"EPIDEMIOLOGICAL STUDIES ON FASCIOLA AND AMPHISTOMES IN BUFFALO IN SOME RIVER BELT DISTRICTS OF BIHAR WITH SPECIAL REFERENCE TO CHEMOTHERAPY".

THESIS

SUBMITTED TO THE

RAJENDRA AGRICULTURAL UNIVERSITY

(FACULTY OF VETERINARY SCIENCE AND ANIMAL HUSBANDRY)

PUSA, SAMASTIPUR (BIHAR)

In Partial fulfilment of the requirements

FOR THE DEGREE OF

Master of Veterinary Science

(PARASITOLOGY)

BY

UPENDRA PANDIT

REG. NO. M/VPA/92/2000-2001.

DEPARTMENT OF VTERINARY PARASITOLOGY

BIHAR VETERINARY COLLEGE

P A T N A – 800014 (BIHAR)

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DEPARTMENT OF VETERINARY PARASITOLOGY,
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RAJENDRA AGRICULTURAL UNIVERSITY,
PUSA (SAMASTIPUR), BIHAR.

CERTIFICATE-I

This is to certify that thesis entitled "**Epidemiological studies on *Fasciola* and *Amphistomes* in Buffalo in some river belt districts of Bihar with special reference to Chemotherapy**" submitted in partial fulfilment of the requirements for the Degree of **Master of Veterinary Science (Veterinary Parasitology)** of the faculty of Post-Graduate Studies, Rajendra Agricultural University, Pusa (Samastipur), Bihar is record of bonafide research work carried out by **Dr. Upendra Pandit,** Registration NO. M/VPA/92/2000-2001 under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

It is further certified that such help or information, received during the course of this investigation and preparation of the thesis has been fully acknowledged.

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CERTIFICATE-II

We the undersigned, members of the Advisory Committee of **Dr. Upendra Pandit**, Registration NO. M/VPA/92/2000-2001 a candidate for the Degree of Master of Veterinary science with Major in **Veterinary Parasitology** have gone through the manuscript of the thesis and agree that the thesis entitled "**Epidemiological studies on *Fasciola* and *Amphistomes* in Buffalo in some river belt districts of Bihar with special reference to Chemotherapy**" may be submitted by **Dr. Upendra Pandit** in partial fulfilment of the requirements for the degree.

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CERTIFICATE-III

This is to certify that thesis entitled "**Epidemiological studies on *Fasciola* and *Amphistomes* in Buffalo in some river belt districts of Bihar with special reference to Chemotherapy**" submitted by **Dr. Upendra Pandit**, Registration NO. M/VPA/92/2000-2001in partial fulfillment of the requirement for the **Degree of Master of Veterinary Science (Veterinary Parasitology)** of the faculty of Post-Graduate Studies, Rajendra Agricultural University, Bihar was examined and approved on 9/2/2004

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Dedicated

to

My

Dear Parents

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Place: patna

Date: 1-9-03

Upendra Pandit

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CHAPTER - I

INTRODUCTION

INTRODUCTION

India possesses the largest livestock population in the world. It accounts for 57% of the world's buffalo population and 15% of the cattle population. According to the livestock census (1992), the country has about 20.5 crore cattle and 8.4 crore buffaloes. The contribution of livestock sector to the food basket in the form of milk and meat has been immense in fulfilling the animals protein requirement of ever growing human population. Animal Husbandry output constitutes about 30% of the country's agriculture output and 7% of its national income. According to estimates of national accounts, the gross value of output for livestock sector during 2000-01 was about Rs 1,67,205 crore as against the total value of output Rs. 5,61,717 crore for the agriculture sector (India-2003).

Gastrointestinal helminthic infestations in livestock cause fairly a gross harm that hampers the productivity of the livestock industry. A number of helminths use to harbour in gastrointestinal tract of buffalo and thus causing severe morbidity and mortality. The effects of treamatodes are more pronounced in cow and buffalo of Indian subcontinent because of having humid and tropical climate, which is ideal condition for growth and propagation of these helminths and their developmental stages. Fascioliasis and amphistomiasis are the two most important helmintic infections of large ruminants.

Fasciolosis usually caused by *Fasciola hepatica* or *F gigantica*, major harmful, widespread and common parasites of Indian livestock, affecting the health of large ruminants. During migration of its

metacercariae and their localization in the biliary passage causes extensive damage to liver tissues viz. portal cirrhosis, obstruction of bile flow causing obstructive jaundice, cystic dilation of bile ducts by thickening its wall and development of fibrous tissue cause extensive damage of liver. The animal becomes inactive and shows distended abdomen, ascites, anaemia, anorexia, bottle jaw (oedema of intermandibulous region), diarrhoea, hypoproteinemia and reduced body weight (Pachauri 1995). It has been estimated that 300 million bovines are exposed to this parasite world wide causing economic losses amounting to more than US $ 3.0 billions per year (FAO, 1994).

Amphistomiasis caused by immature paramphistome which is the main parasite remaining in the rumen and reticulum of cow and bile duct of buffalo (Gigantocotyle explanatum). During heavy infestations necrosis and haemorrhage in duodenum and upper ileum occurs which are responsible for severe pathological changes. Clinical symptoms marked by profuse foetid diarrhoea, gastroentritis and rapid emaciation, result in weakness and frequently death may occur.

The propagation of these parasites depend upon various factors including their intermediate host which is fresh water snails and they are commonly inhabit in the natural water bodies (ponds, lakes, canals, channels, ditches etc.) and paddy fields, there by increasing the chances of natural infections. Infestation in buffaloes is more common as they dwell in these water bodies infested with snails or get the infection by grazing near the water bodies and drinking water from the snail infested water reservoirs. Stallfed animals supplied with paddy straw carrying

encysted metacercariae of these parasites is an important source of transmission of diseases.

These diseases are endemic in high rainfall areas. Post flood situations are quite prevailing for propagations of intermediate hosts. In tropical plain land like India the infection is seasonal and is primarily governed by the onset of Monsoon rain leading to two critical periods of infection based on availability of metacercariae in pasture or fodder, during July to October (Rainy season) and February to March (Spring) but it has also been reported that high incidence of these trematodal infestations was highest in Winter season specially in flooded areas because when flood water receded and the exposed grass or vegetation carrying heavy deposition of infective metacercareae are grazed by grazing animals. Previously Monsoon provides best suitable condition for breeding of snails.

Despite FAO, (1994) recommendation of strategic dosing against these fluke in cattle and buffalo in India, anthelmintic treatment is only practiced in animals showing just clinical symptoms and the control is heavily relied on the use of flukicides which are expensive and reported to be toxic to the hosts. Some of chemotherapeutic agents are effective against immature flukes while others are effective against mature stages and few others are lethal to both mature and immature stages. Thus the emergence of anthelmintic resistant strains is however yet to be documented but the response of some suitable drugs for amphistomiasis and fascioliasis varying in different area according to geoclimatic and field

condition as it has been reported and experienced by various workers of Bihar also.

The land of Bihar is full of rivers and these trematode infestations are regarded as the most pathognomic and dreaded disease in the river belt districts of Bihar for all livestock population including buffalo. Occurrence of flood has been reported every year in the large areas surrounding these rivers and it regulates the natural water environment for breeding of intermediate host. Thus, survival of parasites in host animals become easy because most of the animal owners of this area apply open grazing practices.

The main objective behind the reporting about the prevalence of fascioliasis and amphistomiasis in buffaloes, in river belt areas is to elucidate and correlate fluke infestations with this region, which may help in formulation the new control strategies. The studies are also intended to judge whether any resistant strain of *Fasciola* sp. and *Amphistome* sp. have any impact on the efficacy of common fluikicide responsiveness and effectiveness. Keeping in view the following objectives have been taken:-

1. To study the prevalence of helminthic parasites of buffaloes in some river belt districts of Bihar with special reference to *Fasciola* sp. and *Amphistome* sp.
2. To study the haematological changes in buffaloes infested with *Fasciola* sp. and *Amphistome* sp.
3. To evaluate the efficacy of anthelmintic against infestation of *Fasciola* sp. and *Amphistome* sp. in buffaloes in different river belt districts of Bihar.

CHAPTER - II

REVIEW OF LITERATURE

REVIEW OF LITERATURES

PREVALENCE OF *FASCIOLA* SP. AND *AMPHISTOME* SP.

Varma (1957) observed seasonal occurrence of Paramphistomiasis in Bihar confined between September/October to February/March, when floodwater receded and the animals grazed the exposed grasses or crops carrying heavy deposition of infective metacercariae.

Hassan (1977) collected the most common helminths in cattle were *Mecistocirrus digitatus*, *Haemonchus placei*, *Fasciola gigantica* and *F. hepatica* in peninsular Malasia. The other less common parasite was *Dictyocaulus viviparous* in cattle and *F. gigantica* in bufflalo.

Pal *et. al.* (1978) conducted a study on domestic animals suffering from gastrointestinal disturbances in the rural area of Delhi and the faecal examination revealed the eggs of *Fasciola hepatica* in 5 of 18 buffaloes, 1 of 16 buffalo calves, 2 of 13 cows, 1 of 10 cow calves, while *Fasciola gigantica* occurred in 9, 3, 1 and 2 respectively.

Chiriboga *et al.* (1980) conducted a study on epidemiology of *F. hepatica* infestation in dairy cattle at Dorado, Puerto Rico. The faecal examination revealed that 86% of cattle were found positive for *F. hepatica*. Infection rate was highest (95%) in older cows (9-12 years old) and lowest (71%) in cows 3½ years old. Highest infection of *Fasciola hepatica* occurred in cattle between November and February

because average monthly rainfall was 145 mm and average temp was 24^0c which were the most favourable for snail development.

Sharma and Lal (1983) examined that altogether 80% buffalo of Meerut, district were infected with trematodes. Out of which 70% were found infected with *Paramphistomum explanatum*, 50% with *Gastrothylax* sp. 15% with *F. gigantica*, 20% harboured both amphistomes and *F. gigauntica*, and 60% harboured *Amphistome* sp. only. *Amphistome* sp. and *F. gigantica* were found 60% and 10% in the Summer season, 65% and 15% in Rainy and 80% and 20% in the Winter season.

Gupta *et al.* (1986) observed higher incidence of fascioliasis (*F. gigantica*) in Haryana state's animals due to heavy pasture infestation with metacercarieae, but exception was recorded during May (5.2%) September (27.1%) and October (16.7%) without any seasonal pattern, attributed to the prevalence of infected intermediate host, whereas the low incidence of fascioliasis in the animals was because of absence of natural or artificial large water columns, thus carrying negligible pasture infestation.

Sahay (1987) made studies on the epidemiology and control of paramphistomiasis in bovine in Bihar and reported that 58.39% cattle and 40.53% buffaloes harbouring amphistomes. The highest incidence in bovine was observed in Winter (57.2%) followed by Monsoon (52.3%), Spring (33.5%) and Summer (15.3%). Cattle also shown highest incidence in Winter (73.03%) followed by Monsoon (60.98%), Spring (55.67%) and Summer (25.86%) whereas in buffaloes

the incidence was highest in Monsoon (57.6%) followed by Winter (15.63%), Spring (21.44%) and Summer (13.93%) seasons.

Hafeez and Aasatthi (1987) examined a total of 225 cattle and buffaloes in slaughterhouses in and around Anand, Gujarat. 20.0% and 20.6% of 75 cattle and 150 buffaloes, respectively, harboured gastro-intestinal amphistomes viz. *Paramphistomum epiclitum, Gastrothylax crumenifer, Fischoederius elongatus, F. cobboldi, Calicophoron calicophorum* and *Ceylonocotyle thapari. G. crumenifer* was the most prevalent parasite in cattle and buffaloes.

Coskun (1988) conducted a survey of slaughtered ruminants in the Abattoir Ankara. He found paramphistome infection was highest in cattle (60.98%) followed by in sheep (12.14%). Rate of infection was highest between January and May. *P. cervi* and *Calicophoron daubneyi* were found both in sheep and cattle.

Dhar *et al.* (1988) made some studies on the prevalence of fascioliasis in animals in different parts of Kashmir valley, prevalence ranging from 0 to 100% by faecal examination. Cattle showing the highest percentage, goats the lowest and sheep occupying an intermediate position of infecting animals with predominant species of *Fasciola* sp.

D'souza *et al.* (1988) conducted a study on prevalence of gastrointestinal parasite by examining the faeces, 64.84% positive cases of cattle and 69.54% of buffaloes in Mysore and Mandya districts in Karnataka. They found mixed infections of *Paramphistomum* and *Fasciola. Paramphistomum* and *Buxtonella* were the most common

trematodal species. Prevalence was significantly higher among 8 months old cattle. Prevalence of paramphistomiasis was highest during the south-west and north-east monsoons.

Jagannath *et al.* (1988) conducted a survey of gastrointestinal parasites occurring in dairy cattle and buffaloes in Bangalore and Mysore Union of K.D.C.C. project area. 30.01 and 29.59% and 32.81 and 27.01% infection were recorded from cattle and buffaloes in Bangalore and Mysore Union, respectively. *Buxtonella sulcazta*, *Amphistomes*, *Strongylus*, and *Fasciola* infections were frequent. Parasites were most common in 4-8 years of age group and were least frequent in animals below one year of age.

Bhatia *et al.* (1989) studied the epidemiology of *Fasciola gigantica* in buffaloes, goats and sheep in Tarai region of Uttar Pradesh. Altogether 39% buffaloes had found to be infected in slaughterhouse material. The season wise incidence in buffaloes was recorded to be highest during Winter and lower peaks were observed during June/July and January/February specially in buffaloes.

Krishna *et al.* (1989) examined faecal samples of ruminants in Kangra valley, Himachal Pradesh for gastrointestinal parasites. They found *Fasciola* in 50% of cattle and 64% of buffaloes, along with other parasites.

Sahay *et al.* (1989) conducted a detailed survey of paramphistomiasis in bovine with its seasonal and regional variations in the state of Bihar. The highest incidence was recorded to be 91.6% in cattle of Madhubani district and 59.67% in buffaloes of Begusarai,

where as lowest was observed to be 46.44% in cattle of Ranchi and 20.57% in buffaloes of Dhanbad. The maximum incidence was observed during Winter followed by Monsoon spring and Summer seasons.

Kulkarni (1990) reported the incidence and prevalence of helminthes parasites in buffalo of Andhra Pradesh. Ruminal amphistomes comprised *Cotylophoron cotylopheron, Fischeoderius elongatus, Fischeoderius coboldi, Gastrothylax crumenifer, Carmyrius spathiosus, Paramphistomum cervi, Calicophoron calicophorum* including various other nematodal, cestodal and other trematodal infections. Multiple infection of liver with *Fasciola gigantica, Gastrothylax explanatum, Selistomes* and hydatid cyst were also encountered.

Mohpatra *et al.* (1990) studied prevalence of paramphistomiasis in cattle of Orissa. By the examination of faecal samples the incidence was found to be 66%, 82.5% and 96.66% in animals between the age group of 1 year, 1-3 years and above 3 years of age respectively. Higher incidence (91.66%) of paramphistomiasis was observed in male animals than female (86.33%). Season wise incidence was found to be 100%, 87.50% and 75% in Winter, Rainy and Summer seasons respectively. The incidence was more frequent in coastal areas than of plain and hilly areas.

Seasonal incidence of amphistomes in cattle in Meghalaya was examined by Roy and Tandon (1991), revealed 54.7% overall rate of adult *Amphistome* sp. infection throughout the year with two peak

periods of prevalence, one between June and September and other between January and February.

Ratnaparkhi (1991) made studies on the month wise incidence of *Fasciola* sp. in buffalo, cattle, crossbred cattle, sheep and goat. He found maximum incidence in month of December and lowest in the month of May in buffaloes.

Mahato and Rai (1992) studied prevalence of paramphistomiasis in cattle of Terai and Koshi zone of Nepal. The prevalence of amphistomiasis was recorded to be 32.5% and 22.2% in Terai and Koshi zone respectively.

Chaudhri *et al.* (1993) conducted epidemiological studies and control of *Fasciola gigantica* infection of cattle and buffaloes in eastern Haryana and observed that the ruminants of the eastern region of Haryana were more prone to helminth diseases than those of the western region, Among the digeneans, paramphistomes such as *Paraphistomum cervi* and *Calicophoron calicophorum* were widely prevalent along with fasciolids infection.

Manna *et al.* (1994) conducted the systematic studies on the incidence of amphistomiasis in domestic ruminants in west Bengal and found the overall incidence of 56.5% in cattle and 72.40% in buffaloes. Biliary amphistomiasis was recorded to be 24.31% buffalo, which was highest in Summer.

Rai *et al.* (1996) conducted an epidemiological study on bovine fascioliasis in the Andaman and Nicobar islands; incidence varied from 12.1% to 70% in a year and was higher from September to

April. Ducks broke preliminary fields trials showed that ducks grazing in open range systems could be an effective biological control of the snails, as 32.5 ± 11.3%. Deworming with albendazole twice a year (September/October and February/March) reduced the incidence of *F. gigantica* infection.

Cheema *et al.* (1997) conducted a study on prevalence and pathology of amphistomiasis in buffaloes and cattle in Pakistan and observed that prevalence rate in buffaloes (75.07%) and in cattle (50.7%). Multiple amphistomes were observed, *Gastrothlax crumernifer* located in different parts of the rumen, where as *Gigantocotyle explanatum* in bile duct.

Rajkovic-Janje *et al.* (1997) studied prevalence of endoparasites in 150 cows from 76 small farms in Tuhelj, Croatia. Faecal samples were collected from each cow and examined for endoparasites using modified Mcmaster and sedimentation methods. The common helminth parasites were *Paramphistomum* sp. (63), gastrointestinal nematodes (12), *Eimeria sp.* (8) and *Fasciola hepatica* (6).

Borthakur and Das (1998) conducted epidemiological studies on the gastro-intestinal helminths in dairy animals of Assam and recorded that crossbred cattle were more susceptible than native cattle and buffaloes. *Fasciola* sp. and *Paramphistome* sp. were recorded through out the year in the heifers and cows.

Srivastava *et al.* (1998) made a study on occurance of *Gastrothylax* sp. in Barsingha, M.P and revealed that *G. crumenifer* and *G. glandiformis* were also common in domestic ruminatnts.

Hirani *et al.* (1999) studied the prevalence of gastrointestinal parasitic infections in cattle and buffaloes of Kheda district of Gujrat. They examined 929 faecal samples of cattle and buffaloes and revealed that 38.6% of the samples were positive for gastrointestinal parasites. Significant increase in *amphistomes* was observed between December to June.

Jitendran and Bhath (1999) conducted a systematic survey for the prevalence of parasitosis in particular gastrointestinal nematodes and flukes in dairy animals of Kangra valley of Himachal Pradesh. *Fasciola, Amphistome* sp., *Dicrocoelium* and *strongylus* were the most important parasitic infections. *Fasciola* was also prevalent throughout the year more in buffalo than in cattle. Other flukes and nematodes infections were found in peak in March-April and July-September. The egg counts ranged higher in buffaloes than cattle during Rainy and post rainy seasons.

Prasad and Varma (1999) described the prevalence and community dominance among *paramphistomes* infecting domestic rumininats. They conducted a study to assess the prevalence of rumen *paramphistomes* in domestic ruminants slaughtered at various abattoirs in Barielly, Uttar Pradesh, revealed maximum rate of infection during post monsoon (24.24%) as compared to Summer (23.01%), Winter (20.64%) and Monsoon (19.67%) seasons respectively.

Paramphistomum epictilium and *Gastrothylax Crumenifer* were predominant species in buffalo.

Bedarkar *et al.* (2000) studied the seasonal prevalence of snail borne fluke infections in different ruminants of Marathwada region. Seasonal prevalence of fluke infections caused by *Schistosoma* sp., *Fasciola* sp. and *Amphistome* sp. were observed highest during Monsoon and lowest in Summer. Most fluke infections were more common in sheep (48.05%) followed by buffaloes (46.58%), goats (40.46%) and cattle (36.27%).

Pal *et al.* (2001) reported the prevalence of gastrointestinal parasites in cattle and buffaloes from Chattisgarh region by examination of faecal samples and revealed 23.3% positive for various gastrointestinal parasitic infections. The prevalence of *Amphistome* sp. (18.2%), *Strongylus* (14.2%), *Trichuris* sp. (6.6%), *Eimeria* sp. (3.3%), *Toxocara* sp. (2.4%), *Fasciola* sp. (1.2%) *and Moniezia* sp. (.6%) were recorded. The prevalence rate of the parasitic infections was higher in younger animals (< 6 months) than in adults.

Chowdhury (2002) studied the epidemiology of helminths in livestock of Indian sub-continent and noted that the epidemiology of parasites in a particular area is crucial for the development and implementation of effective management practices. This requires sound epidemiological data of the parasites, range of hosts involved including intermediate hosts transmitting factors etc.

Gupta and Singh (2002) had given a description about fasciolosis in cattle and buffaloes in India. Despite sporadic work on

epizootiological aspects and control of the disease, the prevalence of infection from various parts of the country has been reported to vary from 30 to 80% with almost 70% cattle/Buffalo carrying the infection in Kashmir, to morbid outbreaks in North-East, Andman and Nicobar islands, Central and Western regions of the country. Control of the malady is possible today through chemotherapy using anthelmintics viz. closantel, nitroxynil. oxyclozanide and triclabendazole. The enormity of this meance stresses the need to generate reinforcing date for developing forecasting systems and strategies for control.

Sahoo, *et al.* (2003) studied seasonal prevalence of gastrointestinal helminthic infections in stallfed and grazing cattle in a rain fed district which revealed the highest prevalence of parasites in Rainy (64.70%) followed by Winter (49.74%) and Summer (37.89%) seasons. *Amphistome* sp. and *Fasciola* sp. infections were predominant in 51.47% and 2.06%, 40.31% and 1.53%, and 29.21% & 1.05% in cattle during Rainy, Winter and Summer seasons respectively. Trematodal infections were more prevalent in free grazing (44.90%) than stallfed (13.22%) cattle.

HAEMATOLOGICAL STUDIES

Ogunrinade and Bamgboye (1980) described about haematological indices and their correlation with worm burden in chronic fascioliasis. Significantly ($p<0.01$) lower values were obtained for packed cell volume (PCV), haemoglobin (Hb) and Red blood cell

counts (RBC) while significant ($p<0.05$) higher value was observed for eosinophil counts in infected cattle.

An investigation of the haematological, biochemical and pathological aspects of naturally occurring bovine fascioliasis in India had observed by Kumar *et al.* (1982). Affected animals revealed reduction in total erythrocyte count (TEC), packed cell volume (PCV) and haemoglobin content (Hb); increase in total leukocyte, lymphocyte and eosinophil counts along with a decrease in neutrophil count.

Swarup *et al.* (1987) carried out a study on the prevalence and clinico-pathology of naturally occurring fascioliasis and biliary amphistomiasis (*Fasciola gigantica* and *Gigantocotyle explanatum*) in buffaloes indicated 61.78% infection with either of two or both types of parasites. Highest infection was observed for *F.gigantica* (29.26%) and lowest for *G. explanatum* (19.51%). Significantly low values of total erythrocyte count, haemoglobin, packed cell volume, and accelerated erythrocyte sedimentation rate (ESR), high values of eosinophils, total leucocyte count (TLC), Monocytes were recorded in *F. gigantica* infection. No significant changes indicated in *G. explantum* infected buffaloes.

Chaudhri *et al.* (1988) conducted a study on haematological and biochemical observations in buffaloes naturally infected with *F. gigantica* and there was significant decrease marked in the values of haemoglobin content, packed cell volume, total erythrocyte count of infected buffaloes than normal values.

ANTHELMINTIC EFFICACY OF ALBENDAZOLE, CLOSANTEL AND OXYCLOZANIDE + LEVAMISOLE:

Yakubovskii and Zharikov (1974) evaluated the economic effectiveness of control measures against paramphistomiasis and an attempt was made to assess economic losses attributed to infestation. The results justified the anthelmentic treatment and other control measures directly related in terms of increased productivity.

Georgiev and Gruev (1979) studied efficacy of levamisole and oxyclozanide against *Paramphistomum* in sheep and cattle. Administration of oxyclozanide to cattle at 10mg/kg gave reductions of paramphistomes by 74.84-89.97%. Combined administration of two drugs oxyclozanide 15mg/kg + levamisole 12 mg/kg to cattle gave reduction of paramphistomes by 87.91-99.96%.

Ronald *et al.* (1979) observed a controlled evaluation of albendazole against natural infections with *F. hepatica* and *Fascioloides magna* in cattle. Albendazole administered to cattle at 45mg/kg, 35mg/kg, 25mg/kg or 15 mg/kg body weight reduced the number of viable *F. hepatica* adults by 99.8%, 97.6%, 97.6% and 95.7%, *F. hepatica* immatures by 100%, 98.6%, 98.6% and 91.1% and *Fascioloides magna* by 96.5%, 99.1%, 94.0% and 94% respectively.

Theodorides and Freeman (1980) administered albendazole 10 or 15mg/kg in calves infected with *F. hepatica*, revealed the reduction of eggs by 93 and 95% respectively. In another experiment, 7.5 mg/kg given once, twice with two weeks or three weeks interval reduced the mean fluke burden by 44%, 72% and 90% respectively.

Manstretta and Pera (1981) reported preliminary results on the efficacy of closantel against bovine fascioliasis in the province of Piacenza, Italy at a dose rate 2.5 mg/kg body wt that resulted remission of symptoms rapidly and recovery of performance in terms of body weight, milk production and fertility.

Malone, *et al.* (1982) treated albendazole in drench form to calves as 4.55% (w/v), against mature *F. hepatica*. The efficacies were recorded to be 77.5% with the dose of 7.5mg/kg. 92.3% with 10 mg/kg and 85.9%. with 15mg/kg. Drug efficacies against immature flukes with these doses were found to be 32.7%, 20% and 36.7% respectively. Number of eggs recovered in bile (P.M.) were reduced by 87.8% with the dose 7.5mg/kg followed 91.8% with 10mg/kg and 95.6% with 15mg/kg.

Genchi *et al* (1986) treated closantel at 5 mg/kg body weight and rafoxanide at 7.5 mg/kg body weight in calves, eliminated 99.8 and 98.9% of the parasites. In another trial however the examination of slaughtered material revealed, elimination 81.3% and 89.0% of flukes on 36 day after treated with oxyclozanide (10 mg/kg) and albendazole (10 mg/kg) respectively. The lower efficacy was probably due to less activity of drugs against the immature flukes.

Rolfe and Boray (1987) conducted a study on chemotherapy of paramphistomiasis in cattle-calves. The treatment of levamisole reduced the parasites. The treatment of Oxyclozanide at 18.7 mg./kg combined with levamisole reduced parasite numbers in the small intestine, abomasum and rumen-recticulum by 61-96.1% and 50–

92.6.1% and 56.5-98.1% respectively. When 2 doses of oxyclozanide were given at 3 days apart was found to be effective 99.9%, 100% and 100% respectively in above-mentioned organs. Faecal egg counts were also observed to be reduced on 10-14 days after treatment. Against mature paramphistomes with oxyclozanide as a single dose or 2 doses at 3 days apart with 12.8–18.7 mg/kg was found to be 93.6–97.5% effective.

Kumar and Pachauri (1989) evaluated the efficacy of albendazole against *Fasciola gigantica* infection in buffaloes and observed that milk yield and improvement in milk quality in terms of total solids, protein, fat, SNF, ash content and specific gravity improved after treatment with a single oral dose of albendazole.

Mohapatra *et al.* (1990) observed the anthelmentic efficacy of (Albomar) albendazole and thiophanate against natural paramphistome infection in cattle. On the basis of faecal egg count before and after medication, albomar at 15mg/kg body weight was proven to be 85.40% effective against mature paramphistomes.

Richards *et al.* (1990) assessed the efficacies of triclabendazole, albendazole, closulon, nitroxynil oxyclozanide and rafoxanide against *F. hepatitica* in cattle. The mean efficacies of triclabendazole given orally at 12 mg/kg against 1, 2 and 4 weeks old early immature fluke were 88.1%, 95.3 and 90.7% respectively. Albendazole and oxyclozanide showed no activity against 6 weeks old parasites. Clorsulon, nitroxynil and rafoxanide had only moderate activity. The efficacies of triclabendazole, nitroxynil and rafoxanide

against 10 or 12 week old parasites were 100%, 99.0%, and 99.1% respectively. Albendazole and oxyclozanide showed poor efficacy against 1-12 weeks old infection.

Shrestha *et al.* (1992) examined faecal samples from cattle and buffaloes from Dhankuta district, Nepal. Prevalence of *Fascila gigantica* infection was highest in buffaloes (33.3% in a dry areas and 71.4% in a wet areas) as compared to cattle (24.7% in dry areas and 28.6% in a wet areas). Naturally infected buffaloes with *F. gigantica* were treated with 14 mg/kg of triclabedazole. Cent percent efficacy of drug was recorded on 15th day of post treatment as per the declining rate of egg per gram.

Dorchies (1993) carried out a trial in France on cows with natural infection of *Fasciola hepatica.* Valbazen 5% (albendazole) at single dose rate of 10 mg/kg body wt. was found to be 85% effective at housing, when the treatment was repeated after 8 weeks the efficacy rose to 96.2%. A single treatment with valbazen was as effective as nitroxynil at 10mg/kg given 56 days after panacur (fenbendazole) at housing. Valbazen treatment at housing may be recommended because of its hematicidal activity and efficacy against adult *F. gigantica.*

Mage *et al.* (1993) tested the efficacy of closantel 2.5 mg/kg or 5 mg/kg body wt. against *Fasciola hepatica* under field condition randomly in infected adult cattle. The coprology of these animals revealed the both doses were 100% effective at 60 and 100 days after treatment.

Alam *et al.* (1995) treated some fasciolicides viz. triclabendazole (12 mg/kg p.o), nitroxynil (1.5 ml/50kg s.c.) albendazole (15mg/kg p.o) and hexachlorophene against bovine fascioliasis. All the drugs were given twicely with an interval of 15 days for evaluation of comparative efficacy in groups of 5 dairy cattle (3 to 7 years old) with naturally infected with *Fasciola* sp. The anthelemintic efficacies, measured by reduction in faecal egg counts were 100%, 93.7%, 78.5% and 75.0% on 28 day after treatment respectively.

Yakubovski and Lipnitski (1995) evaluated the efficacy of anthelmintics against fascioliaisis in ruminants. Triclabendazole, albendazole, closantel, rafoxanide were found to be 98-100%, 97-100%, 93-94% and 86-96% effective as per their recommended doses respectively.

Bauer *et al.* (1996) conducted a field trial to evaluate the efficacy of closantel and given orally at a dose rate of 10 mg/kg body wt. against *Fasciola hepatica* and *Haemonchus contortus*. Closantel however, resulted in 100% reduction of *Haemonchus contortus* and 94% *Fasciola hepatica* eggs.

The effect of treating naturally acquired *Fasciola hepatica* *Paramphistomum ichikawai* and *Calicophoron calicophorum* infections in dairy cattle on milk production had studied by Spence *et al.* (1996). Cows were either not treated or treated with 4.5 mg/kg oxfendazole, 16.6mg/kg oxyclozanide or 4.5 mg/kg oxfendazole + 16.6 mg/kg oxyclozanide in March, May and August. A significant increase in milk

production, averaging 0.4 litres per day, was seen when infected cows were treated with combined drug. Cows treated with oxyclozanide alone produced no more milk than untreated cows. However faecal egg counts confirmed that oxyclozanide treatment reduced the paramphistome population.

Aziz *et al.* (1999) treated with anthelmintics against fascioliasis in buffaloes under field conditions. Based on faecal egg counts oxyclozanide was found to be 86.5% effective by day 21 and 92.5% effective by day 28.

Sanyal (2001) described about the control of tropical fasciolosis in cattle and buffaloes in India at the back drop of integrated management and described the limited anthelmentic groups on the same parasite may be responsible for drug resistant cases and regular use of flukicide is very expensive and toxic to the host. He further suggested the development and application of cost effective; environment friendly, non-chemical control strategies should be the priority area of research to combat fascioliasis.

Sahoo and Mohanty (2002) conducted a field trial to evaluate the efficacy of anthelmintics against *Amphistome* sp. infestation of cattle in rain-fed districts of Orissa. The highest percentage of efficacy was recorded with oxyclozanide (91.8%), followed by closantel (75.5%) and albendazole (64.8%) at the dose rate of 10 mg/kg, 15 mg/kg and 15 mg/kg body weight respectively. According to the percentage efficacy and clinical cure, oxycloznide

was considered to be the drug of choice for the treatment of amphistomes infestation in cattle.

Pal *et al.* (2003) conducted study on buffaloes naturally infected with *Fasciola gigantica* and *Amphistome* sp. and observed that oxyclozanide at 10 mg/kg body wt. was 100% and 86.7% effective against patent infection of *F. gigantica* and *Amphistome* sp.

CHAPTER - III

MATERIALS AND METHODS

MATERIALS AND METHODS

Bihar has many important rivers and it has also full of livestock population. Flood is very prone to districts situated on the bank of rivers. The productivity of animal husbandry sector is very poor due to some complex factors mainly with trematodal infection. The geographical conditions and ecology are very much helpful for propagation and habitats of snails, intermediate host of these flukes. So in the present investigation, an attempt was made to study the prevalence of fascioliasis and amphistomiasis, two major trematodal diseases of buffaloes in different seasons, age groups, sex and managemental condition in three river belt districts of Bihar viz. Patna, Khagaria and Lakhisarai. The period of study was between June 2002 to May 2003. Naturally infected buffaloes with *Fasciola* sp. and *Amphistome* sp. were identified and the haematological changes were anyalyzed. Comparative evaluation of chemotherapeutic agents viz. albendazole, closantel, and oxyclozanide + levamisole were carried out in naturally infected buffaloes with *Fasciola* sp. and *Amphistome* sp.

(1) Selection of some river belt districts of Bihar:

Following river belt districts of Bihar had been selected to conduct the present study:

Patna – Ganga river

Khagaria – Koshi river

Lakhisarai – Kiul river

COLLECTION OF MATERIALS

Faecal sample:

A total number of 1285 faecal samples of buffaloes were screened out from three selected districts of Bihar. Out of total number of samples 450, 415 and 420 number of faecal samples were examined from Patna, Lakhisarai and Khagaria districts respectively. Special emphasis were made while keeping the records of positive test result with *Fasciola* sp. and *Amphistome* sp. in different seasons, age, sex and managemental conditions, the details of which are depicted in following table:

Faecal samples of Buffaloes examined

Table – 1

	No. of faecal samples examined		
Season	Patna	Khagaria	Lakhisarai
Summer	140	120	135
Monsoon	160	170	150
Winter	150	130	130
Total	450	420	415

Table – 2

	No. of faecal samples examined		
Age group	Patna	Khagaria	Lakhisarai
< 1 year	145	130	130
1-3 year	145	130	130
> 3 year	16	160	145
Total	450	420	415

Table – 3

	No. of faecal samples examined		
Sex	Patna	Khagaria	Lakhisarai
Male	200	200	185
Female	250	220	230
Total	450	420	415

Table – 4

	No. of faecal samples examined		
Managemental Condition	Patna	Khagaria	Lakhisarai
Stall fed	200	180	190
Free grazing	250	240	225
Total	450	420	415

GASTROINTESTINAL TRACT:

Samples collected from slaughter house Patna were mostly available from overaged buffaloes the records of sex and managemental condition were not possible to accounting due to its unavailability:

Season	No. of samples
Summer	50
Monsoon	60
Winter	40
Total	150

BLOOD:

A total of 30 buffaloes consisting 10 each of healthy, naturally infected with *Fasciola* sp. and naturally infected with *Amphistome* sp. were selected for haematological trials in Patna district. Blood samples collected in vials containing anticoagulant (E.D.T.A.) from the jugular vein of each animal with the help of sterilized disposable syringe after removal of hairs and proper sterilizing the surface with the help of 70% alcohol. For differential leucocyte count (D.L.C), thin and uniform smears were prepared on clean and grease free slides. Smears were dried in air and properly labelled.

THERAPEUTIC TRIALS:

The therapeutic trial against fascioliasis and amphistomiasis were conducted in Patna, Khagaria and Lakhisarai districts separately. A total of 40 buffaloes were selected in each district for this experiment out of positive cases of *Fasciola* sp. and *Amphistome* sp. during the study of incidence. These buffaloes were marked for theraupetic trial. Design and process of experiments were explained to the owners and then permission was taken to conduct the trials on their animals before commencement of experiment.

Albendazole, closantel and oxyclozanide + levamisole were chosen as anthelminthics for the therapeutic trials, to evaluate the comparative efficacies under natural infection of *Fasciola* sp. and *Amphistome* sp. in buffaloes.

EXAMINATION OF COLLECTED MATERIALS:

Faecal samples and samples of G.I. tract:

The faecal samples collected from buffaloes were kept in individual vials and properly labeled. Few drops of 10% formalin were added in the collected samples to preserve the morphological characteristic of helminthic ova, if any delay was made in processing the samples. The samples of gastronintestinal tract were collected from local slaughter houses, Patna and post-mortem section of the Department of pathology, Bihar veterinary college, Patna. Information regarding age, sex. breed etc. of faecal samples were also recorded.

The collected faecal samples were examined by the following methods.

Gross Examination and Direct method:

Collected material was grossly examined for the presence of adult parasite. On a clean glass slide very small quantity of faecal sample is mixed with as much quantity of water so that a uniform emulsion should prepared with the help of matchstick and then covered with a cover slip. This preparation was examined first under low power objective and then under high power objective. For each sample separate stick was used.

Concentration Method:

The following concentration method was done for the detection of ova of *Fasciola* sp. and *Amphistome* sp. in buffaloes.

Sedimentation technique:

This is the most suitable technique for detection of trematode eggs. About 1 or 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 filtrate was centrifuged at 2000 rpm for 10 minutes. The supernatant fluid was discarded and drop of residue was taken by glass rod on a clean microscopic slide and then covered with a cover slip, the preparation was examined first under low and then high power of the microscope.

Identification of *Fasciola* sp. and *Amphistome* sp. Ova

The identification of *Fasciola* sp. and *Amphistome* sp. ova was done by the method as described by Soulsby (1982) and Yamaguti (1959).

GASTRO-INTESTINAL TRACT SCRAPING:

The gastro-intestinal tract was placed in a metal tray containing some lukewarm water. Intestine was cut along with the entire length and all visible helminthes were recovered. The mucosae were carefully examined for trematodal immature stages. The material was collected in petridish and a little portion was examined by taking them in slide for the presence of ova and in watch glass under *Amphistome* sp. These were identified after cleaning them in normal saline as described by Soulsby (1982).

EFFECT OF SEASON, AGE, SEX AND MANAGEMENTAL CONDITION:

Faecal samples of buffaloes were identified for the ova of *Fasciola* sp. and *Amphistome* sp. Record of *Fasciola* sp. and *Ampohistome* sp. infestation in each animal including details such as age, sex,

managemental condition and the month in which the samples collected were maintained and further study was conducted as described below.

Effect of Seasonal Prevalence:

Seasonal variations of *Fasciola* sp. and *Amphistome* sp. in buffaloes were studied in the present investigation according to the regional weather conditions. The period of study was divided into Summer (March to June) Monsoon (July to Oct.) and Winter (Nov. to Feb.) out of 450 cases 140, 160 and 150 were screened from Patna district in Summer, Monsoon and Winter seasons respectively in buffaloes. Followed by 120, 170, and 130 samples were screened out of 420 cases from Khagaria district and 135, 150 and 130 samples were screened out of 415 cases from Lakhisarai district (Table – 1).

Effect of Age group:

According to Table 2 the collected faecal samples were divided in different age sgroups viz below one year, 1-3 year and more than 3 years for the present study to see the prevalence in different age groups.

Effect of Sex:

Out of 450 samples of buffaloes from Patna district, 200 of them were male and 250 of them were female. Similarly 420 samples of buffaloes from Khagaria district 200 were male and 220 were female. Out of 415 samples of buffaloes from Lakhisarai district 185 were male and 230 were female (Table – 3).

Effect of Managemental Condition:

The animals were divided in two groups according to their managemental condition. Samples were collected from stallfed animals

and free grazing animals in Patna, Khagaria and Lakhisarai districts which are depicted in Table 4.

HAEMATOLOGICAL STUDIES:

To evaluate the haematological changes in buffaloes infected with *Fasciolia* sp. and *Amphistome* sp., whole blood samples were collected from 10 apparently healthy buffaloes that served as control and from each 10 buffaloes naturally infected with *Fasciola* sp. and *Amphistome* sp. in Patna district only. It was not possible to conduct the study in Khagaria and Lakhisarai due to unavailability of lab-facilities. Various studies were performed with each blood sample as per the methods described below:

1. Haemaglobin (Hb gram % / dl):

Haemoglobin content of buffaloes naturally infected with *Fasciola* sp. and *Amphistome* sp. and healthy control buffaloes obtained by Hellige-sahlis method described by Sinha (1998).

(i) Erythrocyte sedimentation rate (ESR):

Erythrocyte sedimentation rate per hour of healthy buffaloes and naturally infected animals with *Fasciola* sp. and *Amphistome* sp. were done. The blood was filled with a Pasteur pipette in the wintrobe hematocrit tube from bottom upwards up to zero mark and then the tube was kept in a special ESR stand vertically and level of sedimentation rate per hour was calculated from each sample (Sinha 1998).

(ii) Total Erythrocyte Count (TEC):

The collected blood was taken up to 0.5mark in the R.B.C diluting pipette of Haemocytometer and then diluted with R.B.C. diluting fluid up to 101 mark. The content to the pipette was mixed with twisting motion. A few drops of this diluted blood was is discarded and then a drop of it was allowed to trickle in the gap between cover glass and Neubauer's counting chamber of Haemocytometer. All the cells including touching all the side of the wall of 5 big squares (4 corner and 1 central) or 80 squares for counting cells of R.B.C. were counted and the total number of Erythrocyte count per cubic mm was calculated (Sinha 1998).

(iii) Total Leucocyte Count (TLC):

Blood was sucked up to 0.5 mark of the W.B.C. diluting fluid up to 11 mark taking care that no air bubble was included. The pipette was shaken and the Neubauer's counting chamber was filled as described for R.B.C. counting method. The white cells were counted in the four large corner squares of the chamber and the total number of leucocyte count per cubic mm was calculated (Sinha 1998).

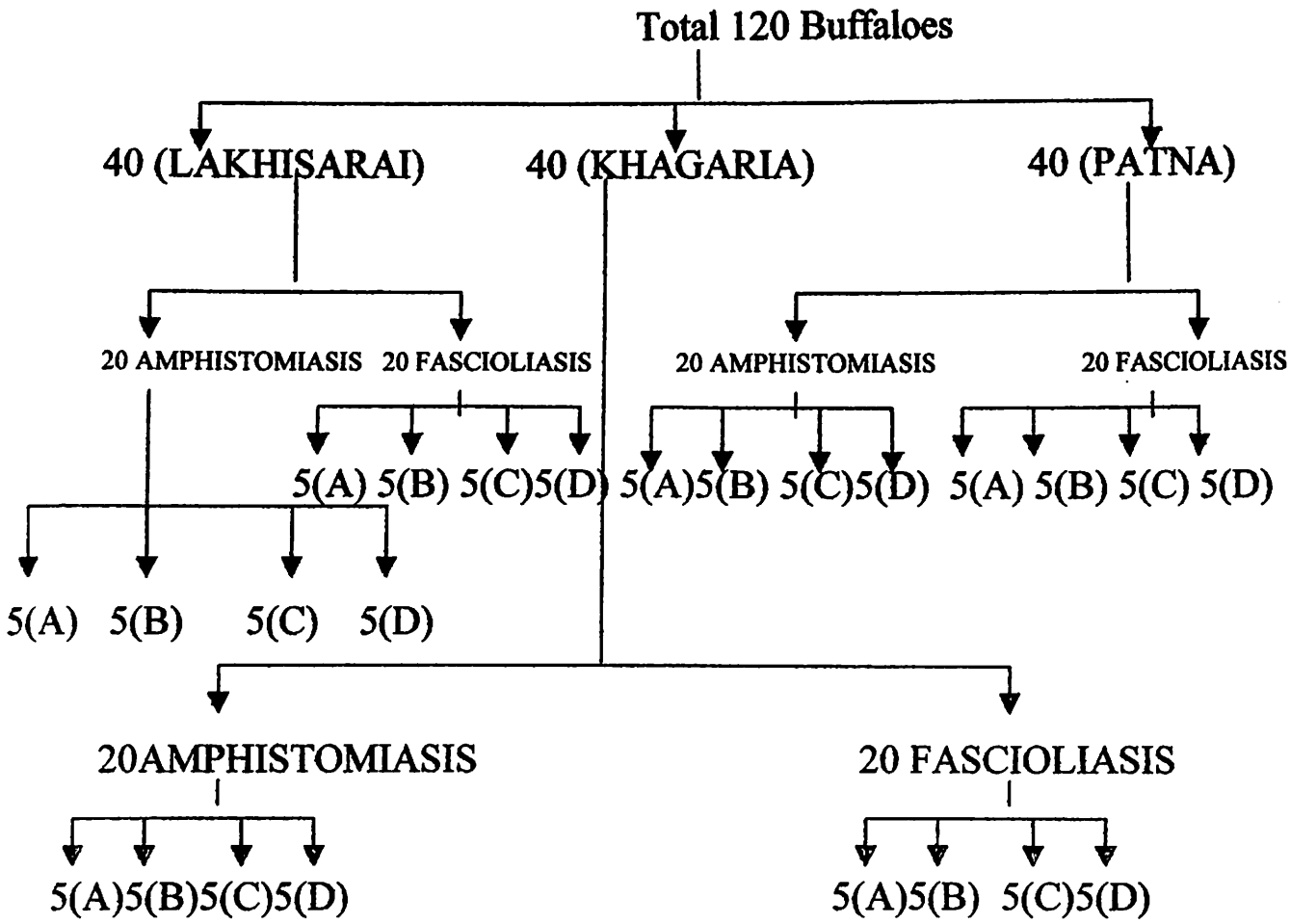
Differential Leucocyte Count (DLC):

For Differential Leucocyte Count, a thin and uniform smear of blood was prepared on clean grease free slide and dried in the air. The smear was stained with leishman's stain. The stained blood film was seen under low power objective of the microscope to see whether the film was homogeneously stained or not and then examined under oil immersion objective of the microscope by placing a drop of cedar wood oil in well separated film. While counting the edges were avoided and the cells

running in strips in the whole length of the film was examined. During examination of the cell, 200 cells were counted and the percentage of different cells were recorded. Leucocytes were differentiated as per standard technique (Schalm *et al.* 1975).

THERAPEUTIC TRIAL:

One hundred and twenty buffaloes were selected for therapeutic trial from all the three districts. Therapeutic trial were conducted on total 120 buffaloes, out of these 40 belongs each districts viz. Patna, Khagaria and Lakhisarai and further as per the experimental plan 20 buffaloes naturally infected with *Fasciola* sp. and rest 20 buffaloes naturally infected with *Amphistome* sp. were selected from each district. Again each group of 20 buffaloes were divided into four groups viz. A, B, C, & D each containing 5 buffaloes and drugs were administered as per the trial programme. The group A remained always as untreated control whereas each group B, C, and D were selected for the administration of drugs viz. albendazole, closantel and combination of oxyclozanide and levamisole respectively.



(A) = Untreated Control, (B) = Albendazole treated, (C) = Closantel treated, (D) = Oxyclozanide and levamisole treated.

The average egg count per gram (EPG) of faeces were evaluated before treatment and on 7th, 14th, 21st day of post treatment and the percent efficacy was calculated on the basis of the decline in the rate of E.P.G. during post treatment.

Group	No.of animals	Drugs used forchemotherapy	Dose in mg/kg	Route
A	5	Untreated control	-	-
B	5	Albendazole Albidol (Bolus 1.5 g) **Concept**	10mg/kg. body wt.	Orally
C	5	Closantel Zycloz (Bolus 1 g) **Sarabhai-Zydus**	7.5mg/kg. body wt.	Orally
D	5	Oxyclozanide + Levamisole Fasmin (Bolus 1.5 g) **Lyka**	22.5 mg/kg. body wt.	Orally

Table – Experimental dosing of anthelmintics trials in buffaloes infected with *Fasciola* sp. and *Amphistome* sp.

The E.P.G. of the faecal samples were calculated according to the Stoll's egg counting techniques.

(i) Approximately 10 to 12 ml. Glass stoppered bottle with 45 ml. Of decinormal caustic soda (N/10 NaOH) solution.

(ii) 3 gram of faeces, stirred thoroughly and were put in the bottle.

(iii) The stopper was fitted to the bottle and shaked thoroughly until all the faecal matters were broken down.

(iv) The mixture were the poured through a fine tea stainer with an aperture of 0.15 mm and the strained fluid was kept in a bowl. The debris left on the filter was discarded.

(v) The filtrate of faeces was well stirred and 0.15 ml of the fluid was taken by means of a graduated pipette.

(vi) The measured quantity of fluid was ejected on to a plain slide and covered with a 22 × 22 mm. cover glass. The whole of the 0.15ml sample of the faecal suspension was examined under low power magnification seen were counted with a hard tally counter.

(vii) The figures obtained from the count (i.e. the total member of eggs present in 0.15ml of diluted faeces) were multiplied by 100 to give the number of eggs per gram of the original faecal samples.

After recording their average EPG just prior treatment different drugs were used as per the experiment plan. The EPG was measured on 7^{th}, 14^{th} and 21^{st}day post drugs treatment for their efficacy.

$$\% \text{ Efficacy} = \frac{\text{Pre-treatment EPG-Post treatment EPG}}{\text{Pre-treatment EPG}} \times 100\%$$

STATISTICAL ANALYSIS:

After generation of data, various statistical analysis was done as per Snedecor and Cochran (1967).

CHAPTER - IV

RESULTS

RESULTS

Gastrointestinal system is very complex and integrated system. It can be disrupted by many infections, which lead to major sign like diarrhoea, dysentery, constipation etc. Fascioliasis and amphistomiasis are the most common diseases in livestock of tropical countries. They cause considerable damage to hepato-biliary system and therefore a great attention is needed to control the parasites causing these diseases. Among various control measures, chemotherapy is most important but due to its indiscriminate use, improper doses and other factors have resulted the drug resistance by the strains. The land of Bihar is full of rivers and districts situated on the bank of these rivers are highly prone towards these diseases in all livestock population including buffaloes. As a result the owners indiscriminate use of anthelmintics practices without any prescribed, suggestion or diagnosis by the veterinarian for control of parasites. So it is necessary to evaluate the degree of frequency of infection of *Fasciola* sp. & *Amphistome* sp. in various districts of Bihar. Keeping in view, the present study was conducted in three river belt districts of Bihar to see the prevalence of fascioliasis and amphistomiasis in buffaloes. Further more, haematological changes were also been recorded in Patna district due to these diseases. Apart from these the efficacy of various anthelmintics viz. albendazole closantel, oxyclozanide + levamisole were evaluated.

STUDY OF PREVALENCE OF *FASCIOLA* SP. AND *AMPHISTOME* SP.:

Altogether 1285 faecal samples of buffaloes were examined from three districts of Bihar. Out of total 450, 420, and 415 samples were from Patna, Khagaria and Lakhisarai districts respectively. The positive samples for fascioliasis as well as amphistomiasis were about 73.77%, 78%, 65.06% in Patna, Khagaria and Lakhisarai districts respectively. The highest percentage of individual infection of *Fasciola* sp. and *Amphistome* sp. was found to be 45.95% and 52.61% respectively in Khagaria district which was followed by Patna (37.55%) for *Fasciola* sp. and 45.33% for *Amphistome* sp., the lowest infection was found in Lakhisarai district where the rate of infection was 34.45% for *Fasciola* sp. and 40.48% for *Amphistome* sp. The range of mixed infection was noted to be 9.33 to 15.00% (Table 1).

The individual infection of *Fasciola* sp. and *Amphistome* sp. and mixed infection of these two infections separately for Patna, Khagaria and Lakhisarai districts have been presented in Table 2, 3 and 4 respectively.

Effect of season on the prevalence of *fasciola* sp. and *amphistome* sp. through examination of faecal samples:

Seasonal prevalence of *Fasciola* sp. and *Amphistome* sp. in buffaloes of Patna district have been depicted in Table 5. 160, 150, and 140 samples were examined in Monsoon, Summer and Winter seasons respectively. The prevalence of *Fasciola* sp. (58.75%) and *Amphistome* sp. (71.25%)was found to be highest in Monsoon, the lowest percent of

infection was recorded in Summer for both *Fasciola* sp. (18.57%) and *Amphistome* sp. (22.85%). However Winter found to be moderately in frequency of infection i.e. 32.66% for *Fasciola* sp. and 39.33% for *Amphistome* sp. The over all seasonal prevalence was found to be highly significant ($p<0.01$) for both the infections and the differences within groups between all seasons were also found to be highly significant ($p<0.01$).

Similarly the seasonal prevalence of *Fasciola* sp. and *Amphistome* sp. for Khagaria district is depicted in Table 6 where the *Fasciola* sp. was found to be 60.00%, 43.07% and 29.16% in the Monsoon, Summer and Winter seasons respectively where as *Amphistome* sp. was recorded to be highest in Monsoon (72.94%) followed by Winter (45.36%) and Summer (31.66%). The overall influence of season was found to be highly significant ($p<0.01$).

Lakhisarai district also showed the similar trend of seasonal influence, which is depicted in Table 7. The highest percent was in Monsoon for *Fasciola* sp. (56.00%) and *Amphistome* sp. (63.33%). The differences within season were also found to be highly significant ($p<0.01$).

Effect of age on the prevalence of *fasciola* sp. and *amphistome* sp. infections in buffalo through examination of faecal samples:

Effect of age on the prevalence of *Fasciola* sp. and *Amphistome* sp. has been presented in Table 8, 9, and 10 for Patna, Khagaria and Lakhisarai district respectively. According to age the buffaloes were divided in three groups and it was found that both *Fasciola*

sp. and *Amphistome* sp. were most prevalent among the buffaloes above three years of age group. The rate of infection due to *Fasciola* sp. was 55.62%, 60.63% and 53.79% in Patna, Khagaria and Lakhisarai districts respectively. Similarly the respective rate of infection was observed to be 68.13%, 71.25% and 62.06% for *Amphistome* sp. The lowest range of infection was observed in the buffalo calves below one year of age where the fascioliasis ranged from 15.86% (Patna) and 27.69% (Khagaria). It was found to be 18.46% in Lakhisarai district. Amphistomiasis was observed to be 22.75%, 31.53% and 19.23% in Patna, Khagaria and Lakhisarai districts. Within the age group of 1-3 years had observed to be moderately infected with *Fasciola* sp. and *Amphistome* sp. in all the three districts. The influence of age was found to be highly significant ($p<0.01$) both in case of fascioliasis and amphistomiasis. The differences within each group age were proven to be highly significant ($p <0.01$).

Effect of sex on the prevalence of *fasciola* sp. and *amphistome* sp. through examination of faecal samples:

The sex wise prevalence of *Fasciola* sp. and *Amphistome* sp. has been presented in Table 11, 12 and 13 for Patna, Khagaria and Lakhisarai districts respectively.

The percentage of infection was higher in female in all the three districts for both *Fasciola* sp. and *Amphistome* sp. except in Patna which has more infection in male with *Fasciola* sp. only. The higher percentage of infection for *Amphistome* sp. were estimated to be 49.27%, 53.63% and 43.04% for Patna, Khagaria and Lakhisarai districts respectively. The infection of *Fasciola* sp. in female was between 37.20%

to 46.82%. The overall percentage of infection was calculated to be non significant for all the 6 observations.

Effect of managemental condition on the prevalence of *Fasciola* sp. and *Amphistomes* sp. through examination of faecal samples:

According to the managemental condition the animals were divided into two groups viz. stallfed and free grazing systems and the data of Patna, Khagaria and Lakhisari districts are presented in the Table 14, 15 and 16 respectively.

In Patna district the free grazing buffaloes show higher degree of infection both in *Fasciola* sp. and *Amphistomes* sp. The percentage of infection was noted to be 46.8 and 52.0% respectively. The stall fed buffaloes had better managemental condition and had reduced incidence of *Fasciola* sp. (26%) and Amphistome sp. (37.50%). The overall influence of managemental practices was found to be highly significant ($p < 0.01$) for both *Fasciola* sp. and *Amphistome* sp. In Lakhisarai the influence of management was found to be non significant for both *Fasciola* sp. and *Amphistome* sp. Where the higher percentage of infection was noted in free grazing system than stallfed conditions.

Unlike to be above observation though Khagaria district showed higher percentage of infection for both in free grazed animals in case of *Fasciola* sp. and *Amphistome* sp. but overall influence of managemental was found to be non-significant in both the cases. The stall fed animals had 45.50% and 42.85% of positive cases for *Fasciola* sp. and *Amphistome* sp. respectively.

Effect of season on the prevalence of *Fasciola* sp. and *Amphistome* sp. through Examination of intestinal scraping in Patna district:

Seasonal prevalence of *Fasciola* sp. and *Amphistome* sp. through examination of intestinal scraping in Patna district depicted in Table 17. The present study on the seasonal prevalence revealed that the higher percentage of infection was found to be Monsoon followed by Winter and Summer seasons. The rate of infection was noted to be 55%, 22% & 32.5% for Monsoon, Summer and Winter seasons respectively in case of *Fasciola* sp. where as 61.66%, 26.00% and 35.00% were noted positive cases for amphistomiasis in Patna district. The overall seasonal influence was found to be highly significant ($p<0.01$). The comparisons among seasonal groups were also noted to be highly significant in Monsoon Vs Summer season for both the infections where as it was found to be only significant ($P < 0.05$) for *Fasciola* sp. Infection in Monsoon Vs Winter season was found to be highly significant ($P<0.01$) for *Amphistome* sp. The difference in rate of infection for both *Fasciola* sp. and *Amphistome* sp. was found to be non-significant in Summer vs Winter season.

HAEMATOLOGICAL CHANGES IN BUFFALOES INFECTED WITH *FASCIOLA* SP. AND *AMPHISTOME* SP.

A total of 30 buffaloes consisting 10 each of healthy, naturally infected with *Fasciola* sp. and naturally infected with *Amphistome* sp. were taken to study the haematological changes.

Haemoglobin:

Mean along with S.E. of healthy and infected buffaloes have been presented in Table 18. Mean ± S.E. value of Hb% were calculated in arcsin value. It was observed that significant decrease in the value of Hb% were observed in both the cases of *Fasciola* sp. (16.97 ± 0.18) and *Amphistome* sp. (15.71 ± 0.27) from control group of buffaloes. Further t value revealed the level of significance between *Fasciola* sp. and *Amphistomes* sp. was statistically non-significant.

Total Erythrocyte Count (TEC):

Mean ± SE of total erythrocyte count in *Fasciola* sp. and *Amphistomes* sp. infected groups were noted to be 4.85 ± 0.12 x 10^6 /mm^3 and 4.86 ± 0.23 x 10^6 /mm^3 respectively. Both the values were significantly ($P < 0.05$) lower than the control group (6.32 ± 0.11 x 10^6 /mm^3). However t value revealed that there were no significant changes within the value of *Fasciola* sp. and *Amphistomes* sp.

Total Leucocyte Count (TLC):

Mean ± S.E of total leucocyte count of control and both the infected groups have been noted to be 7.22 ± 0.06 x 10^3/mm^3, 9.93 ± 0.17 x 10^3/mm^3 and 8.47 ± 0.09 x 10^3/mm^3 respectively. The mean ± S.E value in infected buffaloes of both the groups were significantly ($P<0.01$) found to be higher than the mean value of control. However the difference between *Fasciola* sp. and *Amphistome* sp. infected groups differed significantly ($p < 0.01$) for TLC values.

Packed Cell Volume:

The packed cell volume in all the groups was calculated in Arcsin. It was noted to be 40.92 ± 0.24, 39.45 ± 0.35 and 34.99 ± 0.39 for control, *Fasciola* sp. and *Amphistome* sp. infected groups. It was observed that both the infected groups were significantly ($p<0.01$) decreased from the control. The t value also revealed that the values of *Fasciola* sp. significantly ($p<0.01$) increased from the mean value of PCV in *Amphistome* sp. infected group.

Erythrocyte Sedimentation Rate (ESR):

Mean ± S.E of erythrocyte sedimentation rate value of control (50.56 ± 1.39) and infected groups (81.33 ± 1.17 and 73.57 ± 1.57) have been presented in Table 18. Mean ± S.E values of ESR for both the infected groups were significantly ($p<0.01$) higher than the control.

Mean values of ESR of *Fasciola* sp. group were also found to be significantly ($p < 0.01$) higher than the *Amphistome* sp. infected group.

Diffeential Count (DC) of White Blood Cells:

All the values of differential count were also evaluated in arcsin. The mean value of different cell percentage along with S.E. depicted in table 19.

Neutrophils:

It was found significantly ($p<0.01$) lower (30.10 ± 0.55) value of neutrophil in *Fasciola* sp. infected group than the control (36.84 ± 0.56). However the *Amphistome* sp. infected group was significantly ($p<0.01$) higher values (33.38 ± 0.41) than the *Fasciola* sp. infected group but it was found to be significantly ($p<0.01$) lower than the control.

Lymphocytes:

The estimates of mean ± S.E. of lymphocyte percentage were found to be 50.50 ± 0.56, 54.77 ± 0.55 and 52.42 ± 0.49 respectively of control, *Fasciola* sp. and *Amphistome* sp. infected groups. It was found that both the infected groups differ significantly ($p<0.01$) higher than the control. The mean value of *Fasciola* sp. infected group was also observed to be significantly ($p<0.01$) higher than the *Amphistome* sp. infected group.

Monocytes:

It has been observed that the mean values of monocyte count in *Fasciola* sp. (9.92 ± 1.43) infected groups did not differ significantly with control and *Amphistome* sp. (9.65 ± 0.58) infected groups. Whereas *Amphistome* sp. infected group differ significantly ($p < 0.01$) from control.

Eosinophils:

Significantly ($p<0.01$) higher values were noted in both *Fasciola* sp. (12.13 ± 0.88) infected group and *Amphistome* sp. (11.43 ± 0.55) infected group than the control (9.40 ± 0.71).

Basophils:

Basophils were not found.

CHEMOTHERAPEUTIC TRIALS:

Fascioliasis and Amphistomiasis are the two most important helminthic infections of large ruminants including buffaloes. Economic losses due to reduce production and fatality are enormous by these two diseases. To overcome these losses a number of control measures are used. The chemotherapeutic agents are effectively used to control the flukes.

Some of these are broad-spectrum anthelmintics and effective against a number of mature and immature stages of flukes. They are being used indiscriminately without taking into consideration its toxicity and biodegradability by farmers, especially in suburban and rural areas. Many of these drugs which are extensively used in recent times revealed drug resistance and toxicity in animals. Reduced efficacy of drug also causing emergence of drug resistant. Therefore in the present investigation, a detail study of three drugs viz. albendazole, closantel and oxyclozanide + levamisole were carried out in three districts. These are established wide range anthelmintic and readily available were undertaken for present trial to ascertain their comparative efficacy and also to evaluate their effectiveness in three river belt districts viz. Patna, Khagaria and Lakhisarai. The doses of drugs were administered-

Albendazole - 10 mg/kg body wt.

Closantel - 7.5 mg/kg body wt.

Oxyclozanide + levamisole – 22.5 mg/kg body wt.

EFFICACY OF ANTHELMINTICS AGAINST *FASCIOLA* SP. AND *AMPHISTOME* SP. IN BUFFALOES IN PATNA DISTRICT

Efficacy against *Fasciola* sp.:

Mean ± S.E. of average number of eggs per gram of faeces (E.P.G.) and percent efficacy of different anthelmintics viz. albendazole, closantel and oxyclozanide + levamisole against *Fasciola* sp. in buffaloes in Patna district at different days of interval is presented in Table 20. The analysis of variance for the effect of albendazole, closantel and

oxyclozanide + levamisole against *Fasciola* sp. in buffaloes in Patna district at different days of interval is presented in Table 21.

The estimates of E.P.G. on 0 day in control, albendazole, closantel and oxyclozanide + levamisole treated groups were recorded to be 377.2 ± 14.53, 360.6 ± 14.15, 360.2 ± 14.15 and 361.0 ± 16.02 respectively. On 7 day post-treatment the average estimates of E.P.G. in albendazole, closantel and oxyclozanide + levamisole groups have been reckoned to be decreased by 215.6, 301.8 and 338.6 respectively from mean E.P.G. values of the respective groups on 0 day. The analysis of variance revealed significant ($p<0.01$) difference in mean values of E.P.G. on 7 day post treatment. The mean E.P.G. values in albendazole, closantel and oxyclozanide + levamisole groups were observed to be significantly ($p<0.05$) decreased from the mean value of control by 267.6, 354.2 and 390.2 respectively. The mean E.P.G. value of oxyclozanide + levamisole group was found to be significantly ($p<0.05$) decreased from mean E.P.G. value of albendazole group by 122.6. The mean E.P.G. value of this (oxyclozanide + levamisole) group was though lesser than the closantel group however, the difference was not significant and the mean E.P.G. values between albendazole and closantel groups did not differ significantly. The efficacy percentage in oxyclozanide + levamisole group was estimated to be 93.80 which was higher by 10.01 and 34.01 than the closantel and albendazole groups respectively.

The average estimates of EPG on 14 day post treatment were observed to be further decreased in all the groups except control in which the mean E.P.G. value was found to be increased. The mean E.P.G. values

in albendazole and closantel groups had recorded to be decreased from mean values on 0 day of the respective groups by 267.2 and 346.6. Further, it is stated that no eggs of *Fasciola* sp. could be identified in oxyclozanide + levamisole group. The analysis of variance (Table 21) revealed significant ($p<0.01$) difference in mean values of E.P.G. between the groups. The mean E.P.G. values of both albendazole and closantel groups were estimated to be significantly ($p<0.05$) lesser by 351.6 and 431.4 respectively from the control. It was also reckoned that mean E.P.G. value of closantel group was significantly ($p<0.05$) lesser than the E.P.G. value of albendazole group by 79.8. The percent efficacies in oxyclozanide + levamisole, closantel and albendazole groups were estimated to be 100.00, 96.22 and 74.10 respectively.

The trend on 21 day post-treatment was similar to that of estimates observed on 14 day. The analysis of variance revealed significant differs of mean E.P.G. values on 21 day post-treatment. The average estimates of E.P.G. in albendazole and closantel groups were significantly ($p<0.05$) lesser than the control by 423.0 and 462.2 and mean E.P.G. value of closantel group was significantly ($p<0.05$) lesser by 39.2 from mean value of albendazole group. The efficacy percentages in oxyclozanide + levamisole, closantel and albendazole groups were estimated to be 100.00, 99.94 and 88.57 respectively. The efficacy percent in closantel group was higher than the albendazole group by 11.37.

Efficacy against *amphistome* sp.:

Mean ± S.E. of average number of eggs per gram of faeces (E.P.G.) and percent efficacy of different anthelmintics viz. albendazole,

closantel and oxyclozanide + levamisole against *Amphistome* sp. in buffaloes in Patna district at different days of interval is depicted in Table 22. The analysis of variance for the effect of albendazole, closantel and oxyclozanide + levamisole against *Amphistome* sp. in buffaloes in Patna district at different days of interval is depicted in Table 23.

The estimates of E.P.G. on 0 day in control, albendazole, closantel and oxyclozanide + levamisole groups were noted to be 418.2 ± 6.07, 446.4 ± 18.22, 432.6 ± 16.90 and 449.0 ± 22.93 respectively. On 7 day post-treatment the average estimates of E.P.G. in albendazole, closantel and oxyclozanide + levamisole groups have been recorded to be decreased by 288.6, 300.6 and 355.0 respectively from mean E.P.G. values of the respective groups on 0 day. The analysis of variance revealed significant difference ($p<0.01$) in mean values of E.P.G. on 7 day post-treatment. The mean E.P.G. values in albendazole, closantel and oxyclozanide + levamisole groups were observed to be significantly ($p<0.05$) decreased from the mean value of control by 261.0, 304.8 and 342.8 respectively. The mean E.P.G. values of closantel and oxyclozanide + levamisole groups were found to be significantly ($p<0.05$) decreased from the mean E.P.G. value of albendazole group by 43.8 and 81.8 respectively. The efficacy percentage was estimated to be 74.06 in oxyclozanide + levamisole which was higher by 4.57 and 13.44 than the closantel and albendazole groups respectively.

The average estimates of E.P.G. on 14 day post-treatment had observed to be further decreased in all the groups except control in which the mean E.P.G. value had found to be increased. The mean E.P.G. values

in albendazole, closantel and oxyclozanide + levamisole groups were recorded to be decreased by 351.4, 368.2 and 412.0 from mean values of respective groups on 0 day. The analysis of variance (Table 23) revealed significant ($p<0.01$) difference in mean values of E.P.G. between the groups on 14 day post-treatment. The mean E.P.G. values of albendazole, closantel and oxyclozanide groups were observed to be significantly ($p<0.05$) decreased from the mean E.P.G. value of control by 358.2, 388.8 and 416.2 respectively. The mean E.P.G. value of closantel and oxyclozanide + levamisole groups were found to be significantly ($p<0.05$) decreased from the mean E.P.G. values of albendazole group by 30.6 and 58 respectively. The percent efficacies in oxyclozanide + levamisole, closantel and albendazole groups were noted to be 91.76, 85.11 and 78.72.

The average estimates of E.P.G. on 21 day post-treatment in albendazole, closantel and oxyclozanide + levamisole groups have been recorded to be decreased by 397.6, 418.4 and 445.2 respectively from the mean values of the respective groups on 0 day. The analysis of variance revealed significant ($p<0.01$) difference in mean E.P.G. values of closantel and oxyclozanide groups were found to be significantly ($p<0.05$) decreased by 34.6 and 44.4 from mean E.P.G. value in albendazole group respectively and the mean E.P.G. values between oxyclozanide + levamisole and closantel groups did not differ significantly. The percentage efficacies in oxyclozanide + levamisole, closantel and albendazole groups were estimated to be 99.15, 96.72 and 89.07 respectively.

EFFICACY OF ANTHELMINTICS AGAINST *FASCIOLA* SP. AND *AMPHISTOME* SP. IN BUFFALOES IN KHAGARIA DISTRICT

Efficacy against *Fasciola* sp.:

Mean ± S.E. of average number of eggs per gram of faeces (E.P.G.) and percent efficacy of different anthelmintics viz. albendazole, closantel and oxyclozanide + levamisole against *Fasciola* sp. in buffaloes in Khagaria district at different days of interval is presented in Table 24. The analysis of variance for the effect of albendazole, closantel and oxyclozanide + levamisole against *Fasciola* sp. in buffaloes at different days of interval in Khagaria district is depicted in Table 25.

The estimates of E.P.G. on 0 day in control, albendazole, closantel and oxyclozanide + levamisole groups were noted to be 496.2 ± 36.43, 458.0 ± 23.92, 410.6 ± 12.11 and 414.6 ± 32.83 respectively. On 7 day post-treatment the average estimates of E.P.G. in albendazole, closantel and oxyclozanide + levamisole groups have been recorded to be decreased by 320.2, 391.4 and 410.6 respectively from mean E.P.G. values of the respective groups on 0 day. The analysis of variance (Table 25) revealed significant ($p<0.01$) in mean values of E.P.G. on 7 day post-treatment. The mean E.P.G. values in albendazole, closantel and oxyclozanide + levamisole groups were observed to be significantly ($p<0.05$) decreased by 390.4, 509.0 and 524.2 from the mean E.P.G. value of control respectively. The mean E.P.G. values of closantel and oxyclozanide groups were found to be significantly ($p<0.05$) decreased from mean E.P.G. value of albendazole group by 118.6 and 133.8 respectively. However, mean E.P.G. values between closantel and

oxyclozanide + levamisole groups did not differ significantly. The efficacy percentage of oxyclozanide + levamisole group was estimated to be 99.04 which was higher by 13.58 and 29.13 than closantel and albendazole group.

On 14 day post-treatment the mean E.P.G. value in albendazole, closantel and oxyclozanide + levamisole groups were recorded to be decreased by 418.4, 406.2 and 413.8 respectively from the mean E.P.G. values of respective groups on 0 day. The analysis of variance (Table 25) revealed significant ($p<0.05$) difference in mean E.P.G. values between groups on 14 day post-treatment. The mean E.P.G. values in albendazole, closantel and oxyclozanide + levamisole groups were observed to be significantly ($p<0.05$) decreased from the mean value of control by 522.4, 557.6 and 561.2 respectively. The mean E.P.G. value of oxyclozanide + levamisole group was found to be significantly ($p<0.05$) decreased by (39.6-.8) from the mean E.P.G. value of albendazole group. The mean E.P.G. value of closantel group was observed to be non-significant difference from E.P.G. value of albendazole group. The percent efficacies were noted to be 99.81, 98.69 and 91.35 in oxyclozanide + levamisole, closantel and albendazole groups.

On 21 day post-treatment the mean E.P.G. values of albendazole group was found to be decreased by 452 from value of E.P.G. respective group on 0 day. No eggs could be revealed in closantel and oxyclozanide + levamisole treated groups. The analysis of variance (Table 25) revealed significantly difference in mean E.P.G. values between groups on 21 day post-treatment the mean E.P.G. values of albendazole,

closantel and oxyclozanide + levamisole groups have been estimated to be decreased significantly ($p< 0.05$) from the control. The percent efficacies were recorded to be 100.00, 100.00 and 98.68 in oxyclozanide+ levamisole, closantel and albendazole group.

Efficacy against *Amphistome* sp.:

Mean ± S.E. of average number of eggs per gram of faeces (E.P.G.) and percent efficacy of different anthelmintics namely albendazole, closantel and oxyclozanide + levamisole against *Amphistome* sp. in buffaloes in Khagaria district at different days of interval is presented in Table 26. The analysis of variance for the effect of albendazole, closantel and oxyclozanide + levamisole against *Amphistome* sp. in buffaloes at different days of interval in Khagaria district is presented in Table 27.

The estimates of E.P.G. on 0 day in control, albendazole, closantel and oxyclozanide groups were recorded to be 540.62 ± 13.09, 547.8 ± 19.62, 548.4 ± 19.34 and 543 ± 23.26. On 7 day post-treatment the mean E.P.G. values in albendazole, closantel and oxyclozanide + levamisole groups have been counted to be decreased from mean values of respective groups on 0 day by 288.2, 396.2 and 461.2 respectively. The analysis of variance revealed significant difference ($p<0.01$) in mean E.P.G. values on 7 day post-treatment. The mean E.P.G. values in albendazole, closantel and oxyclozanide + levamisole groups were observed to be significantly ($p<0.05$) decreased from the mean E.P.G. of control by 302.4, 409.8 and 480.2 respectively. The mean E.P.G. values of closantel and oxyclozanide groups had significantly ($p<0.05$) decreased by

107.4 and 177.8 from mean E.P.G. value of albendazole group respectively. The percent efficacies in oxyclozanide were 84.94, which was higher by 12.69 and 32.33 than the closantel and albendazole.

The trend on 14 day post-treatment was similar to that of estimates observed on 7 day. The mean E.P.G. value in albendazole, closantel and oxyclozanide + levamisole groups have been recorded to be decreased by 417.4, 467.2 and 525.2 respectively from mean E.P.G. value of respective groups on 0 day. The analysis of variance (Table 27) revealed significant difference ($p<0.01$) mean E.P.G. values on 14 day post-treatment.

The mean E.P.G. value in albendazole, closantel and oxyclozanide + levamisole groups were found to be decreased significantly ($p<0.05$) from the control by 451.2, 500.4 and 563.8 respectively. The mean E.P.G. values of closantel and oxyclozanide + levamisole groups were observed to be significantly ($p<0.05$) decreased by 49.2 and 112.6 respectively. The percent efficacies in oxyclozanide + levamisole, closantel and albendazole were noted to be 96.72, 85.19 and 76.20 respectively.

Similar trend was also observed on 21 day post-treatment. The mean E.P.G. value in albendazole and closantel groups were found to be decreased by 479.0 and 511.2 from mean values of respective groups on 0 day. No eggs could be recorded in oxyclozanide + levamisole treated group. The analysis of variance (Table 27) revealed significant difference ($p<0.01$) of mean E.P.G. values on 21 day post-treatment. The mean E.P.G. values in albendazole and closantel groups had found to be

significantly ($p<0.05$) decreased by 525.0 and 556.6 respectively from mean value of control. The mean E.P.G. value of closantel group had found to be significantly ($p<0.05$) decreased by (31.60) from mean E.P.G. value of albendazole group. The percentage efficacies in oxyclozanide + levamisole, closantel and albendazole groups were recorded to be 100, 93.22 and 87.57.

EFFICACY OF ANTHELMINTICS AGAINST *FASCIOLA* SP. AND *AMPHISTOME* SP. IN BUFFALOES IN LAKHISARAI DISTRICT

Efficacy against *Fasciola* sp.:

Mean ± S.E. of average number of eggs per gram of faeces (E.P.G.) and percent efficacy of different anthelmintics viz. albendazole, closantel and oxyclozanide + levamisole against *Fasciola* sp. in buffaloes in Lakhisarai district at different days of interval is presented in Table 28. The analysis of variance for the effect of albendazole, closantel and oxyclozanide + levamisole against *Fasciola* sp. in buffaloes at different days of interval is presented in Table 29.

The estimates of E.P.G. on 0 day in control, albendazole, closantel and oxyclozanide + levamisole groups were noted to be 226.8 ± 13.20, 208.6 ± 17.57, 226.0 ± 19.32 and 245.20 ± 22.30 respectively. On 7 day post-treatment the average estimates of E.P.G. in albendazole, closantel and oxyclozanide + levamisole groups were recorded to be decreased by 82.4, 146.4 and 220.8 respectively from mean E.P.G. values of the respective groups on 0 day. The analysis of variance revealed significant difference ($p<0.01$) in mean E.P.G. values on 7 day post treatment. The mean E.P.G. values in albendazole, closantel and

oxyclozanide + levamisole groups were observed to be significantly ($p<0.05$) decreased from the mean value of control by 122.0, 168.6 and 223.8 respectively. The mean E.P.G. value of closantel and oxyclozanide + levamisole group were found to be significantly ($p<0.05$) decreased from mean E.P.G. value of albendazole group by 47 and 101.8 respectively. The percent efficacy of oxyclozanide + levamisole group was 90.04 which was higher by 25.27and 50.54 than the closantel and albendazole groups respectively.

On 14 day post-treatment the mean E.P.G. values in albendazole, closantel and oxyclozanide + levamisole groups were found to be decreased by 125.6, 208.0 and 241.8 respectively from mean values of respective groups on 0 day. The analysis of variance revealed (table 28) revealed significant difference ($p<0.01$) in mean E.P.G. values between the groups on 14 day post-treatment. The mean E.P.G. value of oxyclozanide + levamisole group was found to be significantly ($p<0.05$) decreased by 65 from mean E.P.G. value of albendazole group. Whenever it did not differ significantly from mean E.P.G. value of closantel group. The percent efficacies in oxyclozanide + levamisole, closantel and albendazole groups were recorded to be 98.85, 91.94 and 60.21.

The similar trend was also observed on 21 day post-treatment. The mean E.P.G. values in albendazole, closantel, and oxyclozanide + levamisole have been reckoned to be decreased from mean E.P.G. values of respective groups on 0 day by 187.4, 224.4 and 244.6 respectively. The analysis of variance revealed significant difference ($p<0.01$) in mean E.P.G. values between the groups on 21 day post-treatment. The mean

E.P.G. values in albendazole, closantel, and oxyclozanide + levamisole groups have been noted to be significantly ($p<0.05$) decreased by 279.4, 279.4 and 300.0 respectively from mean E.P.G. value of control. The mean E.P.G. value of oxyclozanide + levamisole was found to be significantly ($p<0.05$) decreased from mean E.P.G. value of albendazole by 20.6 however it did not significantly differ from mean E.P.G. value of closantel group. The percent efficacies in albendazole, closantel and oxyclozanide + levamisole groups were found to be 89.83, 99.29 and 99.75 respectively.

Efficacy against *Amphistome* sp.:

Mean ± S.E. of average number of eggs per gram of faeces (E.P.G.) and percent efficacy of different anthelmintics namely albendazole, closantel and oxyclozanide + levamisole against *Amphistome* sp. in buffaloes in Lakhisarai district at different days of interval is depicted in Table 30. The analysis of variance for the effect of albendazole, closantel and oxyclozanide + levamisole against *Amphistome* sp. in buffaloes at different days of interval in Khagaria district is depicted in Table 31.

The estimates of E.P.G. on 0 day in control, albendazole, closantel and oxyclozanide groups were recorded to be 324.8 ± 9.03, 378.2 ± 8.79, 396.2 ± 5.70 and 376.2 ± 10.94 respectively. On 7 day post-treatment the average estimates of E.P.G. in albendazole, closantel and oxyclozanide + levamisole treated groups were recorded to be decreased from mean E.P.G. value of respective groups on 0 day by 179.4, 305.2 and 358.0 respectively. The analysis of variance (Table 31) revealed

significant difference ($p<0.01$) in mean E.P.G. values between groups on 7 day post-treatment. The estimates of mean E.P.G. values in albendazole, closantel and oxyclozanide + levamisole groups were reckoned to be significantly ($p<0.05$) decreased from mean E.P.G. of control by 153.4, 261.2 and 334.0 respectively. The mean E.P.G. values of closantel and oxyclozanide groups were found to be significantly ($p<0.05$) decreased from mean E.P.G. value of albendazole group by 107.8 and 180.6. The percent efficacy of oxyclozanide + levamisole group was recorded to be 95.16 which was higher by 18.13 and 47.72 than closantel and albendazole groups.

The average estimates of E.P.G. on 14 day post-treatment were observed to be further decreased in all the groups except control, the mean E.P.G. values in albendazole, closantel, and oxyclozanide + levamisole groups were recorded to be decreased by 268.4, 365 and 374.2 respectively from mean E.P.G. values of respective groups on 0 day. The analysis of variance (Table 31) revealed significant difference ($p<0.01$) in mean E.P.G. values between groups on 14 day post-treatment. The estimates of mean E.P.G. values in albendazole, closantel and oxyclozanide + levamisole groups were recorded to be decreased significantly ($p<0.05$) by 263.8, 342.4 and 371.6 respectively from the mean E.P.G. value of control. The mean E.P.G. values of oxyclozanide + levamisole and closantel groups were found to be significantly decreased by 78.6 and 107.8 from mean E.P.G. value of albendazole group. The percent efficacies were noted to be 99.47, 92.13 and 70.97 in oxyclozanide + levamisole, closantel and albendazole groups respectively.

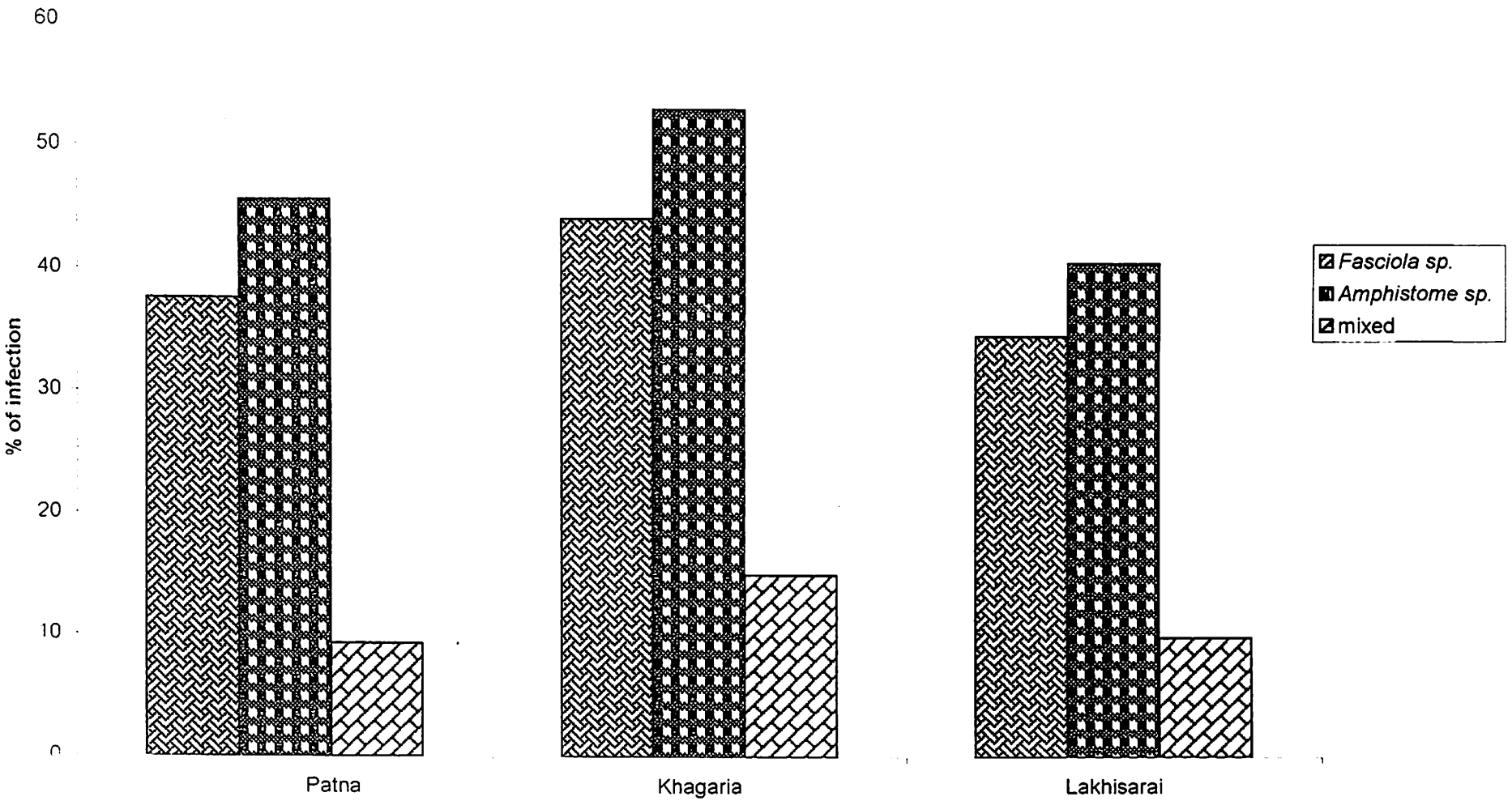
The trend on 21 day post-treatment was similar to that of estimates observed on 14 day. The E.P.G. values in albendazole and closantel groups were found to be decreased by 328.2 and 394.4 from mean E.P.G. values of respective groups on 0 day. No eggs could be recorded in oxyclozanide + levamisole treated group. The analysis of variance (Table 31) revealed significant differ ($p<0.01$) in mean E.P.G. values between groups on 21 day post-treatment. The estimates of mean E.P.G. value in albendazole and closantel groups had observed to be significantly ($p<0.05$) decreased by 339.8 and 388.0 respectively from control. The mean E.P.G. value of closantel group had found to be significantly decreased by 48.2 from mean E.P.G. value of albendazole group. The percent efficacies in oxyclozanide + levamisole, closantel and albendazole group were noted to be 100, 99.55 and 86.78 respectively.

Table-1

Infection of *Fasciola* sp. and *Amphistome* sp. in buffaloes in three different river belt districts of Bihar through examination of faecal samples

Place	No. of samples examined	No. of samples found positive for *Fasciola* sp. & *Amphistome* sp.	Overall percentage of infection	No. of samples found positive for *Fasciola* Sp.	Percentage of infection	No. of samples found positive for *Amphistome* Sp.	Percentage of infection	Mixed infection	Percentage of infection
Patna	450	332	73.77	169	37.55	205	45.56	42	9.33
Khagaria	420	328	78.09	193	45.95	221	52.62	63	15.00
Lakhisarai	415	270	65.06	143	34.46	168	40.48	41	9.88

Fig. 1: Infection of *Fasciola* sp. and *Amphistome* sp. in buffaloes in three river belt districts of Bihar through examination of faecal samples.



Mixed infection of *Fasciola* sp. and *Amphistome* sp. in buffaloes in Patna, Khagaria and Lakhisarai districts

Table-2

Patna	*Amphistome Sp.*	*Fasciola Sp.*	
Amphistome Sp.	163	42	205
Fasciola Sp.	42	127	169
Total	205	169	

Table-3

Khagaria	*Amphistome Sp.*	*Fasciola Sp.*	
Amphistome Sp.	158	63	221
Fasciola Sp.	63	130	193
Total	221	193	

Table-4

Lakhisarai	*Amphistome Sp.*	*Fasciola Sp.*	
Amphistome Sp.	127	41	168
Fasciola Sp.	41	102	143
Total	168	143	

N.B. Diagonal figure represent the number of parasites which occur singly

Table-5

Seasonal Prevalence of *Fasciola Sp.* and *Amphistome Sp.* in buffaloes of Patna district through examination of faecal samples

		Fasciola Sp.			*Amphistome* sp.		
Season	No. of faecal samples Examine	No. of samples found positive	Percentage of infection	$\chi^2$2d.f	No. of samples found positive	Percentage of infection	$\chi^2$2d.f
Monsoon	160	94	58.75		114	71.25	
Winter	150	49	32.67	53.67**	59	39.33	74.00**
Summer	140	26	18.57		32	22.85	
Total	450	169			205		

Comparison within group χ^2 1d.f value

Monsoon Vs Summer
50.22**

Monsson Vs Winter
21.19**

Summer Vs Winter
7.50**

Monsoon Vs Summer
69.99**

Monsson Vs Winter
31.97**

Summer Vs Winter
9.12**

**- Significant at P<0.01

NS - Non-Significant

Fig. 2: Seasonal prevalence of *Fasciola* sp. and *Amphistome* sp. in buffaloes of Patna district through examination of faecal samples.

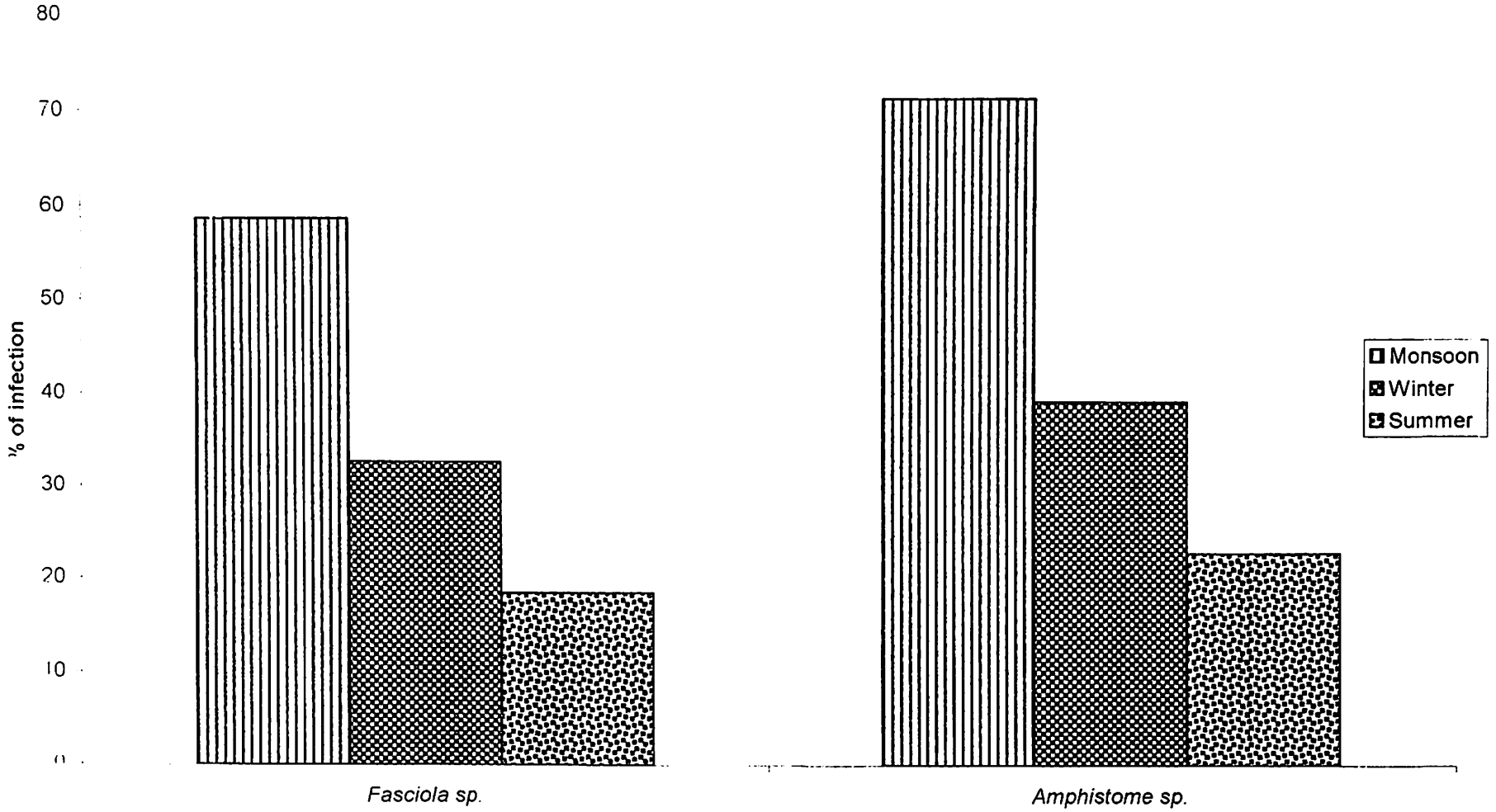


Table-6

Seasonal Prevalence of *Fasciola Sp.* and *Amphistome Sp.* in buffaloes of Khagaria district through examination of faecal samples:-

		Fasciola Sp.			*Amphistome* sp.		
Season	No. of faecal samples examined	No. of samples found positive	Percentage of infection	$\chi^2$2d.f	No. of samples found positive	Percentage of infection	$\chi^2$2d.f
Monsoon	170	102	60.00		124	72.94	
Winter	130	56	43.08	27.37**	59	45.38	51.99**
Summer	120	35	29.17		38	31.67	
Total	420	193			221		

Comparison within group χ^2 1d.f value

Monsoon Vs Summer
26.83**

Monsson Vs Winter
8.46**

Summer Vs Winter
5.21*

Monsoon Vs Summer
49.37**

Monsson Vs Winter
23.51**

Summer Vs Winter
4.94*

*-Significant at $p<0.05$

**-Significant at $P<0.01$

NS-Non Significant

Fig. 3: Seasonal prevalence of *Fasciola* sp. and *Amphistome* sp. in buffaloes of Khagaria district through examination of faecal samples.

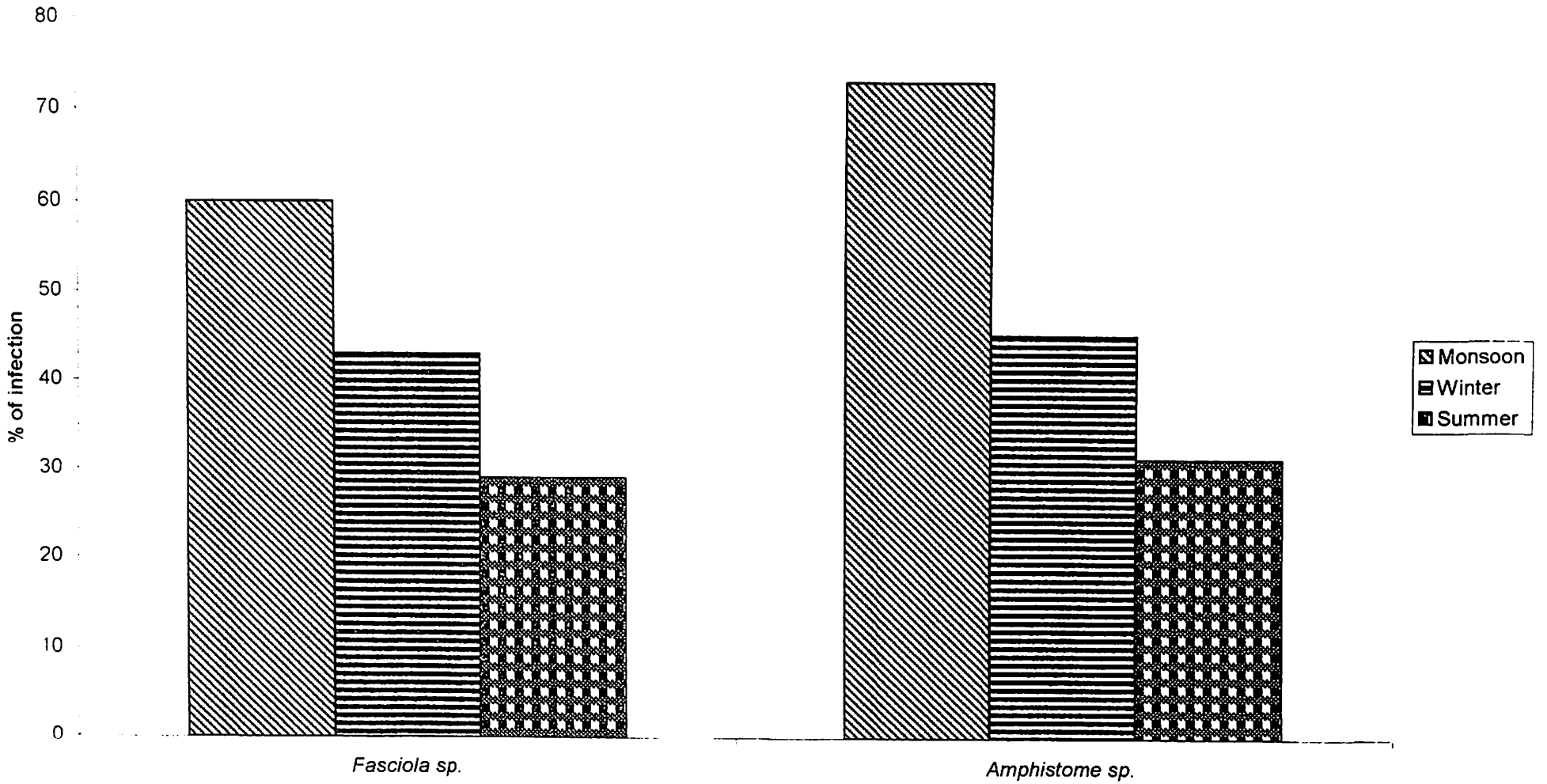


Table-7

Seasonal Prevalence of *Fasciola Sp.* and *Amphistome Sp.* in buffaloes of Lakhisarai district through examination of faecal samples

		Fasciola Sp.			*Amphistome* sp.		
Season	No. of faecal samples examined	No. of samples found positive	Percentage of infection	$\chi^2$2d.f	No. of samples found positive	Percentage of infection	$\chi^2$2d.f
Monsoon	150	84	56.00	56.43**	95	63.33	56.17**
Winter	135	19	14.07		28	20.74	
Summer	130	40	30.77		45	34.62	
Total	415	143			168		

Comparison within group χ^2 1d.f value

Monsoon Vs Summer
54.11**

Monsson Vs Winter
17.96**

Summer Vs Winter
10.66*

Monsoon Vs Summer
52.54**

Monsson Vs Winter
22.97**

Summer Vs Winter
6.38*

*-significant at P<0.05

**-Significant at P<0.01

NS-Non Significant

Fig. 4: Seasonal prevalence of *Fasciola* sp. and *Amphistome* sp. in buffaloes of Lakhisarai district through examination of faecal samples.

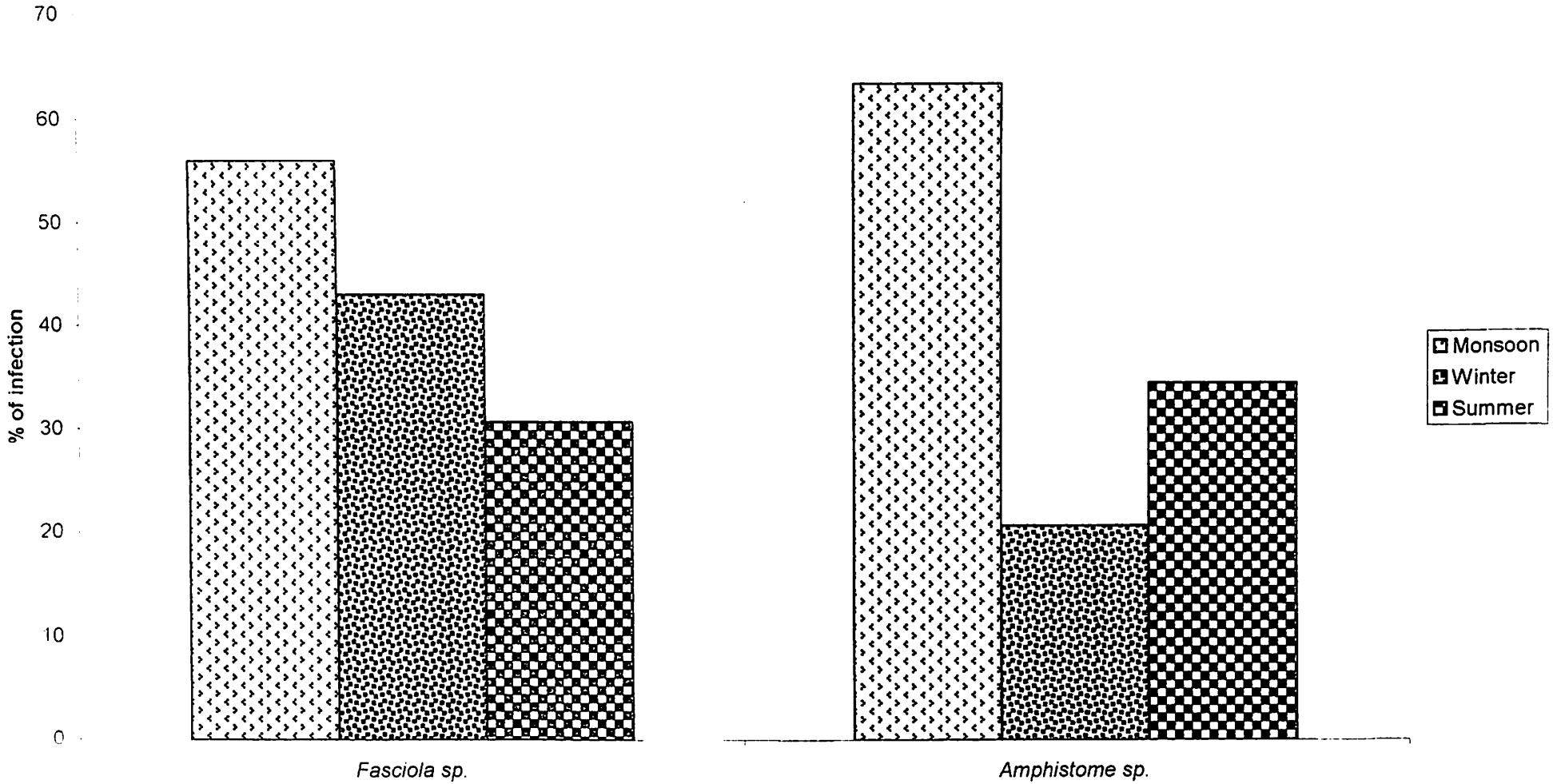


Table-8

Prevalence of *Fasciola Sp.* and *Amphistome Sp.* infection in different age group of buffaloes of Patna district through faecal samples examination:-

		Fasciola Sp.			*Amphistome* sp.		
Age group	No. of faecal samples examined	No. of samples found positive	Percentage of infection	$\chi^2$2d.f	No. of samples found positive	Percentage of infection	$\chi^2$2d.f
< 1 year	145	23	15.86		33	22.76	
1-3 year	145	57	39.31	51.56**	63	43.45	63.49**
> 3 year	160	89	55.63		109	68.13	
Total	450	169			205		

Comparison within group χ^2 1d.f value

< 1 year Vs 1-3 year	< 1 year Vs 1-3 year
19.95**	14.01**
< 1 year Vs > 3 year	< 1 year Vs > 3 year
51.75**	62.91**
1-3 year Vs > 3 year	1-3 year Vs > 3 year
8.11**	18.83**

** -Significant at P<0.01

NS - Non-Significant

Fig. 5: Prevalence of *Fasciola* sp. and *Amphistome* sp. infection in different age group of buffaloes of Patna district through faecal samples examination.

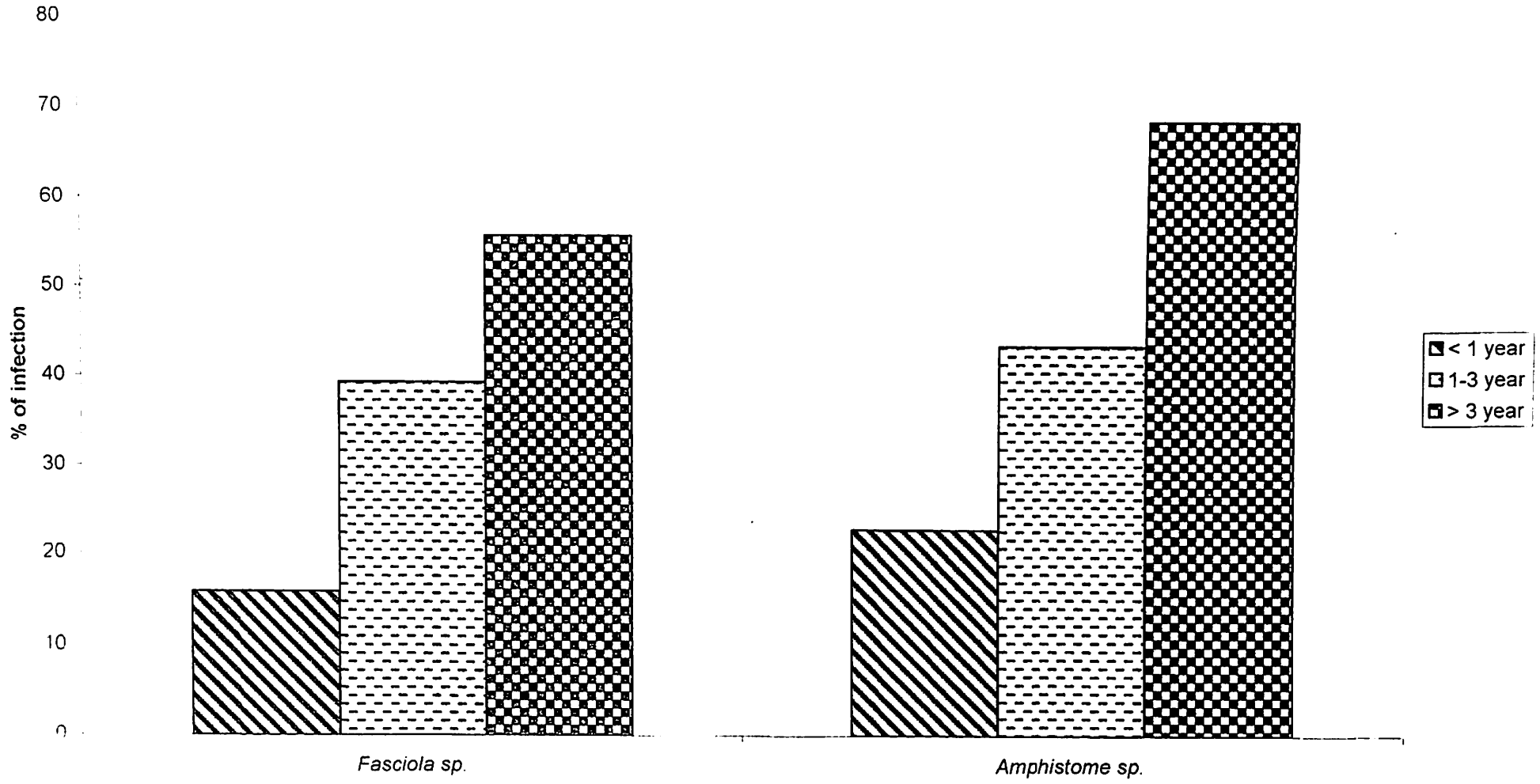


Table-9

Prevalence of *Fasciola Sp.* and *Amphistome Sp.* infection in different age group of buffaloes of Khagaria district through faecal samples examination

		Fasciola Sp.			*Amphistome* sp.		
Age group	No. of faecal samples examined	No. of samples found positive	Percentage of infection	$\chi^2$2d.f	No. of samples found positive	Percentage of infection	$\chi^2$2d.f
< 1 year	130	36	27.69		41	31.54	
1-3 year	130	60	46.15	31.20**	66	50.77	45.59**
> 3 year	160	97	60.63		114	71.25	
Total	420	193			221		

Comparison within group χ^2 1d.f value

< 1 year Vs 1-3 year
9.51**

< 1 year Vs > 3 year
31.33**

1-3 year Vs 1 year
6.04*

< 1 year Vs 1-3 year
9.92**

< 1 year Vs > 3 year
45.45**

1-3 year Vs 1 year
12.77**

*-Significant at P<0.05

**-Significant at P<0.01

NS-Non-Significant

Fig. 6: Prevalence of *Fasciola* sp. and *Amphistome* sp. infection in different age group of buffaloes of Khagaria district through faecal samples examination.

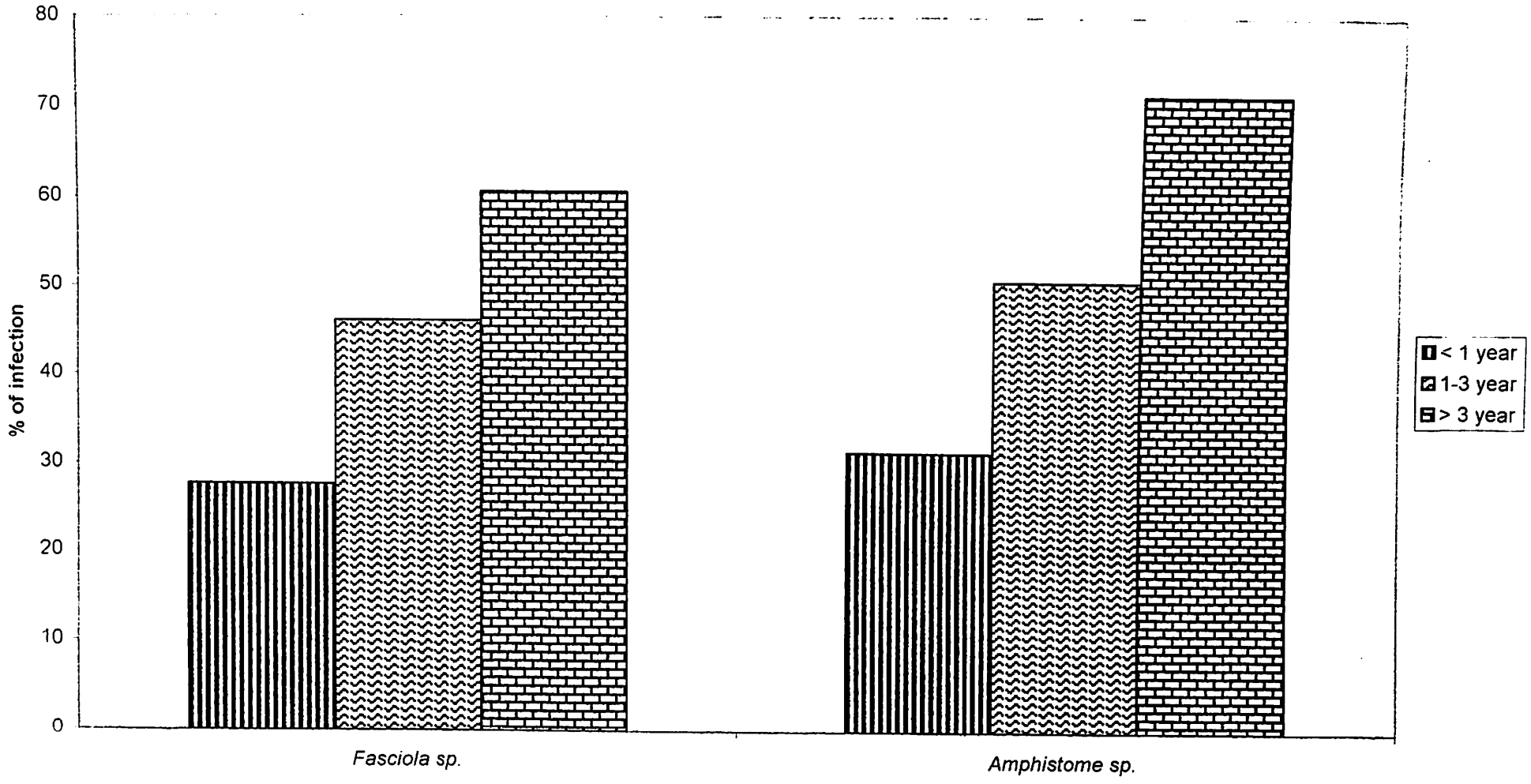


Table-10

Prevalence of *Fasciola Sp.* and *Amphistome Sp.* infection in different age group of buffaloes of Lakhisarai district through faecal samples examination:-

		Fasciola Sp.			*Amphistome* sp.		
Age group	No. of faecal samples examined	No. of samples found positive	Percentage of infection	$\chi^2$2d.f	No. of samples found positive	Percentage of infection	$\chi^2$2d.f
< 1 year	130	24	18.46		25	19.23	
1-3 year	140	41	29.29	40.29**	53	37.86	52.80**
> 3 year	145	78	53.79		90	62.07	
Total	415	143			168		

Comparison within group χ^2 1d.f value

< 1 year Vs 1-3 year
4.32**

< 1 year Vs > 3 year
36.67**

1-3 year Vs > 3 year
17.59**

< 1 year Vs 1-3 year
11.38**

< 1 year Vs > 3 year
51.69**

1-3 year Vs > 3 year
16.70**

**-Significant at P<0.01

NS-Non-Significant

Fig. 7: Prevalence of *Fasciola* sp. and *Amphistome* sp. infection in different age group of buffaloes of Lakhisarai district through faecal samples examination.

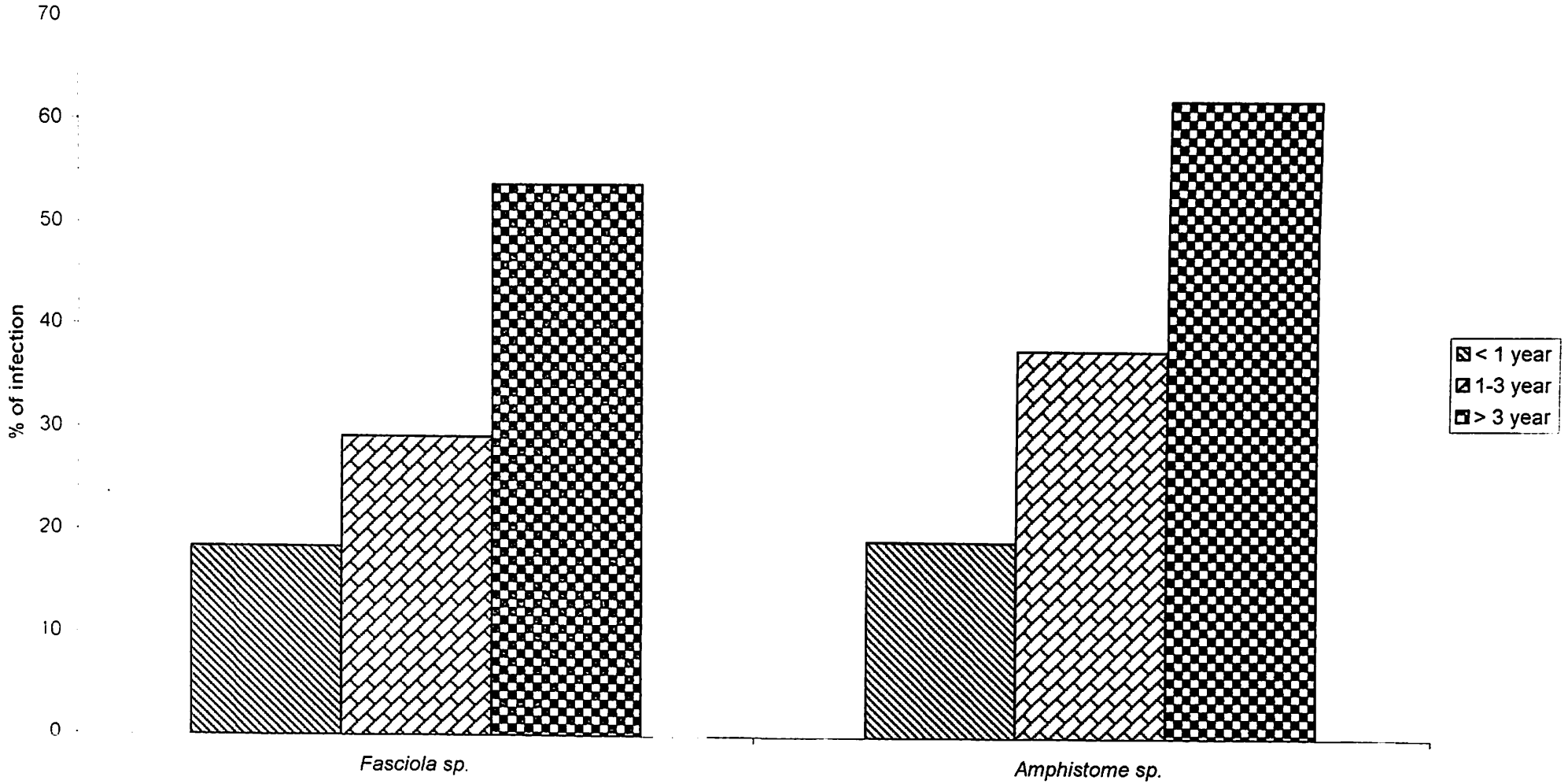


Table-11

Influence of Sex on the Prevalence of *Fasciola Sp.* and *Amphistome Sp.* infection in buffaloes of Patna district through faecal samples examination

		Fasciola Sp.			*Amphistome* sp.		
Sex	No. of faecal samples examind	No. of samples found positive	Percentage of infection	$\chi^2$1d.f	No. of samples found positive	Percentage of infection	$\chi^2$1d.f
Male	200	76	38.00		82	41.00	
Female	250	93	37.20	0.03^{NS}	123	49.20	3.01^{NS}
Total	450	169			205		

**-Significant at P<0.01

NS-Non-Significant

Fig. 8: Influence of sex on the prevalence of *Fasciola* sp. and *Amphistome* sp. infection in buffaloes of Patna district through faecal samples examination.

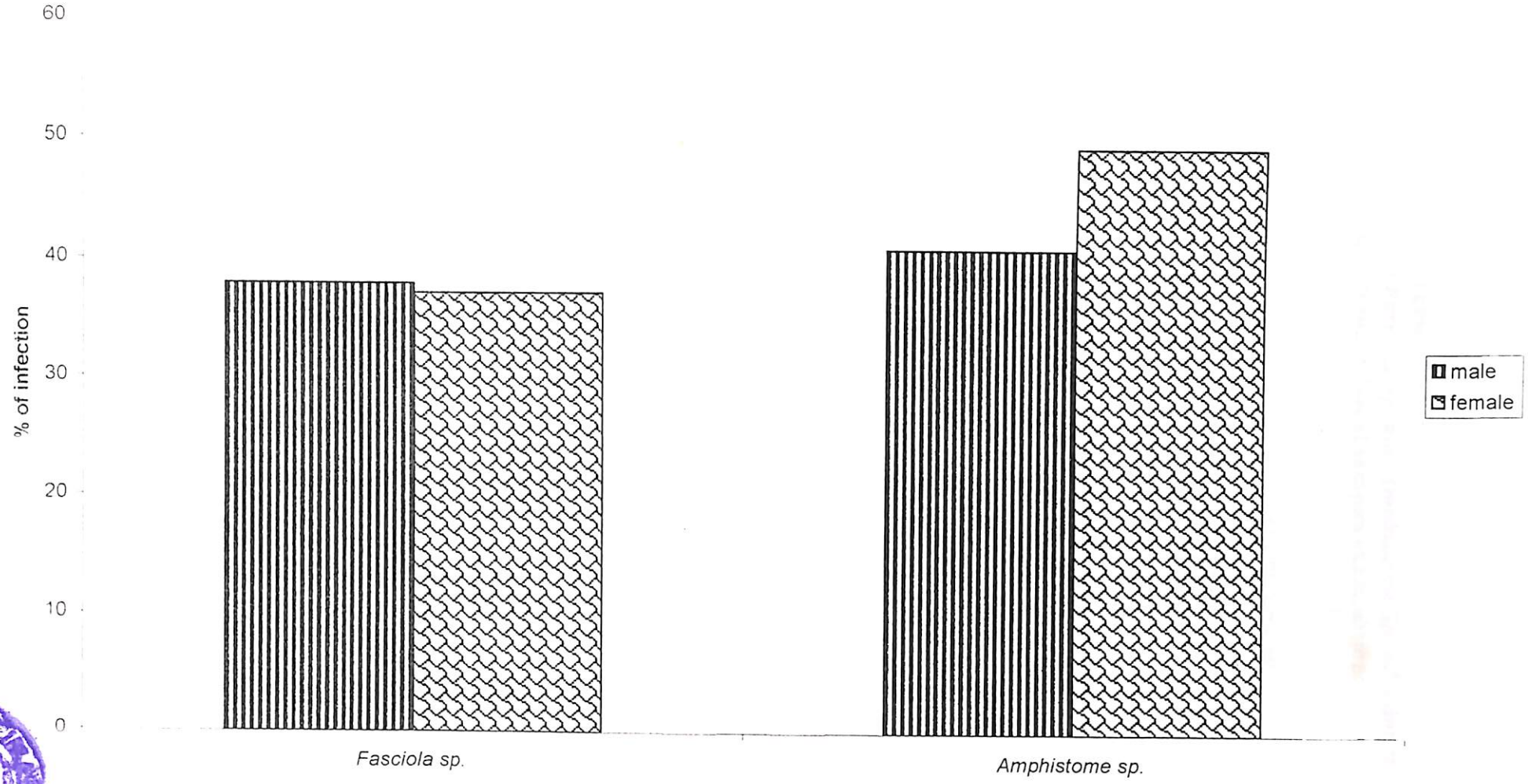


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Table-12

Influence of Sex on the Prevalence of *Fasciola Sp.* and *Amphistome Sp.* infection in buffaloes of Khagaria district through faecal samples examination:-

		Fasciola Sp.			*Amphistome* sp.		
Sex	No. of faecal samples examined	No. of samples found positive	Percentage of infection	$\chi^2$1d.f	No. of samples found positive	Percentage of infection	$\chi^2$1d.f
Male	200	90	45.00		103	51.50	
Female	220	103	46.82	0.054^{NS}	118	53.64	0.191^{NS}
Total	420	193			221		

**-Significant at P<0.01

NS-Non-Significant

Fig. 9: Influence of sex on the prevalence of *Fasciola* sp. and *Amphistome* sp. infection in buffaloes of Khagaria district through faecal samples examination.

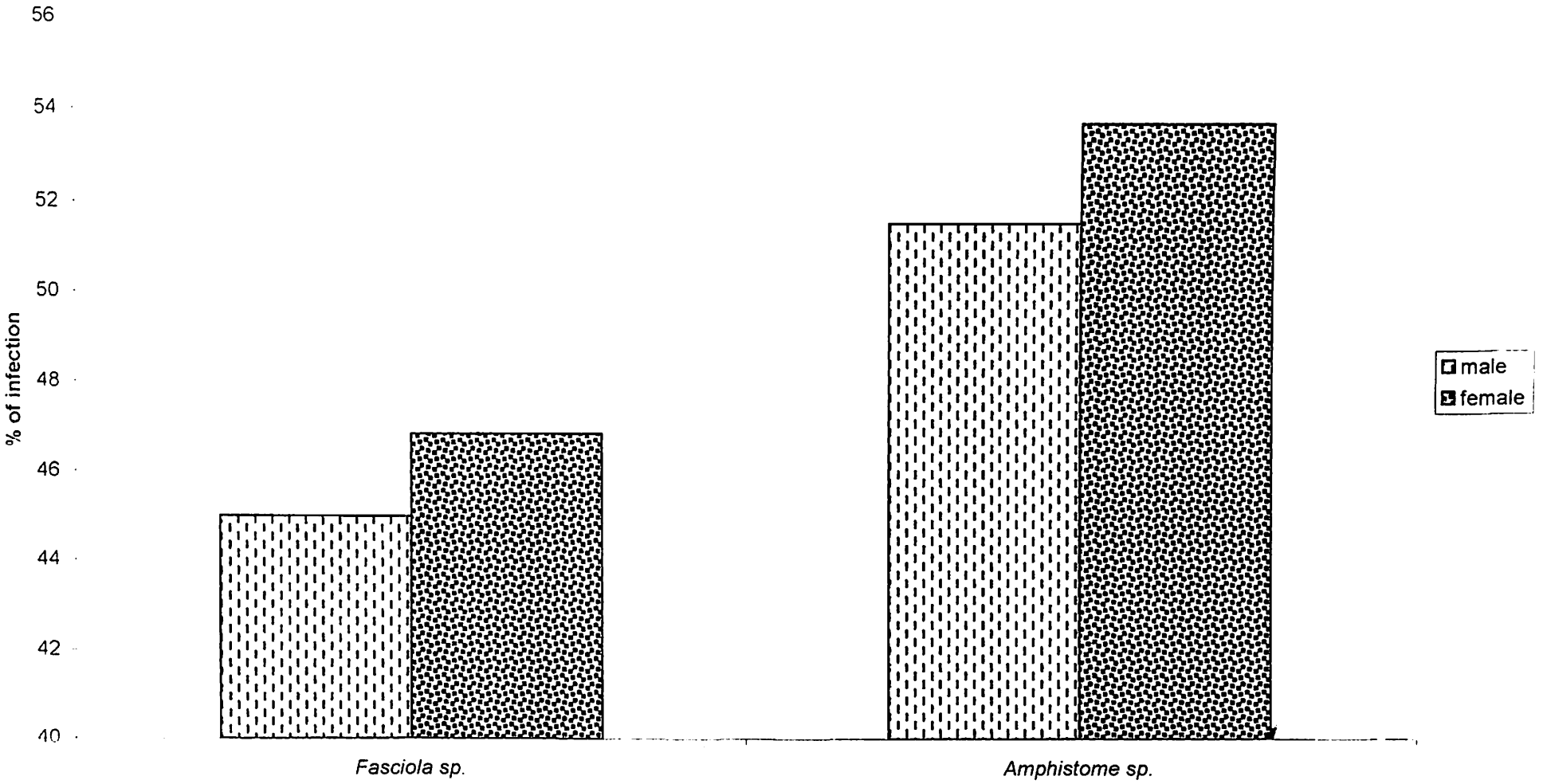


Table-13

Influence of Sex on the Prevalence of *Fasciola Sp.* and *Amphistome Sp.* infection in buffaloes of Lakhisarai district through faecal samples examination:-

		Fasciola Sp.			*Amphistome* sp.		
Sex	No. of faecal samples examined	No. of samples found positive	Percentage of infection	$\chi^2$1d.f	No. of samples found positive	Percentage of infection	$\chi^2$1d.f
Male	185	57	30.81		69	37.29	
Female	230	86	37.39	1.96^{NS}	99	43.04	1.40^{NS}
Total	415	143			168		

**-Significant at P<0.01

NS-Non-Significant

Fig. 10: Influence of sex on the prevalence of *Fasciola* sp. and *Amphistome* sp. infection in buffaloes of Lakhisarai district through faecal samples examination.

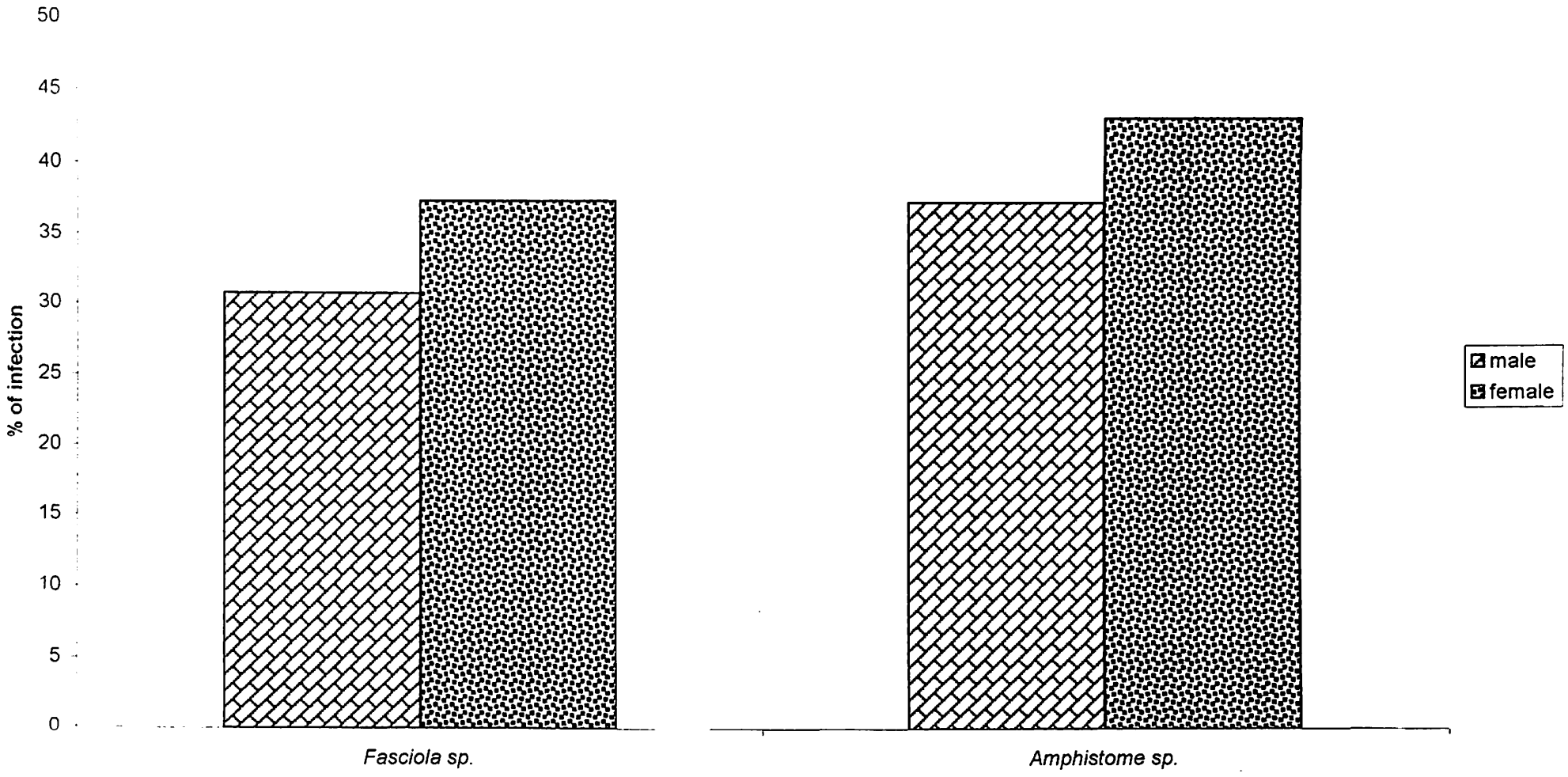


Table-14

Prevalence of *Fasciola Sp.* and *Amphistome Sp.* between stallfed and free grazing buffaloes of Patna district through faecal samples examination:-

		Fasciola Sp.			*Amphistome* sp.		
Managemental Condition	No. of faecal samples examined	No. of samples found positive	Percentage of infection	$\chi^2$1d.f	No. of samples found positive	Percentage of infection	$\chi^2$1d.f
Stallfed	200	52	26.00		75	37.50	
Free-grazing	250	117	46.80	20.49**	130	52.00	9.41**
Total	450	169			205		

**-Significant at P<0.01

NS-Non-Significant

Fig. 11: Prevalence of *Fasciola* sp. and *Amphistome* sp. between stallfed and free-grazing buffaloes of Patna district through faecal samples examination.

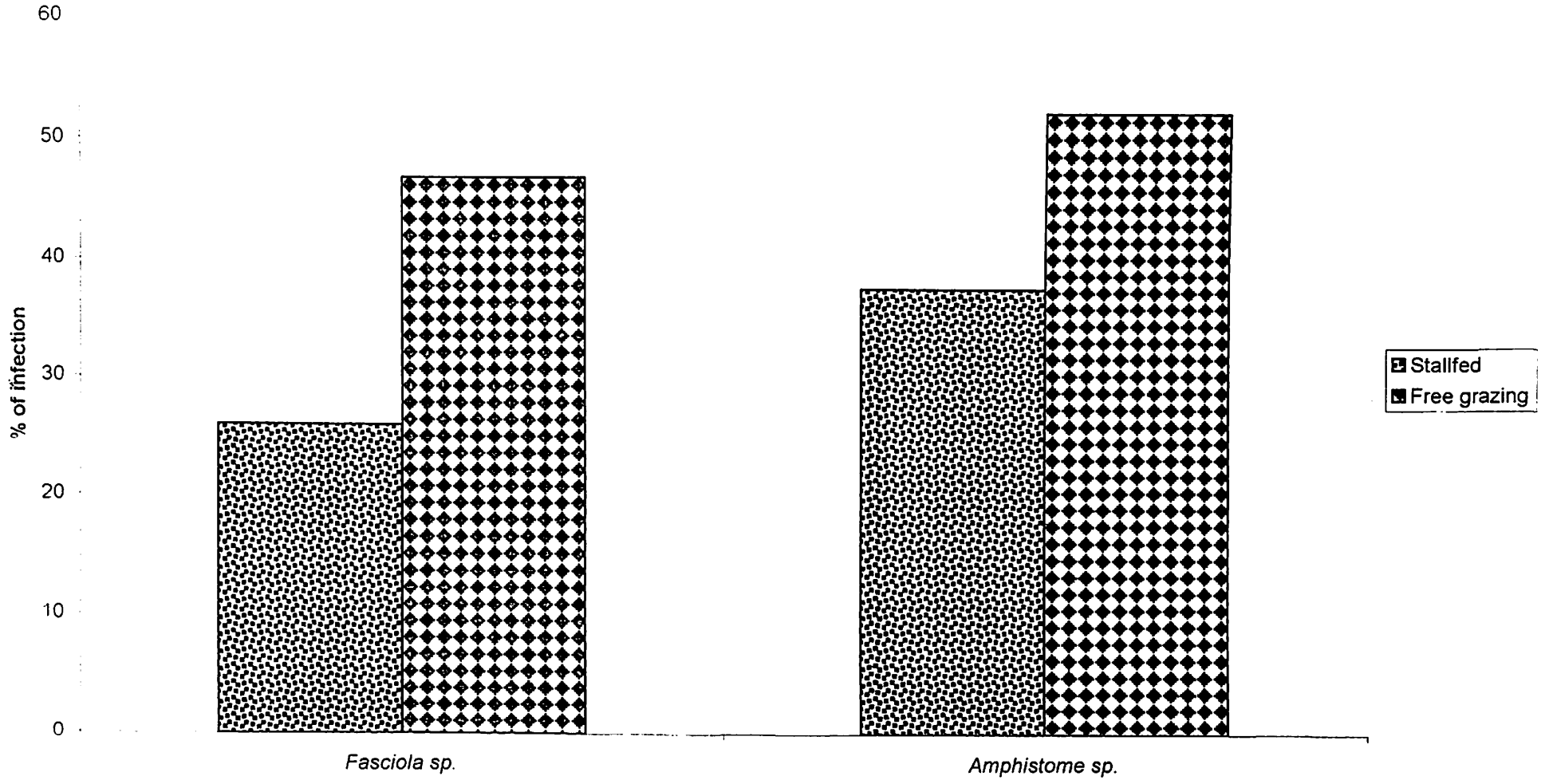


Table-15

Prevalence of *Fasciola Sp.* and *Amphistome Sp.* between stallfed and free grazing buffaloes of Khagaria district through faecal samples examination:-

		Fasciola Sp.			*Amphistome* sp.		
Managemental Condition	No. of faecal samples examined	No. of samples found positive	Percentage of infection	$\chi^2$1d.f	No. of samples found positive	Percentage of infection	$\chi^2$1d.f
Stallfed	180	82	45.55		92	51.11	
Free-grazing	240	111	46.25	0.019^{NS}	129	53.75	0.287^{NS}
Total	420	193			221		

**-Significant at P<0.01

NS-Non-Significant

Fig. 12: Prevalence of *Fasciola* sp. and *Amphistome* sp. between stallfed and free-grazing buffaloes of Khagaria district through faecal samples examination.

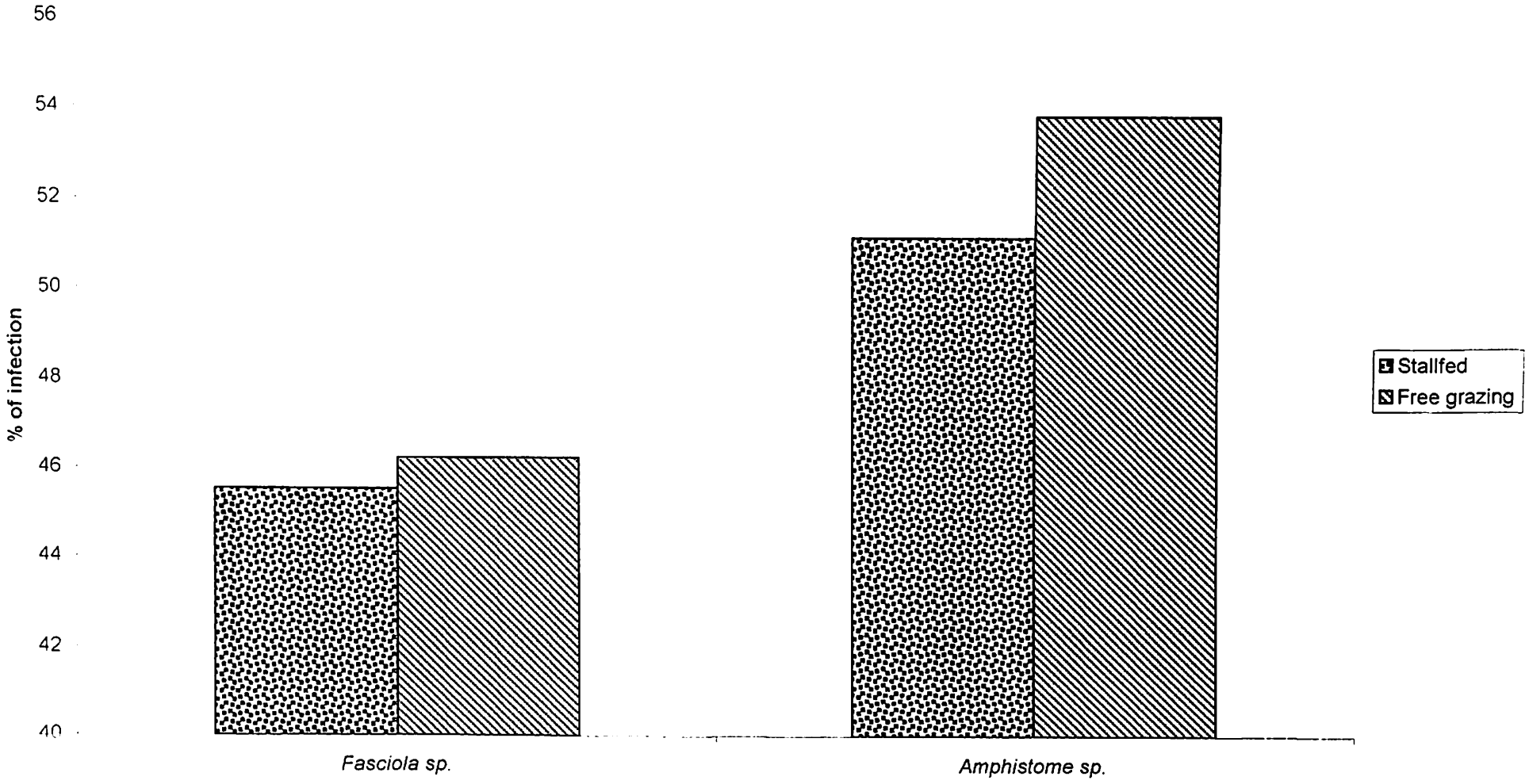


Table-16

Prevalence of *Fasciola Sp.* and *Amphistome Sp.* between stallfed and free grazing buffaloes of Lakhisarai district through faecal samples examination:-

		Fasciola Sp.			*Amphistome* sp.		
Managemental Condition	No. of faecal samples examined	No. of samples found positive	Percentage of infection	$\chi^2$1d.f	No. of samples found positive	Percentage of infection	$\chi^2$1d.f
Stallfed	190	64	33.68		73	38.42	
Free-grazing	225	79	35.11	0.092^{NS}	95	42.22	0.936^{NS}
Total	415	143			168		

**-Significant at P<0.01

NS-Non-Significant

Fig. 13: Prevalence of *Fasciola* sp. and *Amphistome* sp. between stallfed and free-grazing buffaloes of Lakhisarai district through faecal samples examination.

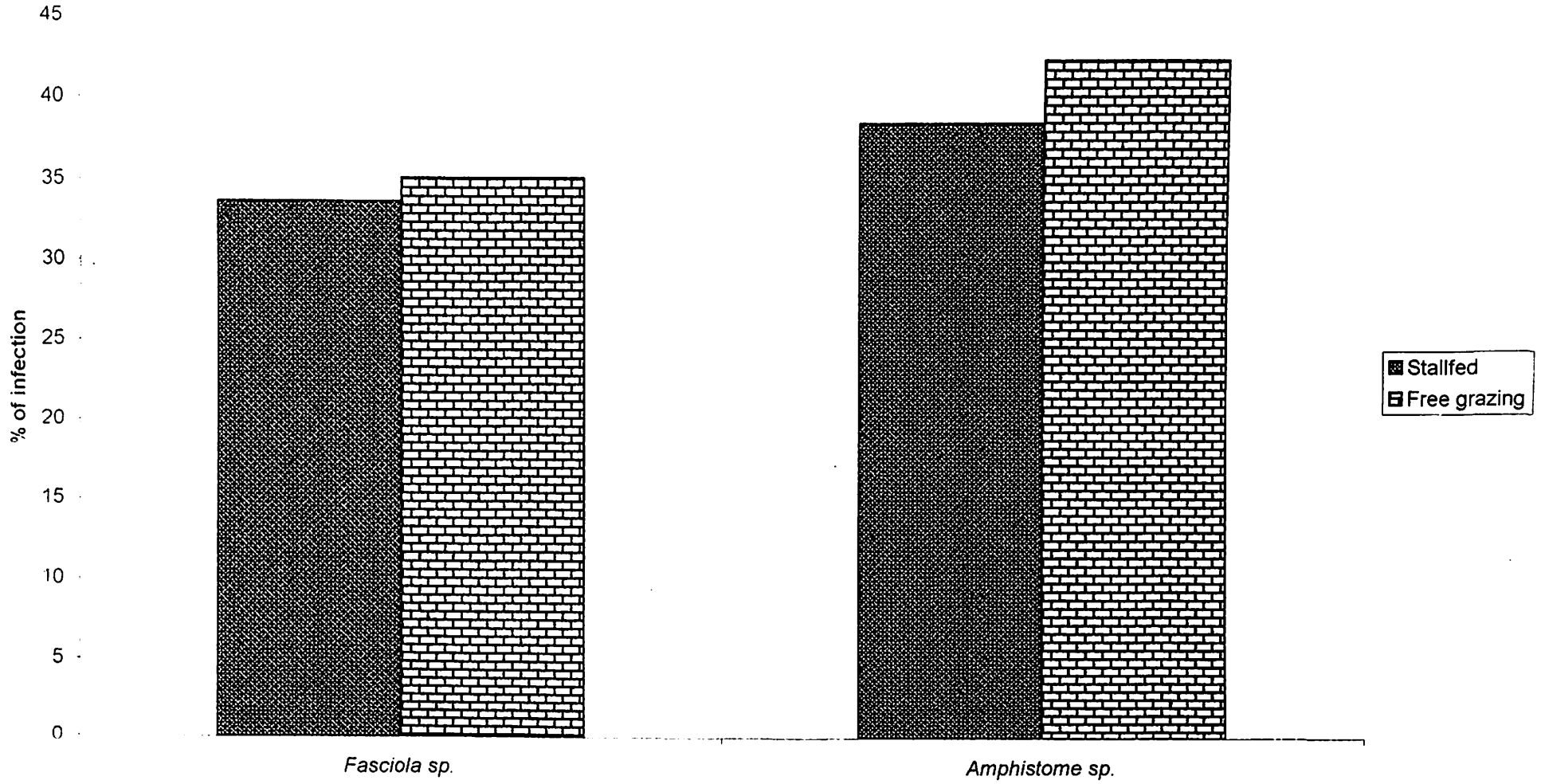


Table-17

Seasonal Prevalence of *Fasciola Sp.* and *Amphistome Sp.* of buffaloes in Patna district through examination of intestinal scraping:-

		Fasciola sp.			*Amphistome* sp.		
Season	No. of samples Examined	No. of samples found positive	Percentage of infection	χ^2 2df	No. of samples found positive	Percentage of infection	χ^2 2d.f
Monsoon	60	33	55.00		37	61.66	
Winter	50	11	22.00	11.22**	13	26.00	15.47**
Summer	40	13	32.50		14	35.00	
Total	150	57			64		

Comparison within group χ^2 1d.f value

Monsoon Vs Summer
12.37**

Monsson Vs Winter
4.89**

Summer Vs Winter
1.25^{NS}

Monsoon Vs Summer
13.99**

Monsson Vs Winter
6.82**

Summer Vs Winter
0.86^{NS}

**-Significant at P<0.01

NS-Non-Significant

Fig. 14: Seasonal prevalence of *Fasciola* sp. and *Amphistome* sp. in buffaloes of Patna district through examination of faecal samples.

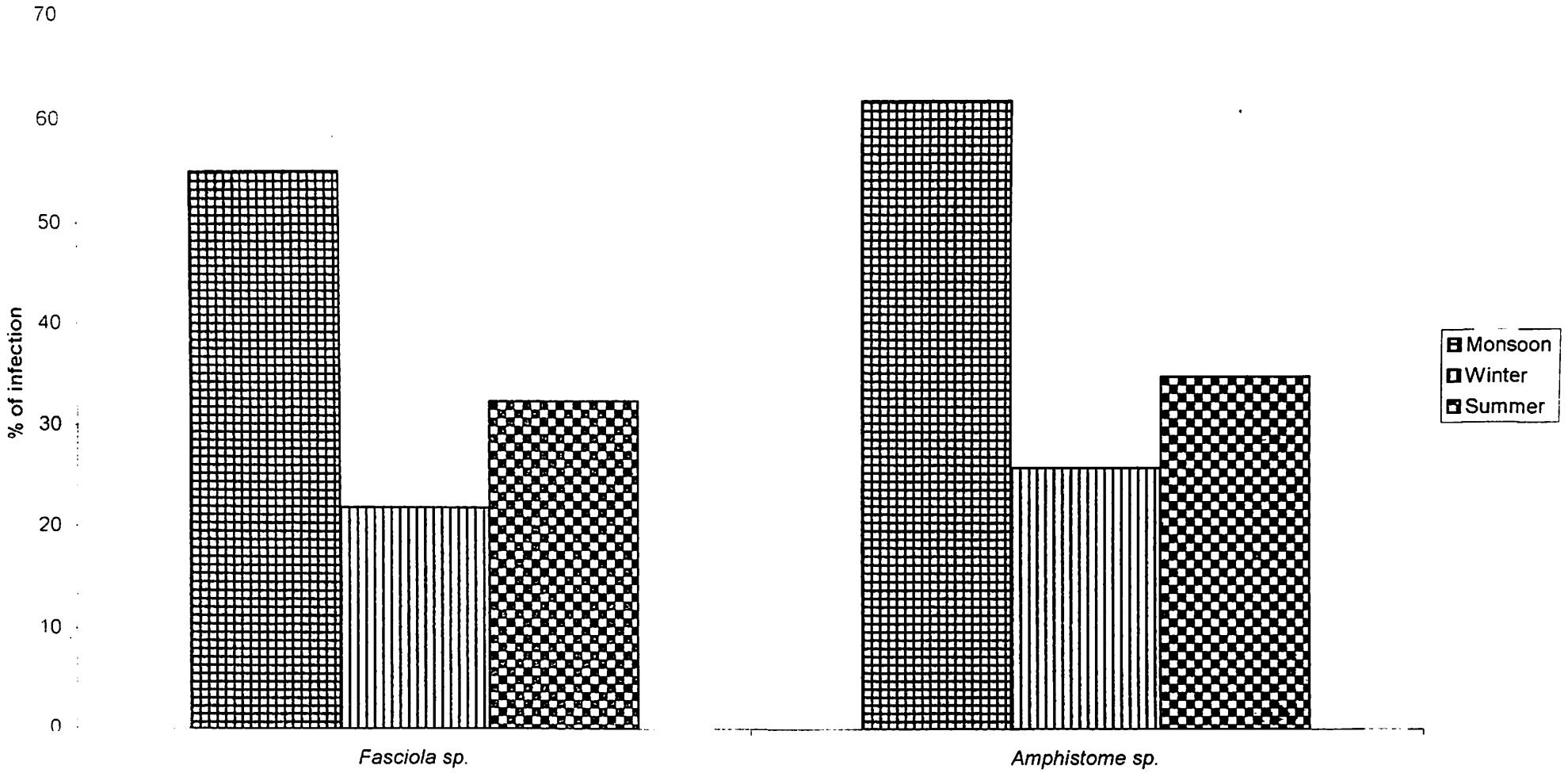


Table - 18

Mean ± S.E. along with 't' value of haematogical parameters in control, *Fasciole sp.* infected and *Amphistome sp.* infected buffaloes of Patna district.

	Mean ± S.E.			't' value and level of significance		
Parameter	Control	*Fasciole sp.* Infected	*Amphistomic sp.* infected	A	B	C
Hb (gm%)	20.60^{a}±0.14 (13.29)	16.97^{b}±0.18 (8.53)	15.71^{b}±0.27 (7.35)	15.72**	16.05**	2.01^{NS}
PCV(%)	40.92^{a}±0.24 (42.89)	39.45^{b}±0.35 (36.99)	34.99^{c}±0.39 (32.91)	8.09**	12.90**	4.66**
TEC X $10^{6}/mm^{3}$	6.32^{a}±0.11	4.85^{b}±0.12	4.86^{d}±0.23	8.94**	5.81**	0.04^{NS}
TLC X $10^{3}/mm^{3}$	7.22^{a}±.0.06	9.93^{b}±0.17	8.47^{c}±0.09	14.61**	10.96**	8.69**
ESR	50.56^{a}±1.39	81.33^{b}±1.17	73.57^{a}±1.57	16.86**	10.92**	3.94**

A- Between control and *Fasciola sp.* infected
B- Between control and *Amphistome* sp. infected.
C- Between two infected group.

*-Significant at P<0.05
** - Significant at P<0.01
NS-Non-significant.

Values of Hb (gm%) and PCV (%) are the values of angles corresponding to percentages (Angle = Arcsin percentage)
Figures in parenthesis indicate mean percentages of original value.
N.B. Means (row-wise) with different superscripts differ significantly p<0.01 from control.

Fig. 15: Mean values of haematological parameters in control, *Fasciola* sp. infected and *Amphistome* sp. infected buffaloes.

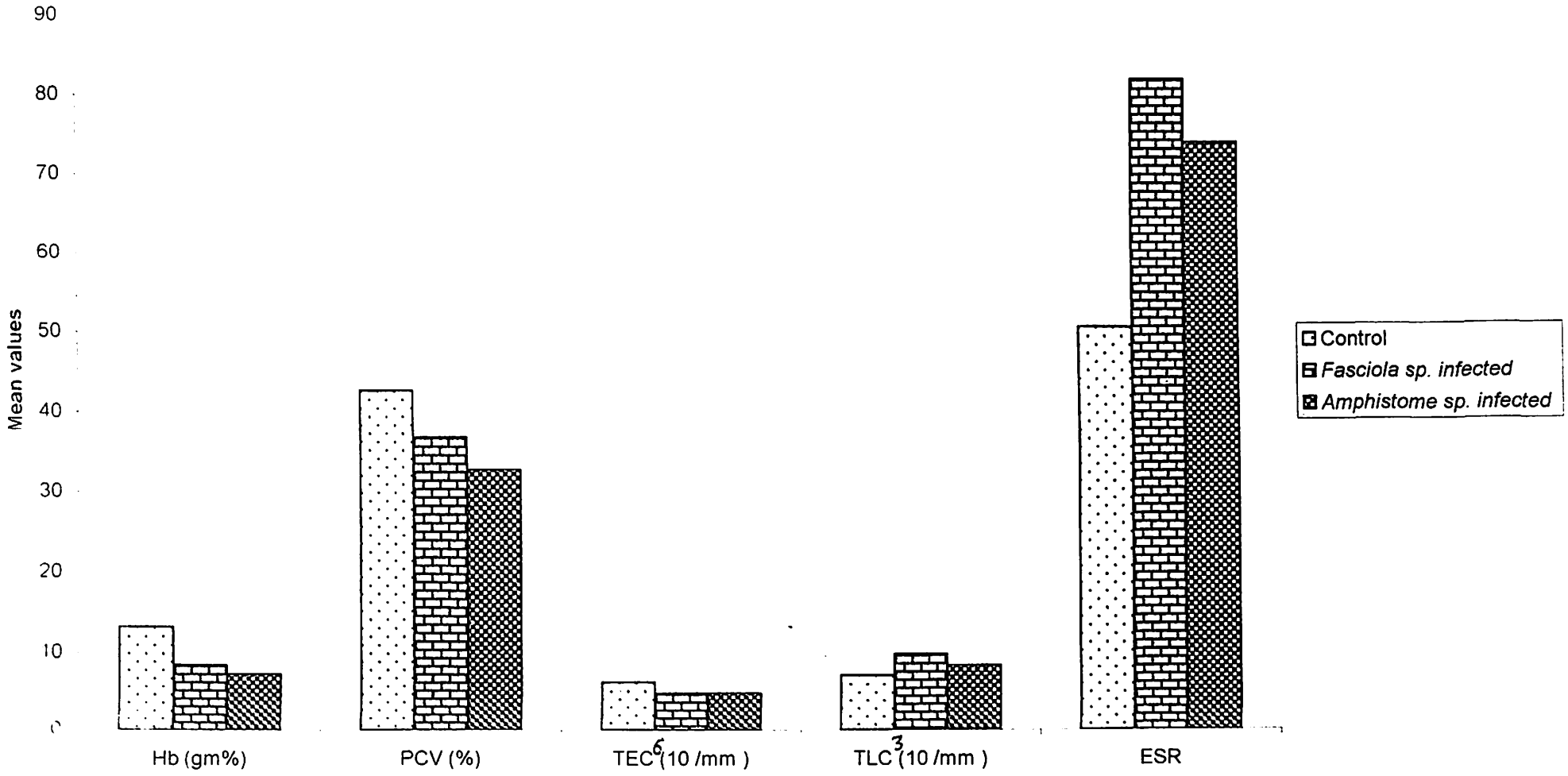


Table - 19

Mean ± S.E. along with 't' value of haematogical parameters in control, *Fasciole sp.* infected and *Amphistome sp.* infected buffaloes of Patna district.

	Mean ± S.E.			't' value and level of significances		
Parameter	Control	*Fasciole sp.* Infected	*Amphistomic sp.* infected	A	B	C
Neutrophils(%)	$36.84^{a}\pm0.56$ (35.80)	$30.10^{b}\pm0.55$ (25.20)	$33.38^{c}\pm0.41$ (30.32)	8.49**	4.93**	4.71**
Lymphocytes(%)	$50.50^{a}\pm0.56$ (59.5)	$54.77^{b}\pm0.55$ (66.7)	$52.42^{c}\pm0.49$ (62.8)	5.38**	2.55*	3.13**
Menocytes (%)	$7.73^{a}\pm0.59$ (1.9)	$9.92^{ac}\pm1.43$ (3.5)	$9.65^{bc}\pm0.58$ (2.9)	1.41^{NS}	2.30*	0.17^{NS}
Eosinophils(%)	$9.40^{a}\pm0.71$ (2.8)	$12.13^{b}\pm0.88$ (4.6)	$11.43^{c}\pm0.55$ (4.0)	2.45*	2.25*	2.19*

A - Between control and *Fasciola sp.* Infected
B- Between control and *Amphistome* sp. infected.
C- Between two infected group.

*-Significant at P<0.05
**-Significant at P<0.01
NS-Non-significant

All values are in angles corresponding to percentages.(Angle=Arcsin Percentage)
Figures in parenthesis indicate mean percentages of original value.
N.B. Means (row-wise) with different superscripts differ significantly p<0.01 from control.

Fig. 16: Mean values of haematological parameters in control, *Fasciola* sp. infected and *Amphistome* sp. infected buffaloes.

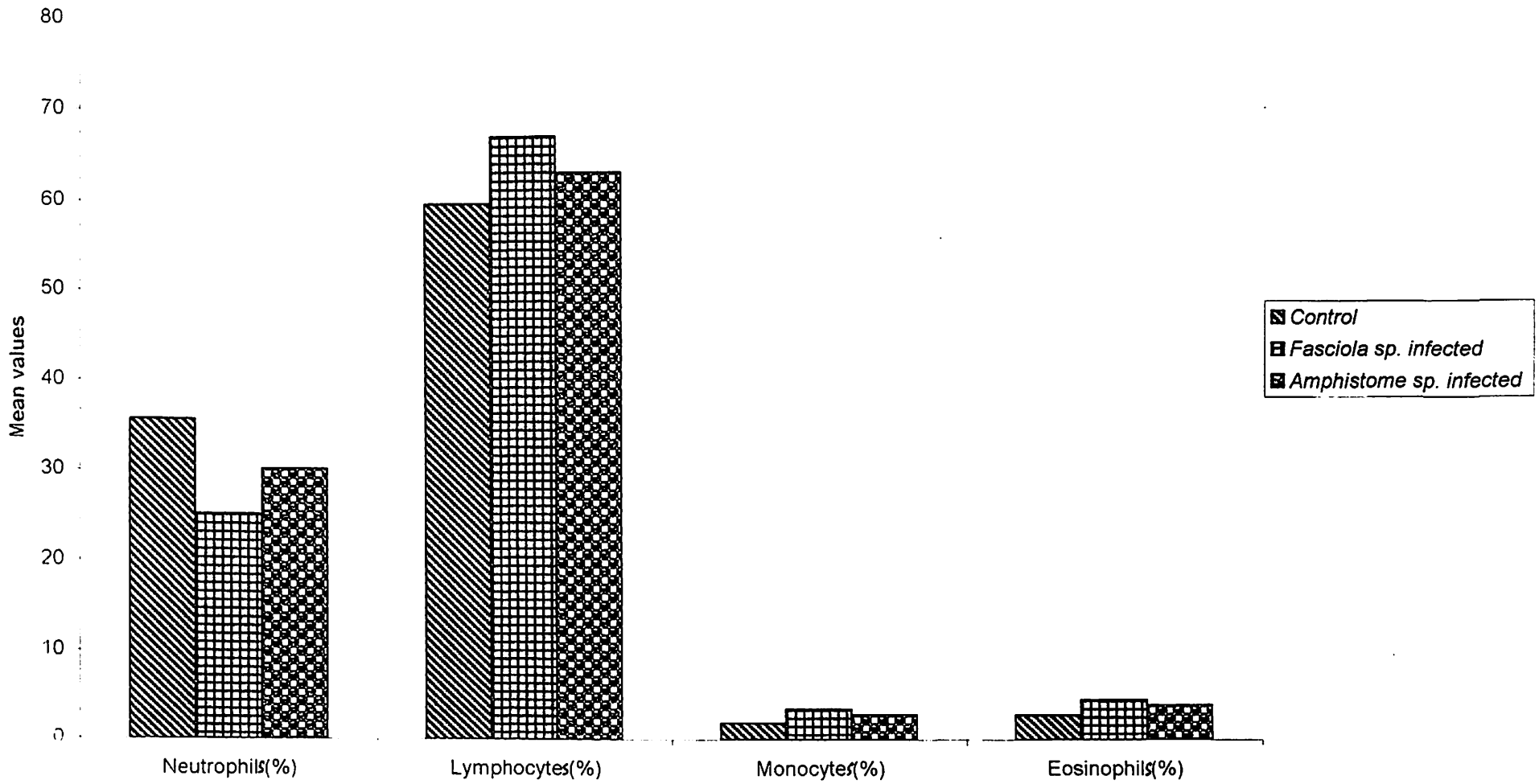


Table - 20

Mean ± S.E. of E.P.G. and percent efficacy of anthelmintics against *Fasciola* sp. in buffaloes at different days of interval in Patna district

Drug used	Dose	Average EPG of faeces post-treatment (Eggs per gram)						
		0 Day	7 Day	% Efficacy	14 Day	% Efficacy	21 Day	% Efficacy
Untreated control	-	377.2±14.53	412.6^{a}±17.78		445.0^{a}±16.05		464.2^{a}±19.09	
Albendazole	10 mg/kg	360.6±14.15	145.0^{b}±7.28	59.79	93.4^{b} ±2.71	74.10	41.2^{b} ±6.89	88.57
Closantel	7.5 mg/kg	360.2±14.15	58.4^{bc}±13.21	83.79	13.6^{c}±3.23	96.22	2^{c}±0.2	99.94
Oxiclozanide Levamisole	22.5 mg/kg	361.0±16.02	22.4^{c}±1.89	93.80	0	100.00	0	100.00

N.B. values with same superscript (column wise) do not differ significantly.

Table - 21

Analysis of variance for the effect of different anthelminthic drugs against *Fasciola* sp. in buffaloes at different days of interval in Patna district.

Source of variation	Periods											
	0 day			7 day			14 day			21 day		
	DF	MS	F	DF	MS	F	DF	MS	F	DF	MS	F
Between treatment	3	344.98	0.32^{NS}	3	121761.8	17.38**	2	325513.8	708.87**	2	383178.2	558.37**
Error (within treatment)	16	1085.43		16	7005.96		12	459.2		12	686.37	

**-Significant P<0.01

NS- Non-significant

Fig. 17: percent efficacy of anthelmintics against *Fasciola* sp. in buffaloes at different days of interval in Patna district.

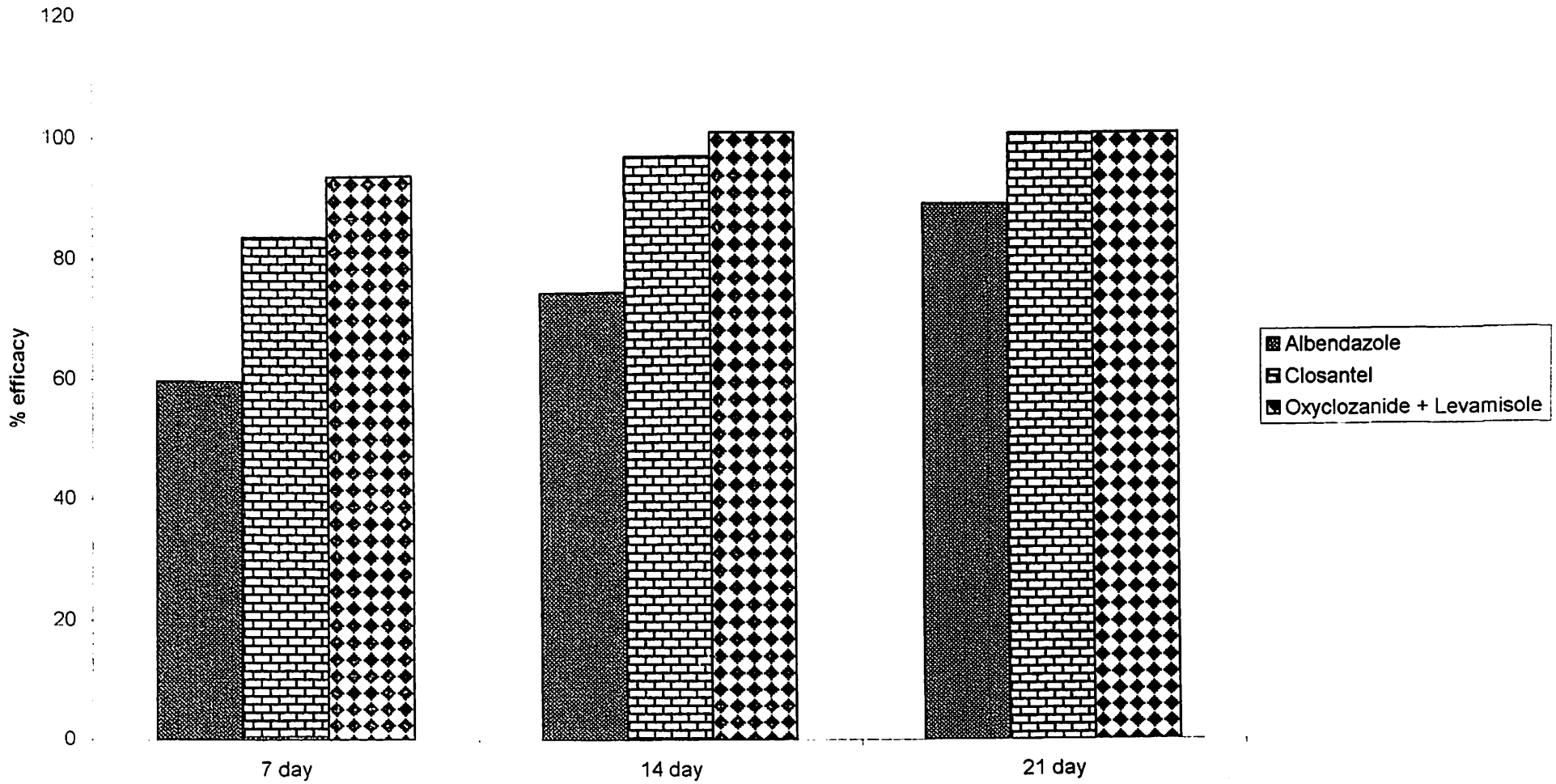


Table - 22

Mean ± S.E. of E.P.G. and percent efficacy of anthelmintics against *Amphistome* sp. in buffaloes at different days of interval in Patna district

Drug used	Dose	Average EPG of faeces post-treatment (Eggs per gram)						
		0 Day	7 Day	% Efficacy	14 Day	% Efficacy	21 Day	%Efficacy
Untreated control	-	418.2±6.07	436.8^{a}±6.72		453.2^{a}±7.08		475.4^{a}+8.93	
Albendazole	10 mg/kg	446.4±18.22	175.8^{b}±7.77	60.62	95.0^{b}±2.17	78.72	48.8^{b}±2.99	89.07
Closantel	7.5 mg/kg	432.6±16.90	132.0^{c}±9.34	69.49	64.4^{c}±10.78	85.11	14.2^{c}±2.84	96.72
0xiclozanide Levamisole	22.5 mg/kg	449.0±22.93	94.0^{d}±5.97	74.06	37.0^{d}±6.32	91.76	3.8^{c}±1.36	99.15

N.B. values with same superscript (column wise) do not differ significantly.

Table - 23

Analysis of variance for the effect of different anthelminthic drugs against *Amphistome* sp. in buffaloes at different days of interval in Patna district.

Source of variation	Periods											
	0 day			7 day			14 day			21 day		
	DF	MS	F	DF	MS	F	DF	MS	F	DF	MS	F
Between treatment	3	1007.25	0.68^{NS}	3	118847.98	416.13**	3	189944.93	719.83**	3	257608.78	2092.60**
Error (within treatment)	16	1475.45		16	285.6		16	263.88		16	123.1	

**-Significant at p<0.01

NS-Non-significant

Fig. 18: percent efficacy of anthelmintics against *Amphistome* sp. in buffaloes at different days of interval in Patna district.

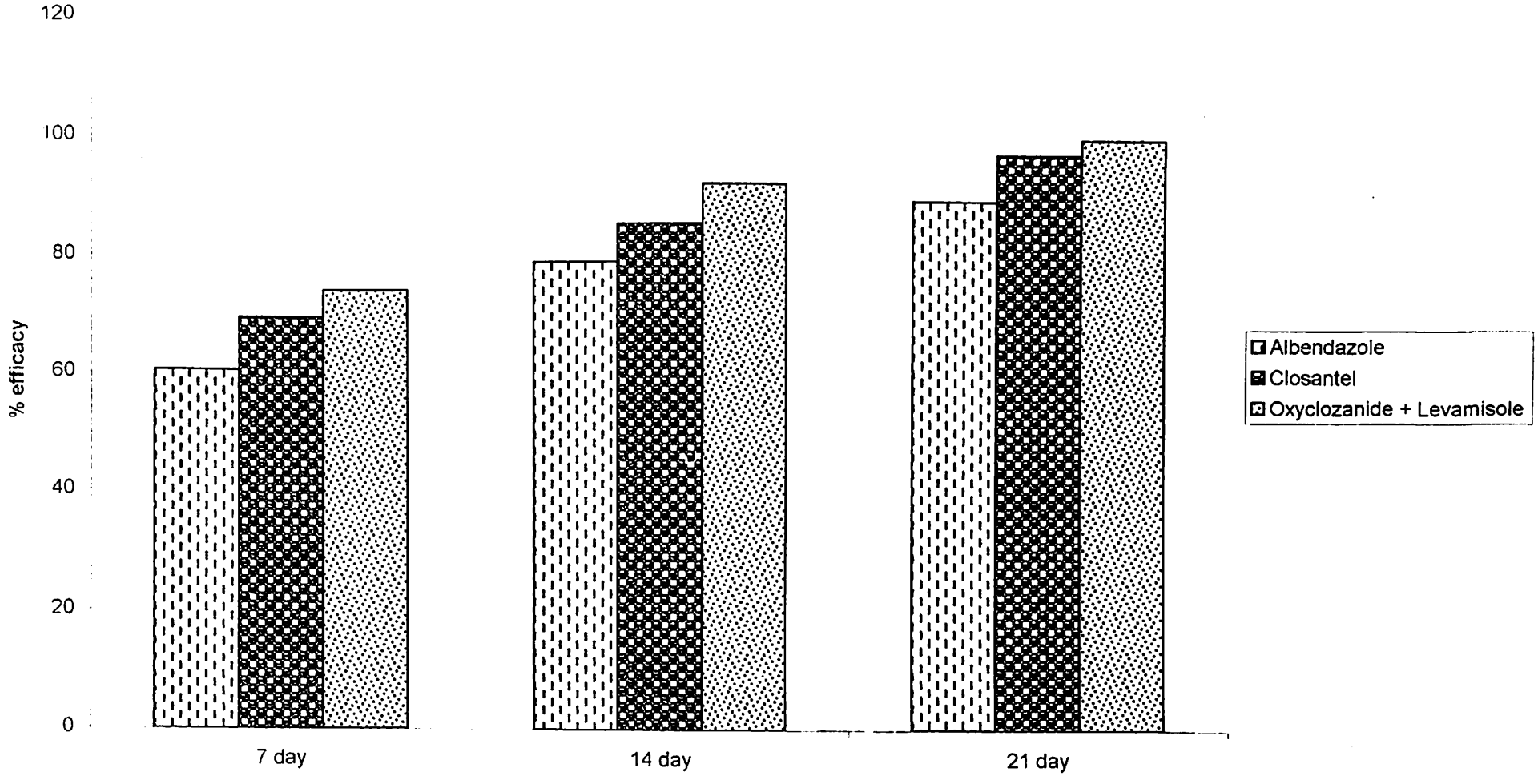


Table - 24

Mean ± S.E. of E.P.G. and percent efficacy of anthelmintics against *Fasciola* sp. in buffaloes at different days of interval in Khagaria district

Drug used	Dose	Average EPG of faeces post-treatment (Eggs per gram)						
		0 Day	7 Day	% Efficacy	14 Day	% Efficacy	21 Day	% Efficacy
Untreated control	-	496.2±36.43	528.2^{a}±28.59		562.0^{a}±23.83		601.0^{a}±23.82	
Albendazole	10 mg/kg	458.0±23.92	137.8^{b}±13.31	69.91	39.6^{b}±6.24	91.35	6.0^{b}±1.14	98.68
Closantel	7.5 mg/kg	410.6±12.11	19.2^{c}±.0.73	85.46	4.4^{bc}±1.57	98.69	0	100.00
Oxiclozanide+ Levamisole	22.5 mg/kg	414.6±32.83	4.0^{c}±1.14	99.04	0.8^{c}±.0.8	99.80	0	100.00

N.B. values with same superscript (column wise) do not differ significantly.

Table - 25

Analysis of variance for the effect of different anthelminthic drugs against *Fasciola* sp. in buffaloes at different days of interval in Khagaria district.

Source of variation	Periods											
	0 day			7 day			14 day			21 day		
	DF	MS	F	DF	MS	F	DF	MS	F	DF	MS	F
Between treatment	3	8163.12	2.09^{NS}	3	298217.26	234.44**	3	375634.33	492.75**	1	1354683.75	1071.93**
Error (within treatment)	16	3905.83		16	1272.03		16	762.325		9	1263.77	

**-Significant at $p<0.01$

NS-Non-significant

Fig. 19: percent efficacy of anthelmintics against *Amphistome* sp. in buffaloes at different days of interval in Khagaria district.

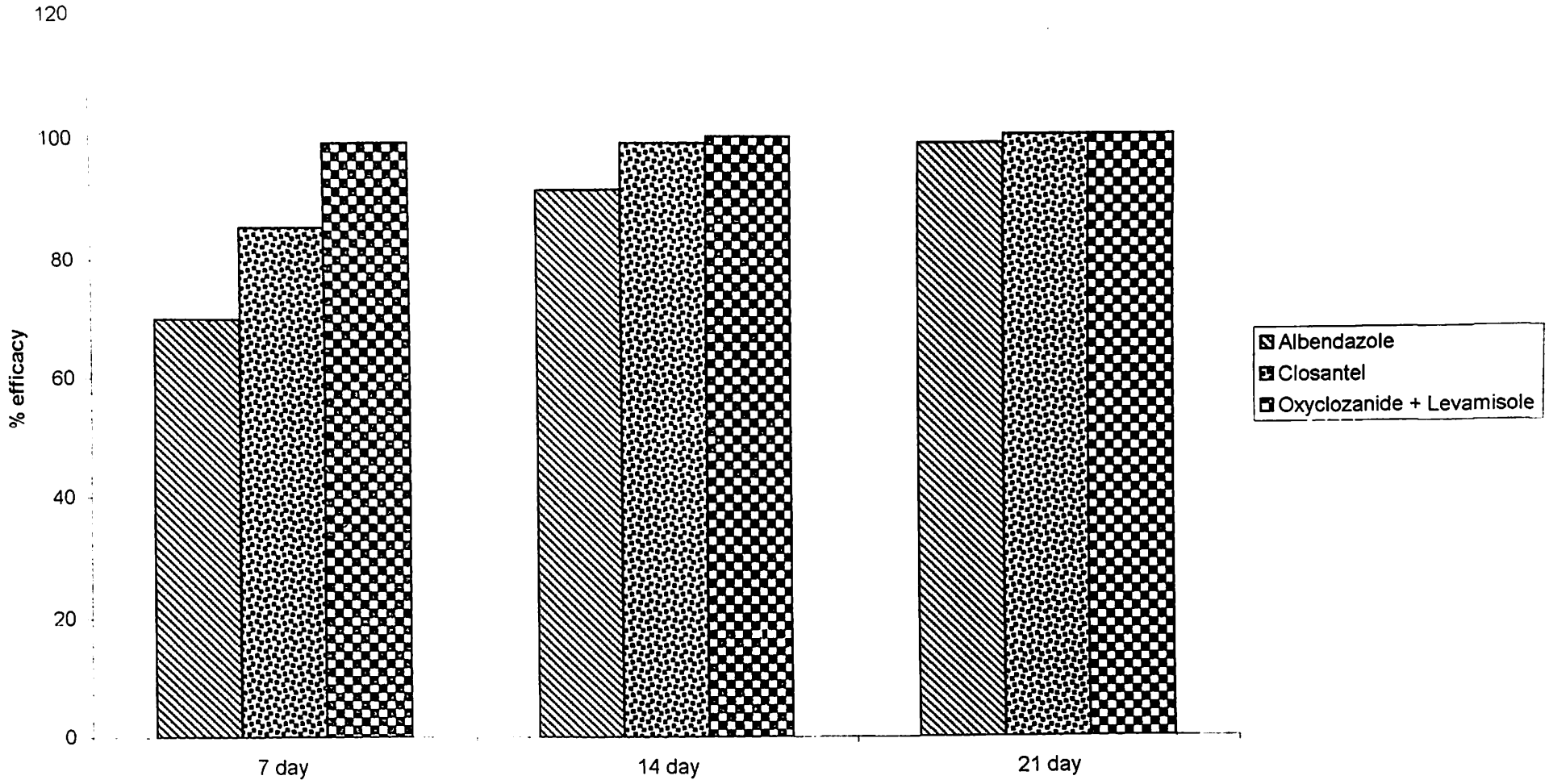


Table - 26

Mean ± S.E. of E.P.G. and percent efficacy of anthelmintics against *Amphistome* sp. in buffaloes at different days of interval in Khagaria district

Drug used	Dose	Average EPG of faeces post-treatment (Eggs per gram)						
		0 Day	7 Day	% Efficacy	14 Day	% Efficacy	21 Day	% Efficacy
Untreated control	-	540.6±13.09	562.0^{a}±10.39		581.6^{a}±13.05		593.8^{a}±10.12	
Albendazole	10 mg/kg	547.8±19.62	259.6^{b}±15.21	52.61	130.4^{b}±5.73	76.19	68.8^{b}±4.28	87.57
Closantel	7.5 mg/kg	548.4±19.34	152.2^{c}±5.99	72.25	81.2^{c}±4.07	85.19	37.2^{c}±7.96	93.22
Oxiclozanide+ Levamisole	22.5 mg/kg	543.0±23.26	81.8^{d}±5.97	84.94	17.8^{d}±2.82	96.72	0	100.00

N.B. values with same superscript (column wise) do not differ significantly.

Table - 27

Analysis of variance for the effect of different anthelminthic drugs against *Amphistome* sp. in buffaloes at different days of interval in Khagaria district.

Source of variance	Periods											
	0 day			7 day			14 day			21 day		
	DF	MS	F	DF	MS	F	DF	MS	F	DF	MS	F
Between treatment	3	71.25	$.039^{NS}$	3	222439.66	433.04**	3	328219.59	1153.67**	2	588964.78	1918.27**
Error (within treatment)	16	1838.83		16	513.68		16	284.5		12	307.03	

**-Significant at $p<0.01$

NS-Non-significant

Fig. 20: percent efficacy of anthelmintics against *Amphistome* sp. in buffaloes at different days of interval in Khagaria district.

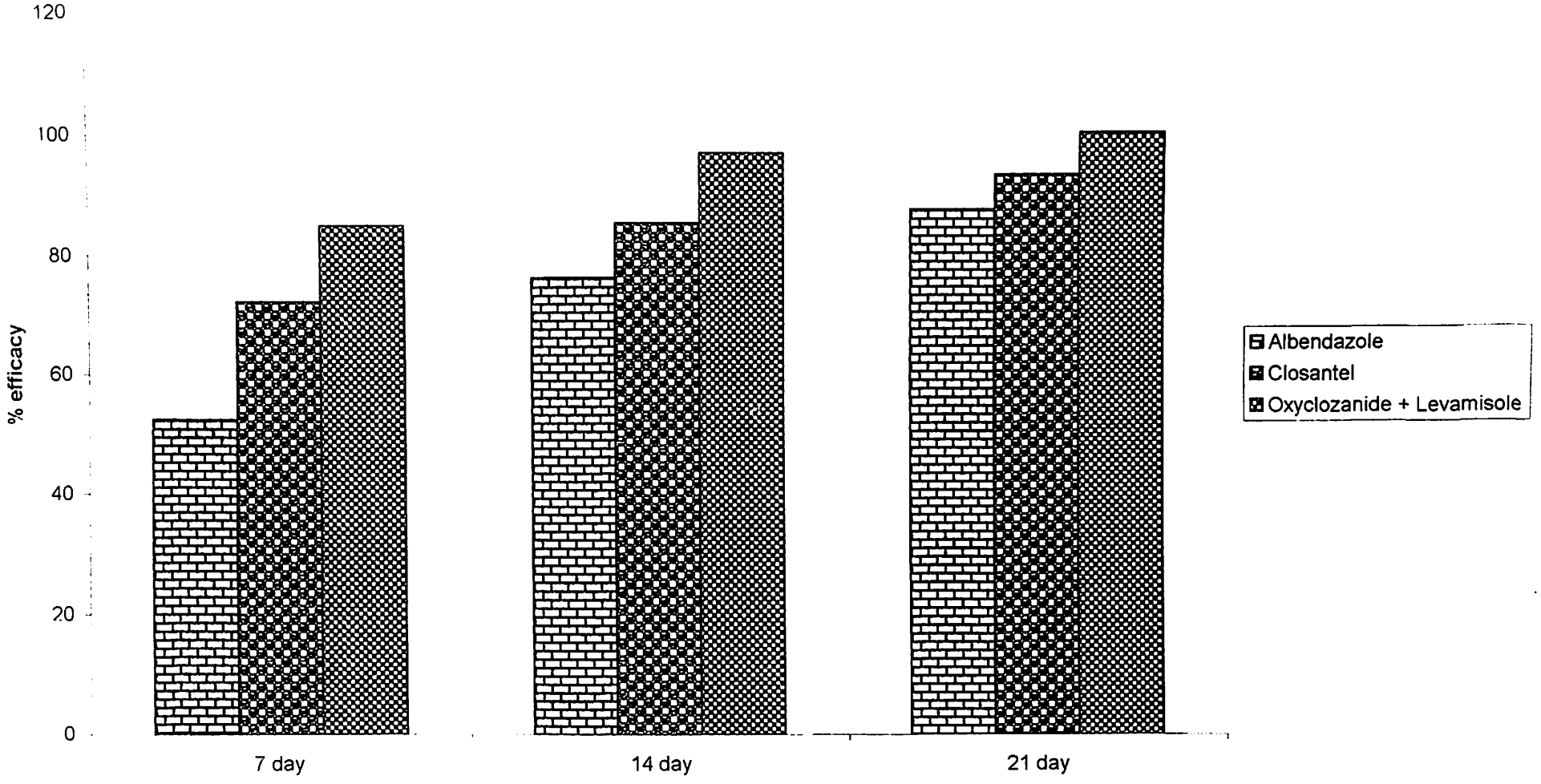


Table - 28

Mean ± S.E. of E.P.G. and percent efficacy of anthelmintics against *Fasciola* sp. in buffaloes at different days of interval in Lakhisarai district

Drug used	Dose	Average EPG of faeces post-treatment (Eggs per gram)						
		0 Day	7 Day	% Efficacy	14 Day	% Efficacy	21 Day	% Efficacy
Untreated control		226.8±13.20	248.2^{a}±14.43		277.6^{a}±11.66		300.6^{a}±5.71	
Albendazole	10 mg/kg	208.6±17.57	126.2^{b}±5.24	39.50 %	83.0^{b}±5.76	60.21	21.2^{b}±3.31	89.83
Closantel	7.5 mg/kg	226.0±19.32	79.6^{c}±1.50	64.77 %	18.0^{c}±3.36	91.94	1.6^{c}±0.51	99.29
Oxiclozanide+ Levamisole	22.5 mg/kg	245.2±22.30	24.4^{d}±4.95	90.04 %	3.2^{c}±2.04	98.85	0.6^{c}±0.40	99.75

N B. values with same superscript (column wise) do not differ significantly.

Table - 29

Analysis of variance for the effect of different anthelminthic drugs against *Fasciola* sp. in buffaloes at different days of interval in Lakhisarai district.

Source of variation	Periods											
	0 day			7 day			14 day			21 day		
	DF	MS	F	DF	MS	F	DF	MS	F	DF	MS	F
Between treatment	3	1117.25	$.66^{NS}$	3	44610.26**	135.96**	3	79172.67**	347.94**	3	107074.53**	1945.00**
Error (within treatment)	16	1192.18		16	328.13		16	227.55		16	55.03	

**-Significant at $p<0.01$

NS-Non-significant

Fig. 21: percent efficacy of anthelmintics against *Amphistome* sp. in buffaloes at different days of interval in Lakhisarai district.

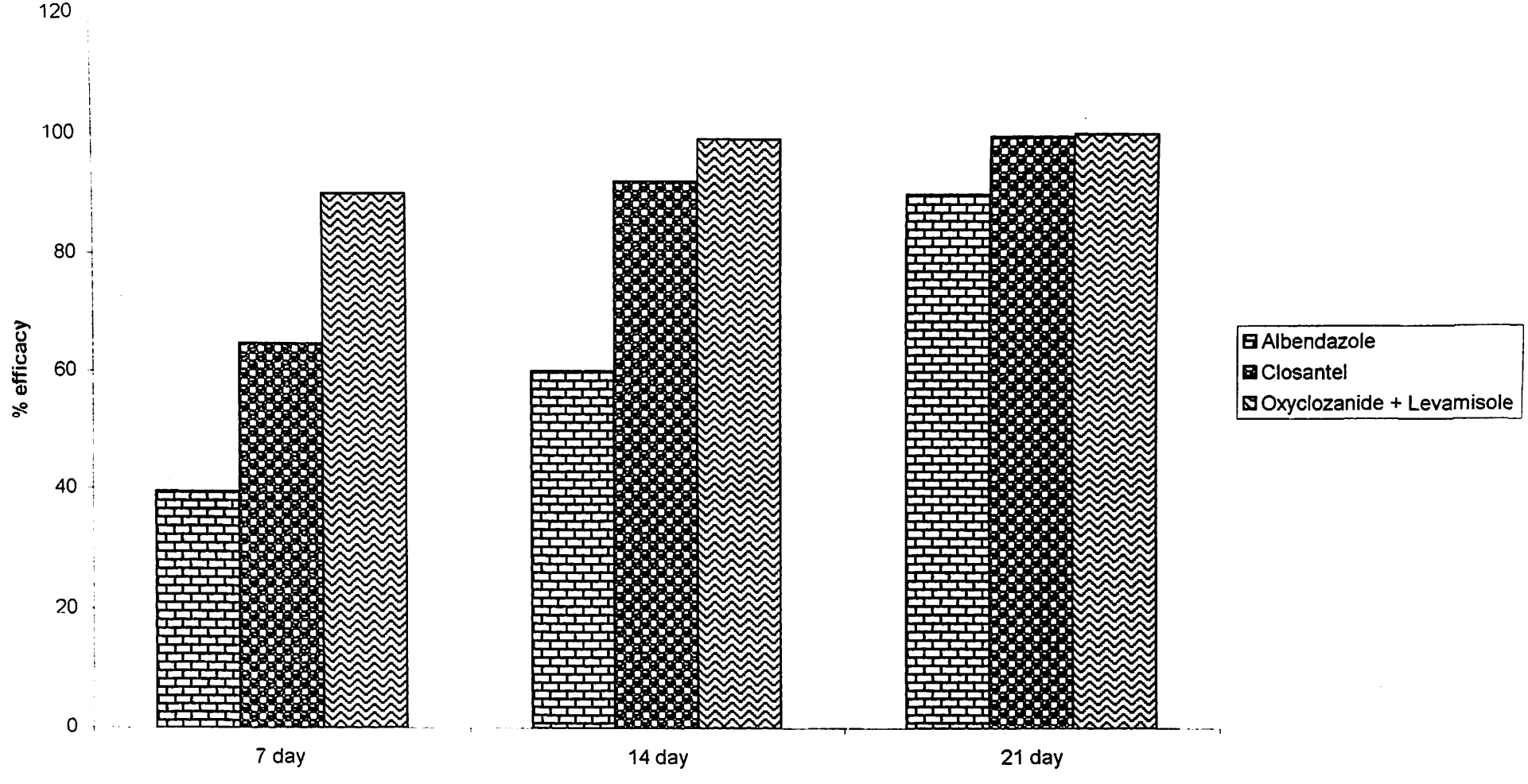


Table - 30

Mean ± S.E. of E.P.G. and percent efficacy of anthelmintics against *Amphistome* sp. in buffaloes at different days of interval in Lakhisarai district

Drug used	Dose	Average EPG of faeces post-treatment (Eggs per gram)						
		0 Day	7 Day	% Efficacy	14 Day	% Efficacy	21 Day	% Efficacy
Untreated control	-	324.8±9.03	352.2^{a}±7.08		373.6^{a}±2.80		389.8^{a}±2.29	
Albendazole	10 mg/kg	378.2±8.79	198.8^{b}±5.60	47.44	109.8^{b}±7.92	70.97	50.0^{b}±2.51	86.78
Closantel	7.5 mg/kg	396.2±5.70	91.0^{c}±5.93	77.03	31.2^{c}±2.60	92.13	1.8^{c}±1.56	99.55
Oxiclozanide+ Levamisole	22.5 mg/kg	376.2±10.49	18.2^{d}±2.52	95.16	2.0^{d}±0.63	99.47	0	100.00

N.B. values with same superscript (column wise) do not differ significantly.

Table - 31

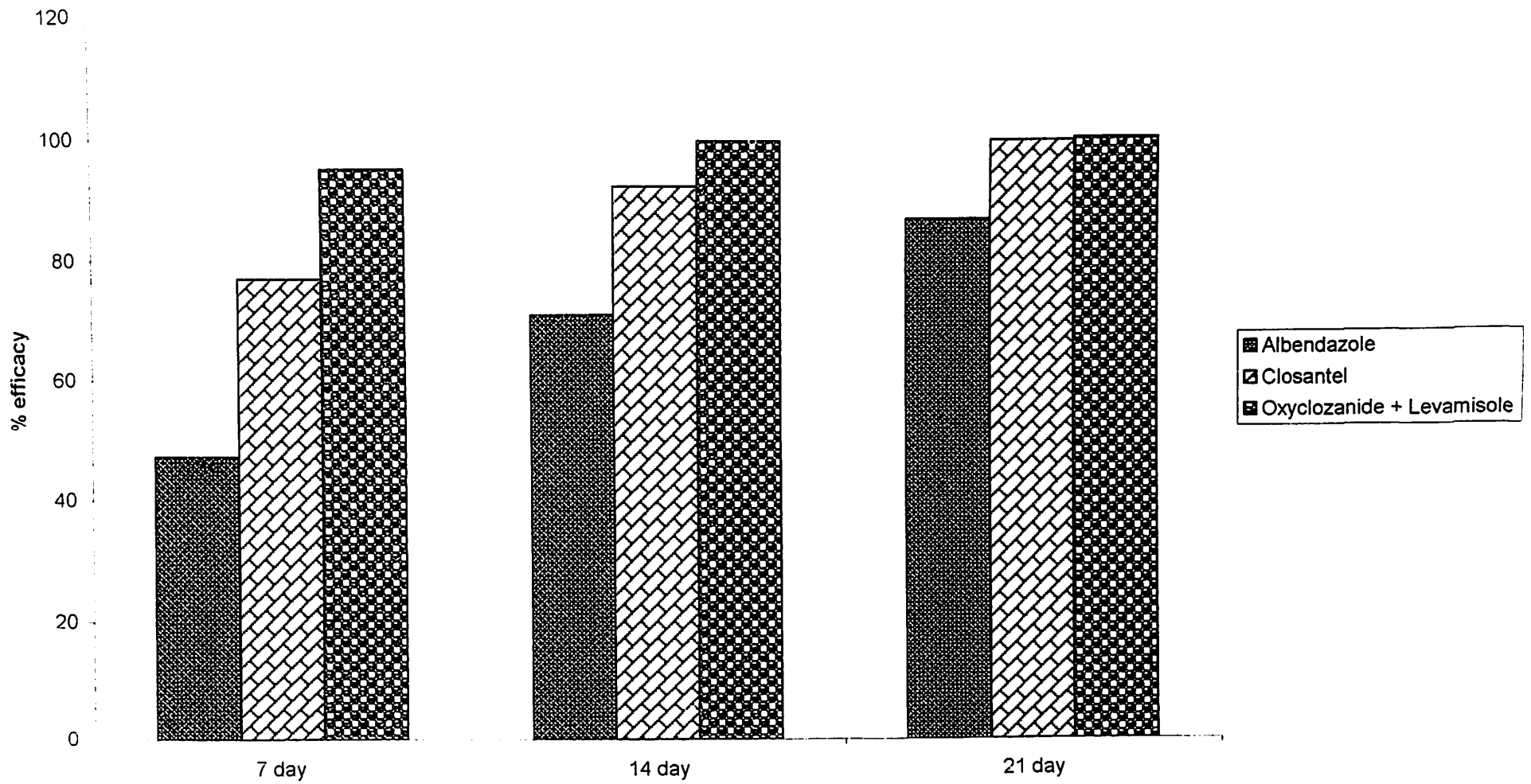
Analysis of variance for the effect of different anthelminthic drugs against *Amphistome* sp. in buffaloes at different days of interval in Lakhisarai district.

Source of variation	Periods											
	0 day			7 day			14 day			21 day		
	DF	MS	F	DF	MS	F	DF	MS	F	DF	MS	F
Between treatment	3	376.83	0.34^{NS}	3	104253.78	677.9**	3	142291.5	1464.10**	2	264236.6	11340.63**
Error (within treatment)	16	1116.45		16	153.79		16	97.19		12	23.3	

**-Significant at $p<0.01$

NS-Non-significant

Fig. 22: percent efficacy of anthelmintics against *Amphistome* sp. in buffaloes at different days of interval in Lakhisarai district.



CHAPTER - V

DISCUSSION

DISCUSSION

Fascioliasis and amphistomiasis are the commonest problem of cattle and buffaloes of Bihar. Prevalence of these infections is reported in the most part of India. In nature, fresh water aquatic snails act as intermediate host, which are commonly, inhabit in natural bodies like river, lakes, ponds, canals etc. The paddy fields there by also increase the chances of natural infection to these animals. Infection in buffalo is more common as they wallow in these water bodies having snails population and also due to their grazing habit and drinking of water near to the snail infested water reservoirs. Stallfed buffalo supplied with paddy straws carrying infection of these parasites is also important source of transmission of these diseases.

The topography of Bihar comprises of many rivers and Bihar is often flooded with water in Rainy season. When flood water receded the banks of these river become major source of heavy infection because the grasses and banks pasture carrying heavy deposition of infective snail and larval stages of parasites which are ingested by animals during grazing. Therefore present study was undertaken to see the prevalence of *Fasciola* sp. and *Amphistome* sp. in three river belt districts of Bihar viz. Patna, Khagaria and Lakhisarai.

The overall prevalence of *Fasciola* sp. and *Amphistome* sp. by examination of the faecal samples have depicted in table 1, 2, 3 and 4. The overall fluke infection was found to be highest in Khagaria district (78.09%) followed by Patna (73.77%) and Lakhisarai (65.06%). Similar

trends of observation were found to be separately for *Fasciola* sp. and *Amphistome* sp. infections. The infection of *Amphistome* sp. was higher than *Fasciola* sp. infection in all the three districts and the mixed infection was ranged between 9.33 to 15.00%. This could be possible because incidences of these parasites are directly related to availability of intermediate host and their metacercariae. The meterological data of these areas and available of flood prone rivers easily provide the best suitable condition for breeding of snails and multiplication of flukes within a very limited time even metacercariae can also transmit easily at this environment because of grazing practices of buffaloes on newly settle metacercariae in near by grass belt. Further, infections are also carried out in buffaloes that dwell, drink or graze from these areas. The relative humidity and temperature of Khagaria district including Koshi river belt were more favourable for propagation of these parasites. This may be the cause for higher infection in Khagaria. The Kiul river of Lakhisarai district was found to be constricted and it is less prone to flood that is why infection was lower than other two districts. The present finding also related with Sahay *et al.* (1989) where the percentage of infection of paramphistomes was found to be highest in Begusarai districts, which is near to Khagaria district.

Manna *et al.* (1994) reported 72.40% amphistomiasis in buffalo. Sharma *et al.* (1983) reported 60% buffaloes harboured both *Fasciola* sp. and *Amphistome* sp. in Meerut district.

The prevalence of *Amphistome* sp. (16.2%) and *Fasciola* sp. (1.2%) was recorded by Pal *et al.* (2001) in cattle and buffaloes from

Chhatisgarh region which supports the finding of present investigation. On contrary to this Bedarkar *et al.* (2000) reported higher prevalence of *Fasciola* sp. (23.79%) than of *Amphistome* sp. (2.24%) but Sahoo *et al.* (2003) showed predominance of *Amphistome* sp.over *Fasciola* sp. in all the three seasons, which highly confirms the finding of present observation.

EFFECT OF SEASON ON THE PREVALENCE OF *FASCIOLA* SP. AND *AMPHISTOME* SP. IN BUFFALOES

The seasonal prevalence of Patna, Khagaria and Lakhisarai districts are presented in Table 5, 6 and 7 where highest infection was noted in Monsoon season. The range of infection for *Fasciola* sp. in Monsoon was found to be 56.00 to 60.00%. Similarly the amphistomiasis was recorded to be 63.33% in Lakhisarai and 71.25% in Patna district. Khagaria district had highest prevalence (72.94%) of amphistomiasis in monsoon season. In the Winter season the infection ranges for *Fasciola* sp. from 30.77% (Lakhisarai) to highest in Khagaria 43.08%. In the summer season the infection of *Fasciola* sp. was lowest in Lakhisarai and highest in Khagaria district. Similarly the infection of *Amphistome* sp. had 45.38% in Khagaria and Minimum of 20.74% in Lakhisarai. Although the luke infections had recorded to be highest in Monsoon. But infections were persisted in buffaloes through out the year. The infections were significantly ($p<0.01$) lower in Winter and least in Summer. It has been observed that there was no difference in the seasonal distributions pattern of fluke infection among the three districts chosen for the study. The

maximum infections in Monsoon were due to bright sunshine, rainfall, humidity and vapour pressure, which favoured the prevalence of helminthic infection in Monsoon season. The full-fledged rivers act as a reservoir source for the propagation of snails on the banks and high relative humidity including intermittent rain fall strongly favoured the development of larval stages in Monsoon. Seasonal prevalence of *Fasciola* sp. and *Amphistome* sp. of intestinal scraping of buffaloes in Patna district is presented in Table 17.which revealed higher rate of both infection in Monsoon followed by Winter and Summer season. Sahoo *et al.* (2003) also reported maximum gastrointestinal helminthic infections in Rainy (64.70%) season followed by Winter (49.74%) and Summer seasons (37.89%). Other workers reported the highest infection of flukes in Winter. Sahai *et al.* (1986) reported highest incidence of *Amphistome* sp. was in winter season followed by monsoon summer season in different district of Bihar. Sharma *et al* (1983) also reported 80% and 20% highest prevalence of *Amphistome* sp. and *Fasciola* sp. respectively in winter season. Gupta *et al.* (1986), Roy and Tondon (1991), Hirani *et al.* (1999) reported higher prevalence of *Amphistome* sp. in Winter and Summer season. Chiriboga *et al.* (1980) reported that the winter months are more favourable for highest infection of *F. hepatica* due to average of rainfall 145 mm and 24°C temperature, which were more favourable for snail development. D'souza *et al.* (1988) however reported that the prevalence of paramphistomiasis was highest in buffaloes during South-West and South-East Monsoon but Bhatia *et al.* (1989) found highest peak of *Fasciola* sp. infection in buffalo during Winter. Mohpatra *et al.* (1990)

also found 100%, 87.5% and 75% infection of paramphistome in Winter, Rainy and Summer seasons respectively. Prasad and Verma *et al.* (1999) reported that percentage of paramphistomiasis during post monsoon season was observed to be highest as compared to Summer in buffaloes.

PREVALENCE OF *FASCIOLA* SP. AND *AMPHISTOME* SP. IN DIFFERENT AGE GROUPS

The study of influence of age on the incidence of *Fasciola* sp. and *Amphistome* sp. has been presented in Table 8, 9 and 10. The study was carried out in three age groups viz. below 1 year, 1-3 years and above 3 years. It had been estimated that influence of age has highly significant ($p<0.01$) of age is highly significant on the prevalence of *Fasciola* sp. and *Amphistome* sp. in all the three districts. The comparisons within age groups were also found to be highly significant ($p<0.01$) almost between all age groups in all the three districts. The maximum infection was noted in the age group of above 3 years in all the three districts. It ranged between 53.79% to 60.63% for fascioliasis and 62.07% to 71.25% for amphistomiasis which is followed by the age group of 1-3 years and less than 1year of age group for both the infections in all the districts. The infection was found to be highest in Khagaria district within all age groups for both infections. It can be presumed that older animals had relatively higher prevalence of infection with *Fasciola* sp. and *Amphistome* sp. in comparison to lower age group because longer period of exposure of these animals to infected pasture and also colostrum feeding in young animals may reduce the rate of infections. It had also been seen during survey that

the calves were cared with more precaution during provision of their food and water, which also reduces the chances of infection. More over adult animals were mostly dependent on the open grazing system especially in river belt areas, without taking any care and control practices may lead higher infections in adult animals. The present finding has analogous to the finding of Dhar *et al.* (1988) and Pal *et al.* (2001). By examination of faecal samples the incidence of amphistomiasis was reported by Mohpatra (1990) as 66%, 82.5% and 96.66% in the animals between below 1year, 1-3 year and more than 3 years of age respectively. Chiriboga *et al.* (1980) also found highest infections of *Fasciola* sp. (95%) in older cows (9½ years) and lowest (71%) in 3½ years old. No contradictory report was found, so it can be inferred in the present investigation that infections of *Fasciola* sp. and *Amphistome* sp. are common in aged buffaloes in all the three districts of Bihar under their regional weather conditions.

INFLUENCE OF SEX ON THE PREVALENCE OF *FASCIOLA* SP. AND *AMPHISTOME* SP. INFECTION IN BUFFALOES

Influence of sex on the prevalence of *Fasciola* sp. and *Amphistome* sp. in three districts has been presented in Table 11, 12, and 13. The range of *Amphistome* sp. infections in Khagaria district was recorded to be 51.50 to 53.64%, which was highest in all the three districts. Similarly the infection of *Fasciola* sp. was also ranged highest in Khagaria district 45% in male and 46.82% in female. This trend was followed by Patna and later Lakhisarai district. It has been observed that females carried higher rate of infection both *Fasciola* sp. and *Amphistome*

sp. in all the three districts though the difference was not found to be significant in all the cases. This result suggests that both male and females are equally susceptible towards both the infection and as because regional farmer of this area mostly domesticates female buffaloes. So the rate of infection may be higher towards female. More over continuous untoward practices applied by the farmers for the better milk production may reduce the immunity of female buffaloes and so they become much more susceptible towards infection. The present result was contradicted to the finding of Mohpatra *et al.* (1990) as they found highest incidence of paramphistomiasis in male animal 91.66% than 86.33% in female. There was no other work of same type has came across the author.

RATE OF PREVALENCE OF FASCIOLA SP. AND AMPHISTOME SP. BETWEEN STALLFED AND FREEGRAZING BUFFALOES

Study of trematodal infection occurring in buffaloes under two pattern of rearing was evaluated. According to this the buffaloes reared under stallfed condition and freegrazing or partially stallfed have been grouped separately. The observations on the rate of prevalence of *Fasciola* sp. and *Amphistome* sp. between stallfed and freegrazing buffaloes in three districts have been presented in Table 14, 15 and 16. The results broadly underline that the prevalence of both species was more virulent or epidemic in freegrazing buffaloes. The causative factors related to these observations repeated infestation of parasites in freegrazing practice and continuous exposure of buffaloes to contaminated pasture including

condusive environment for the propagation of intermediate host, which usually harbour the infective larval stages. The dispersion of immature stages in river bank pasture resulted the chances of contact between host and parasite in freegrazing system. The topographical conditions of these districts are very much suitable for the chances of repeated infection. The catch areas of receding flood of Koshi, Ganga and Kiul river also falling prey to chronic parasitic infestation.

More over the absence of literacy psychological fads, discomposing accessibility of expertise management and lack of swift procurement of medical help intensified the situation.

Incidentally the degree of infection found in stallfed animal is equally alarming as it caused by transmission through drinking water and contaminated feed, hay which usually act as carrier for the transmission of metacercaria.

The condition is not so visious in Patna naturally due to easy accessibility of owner to the control practices and preventive measures. The rate of awareness is higher than other two districts about scientific management practices especially under stallfed condition. In Khagaria and Lakhisarai district both stalled and freegrazing animals almost equally suffered (non-significantly) with fascioliasis and amphistomiasis. The lack of awareness in control practices and medical consultation attributed with the higher rate of infection in both the pattern of rearing. Owners of freegrazing animals are too poor to afford anthelmintic and medical guidance.

The higher rate of infection in free grazing cattle had observed by Sahoo *et al.* (2003). Gupta and Singh (2002) also suggested the various above mentioned reasons for the higher rate of fluke infection in free grazing system. However Borthakur and Das (1998) suggested that in free grazing system animals acquired resistance towards various infections.

HAEMATOLOGICAL OBSERVATIONS

Studies on the various haematological changes in naturally infected buffaloes with *Fasciolia* sp. and *Amphistome* sp. has been presented in Table 17 and 18.

Fasciola gigantica is the most common liver fluke of buffaloes of Indian subcontinent and the *Gigantocotyle explantum* is the most common Indian *Paramphistomum* species of buffaloes. Both these trematodes usually affect the hepatobiliary system of the animals. It has been recognized that immature stages of flukes cause severe haemorrhage to liver and bile ducts. This cause deleterious affect on vascular systems as the most of the clinical symptoms usually associated with various gastrointestinal disturbances. Therefore the present study undertaken to know the various haematological changes found in naturally occurring infection of *Faciola* sp. and *Amphistome* sp. in buffaloes of Patna district only in comparison with healthy group of buffaloes.

It is evident from the Table 17 that significant ($p<0.01$) reduction was found in haemoglobin percentage, Total Erythrocyte Count and Packed Cell Volume in both the cases of infection from control to healthy animals. More over the 't' value revealed marked difference

between the infection of *Amphistomes* sp. and *Fasciola* sp. Total leucocyte count (TLC) increased significantly ($p<0.01$) both in cases of *Amphistome* sp. and *Fasciola* sp. infection from control group and in case of *Fasciola* sp. it was also significantly ($p<0.01$) higher than amphistomiasis infected buffaloes. The accelerated values of Erythrocyte Sedimentation Rate (ESR) were also noted in both cases and more significantly ($p<0.01$) was found in fascioliasis than amphistomiasis.

The migrating behaviour of immature and adult stages of these parasite in bile duct, gall bladder, duodenum, peritoneal cavity and intestinal wall caused marked perforation in this areas which cause erythropoesis and other physiological changes to the host. In the present study the buffaloes infected with *Fasciola* sp. and *Amphitome* sp. revealed reduced values of Total Erythrocyte Count (TEC), Haemoglobin (Hb) and Packed Cell Volume (PCV) which indicated anaemia in both the cases, the low PCV also associated with low level of haemoglobin. The first degree defence mechanism was attributed with the increased level of Total Leucocyte Count (TLC) which is evident in case of any infection. The higher value of Erythrocyte Sedimentation Rate (ESR) usually associated either with the chronicity of any infection or influence in the level of many infectious diseases. In certain cases severe anaemia is responsible for increasing the rate of erythrocytes sedimentation (Sinha 1998).

The possibility of increased level of ESR in present study was also related with these facts. Swarup *et al.* (1987) also reported the same finding in naturally occurring fascioliasis and biliary amphistomiasis in buffaloes. Gunrinade and Bang bo ye (1980) also described lower level of

Hb%, RBC, PCV during fascioliasis. The low PCV also associated with low level of Hb%. The differential count of W.B.C. of *Fasciola* sp. and *Amphistome* sp. infected group of animals and its comparison with healthy group of animals have revealed that the neutrophil was significantly ($p<0.01$) more decreased in case of *Fasciola* sp. than *Amphistome* sp. Percentage of Lymphocytes was significantly ($p<0.01$) more high in *Fasciola* sp. group than *Amphistome* sp. group. However significant increase in Monocyte count was found in case of amphistomiasis though it was higher side for *Fasciola* sp. also. Eosinophil was markedly higher in *Fasciola* sp. infected buffaloes than amphistomiasis cases as compare to healthy group of animals. Similar observations had noted by Swarup *et al.* (1987), Kumar *et al.* (1982). They suggested that eosinophilia is an indication of immuno responses of proteins during parasitism. Reduction in the number of neutrophils may be due to the invasion of liver and other organs during the migratory action of the flukes. The increased lymphocytes usually appeared during intermediary defence process. These values also increase the TLC, which also reflected the perpheral leucocyte response to chronic infection. Chaudhari *et al.* (1988) also found significant ($p<0.01$) decrease in the values of Hb%, PCV and TEC in infected buffaloes with fascioliasis, the present finding also corresponds the above facts.

EFFICACY OF ALBENDAZOLE, CLOSANTEL, OXYCLOZANIDE + LEVAMISOLE AGAINST *FASCIOLA* SP. AND *AMPHISTOME* SP.

Fascioliasis and amphistomiasis are causing major economic losses in domestic ruminants including buffalo. In Bihar the control practices applied by the owner and the strategic dosing of anthelmintic against flukes in ruminants varying as per the interprising nature of the owner. The anthelmentic treatments during the month of Monsoon season and post monsoon months have been recommended for this area but the application of anthelmintics under field condition not only dependent upon requirement facilitated upon but also upon cost for effective treatment of diseases. Without provision of any proper anthelmintic devices during administration of drugs also creates the problem of over dosing or under dosing. Farmers use to Indiscriminate practices of anthelmintics which may be responsible for creating a very less but a certain extent of drug resistance against flukes. Keeping in view in the present investigation three most common and widely chosen drugs viz. albendazole, closantel, oxyclozanide + levamisole against fluke infection has been evaluated and their comparative efficacy were also evaluated on the basis of declining rate of EPG of faeces in the three districts of Bihar.

In Patna district albendazole was found to be 88.57% effective after 21 day against fascioliasis where as closantel had shown over 96.22% efficacy within 14 day. The combination of oxyclozanide + levamisole was found to be 93.80% effective only within 7 days. So it can be determined from the present observation that combination of

oxyclozanide + levamisole at 22.5 mg/kg (15 mg/kg + 7.5 mg/kg respectively) single oral dose is the most effective than other two drugs. However closantel can be recommended as it was found more than 95% effective within 14 day. Similar observation was found by Sahoo *et al.* (2002) in the field trial on anthelminthic viz. albendazole closantel and oxyclozanide. The highest percentage of efficacy was recorded with oxyclozanide (91.8%) followed by closantel (75.5%) and albendazole (64.8%).

The oxyclozanide + levamisole was however found to be only 74.06% effective within 7 days in case of amphistomiasis in Patna district which had been increased 91.76% on 14 day and 99.15% on 21 day. Though it was found the most powerful than other two drugs taken for trial but its effectiveness was lower than fascioliasis treatment. Closantel had also 96.72% efficacious within 21 day. The trend of results for all drugs followed the trend of fascioliasis treatment in Patna district. By the examination of slaughtered calves Genchi *et al.* (1986) found on 36 day after treatment that oxyclozanide (10 mg/kg body wt.) had lower efficacy than albendazole against immature flukes. But closantel 5 mg/kg body wt. eliminated 99.8% of parasites and found superior than rafloxanide for the treatment of fascioliasis.

In Khagaria district all the drugs had shown 98-100% efficacy within 21 day and the oxyclozanide + levamisole had its efficacy of 99.04% even within 7 day, albendazole had more than 90% efficacy within 14 day for the treatment of fascioliasis. Similar trend of result were also observed in the case of treatment of amphistomiasis but the effective

efficacy was reached to be 96.72% within 14 day for oxyclozanide + levamisole and closantel was in 21 day (93.22%) The efficacy of albendazole was recorded to be lowest (87.57%) on 21 day, which is less to even Patna district. It can be estimated that all the drugs can be recommended against fascioliasis but closantel and oxyclozanide + levamisole had better choice for the treatment of amphistomiasis in Khagaria district.

Rolphe and Boray (1987) suggested that oxyclozanide (18.7 mg/kg) + levamisole reduce parasite number in small intestine, abomasum and reticulum upto 96.1%, 92.61% and 98.1% respectively, where as faecal egg count of paramphistomes were reduced by 96.3%, 97.5% on administration of single or double dose at the rate of 12.8 to 8.7 mg/kg body wt. a for oxyclozanide.

In Lakhisarai district both closantel and oxyclosanide + levamisole were found to be almost 90% and cent percent effective within 14 and 21 days respectively both in case of fascioliasis and amphistomiasis. The performance of albendazole found to be less effective.

By the comparison of each drug in all the three districts against fascioliasis and amphistomiasis had recorded. The efficacy of albendazole was found to be lowest and ranges between 88 to 98% for fascioliasis and 86.78 to 89% for amphistomiasis. Closantel showed better performance in all the three districts than albendazole. The combination of drug (oxyclozanide + levamisole) was found to be the most efficacious as

per its dose rate at 22.5 mg/kg for the treatment of both infections in all the districts.

All the drugs were found to be more effective against fascioliasis than amphistomiasis. It can be suggested that farmers use to indiscriminate practices of anthelmintics which may be responsible for creating a very less but a certain extent of drug resistance against amphistomiasis than fascioliasis in all the river belt districts of Bihar. However on the basis of 21 day observation the trend of drug performance was found almost same in all the three districts of Bihar.

Georgiev and Gruev (1979) reported that the oxyclozanide (10 mg/kg) was responsible for reduction of 74.84 to 89.87% of *Paramphistomum* but combined administration of oxyclozanide + levamisole (15 + 12 mg/kg body wt.) was 87.91 to 99-96% effective. Ronald *et al.* (1979) evaluated that 35-45 mg/kg albendazole was 99-100% effective against immature and mature stages of *F. hepatica* in cattle. Theodorides and Freeman (1980) suggested that only 10 mg/kg albendazole was safe and effective (93 to 95%) against egg and larval stages of *F. hepatica.* Malone *et al.* (1982) also found 10-15 mg/kg body wt. of albendazole had 86 to 93% effective against immature flukes. These all findings have close concurrence with present observations. However Genchi *et al.* (1986) found that albendazole (10 mg/kg) had higher efficacy than oxyclozanide (10 mg/kg) against immature flukes and closantel (5 mg/kg body wt.) responsible for clinical of 99.8% of adult flukes. Monstretta and Pera (1981) also reported that administration of closantel resulted the better performance. The present study is also

supported by the reports of Rolfe and Boray (1987) who observed that 2 doses at 3 days apart of oxyclozanide + levamisole was 100% effective against paramphistomiasis. Mohpatra *et al.* (1990) also suggested that Albomar (albendazole) 15 mg/kg body wt. was proven to be 85.40% effective against mature paramphistomes. Alan *et al.* (1995) also found 78.5% efficacy of albendazole against fluke infestation. On the contrary of present finding Yakubovski and Lipnitski (1995) evaluated that albendazole (97 to 100%) was superior to closantel (93-94%) at their performances against fascioliasis in ruminants but Mage *et al.* (1993) reported that closantel (2.5 or 5 mg/kg) was found to be 100% effective at 60 and 100 days after treatment and Aziz *et al.* (1999) reported 92.5% effectiveness of oxyclozanide against fluke infection.

The findings of present observation has close resemblance with reports of Sahoo and Mohanty (2002) who received the percent efficacy of oxyclozanide (91.8%) followed by closantel (75.5%) and albendazole (64.8%) at the dose rate of 10 mg/kg, 15 mg/kg and 15 mg/kg body wt. respectively in naturally infected cattle with amphistomiasis. The present results are also similar to the study of Pal *et al.* (2003) who revealed that buffaloes carrying naturally infection of *F. gigantica* and *Amphistome* sp. treated with oxyclozanide at 10 mg/kg body wt. were 100% and 86.7% effective against respective infections.

CHAPTER - VI

SUMMARY AND CONCLUSION

SUMMARY

The problems of fascioliasis and amphistomiasis are widely prevalent among buffaloes of the riverbelt districts of Bihar due to inundation of large parts which serve as main source of propagation of snails and influence, the conducive development of infective stages of flukes. The free grazing practices and wallowing nature of buffaloes increase the chances to get more infection in river belt. Therefore the present investigation was conducted to study the prevalence of *Fasciola* sp. and *Amphistome* sp. among buffaloes in three river belt districts of Bihar viz. Patna, Khagaria and Lakhisarai which are situated either banks of Ganga, Koshi and Kiul rivers respectively.

The prevalence of both fluke species were found to be highest in Khagaria followed by Patna and Lakhisarai districts. The seasonal prevalence of *Fasciola* sp. and *Amphistome* sp. were also found to be highest during Monsoon season in Khagaria district followed by Winter and Summer seasons. However the trend of seasonal prevalence was observed to be same in other two districts. But it was depicted that high percentage of relative humidity and temperature, near Koshi river belt in Khagaria was more favourable condition for the propagation of these parasites whereas Kiul river of Lahkisarai district has constricted and thus chances to get low infection. The study of influence of age indicates that these two parasitic species were most prevalent among the age group of more than three years whereas 1 to 3 years and below one year of age group were found to be comparatively less susceptible towards these

diseases. The trend was found to be almost same in all the three examined districts. It is concluded that animals supplied with longer period of exposure to contaminated pasture increase the chances of repeated infection in old animals, whereas colostrum feeding and extra precautions to the buffalo calves and young animals may chance to get less infection.

The influence of sex was found to be non-significant in all the three districts. It is suggested that both male and female are equally susceptible for both infections but female buffaloes were mostly domesticated by regional farmers of these area. So the rate of infection was found to be high in female in all the three districts.

The influence of management was quite varied in three examined districts, but in overall it was concluded that free grazing buffaloes suffer more than stallfed animals for both infections. It was attributed that the rate of incidence of both infection in stallfed and free grazing buffaloes totally dependent upon the awareness of healthy management practices, regular deworming and other control and preventive practices applied by the owners and farmers of a particular area. This awareness was quite profound in owners of Patna district where both amphistomiasis and fasciliasis were significantly more prevalent among free grazed buffaloes which are mostly domesticated by poor and illeterate farmers without having much awareness of control measures. The reasons were same for Khagaria district where both stallfed and free grazing animals almost equally suffer for both infections as it may concluded that it was because of having least awareness among local owners of that area and moderate in Lakhisarai district.

F. gigantica is the most common sp. of *Fasciola* and *Gigantocotyle explanatum* is the most common prevalent species of paramphistome in buffaloes in Indian subcontinent. Both trematodes greatly affect the hepatobiliary system of an animal by the migratory behaviour of their immature stages and thus causing haemorrhage in many areas which attributed with the increase number of leucocytes, anaemia and esonophilia in the haematological examination of the present study.

In present investigation the efficacy of three anthelmintic drugs viz. albendazole (10mg/kg) closantel (7.5mg/kg) and oxyclozanide + levamisole (22.5mg/kg) against both fluke infections had been evaluated on the basis of declining rate of EPG in all the three districts. All the drugs were found to be more effective against fascioliasis than amphistomiasis. It can be suggested that the high rate of incidence of *Amphistome* sp. than *Fasciola* sp. due to indiscriminate use of anthelmintics by owners which may be responsible for creating a very less but a certain extent of drug resistance against amphistomiasis than fascioliasis in all the river belt districts of Bihar. On the basis of 21 day observation the trend of drugs performance were found to be almost same i.e. 95% to 100% effective in all the three districts but on the basis of observation on 7 day and 14 day. It can be suggested that performance of oxyclozanide + levamisole was the best drug against fascioliasis and amphistomiasis followed by closantel and albendazole at their suggested doses in the present experiment. However closantel had its peek performance in Khagaria district for fascioliasis and in Lakhisarai district for amphistomiasis. Albendazole had lowest efficacy than other two drugs and found to be its better

performance in Khagaria district for fascioliasis and in Patna for amphistomiasis. Oxyclozanide + levamisole was proven to be best at its dose rate in all the three districts for both infections.

CONCLUSION

On the basis of present investigation, following conclusions were drawn:-

1. Amphistomiasis was more prevalent than fascioliasis in all the three examined districts viz. Patna, Khagaria and Lakhisarai. The most prone area was found Khagaria for both infections, followed by Patna and Lakhisarai.

2. Both diseases were the most prevalent in Monsoon season and in the age group of more than 3 years in three examined districts. So it is advisable to owners to take extra preventive measures before onset of Monsoon and after Monsoon because intermittent rain and receding of flood water cause grow of fresh green vegetation which are grazed by buffaloes and get infected with diseases, as the infective stages heavily deposited in vegetation.

3. Intermediate host snails should also be destroyed by common methods. It had seen that the animal kept on free grazing system as well as partially stallfed were highly affected with these parasites. So farmers should take adequate precautionary measures and immediately consult veterinary physician with the onset of diseases.

4. Amphistomiasis is commonly known as "Chhera" in local language because semisolid or watery faeces of affected animals spread in a wide area whereas fascioliasis is known as "Sara" as it cause rottening of liver.

5. *Fasciola* sp. and *Amphistome* sp. markedly hamper the normal function of not only gastrointestinal system but also affect the hepato-biliary mechanism. The migratory stages cause to damage of different organs and marked decrease in the level of haemoglobin and eosinophils resulting into anaemia and eosinophilia. Weakness and dehydration leads to many secondary infections, usually cause death of animals.

6. The comparative efficacy of albendazole (10mg/kg) closantel (7.5mg/kg) oxyclozanide + levamisole (22.5mg/kg) against naturally infected buffaloes with *Fasciola* sp. and *Amphitome* sp. did not reveal any chances of drug resistance in all the three districts. The combination of drug (oxyclozanide + levamisole) was found to be the most effective for both infections and had almost 100% efficacy in all the three districts. The performance of closantel was next to above drug and had more than 95% efficacy in all the districts. However albendazole was found to be least effective than these two drugs.

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APPENDIX