of infective stage of the parasite and of the intermediate host, managerial condition and deworming practices.

Table 1: EPG of control group at day zero and at day 14th

Calves	AGE (in months)	EPG (0 day)	EPG (14th day)
Calf 1	3	100	100
Calf 2	1	50	0
Calf 3	2	50	150
Calf 4	4	150	100
Calf 5	3.5	100	150
Calf 6	2	50	100
Calf 7	6	200	150
Calf 8	1.5	100	100
Calf 9	5	150	200
Calf 10	4.5	100	150
		Mean EPG = 105	Mean EPG = 120

This is the first group of 10 out of 40 infected calves. The calves of this group were not given any medicine for treatment. As it can be seen from the table, in calf 2, calf 4 and calf 7 of this group, helminth infestation has decreased naturally. This could be due to the strong immunity of the individual calf. In all the remaining 7 calves, the level of helminths infestation was found to be increased in the absence of treatment. Similarly, the mean EPG of this group has also increased from 105 to 120 in the absence of any treatment. This clearly indicates that appropriate treatment is needed to fight against helminths infestation or else the level of helminths infestation may rise.

Table 2: EPG of groupT1 at day zero and at day 14th

Calves	AGE (in months)	EPG (0 day)	EPG (14th day)
Calf 1	2	50	0
Calf 2	6	250	150
Calf 3	3.5	100	0
Calf 4	2	50	0
Calf 5	2.50	100	50
Calf 6	1	50	0
Calf 7	4.5	150	100
Calf 8	6	200	100
Calf 9	4	100	50
Calf 10	3.5	150	50
		Mean EPG = 120	Mean EPG = 50

$$\text{Percent reduction in EPG (FECRT)} = \frac{\text{Pre-treatment mean EPG} - \text{Post treatment mean EPG}}{\text{Pre-treatment mean EPG}} \times 100$$

$$\begin{aligned} \text{(Fenbendazole) FECR \%} &= \frac{120 - 50}{120} \times 100 \\ &= 58.33\% \end{aligned}$$

<90% Indicates resistance

This is the second group of 10 infected calves, which was given Anthelmintic drug named Fenbendazole for treatment. Looking at the table, we get to know that after treatment, calf1, calf3, calf4 and calf6 of this group have become completely free of helminths infestation. Helminths infestation has decreased in the remaining 6 calves but they are not completely infection free. The mean EPG of this group is reduced from 120 to 50. The value of FECR is 58.33% which shows that the resistance is developed in Helminths against the fenbendazole.

In majority of study, it has been found that fenbendazole is insufficiently active. FECR is 58.33% against nematodes parasites of calves. This could be due to frequent use of fenbendazole as an anthelmintic drug in and around Patna. Similar kind of finding has been reported by Lacey *et. al.*, (1994) who has found resistance against fenbendazole on basis of FECR.

Table 3: EPG of group T2 at day zero and at day 14th

Calves	AGE (in months)	EPG (0 day)	EPG (14th day)
Calf 1	4	100	50
Calf 2	1.5	50	00
Calf 3	5	150	50
Calf 4	2	50	00
Calf 5	1	50	00
Calf 6	2.5	100	50
Calf 7	3	100	00
Calf 8	5.5	200	100
Calf 9	1.5	50	00
Calf 10	4	150	100
		Mean EPG = 100	Mean EPG = 35

$$\text{Percent reduction in EPG (FECRT)} = \frac{\text{Pre-treatment mean EPG} - \text{Post treatment mean EPG}}{\text{Pre-treatment mean EPG}} \times 100$$

$$\begin{aligned} \text{(Ivermectin) FECR \%} &= \frac{100 - 35}{100} \times 100 \\ &= 65 \% \end{aligned}$$

<90% Indicates resistance

This is the third group and the calves of this group were given ivermectin for treatment. Looking at the table, it can be seen that on 14th day after treatment calf2, calf4, calf5, calf7 and calf9 of this group have become completely free of helminths infestation. Helminths infestation has decreased in the remaining 5 calves but they are not infection free. The mean EPG of this group is reduced from 100 to 35. The value of FECR is 65% which shows that the resistance is developed in helminths against the ivermectin. Kudo *et al.* 2014 found in his research that the range of FECR for ivermectin was 16-87 % in 2009 and 24-96 % in 2010. Finding of McMahon *et al.*, 2013 showed that resistance to ivermectin was 50 % in Northern Ireland. In the similar study, Veríssimo *et al.*, 2012 reported 54 % resistance to ivermectin in Brazil. Waghorn *et al.* 2006b also reported that FECR for Ivermectin was 25 % which shows complete resistance. And according to Van Wyk *et al.*, 1999 resistance to ivermectin was 73 % in South Africa.

Table 4: EPG of groupT3 at day zero and at day 14th

Calves	AGE (in months)	EPG (0 day)	EPG (14th day)
Calf 1	2	50	0
Calf 2	3	50	0
Calf 3	4	150	50
Calf 4	1.5	50	0
Calf 5	3	100	0
Calf 6	6	200	50
Calf 7	2.5	100	0
Calf 8	3	50	0
Calf 9	1	50	0
Calf 10	5	150	50
		Mean EPG = 95	Mean EPG = 15

$$\text{Percent reduction in EPG (FECRT)} = \frac{\text{Pre-treatment mean EPG} - \text{Post treatment mean EPG}}{\text{Pre-treatment mean EPG}} \times 100$$

$$\begin{aligned} \text{(Levamisole) FECR \%} &= \frac{95 - 15}{95} \times 100 \\ &= \mathbf{84.21 \%} \end{aligned}$$

<90% Indicates moderately effective

This is the last group of calves and the calves of this group were given Levamisole for treatment. Looking at the table, it can be seen that after treatment, 7 calves became completely free from infection while 3 calves still have helminths infection. Although after treatment, a significant improvement in the level of infection can be seen in these 3 calves, but they're not getting infection-free is a cause for concern. The mean EPG of this group decreased from 95 to 15 which shows a significant decrease. As a result the value of FECR has come 84.21% and it is very close to 90%. This indicates that the level of resistance in helminths against Levamisole is low. Keegan *at al.*, 2017 showed in their research that resistance to levamisole was 47 % in Ireland. And in another research, Easwaran *et.al.* 2009 reported that the range of FECR was 70-82 % which is quite similar to our findings.

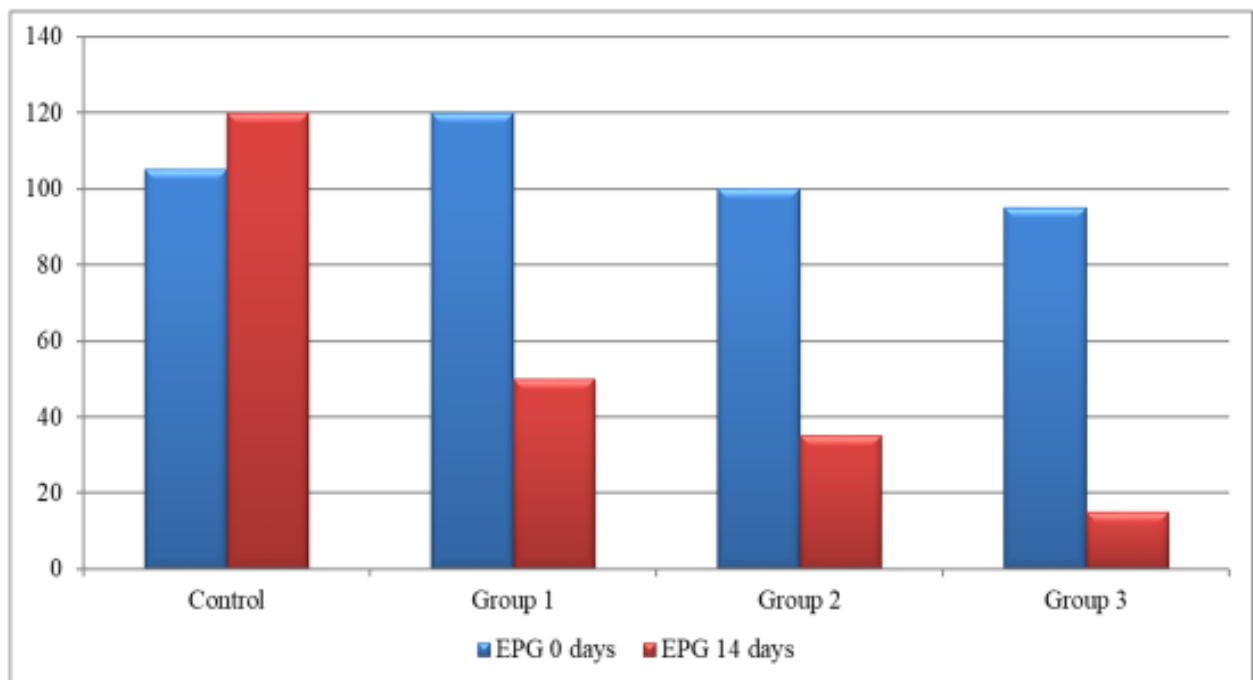


Fig. 8. Graphical representation of comparative EPG among the groups.

Table 5: Egg hatch assay for fenbendazole

	Concentration 1	Concentration 2	Concentration 3
	1μg/ml	5μg/ml	10μg/ml
RESULT	Hatching seen (2 Seen)	Hatching seen (1 Seen)	No hatching seen (2 Seen-non hatched)
CONCLUSION	Resistant	Resistant	Not Resistant

As it can be seen from the table, For EHA, I used three different concentrations of fenbendazole i.e. 1 μ g/ml (concentration1), 5 μ g/ml (concentration2) and 10 μ g/ml (concentration3). Different results are obtained for all the three concentrations. After treatment with fenbendazole with a concentration of 1 μ g/ml, the result was 2 eggs when viewed under a microscope and in both eggs we found hatching. In the case of a solution with concentration of 5

$\mu\text{g/ml}$, I got one egg as a result and hatching is seen in it. On the other hand, I got 2 eggs as a result of fenbendazole solution with a concentration of $10 \mu\text{g/ml}$, but both eggs are found to be unhatched.

So, in conclusion, I find that helminths have developed resistance to concentration1 and concentration2 of fenbendazole while resistance to concentration3 is not developed.

Table 6: Egg hatch assay for ivermectin

	Concentration 1	Concentration 2	Concentration 3
	1 $\mu\text{g/ml}$	5 $\mu\text{g/ml}$	10 $\mu\text{g/ml}$
RESULT	Hatching seen (2 Seen)	50% Hatching seen (2 Seen – 1 hatched & 1 no hatching)	No hatching seen (2 Seen-non hatched)
CONCLUSION	Resistant	Resistant	Not Resistant

For EHA, I used three different concentrations of ivermectin also i.e. $1\mu\text{g/ml}$ (concentration1), $5\mu\text{g/ml}$ (concentration2) and $10\mu\text{g/ml}$ (concentration3). As, it can be seen from the table, different results obtained for all the three concentrations. After treatment with concentration1 of ivermectin, 2 eggs were found when observed under a microscope and in both eggs hatching found. In the case of concentration2, I got 02 eggs as result and hatching was seen in 01 egg while other 01 egg is unhatched. On the other side, I got 02 eggs as a result of concentration3 of ivermectin, but both eggs were found to be unhatched.

So in conclusion, I find that helminths have developed resistance to concentration1, partially resistance to concentration2 but resistance to concentration3 is not developed.

Table 7: Egg hatch assay for levamisole

	Concentration 1	Concentration 2	Concentration 3
	1 $\mu\text{g/ml}$	5 $\mu\text{g/ml}$	10 $\mu\text{g/ml}$
RESULT	Hatching seen (1 Seen)	No hatching seen (3 Seen – none has hatched)	No hatching seen (2 Seen-none has hatched)
CONCLUSION	Resistant	Not Resistant	Not Resistant

For EHA, I used three different concentrations of Levamisole also i.e. 1µg/ml (concentration1), 5µg/ml (concentration2) and 10 µg/ml (concentration3). As I can see from the table, different results are obtained for all the three concentrations. After treatment with concentration1 of levamisole, 1 egg were found when observed under a microscope and I found hatching in it. In the case of concentration2, I got 03 eggs as result and no hatching is seen in any egg. I got 02 eggs in concentration3 of levamisole, but both eggs are found to be unhatched.

So in conclusion, I find that helminths have developed resistance to concentration1 but resistance to concentration2 and concentration3 is not developed.

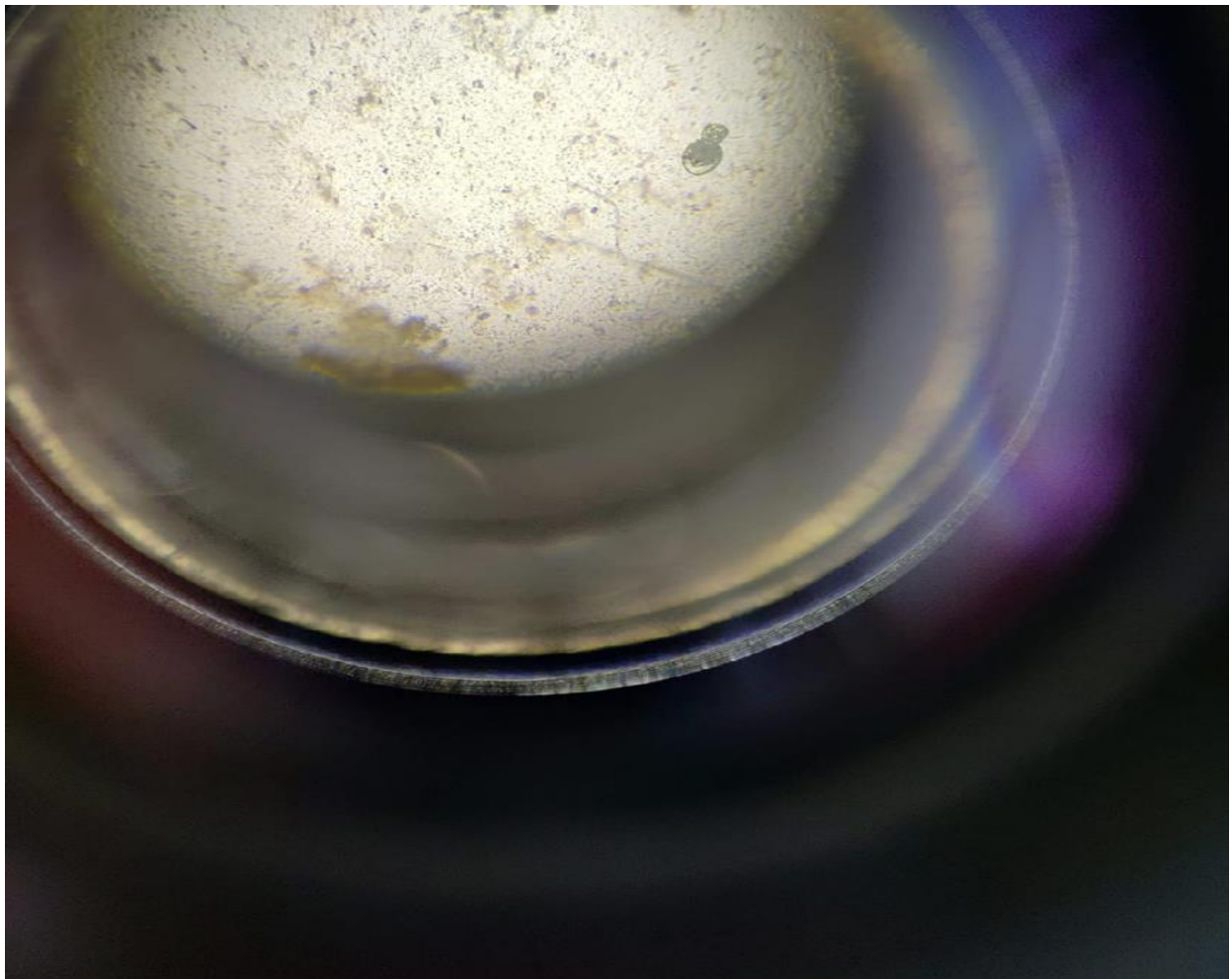


Fig.9. Hatched egg seen after 48 hrs of incubation

Table 8: Percentage reduction in EPG of control group

Calf no.	EPG (0 day)	EPG (14th day)	% reduction
1	100	100	0
2	50	0	100
3	50	150	-200
4	150	100	33
5	100	150	-50
6	50	100	-100
7	200	150	25%
8	100	100	0
9	150	200	-33
10	100	150	-50
	1050	1200	-14%
	9	Less than or equal to 90%	
	1	More than 90%	

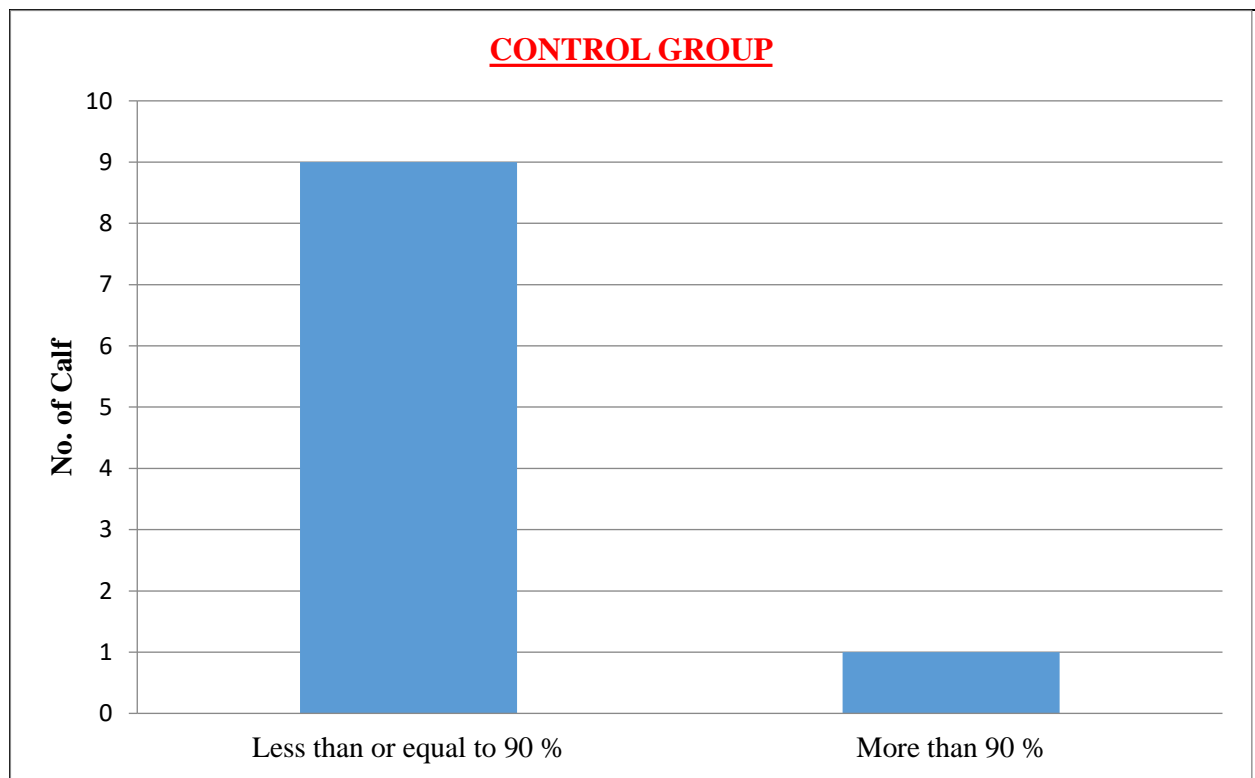


Fig. 10. Graphical representation of % reduction in EPG after 14 days in control group.

Table 9: Percentage reduction in EPG of T1 group after treatment (given fenbendazole)

Calf No.	EPG (0 day)	EPG (14th day)	% reduction
1	100	50	50%
2	250	150	40%
3	100	0	100%
4	200	50	75%
5	100	50	50%
6	50	0	100%
7	150	100	33%
8	200	100	50%
9	100	50	50%
10	150	50	67%
	1400	600	57%
	8	Less than or equal to 90 %	
	2	More than 90 %	

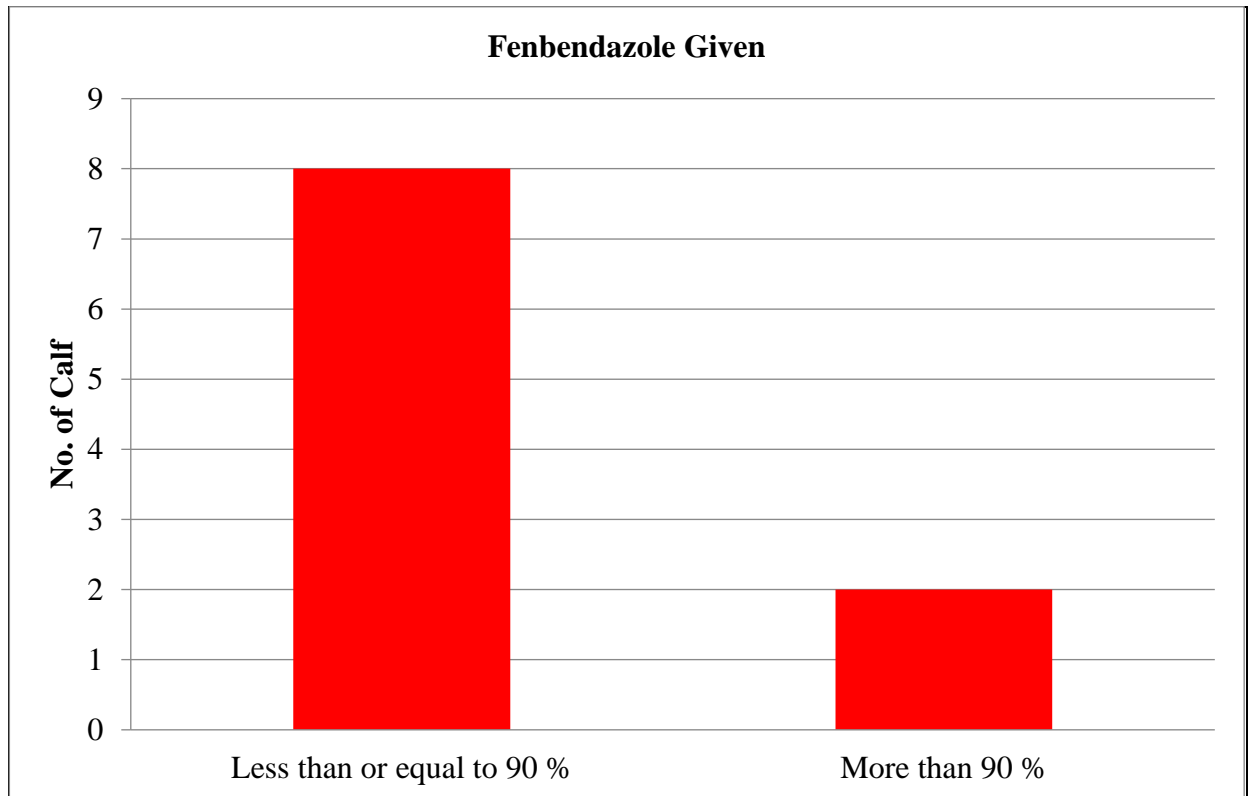


Fig. 11. Graphical representation of % reduction in EPG after 14 days in T1 group.

Table 10: Comparison between observed and expected values of % reduction in EPG of control group and T1 group.

Observed	No. of calves in which EPG reduction- less than or equal to 90%	No. of calves in which EPG reduction- More than 90%
Control group	9	1
Fenbendazole Gives	8	2
	17	3
Expected	No. of calves in which EPG reduction- less than or equal to 90%	No. of calves in which EPG reduction- More than 90%
Control group	8.5	1.5
Fenbendazole Gives	8.5	1.5

Table 11: Value of chi square for fenbendazole.

Chi square calculated =	0.4	
Chi square under probability 0.05	3.841	
Difference =	-3.441	
As the calculated Chi square value is lesser than the Chi square value under probability 0.05, the % reduction in the EPG after 14 days is insignificant and the drug is <u>inefficient</u> .		

Table 12: Percentage reduction in EPG of T2 group after treatment (given ivermectin)

Calf No.	EPG (0 days)	EPG (14 days)	% reduction
1	100	50	50%
2	50	0	100%
3	150	50	67%
4	100	50	50%
5	200	50	75%
6	100	50	50%
7	100	0	100%
8	200	100	50%
9	50	0	100%
10	150	100	33%
	1200	450	63%
	7	Less than or equal to 90 %	
	3	More than 90 %	

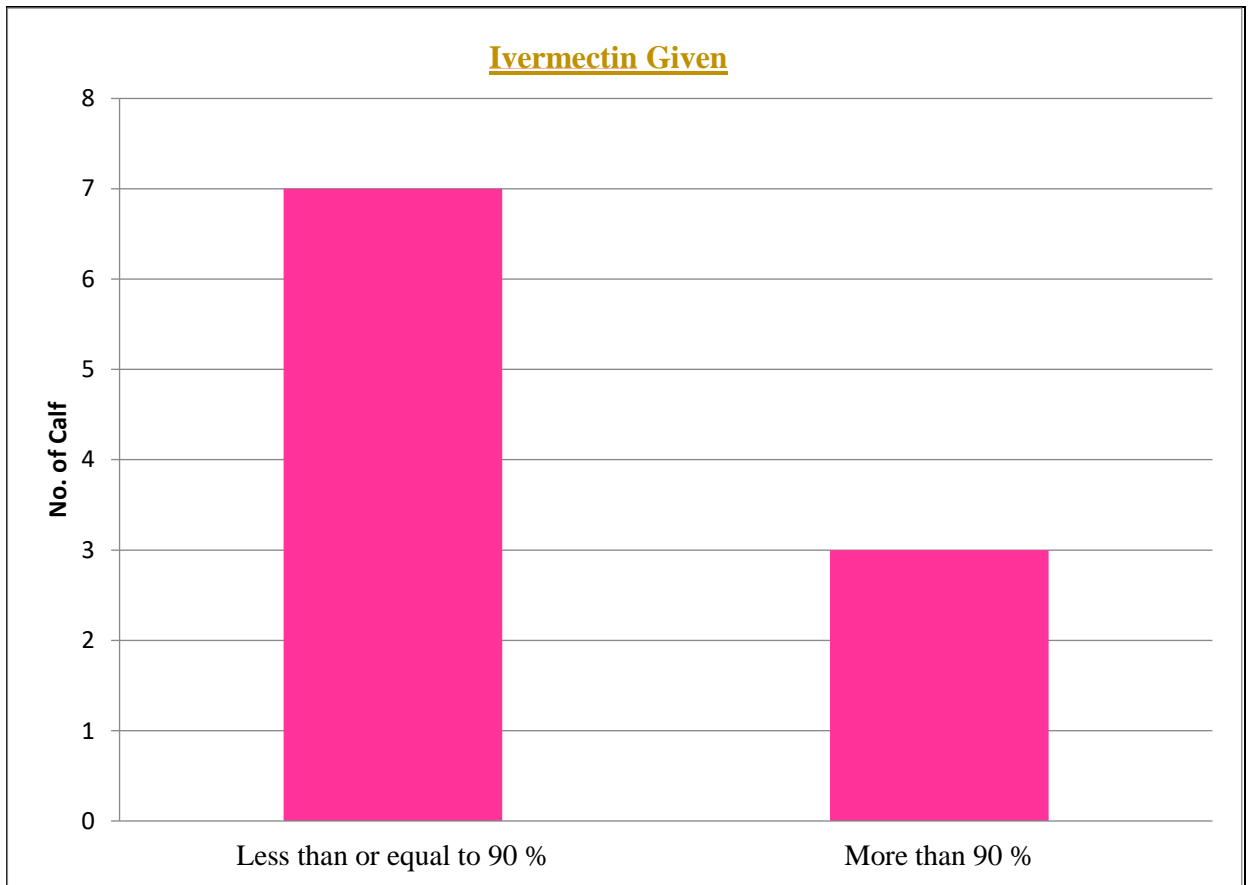


Fig. 12. Graphical representation of % reduction in EPG after 14 days in T2 group.

Table 13: Comparison between observed and expected values of % reduction in EPG of control group and T2 group.

<u>Observed</u>	No. of calves in which EPG reduction- less than or equal to 90%	No. of calves in which EPG reduction- More than 90%
Control group	9	1
Ivermectin Given	7	3
	16	4
<u>Expected</u>	No. of calves in which EPG reduction- less than or equal to 90%	No. of calves in which EPG reduction- More than 90%
Control group	8	2
Ivermectin Given	8	2

Table 14: Value of chi square for ivermectin.

Chi square calculated =	1.26
Chi square under probability 0.05	3.841
Difference =	-2.581
As the calculated Chi square value is lesser than the Chi square value under probability 0.05, the % reduction in the EPG after 14 days is insignificant and the drug is inefficient .	

Table 15: Percentage reduction in EPG of T3 group after treatment (given Levamisole)

Calf No.	EPG (0 days)	EPG (14 days)	% reduction
1	100	0	100%
2	50	0	100%
3	200	50	75%
4	100	50	50%
5	150	50	67%
6	200	50	75%
7	100	0	100%
8	150	50	67%
9	50	0	100%
10	150	50	67%
	1250	300	76%
	6	Less than or equal to 90 %	
	4	More than 90 %	

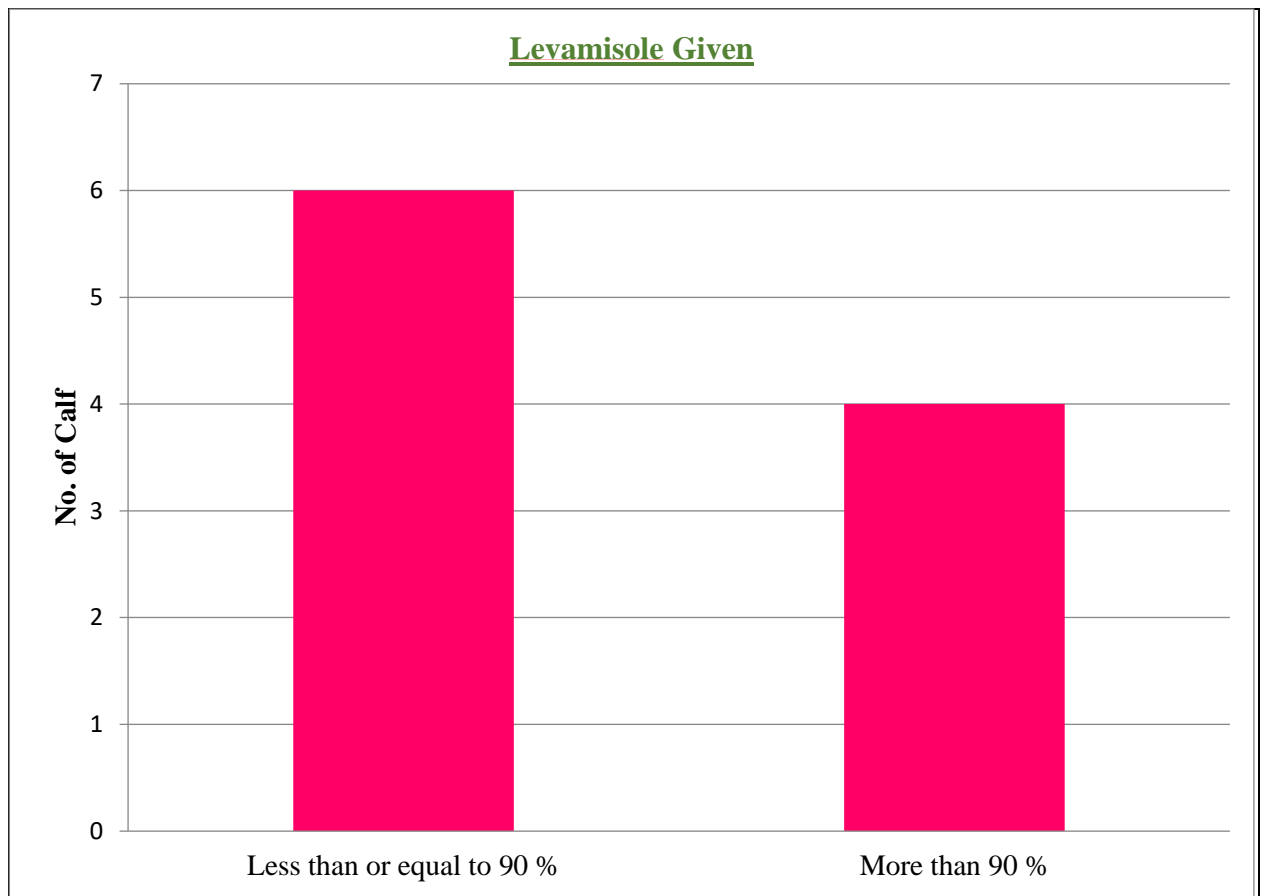


Fig. 13. Graphical representation of % reduction in EPG after 14 days in T3 group.

Table 16: Comparison between observed and expected values of % reduction in EPG of control group and T3 group.

<u>Observed</u>	No. of calves in which EPG reduction- less than or equal to 90%	No. of calves in which EPG reduction- More than 90%
Control group	9	1
Levamisole Given	6	4
	15	5
<u>Expected</u>	No. of calves in which EPG reduction- less than or equal to 90%	No. of calves in which EPG reduction- More than 90%
Control group	7.5	2.5
Levamisole Given	7.5	2.5

Table 17: Value of chi square for Levamisole.

Chi square calculated =	2.4
Chi square under probability 0.05	3.841
Difference =	-1.441
As the calculated Chi square value is lesser than the Chi square value under probability 0.05, the % reduction in the EPG after 14 days is insignificant and the drug is <u>inefficient</u> .	

Table 18: Difference in values of chi square for three drugs.

<u>Drugs Given</u>	<u>Fenbendazole</u>	<u>Ivermectin</u>	<u>Lavamosole</u>
Difference in the calculated chi square value and the chi square value under probability 0.05	-3.441	-2.581	-1.441
Note:- From the above table, it can be observed that the value of difference in the calculated Chi square value and the Chi square value under probability 0.05 is negative for all three drugs, it is understood that all are non-efficient.			

5

SUMMARY AND CONCLUSION

SUMMARY:

The research is focused mainly on "Helminth Infestation and Anthelmintic Resistance in calves". Since it is known that helminth infestation in calves is a common problem and its spread is also very high. In rural areas or farms, helminth infestation occurs through various means in the calves, especially in young calves. Due to this, the health of the calves does not develop properly and in many cases the calves die. The development of healthy generation is also stunted due to helminth infestation. As a result, the livestock owners have to suffer a lot of economic loss.

Although treatment for helminth infestation is readily available in veterinary science; but what is of great concern is that over time the effectiveness of the major anthelmintic drugs such as fenbendazole, ivermectin and levamisole are eroding. Of greater concern is that helminth is developing resistance to these drugs. The results of research clearly indicate that fenbendazole, ivermectin and levamisole have very low efficacy at normal concentrations (1µg/ml and 5µg/ml) and resistance has also developed against them.

In this research, there were 4 groups of 40 infected calves setting in the number of 10-10 in this way- 1. Control group 2. T1 3. T2 and 4. T3. No any medicine was given to the control group for treatment. Groups T1, T2 and T3 were given fenbendazole, ivermectin and levamisole respectively. After that, on day zero and on day 14th, the efficacy of these popular anthelmintic drugs was checked through EPG (Egg Per gram) and FECRT (Faecal egg count reduction test). The EPG value was calculated before and after the treatment for calves found infected. The value of FECRT was driven by means of percent reduction in the value of EPG. It is known that anthelmintic drugs are not effective when the percentage value of FECRT is less than 90%. And in research, FECRT values were found for fenbendazole, ivermectin and levamisole to be 58.33%, 65% and 84.21%, respectively, indicating that these anthelmintic drugs are becoming less effective.

Similarly, in the next phase of my research, using the method of EHA (Egg Hatch Assay), it was the aim to find out the level of developing resistance in helminths against these three anthelmintic drugs. At three different **concentrations (concentration1, concentration2 and concentration3)** of these three medicines were used whose quantities were 1 µg/ml, 5µg/ml and

10 µg/ml respectively. Helminths were found to develop resistance to **concentration1** (1µg/ml) of all three drugs. Resistance to **concentration2** (5µg/ml) of fenbendazole and levamisole were found.

Despite all this, some positive and encouraging results have also emerged from research. In EHA, it was found that the resistance against the **concentration3** (10µg/ml) of these drug was not developed in all the three anthelmintic drugs and these medicines are still effective. In EHA, it was also found that the resistance against the **concentration2** (5µg/ml) of levamisole did not develop.

Therefore, the results of research can be proved to be very helpful in dealing with helminth infestation and anthelmintic resistant in future. It is need of time to pay more attention to the dosage and concentrations of anthelmintic drugs while treating the helminth infestation so that the spread of helminth can be controlled and anthelmintic resistance also does not develop.

CONCLISION:

- It has been found that there is a rampant helminth infestation in calves.
- The prevalence of helminth infestation was found to be 74.07%.
- Only levamisole (84.21%) was found moderately effective and other two drugs fenbendazole (58.33%) and ivermectin (65%) were found resistant.

FUTURE SCOPE:

- Despite the development of strategies to minimize the development of anthelmintic resistance, the development of novel molecules for use in livestock should remain apriority.
- AR caused by a change in the gene frequency of that population resulting from drug selection ([Blackhall et al., 1998](#)). So, working on molecular lever might be beneficial

6

BIBLIOGRAPHY

- Abbas, A., R. Z. Abbas, M. K. Khan, M. A. RAZA, M.S. Mahmood, M. K. Saleemi, T. Hussain, J. A. Khan, Z.D. Sindhu (2019): Anticoccidial effects of *Trachyspermum ammi* (Ajwain) in broiler chickens. *Pak. Vet. J.* 39, 301-304. DOI: 10.29261/pakvetj/2019.056
- Abbas, A., R. Z. Abbas, S. Masood, Z. Iqbal, M. K. Khan, M. K. Saleemi, M. A. Raza, M. S. Mahmood, J. A. Khan, Z.D. Sindhu (2018): Acaricidal and insecticidal effects of essential oils against ectoparasites of veterinary importance. *Bol. Latinoamerican. Y. Del Caribe. De. Plant. Med. Y. Aromát.* 17, 441-452.
- Abbas, A., Z. Iqbal, R. Z. Abbas, M. K. Khan, J. A. Khan (2017b): Immunomodulatory activity of *Pinus radiata* extract against coccidiosis in broiler chicken. *Pak. Vet. J.* 37, 145-149.
- Abbas, A., Z. Iqbal, R. Z. Abbas, M. K. Khan, J.A. Khan, Z. D. Sindhu, M. S. Mahmood, M. K. Saleemi (2017a): In vivo anticoccidial effects of *Beta vulgaris* (sugar beet) in broiler chickens. *Microb. Path.* 111, 139-144. DOI: 10.1016/j.micpath.2017.07.052
- Ahmed, M. M, M. W. Akram, M. H. N. Tahir, S. B. Hussain, J. Ali, A.A. Keerio, A. Abbas (2020): Genetics and association of yield contributing characters T. Rehman et al.: Anthelmintic efficacy of *Citrullus colocynthis* 314 *Vet. arhiv* 91 (3), 309-318, 2021 for achene yield in sunflower (*Helianthus annuus* L.). *Agrobiol. Records.* 1, 26-30. DOI:10.47278/journal.abr/2020.005.
- Amarante, A.F.T., 2014. *Os parasitas de ovinos*, first ed. Unesp, São Paulo. Anziani, O.S., Suarez, V., Guglielmone, A.A., Warnke, O., Grande, H., Coles, G.C., 2004.
- Anon. *The World Factbook*. CIA Directorate of Intelligence. 2007. Available from: <http://www.cia.gov/ciapublicatins/pubs.html> (Accessed: March 2007).
- Baiak, B.H.B., Lehnen, C.R., and da Rocha, R.A., 2018. Anthelmintic resistance in cattle: A systematic review and meta-analysis. *Livestock Science* 217 (2018), 127-135.
- Barnes, E.H., Dobson, R.J., Barger, I.A., 1995. Worm control and anthelmintic resistance: adventures with a model. *Parasitol. Today.* 11, 56–63.
- Batool. M., S. Nasir, A. Rafiq, I. Yousaf, M. Yousaf (2019): Prevalence of tick infestation in farm animals from Punjab, Pakistan. *Pak. Vet. J.* 39, 406-410. DOI: 10.29261/pakvetj/2019.089.

- Blackhall, W.J., Liu, H.Y., Xu, M., Prichard, R.K., Beech, R.N., 1998. Selection at a P-glycoprotein gene in ivermectin and moxidectin-selected strains of *Haemonchus contortus*. *Mol. Bioch. Parasitol.* 95, 193–201.
- Borges, F.A., G.D. Almeida., R.P Heckler., R.T. Lemes., M.K. Onizuka., D.G. Borges, D.G. Anthelmintic resistance impact on tropical beef cattle productivity: effect on weight gain of weaned calves. *Trop. Anim. Health Prod*, 45(3), 723-7 (2013).
- Borges, S.L., Oliveira AA, Mendonça LR, Lambert SM, Viana JM, Nishi SM, et al. Resistência anti-helmíntica em rebanhos caprinos nos biomas Caatinga e Mata Atlântica. *Pesq Vet Bras.* 2015;35:643–8.
- Borgsteede, F.H., Geerts S, De Deken R, Kumar V, Brandt J (1992) Studies on an *Ostertagia ostertagi* strain suspected to be resistant to benzimidazoles. *Vet Parasitol* 41:85–92.
- Burke J. Management of Barber pole Worm in Sheep and Goats in the Southern U.S. Booneville AR. Dale Bumpers Small Farms Research Update, n. p. 2005.
- Calvete, C., Calavia, R., Ferrer, L.M., Ramos, J.J., Lacasta, D. and Uriarte, J. 2012. Management and environmental factors related to benzimidazole resistance in sheep nematodes in Northeast Spain. *Vet. Parasitol.* 184: 193-203.
- Campbell, W.C. and Benz, G.W., 1984. Ivermectin: a review of efficacy and safety. *J. Vet. Phannacol. Ther.* 7, 1–16.
- Castro, G.A., 1996. Helminth: structure, classification, growth and development. In S. Baron (Ed.), *Medical Microbiology* (fourth ed.), University of Texas Medical Branch, Galveston, Texas. <http://www.ncbi.nlm.nih.gov/books/NBK8282>
- Čerňanská D, Várady M, Čudeková P, Čorba J. Worm control practices on sheep farms in the Slovak Republic. *Veterinary Parasitology.* 2008; **154**:270-276. DOI: 10.1016/j.vetpar.2008.03.026.
- Charlier J, Morgan ER, Rinaldi L, van Dijk J, Demeler J, Höglund J, et al. Practices to optimize gastrointestinal nematode control on sheep, goat and cattle farms in Europe using targeted (selective) treatments. *Vet Rec.* 2014; 175:250–5.
- Charlier J, Thamsborg SM, Bartley DJ, Skuce PJ, Kenyon F, Geurden T, et al. Mind the gaps in research on the control of gastrointestinal nematodes of farmed ruminants and pigs. *Transbound Emerg Dis.* 2018;65 (Suppl. 1):217–34.
- Coles, G.C., Bauer C, Borgsteede FH, Geerts S, Klei TR, Taylor MA, et al. World Association for the Advancement of veterinary parasitology (W.A.A.V.P.) methods for the detection

- of anthelmintic resistance in nematodes of veterinary importance. *Vet Parasitol.* 1992; 44:35–44.
- Coles, G.C., Bauer, C., Borgsteede, F.H., Geerts, S., Klei, T.R., Taylor, M.A. and Waller, P.J. World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) methods for the detection of anthelmintic resistance in nematodes of veterinary importance. *Vet Parasitol*, 44, 35-44 (1992).
- Craven, J., Bjorn H, Barnes EH, Henriksen SA, Nansen P (1999). A comparison of in vitro tests and a faecal egg count reduction test in detecting anthelmintic resistance in horse strongyles. *Vet Parasitol* 85:49–59.
- da Cruz DG, da Rocha LO, Arruda SS, Palieraqui JG, Cordeiro RC, Santos E Jr, et al. Anthelmintic efficacy and management practices in sheep farms from the state of Rio de Janeiro, Brazil. *Vet Parasitol.* 2010; 170:340–3.
- Deepa, C.K. and Devada, K. 2007. Anthelmintic resistance in GI nematodes of goats. *J. Vet. Anim. Sci.* 38: 52-54.
- Demeler, J. et al. (2010) Adaptation and evaluation of three different in vitro tests for the detection of resistance to anthelmintics in gastro intestinal nematodes of cattle. *Vet. Parasitol.* 170, 61–70.
- Devi, K., S. Vasantha, P. Manogar, R. Kalpana (2014): In vitro anthelmintic activity of *Actinopteris radiata* (sw.) link. *Afric. J. Sci. Res.* 3, 9-11.
- Easwaran C, Harikrishnan TJ, Raman M. Multiple anthelmintic resistance in gastrointestinal nematodes of sheep in Southern India. *Veterinarski Arhiv.* 2009; 79:611-620.
- Edmonds, M.D., Johnson, E.G., Edmonds, J.D., 2010. Anthelmintic resistance of *Ostertagia ostertagi* and *Cooperia oncophora* to macrocyclic lactones in cattle from the western United States. *Vet. Parasitol.* 170, 224–229.
- Fayaz, M. R., R. Z. Abbas, A. ABbbas, M. K. Khan, M. A. Raza, M. Israr, J. A. Khan, M. S. Mahmood, M. K. Saleemi, T. Rehman, M. A. Zaman, Z. D. Sindhu (2019): Potential of botanical driven essential oils against *Haemochus contortus* in small ruminants. *Bol Latinoam. Caribe. Plant. Med. Aromat.* 18, 533-543. DOI: 10.35588/blacpma.19.18.6.36.
- Fikru, R., S. Teshale, D. Reta, and K. Yosef, “Epidemiology of gastrointestinal parasites of ruminants in western Oromia, Ethiopia,” *International Journal of Applied Research in Veterinary Medicine*, vol. 4, pp. 51–57, 2006.
- Gasbarre, L.C. (2014) Anthelmintic resistance in cattle nematodes in the US. *Veterinary Parasitology* 204, 3–11.

- Gasbarre, L.C. et al. (2009) The identification of cattle nematode parasites resistant to multiple classes of anthelmintics in a commercial cattle population in the US. *Vet. Parasitol.* 166, 281–285.
- Gasbarre, L.C., Smith, L.L., Hoberg, E., Pilitt, P.A., (2009a). Further characterization of a cattle nematode population with demonstrated resistance to current anthelmintic. *Vet. Parasitol.* 166, 275–280.
- GMC/Mercosul. Resolução 76/1996 – Regulamento Técnico para Registro de Produtos Antiparasitários de Uso Veterinário. Brasília: Grupo Mercado Comum; 1996.
- Gupta A, Dixit A.K, Dixit P, Mahajan C. Prevalence of gastrointestinal parasites in cattle and buffaloes in and around Jabalpur, Madhya Pradesh. *J. Vet. Parasitol.* 2012;26(2):186–188.
- Hanna, R.E.B., Edgar, H.W.J., McConnell, S., Toner, E., McConville, M., Brennan, G.P., Devine, C., Flanagan, A., Halferty, L., Meaney, M., Shaw, L, Moffett, D., McCoy, M., Fairweather, I., 2010. *Fasciola hepatica*: histological changes in the reproductive structures of triclabendazole (TCBZ)-sensitive and TCBZ-resistant flukes after treatment in vivo with TCBZ and the related benzimidazole derivative. *Compound Alpha. Vet. Parasitol.* 168, 240-254.
- Haryuningtyas, D., Beriajayadan, G.D., Gray. Resistensiantelmentikagolonganbenzimidazolepadadomba dankambing di Indonesia. *Pros. Seminar Nasional TeknologiPeternakandanVeteriner. Bogor. PuslitbangPeternakan, Bogor*, pp. 509 – 518. (2001).
- Hepworth, K., M. Neary, and T. Hutchens, “Managing intestinal parasitism in sheep and goats,” *Purdue Extension AS- 573-W*, 2015, [https://www.extension.org/mediawiki/files/8/8a/purdue parasite control.pdf](https://www.extension.org/mediawiki/files/8/8a/purdue%20parasite%20control.pdf).
- Howell, S.B., J.M. Burke, J. E. Miller et al., “Prevalence of anthelmintic resistance on sheep and goat farms in the southeastern United States,” *Journal of the American Veterinary Medical Association*, vol. 233, no. 12, pp. 1913–1919, 2008.
- Humbert, J.F., Cabaret J, Elard L, Leignel V, Silvestre A. Molecular approaches to studying benzimidazole resistance in trichostrongylid nematode parasites of small ruminants. *Vet Parasitol.* 2001;101:405–14.
- Hussain, K., Z. Iqbal, R. Z. Abbas, M. K. Khan, M. K. Saleemi (2017): Immunomodulatory activity of *Glycyrrhiza glabra* extract against mixed *Eimeria* infection in chickens. *Int. J. Agric. Biol.* 19, 928–932. DOI: 10.17957/IJAB/15.0397.

- Idris, M, R. Z. Abbas, S. Masood, T. Rehman, U. Farooq, W. Babar, R. Hussain, A. Raza, U. Riaz, T. Rehman et al.: Anthelmintic efficacy of *Citrullus colocynthis* Vet. arhiv 91 (3), 309-318, 2021 315 (2017): The potential of antioxidant rich essential oils against avian coccidiosis. World. Poult. Sci. J. 73, 89-104. DOI: 10.1017/S0043933916000787.
- Ijaz, M., M. A. Zaman, F. Mariam, S. H. Farooqi, A. I. Aqib, S. Saleem, A. Ghaffar, A. Ali, R. Akhtar (2018): Prevalence, hematology and chemotherapy of gastrointestinal helminths in camels. Pak. Vet. J. 38, 81-85. DOI: 10.29261/pakvetj/2018.016.
- Jackson, F. and Coop RL. The development of anthelmintic resistance in sheep nematodes. Parasitology. 2001; **120**:95-107. DOI: 10.1017/ S0031182099005740.
- Kaplan, R.M. and Vidyashankar, A.N. (2012) An inconvenient truth: global warming and anthelmintic resistance. *Vet. Parasitol.* 186, 70–78.
- Kaplan, R.M., 2002. Anthelmintic resistance in nematodes of horses. *Vet. Res.* 33, 491–507.
- Kashyap Z, Sisodia R.S, Shukla P.C. Incidence of gastrointestinal parasites in cattle and buffaloes in Malwa region of Madhya Pradesh. *Haryana Vet.* 1997;36:34–36.
- Keegan, J.D., Keane, O.M., Good, B., De Waal, T., Denny, M., Hanrahan, J.P., Fitzgerald, W., Sheehan, M., 2017. A nationwide survey of anthelmintic treatment failure on sheep farms in Ireland. *Ir. Vet. J.* <http://dx.doi.org/10.1186/s13620-017-0086-9>.
- Khajuria, J. K., R. Katoch, A. Yadav, R. Godara, S. K. Gupta, A. Singh (2013): Seasonal prevalence of gastrointestinal helminths in sheep and goats of middle agro-climatic zone of Jammu province. *J. Parasit. Dis.* 37, 21-25. DOI: 10.1007/s12639-012-0122-3.
- Khater, H. F., A. M. Ali, G. A. Abouelella, M. A. Marawan, M. Govindarajan, K. Murugan, R. Z. Abbas, N. P. Vaz, G. Benelli (2018): Toxicity and growth inhibition potential of vetiver, cinnamon, and lavender essential oil and their blends against larvae of the sheep blowfly, *Lucillia sericata*. *Int. J. Dermatol.* 57, 449-457. DOI: 10.1111/ijd.13828.
- Khater, H. F., H. Ziam, A. Abbas, R. Z. Abbas, M. A. Raza, K. Hussain, E. Z. Younis, I. T. Radwan, A. Selim (2020): Avian coccidiosis: Recent advances in alternative control strategies and vaccine development. *Agrobiol. Records.* 1, 11-25. DOI: 10.47278/journal.abr/2020.004.
- Kochapakdee, S., Pandey, V.S., Pralomkarm, W., Choldumrongkul, S., Ngampongsai, W. and Lawpetchara, A. (1995) Anthelmintic resistance in goats in Southern Thailand. *Veterinary Record* 137, 124-125.
- Kohler, P., (2001) The biochemical basis of anthelmintic action and resistance. *International Journal for Parasitology.* **31**:336-345.

- Kudo, N., T. Yoshioka., Y. Watanabe., Y. Terazono., S. Takenouchi., T. Donomoto., K. Nakajima., K. Hitosugi. R. Tsukada., H. Ikadai and T. Oyamada. Reduced Efficacy of Ivermectin Treatments in Gastrointestinal Nematode Infections of Grazing Cattle in Japan. *J. Vet. Med. Sci*, 76(11), 1487–1491 (2014).
- Lacey, E., Gill, J.H., Biochemistry of benzimidazole resistance. *Acta Trop.* 1994; 56:245–62.
- Lawal, O. A. and Adebawale, 2012 “Dynamics of ruminant livestock management in the context of the Nigerian agricultural system,” in *Livestock Production*, K. Javed, Ed., InTech,Rijeka, Croatia.
- Leathwick, D.M., 2012. Modelling the benefits of a new class of anthelmintic in combination. *Vet. Parasitol.* 186, 93–100.
- Leathwick, D.M. and Luo, D., 2017. Managing anthelmintic resistance—Variability in the dose of drug reaching the target worms influences selection for resistance? *Vet. Parasitol* 243, 29–35.
- Lespine, A., Chartier, C., Hoste, H., Alvinerie, M., 2012. Endectocides in goats: Pharmacology, efficacy and use conditions in the context of anthelmintics resistance. *Small Ruminants. Res.* 103, 10–17.
- LI, K., H. Luo, K. Mehmood, H. Zhang, M. Shahzad, R. Z. Abbas (2019a): Sarcosporidiosis: An emerging disease in Yaks (*Bos grunniens*) on the Qinghai Tibetan Plateau (QTP), China. *Acta. Parasitologica.* 64, 246-250. DOI: 10.2478/s11686-019-00032-0.
- LI, K., H. LUO, K. Mehmood, M. Shahzad, J. LI (2020): Exploring the potential parasitic pathogens causing diarrheal death to yak calves with bloody excrement through high-throughput sequencing. *Agrobiol. Records.*1, 1-5. DOI: 10.47278/journal.abr/2020.002
- LI, K., L. Zhang, H. Luo, K. Mehmood, M. Shahzad, M. U. Naseer (2019b): Besnoitiosis: An emerging parasitic disease in yaks (*Bos grunniens*) and tibetan sheep (*Ovis aries*) on the qinghai tibetan plateau, China. *Pak. Vet. J.* 39, 447-450. DOI: 10.29261/pakvetj/2019.086.
- Maciel, S., Giménez, A.M., Gaona, C., Waller, P.J., Hansen, J.W., 1996. The prevalence of anthelmintic resistance in nematode parasites of sheep in Southern Latin America:Paraguay. *Vet. Parasitol.* 62, 207–212.
- Mansour, T.E. *Chemotherapeutic Targets in Parasites: Contemporary Strategies.* Cambridge: Cambridge University Press; 2002. pp. 156-188.
- McMahon, C., Bartley, D.J., Edgar, H.W.J., Ellison, S.E., Barley, J.P., Malone, F.E., Hanna, R.E.B., Brennan, G.P., Fairweather, I., 2013. Anthelmintic resistance in Northern Ireland

- (I): prevalence of resistance in ovine gastrointestinal nematodes, as determined through faecal egg count reduction testing. *Vet. Parasitol.* 195, 122–130.
- Mehmood, K., H. Zhang, A. J. Sabir, R. Z. Abbas, M. Ijaz, A. Z. Durrari, M. H. Saleem, M. U. Rehman, M. K. Iqbal, Y. Wang, H. I. Ahmad, T. Abbas, R. Hussain, M. T. Ghori, S. Ali, A. U. Khan, J. LI (2017): A review on epidemiology, global prevalence and economical losses of fasciolosis in ruminants. *Microb. Path.* 109, 253-262. DOI: 10.1016/j.micpath.2017.06.006.
- Monteiro, S.G., *Parasitologia na medicina veterinária*. 1st ed. São Paulo: ROCA; 2011.
- Nasir, A., M. A. Zaman, A. Shakoor, T. U. Rehman, I. Khan, S. Ehtisham-ul-haque, A. Sikandar, S. Masood, A. Akbar (2018): Prevalence of *Neospora caninum* using milk and serum ELISA and its hematological effect in dairy buffaloes. *Pak. Vet. J.* 38, 281-285. DOI: 10.29261/pakvetj/2018.039.
- Nielsen, M.K., *Internal Parasite Screening and Control*. Robinson's Current Therapy in Equine Medicine (seventh edition) 2015, 336-340. <https://doi.org/10.1016/B978-1-4557-4555-5.00077-7>.
- Prichard RK. Mechanisms of anthelmintic resistance: implications for the future of parasite control. *Proc. 15th Congr. Bras.Parasit. Vet. Brazil.* 2008;21:62–8.
- Qadir, S., A. K. Dixit, P. Dixit (2010): Use of medicinal plants to control *Haemonchus contortus* infection in small ruminants. *Vet. World.* 3, 515-518. DOI: 10.5455/vetworld.2010.515-518.
- R.Verma., K. Lata and G. Das. An overview of anthelmintic resistance in gastrointestinal nematodes of livestock and its management: India perspectives. *International Journal of Chemical Studies*, 6(2), 1755-1762 (2018).
- Rajagopal, A., Sabu, L., Devada, K., Radhika, R. and Gleeja V.L. (2017): Assessment of benzimidazole resistance status in an organized goat farm by egg hatch assay. *International Journal of Science, Environment and Technology*, Vol.6, No.3, 2017. 2112-2117.
- Roos, M.H., Boersema, J.H., Borgsteede, F.H., Cornelissen, J., Taylor, M., Ruitenber, E.J. Molecular analysis of selection for benzimidazole resistance in the sheep parasite *Haemonchus contortus*. *Mol Biochem Parasitol.* 1990;43:77–88.
- Sangster, N.C., 1999. Pharmacology of anthelmintic resistance in cyathostomes: will it occur with the avermectin/milbemycins? *Vet. Parasitol.* 85, 189–204.

- Sangster, N.C., Cowling A, Woodgate RG. Ten events that defined anthelmintic resistance research. *Trends in Parasitology*. 2018; **34**:553-563. DOI: 10.1016/j.pt.2018.05.001.
- Sargison, N., Wilson, D., Scott, P., (2009). Relative inefficacy of pour-on macrocyclic lactone anthelmintic treatments against *Cooperia* species in Highland calves. *Vet. Rec.* 164, 603-604.
- Shalaby, H. A., “Anthelmintic resistance; how to overcome it?” *Iranian Journal of Parasitology*, vol. 8, no. 1, pp. 18–32, 2013.
- Silvestre, A., Leignel V, Berrag B, Gasnier N, Humbert JF, et al. (2002) Sheep and goat nematode resistance to anthelmintics: pro and cons among breeding management factors. *Vet Res* 33: 465-480.
- Singh, G., R. Singh, P. K. Verma, R. Singh, A. Anand (2015): In vitro anthelmintic efficacy of aqueous extract of *Scindapsus officinalis* (Roxb) schott fruits against *Haemonchus contortus* of small ruminants. *Proc. Natl. Acad. Sci. India. Sect. B. Biol. Sci.* 85, 705-709. DOI: 10.1007/s40011-014-0389-5.
- Soutello, R.G.V., Seno, M.C.Z., Amarante, A.F.T., (2007). Anthelmintic resistance in cattle nematodes in northwestern Sao Paulo state, Brazil. *Vet. Parasitol.* 148, 360–364.
- Speedy, A., (1992). *Progress in sheep and goat research*. CAB Int Wallingford, Oxon, 179-188.
- Stafford, K., Morgan, E., Coles, G., 2010. Sustainable anthelmintic use in cattle. *Vet. Rec.* 167, 309.
- Suarez, V.H. and Cristel, S.L., (2007). Anthelmintic resistance in cattle nematode in the western Pampeana Region of Argentina. *Vet. Parasitol.* 144, 111–117.
- Sutherland, I.A. and Scott, I., (2009) Anthelmintics. In *Gastrointestinal Nematodes of Sheep and cattle: Biology and Control*, pp. 95–116, Wiley- Blackwell.
- Sutherland, I.A., Leathwick, D.M., 2011. Anthelmintic resistance in nematode parasites of cattle: a global issue? *Trends. Parasitol* 27, 176–181.
- Taylor, M.A. et al. (2002) Anthelmintic resistance detection methods. *Vet. Parasitol.* 103, 183–194.
- Torres-Acosta JFJ, Mendoza-De- Gives P, Aguillar-Caballero AJ, Cuellarordaz JA. Anthelmintic resistance in sheep farms: Update of the situation in the American continent. *Veterinary Parasitology*. 2012; **189**:89-96.
- Van Wyk, J.A., Stenson, M.O., Van der Merwe, J.S., Vorster, R.J., Viljoen, P.G., 1999. Anthelmintic resistance in South Africa: surveys indicate an extremely serious situation in sheep and goat farming. *Onderstepoort J. Vet. Res.* 66, 273–284.

- Varshney, T.R. and Y.P. Singh. 1976. A note on the development of resistance of *Haemonchus contortus* worms against phenothiazine and thiabendazole in sheep. *Ind. J. Anim. Sci.* 46: 666-668.
- Vercruyse J, Charlier J, Van Dijk J, Morgan ER, Geary T, von Samson-Himmelstjerna G, et al. Control of helminth ruminant infections by 2030. *Parasitology.* 2018;145:1655–64.
- Veríssimo, C.J., Méo Niciura, S.C., Luz Alberti, A.L., Carvalho Rodrigues, C.F., Pacheco Barbosa, C.M., Chiebao, D.P., Cardoso, D., da Silva, G.S., Pereira, J.R., Margatho, L.F.F., Lopes Dias da Costa, R., Fernandes Nardon, R., Hayama Ueno, T.E., Magalhaes Curci, V.C., Molento, M.B., 2012. Multidrug and multispecies resistance in sheep flocks from São Paulo state, Brazil. *Vet. Parasitol.* 187, 209–216.
- Verma, R., Lata, K. and Das, G., An overview of anthelmintic resistance in gastrointestinal nematodes of livestock and its management: India perspectives. *International Journal of Chemical Studies*, 6(2), 1755-1762 (2018).
- Vijayasarithi, M.K., Sreekumar, C., Venkataramanan, R., Raman, M. Influence of sustained deworming pressure on the anthelmintic resistance status in strongyles of sheep under field conditions. *Tropical Animal Health and Production.* 2016;**48**:1455-1462.
- Waghorn, T.S., Leathwick DM, Rhodes AP, Jackson R, Pomroy WE, West DM, Moffat JR (2006a) Prevalence of anthelmintic resistance on 62 beef cattle farms in the North Island of New Zealand. *N Z Vet J* 54:278–282.
- Waghorn, T.S., Leathwick, D.M., Rhodes, A.P., Lawrence, K.E., Jackson, R., Pomroy, W.E., West, D.M., Moffat, J.R., (2006b). Prevalence of anthelmintic resistance on sheep farms in New Zealand. *N. Z. Vet. J.* 54, 271–277.
- Waller, P. J., “Anthelmintic resistance and the future for roundworm control,” *Veterinary Parasitology*, vol. 25, no. 2, pp. 177– 191, 1987.
- Williams, J.C. and Loyacano, A.F., 2001. Internal Parasite of Cattle in Louisiana and Other Southern States. Louisiana State University. Research Information Sheet 104: 1-19.
- Zafar, A., M. K. Khan, Z. U. D Sindhu, R. Z. Abbas, S. Masood, Z. Abbas, M. S. Mahmood, M. K. Saleemi, J. A.; Khan, R. Hussain, M. U. Naseer, Z. Iqbal, H. Javed (2019): Seroprevalence of *Fasciola hepatica* in small ruminants of District Chakwal, Punjab, Pakistan. *Pak. Vet. J.* 39, 96-100. DOI: 10.29261/pakvetj/2018.024.
- Zaman, M. A., Z. Iqbal, Z. U. D. Sindhu, R. Z. Abbas, M. F. Qamar (2017): An overview of plants with acaricidal and anthelmintic properties. *Int. J. Agric. Biol.* 19, 957–968. DOI: 10.17957/IJAB/15.0289

7

BRIEF RESUME OF STUDENT

Name : Pinky Rani
 Father's Name : Nemu Lal Ram
 Mother's Name : Urmila Devi
 Date of Birth : 06-07-1993
 Email : deepa4upcs@gmail.com
 Contact No. : 9128870084

Academic Qualification

Qualification	School / Collage	Board / University	Passing year	Percentage / CGPA
M.V. Sc. (Veterinary Medicine)	BIHAR VETERINARY COLLEGE, PATNA	BASU, PATNA	2021	8.104/10
B.V. Sc. & A.H.	BIHAR VETERINARY COLLEGE, PATNA	BASU, PATNA	2019	7.154/10
INTERMEDIATE	J.D. WOMEN'S COLLEGE, PATNA	BSEB, PATNA	2010	65.60 %
MATRIC	PATNA CENTRAL SCHOOL, PATNA	CBSE, DELHI	2008	76.33 %