" Studies on the prevalence of helminth parasites with special reference to *Gigantocotyle explanatum* in buffaloes in and around Begusarai district "

THESIS

SUBMITTED TO THE

BIHAR AGRICULTURAL UNIVERSITY

(FACULTY OF POST-GRADUATE STUDIES)

SABOUR (BHAGALPUR) BIHAR

IN PARTIAL FULFILLMENT OF THE REQUIREMENT

FOR THE DEGREE OF

MASTER OF VETERINARY SCIENCE

(Veterinary Parasitology)

By

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BIHAR VETERINARY COLLEGE

PATNA (BIHAR)

2017

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BIHAR VETERINARY COLLEGE, PATNA-800014
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CERTIFICATE-I

This is to certify that the thesis entitled **"Studies on the prevalence of helminth parasites with special reference to *Gigantocotyle explanatum* in buffaloes in and around Begusarai district "** submitted in partial fulfillment of the requirement for the award of **Master of Veterinary Science (VeterinaryParasitology)** of the Faculty of Post-Graduate Studies, Bihar Agricultural University, Sabour, (Bhagalpur), Bihar is the bona fide research work carried out by **Dr. Mritunjay Kumar, Registration No.: M/VPA/223/BVC/2014-15** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

This is further certified that the assistance and help received during the course of this investigation and preparation of this thesis have been fully acknowledged.

Dr. Pankaj Kumar
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CERTIFICATE-II

We the undersigned members of the advisory committee of **Dr. Mritunjay Kumar**, **Registration No.: M/VPA/223/BVC/2014-15,** a candidate for the award of degree of Master of Veterinary Science with major in **Veterinary Parasitology,** have gone through the manuscript of thesis and agree that the thesis entitled **"Studies on the prevalence of helminth parasites with special reference to *Gigantocotyle explanatum* in buffaloes in and around Begusarai district "** may be submitted by **Dr. Mritunjay Kumar**, in partial fulfillment of the requirement for the degree.

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CERTIFICATE-III

This is certified that the thesis entitled **"Studies on the prevalence of helminth parasites with special reference to *Gigantocotyle explanatum* in buffaloes in and around Begusarai district "**submitted by **Dr. Mritunjay Kumar**, **Registration No.: M/VPA/223/BVC/2014-15,** in partial fulfillment of the requirement for the Degree of **Master of Veterinary Science (Veterinary Parasitology)** of the faculty of Post-Graduate Studies, Bihar Agricultural University Sabour, Bhagalpur, Bihar was examined and approved on 15th Feb.2017.

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ACKNOWLEDGMENT

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 endeavour. Words alone are insufficient to convey my sincere feelings of indebtedness and fathomless gratitude towards my major advisor, ***Dr. Pankaj Kumar,*** *Major Advisor, Assistant Professor, Department of Veterinary Parasitology, Bihar Veterinary College, Patna for his learned guidance repleted with stimulating counsel, close supervision, constant encouragement, and healthy criticism.*

Heartiest gratefulness to express my profound sense of gratitude and sincere regards to members of my advisory committee ***Dr. S.R.P. Sinha,*** *University Professor and Head, Department of Veterinary Parasitology,* ***Dr. Purushottam Kaushik,*** *Assistant Professor, Department of Veterinary Public Health,* ***Dr. Pallav Shekhar,*** *Assistant Professor, Department of Veterinary Medicine,* ***Dr. Ramesh Kumar Singh,*** *Assistant Professor, Department of Animal Breeding and Genetics, Bihar Veterinary College, Patna for their valuable suggestion and sincere guidance which made this work possible in stipulated time.*

I am highly obliged to ***Dr. Archana, Nominee Dean PGS,*** *Assistant Professor, Department of Veterinary* ***Pharmacology and Toxicology****, Bihar Veterinary College, Patna for valuable suggestions and innumerable cooperation during the entire research work.*

I am particularly obliged and thankful to ***Dr. S. Samantaray****, Principal, Bihar Veterinary College, Patna for his parental care and suggestions as well providing necessary facilities during the research period.*

I would like to express my honest heartfelt gratitude to ***Dr. Sucheta Sinha,*** *J.R.A., Department of Veterinary Parasitology cum Nodal officer, PIM/ Placement Cell and* ***Dr. K. G. Mandal,*** *University Professor, Department of Animal Breeding and Genetics, Bihar Veterinary College Patna for their valuable suggestions, untiring help and rendering all necessary facilities to carry out the research work successfully.*

I am very thankful to ***Dr. Ajit Kumar*** *and* ***Dr. Anil Gattani*** *Assistant Professor Department of Biochemistry, Bihar Veterinary College, Patna for ever willing help rendered by them in critical moments of my research work. I am extremely grateful for valuable suggestions, keen interest, constant inspiration, generous help in extending necessary facilities and sincere guidance given by them during my research work.*

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

I greatly acknowledge many thanks to ***Dr. A. K. Das, Dr. Bipin Kumar, Dr. Ranveer Kumar, Dr. Anil Kumar,*** *Assistant Professor, Department of Veterinary Medicine and* ***Dr. Anjay****, Assistant Professor, Department of VPH, Bihar Veterinary College, Patna for their immense help during the course of investigation and thesis writing.*

I would also like to thank all the scientific and supporting staff members of Department of Veterinary Parasitology, Bihar Veterinary College, Patna for their support and encouragement during dark days of my research. Thanks are also due to campus librarian Dr. Deepak Kumar, Sri Naresh Yadav, Sri Sanjay Jee and all other library staff for taking the pain and rendering their help in consulting research Journals, text books etc.

I thank Bihar Agricultural University, Sabour, for providing financial assistance in the form of ***B.A.U. Fellowship*** *to me for pursuing Masters Degree at Bihar Veterinary College, Patna Bihar successfully. A deep sense of gratitude is also due to B.A.U., Sabour for providing facilities for completion of the present investigation.*

I must express my sincere thanks to ***Dr. Bamshankar Singh,*** *Block Animal Husbandry Officer and nearby buffalo owners of Patna namely Sri Gopal Prasad Singh, Sri Ajay Kumar, Sri Ramji Singh, Sri B. K. Roy and all other dog owners for their active cooperation during the research work.*

I am particularly grateful to my colleagues ***Dr. Deepak Kumar****,* ***Dr. Dharamveer Kumar,*** ***Dr. Alok Kumar,*** *and* ***Dr. Rajeev Kumar*** *for their extreme patience, moral support and emotional security rendered to me during the course of this study.*

I am flooded with deep emotions and blow of my head, expressing profound sense of gratitude to all members of my family especially my parents ***Mrs. Shanti Devi*** *and* ***Mr. Anil Prasad Singh,*** *younger brother* ***Mr. Vikash Kumar Singh*** *for their affectionate care, moral support, constant encouragement blessings, love, gracious sacrifice and inspiration to pursue higher education.*

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

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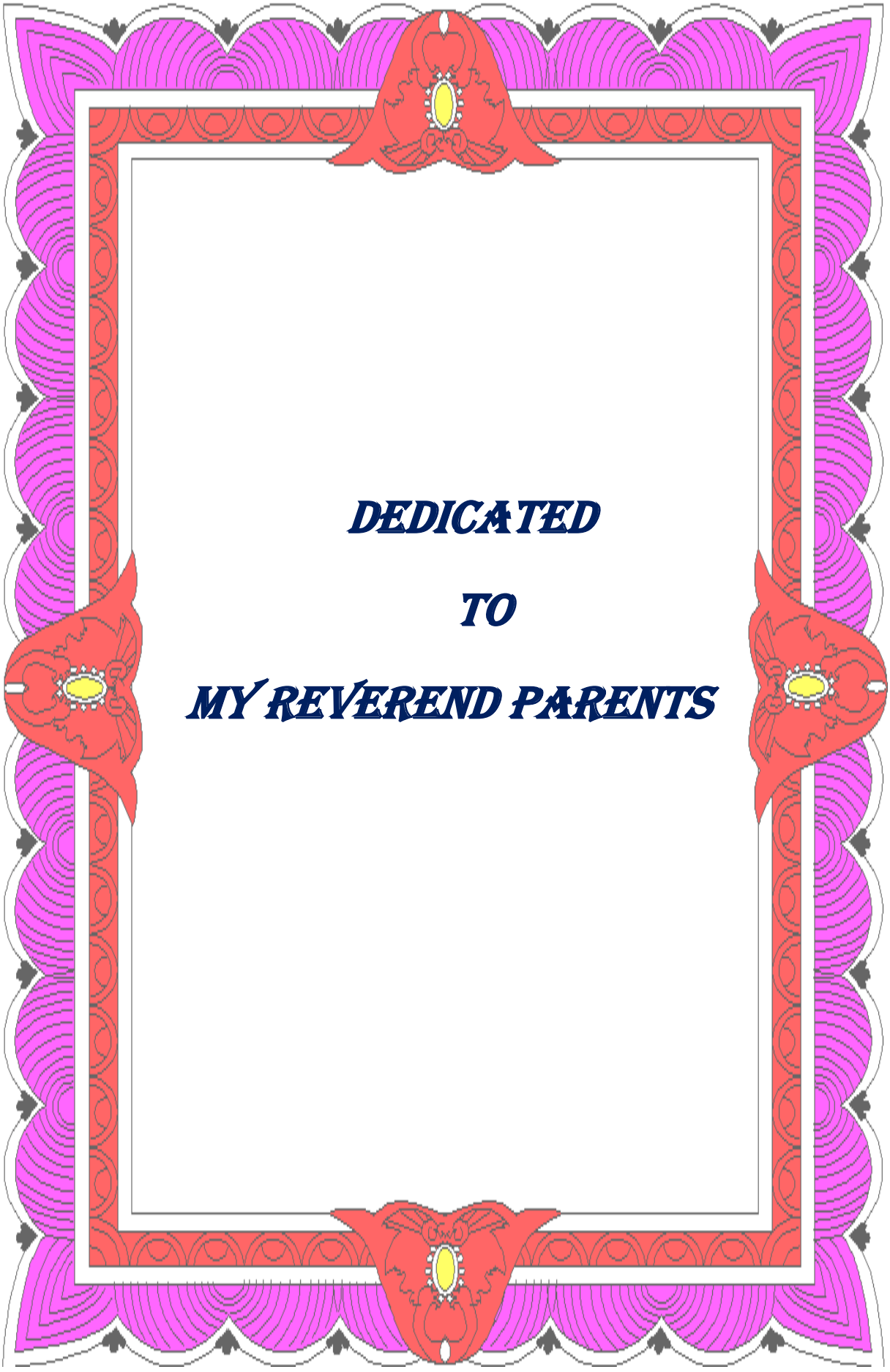
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Abbreviations

%	:	Percentage
AST	:	Aspartate aminotransferase
ALT	:	Alkaline Transaminase
TSP	:	Total Serum Protein
SGOT	:	Serum Glutamic-Oxaloacetic Transaminase
SGPT	:	Serum Glutamate Pyruvate Transaminase
b.w.	:	body weight
conc.	:	Concentration
C	:	Control
cc	:	cubic centimeter
et al	:	et alibi
FAO	:	Food and Agriculture Organization
g	:	Gram
Hb	:	Hemoglobin
i.e.	:	That is
IU	:	International Unit
Kg	:	Kilogram

mg	:	Milligram
ml	:	Milliliter
PCV	:	Packed cell volume
pH	:	Log hydrogen ion concentration
Sig	:	Significant
s/c	:	Subcutaneous
DLC	:	Differential leukocyte Count
TEC	:	Total Erythrocyte Count
TLC	:	Total Leukocyte Count
PCV	:	Packed Cell Volume
MCV	:	Mean Corpuscular Volume
MCH	:	Mean Corpuscular Hemoglobin
MCHC	:	Mean Corpuscular Hemoglobin Concentration
EDTA	:	Ethylene diamine tetra-acetic acid
T/t	:	Treatment
viz.	:	Namely
T_1	:	Treatment Group 1
T_2	:	Treatment Group 2
T_3	:	Treatment Group 3

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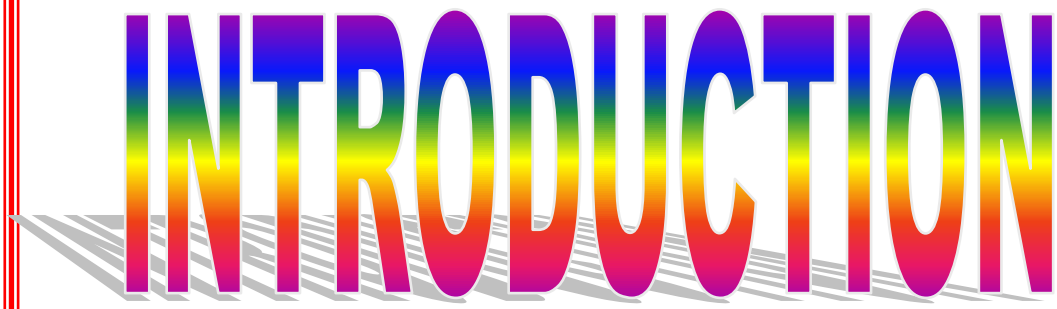


INTRODUCTION

Livestock are an essential part of existing systems and offer opportunities for high value production. It is a major source of income in irrigated, arid, semiarid, and rain-fed areas of Indian sub-continent. *Bubalus bubalis* (buffalo) is one of the most important species of domestic livestock as a source of dairy, meat, manure and drought power and plays an important role in Indian rural economy. According to the latest FAO statistics (2008), world buffalo population is estimated as 185.29 million, spread in some 42 countries, of which 179.75 million (97%) are in Asia. India has 108.7 million buffaloes and they comprise approximately 56.7 per cent of the total world buffalo population. The contribution of buffalo is 12.1% to the world's total milk production, 38.0% in Asia, 55.0% in India, 16.4% in China, 50.8% in Egypt, 65.2% in Nepal and 66.6% in Pakistan (Sohail *et al.*, 2009). In India, the majority of small and marginal farmers are more dependent on buffaloes than cattle for their livelihood as they also serve as an insurance against the risk of crop failure due to natural calamities (Dhanda, 2004).

Buffaloes are raised as economically important animals because they are multipurpose; providing milk, meat and good quality hides. They are also used as draft animals ("tractors" in Southeast Asia) in agriculture farms, means of transportation, and their dung act as a good fertilizer (Liu *et al.*, 2009).

Infections by gastrointestinal (GI) helminth parasites of livestock are among the most common and economically important diseases of grazing livestock (Perry *et. al.*, 2002). It is distributed ubiquitously throughout the world, but the highest prevalence has been reported in tropical and subtropical regions, particularly in Africa, Asia, Australia, Eastern Europe and Russia. The disease is widely prevalent in India and causes economic

loss to the tune of several thousand crores annually (Khan *et al*., 2008). Gastrointestinal parasitic infections of buffaloes are common, which cause considerable global economic losses to the buffalo industry and farming communities as a consequence of mortality in infected animals, reduced weight gain and the condemnation of the affected organs during meat inspection in slaughterhouses (Singh *et. al*, 2012)

They are characterised by lower outputs of animal products (meat, milk, hides and skins) and manure and traction, which all impact on the livelihood of small holder farmers (Perry & Randolph, 1999).

In most areas in the tropics, animals continuously graze on pasture all year round. This exposes the animals to continuous parasite pressure when climatic conditions are favourable for the development and survival of free living stages (Dinnik & Dinnik, 1958). The parasitic diseases like gastro-intestinal helminthiasis, coccidiosis, fasciolosis and mange are not less important in buffaloes than other infectious diseases (Griffiths, 1974). Physiological condition and the habitats makes buffaloes more prone to a variety of parasitic diseases. Parasitic diseases are common and widespread, and an important factor causing low productivity. A variety of parasites particularly helminths harbour the gastrointestinal tract (GIT) of animals affecting the health status of animals and cause enormous economic losses to the livestock industry (Rafiullah *et al.*, 2011).

Helminths are one of the important parasites of livestock including buffaloes. *Gigantocotyle explanatum* is a very common digenean trematode parasite affecting the domesticated animals usually present in the liver, bile duct and gallbladder. *G. explantum* belongs to phylum platyhelminthes, class trematoda and subclass digenea; have oral sucker and acetabulum (Miller and Harly, 2010). It is present in the bile ducts of buffaloes form plugs on the luminal surface by their acetabulum (Malik, 2010). The locations occupied by the adult worms and the ecology of the mollusc can

intermediate hosts are important to study the implications of the contrasting reproductive strategies in the rumen and bile duct inhabiting species (Hanna *et al.,* 1988). The immature stage causes serious enteritis and health hazard in young buffaloes (Soulsby, 1982). The fresh water snails predominantly *Gyrulus convexiusculus* serve as the intermediate host for *G. explanatum* (Patzelt, 1993).

The variation in the prevalence of parasitic intensity depends upon the geographical locations, environment, grazing habits, immunological and nutritional status of the host, presence of intermediate host and number of infective larvae or eggs ingested by the animals (Blood and Radostits, 1989). Direct and indirect losses due to nematode infections are estimated to be high and control of these parasites is therefore considered important. Infections caused by parasites are responsible for decrease in milk production, reduced product quality and quantity and increase mortality rate (Soulsby, 1982). Anorexia and reduction in feed intake, loss of blood and plasma protein in GIT, alteration in protein metabolism, decrease in mineral level enzymes and diarrhoea, all contribute to loss in weight gain (Soulsby, 1982).

Synthetic anthelmintics are widely used in controlling parasitic infections. However, these are expensive, have developed resistance against various anthelmintic compounds and their residues and toxicity problems (Kaemmerer and Buttenkoter, 1973) pose hazards to livestock development and public health. Hence to overcome these problems it is felt necessary to investigate the anthelmintic properties of indigenous herbal plant products in controlling the helminth parasites of buffaloes. It has been reported that neem (*Azadirachta indica)* and garlic (*Allium sativa*) can be used as deworming agents in place of synthetic chemical dewormers against various ailments including helminth parasites. Neem and garlic

cloves contain active components- azardichtin and allicin, respectively. The components have anthelmintic property against many parasites and also enhances natural immunity of the host.

For these reasons, interest for the screening of medicinal plants to evaluate their anthelmintic activity has been a great scientific interest despite extensive use of synthetic chemicals in clinical practices. Till today very little works have been reported in our country in the subject to investigate the anthelmintic properties of indigenous medicinal plants in buffaloes. Considering all of these constraints, this work was undertaken with following objectives:

Objectives

1. To study the incidence of helminth parasites of buffalo with special reference to amphistomes.
2. To study the various haematological and biochemical changes in buffalo infected with helminth parasites including amphistomes.
3. To study the efficiency of some herbal drugs in the control of amphistomes in buffalo.

Chapter-2

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Worm infestation is one of the major constraints in the development of a profitable livestock industry. Gastro-intestinal helminthiasis syndrome is always caused by a mixture of species of helminth parasites in the alimentary tract (Chaudhry *et al.*, 1984).

Internal parasites adversely affect the health and productivity of animal and also decrease the resistance of animal to various diseases, which may ultimately lead to higher mortality. Parasitic infestation is a great economic loss to dairy industry by way of retarded growth, low productivity and increased susceptibility of animals to other infections (Yadav *et al.*, 2004). The grazing habit of buffaloes in low lying areas expose them to more risk of snail worm trematode infections especially *G. explanatum* (Alim *et al.*, 2005).

PREVALENCE OF HELMINTHIC PARASITES IN BUFFALOES

India

Gupta *et al.* (1985) had encountered helminthic infections in 55.80 per cent of cow claves and 62.90 per cent of buffalo calves in Haryana; the strongyle species were more prevalent (44.20 per cent). He also observed a positive correlation between the incidence of strongyle infection and the age of the host.

Jagannath *et al*. (1988) reported 42.12 percent 38.86 percent of incidence of gastrointestinal helminthiasis in buffaloes in Karnataka.

Sanyal and Singh (1995) indicated an increased parasitic burden in hosts and pastures during the rainy season, based on nationwide survey on parasitic epidemiology in dairy animals in seven different agro climatic zones of India. It was observed that with the onset of winter, the infection rate gradually decreased.

Hirani *et al*. (1999) reported 38.86 percent of incidence of gastrointestinal helminthiasis in buffaloes in Gujarat.

Soundararajan (2000) studied the epidemiology gastrointestinal nematodes in Toda buffaloes, crossbred cattle, Nilagiri sheep and non-descript goats in Nilagiri sheep and Crossbred goat in Tamil Nadu. All the four species of animals passed highest number of strongyle eggs during October months. Highest egg per gram was recorded during the northeast monsoon, moderate during southwest monsoon and lowest during the winter and summer months.

Yadav *et al*. (2004) observed slightly higher prevalence of GI nematodes i.e. 60.5 percent in buffaloes reared in Jammu. During this study, higher prevalence rate was observed in adults than young buffaloes.

Muraleedharan (2005) recorded the lower percentages of incidence of gastrointestinal helminthiasis i.e. 20.45 percent in buffaloes of Karnataka.

Hassan and Juyal (2006) reported that 3.99 per cent of prevalence rate of paramphistomosis in ruminants in Punjab. The incidence rate was highest in buffaloes (5.42%) followed by cattle (3.71%), sheep (1.79%) and goats (0.85%). Highest incidence was recorded during monsoon and post monsoon (July to October) with the prevalence rate of 8.06% followed by 2.92% in summer (March to June) and 0.49% in winter (November to December).

Samanta and Santra (2009) reported high prevalence rate in the below one year age group of buffalo population. It was also observed that with the onset of winter, the infection rate gradually decreased.

Wadhwa *et al*. (2011) recorded the lower percentages of incidence of gastrointestinal helminthiasis i.e. 13 percent in buffaloes in Rajastan.

Reddy *et al*. (2012) reported the highest incidence of amphistomes 180 (22.5%) followed by Coccidia 65(8.1%), Strongyles 61 (7.6%) and *Fasciola* 30 (3.8%) among cattle and buffaloes in Piler, Chittoor district of Andhra Pradesh. In this study, younger animals (<12 months) had higher infection of nematodes.

Saha *et al.* (2013) reported highest prevalence of gastrointestinal helminthes in buffaloes of 3years to 6 years of age (40.19%), second most prevalence was found among buffaloes which are 7 years and above age (0-6 months of age (65%) and lowest prevalence(12.15%).

Sreedevi and Hafeez (2014) reported prevalence of gastro-intestinal parasites of buffaloes out of which amphistomes were 15.42%. Mixed infections of *Toxocara vitulorum, Strongyloides papillosus* and *Eimeria sp.* were observed in below-one-year age but ova of Amphistomes and cysts of Buxtonella were observed only in animals above one year of age buffaloes. Seasonal effect on prevalence of G. I. parasites was revealed and the infection rate was significantly higher ($P<0.05$) during the rainy season (44.50%) followed by the summer (35.46%) and winter (33.58%) seasons.

Patel *et al.,* (2015) reported the general prevalence rate of helminth parasites in buffaloes 64.67%. Study revealed that 64 % cases are of trematodes- *Gigantocotyle explanatum* -11.33%; the prevalence of helminth was maximum (46.39%) in young age group followed by adult (27.83%) and old animals (25.77%). Also the rainy season showed highest prevalence of 51.54%, followed by winter at 34.02% and summer at 14.43%.

Vanisri *et al.* (2016) conducted his study in Tamil Nadu over a period of six months and found overall prevalence of parasitic eggs and oocysts 76 per cent. Of this overall prevalence of 76.0%, 40.0% was infected by nematodes, 36.09% by trematodes, 16% by protozoan parasite (*Eimeria sp.*) and 10.00% by cestodes. The most common gastrointestinal nematodes observed in this study was the strongyle (21.05%) compared to Strongyloides (2.63%). Younger animals had higher infection of nematodes (24.0%) and trematode (20.00%) than the adult cattle. Male cattle had higher infection of nematodes (24.0%), tremaotode (20.00%) and *Eimeria sp*. (8.0%) while females had higher infection of cestodes (8.0%). Cattle had heavier infection of trematodes only during summer months (32.00%) whereas cattle had heavy infection of nematodes (30.00%), *Eimeria sp* (10.0%) and cestodes (10.0%) during monsoon months. As compared to females, male cattle had higher infection of nematodes, trematode and *Eimeria sp.* while female cattle had higher infection of cestodes (6.00%).

Abroad

Chaudhry *et al.* (1984) reported *Fasciola hepatica* (70%), *Cotylophoron cotylophorum* (5%), *Ostertagia ostertagi* (64.6%), *Haemonchus contortus* (58.5%) and *Ascaris vitulorum* (48%) infecting cattles and buffaloes.

Islam *et al.* (1992) reported trematodes i.e. Paramphistomes (*G.explanatum* and others) 29.5-48.3 % in the water buffaloes in Bangladesh.

Javed *et al.* (1993) recorded higher (14.43%) prevalence of endoparasites in buffalo and cattle calves under one year of age compared with that (7.15%) from 1-3 years of age at Bahadumagar (Okara) and Qadirbad (Sahiwal); species identified were *Oesophagostomum, Strongyloides spp*. and *H. Contortus*.

Anwar *et al.* (1996) reported 63.8% buffalo calves having gastrointestinal helminthiasis at Faisalabad; the species recorded were *S. Papillosus , Toxocara vitulorum, Trichostrongylus species, O. Ostertagi, Oe. Radiatum, Cooperia spp, M.benedeni, B. Phlebotomum, M. expansa* and *Nematodirus spp.*

Zar (1996) reported paramphistomes in the rumen (38.4%) and gall bladder (0.2%), incidence being highest (73%) in August and the lowest in November(5%); the species recorded were *P. Cervi* (55%), *C. Cotylophorum* (27%), *G. Crumenifer* (15%) and *Gigantocotyle explanatum* (3%) in Lahore, Pakistan.

Cheema *et al.* (1997) reported 75.07% buffaloes having amphistmiasis in Pakistan.

Ibrahim (1997) reported the higher prevalence of helminthic infection was in male buffaloes than female buffaloes.

Ngugen *et al. (*1997) reported the prevalence rate of *G. explanatum* in Vietnam at 12.5 percent .

Bhutto *et al.* (2002) observed that faecal samples of buffalo calves of different age and sex groups, 47 % were found infected with different species of gastro-intestinal helminthes in Sindh area, Pakistan. Forty three percent and 4 percent were positive for nematodes and trematodes, respectively. Mixed infections with nematodes and trematodes where observed in 1.5 % faecal samples. However, cestodes were found absent in all the faecal samples. A slightly higher prevalence of helminthes was observed in females than males, as 48.30 and 45.12%, respectively.

Keyyu *et al.* (2006) observed that prevalence of both *Fasciola* and *Amphiostomes* was higher i.e. 85.5%, 75.2% in adults buffaloes.

Ahmedullah *et al.* (2007) as demonstrated 31.25% prevalence of *G. explanatum* in buffaloes in Bangladesh.

Khalil-ur-Rehman *et al.* (2009) reported the prevalence of trematodes infestation in buffaloes was 49.01% in Punjab, Pakistan.

Mamun *et al*. (2011) recorded higher prevalence of gastrointestinal helminthiasis (61.0 percent) in buffaloes of Bangladesh. It was found that the infection rate was significantly higher during the rainy season (44.50%) followed by the summer (35.46%) and winter (33.58%) seasons.

Iqbal *et al.* (2014) observed that 25% prevalence of *Gigantocotyle explanatum*, in Rawalpindi and Islamabad, Pakistan. From age related distribution, highest prevalence was found in buffalos having age of 22 years (56.4%), followed by 20 years (24%), then 18 years (17.2%) and least was observed in 16 years buffalos (2.29%). Highly significant correlation was found between worm burden and age indicating that the worm burden influenced by the age of animal.

Muhammad *et al.* (2015) conducted his studies at Rawalpindi, Pakistan. Three hundred livers of buffaloes were examined and sixty three livers were found infected by *G. explanatum.* The prevalence of *G. explanatum* was 21.24%. These results showed significant range of damage in buffaloes by this parasite, which in turn causes heavy economic loss in dairy industry.

HEMATOLOGICAL CHANGES

Panda and Mishra (1980) who reported reduction of hemoglobin content and erythrocyte count with eosinophilia in clinical pathological studies of the buffalo calf affected with amphistomosis.

Singh *et al*. (1984) also observed that there was a significant drop in TEC ($p<0.05$), Hb ($p<0.01$), and PCV ($p<0.01$) in biliary amphistomiasis and significant increase in eosinophilic count ($p>0.01$) as compared to the healthy animals.

Lukes (1985) observed marked increase in leucocytes, eosinophils and neutrophils in rabbits infected with 5000 *T. canis* eggs. Eosinophilia was highest on week 2 and 3 of infection.

Saheb and Hafeez (1995) who documented significant decrease ($p<0.05$) in the hematological values of TEC, PCV and Hb and increased eosinophilic count ($p>0.05$) in ruminal amphistomiasis as compared to healthy buffaloes.

Umar *et al.* (1998) studies also indicate that chronic and excessive intake of garlic may cause less desirable effects, such as anemia.

Vatta *et al.* (2002) showed that garlic did not have a significant effect on packed cell volume in his study on boer goats in USA. All groups varied within the natural range for goats. Packed cell volume values are directly related to anemia, which is an important tool in the diagnosis of parasitic infections.

Landmann and Prociv (2003) studied a marked blood eosinophilia followed a single oral exposure to 100 infective larvae, while faecal examination remained negative. Eosinophil counts then declined gradually, although a rapid, spontaneous rise several months later, at the beginning of spring, possibly indicated reactivation of dormant larvae. Blood eoshinophil numbers did not rise significantly after percutaneous infection with 200 larvae. A subsequent, smaller, oral inoculum of 20 larvae provoked an eosinophil response similar to that of the first experiment. Finding suggests that, following ingestion, some infective larvae of *A. caninum* develop directly into adult worms in the human gut (as they do in dogs). While the percutaneous route be the most common means of human exposure to canine hookworm larvae, leading generally to sub clinical

infection, oral infection may be more likely to provoke symptomatic eosinophilic enteritis.

Molina, E.C. (2005) The red blood cell (RBC) count was significantly higher in infected than in non-infected swamp buffaloes ($p< 0.05$) while there was no significant difference in packed cell volume (PCV) and haemoglobin values between infected and non-infected buffaloes. Red blood cell count was significantly higher in buffaloes with high flukes burdens than those with no flukes or with medium fluke burden(21-70 flukes) ($p< 0.05$).Significantly higher PCV value was also observed in buffaloes with high fluke burdens compared with those with low or medium worm loads. Haemoglobin values did not differ significantly between buffaloes with low, medium, high or no fluke burdens($p>0.05$)

Nath (2007) conducted hemato-biochemical changes in cattle with paramphistomiasis and showed a significant decrease in Hb, PCV, TEC and Lymphocyte count and a significant increase in neutrophil and eosinophil count in the diseased animals respectively.

Thakur *et al*. (2007) studied clinico-hematology on amphistomosis in cattle and revealed eosinophilia.

Clark (2008) stated that the acute eosinophilic enteritis is a difficult to diagnose. Insufficient consideration of eosinophilia may commit patients to surgical treatment when medical therapy may be appropriate. The aim of the study was to determine whether the eosinophil count was considered in the diagnostic evaluation of patients presenting with acute abdominal pain who subsequently underwent appendectomy and whether eosinophilia was related to subsequent histology. Eosinophilia may be underutilized and helminth infection may not be considered in the differential diagnosis of abdominal pain. A normal eosinophil count in the setting of clinically

suspected appendicitis may make the diagnosis of eosinophilic enteritis less likely, but does not exclude it. Patients with abdominal pain and peripheral eosinophilia appear less likely to have acute appendicitis on subsequent histology; however, further study is required to validate these findings. The decision to operate remains one of clinical judgement.

Mavenyengwa *et al*. (2010) found significant decrease ($p<0.05$) in the PCV, Hb and RBC levels occurred from 21 day post-infection, while a significant increase ($p<0.05$) in the circulating eosinophils occurred between 7 and 21 days post-infection of amphistomosis in cattle.

Balasasirekha and Lakshmi (2011). A gradual increase in the blood Hb levels was observed among garlic (14.32 mg/dl) supplemented group over the three months period which significant at 5% level. There was slight increase in haemoglobin levels among the other groups but not at a significant level. A comparison of supplemented groups with control group revealed a high significant (1%) change in haemoglobin levels among garlic supplemented group and no significant change among the other group. This clearly indicate that over a period of three months of time garlic supplementation was effective in raising the blood haemoglobin levels with a minimum increase among cinnamon supplementation.

Ugwu and Omale (2011). The relationship between some haematological and lipid indices were studied and compared in white albino rats using aqueous garlic (*Allium sativum*) and onion (*Allium cepa*) extracts. The effect of garlic and onion extracts were each tested with 0.5, 1.0, and 1.5 mg/kg body wt. concentrations for 28 days. Biochemical parameters were assayed using standard methods.

Biswas *et al*. (2013) documented hemato-biochemical changes in cattle with naturally acquired paramphistomiasis in which the levels of Hb,

TEC and PCV were significantly decreased and increased levels of eosinophil and neutrophil were reported.

Chauhan *et al* (2015) The study was undertaken for the period of 12 months from March-2013 to February-2014 in Anand and Ahmedabad district of Gujarat, India The infected buffaloes in this study showed a highly significant reduction ($p<0.01$) in the mean Hb, TEC and PCV. There was a significant increase ($p<0.05$) in the neutrophils count and eosinophil count of infected buffaloes as compared to the non-infected buffaloes. The total leukocyte, monocyte and lymphocyte count decreased non-significantly, and basophil counts increased non-significantly in infected buffaloes. There was no significant change in the values of MCV, MCH and MCHC of control (apparently healthy) and infected buffaloes.

PHYTOTHERAPEUTIC CONTROL

In vivo anthelmintic activity

Ibrahim *et al.* (1984) screened eighteen plants traditionally used for the treatment of human and animals helminthiasis in Africa for anthelmintic activity using the *Nippostrongylus*-rat model. *Aloi barteri, Terminalia aviceioides, Annona senegalensis, Cassia occidentalis, Angeissus leicarpus* and *Diospyros mespiliformis* showed significant activity, giving deporasitizations of 92, 89, 75, 69, 60 and 58% respectively compared to untreated controls.

Soffar *et al.* (1991). The effect of serial dilutions of crude garlic (*Allium sativum*) extract on adult *Hymenolepis nana* was studied to detect the minimal lethal concentration which was found to be 1/20. *A. sativum* was tried in the treatment of 10 children infected with *H. nana* and 26 infected with *G. lamblia* as 5 ml crude extract in 100 ml water in 2 doses

per day, or commercial preparation (0.6 mg capsules) 2 capsules twice/day for 3 days. *A. sativum* was found to be efficient, safe and shortens the duration of treatment. The possible mode of action of *A. sativum* and the correlation between the clinical and parasitological findings were discussed.

Schillhorn van Veen (1997) claimed that some ethnoveterinary remedies are efficacious in controlling parasitic diseases; other remedies have complementary value, while some remedies have little or no value.

Abells and Haik (1999) reported that garlic was ineffective in decreasing faecal strongly an ovum counts in donkey two weeks after treatment with garlic. Alternatively, garlic may function by stimulating the immune system. Allicin is extracted in the organic layer of raw garlic homogenate. It can be found in dry garlic Powder and can also be synthesized in the laboratory. Allcin was found to be the successful anthelmintic.

Guarrera (1999) Garlic has been used to treat animals that suffer from gastrointestinal parasitism.

Akhtar *et al*. (2000) examined that helminthiasis is one of the most important group of parasitic diseases in Indo-Pakistan subcontinent resulting in heavy production losses in livestock. A wide variety of anthelmintics is used for the treatment of helminths in animals. However, the developments of resistance in helminths against commonly used anthelmintics have always been a challenge faced by the animal health care professionals. Therefore, exploitation of anthelmintic potential of plants indigenous to Indo-Pak subcontinent is an area of research interest. This paper reviews the use of some indigenous plants as anthelmintics in animals.

Ketzis *et al*. (2001) reported that there is considerable data on plants used in traditional veterinary and human medicine for endo and ecto-parasite infections. In addition, zoopharmacognosy observations are providing information on potential endo- and ecto-parasite treatments. However, little efficacy and safety data are available for these treatments. These plants show activity in *in vitro* tests. *In vivo* and toxicity tests are planned for the future.

Arunachal *et al.* (2002) noted that Neem leaves, seeds and bark were 53%, 49% and 38% effective against gastrointestinal helminths in the sheep respectively.

Rahman (2002) found the effects of water extract of Neem, betal, leaf and jute leaves were 62%, 58% and 42% respectively in goat on 21st day post treatment against gastrointestinal nematodes.

Noon (2003). Studies from organic sheep producers in the US reported the use of garlic as a viable alternative to commercial anthelmintics .

Susan *et al.* (2004) reported that garlic (*Allium sativum*) is one of the best known herbs around the world. The Herb society of America has designated garlic as their Herb of the year 2004. Garlic has also been promoted to treat colds, coughs, high blood pressure, arteriosclerosis, fungal infection, cancer, hyperglycemia, high cholesterol, roundworms and hookworms.

Biffa *et al.* (2004) studied the antihelmintic effect of *Halothamnus somalensis* on gastrointestinal parasites of goats showed a 50% reduction in EPG which was similar to the 52% reduction in EPG count reported for the water extract of *Albizia gummifera* in Ethiopia.

Anthony *et al*. (2005). Garlic oil has a broad-antimicrobial spectrum; as it has antibacterial, antifungal, antiviral, and antiparasitic effects. Further, it influences the growth of at least 12 different human and nonhuman parasites and has immunomodulatory activity.

Chandrawathani *et al*. (2006) reported the anthelmintic effect of neem on nematode parasites of sheep through the faecal egg counts using the modified Mc Master technique. Faecal egg count showed no significant difference between the control and treated group ($p = 0.081$). However worm burden estimation revealed that number of parasites was significantly reduced in treated group ($p < 0.05$) as compared to untreated group of sheep and even highly pathogenic parasite, *Haemoncus contortus* appears particularly sensitive to the intake of fresh neem leaves by the animals.

Githiori *et al*. (2006) reviewed many plants used as anthelmintics such as garlic, onion, mint, walnuts, dill, and parsley all used for gastrointestinal parasitism.

Hoste *et al.* (2006) studied that apart from the obvious role of plants in herbivore nutrition, they are also a rich source of bioactive products that can operate either to the benefit or the detriment of grazing animals. Here, we review the available evidence for the potential beneficial effects that plant-derived bioactive substances can have on gastrointestinal parasites. Tannin-rich plants have attracted most attention for their effect on internal nematodes in ruminants. These plants could act through direct antiparasitic activity but might also act indirectly by increasing host resistance. The effects vary with the species of plant, parasite and host. More research is required to understand better the mechanisms of action, and therefore make more pertinent use of these bioactive plants in livestock systems.

Lans *et al.* (2007) stated that medicinal plants used to treat endoparasites and stomach problems in dogs, cats and pigs in British Columbia, Canada. Ethnoveterinary data was collected over a 6-month period in 2003. The majority of the information on pets came from 2 naturopaths, 10 herbalists, 5 dog trainers, breeders and pet shop owners, 9 holistic veterinarians and 6 of 27 organic farmers. Two pig farmers joined the study in the final stages. The following plants were used as anthelmintics: *Artemisia cina* O. Berg and C.F. Schmidt, *Artemisia vulgaris,* L. *Artemisia annua*, *Calendula officinalis* L., *Echinacea purpurea* (L.) Moench (all Asteraceae), *Mentha piperita* L. and *Salvia officinalis* L. (Lamiaceae) (*Allium sativum* L. (Alliaceae), *Cucurbita pepo* L. (Cucurbitaceae), *Eugenia caryophyllata* Thunb (Myrtaceae), *Gentiana lutea* L. (Gentianaceae), *Hydrastis canadensis* L. (Ranunculaceae), *Juglans nigra* L. (Juglandaceae), *Olea europaea* L. (Oleaceae) and *Ruta graveolens* L. (Rutaceae)). There is insufficient information available to assess the anthelmintic efficacies of *C. officinalis*, *Salvia officinalis*, *Eugenia caryophyllata* and *O. europaea*; the other plants have mid- to high-level validity for their ethnoveterinary uses.

Toulah and Al-Rawi (2007). The efficacy of garlic on Coccidia infections has been reported in rabbits.

Amin *et al.* (2008) reported that neem (10% water extract of leaves) reduced significantly ($p<0.01$) EPG count 62.23%, 65.77%, 56.70%, and 48.05% on 3rd, 10th, 17th and 28th day, respectively in cattle.

Erol *et al.* (2008). Availability, improved animal health, and comparable cost to commercial preparations are cited as attributes that make garlic attractive to producers. An anthelmintic effect of garlic in mice has been patented.

Swarnkar *et al*. (2008) have reported that alcoholic extract of *A. indica* bark was evaluated in for anthelmintic activity against pre-parasitic and parasitic stages of *H.contortus*, no anthelmintic activity was noticed. However, oral administration of *extract* of *A. indica* bark at the rate of (50 mg/kg body weight) *was* found to cause gradual reduction in faecal egg count in sheep infected with *H. contortus*. The corrected faecal egg count reductions on day 7 and 10, post treatment, were 44.1 and 56.9%, respectively, indicating the presence of moderate level of anthelmintic property in the extract.

Bachaya *et al.* (2009) evaluated the anthelmintic activity of *Terminalia arjuna* (Roxb.) bark locally used as an anthelmintic. Lethal median concentration (LC_{50} values) of methanolic extract of *T. arjuna* bark in egg hatch and larval development tests against *Haemonchus contortus* ova and larva were found to be 645.65 and 467.74 μg/ml, respectively while In adult motility assay, efficacy of the extract was evident by the mortality of *H. contortus* at different hours post exposure. *In vivo* results revealed maximum (87.3%) egg count percent reduction in sheep treated with crude methanolic extract at the rate of 3 g/kg body weight on day 11 post-treatment. The data revealed dose-dependent anthelmintic activity both in the *in vitro* and *in vivo* studies.

Nahed *et al.* (2009) carried a study designed to evaluate the prophylactic and therapeutic values of garlic treatment against *Schistosoma mansoni.* Albino mice were infected with *S. mansoni* cercariae and were classified into: (a) treated with garlic before infection (prophylactic group), (b) treated with garlic after infection (therapeutic group), (c) treated with garlic before and after infection; (d) infected non-treated (control) group. Seven weeks post infection, all mice were necropsied, and their livers and ilea were obtained for parasitological assessments. Schistosomes recovered

from all groups were processed for ultrastructural investigations. Garlic treatment significantly evoked a reduction in the egg and worm burden. Garlic efficacy was highest in the group treated with garlic before and after bilharzial infection. However, the statistical difference between the three treated groups was not significant. Garlic also resulted in various ultrastructural alterations in the tegument of the surviving worms including tubercular disruption, oedema, blebbing, ulcers, and vacuolization of other tegumental structures. Our findings suggest that garlic is a convenient prophylactic and a promising therapeutic agent for *Schistosoma mansoni* infection.

Worku *et al.* (2009) evaluated the effect of extracts from neem (*Azadirachta indica*), Wormwood (*Artemisia absinthium*) and tobacco (*Nicotina tabacum*) with added copper sulphate, on female Boar goats infected artificially with gastrointestinal parasites with a mix containing approximately 80% *Haemonchus contortus* and 20% *Trichostrongylus* spp. After 21 days. The three plants aqueous extracts tested did not have any anthelmintic effect in female Boer goats infected with internal parasites. No significant differences were observed in fecal egg count between treatment groups ($p>0.05$). The highest number of eggs per gram of feces was observed in week two. The highest fecal egg count was observed in untreated animals.

Amin *et al.* (2010) observed the effect of neem, betel leaf, devil's tree, jute and turmeric against gastrointestinal nematodes in sheep through EPG examination. Fecal sample was examined before treatment and on 7^{th}, 14^{th}, 21^{th} and 28^{th} day. A significant ($p<0.01$) reduction in EPG was found following administration of neem, (37.60-47.03%), betel leaf (6.43-14.00%), devil's tree (3.04-11.04%), jute (0.50-5.26%) and turmeric (0.46-

8.30%) in sheep. The EPG count of the control group were significantly ($p<0.01$) increased up to the last day of experiment.

Rabiu and Subhashish (2011) were investigated the aqueous extract of *A. indica* leaves for anthelmintic activity using earthworms (Phertima posthuma), tapeworms (*Retillietina spiralis*) and roundworms (*Ascaridia galli*). Various concentrations (10-70 mg/ml) of plant extract were tested in the bioassay. Extract exhibited significant anthelmintic activity at the conc. of 40 mg/ml. the result shows that aqueous extract possesses vermicidal activity and found to be effective as an anthelmintic.

Shaziya and Goyal (2012) investigated that gastrointestinal parasite is serious threat to the productivity of livestock in developing nation. Neem has been shown to possess many medicinal properties including dewormer property. The purpose of this experiment was to study the dewormer activity of neem against *Ancylostoma caninum* in infected mice. Two groups of mice were infected with *A. caninum* infective larvae. Before infection one group of mice were given neem extract at dose level of 0.2ml/ mouse. One group of mice served as non treated group. The dewormer activity was determined by larval reduction, mast cell and eosinophil cell level. Neem extract were highly effective in reducing the number of *A. caninum.* Larval reduction showed that the number of larvae reduced was higher in the treated and infected group compared to the infected group within 72 and 96 hours after challenge infection. Mast cell result suggest that on day 16 and 24 in mice infected with *A. caninum* larvae developed higher mastocytosis in comparison to treated and infected group. Decline level of eosinophil cell recorded on day 16 and 24 in treated and infected group when compared with infected group. The result suggests that the number of larvae correlated with number of mast cells and eosinophil cell

and a potential role of neem extract as a dewormer activity against *A. caninum* in mice.

Molefe *et al.* (2013) investigated the anthelmintic activity of acetone and water extracts from the shoots of *Cotyledon orbiculata, Hermannia depressa* and *Nicotiana glauca* were investigated using the egg hatch, larval development and larval mortality assays. In this study, the nematode species specification was made according to the McMaster technique which included *Haemonchus*, *Oesophagostomum*, *Trichostrongylus* or *Teladorsagia*. The water extracts of *C. orbiculata* showed anthelmintic qualities like inhibition of egg hatch, larval development and for the same kind of extracts, *H. depressa*, also there was an inhibition qualities for the egg hatching and larval development but performance of previous extract was found better than later mentioned extracts.

Nawaz *et al.* (2014) evaluate the anthelmintic activity of water extract prepared from leaves of *Azadirachta indica*, *Dalbergia sisso* and *Morus alba* against ova and adult worms of *Haemonchus contortus*. Anthelmintic activity of water extract of *Azadirachta indica*, *Dalbergia sisso* and *Morus alba* was determined using fecal egg count reduction test, adult motility assay and egg hatch test. In fecal egg count reduction test, water extract of plants (*Azadirachta indica*, *Dalbergia sisso* and *Morus alba*) was administrated to the animals (sheep) at the dose rate of 02, 04 and 8 ml/kg b. wt. After 12 days of treatment, the plants extract (*Azadirachta indica*, *Dalbergia sisso* and *Morus alba*) induced 89%, 87% and 36% reduction in EPG, respectively Results indicated that extracts of *Azadirachta indica*, *Dalbergia sisso* and *Morus alba* are capable of inducing anthelmintic activity.

Priscilla *et al.* (2014) observed the comparative efficiency of methanol extracts of neem (*Azadirachta indica*), bitter gourd (*Mimordica charantia*) and chemical anthelmintic Albendazole on gastrointestinal nematodes infected goats were evaluated by egg per gram (EPG) count for a period of approximately three weeks. They found that methanol extract of *Azadirachta indica* exhibited significant ($P<0.01$) reduction in EPG count in gastrointestinal nematodes infected goats on post treatment days of observation. Goats treated with methanolic extract of *Azadirachta indica* could successfully maintained gastrointestinal nematodes at safe level until 3 weeks post treatment.

***In vitro* anthelmintic activity**

Neogi *et al.* (1964) evaluated the *in vitro* anthelmintic activity of the aqueous and alcoholic extracts of *Melia azedarach*, *Ananas comosus*, A. *Sativus., Embelia ribes* and *Mucuna prurita* against *Taenia canina* and *Phamphistomum cervi. M. Prurita* was found more active against the trematodes.

Prakash *et al.* (1980) reported that the root and stem bark of the plant *Punica granatum* (Anar) is used as the astringent and anthelmintic in the indigenous system of medicine. The alcoholic extracts of its stem bark was evaluated for its proclaimed anthelmintic potential. The activity was found dose dependent, inhibiting transformation of eggs to filariform larvae of *Haemonchus contortus.*

Khobragade *et al.* (1994). In vitro anthelmintic trial of aq. extract of *Allium sativum* was conducted against *B. trigonocephalum* in goat. The cessation of motility of worms was observed at 2, 6, 8, 12 hr and mortality at 6, 12, 16, 20 hr of exposure to different concentratios (200, 100, 50, 25 mg/ml) of *A. sativum.*

Roy and Tondon (1997) studied on efficacy of crude extract of *Cannabis sativa* leaves on the mortality and morphology of *Fasciolopsis buski* by using scanning electron microscopy. *In vitro* treatment with 5, 10, and 20 mg/ml of crude extract in phosphate buffer saline caused paralysis of worm followed by death and found to be more leathal than the commercial fluckicide, Oxyclozanide.

Asuzu *et al.* (1999), isolated and prepared methanolic extract from stem bark of the plant *Piliostigma thonningii* (Milne – Redh) and screened for anthelmintic activity by larval paralysis using Levamisole as a reference drug. Third stage larvae of *Haemonchus contortus* from fecal sample of infected lamb were used in the drug study. Plant preparation induced approximately 60% larvae paralysis within 24 hr. at 4.4 mg/ml concentration.

Asha *et al.* (2001) showed the essential oil and Eugenol (cheif constituents of Tulsi) possesses potent *in vitro* anthelmintic acitivity against *Coenorhabditis elegans* (nematode). During experiment various concentration of essential oil and Eugenol were using tested using Levamisole as reference standard. Both exhibited LD_{50} of 237.9 and 62.1μg/ml, respectively.

Berlin (2002) found that fresh Neem leaves significantly reduce *Hemonchus contortus* in the abomasum of the treated sheep.

Raja and Jandge (2003) found *in vitro* anthelmintic activity of decoction of *Nicotina tobaccum* against *Haemoncus contortus* in goats.

Iqbal *et al.* (2006) observed *in vitro* anthelmintic activity of *Nicotina tabacum* leaves against gastrointestinal nematodes of sheep. Live *Haemonchus contortus* were used to assess *in vitro* anthelmintic effects of crude aqueous extract and methanol extracts were administered in

increasing doses (1.0-3.0 g/kg) to sheep naturally infected mixed species of gastrointestinal nematodes. *In vitro* inhibitory effect was evident as paralysis and/or mortality of worms noted at 6 hours post-exposure. The result of the study showed that both the extracts possess dose- dependent anthelmintic activity, justifying the used of plant in traditional system of medicine.

Eguale *et al*. (2007) investigated *in vitro* anthelmintic activities of crude aqueous and hydro- alcoholic extracts of the seeds of *Coriandrum sativum* (*Apiaceae*) on the egg and adult nematode parasite *H. contortus.* The aqueous extract of *C. sativum* was also investigated for *in vivo* anthelmintic activity in sheep infected with *H. contortus*. Both extract types of *C. sativum* inhibited hatching of eggs completely at a concentration less than 0.5 mg/ml. The hydro-alcoholic extract showed better in vitro activity against adult parasites than the aqueous one. For the *in vivo* study, significant ($p < 0.05$) total worm count reduction was detected only for higher dose of *C. sativum.* Reduction in male worms was higher than female worms.

Costa e*t al*. (2008) conducted an *in vitro* test using ethyl acetate and ethanol extracts of *A. indica* on *H. contortus* eggs and larvae. The ethanol extract was found to be more effective inhibiting larval development by 87.11% as compared to 68.10% by ethyl acetate extract at 50mg/ml. The ethanol extract inhibited egg hatching by 99.77% at 3.12mg/ml. These results suggest that *A. indica* extracts may be useful in the control of gastro-intestinal nematodes of small ruminants.

Sujon *et al.* (2008) found that the efficacy of alcoholic extract of 10 plants such as, Anaros, Ata, Durba grass, Karola, Katakhura, Labanga, Neem, Pan, Pat and Tobacco against the gastrointestinal nematodes of goat were studied *in vitro* at different concentration at the rate of 1% (10mg/ml),

2% (20mg/ml), 5% (50mg/ml) and 10% (100mg/ml). The ethanol extract of 10 plants showed that all of them have more or less wormicidal effect. Four out of selected ten indigenous medicinal plants showed potential *in vitro* activity against gastrointestinal nematodes. Within these ten (10) plants 4 showed 60-80% and other showed 50% at a concentration of 100 mg / ml. The most common plants had highly significant activity against adult g/i nematodes *in vitro* were Anaros (Pineapple leaves), Karolla (fruit), Labanga (flower bud) and Neem (leaves). Goat treated with ethanol extract of Pineapple leaves, Karolla, Labanga and Neem at the dose of 100mg/kg body weight showed 73%, 78%, 85% and 81% efficacy on 9^{th} day respectively. They observe in goats the ethanol extract of Neem, at the dose of 100 mg/kg showed a 100% killing of parasites.

Adama *et al.* (2009) was examined the *in vitro* tests of *Anogeissus leiocarpus* leaf and *Daniellia oliveri* stem barks extracts as effective anthelmintic activity on eggs, first stage larvae and adults of *Haemonchus contortus*. The extracts were prepared to obtain six increasing concentrations. This was done with Phosphate Buffered Saline (PBS) for egg hatch, embryonated egg assays (75, 150, 300, 600, 1 200 and 2 400 μg/ml) and adult inhibition of motility assay (0.25, 0.5, 1, 2, 4 and 8 mg/ml). PBS and levamisole (at 0.125 μg/ml in PBS) were used as negative and positive control groups, respectively. Both plant extracts induced anthelmintic effects on the three life-cycle stages of *H. contortus* and these effects were significantly different when they were compared to the negative control group (PBS) ($P<0.05$).

Amin *et al.* (2009) also found that water extract of some indigenous plants like Neem, Tulsi, Tobacco, Jute etc. shows potential *in vitro* activities against adult parasites in cattle. The efficacy of water extract of these plants at concentration of 25 mg/ml and 50mg/ml was much lower

than that of conc. of 100mg/ml except Tobacco plant. Tobacco plants (25mg/ml and 50mg/ml) showed 100% in vitro efficacy against gastrointestinal nematodes of cattle. Neem, Barbados lilac, betel leaf, papaya, dodder, bitter gourd and white verticillia (50 mg/ml) showed 70-80% *in vitro* efficacy against gastrointestinal nematodes of cattle. Turmeric (50 mg/ml) was 88% effective in vitro against gastrointestinal nematodes of cattle. The plants (100mg/ml) had highly significant activity (90-100%) against adult gastrointestinal nematodes in vitro were: neem (leaves and bark), tobacco plant (leaves), Barbados lilac (leaves and bark), betel leaf (leaves), pineapple (leaves), jute (leaves), turmeric (rhizome), garlic (bulbs), devil's tree (leaves), papaya (leaves), dodder (whole plant), bitter gourd (leaves and seeds), white verticillia (leaves) and chaste tree (leaves).

Jeyathilakan *et al.* (2010) studied the flukicidal property of ethno-medicinal plant extracts of *Areca catechu*, *Erythrina indica* and *Zingiber officinale* against adult stage of *Fasciola gigantica in vitro.*Based on gross visual motility and mortality with score index, gross changes and histopathology of treated flukes, the *Areca catechu* extract had 100% lethal effect at 1% , 2.5% and 5% concentration. *Zingiber officinale* extract was effective only at 5% concentration and *Erythrina indica* ineffective in the concentrations.

Hussain *et al.* (2011) were evaluated ovicidal efficacy of *Ziziphus mauritiana* and *Terminalia arjuna* leaves. For this purpose, egg hatch test (EHT) was conducted on nematode ova to investigate the *in vitro* ovicidal effects of crude aqueous extract (CAE) and crude aqueous methanolic extracts (CAME) of the leaves of the plants. Lethal concentration 50 (LC_{50}) values of CAE and CAME of *Ziziphus mauritiana* leaves were 0.1773 and 0.6778 while of *Terminalia arjuna* leaves were 1.502 and 3.002

respectively. This study shows that *Ziziphus mauritiana* and *Terminalia arjuna* leaves possess *in vitro* anthelmintic activity.

Ahmed *et al.* (2012) reported ethanol extracts of 25 plant species were screened for anthelmintic effects against *Haemonchus contortus.* Ethanol extracts of each plant were used at various concentrations (10, 20 and 30%) to treat 10-day faecal cultures, incubated at 278 $^{\circ}$C with control cultures which were treated with ethanol for 48 h. Five plants with high efficacies (*Ananas comosus, Aloe ferox, Allium sativum, Lespedeza cuneata* and *Warburgia salutaris)* were selected from the first screening for further investigation using ethanol, dichloromethane and water extracts at four concentrations (2.5, 5, 10 and 20%). Ethanol was the most effective solvent. Larval counts decreased with increasing extract concentrations, of which 10 and 20% had similar effects. *Lespedeza cuneata* caused more than 70% mortality at all concentrations. However, there remains a need to assess in vivo efficacy of these plants.

Goswami *et al.* (2013) have been reported for the *in-vitro* anthelmintic activity of 30 plant seeds. The families of the plants have also been reported in which Fabaceae was found as most common plant family. Combretaceae, Meliaceae, Cucurbitaceae, Myrsinaceae and Anacardiaceae families were also found possessing anthelmintic activity. The current review also clears the picture about choosing the parasites which have been used for the activity. Ascaris Sp (35%), such as *Ascaris lumbricoides, Ascaris suum* etc, *Ascardia Galli* (29%), Tapeworm (18%), Earthworm *i.e. Pheritima posthuma* (18%), *Nippostrongyllus braziliensis, Haemonchus contours*, Roundworms, *Trichostrongylus colubrifromis* etc have been reported as different targeted parasites for the evaluation of anthelmintic activity. This review on the plant seeds which have been used in antelmintic activity will help in ethnopharmacology by evaluating possible

molecular level mechanism and isolation of active constituents by chromatography.

Joshi *et al.* (2013) selected *Ocimum sanctum* for its considerable Phenolic content and an attempt was made to evaluate anthelmintic potential against *Pheretema posthuma*. The aqueous extract and ethanolic extracts of *Ocimum sanctum* showed dose dependent anthelmintic action. Aqueous extract is more potent than ethanolic extract. Aqueous extract at concentration 2, 4 and 10 mg showed paralysis and consequent death of the organism.

Verma *et al.* (2013) investigated the anthelmintic efficacy an herbal drug of *Ocimum sanctum* in *Syphacia muris* infected mice. The drugs were administrated to the infected mice on 18th, 19th and 20th post infection days. Larval and Adult worm recovery were found to be directly proportional to the doses of drug. Significant decrease in worm recovery (larval and adult) due to test drug indicated the anthelmintic efficacy of *Ocimum sanctum*. Obtained results indicate that studied drug can be good anthelmintic/ nematocidal agent.

Swarnakar and Kumawat (2014) tested the alcoholic fruit pulp extract of *Citrullus colocynthis* was tested *in vitro* against amphistome parasites. The treated parasites showed complete loss of activity and paralysis followed by complete mortality at 5 hrs of exposure to 40mg/ml dose of fruit extract. During investigation, number of tests was carried out on *Orthocoelium scoliocoelium* with the fruit extract of *Citrullus colocynthis* to observe the anthelmintic efficacy of plant. The treated worms became agglutinated, shrunken, paralysed and dead after 5 hours at 40 mg/ml concentration of alcoholic fruit extract of *Citrullus colocynthis.*

Swarnakar et *al.* (2014) evaluated *in vitro* efficacy of medicinal plant aqueous extract of *Trigonella foenum-graecum* on *Gastrothylax crumenifer.* Treated worms become slender, shrunken, paralyzed and then finally died after 5 hours at 130 mg/ml concentration of seed of *Trigonella foenum-graecum.*

Akhtar *et al.* (2015) determine the anthelmintic activity of eight medicinal plants against adult and larvae (L3) of *Haemonchus contortus* during July to November, 2013. *In vitro* adulticidal and larvicidal activiies were screened by preparing aqueous and methanol extract of some plants at 1%, 5% and 10% concentration with respect to control by PBS. The results revealed that neem showed best effect within 3 hours at 5% concentration (100%) and 10% concentration (100%) with methanol extract on adult *Haemonchus*. Rest of the ingredient showed their efficacy after 3 hours. Methanol extracts showed better effect than aqueous extracts. In case of L3 stage larvae, the highest efficacy was observed at 10% concentration in neem (100%) and korolla fruits (100%), followed by korolla leaves (80%), sharna lata (80%) and lazzabati (60%) less than 3 hours. It is concluded that neem, korolla, sharna lata and lazzabati extract give a better approach against *Haemonchus contortus* and can be used against the treatment of haemonchosis in goat as an alternative of patent drug.

Sirama *et al.* (2015) investigated the anthelmintic activity of *Vernonia amygdalina* (Asteraceae) which is used by traditional medicine practitioners in Migori County, Kenya using adult *Haemonchus contortus* worm as a model.Death of *Haemonchus contortus* worm was determined within a period of 24 hrs. *Vernonia amygdalina* (roots) extract had mean mortality of 20-33.3% at 6.25 mg/ml; 23.3-46.7% at 12.5 mg/ml and 26.7-56.7% at 25 mg/ml. The result indicated that *Vernonia amygdalina*

contains tannins, saponins and cardiac glycosides which are anthelmintic agents this justifies its traditional use in the treatment of helimnthiosis.

Swarnakar *et al.* (2015) evaluated the *in vitro* anthelminthic activity of alcoholic extract of *Balanites aegyptica* (hingot) on amphistome *Paramphistomum cervi* of buffalo. 125 mg/ml concentrations of alcoholic extract gave total mortality at 5 hours. The alcoholic extract of hingot showed discontinuous, damaging cells of tegument, vacuolization & breakage in oral sucker and acetabulum of *Paramphistomum cervi.* This study revealed that the potential role of hingot fruit extract as an anthelminthic activity against *Paramphistomum cervi.*

Chapter-3

MATERIALS AND METHODS

MATERIALS AND METHODS

I. PREVALENCE OF AMPHISTOMES

❖ Area of Study

Study was conducted in buffaloes and their calves from privately organized khatals and local unorganized buffalo rearing farmers in and around Begusarai district with or without clinical signs of gastrointestinal disorders. Faecal samples were collected and examined.

❖ Duration of Study

The study was conducted for a period of one year from 1st June 2016 to 31st May 2017 in different villages of Begusarai district.

❖ Prevalence of amphistomiasis in buffaloes and their calves

To record the prevalence of amphistomiasis in buffaloes and their calves, a total of 426 buffaloes were surveyed during the period from 1st June 2016 to 31st May 2017 for the presence of different GI parasites including *Gigantocotyle explanatum* eggs. The prevalence of infection was assessed in terms of month-wise, season wise, age-wise, sex-wise, breed-wise and managemental condition. The prevalence of the disease was recorded as per the formula described below.

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

❖ Collection of Faecal Samples

Faecal samples were collected directly from the rectum by inserting and screwing moistened cotton swabs and kept in labled stoppered wide mouthed bottles of 20-30 ml capacity. In cases when samples were to be examined later few drops of 10% formalin were added to preserve the morphological characteristics of parasitic eggs.

❖ Examination of Faecal Samples

The examination of faecal samples were conducted in the PG laboratory of Department of Parasitology, Bihar Veterinary College, Patna.

(a) Gross Examination of faeces: First the collected samples were examined for the presence of adult and larval stages of parasites.

(b) Direct Smear Method: A small quantity of fresh faeces 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 40x.

(c) Concentration Methods: The following concentration methods were performed for detection of ova of different gastrointestinal helminth parasites.

(i) Sedimentation technique: About 1 to 2 grams of faecal sample was triturated in mortar and pestle with the addition of distilled water. The suspension was then filtered through wire mesh and the filtrate was centrifuged at 2000 rpm for 10 minutes. The supernatant fluid was discarded and a drop of residue was taken by glass rod on a clean microscopic slide and then covered with a coverslip. The preparation was examined first under low and then high power of microscope.

(ii) Salt floatation technique: About 1 to 2 grams of faecal sample was triturated in mortar and pestle by adding 2-3 ml of saturated salt solution (sp. gr. nearly 1.20). It was strained through fine tea strainer. This process was repeated 2 to 3 times to obtain clear solution without any debris and then filtrate was filled in glass vial upto its mouth. The final filling was carried out by means of dropper until a convex meniscus was formed. A clean slide was kept on the vial in such a way that it should touch the upper convex meniscus of the fluid. This preparation was allowed to stand for about 30 minutes and after that microscopic slide was quickly lifted, turned over smoothly to avoid spilling of the liquid and immediately covered with a coverslip. This process was conducted within 25-30 minutes, so that ova may not start distorting due to osmosis.

To obtain the accurate information with regard to the severity of infection, the no. of eggs per gram of faeces (epg) was determined by Stoll`s egg counting technique.

**(d) Stoll`s egg counting technique:** The E.P.G. of the faecal sample was calculated according to Stoll`s egg counting technique (1923).

a) Approximately 10-12 small sized (3-5mm diameter) glass beads were put in 120 ml glass stoppered bottle with 45 ml of N/10 caustic soda solution.
b) Three grams of faecal sample was added, stirred thoroughly in the bottle.
c) The stopper was fitted to the bottle and shaked thoroughly until all the faecal matter was broken down.
d) The mixture was then poured through a wire mesh screen with an aperture of 0.15 mm and the strained fluid was kept in a bowl. The debris left on the wire mesh was discarded.
e) The filtrate of the faeces was well stirred and 0.15 ml of the fluid was taken by means of a graduated pipette.
f) The measured quantity (0.15 ml) of fluid was ejected onto a slide and covered with a 22 x 10 mm cover glass.
g) The eggs of whole of the 0.15 ml sample of faecal suspension was examined under low power magnification of the microscope and all the eggs seen were counted with a hand tally counter.
h) The figures obtained from the count (i.e. the total number of eggs present in 0.15 ml of diluted faeces) were multiplied by 100 to give the no. of eggs per gram of the original faecal samples.

❖ **Identification of ova of *Gigantocotyle explanatum***

The eggs of amphistomes were identified on the basis of morphological characteristics as described by Soulsby, 1982.

2. COLLECTION OF BLOOD SAMPLES AND ESTIMATION OF BLOOD PARAMETERS:

Blood samples from five buffalo calves naturally infected by *Gigantocotyle explanatum* and five healthy buffalo calves (control) were collected. Three to five ml of blood was collected in EDTA coated vials from the jugular vein of each buffalo calf with help of disposable syringes after proper sterilization with spirit.

The blood samples were also collected on 0^{th}, 7^{th}, 14^{th} and 21^{st} post treatment days during clinical trial to evaluate the various haematological changes and blood biochemical levels in buffalo calves infected with *Gigantocotyle explanatum* in experimental calves.

(A). HAEMATOLOGICAL STUDIES:

The haematological studies in five infected and five control (healthy) calves were conducted soon after blood collection. Different blood parameters were studied as per the method described below.

Haemoglobin (Hb%):- Haemoglobin content of buffalo calves naturally infected with amphistomiasis i.e. *G. explanatum* and healthy control buffalo calves were estimated by Sahlis acid hematin method (Sinha, 1998).

Total Erythrocyte Count (TEC):- The total erythrocyte count values were counted by Neubauer`s haemocytometer counting chamber method (Sinha, 1998).

Total Leucocyte Count (TLC): The TLC values were determined by Neubauer`s haemocytometer counting chamber method as described by Sinha, 1998.

Packed Cell Volume (PCV): The PCV of all the blood samples were carried with the help of Wintrobe haematocrit method (Schalm *et al.* 1975).

Differential Leucocyte Count (DLC): The DLC was determined by Leishman`s stain method (Schalm *et al.* 1975).

SEPARATION OF SERUM AND ITS PRESERVATION:

The blood collected in a dried test tubes were allowed to clot in slanting position for about half an hour at room temperature. Then it was transferred into a centrifuge tube and centrifuged for 20 minutes at 3000 rpm. The clean serum was pipette out with the help of sterilized pasture pipette and kept in 10 ml clean vial. The serum was stored in deep freezer at -20^0C till further analysis.

(B) ESTIMATION OF SERUM BILE ENZYMES:

Estimation of serum bile enzymes were determined by method as described by Sinha, 1998.

3. INTENSITY OF INFECTION OF *Gigantocotyle explanatum* IN BUFFALOES AND THEIR CALVES:

A total of 426 faecal samples for amphistome infection were screened and classified for the range of intensity of infection. The no. of

eggs were counted of different twenty randomly selected fields of cover slip for each sample and categorized after counting mean or average number ova per five field. All the samples were examined by direct method and observed under 10x power of microscope. The range of intensity were classified as follows :

(+) = 2-4 ova per five field (Light).

(++) = 4-8 ova per five field (Medium or Moderately)

(+++) = 8-15 ova per five field (Heavy)

(++++) = More than 15 ova per field (Very Heavy)

4. THERAPEUTIC TRIALS:

The experiment was conducted to determine the efficacy of 2 different herbs viz. *Allium sativum* (garlic) and of *Azadirachta indica* (neem) in an attempt to control the amphistome infection in buffaloes. A total of 20 buffalo calves (3-6 months old), naturally infected with amphistomes, without any previous history of use of anthelmintics were randomly selected for this experiment.

❖ Experimental Design

The experimental buffalo calves were randomly divided into four groups viz. $\mathbf{T_1}$, $\mathbf{T_2}$, $\mathbf{T_3}$ and **C** five calves in each group. The calves in group **C** were kept control and were not treated with any medicine. The group $\mathbf{T_1}$ was treated with aqueous neem leaves powder orally at the dose rates 100 mg/kg b.w./day (200mg/kg b.w./day) of neem leaves powder and molasses preparation) in 1:1 ratio for twenty one days. The calves in group $\mathbf{T_2}$ were administered with aq. garlic cloves extract at the dose rate of 100mg/kg b.w./day. for twenty one days. The calves in group $\mathbf{T_3}$ were administered with both aq. garlic cloves extract and neem leaves powder at the dose rate of (100mg + 100mg) /kg b.w./day for twenty one days. The aq. garlic cloves extract and neem leaves powder were prepared as described below.

❖ **Methods of preparation of neem leaves powder:**

Powder was prepared by pulverizing the dried leaves of neem tree with the help of manual grinder. A 25 mesh diameter sieve was used to obtain fine dust and preserved them into airtight plastic container till their use in extract preparation. One hundred grams of powder was mixed thoroughly with 100 gm of molasses to make it 200g preparation.

❖ **Methods of preparation of aqueous garlic cloves extract:** Aqueous extract of *Allium sativum* was prepared by the method as described by Handa (1990).

- One hundred grams of powdered garlic was taken in a flask to which one litre of distilled water was added.
- This was boiled till the contents were reduced to half.
- Subsequently, it was cooled and filtered through a muslin cloth to remove insoluble material.
- The filtrate was again filtered through ordinary filter paper and then poured in a clean dry petridish and heated for complete evaporation.
- Care was taken to avoid charring.
- The petridish was then allowed to cool at room temperature. Thus, each ml of this preparation contains 200mg of garlic cloves extract.

❖ **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 0^{th}, 7^{th}, 14^{th} and 21^{st} day post treatment using Stoll`s egg counting technique as described before. The blood was also collected on the day of treatment as well as on the final day of experiment (day 15^{th}). The blood was processed for normal haematological indexes as described by Latimer *et al.* (2003). The efficacy

of both of these herbs were evaluated on the basis of negative faecal sample (i.e. declining rate of epg) found and improvement in blood parameters on post supplementation days.

$$\text{Percent Efficacy} = \frac{\text{Pretreatment epg} - \text{Post treatment epg}}{\text{Pretreatment epg}} \times 100$$

Experimental design of anthelmintic trials in buffalo infected with *Giganocotyle explanatum*

Group	No. of infected buffaloes	Supplementation with herbs at specified dose	Days of Observation			
			0th day	7th day	14th day	21st day
T 1	5	Neem extract **(100mg/kg b.w./day)**	(i) Faecal sample examination. (ii) Haematological parameters.			
T 2	5	Garlic clove extract **(100 mg/kg b.w./day)**				
T 3	5	Both neem extract and garlic clove extract **(100mg + 100mg) /kg b.w/day)**				
C	5	Control (untreated)				

STATISTICAL ANALYSIS: Statistical analysis were done with the method of Snedecor and Cochran (1991) analysis.

COLLABORATION WITH OTHER DEPARTMENTS

Department of Veterinary Biochemistry, Veterinary Pathology, Veterinary Public Health, Veterinary Medicine, Teaching Veterinary Clinical Complex, Instructional Composite Livestock Farm (ICLF) of Bihar Veterinary College, Patna and Baliya (Begusarai) and Barauni (Begusarai) Block Animal Husbandry Offices.

Chapter- 4

RESULTS AND DISCUSSION

RESULTS

Bubalus bubalis (buffalo) is one of the most important species of domestic livestock as a source of dairy, meat, manure and drought power and plays an important role in Indian rural economy. In India, the majority of small and marginal farmers are more dependent on buffaloes than cattle for their livelihood as they also serve as an insurance against the risk of crop failure due to natural calamities (Dhanda, 2004). Buffalo diseases have been considered as one of the major constraints for the development of the dairy and meat industry in the developing countries causing substantial economic loss to poor subsistence farmers. In India, many factors like diseases, genetic makeup, poor nutritional and management practices, environmental stress etc. are major constraints responsible for the low productivity of buffalo. The parasitic diseases, gastro-intestinal helminthiasis, coccidiosis, fasciolosis and mange are not less important in buffaloes than other infectious diseases (Griffiths, 1974). Epidemiological survey of parasitic infection is an important aid to combat infections more effectively and in controlling economic losses by adopting effective control measures. The incidence of G.I. parasites in cattle and buffaloes from different parts of India has been published from time to time for this purpose (Sanyal and Singh, 1995;; Muraleedharan, 2005; Wadhwa *et al*., 2011).

Helminth parasitism especially gastrointestinal parasitism is one of the major health problems severely limiting the animal productivity in dairy animals. Chemotherapy is a major treatment modality used for the control of helminth infection in livestock. However, due to increasing

development of anthelmintic resistance and the limited availability of commercial drugs to the rural people as well as the high cost of such synthetic medicines, a growing interest in the ethno-veterinary approach to examine the anthelmintic properties of plants traditionally used by local farmers in different parts of the globe is emerging. Medicinal plants are resources of new drugs and have served through ages, as a constant source of medicaments for the treatment of a variety of diseases and are known to provide a rich source of botanical anthelmintics. A large number of plant products are being used to combat gastro-intestinal parasites of livestock and also humans. Phytotherapeutic drugs are safe, non-toxic, biodegradable and do not leave residues in animals products. This study summarizes the anthelmintic activities of the medicinal plants viz. neem and garlic.

❖ OVERALL PREVALENCE OF GASTRO-INTESTINAL PARASITES

During this study period, a total of 426 fecal samples were examined. More than three fourth samples (82.18%) were found to be infected with one or more species of gastro-intestinal parasites. Five species of gastro-intestinal parasites were detected. Among them, two species were trematode, namely *Fasciola gigantica* (21.13%) and Amphistomes (including *Gigantocotyle explanatum)* (46.24%). Two species were nematode viz. *Haemonchus contortus* (17.84%) and *Toxocara vitulorum* (11.74%) and one species of protozoa, *Balantidium coli* (44.86%). (Table- 1, Graph-1).

❖ AGE RELATED PREVALENCE OF GASTRO-INTESTINAL PARASITES

In this study, buffaloes of all age groups were found infected with five species of gastrointestinal parasites with single or combined infections.

Amphistomes was the highest (57.14%) in buffalo calves group whereas in adult and young groups, it was 46.26% and 40.00%, respectively. Prevalence of *Toxocara vitulorum* (40.00%) was recorded highest in buffalo calves whereas in adult and young buffaloes it was 15.38% and 9.23%, respectively. So, *Toxocara vitulorum* was lowest in young age group. *Haemonchus contortus* (6.16%) was lowest in young group whereas in adult it was highest (50.00%) among buffalo calves. Faecal samples of sixty percent buffalo calves were found positive for *B. coli* infection. *Fasciola gigantica* was recorded at highest level among adult buffalo population followed by young and calves. (Table 2, Graph 2).

- **SEX RELATED PREVALENCE OF GASTRO-INTESTINAL PARASITES**

Fasciola gigantica and *Haemonchus contortus* positive samples were more among female buffaloes (25.71% and 18.21%) population than males (12.33% and 17.21%) as represented in Table- 3. Amphistomes (50.68%), *Toxocara vitulorum* (12.33%) and *B. coli* (57.33%) infection prevalence was higher among male buffalo population than female buffaloes i.e. 43.93, 18.21 and 38.21 percent, respectively. (Graph-3)

- **NUTRITIONAL AND MANGEMENTAL STATUS RELATED TO PREVALENCE OF GASTRO-INTESTINAL PARASITES**

It was revealed both medium and poor body conditioned buffaloes were infected with gastrointestinal parasites and every individual was infected with at least one species of parasite. In poor body conditioned buffaloes the highest infection was with amphistomes (62.28%), followed by *B. coli* (50.00%), *F. gigantica* (28.07%), *H. contortus* (25.44%) and *T. vitulorum* (18.42%), respectively. In medium body conditioned buffalo, the prevalence was highest in *T. vitulorum* (40.40%) followed by *B. coli*

(38.89%), amphistomes (27.78%), *Fasciola gigantica* (13.13%) and *H. contortus* (9.09%), respectively. (Table 4, Graph-4).

- **SEASONAL PREVALENCE OF GASTRO-INTESTINAL PARASITES**

Table-5 and Graph-5 shows the seasonal prevalence of gastro-intestinal parasites. It was observed that rainy season (July - October) registered highest prevalence of all the five species of G.I. parasites. Prevalence of amphistomes (40.00%) was found higher in summer season than winter season i.e. 32.14%. On the other hand, *Fasciola gigantica* (19.29%), *T. vitulorum* (11.43%) and *B. coli* (43.57%) were observed more in winter season. Fifteen percent faecal samples were found positive for *H. contortus* infection in both winter and summer seasons.

- **MONTHWISE AND SEASONWISE PREVALENCE OF AMPHISTOMES (INCLUDING *G. explanatum)***

It was observed that, the monthwise and seasonwise effect on amphistomes parasitism in buffaloes was significant ($p<0.01$). A total of 197 samples out of 426 faecal samples were found positive for amphistomes (including *Gigantocotyle explanatum.)* Highest prevalence of amphistomes was registered in the month of July (86.48%), whereas lowest positive cases (25.00%) were registered in the month of April. (Table-6, Graph-6). Highest prevalence of amphistomes was observed in the rainy season followed by summer and winter seasons i.e. 65.75, 40.00 and 32.14 percent, respectively. (Table-7, Graph-7).

- **AGEWISE AND SEXWISE PREVALENCE OF AMPHISTOMES (INCLUDING *G. explanatum*)**

Buffalo calves (57.14%) were found positive for amphistomes followed by adult (46.46%) and young (40.00%) buffaloes, respectively. Amphistomes affected more than fifty percent male buffaloes whereas about forty four percent female buffaloes were affected, as represented in Tables-8 and 9.

- **NUTRITIONAL AND MANAGEMENTAL STATUS RELATED PREVALENCE OF AMPHISTOMES (INCLUDING *G. explanatum*)**

It was revealed that nutritional and managemental status of buffaloes had significant ($p<0.01$) effect on amphistomes (including *G. explanatum*) parasitic infection. In buffaloes kept in poor nutritional and managemental conditioned the rate of infection was 62.28% whereas in buffaloes kept in medium nutritional and managemental conditions it was 27.78%. (Table-10, Graph-10).

- **HAEMATOLOGICAL PARAMETERS IN CONTROL AND *G. explanatum* INFECTED GROUPS :**

Mean $\pm$ S.E. of haematological parameters in control and *G. explanatum* infected groups were studied as represented in Tables - 11,12 and Graphs – 11, 12,13. Mean $\pm$ S.E. values of Haemoglobin (9.075 $\pm$ 0.160 to 8.112 $\pm$ 0.069), Total Erythrocyte Count (TEC) (7.607 $\pm$ 0.156 to 5.195 $\pm$ 0.086) and Packed Cell Volume (32.653 $\pm$ 0.180 to 26.317 $\pm$ 0.573) showed significant reduction in *G. explanatum* infected buffalo groups. On the other hand, Total Leucocyte Count (TLC) (8.072 $\pm$ 0.0551 to 8.089 $\pm$ 0.0573), Mean Corpuscular Volume (MCV) (42.89 $\pm$ 0.240 to 50.67$\pm$ 0.320), Mean Corpuscular Hemoglobin (MCH) (11.92 ± 0.194 to 15.61 ± 0.218) and Mean Corpuscular Hemoglobin Concentration (MCHC) (27.79

± 0.128 to 30.82 ± 0.186) showed non-significant rise in Mean ± S.E. values in *G. explanatum* infected groups.

In Differential Leucocyte Count (DLC), it was observed that there was significant rise in Mean ± S.E. values of neutrophils (29.420 ± 0.127 to 29.909± 0.181), eosinophils (4.588 ± 0.193 to 5.177 ± 0.347) and monocytes (4.817 ± 0.142 to 5.096 ± 0.032). A non- significant rise in Mean ± S.E. value of basophils (0.240 ± 0.037 to 0.302 ± 0.043) was observed, whereas reduction in Mean ± S.E. value of lymphocytes (56.486 ± 0.241 to 55.870 ± 0.216) was recorded in *G. explanatum* infected groups. (Tables - 11, Graphs-12).

Table 12 and Graph-13 shows Mean ± S.E. of Serum enzyme level (bile biochemical analysis) in healthy and *G. explanatum* infected groups. There was a significant rise in Mean ± S.E. value of Aspartate Aminotransferase (AST) enzyme level (90.52 ± 137.2 to 238.2 ± 319.2), formerly known as Serum Glutamic-Oxaloacetic Transaminase (SGOT). The rise in Total Serum Protein (TSP) (0.79 ± 0.0 to 0.84 ± 0.0) and Alanine Transaminase (ALT) (5.8 ± 0.0 to 6.86 ± 1.39) formerly known as, Serum Glutamate Pyruvate Transaminase (SGPT) was non- significant.

- **Post treatment changes in Mean ± S.E. of eggs per gram (epg) and percent efficacy of herbal preparations in *G. explanatum* infected buffaloes.**

The buffaloes in **T_1** group treated with neem extract showed significant reduction in epg. The percent efficacy recorded was 3.99, 9.32 and 17.88 percent on 7^{th}, 14^{th} and 21^{st} days, respectively. The buffaloes in **T_2** group treated with garlic extract showed significant reduction in epg. The percent efficacy recorded was 7.06, 9.84 and 18.59 percent on 7^{th}, 14^{th} and 21^{st} days, respectively. But, in **T_3** group treated with both neem and

garlic extract significant reduction in epg was recorded. The percent efficacy registered was 7.65, 16.67 and 23.98 percent on 7^{th}, 14^{th} and 21^{st} days, respectively. This indicated that these herbs in combination are much efficient in amphistome control than used alone. The buffaloes in untreated control group (**C**) did not show any significant change in epg values as represented in Table-13, Graph-14.

- **Post treatment changes in Mean ± S.E. of Haemoglobin (%), Total Erythrocyte Count (TEC), Total Leucocyte Count (TLC) and Packed Cell Volume (PCV) of *G. explanatum* infected buffaloes treated with different herbal preparations at different time intervals.**

The post treatment changes in Mean ± S.E. of Haemoglobin (%) and Total Erythrocyte Count (TEC) in treatment groups $\mathbf{T_1}$, $\mathbf{T_2}$ and $\mathbf{T_3}$ were observed significant on 7^{th}, 14^{th} and 21^{st} days, respectively. But, in control group (**C**) the changes in Haemoglobin (%) and Total Erythrocyte Count (TEC) was non-significant on post treatment days. (Table-15,16 and Graph-16,17). The buffaloes in $\mathbf{T_1}$ and $\mathbf{T_2}$ group buffaloes treated with herbs showed non-significant increase in Total Leucocyte Count (TLC) on 7^{th}, 14^{th} and 21^{st} days, respectively, whereas in $\mathbf{T_3}$ and **C** group it showed significant rise. (Table-17, Graph-18).

Table-18 showed the significant rise in Packed Cell Volume (PCV) in treatment groups $\mathbf{T_1}$, $\mathbf{T_2}$ and $\mathbf{T_3}$ on 7^{th}, 14^{th} and 21^{st} days, respectively. But, in control group (**C**) no significant change in Packed Cell Volume (PCV) was recorded.

- **Post treatment changes Mean ± S.E. of Serum Enzyme Level (bile biochemical analysis) in *G. explanatum* infected buffaloes**

treated with different herbal preparations at different time intervals.

Table-19 and Graph-20 represented significant rise in Mean ± S.E. value of Aspartate Aminotransferase (AST) enzyme level was observed in treatment groups **T_1**, **T_2** and **T_3** on 7^{th}, 14^{th} and 21^{st} days, respectively. But, in control group (**C**) no significant change in Packed Cell Volume (PCV) was recorded. The significant rise in treatment group **T_3** was higher than recorded in **T_1** and **T_2** groups. This rise was suggestive of the regeneration of liver damage.

Table -1

Overall prevalence of gastrointestinal parasites in buffaloes at Begusarai, Bihar (n=426).

Categories	Name of parasites	No. of animal affected	Prevalence (%)
Buffaloes and their calves (N=426)	*Fasciola giganitca*	90	21.13
	Amphistomes (including *Gigantocotyle explanatum*)	197	46.24
	Toxocara vitulorum	50	11.74
	Haemonchus contortus	76	17.84
	B. coli	191	44.86
	Sub Total	**604***	**141.81***

***** = Total no. of animals affected is less than the summation of individual infection because same animal was infected with more than one type of gastro-intestinal parasites

Graph – 1

Overall prevalence of gastrointestinal parasites in buffaloes at Begusarai, Bihar (n=426).

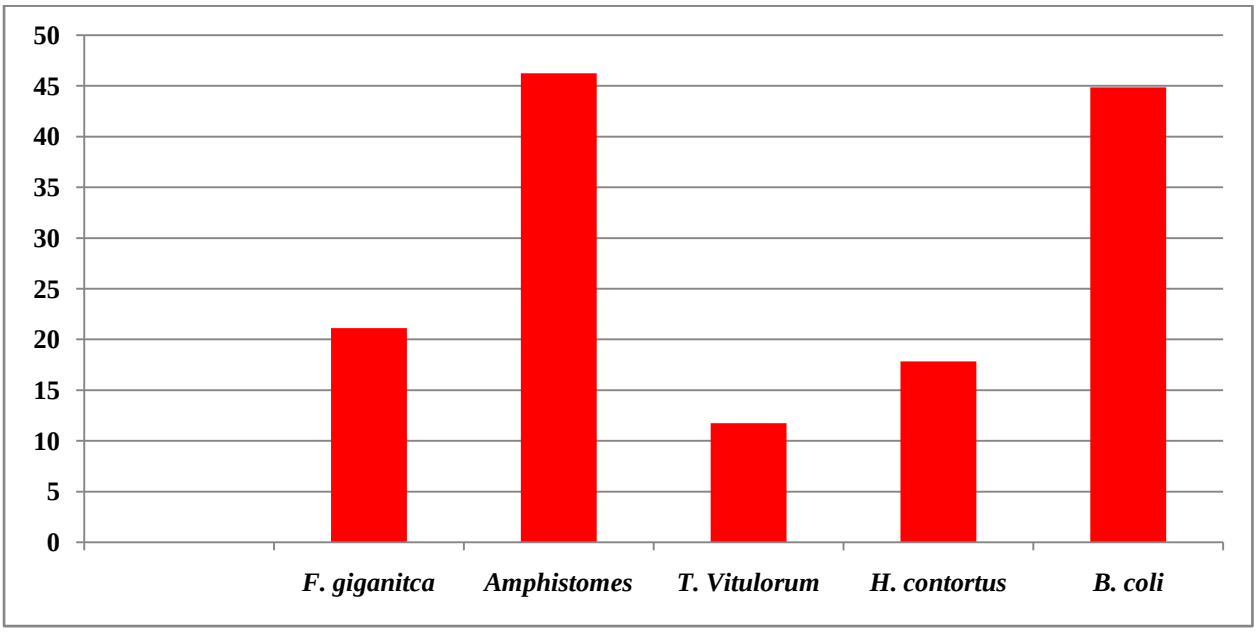


Table - 2

Age related prevalence of gastrointestinal parasites in buffaloes at Begusarai, Bihar (n=426).

Categories	Name of parasites	No. of animal affected	Prevalence (%)
Buffalo calves <2 years (N= 70)	*Fasciola giganitca*	5	7.14
	Amphistomes (including *Gigantocotyl explanatum*)	40	57.14
	Toxocara vitulorum	28	40.00
	Haemonchus contortus	35	50.00
	B. coli	42	60.00
	Sub Total	**70**	**100**
Young (2-5) years (N=130)	*Fasciola giganitca*	11	8.46
	Amphistomes (including *Gigantocotyl explanatum*)	52	40.00
	Toxocara vitulorum	12	9.23
	Haemonchus contortus	8	6.16
	B. coli	76	58.46
	Sub Total	**130**	**100**
Adult >5 years (N=226)	*Fasciola giganitca*	74	32.74
	Amphistomes (including *Gigantocotyl explanatum*)	105	46.46
	Toxocara vitulorum	20	15.38
	Haemonchus contortus	33	14.60
	B. coli	73	32.30
	Sub Total	**226**	**100**

Graph - 2

Age related prevalence of gastrointestinal parasites in buffaloes at Begusarai, Bihar (n=426).

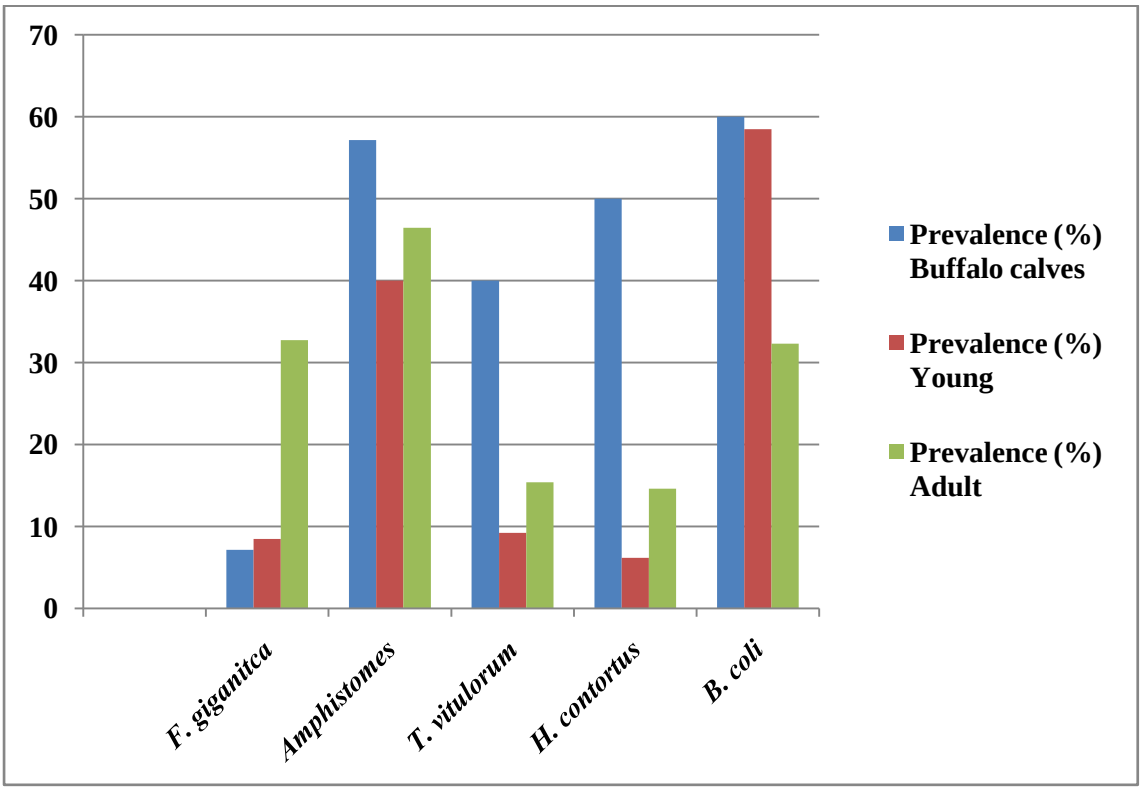


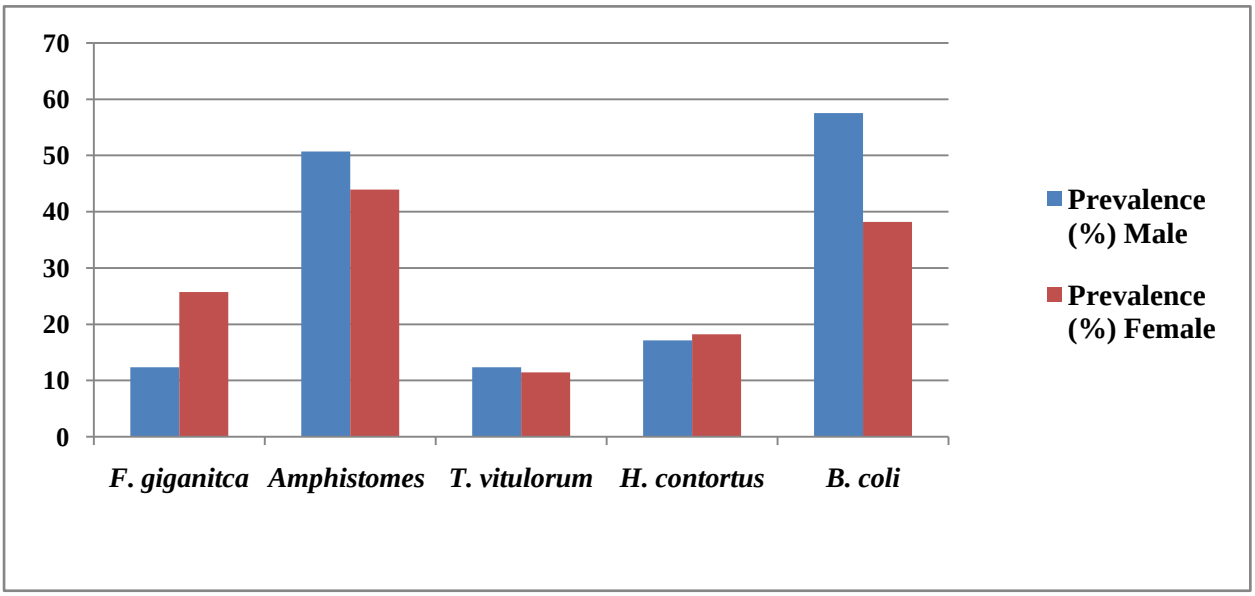
Table 3

Sex related prevalence of gastrointestinal parasites in buffaloes at Begusarai, Bihar (n=426).

Categories	Name of parasites	No. of animal affected	Prevalence (%)
Male (N=146)	*Fasciola giganitca*	18	12.33
	Amphistomes (including *Gigantocotyl explanatum*)	74	50.68
	Toxocara vitulorum	18	12.33
	Haemonchus contortus	25	17.12
	B. coli	84	57.53
	Sub Total	**146**	**100**
Female (N=280)	*Fasciola giganitca*	72	25.71
	Amphistomes (including *Gigantocotyl explanatum*)	123	43.93
	Toxocara vitulorum	32	11.43
	Haemonchus contortus	51	18.21
	B. coli	107	38.21
	Sub Total	**280**	**100**

Graph - 3

Sex related prevalence of gastrointestinal parasites in buffaloes at Begusarai, Bihar (n=426).



Graph - 4

Nutritional and Managemental Status related prevalence of gastrointestinal parasites in buffaloes at Begusarai, Bihar (n=426).

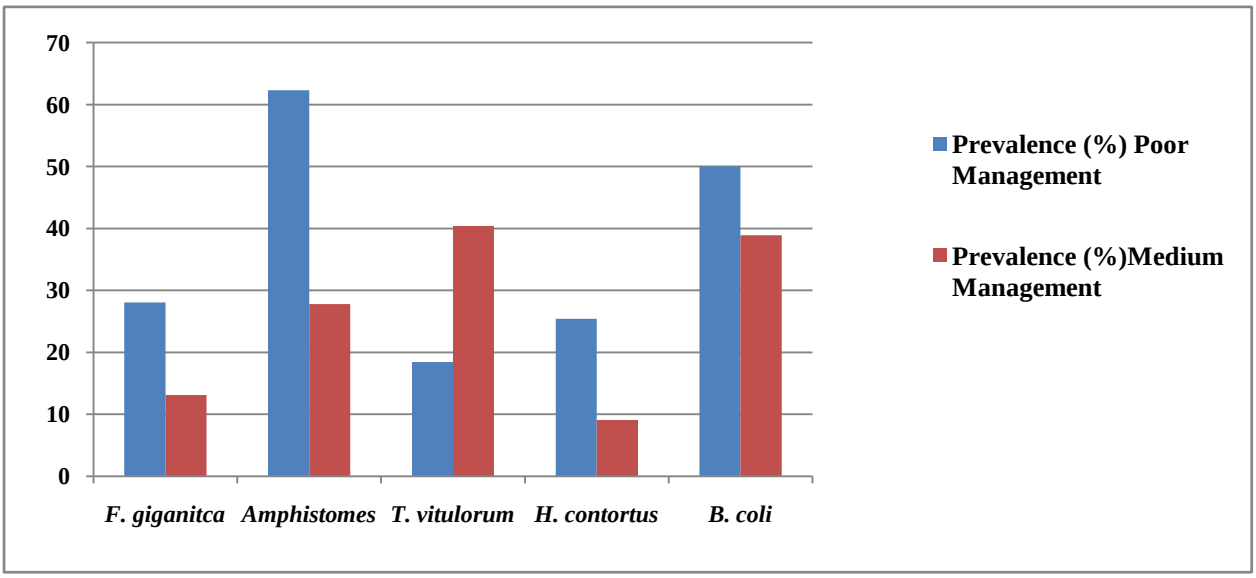


Table – 4

Nutritional and Managemental Status related prevalence of gastrointestinal parasites in buffaloes at Begusarai, Bihar (n=426).

Categories	Name of parasites	No. of animal affected	Prevalence (%)
Poor (N=228)	*Fasciola giganitca*	64	28.07
	Amphistomes including *Gigantocotyl explanatum*)	142	62.28
	Toxocara vitulorum	42	18.42
	Haemonchus contortus	58	25.44
	B. coli	114	50.00
	Sub Total	**228**	**100**
Medium (N=198)	*Fasciola giganitca*	26	13.13
	Amphistomes (including *Gigantocotyl explanatum*)	55	27.78
	Toxocara vitulorum	8	40.40
	Haemonchus contortus	18	9.09
	B. coli	77	38.89
	Sub Total	**198**	**100**

Table - 5

Seasonal status related prevalence of gastrointestinal parasites in buffaloes at Begusarai, Bihar (n=426).

Categories	Name of parasites	No. of animal affected	Prevalence (%)
Summer (March- June) (N=140)	*Fasciola giganitca*	23	16.43
	Amphistomes (including *Gigantocotyl explanatum*)	56	40.00
	Toxocara vitulorum	15	10.71
	Haemonchus contortus	21	15.00
	B. coli	57	40.71
	Sub Total	**140**	**100**
Rainy (July- Oct.) (N=146)	*Fasciola giganitca*	40	27.40
	Amphistomes (including *Gigantocotyl explanatum*)	96	65.75
	Toxocara vitulorum	19	13.01
	Haemonchus contortus	34	23.29
	B. coli	73	50.00
	Sub Total	**146**	**100**
Winter (Nov.- Feb.) (N=140)	*Fasciola giganitca*	27	19.29
	Amphistomes (including *Gigantocotyl explanatum*)	45	32.14
	Toxocara vitulorum	16	11.43
	Haemonchus contortus	21	15.00
	B. coli	61	43.57
	Sub Total	**140**	**100**

Graph - 5

Seasonal status related prevalence of gastrointestinal parasites in buffaloes at Begusarai, Bihar (n=426).

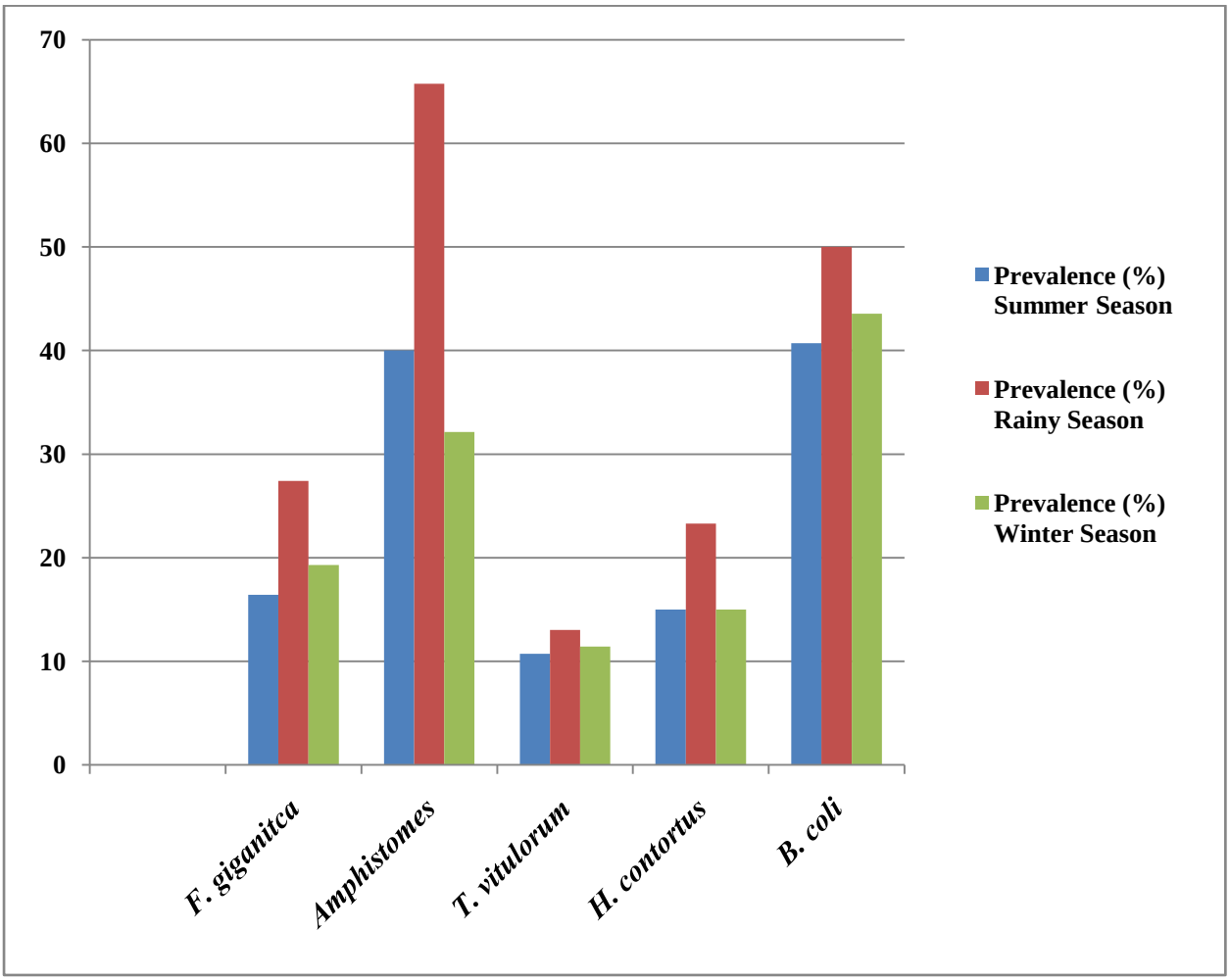


Table - 6

Monthwise prevalence of amphistomes (including *Gigantocotyle explanatum*) in buffaloes at Begusarai, Bihar (n=426).

Month	No. of animals screened	No. of animals affected	Prevalence (%)	$\chi 2$ at 11 df
June	35	27	77.14	
July	37	32	86.48	
August	40	30	75.00	
September	34	23	67.64	
October	35	13	37.14	
November	35	11	31.42	25.37^{**}
December	33	11	33.33	
January	37	12	32.43	
February	35	9	25.71	
March	35	6	17.14	
April	36	9	25.00	
May	34	14	41.17	
Subtotal	**426**	**197**		

**** = significant at (P<0.01)**

Graph – 6

Monthwise prevalence of amphistomes (including *Gigantocotyle explanatum*) in buffaloes at Begusarai, Bihar (n=426).

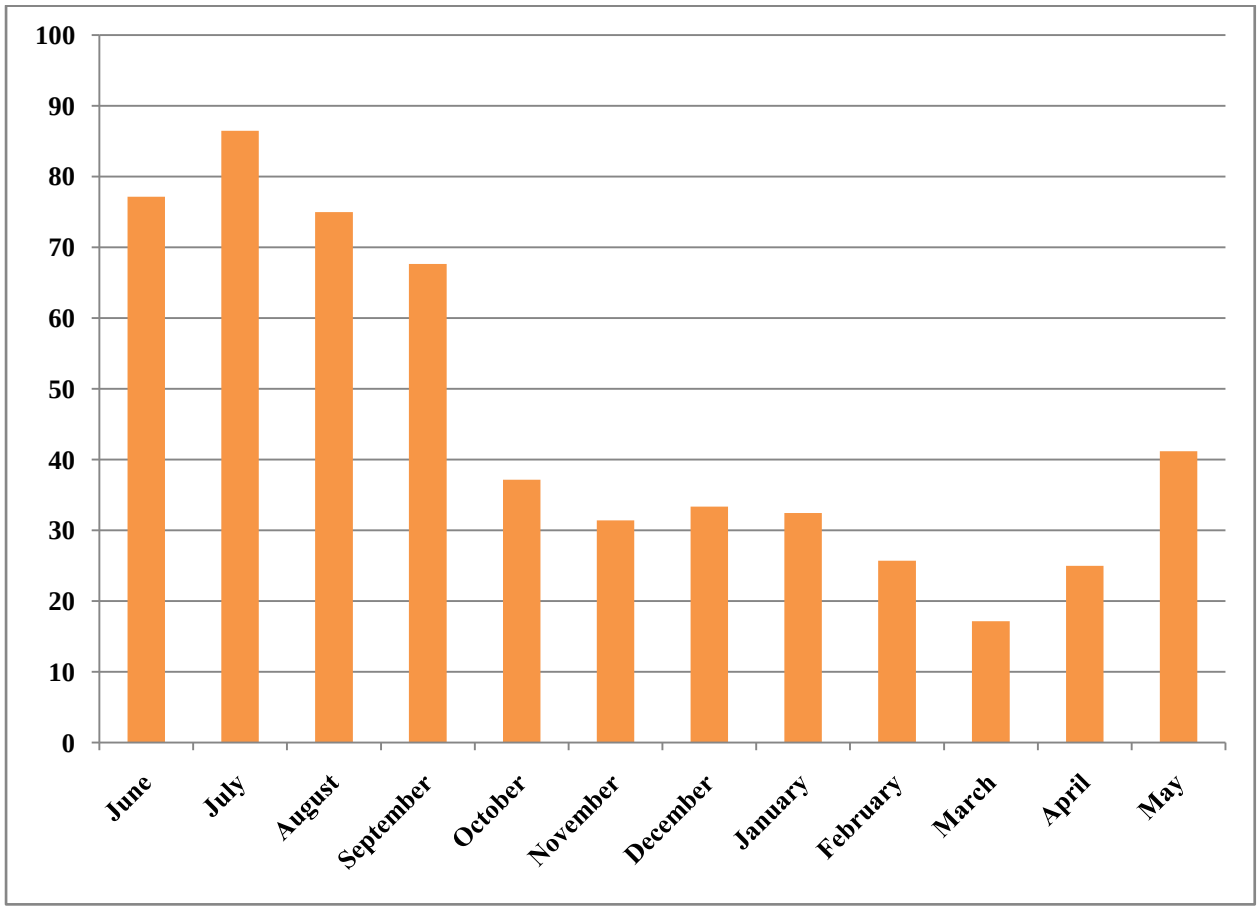


Table –7.

Seasonwise prevalence of Amphistomes (including *Gigantocotyle explanatum*) in buffaloes at Begusarai, Bihar (n=426).

Season	No. of samples	No. of positive samples	Percentage of infections	$\chi 2$ at 2 df
Winter (Nov.-Feb.)	140	45	32.14	21.18^{**}
Summer(March-June)	140	56	40.00	
Rainy (July- October)	146	96	65.75	
Total	**426**	**197**		

**** = significant at (P<0.01)**

Graph –7.

Seasonwise prevalence of Amphistomes (including *Gigantocotyle explanatum*) in buffaloes at Begusarai, Bihar (n=426).

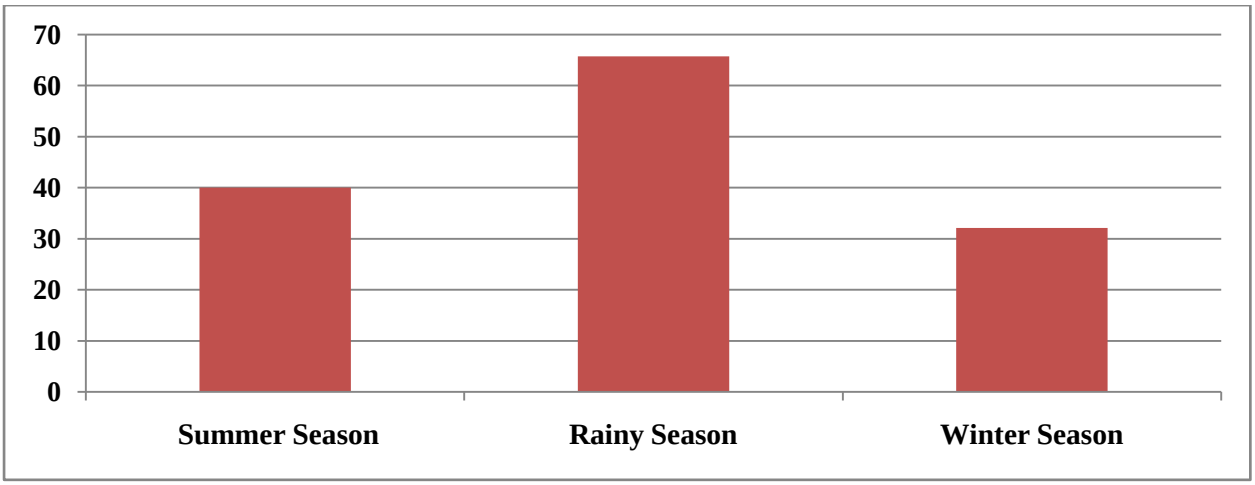


Table -8.

Agewise prevalence of Amphistomes (including *Gigantocotyle explanatum*) in buffaloes at Begusarai, Bihar (n=426).

Age group	Faecal samples examined	Samples found positive	Percentage of infections	$\chi 2$ at 2 df
Buffalo calves (<2 years)	70	40	57.14	34.57**
Young (2-5 years)	130	52	40.00	
Adult (>5 years)	226	105	46.46	
Total	**426**	**197**		

**** = significant at (P<0.01)**

Graph – 8

Agewise prevalence of Amphistomes (including *Gigantocotyle explanatum*) in buffaloes at Begusarai, Bihar (n=426).

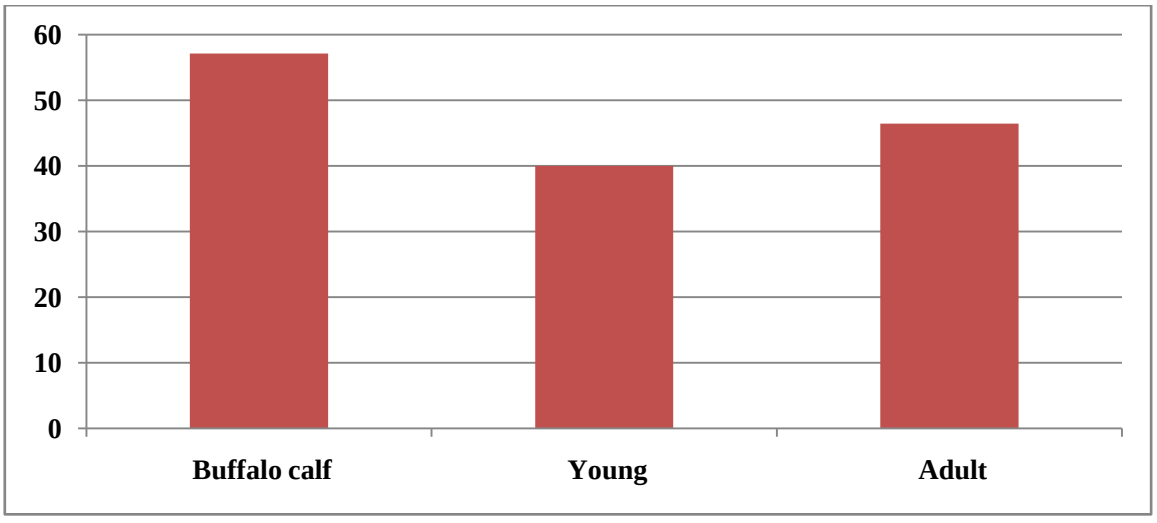


Table –9

Sexwise prevalence of Amphistomes (including *Gigantocotyle explanatum*) in buffaloes at Begusarai, Bihar (n=426).

Sex	No. of faecal Samples examined	Samples found positive	Percentage of infections	$\chi 2$ at 1 df
Female	280	123	43.93	19.35^{**}
Male	146	74	50.68	
Total	**426**	**197**		

**** = significant at (P<0.01)**

Graph –9

Sexwise prevalence of Amphistomes (including *Gigantocotyle explanatum*) in buffaloes at Begusarai, Bihar (n=426).

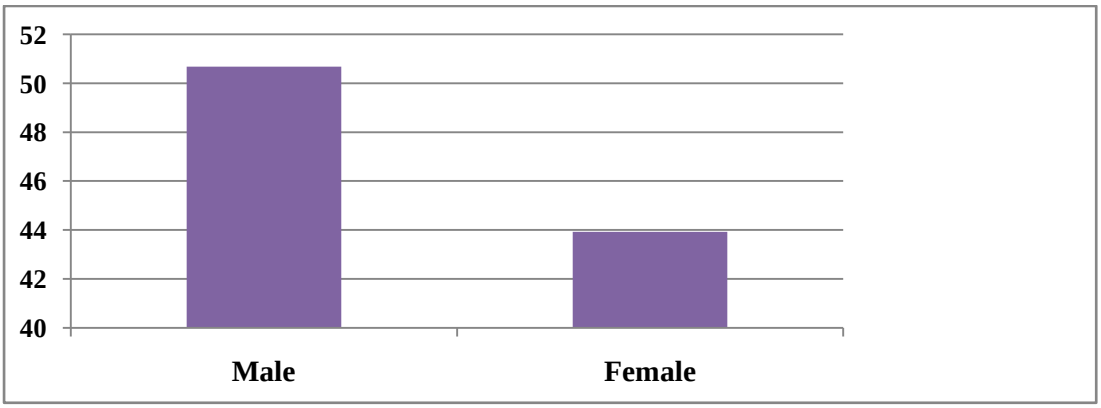


Table – 10

Nutritional and Managemental Status related prevalence of Amphistomes (including *G. explanatum*) in buffaloes at Begusarai, Bihar (n=426).

Categories	Samples examined	Samples Found positive	Percentage of infection	$\chi 2$ at 1 df
Poor	228	142	62.28	20.397**
Medium	198	55	27.78	
Total	**426**	**197**		

**** = significant at (P<0.01)**

Graph – 10

Nutritional and Managemental Status related prevalence of Amphistomes (including *G. explanatum*) in buffaloes at Begusarai, Bihar (n=426).

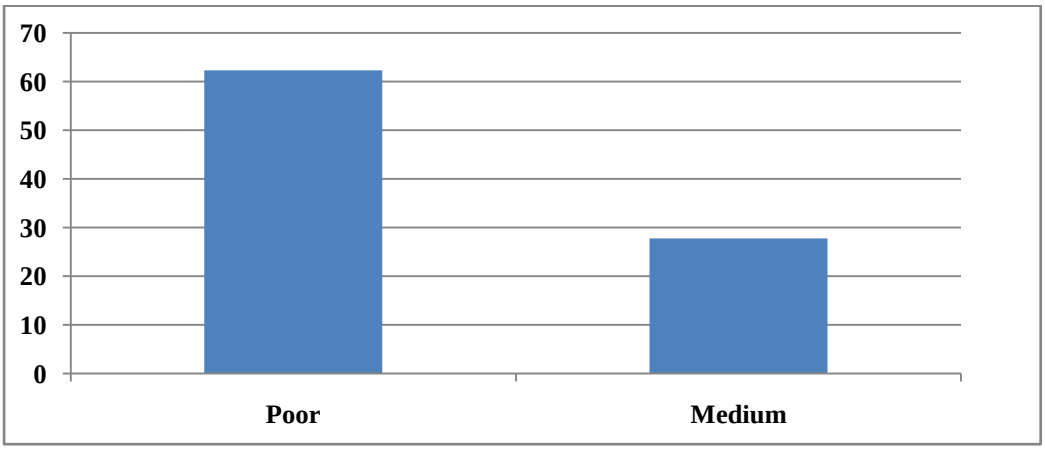


Table 11.

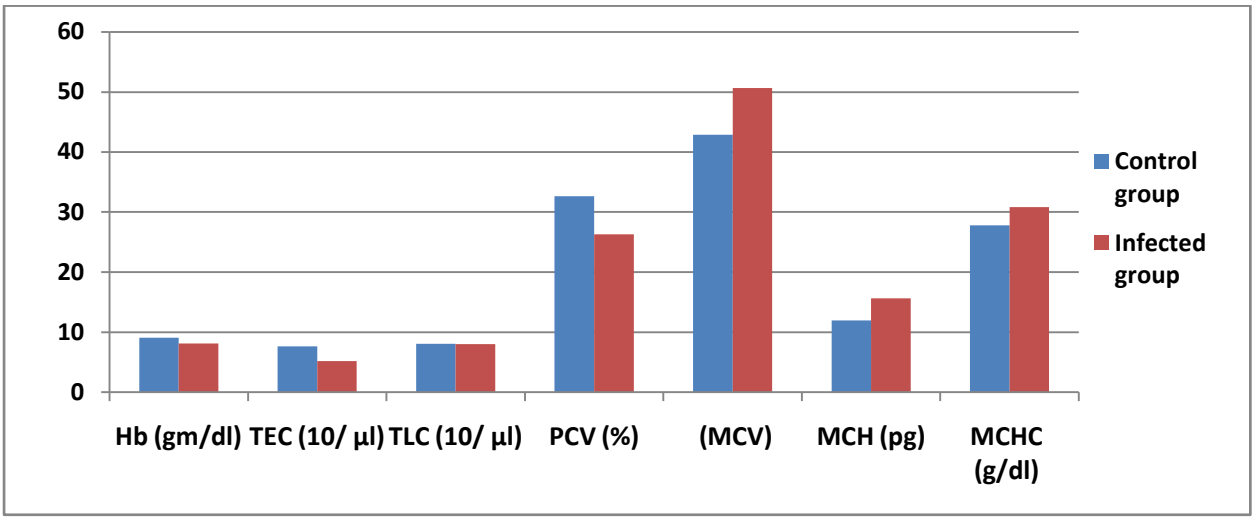
Mean ± S.E. of haematological parameters in control and *G. explanatum* infected groups

Parameter	Control group	Infected group
	Mean ± S.E.	Mean ± S.E.
Haemoglobin (gm/dl)	$9.075^{a} \pm 0.160$	$8.112^{b} \pm 0.069$
Total Erythrocyte Count (TEC) 10^{6}/ µl	$7.607^{a} \pm 0.156$	$5.195^{b} \pm 0.086$
Total Leucocyte Count (TLC) 10^{6}/ µl	$8.072^{a} \pm 0.055$	$8.019^{a} \pm 0.055$
Packed Cell Volume (%) (PCV)	$32.653^{a} \pm 0.180$	$26.317^{b} \pm 0.573$
Mean Corpuscular Volume (MCV)	$42.89^{a} \pm 0.240$	$50.67^{a} \pm 0.320$
Mean Corpuscular Hemoglobin (pg) (MCH)	$11.92^{a} \pm 0.194$	$15.61^{a} \pm 0.218$
Mean Corpuscular Hemoglobin Concentration (g/dl) (MCHC)	$27.79^{a} \pm 0.128$	$30.82^{a} \pm 0.186$
Neutrophils (%)	$29.420^{a} \pm 0.127$	$29.909^{b} \pm 0.181$
Lymphocytes (%)	$56.486^{a} \pm 0.241$	$55.870^{a} \pm 0.216$
Eosinophils (%)	$4.588^{a} \pm 0.193$	$5.177^{b} \pm 0.347$
Monocytes (%)	$4.817^{a} \pm 0.142$	$5.096^{b} \pm 0.032$
Basophils (%)	$0.240^{a} \pm 0.037$	$0.302^{a} \pm 0.043$

- Values of Hb (gram%), PCV (%), Neutrophils (%), Lymphocytes (%), Eosinophils (%), Monocytes (%) and Basophils (%) are the values of angles corresponding the percentage (Angle =Arc sin √Percentage).
- Mean (row wise) with different superscripts differ significantly.

Graph - 11.

Mean of haematological parameters of haematological parameters in control and *G. explanatum* infected groups



Graph - 12

Mean of haematological parameter changes (DLC) in control and *G. explanatum* infected groups

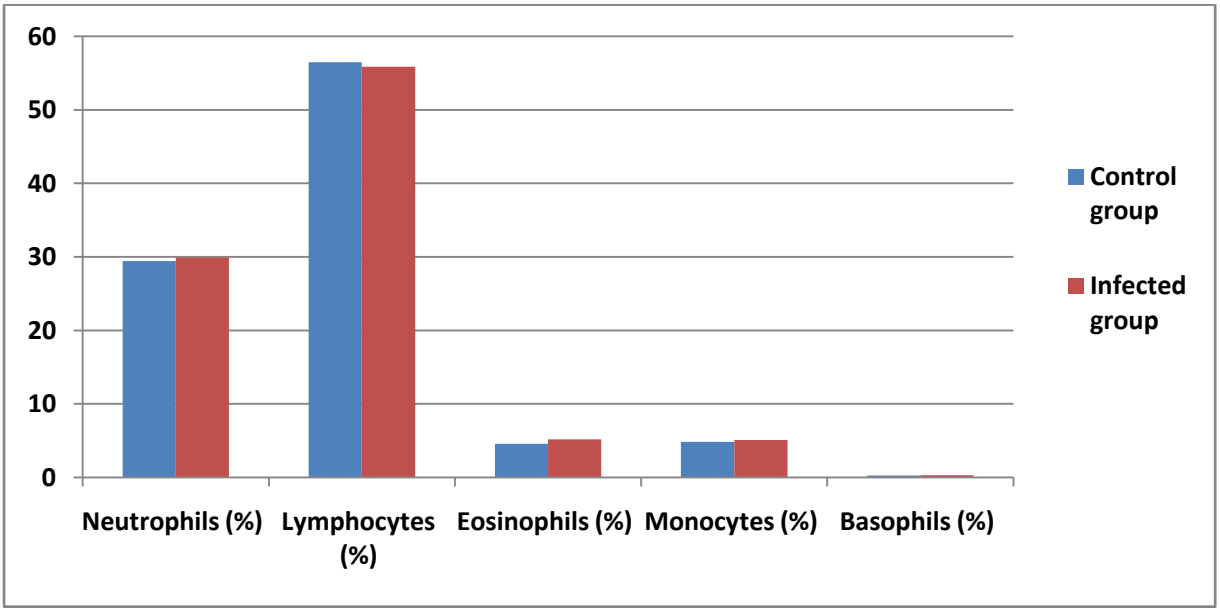


Table 12.

Mean ± S.E. of Serum enzyme level (bile biochemical analysis) haematological parameters in control and *G. explanatum* infected groups

Parameter	Control group	Infected group
	Mean ± S.E.	**Mean ± S.E.**
Alanine transaminase (IU/L) **(ALT)** / Serum Glutamate Pyruvate Transaminase **(SGPT)**	5.8 ± 0.0^{a}	6.86 ± 1.39^{a}
Aspartate Aminotransferase (IU/L) **(AST)** / Serum Glutamic-Oxaloacetic Transaminase **(SGOT)**	90.52 ± 137.2^{a}	238.2 ± 319.2^{b}
Total Serum Protein (IU/L) **(TSP)**	0.79 ± 0.0^{a}	0.84 ± 0.0^{a}

Graph -13.

Mean ± S.E. of Serum enzyme level (bile biochemical analysis) haematological parameters in control and *G. explanatum* infected groups

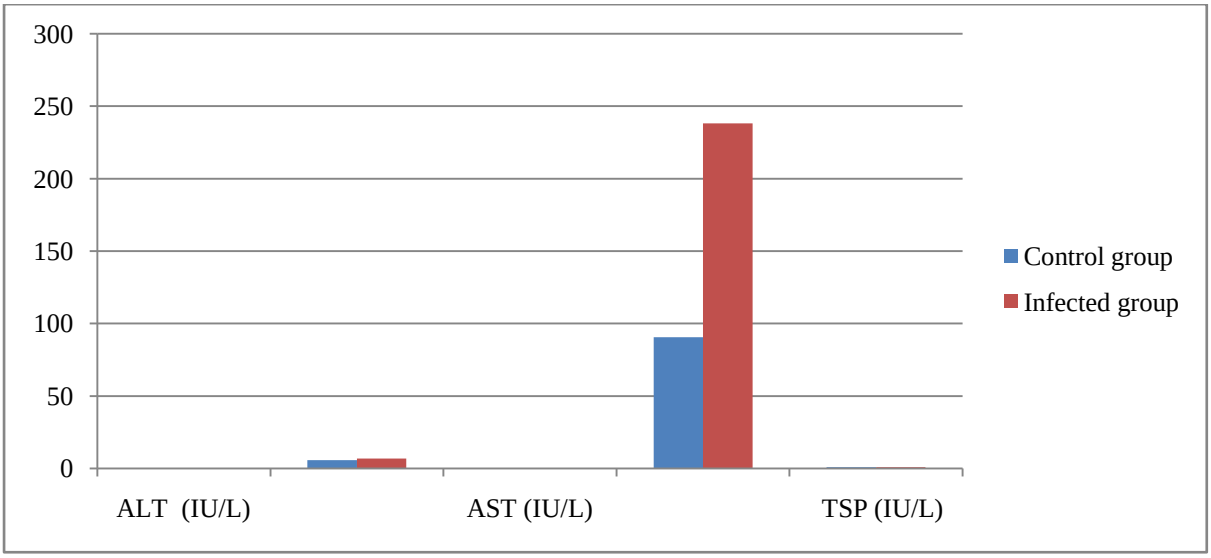


Table 13. Post treatment changes in mean ± S.E. of eggs per gram (epg) and percent efficacy of herbal preparations in *G. explanatum* infected buffaloes.

	Average EPG of faeces post treatment (eggs per gram)							
T/t Group	**Dose**	**0th day of T/t**	**7th day of T/t**	**% efficacy**	**14th day of T/t**	**% efficacy**	**21st day of T/t**	**% efficacy**
Neem extract **(T_1)**	100mg/kg b.w./day	1202^{aA} ± 153.576	1154^{aA} ± 146.284	3.99	1090.6^{aB} ± 123.498	9.32	987^{aC} ± 89.184	17.88
Garlic clove extract **(T_2)**	100 mg/kg b.w./day	1189^{aA} ± 155.569	1105^{aB} ± 149.403	7.06	1072^{aB} ± 159.957	9.84	968^{aC} ± 100.170	18.59
Both neem extract and garlic clove extract **(T_3)**	(100mg + 100mg) /kg b.w./ day	$1176.^{abA}$ ± 143.156	$1086.^{abB}$ ± 117.917	7.65	980^{abC} ± 109.334	16.67	894^{abD} ± 91.252	23.98
Control (untreated) **(C)**	------	1206^{aA} ± 118.055	1174^{aA} ± 108.443	--------	1197^{aA} ± 109.056	-------	1208^{aA} ± 111.562	-------

- Mean (row wise) with similar superscripts (row-wise A,B,C and column-wise a,b,c) did not differ significantly.

Graph- 14 **Post treatment changes in Mean ± S.E. of eggs per gram (epg) and percent efficacy of herbal preparations in *G. explanatum* infected buffaloes.**

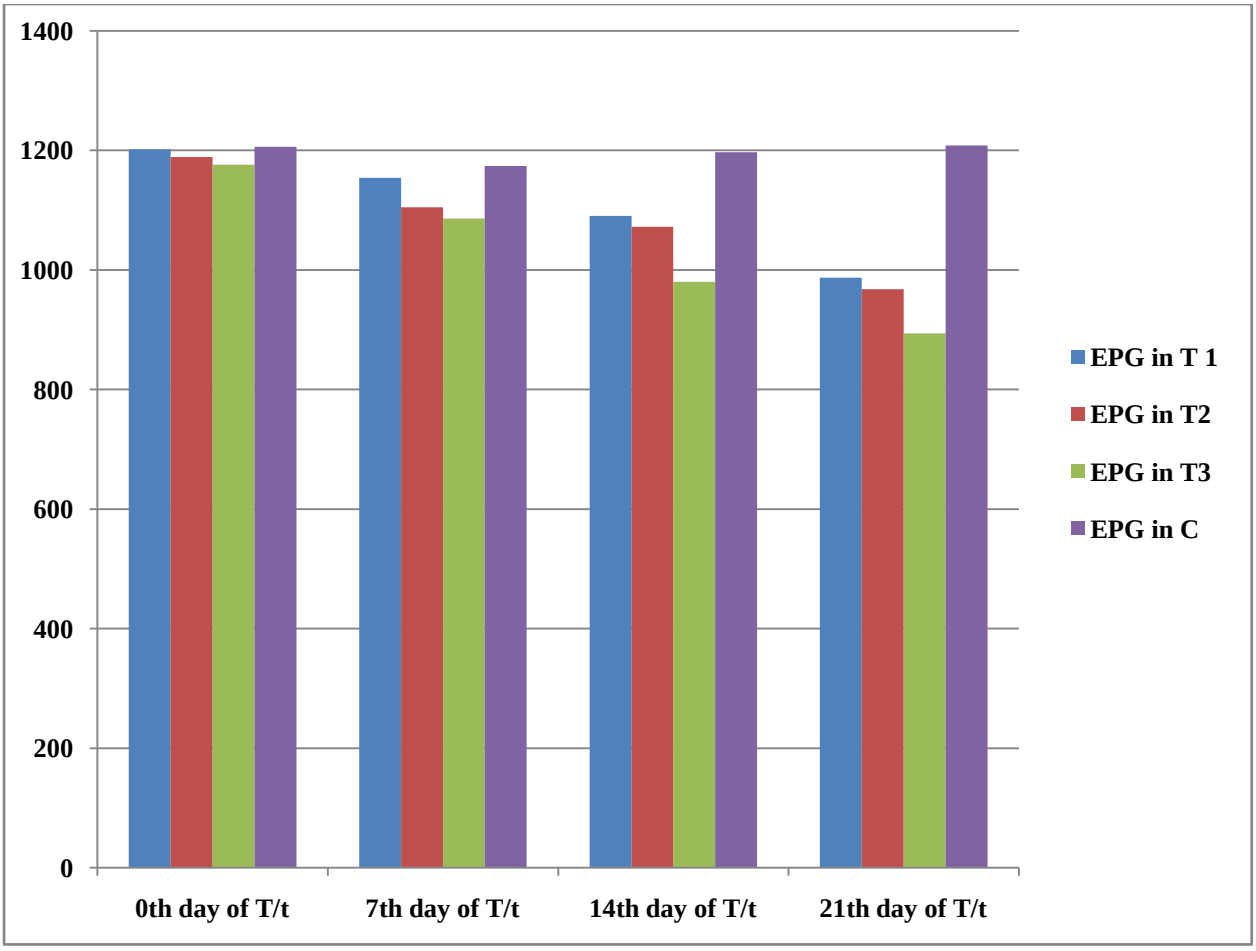


Table 14. Post treatment changes in mean ± S.E. of Haemoglobin (%) of *G. explanatum* infected buffaloes treated with different herbal preparations at different time intervals.

T/t Group	Dose	0th day of T/t	7th day of T/t	14th day of T/t	21th day of T/t
Neem extract **(T_1)**	100mg/kg b.w./day	$8.116^{aA} \pm 0.233$	$8.163^{aB} \pm 0.299$	$8.336^{aC} \pm 0.246$	$8.425^{aD} \pm 0.246$
Garlic clove extract **(T_2)**	100 mg/kg b.w./day	$8.121^{bA} \pm 0.172$	$8.208^{bB} \pm 0.205$	$8.347^{bD} \pm 0.142$	$8.491^{bD} \pm 0.142$
Both neem extract and garlic clove extract **(T_3)**	(100mg + 100mg) /kg b.w./ day	$8.111^{cA} \pm 0.244$	$8.170^{cB} \pm 0.345$	$8.571^{cD} \pm 0.248$	$8.621^{cD} \pm 0.248$
Control (untreated) **(C)**	------	$8.114^{dA} \pm 0.192$	$8.121^{dB} \pm 0.237$	$8.122^{dD} \pm 0.263$	$8.197^{dD} \pm 0.263$

- Mean (row wise) with similar superscripts (row-wise A,B,C and column-wise a,b,c) did not differ significantly.

Graph- 15 **Post treatment changes in mean ± S.E. of Haemoglobin (%) of *G. explanatum* infected buffaloes treated with different herbal preparations at different time intervals.**

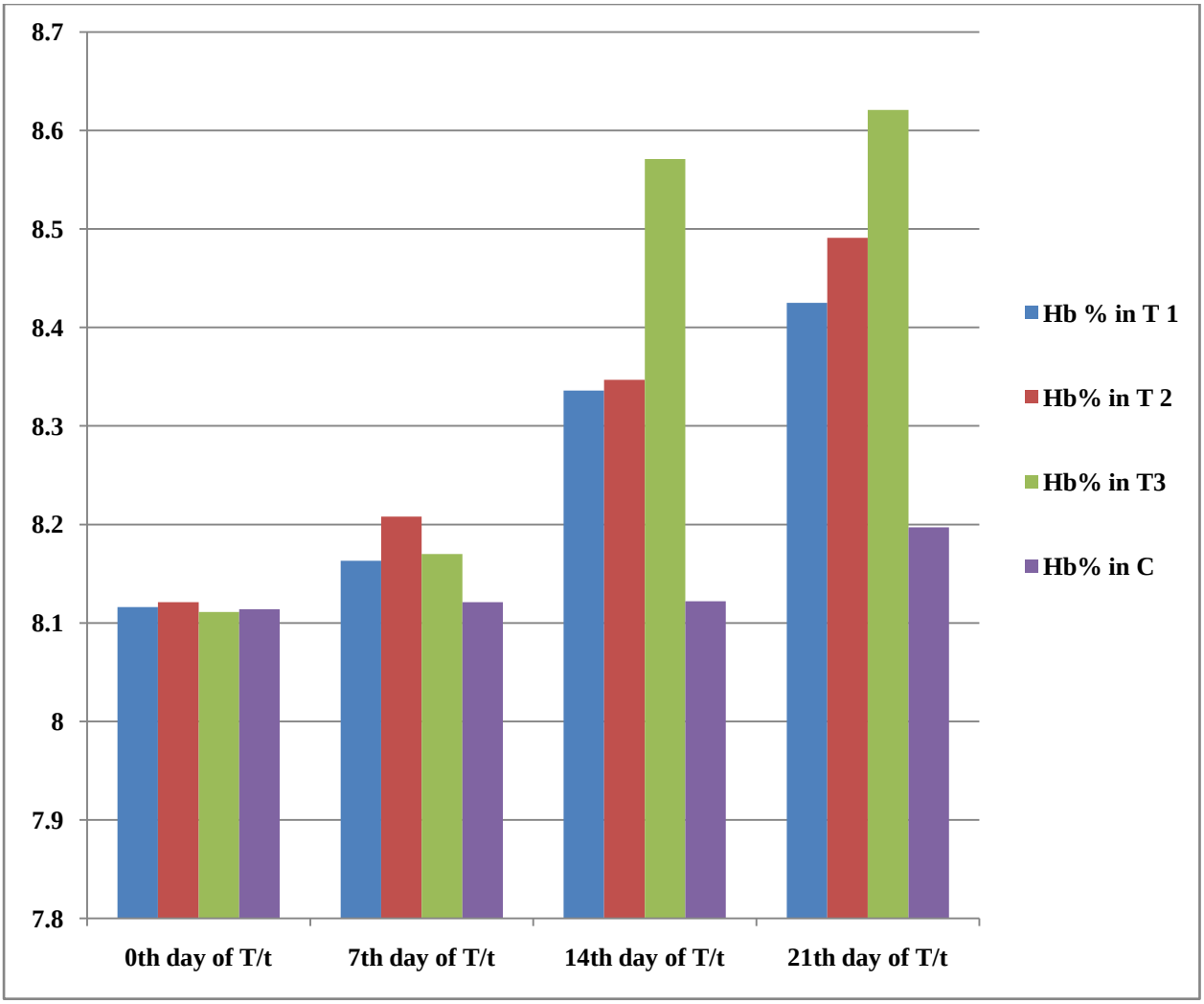


Table 15. Post treatment changes in mean ± S.E. Total Erythrocyte Count (TEC) 10^6/ mm^3 of of *G. explanatum* infected buffaloes treated with different herbal preparations at different time intervals.

T/t Group	Dose	0th day of T/t	7th day of T/t	14th day of T/t	21th day of T/t
Neem extract (T_1)	100mg/kg b.w./day	$5.195^{aA} \pm 0.233$	$5.297^{aB} \pm 0.299$	$5.353^{aD} \pm 0.246$	$5.446^{aD} \pm 0.246$
Garlic clove extract (T_2)	100 mg/kg b.w./day	$5.206^{bA} \pm 0.172$	$5.357^{bB} \pm 0.205$	$5.455^{bD} \pm 0.142$	$5.538^{bD} \pm 0.142$
Both neem extract and garlic clove extract (T_3)	(100mg + 100mg) /kg b.w./ day	$5.208^{cA} \pm 0.244$	$5.498^{cB} \pm 0.345$	$5.746^{cD} \pm 0.248$	$6.290^{cD} \pm 0.248$
Control (untreated) (C)	------	$5.196^{dA} \pm 0.192$	$5.190^{dB} \pm 0.237$	$5.188^{dD} \pm 0.263$	$5.195^{dD} \pm 0.263$

- Mean (row wise) with similar superscripts (row-wise A,B,C and column-wise a,b,c) did not differ significantly.

Graph - 16 **Post treatment changes in mean ± S.E. Total Erythrocyte Count (TEC) 10^6/ mm^3 of of *G. explanatum* infected buffaloes treated with different herbal preparations at different time intervals.**

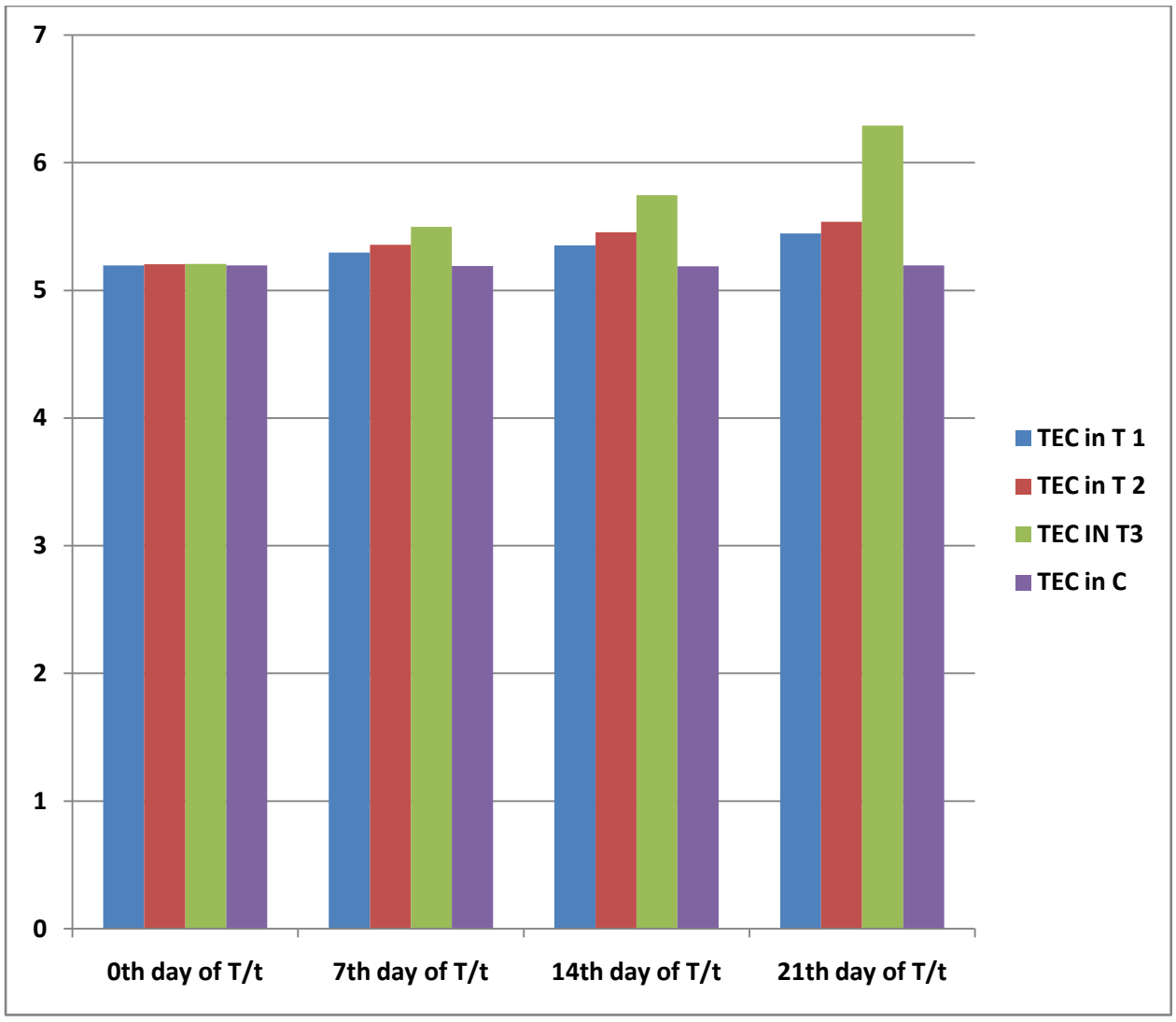


Table 16. Post treatment changes in mean ± S.E. of Total Leucocyte Count (TLC) 10^6/ µl *G. explanatum* infected buffaloes treated with different herbal preparations at different time intervals.

T/t Group	Dose	0th day of T/t	7th day of T/t	14th day of T/t	21st day of T/t
Neem extract **(T_1)**	100mg/kg b.w./day	$8.017^{aA} \pm 0.243$	$8.020^{aA} \pm 0.233$	$8.022^{aA} \pm 0.142$	$8.023^{aA} \pm 0.219$
Garlic clove extract **(T_2)**	100 mg/kg b.w./day	$8.012^{aA} \pm 0.233$	$8.018^{aA} \pm 0.174$	$8.022^{aA} \pm 0.185$	$8.027^{aA} \pm 0.213$
Both neem extract and garlic clove extract **(T_3)**	(100mg + 100mg) /kg b.w. / day	$8.021^{aA} \pm 0.115$	$8.032^{aB} \pm 0.129$	$8.041^{aC} \pm 0.108$	$8.054^{aC} \pm 0.129$
Control (untreated) **(C)**	------	$8.015^{aA} \pm 0.160$	$8.017^{aA} \pm 0.158$	$8.019^{aA} \pm 0.162$	$8.018^{aA} \pm 0.157$

- Mean (row wise) with similar superscripts (row-wise A,B,C and column-wise a,b,c) did not differ significantly.

Graph - 17 **Post treatment changes in mean ± S.E. of Total Leucocyte Count (TLC) 10^3/ mm^3 *G. explanatum* infected buffaloes treated with different herbal preparations at different time intervals.**

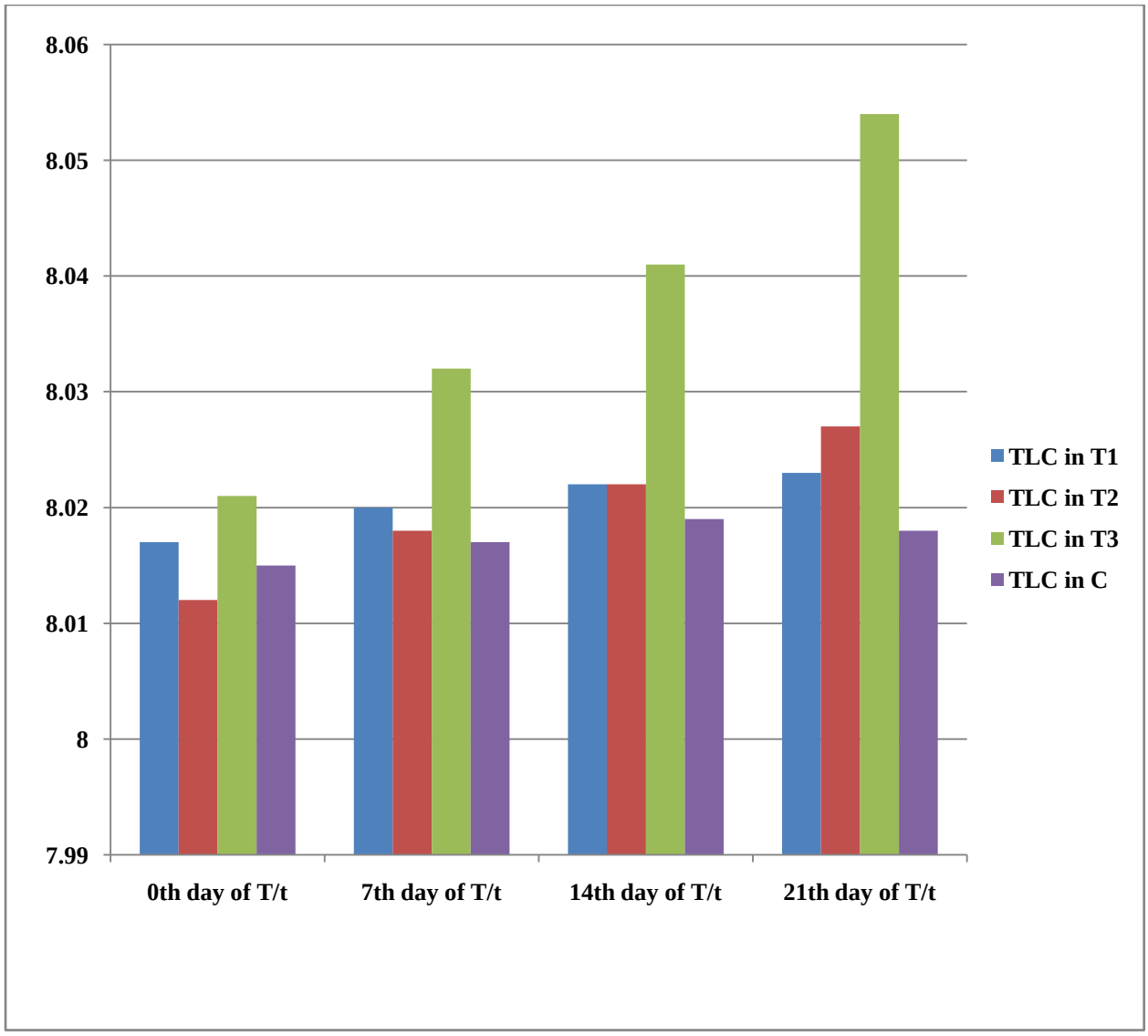


Table - 17 **Post treatment changes in mean ± S.E. of Packed Cell Volume (PCV) in *G. explanatum* infected Buffaloes treated with different herbal preparations at different time intervals.**

T/t Group	Dose	0th day of T/t	7th day of T/t	14th day of T/t	21th day of T/t
Neem extract (T_1)	100mg/kg b.w./day	26.317^{aA} ± 0.381	26.788^{aB} ± 0.436	27.521^{aC} ± 0.946	27.927^{aC} ± 0.373
Garlic clove extract (T_2)	100 mg/kg b.w./day	26.323^{bA} ± 0.352	27.217^{bC} ± 0.345	28.418^{aC} ± 0.478	29.327^{bD} ± 0.353
Both neem extract and garlic clove extract (T_3)	(100mg + 100mg) /kg b.w./ day	26.334^{bA} ± 0.281	27.747^{bC} ±0.224	28.318^{aC} ± 0.196	29.533^{bD} ± 0.282
Control (untreated) (C)	------	26.323^{aA} ± 0.315	26.337^{aB} ± 0.340	26.330^{aC} ± 0.361	26.351^{aC} ± 0.328

- Mean (row wise) with similar superscripts (row-wise A,B,C and column-wise a,b,c) did not differ significantly.

Graph - 18 **Post treatment changes in mean ± S.E. of Packed Cell Volume (PCV) in *G. explanatum* infected Buffaloes treated with different herbal preparations at different time intervals.**

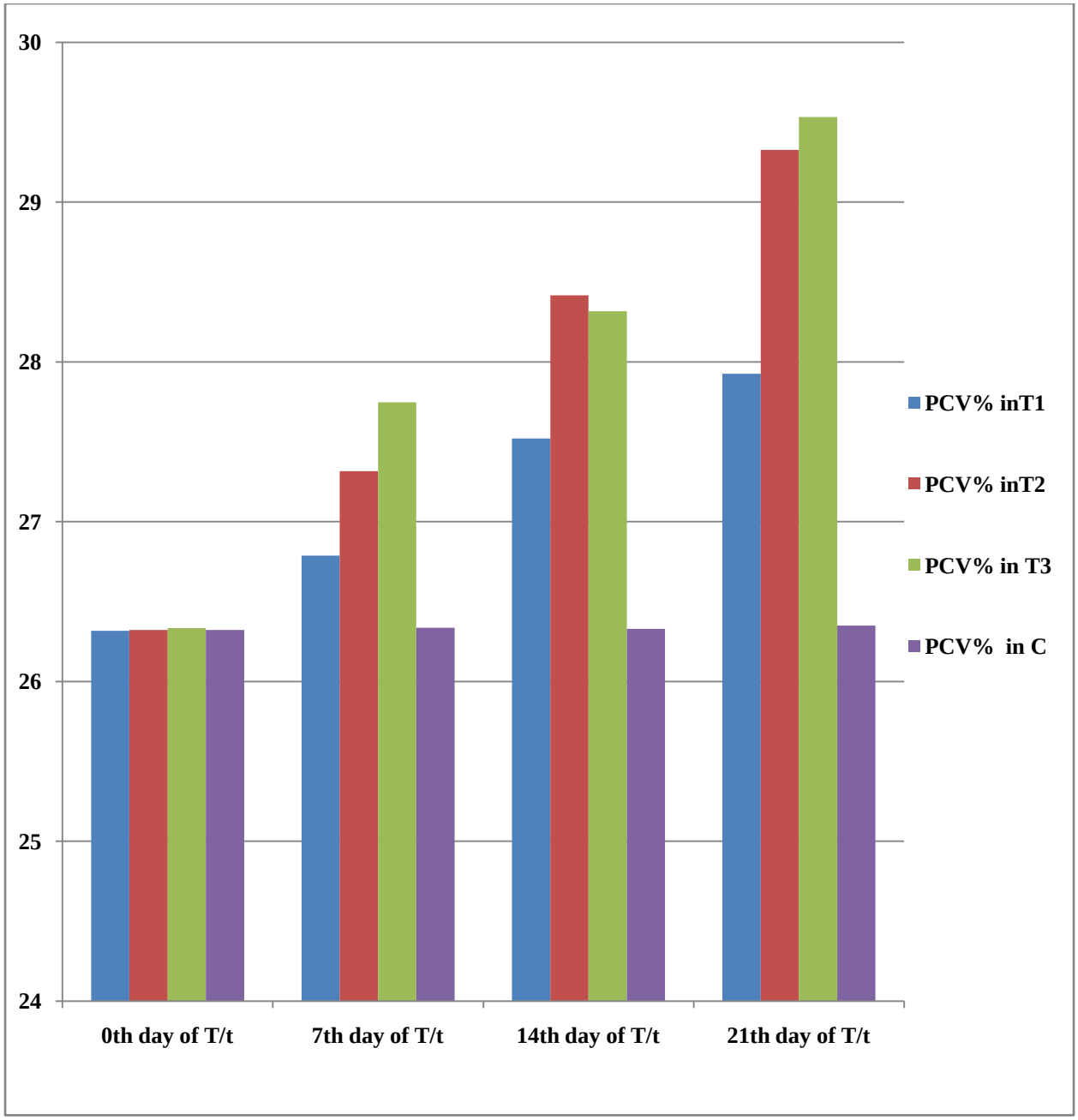


Table - 18.

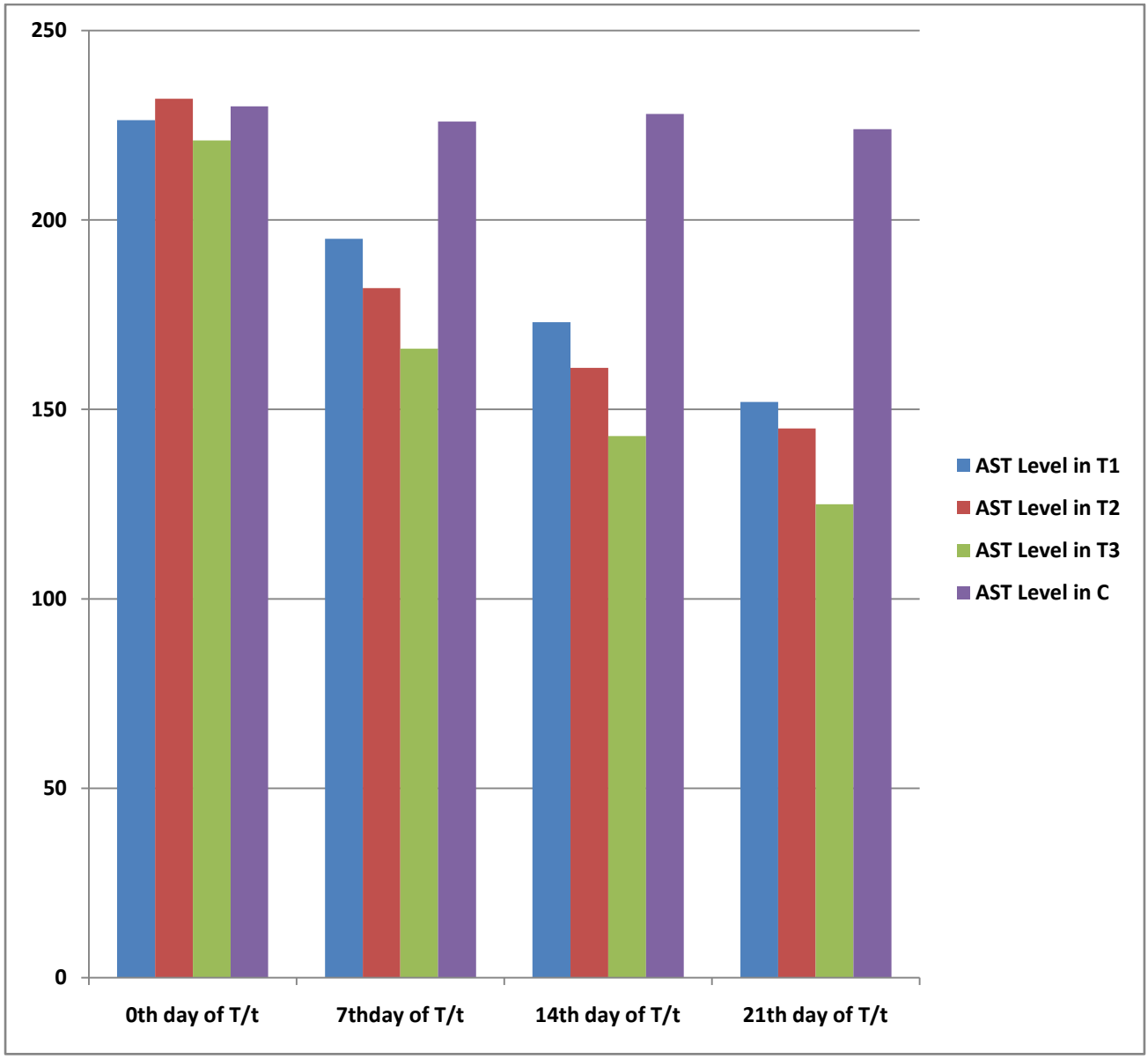
Post treatment changes Mean ± S.E. of Aspartate Aminotransferase (AST) level (bile biochemical analysis) in control and *G. explanatum* infected groups

T/t Group	Dose	0th day of T/t	7thday of T/t	14th day of T/t	21th day of T/t
Neem extract **(T1)**	100mg/kg b.w./day	226.317^{aA} + 0.381	195.728^{aB} + 0.436	173.521^{aC} + 0.946	152.927^{aC} + 0.373
Garlic clove extract **(T2)**	100 mg/kg b.w./day	232.303^{aA} + 0.352	182.217^{aB} + 0.345	161.418^{aC} + 0.478	145.532^{aD} + 0.353
Both neem extract and garlic clove extract **(T3)**	(100mg + 100mg) /kg b.w./ day	221.322^{bA} + 0.281	166.747^{bC} + 0.224	143.318^{aC} + 0.196	125.533^{bD} + 0.282
Control (untreated) **(C)**	------	230.323^{aA} + 0.315	226.337^{aA} + 0.340	228.330^{aA} + 0.361	224.351^{aA} + 0.328

- Mean (row wise) with similar superscripts (row-wise A,B,C and column-wise a,b,c) did not differ significantly.

Graph - 19.

Post treatment changes Mean ± S.E. of Aspartate Aminotransferase (AST) level (bile biochemical analysis) in control and *G. explanatum* infected groups



DISCUSSION

Buffalo is one of the potential animals that can boost dairy, meat and leather industry in India (Muhammad *et al.,* 2015). The total Buffalo contributes around 21.23% of the total livestock population. The total number of Buffalo in the country as per 2012 Census is 108.7 million numbers. Bihar is homeland to 6.96 percent of total buffalo population of India. Buffaloes are not only the main source of animal proteins but also their products such as bones, skins and goods made from them are of great economic importance. Buffalo meat is the healthiest meat among red meats known for human consumption since it is low in calories and cholesterol. Buffalo meat and milk are well comparable to cattle in many of the physicochemical, nutritional, functional properties and palatability attributes. Buffaloes are usually raised in extensive system in the coastal areas where large scale pasture land is available. There is no definite ideal management system of buffalo in coastal areas i.e. about housing, breeding, de-worming, vaccination, animal identification and record-keeping. Factors like diseases, genetic makeup, poor nutritional and management practices, environmental stress etc. are major constraints of livestock responsible for the low productivity of buffalo. Among all parasitic infections, mainly those caused by gastrointestinal helminthes are the major problems of our livestock and causes great economic loss to dairy industry by way of retarded growth, low productivity and increased susceptibility of animals to other infections. Gastrointestinal parasitic infections of buffaloes are common, which cause considerable global economic losses to the buffalo industry and farming communities as a consequence of mortality in infected animals, reduced weight gain and the condemnation of the affected organs during meat inspection in slaughterhouses (Singh *et. al,* 2012)

Chowdhury and Tada (1994) described the prevalence and other factors associated with helminthes of domestic animals in Indian subcontinent causing parasitic gastroenteritis. Watery diarrhea, weakness, weight loss, decreased milk production, reduced product quality, mortality and other secondary infections are caused by trematode parasites (Soulsby, 1982). The variation in the prevalence of parasitic intensity depends upon the geographical locations, environment, grazing habits, immunological and nutritional status of the host, presence of intermediate host and number of infective larvae or eggs ingested by the animals (Blood and Radostits, 1989).

Synthetic anthelmintics are widely used in controlling parasitic infections. However, these are expensive, have developed resistance against various anthelmintic compounds and their residues and toxicity problems (Kaemmerer and Buttenkoter, 1973), pose hazards to livestock development and public health. Hence to overcome these problems it was felt necessary to investigate the anthelmintic properties of indigenous herbal plant products in controlling the helminth parasites of buffaloes. Very few available journals, research papers and other literatures indicate the incidence of *Gigantocotyle explanatum* infection in local buffalo population of Begusarai district and its surroundings, but it is yet to be documented. A little work has been done on the use of herbal preparations i.e. neem and garlic in control of *G. explanatum* infection in buffaloes.

The present study was conducted in buffaloes and their calves from privately organized khatals and local unorganized buffalo rearing farmers in and around Begusarai district with or without clinical signs of gastrointestinal disorders. Faecal samples were collected and examined. To record the prevalence of amphistomiasis in buffaloes and their calves, a total of 426 buffaloes were surveyed during the period from 1st June 2016 to 31st May 2017 for the presence of different GI parasites including

Gigantocotyle explanatum eggs. The prevalence of infection was assorted in terms of month-wise, season wise, age-wise, sex-wise, breed-wise and managemental condition. The efficacy of each drug was assessed in term of reduction in the number of ova following treatment.

❖ OVERALL PREVALENCE OF GASTRO-INTESTINAL PARASITES

The prevalence of various gastrointestinal parasitic infections in buffaloes is summarized in Table 1. Faecal examination of 426 buffaloes revealed 82.18 percent incidence of parasitic infections. Vanisri *et al.* (2016) reported overall prevalence of parasitic eggs and oocysts 76 per cent in Tamil Nadu. Slightly lower prevalence was observed by Yadav *et al.* (2004), Gupta *et al.* (1985) and Mamun *et al.* (2011) who recorded 60.51%, 62.90%, 64.41% and 61.02% in Jammu, Haryana, Pakistan and Bangladesh. Patel *et al.* (2015) reported the general prevalence rate of helminth parasites in buffaloes 64.67%. Ahmad (1995) and Anwar *et al.* (1996) reported 65.79% and 63.8% buffalo calves suffering from helminthiasis in Pakistan. Whereas, lower prevalence was reported by Jagannath *et al.* (1988) and Hirani *et al.* (1999) who observed 42.12 percent and 38.86 percent of incidence of gastrointestinal helminthiasis in buffaloes in Karnataka and Gujarat, respectively. Low and high prevalence of gastrointestinal helminthiasis from different parts of India could be due to the deworming of buffaloes and the managemental practices followed in the particular area.

In the present study, prevalence of five species were studied :- Two were trematode viz. *Fasciola gigantica* (21.13%) and Amphistomes (including *G. explanatum)* (46.24%); Two species were nematode viz. *H. contortus* (17.84%) and *T. vitulorum* (11.74%) and one species of protozoa, *B. coli* (44.86%). The result of present study is

similar to that found by various researchers. Sreedevi and Hafeez, (2014) reported prevalence of gastro-intestinal parasites of buffaloes out of which amphistomes were 15.42%. Mixed infections of *T. vitulorum*, *Strongyloides papillosus* and *Eimeria sp*. were observed. Patel *et al.* (2015) revealed that 64 % cases are of trematodes- *G. explanatum* -11.33%. Chaudhry *et al.* (1984), Anwar *et al.* (1996), Zar (1996), Bhutto *et al.* (2002) and Reddy *et al*. (2012) also reported mixed parasitic infections in buffalo population.

❖ AGE RELATED PREVALENCE OF GASTRO-INTESTINAL PARASITES

Higher prevalence of amphistomes (57.14%) and *Toxocara vitulorum* (40.00%) was reported in buffalo calves than in adult and young groups as shown in Table-2. Samanta and Santra (2009) and Reddy *et al*. (2012) also reported high prevalence rate in the below one year age group of buffalo population. Findings of Javed *et al.* (1993) and Saha *et al.* (2013) in relation to age dependent helminth infection is in accordance with findings of this study. Similarly, Patel *et al.* (2015) revealed the prevalence of helminth was maximum (46.39%) in young age group followed by adult (27.83%) and old animals (25.77%). But, Iqbal *et al.* (2014) observed contrasting result in Pakistan. Likewise, Keyyu *et al.* (2006) also observed higher prevalence of both *Fasciola* and amphiostomes in adults buffaloes.

❖ SEX RELATED PREVALENCE OF GASTRO-INTESTINAL PARASITES

It was observed that amphistomes (50.68%), *Toxocara vitulorum* (12.33%) and *B. coli* (57.33%) infection was higher among male buffalo population than female buffaloes i.e. 43.93, 18.21 and 38.21 percent,

respectively as shown in Table-3. The result of present study is similar to that found by various researchers. Ibrahim (1997) and Vanisri *et al.* (2016) reported the same finding of higher prevalence of helminthic infection in male buffaloes than in female buffaloes. In contrast to this, Bhutto *et al.* (2002) reported a slightly higher prevalence of helminthes in females than males.

❖ NUTRITIONAL AND MANGEMENTAL STATUS RELATED TO PREVALENCE OF GASTRO-INTESTINAL PARASITES

Table - 4 represents the nutritional and mangemental status related prevalence of gastro-intestinal parasites in buffaloes. In poor body conditioned buffaloes the higher rate of gastro-intestinal parasites infection was recorded than in medium body conditioned buffaloes. Blood and Radostits (1989) also reported the similar effect of nutritional and mangemental condition on rate of incidence of helmnthic infections.

❖ SEASONAL PREVALENCE OF GASTRO-INTESTINAL PARASITES

Seasonal prevalence of gastro-intestinal parasites is shown in Table -5. It was observed that rainy season (July - October) registered highest prevalence of all the five species of G.I. parasites. Sreedevi and Hafeez, (2014) reported prevalence of G. I. parasites the infection rate was significantly higher ($P<0.05$) during the rainy season (44.50%) followed by the summer (35.46%) and winter (33.58%) seasons. Similarly, Sanyal and Singh (1995), Zar (1996), Soundararajan (2000), Hassan and Juyal (2006) and Vanisri *et al.* (2016) indicated an increased parasitic burden in hosts and pastures during the rainy season.

Prevalence of amphistomes (40.00%) was found higher in summer season than winter season i.e. 32.14%. On the other hand,

Fasciola gigantica (19.29%), *T. vitulorum* (11.43%) and *B. coli* (43.57%) were observed more in winter season. Soundararajan (2000), Hassan *et al.* (2005) and Mamun *et al*. (2011) also reported moderate helminthiasis during monsoon and lowest during the winter and summer months. Similarly, Samanta and Santra (2009) observed that with the onset of winter, the infection rate gradually decreased.

❖ MONTHWISE AND SEASONWISE PREVALENCE OF AMPHISTOMES (INCLUDING *G. explanatum)*

Monthwise and Seasonwise prevalence of amphistomes (including *G. explanatum)* is shown in Tables - 6 and 7. It was observed that, the monthwise and seasonwise effect on amphistomes parasitism in buffaloes was significant ($p<0.01$). Highest and lowest prevalence of amphistomes was registered in the month of July (86.48%) and April (25.00%), respectively. Highest prevalence of amphistomes was observed in the rainy season followed by summer and winter seasons. This seasonal variation is in accordance with the results observed by Hassan and Juyal (2006) and Sreedevi and Hafeez (2014).

❖ AGEWISE AND SEXWISE PREVALENCE OF AMPHISTOMES (INCLUDING *G. explanatum*)

Agewise and sexwise prevalence of amphistomes (including *G. explanatum*) is represented in Tables - 8. Buffalo calves (57.14%) were found positive for amphistomes followed by adult (46.46%) and young (40.00%) buffaloes, respectively. Amphistomes affected more than fifty percent male buffaloes whereas about forty four percent female buffaloes were affected. Iqbal *et al.* (2014) observed significant correlation found between amphistome burden and age indicating that

the amphistome burden influenced by the age of animal. This result was similar to the findings of present study.

❖ HAEMATOLOGICAL PARAMETERS IN CONTROL AND *G. explanatum* INFECTED GROUPS :

Tables - 11 exhibits the Mean ± S.E. values of haematological parameters in control and *G. explanatum* infected groups. Mean ± S.E. values of Haemoglobin, Total Erythrocyte Count (TEC) and Packed Cell Volume showed significant reduction in *G. explanatum* infected buffalo groups The result of present study is similar to that found by various researchers. Panda and Mishra (1980), Singh *et al*. (1984), Saheb and Hafeez (1995), Molina, E.C. (2005), Nath (2007), Mavenyengwa *et al*. (2010), Biswas *et al*. (2013) and Chauhan *et al* (2015) showed a highly significant reduction ($p<0.01$) in the mean Hb, TEC and PCV in amphistome infected buffaloes.

On the other hand, Total Leucocyte Count (TLC), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) showed non-significant rise in Mean ± S.E. values in *G. explanatum* infected groups. Chauhan *et al* (2015) also reported no significant change in the values of MCV, MCH and MCHC of control (apparently healthy) and infected buffaloes.

The possible cause of anemia in the present study may be assigned to the parasitic infection, which feed on host nutrition thus resulting in the depletion of nutrients and improper digestion. Furthermore, due to obstruction of bile duct by these parasites, the flow of bile is not possible and the outcome is a problem in digestion. This

over all affects the animal efficiency including the hematological parameters. The non-significant change in the values of TEC and PCV may be due to haemoconcentration resulting from diarrhoea.

In Differential Leucocyte Count (DLC), it was observed that there was significant rise in Mean $\pm$ S.E. values of neutrophils, eosinophils and monocytes. A non- significant rise in Mean $\pm$ S.E. value of basophils was observed, whereas reduction in Mean $\pm$ S.E. value of lymphocytes was recorded in *G. explanatum* infected groups. These findings are in coincidence with the findings of Chauhan *et al* (2015) who also reported a significant increase ($p<0.05$) in the neutrophils count and eosinophil count of infected buffaloes as compared to the non-infected buffaloes. The total leukocyte, monocyte and lymphocyte count decreased non-significantly, and basophil counts were found increased non-significantly in infected buffaloes. Similarly, Nath (2007) and Biswas *et al*. (2013) reported increased levels of eosinophil and neutrophil. Studies of Saheb and Hafeez (1995), Thakur *et al*. (2007), Clark (2008) and Mavenyengwa *et al*. (2010) also reported eosinophilia amphistome infected buffaloes.

There was a significant rise in Mean + S.E. value of Aspartate Aminotransferase (AST) enzyme level as shown in Table-12. The rise in Total Serum Protein (TSP) and Alanine Transaminase (ALT) was non-significant). The changes in the level of these serum enzymes is because G. explanatum is present in the bile ducts of buffaloes form plugs on the luminal surface by their acetabulum as reported by Malik (2010). Cheema *et al.* (1997) also reported *G. explanatum* in the common bile duct, cystic and hepatic duct. The decrease in total plasma protein levels observed in this study is probably related to the pathogenic mechanism of amphistomes which, upon infection and excystment in the small intestine, cause tissue destruction during the migratory phase through

the tunica muscularis to the submucosa (Singh & Lakra, 1971). During the migratory phase, the immature flukes attach to the mucosa using acetabula (Dutt, 1980; Rolfe *et al*., 1994). The decrease in total plasma protein concentration appeared to be almost entirely due to a drop in plasma albumin concentration, which decreased simultaneously.

In current study the possible reason for high level of ALT and AST in the bile juice in the case of infected animals is the increase production of these enzymes by the cells due to inflammatory reactions and release in to the bile. These three enzymes are considered as a measure of tissue necrosis.

❖ **Post treatment changes in Mean ± S.E. of eggs per gram (epg) and percent efficacy of herbal preparations in *G. explanatum* infected buffaloes.**

The buffaloes in $\mathbf{T_1}$ and $\mathbf{T_2}$ group treated with neem and garlic extract, respectively, showed significant reduction in epg. But, in $\mathbf{T_3}$ group treated with both neem and garlic extract high significant reduction in epg was recorded. This indicated that these herbs in combination are much efficient in amphistome control than used alone. The buffaloes in untreated control group (**C**) did not show any significant change in epg values as represented in Table-13. Abells and Haik (1999) had reported reduction in epg count on garlic extract administration in helminth infested donkeys. Guarrera (1999), Susan *et al.* (2004), Toulah and Al-Rawi (2007), Erol *et al.* (2008), Nahed *et al.* (2009) also reported anthelmintic property of garlic. Amin *et al.* (2009) reported that neem (10% water extract of leaves) reduced significantly ($p<0.01$) EPG count 62.23%, 65.77%, 56.70%, and 48.05% on 3rd, 10th, 17th and 28th day, respectively in cattle. Similarly, Arunachal *et al.*

(2002), Rahman (2002), Chandrawathani *et al*. (2006), Worku *et al*. (2009), Rabiu and Subhashish (2011), Nawaz *et al.* (2014) and Priscilla *et al.* (2014) had also reported reduction in epg count on neem extract administration in helminth infested cattle and other ruminants.

- **Post treatment changes in Mean ± S.E. of Haemoglobin (%), Total Erythrocyte Count (TEC), Total Leucocyte Count (TLC) and Packed Cell Volume (PCV) of *G. explanatum* infected buffaloes treated with different herbal preparations at different time intervals.**

The post treatment changes in Mean + S.E. of Haemoglobin (%) and Total Erythrocyte Count (TEC) in treatment groups T1, T2 and T3 were observed significant on 7th, 14th and 21st days, respectively. But, in control group (C) the changes in Haemoglobin (%) and Total Erythrocyte Count (TEC) was non-significant on post treatment days as represented inTables-15, 16. Similar to the result of present study, Balasasirekha and Lakshmi (2011) also reported a gradual increase in the blood Hb levels among garlic supplemented group over the three months period. Umar *et al.* (1998) revealed that chronic and excessive intake of garlic caused less desirable effects in anemia. Contrary to this, Vatta *et al.* (2002) showed that garlic did not have a significant effect on packed cell volume in his study on boer goats in USA. Mulumebet *et al.* (2009) also found that packed cell volume did not show significant changes in garlic extract treated adult goats infected with coccidia.

The buffaloes in **T_1** and **T_2** group buffaloes treated with herbs showed non-significant increase in Total Leucocyte Count (TLC) on 7^{th}, 14^{th} and 21^{st} days, respectively, whereas in **T_3** and **C** group it showed significant rise as shown in Table-17. Shaziya and Goyal (2012) had studied the dewormer activity of *A. indica* by decline level of eosinophil cell recorded on day 16 and 24 in treated and infected group.

Table-18 exhibits the significant rise in Packed Cell Volume (PCV) in treatment groups **T_1**, **T_2** and **T_3** on 7^{th}, 14^{th} and 21^{st} days, respectively. But, in control group (**C**) no significant change in Packed Cell Volume (PCV) was recorded. There are a little work on the improvement in haematological parameters of helminth infested buffaloes or other ruminants. But, the positive changes in hemato-biochemical changes are suggestive of the reduction in worm load of infected buffaloes.

- **Post treatment changes Mean $\pm$ S.E. of Aspartate Aminotransferase (AST) level (bile biochemical analysis) in control and *G. explanatum* infected groups**

The significant rise in Mean $\pm$ S.E. of Aspartate Aminotransferase (AST) level treatment group **T_3** was higher than recorded in **T_1** and **T_2** groups. This rise was suggestive of the regeneration of liver damage. (Table-19). In current study the possible reason for high level of ALT and AST in the bile juice in the case of infected animals is the increase production of these enzymes by the cells due to inflammatory reactions and release in to the bile. Both enzymes are considered as a measure of tissue necrosis. Tanritanir *et al*. (2009) recorded that pathological changes in tissues and organ are associated with differences in actions of liver enzymes like AST, ALT, GGT, ALP, LDH and GLDH. The hepatocellular damage and hepatocyte

proliferation elevates the plasma concentration of ALT and AST (Hodzic *et al*., 2013; Salem and Hassan, 2011). Rumosa *et al*. (2012) reported non-significant differences in serum level of both AST and ALT enzymes among goats suffering with liver infections which are partially consistent with current results.

Reduction of ova in faeces, apparent clinical recovery and mild improvement in haematological and blood biochemical enzyme levels revealed that anthelmintic efficacy of combination of garlic clove extract and neem leaves powder was superior than using these herbs alone. However, garlic was found superior than neem in treating amphistome laden buffalo calves.

Chapter- 5

SUMMARY AND CONCLUSIONS

SUMMARY

India is a predominantly agrarian country with more than 70% of its population living in villages, 80 % of which depending on agriculture and its allied actitivities for their livelihood. Rural dairying constitutes an integral and interwoven part of agriculture farming. The parasitic especially the helminthes infection result in great economic losses to livestock industry due to deterioration on health and reduced production of animal. Helminth infections are prevalent throughout the world, affecting both humans as well as livestock animals and cause huge economic losses. Amphistomes that have been identified in ruminants encompass more than 70 species. Most of these species are pathogens that cause serious morbidity in ruminants. *Gigantocotyle explanatum* is one of neglected amphistome parasite infecting the bile duct of water buffalo. The fresh water snail *Gyrulus convexiculus* serve as the intermediate host. The prevalence of infection is very high in Indian subcontinent. The exact estimates of economic loss in the world due to *G. explanatum* are not available. Adult amphistomes remain attached to the epithelium of the bile duct of domestic ruminants where they inflict sever damage.

Synthetic anthelmintics are widely used in controlling parasitic infections. However, these are expensive, have developed resistance against various anthelmintic compounds and their residues and toxicity problems, pose hazards to livestock development and public health. Hence to overcome these problems it was felt necessary to investigate the anthelmintic properties of indigenous herbal plant products in controlling the helminth parasites of buffaloes. Very few available journals, research papers and other literatures indicate the incidence of *Gigantocotyle explanatum* infection in local buffalo population of Begusarai district and

its surroundings, but it is yet to be documented. A little work has been done on the use of herbal preparations i.e. neem and garlic in control of *G. explanatum* infection in buffaloes. To record the prevalence of amphistomiasis in buffaloes and their calves, a total of 426 buffaloes were surveyed round the year for presence of different GI parasites including *Gigantocotyle explanatum* eggs. The prevalence of infection was assorted in terms of month-wise, season wise, age-wise, sex-wise, breed-wise and managemental condition. The efficacy of each drug was assessed in term of reduction in the number of ova following treatment.

Faecal examination of 426 buffaloes revealed 82.18 percent incidence of parasitic infections. Five species of gastro-intestinal parasites were detected. Among them, two species were trematode, namely *Fasciola gigantica* (21.13%) and Amphistomes (including *Gigantocotyle explanatum)* (46.24%). Two species were nematode viz. *Haemonchus contortus* (17.84%) and *Toxocara vitulorum* (11.74%) and one species of protozoa, *Balantidium coli* (44.86%).

Buffaloes of all age groups were found infected with five species of gastrointestinal parasites. Amphistomes was the highest (57.14%) in buffalo calves group whereas in adult and young groups, it was 46.26% and 40.00%, respectively.

Amphistomes affected more than fifty percent male buffaloes whereas about forty four percent female buffaloes were affected. It was observed that amphistomes (50.68%), *Toxocara vitulorum* (12.33%) and *B. coli* (57.33%) infection was higher among male buffalo population than female buffaloes. It was revealed both medium and poor body conditioned buffaloes were infected with gastrointestinal parasites and every individual was infected with at least one species of parasite. In poor body conditioned buffaloes the higher rate of gastro-intestinal parasites infection was recorded than in medium body conditioned buffaloes.

Rainy season (July - October) registered highest prevalence of all the five species of G.I. parasites. Prevalence of amphistomes was observed highest in the rainy season followed by summer and winter seasons i.e. 65.75, 40.00 and 32.14 percent, respectively. Highest prevalence of amphistomes was registered in the month of July (86.48%), whereas lowest positive cases (25.00%) were registered in the month of April.

Mean $\pm$ S.E. values of Haemoglobin, Total Erythrocyte Count (TEC) and Packed Cell Volume showed significant reduction in *G. explanatum* infected buffalo groups. On the other hand, Total Leucocyte Count (TLC), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) showed non-significant rise in Mean $\pm$ S.E. values in *G. explanatum* infected groups. The possible cause of anemia in the present study may be assigned to the parasitic infection, which feed on host nutrition thus resulting in the depletion of nutrients and improper digestion. Significant rise in Mean $\pm$ S.E. values of neutrophils, eosinophils and monocyte are suggestive of the gastrointestinal infestation. Eosinophilia is a well known and marked feature for any allergic reaction and observed in most of the cases of helminthic diseases

In current study the possible reason for high level of ALT and AST in the bile juice in the case of infected animals is the increase production of these enzymes by the cells due to inflammatory reactions and release in to the bile. These three enzymes are considered as a measure of tissue necrosis.

In the present investigation, efficacy of two locally available herbs viz. Aqueous garlic cloves extract (100mg/kg b.w./day) and neem leaves powder in molasses preparation (200mg/kg b.w./day) were given to the buffalo calves individually and in combination at previously mentioned dose rate, were tested by evaluating declining rate of eggs per gram (epg)

in calves naturally infected with *G. explanatum*. The epg decreased sharply in garlic treated calves and calves treated with combination of garlic and neem leaves powder treated calves. While longer period observed in declining epg neem leaves powder treated calves. The observed percent efficacy revealed that treatment of garlic in combination with neem leaves powder was most effective.

The post treatment changes in values of Haemoglobin (%), Total Erythrocyte Count (TEC) and Packed Cell Volume (PCV) in treatment groups $\mathbf{T_1}$, $\mathbf{T_2}$ and $\mathbf{T_3}$ were observed significant on 7th, 14th and 21st days, respectively. The significant rise in Aspartate Aminotransferase (AST) level treatment group $\mathbf{T_3}$ was higher than recorded in $\mathbf{T_1}$ and $\mathbf{T_2}$ groups. This rise was suggestive of the regeneration of liver damage.

Reduction of ova in faeces, apparent clinical recovery and mild improvement in haematological and blood biochemical enzyme levels revealed that anthelmintic efficacy of combination of garlic clove extract and neem leaves powder was found superior than using these herbs alone. However, garlic was found superior than neem in treating amphistome laden buffalo calves.

CONCLUSION

1. The total Buffalo contributes around 21.23% of the total livestock population. The total number of Buffalo in the country as per 2012 Census is 108.7 million numbers. Bihar is homeland to 6.96 percent of total buffalo population of India. Among all parasitic infections, mainly those caused by gastrointestinal helminthes are the major problems of our livestock and causes great economic loss to dairy industry by way of retarded growth, low productivity and increased susceptibility of animals to other infections.
2. Common gastrointestinal parasites detected in buffalo population are *Fasciola gigantica, Gigantocotyle explanatum, Haemonchus contortus, Toxocara vitulorum* and *Balantidium coli* etc.
3. Buffaloes of all age groups were found infected with five species of gastrointestinal parasites. Amphistomes was the highest (57.14%) in buffalo calves group whereas in adult and young groups, it was 46.26% and 40.00%, respectively.
4. Amphistomes affected more than fifty percent male buffaloes whereas about forty four percent female buffaloes were affected. It was observed that amphistomes (50.68%), *Toxocara vitulorum* (12.33%) and *B. coli* (57.33%) infection was higher among male buffalo population than female buffaloes.. In poor body conditioned buffaloes the higher rate of gastro-intestinal parasites infection was recorded than in medium body conditioned buffaloes.
5. Rainy season (July - October) registered highest prevalence of all the five species of G.I. parasites. Prevalence of amphistomes was observed highest in the rainy season followed by summer and winter

seasons. Highest prevalence of amphistomes was registered in the month of July (86.48%), whereas lowest positive cases (25.00%) were registered in the month of April.

6. Values of Haemoglobin, Total Erythrocyte Count (TEC) and Packed Cell Volume showed significant reduction in *G. explanatum* infected buffalo groups. The possible cause of anemia in the present study may be assigned to the parasitic infection, which feed on host nutrition thus resulting in the depletion of nutrients and improper digestion. Significant rise in values of neutrophils, eosinophils and monocyte are suggestive of the gastrointestinal infestation. Eosinophilia is a well known and marked feature for any allergic reaction and observed in most of the cases of helminthic diseases
7. In current study the possible reason for high level of ALT and AST in the bile juice in the case of infected animals is the increase production of these enzymes by the cells due to inflammatory reactions and release in to the bile. These three enzymes are considered as a measure of tissue necrosis.
8. Reduction of ova in faeces, apparent clinical recovery and mild improvement in haematological and blood biochemical enzyme levels revealed that anthelmintic efficacy of combination of garlic clove extract and neem leaves powder was found superior than using these herbs alone. However, garlic was found superior than neem in treating amphistome laden buffalo calves.

Chapter-6

FUTURE SCOPE OF STUDY

The present investigation was carried out to evaluate the use and efficacy of aqueous extract of garlic and neem leaves powder as anthelmintic in buffaloes infected with *Gigantocotyle explanatum.*

The herbs were prepared in very scientific way. The prepared aqueous extract of garlic was easy to administer orally dropwise and neem leaves powder was mixed with molasses to make it palatable so that the buffaloes consume it readily.

The present findings clearly indicates the importance of natural herbs to eliminate the amphistomes load from buffalo population, but these are to be fed for longer time. The use of these natural herbs may replace the conventional chemical dewormers which are costly and developing resistance against gastrointestinal parasitic helminths, posing to several deleterious side effects.

After the achievement it may be enlisted as feed resources and will serve the buffalo owners for economic deworming practices.

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"STUDIES ON THE PREVALENCE OF HELMINTH PARASITES WITH SPECIAL REFERENCE TO GIGANTOCOTYLE EXPLANATUM IN BUFFALOES IN AND AROUND BEGUSARAI DISTRICT"

ABSTRACT OF THESIS

SUBMITTED TO THE

BIHAR AGRICULTURAL UNIVERSITY

(FACULTY OF POST-GRADUATE STUDIES)

SABOUR (BHAGALPUR) BIHAR

IN PARTIAL FULFILLMENT OF THE REQUIREMENT

FOR THE DEGREE OF

MASTER OF VETERINARY SCIENCE

(Veterinary Parasitology)

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2017

India is a predominantly agrarian country with more than 70% of its population living in villages, 80 % of which depending on agriculture and its allied actitivities for their livelihood. Rural dairying constitutes an integral and interwoven part of agriculture farming. The parasitic especially the helminthes infection result in great economic losses to livestock industry due to deterioration on health and reduced production of animal. Helminth infections are prevalent throughout the world, affecting both humans as well as livestock animals and cause huge economic losses. Amphistomes that have been identified in ruminants encompass more than 70 species. Most of these species are pathogens that cause serious morbidity in ruminants. *Gigantocotyle explanatum* is one of neglected amphistome parasite infecting the bile duct of water buffalo. The fresh water snail *Gyrulus convexiculus* serve as the intermediate host. The prevalence of infection is very high in Indian subcontinent. The exact estimates of economic loss in the world due to *G. explanatum* are not available. Adult amphistomes remain attached to the epithelium of the bile duct of domestic ruminants where they inflict sever damage.

Synthetic anthelmintics are widely used in controlling parasitic infections. However, these are expensive, have developed resistance against various anthelmintic compounds and their residues and toxicity problems, pose hazards to livestock development and public health. Hence to overcome these problems it was felt necessary to investigate the anthelmintic properties of indigenous herbal plant products in controlling the helminth parasites of buffaloes. Very few available journals, research papers and other literatures indicate the incidence of *Gigantocotyle explanatum* infection in local buffalo population of Begusarai district and its surroundings, but it is yet to be documented. A little work has been done on the use of herbal preparations i.e. neem and garlic in control of *G. explanatum* infection in buffaloes. To record the prevalence of amphistomiasis in buffaloes and their calves, a total of 426 buffaloes were surveyed round the year for presence of different GI parasites including *Gigantocotyle explanatum* eggs. The prevalence of infection was assorted in terms of month-

wise, season wise, age-wise, sex-wise, breed-wise and managemental condition. The efficacy of each drug was assessed in term of reduction in the number of ova following treatment. Faecal examination of 426 buffaloes revealed 82.18 percent incidence of parasitic infections. Five species of gastro-intestinal parasites were detected. Among them, two species were trematode, namely *Fasciola gigantica* (21.13%) and Amphistomes (including *Gigantocotyle explanatum)* (46.24%). Two species were nematode viz. *Haemonchus contortus* (17.84%) and *Toxocara vitulorum* (11.74%) and one species of protozoa, *Balantidium coli* (44.86%).

Buffaloes of all age groups were found infected with five species of gastrointestinal parasites. Amphistomes was the highest (57.14%) in buffalo calves group whereas in adult and young groups, it was 46.26% and 40.00%, respectively.

Amphistomes affected more than fifty percent male buffaloes whereas about forty four percent female buffaloes were affected. It was observed that amphistomes (50.68%), *Toxocara vitulorum* (12.33%) and *B. coli* (57.33%) infection was higher among male buffalo population than female buffaloes. It was revealed both medium and poor body conditioned buffaloes were infected with gastrointestinal parasites and every individual was infected with at least one species of parasite. In poor body conditioned buffaloes the higher rate of gastro-intestinal parasites infection was recorded than in medium body conditioned buffaloes.

Rainy season (July - October) registered highest prevalence of all the five species of G.I. parasites. Prevalence of amphistomes was observed highest in the rainy season followed by summer and winter seasons i.e. 65.75, 40.00 and 32.14 percent, respectively. Highest prevalence of amphistomes was registered in the month of July (86.48%), whereas lowest positive cases (25.00%) were registered in the month of April.

Mean $\pm$ S.E. values of Haemoglobin, Total Erythrocyte Count (TEC) and Packed Cell Volume showed significant reduction in *G. explanatum* infected buffalo groups. On the other hand, Total Leucocyte Count (TLC), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) showed non-significant rise in Mean $\pm$ S.E. values in *G. explanatum* infected groups. The possible cause of anemia in the present study may be assigned to the parasitic infection, which feed on host nutrition thus resulting in the depletion of nutrients and improper digestion. Significant rise in Mean $\pm$ S.E. values of neutrophils, eosinophils and monocyte are suggestive of the gastrointestinal infestation. Eosinophilia is a well known and marked feature for any allergic reaction and observed in most of the cases of helminthic diseases

In current study the possible reason for high level of ALT and AST in the bile juice in the case of infected animals is the increase production of these enzymes by the cells due to inflammatory reactions and release in to the bile. These three enzymes are considered as a measure of tissue necrosis.

In the present investigation, efficacy of two locally available herbs viz. Aqueous garlic cloves extract (100mg/kg b.w./day) and neem leaves powder in molasses preparation (200mg/kg b.w./day) were given to the buffalo calves individually and in combination at previously mentioned dose rate, were tested by evaluating declining rate of eggs per gram (epg) in calves naturally infected with *G. explanatum*. The epg decreased sharply in garlic treated calves and calves treated with combination of garlic and neem leaves powder treated

calves. While longer period observed in declining epg neem leaves powder treated calves. The observed percent efficacy revealed that treatment of garlic in combination with neem leaves powder was most effective.

The post treatment changes in values of Haemoglobin (%), Total Erythrocyte Count (TEC) and Packed Cell Volume (PCV) in treatment groups $\mathbf{T_1}$, $\mathbf{T_2}$ and $\mathbf{T_3}$ were observed significant on 7th, 14th and 21st days, respectively. The significant rise in Aspartate Aminotransferase (AST) level treatment group $\mathbf{T_3}$ was higher than recorded in $\mathbf{T_1}$ and $\mathbf{T_2}$ groups. This rise was suggestive of the regeneration of liver damage.

Reduction of ova in faeces, apparent clinical recovery and mild improvement in haematological and blood biochemical enzyme levels revealed that anthelmintic efficacy of combination of garlic clove extract and neem leaves powder was found superior than using these herbs alone. However, garlic was found superior than neem in treating amphistome laden buffalo calves.

CONCLUSION

1. The total Buffalo contributes around 21.23% of the total livestock population. The total number of Buffalo in the country as per 2012 Census is 108.7 million numbers. Bihar is homeland to 6.96 percent of total buffalo population of India. Among all parasitic infections, mainly those caused by gastrointestinal helminthes are the major problems of our livestock and causes great economic loss to dairy industry by way of retarded growth, low productivity and increased susceptibility of animals to other infections.
2. Common gastrointestinal parasites detected in buffalo population are *Fasciola gigantic, Gigantocotyle explanatum, Haemonchus contortus, Toxocara vitulorum* and *Balantidium coli* etc.
3. Buffaloes of all age groups were found infected with five species of gastrointestinal parasites. Amphistomes was the highest (57.14%) in buffalo calves group whereas in adult and young groups, it was 46.26% and 40.00%, respectively.
4. Amphistomes affected more than fifty percent male buffaloes whereas about forty four percent female buffaloes were affected. It was observed that amphistomes (50.68%), *Toxocara vitulorum* (12.33%) and *B. coli* (57.33%) infection was higher among male buffalo population than female buffaloes.. In poor body conditioned buffaloes the higher rate of gastro-intestinal parasites infection was recorded than in medium body conditioned buffaloes.
5. Rainy season (July - October) registered highest prevalence of all the five species of G.I. parasites. Prevalence of amphistomes was observed highest in the rainy season followed by summer and winter seasons. Highest prevalence of amphistomes was registered in the month of July (86.48%), whereas lowest positive cases (25.00%) were registered in the month of April.
6. Values of Haemoglobin, Total Erythrocyte Count (TEC) and Packed Cell Volume showed significant reduction in *G. explanatum* infected buffalo groups. The possible cause of anemia in the present study may be assigned to the parasitic infection, which feed on host nutrition thus resulting in the depletion of nutrients and improper digestion. Significant rise in values of neutrophils, eosinophils and monocyte are suggestive of the gastrointestinal infestation. Eosinophilia is a well known and marked feature for any allergic reaction and observed in most of the cases of helminthic diseases

7. In current study the possible reason for high level of ALT and AST in the bile juice in the case of infected animals is the increase production of these enzymes by the cells due to inflammatory reactions and release in to the bile. These three enzymes are considered as a measure of tissue necrosis.
8. Reduction of ova in faeces, apparent clinical recovery and mild improvement in haematological and blood biochemical enzyme levels revealed that anthelmintic efficacy of combination of garlic clove extract and neem leaves powder was found superior than using these herbs alone. However, garlic was found superior than neem in treating amphistome laden buffalo calves.

FUTURE SCOPE OF STUDY

The present investigation was carried out to evaluate the use and efficacy of aqueous extract of garlic and neem leaves powder as anthelmintic in buffaloes infected with *Gigantocotyle explanatum.*

The herbs were prepared in very scientific way. The prepared aqueous extract of garlic was easy to administer orally dropwise and neem leaves powder was mixed with molasses to make it palatable so that the buffaloes consume it readily.

The present findings clearly indicates the importance of natural herbs to eliminate the amphistomes load from buffalo population, but these are to be fed for longer time. The use of these natural herbs may replace the conventional chemical dewormers which are costly and developing resistance against gastrointestinal parasitic helminths, posing to several deleterious side effects.

After the achievement it may be enlisted as feed resources and will serve the buffalo owners for economic deworming practices.