1. INTRODUCTION

1.1 Background Information

The most of the digenetic trematodes are vertebrate endoparasites. Their adult stages are found in intestine, lungs, liver, gall-bladder, bile passages, and urinary bladder. The parasites are also found in kidneys and ureters, air sacs of birds, other parts of the respiratory system, coelom, eye and various head cavities. Some genera like *Sanguinicola*, *Schistosoma* inhabit the blood of their definitive host (Hyman, 1993).

The Digenea, have complicated life cycle. The "typical" life cycle includes four larval forms which are found in two, three, four or sometimes more as definitive and intermediate or paratenic hosts (Cheng, 2006). The parasite passed its adult stage and completes sexual reproduction in the definitive hosts. In the intermediate hosts, the parasite assumes various larval forms, each developing asexually from the preceding generation. The paratenic host (also called carrier host) is not necessary for the completion of the parasite's lifecycle, but is utilized as a temporary refuge and vehicle for reaching on obligatory host (usually the definitive host).

The freshwater snails like *Biomphalaria* spp; *Bulinus* spp; *Lymanea* spp; *Indoplanorbis exustus* involved as intermediate hosts of many digenetic trematodes. Some of them like *Schistosoma* spp; *Fasciola* spp; *Clonorchis sinensis, Paragonimus westermani* and *Paramphistomum cervi* are global health problem of human, livestock and wildlife. The blood flukes infecting warm blooded animals belong to the family Schistosomatidae (Loss, 1899 cited in Cheng, 2006). There are 13 recognized genera and approximately 100 species of schistosomes, which infect both pulmonates and caenogastropod snails. Adults of these parasites parasitize crocodiles, birds and mammals (Basch, 1991; Platt and Brooks, 1997). Some flukes belonging to families Sanguinicolidae and Spirorchiidae also infect fishes and chelonians (Graff, 1907; Stunkard, 1921) respectively. These blood flukes inhabit the hepatic portal system and pelvic veins of their vertebrate hosts.

Schistosomiasis is one of the most widely distributed and interactable human disease (Snynder and Loker, 2000) and currently infect 200 million people in 74 tropical and subtropical countries in the world (Savioli et al; 1997). Some species are destructive parasites of domestic animals in Africa and Asia (Rollinsom and Southgate, 1987). Schistosomes are causing agents of "Swimmer's Itch" which is a common global problem for users of recreational swimming areas and can be contracted in fresh, brackish and salt water (Hurley et al., 1994). Schistosomiasis is also common among the Asian elephants. A number of adult worms of schistosomes were recovered from a domestic elephant, which died in 1999 in Sri Lanka (Agatsuma et al; 2004).

The trematodes, *Fasciola hepatica* and *F. gigantica* are parasites of herbivores and also infect humans accidentally. Human fascioliasis are found in areas where sheep and cattle are raised, and where humans consume raw watercress, including Europe, Middle East and Asia. Esteban et al. (2003) detected 5.2-19 percent human fascioliasis in Egypt.

Fascioliosis in cattle and sheep is a next serious economic problem of the agricultural field in the world. Olsen reported that the livers of 1,400,000 cattle and 60,500 calves have been condemned as unfit for human consumption due to fascioliasis in USA. Approximately 1 million pounds of liver were involved (Cheng, 2006). Fascioliasis among Asian elephants is very common and produce signs of anorexia, constipation or diarrhoea, anaemia, elevation of intrahepatic blood pressure and hypoproteinemia

(Fowler and Mikota, 2006) and young elephants were the most affected (Saidul, 1997).

Infection by adult members of the family Paramphistomatidae may be found in sheep, goats, cattle and water buffalo. The disease paramphistomiasis confined to Africa, Asia, Australia, Eastern Europe and Russia caused by massive infection of the small intestine with immature paramphistomes (Horak, 1971)

In Africa many cattle and sheep are infected with adult paramphistomes (Eisa, 1963; Dinnik, 1964 cited in Horak, 1971). These infections acquired by the ingestion of small numbers of metacercariae on one or several occasions cause no harm to the host, mature rapidly and serve as a source of infection for successive generations of snails. Paramphistomes may survive for some years in their cattle hosts. The source of infection is virtually constant (Dinnik, 1964 cited in Horak, 1971). In Northern Thailand, approximately 41 percent of the animals were found to be infected with trematodes, *Paramphistomum* spp. accounting most of the cases. The parasite does not generally cause severe clinical disease, but heavy infestation affects an animal's production and growth and an athelmintic treatment is recommended (Padungtod et al., 2001 cited in Perry et al., 2002). Paramphistomiasis is reported to cause serious economic losses among goats in India (Singh, 1993 cited in Perry et al, 2002).

The strigeoid digeneans are parasites of aquatic reptiles, aquatic birds, and mammals that feed on aquatic amphibians, fish, and invertebrates. The biology of this group of flukes has been extensively reviewed and studied by Dubois (Cheng, 2006).

1.2 Lifecycle Pattern of Digenean Trematodes

The adult digenean trematodes lay eggs in a definitive host. Eggs are passed out with feces which are directly hatched in water or ingested by the freshwater snails then hatched in miracidium in gut. The miracidium has developed into sporocyst. The sporocyst either developed into daughter sporocyst as in *Schistosoma* spp. or developed into redia as in *Fasciola* spp. The redia sometimes develops into daughter redia. The daughter sporocyst, redia or daughter redia are developed into cercaria which in some case don't leave the molluscan host and ingested with snail by a definitive host as in *Leucochloridium macrostoma.* In some cases the cercaria develops into free swimming cercaria in water. The cercaria further develops into encysted metacercaria attached with vegetation as in *Fasciola* sp. Upon grazing definitive hosts swallow metacercaria along with grasses and developed into adult. Some cercariae penetrate body of fish or arthropods and develop into metacercaria in second intermediate host as in *Clonorchis sinensis.* The second intermediate host again ingested by definitive host and the metacercaria released in the intestine and developed into an adult. In some cases like *Schistosoma* spp. the free swimming cercaria directly penetrates the definitive host and develop into an adult (Figure 1).

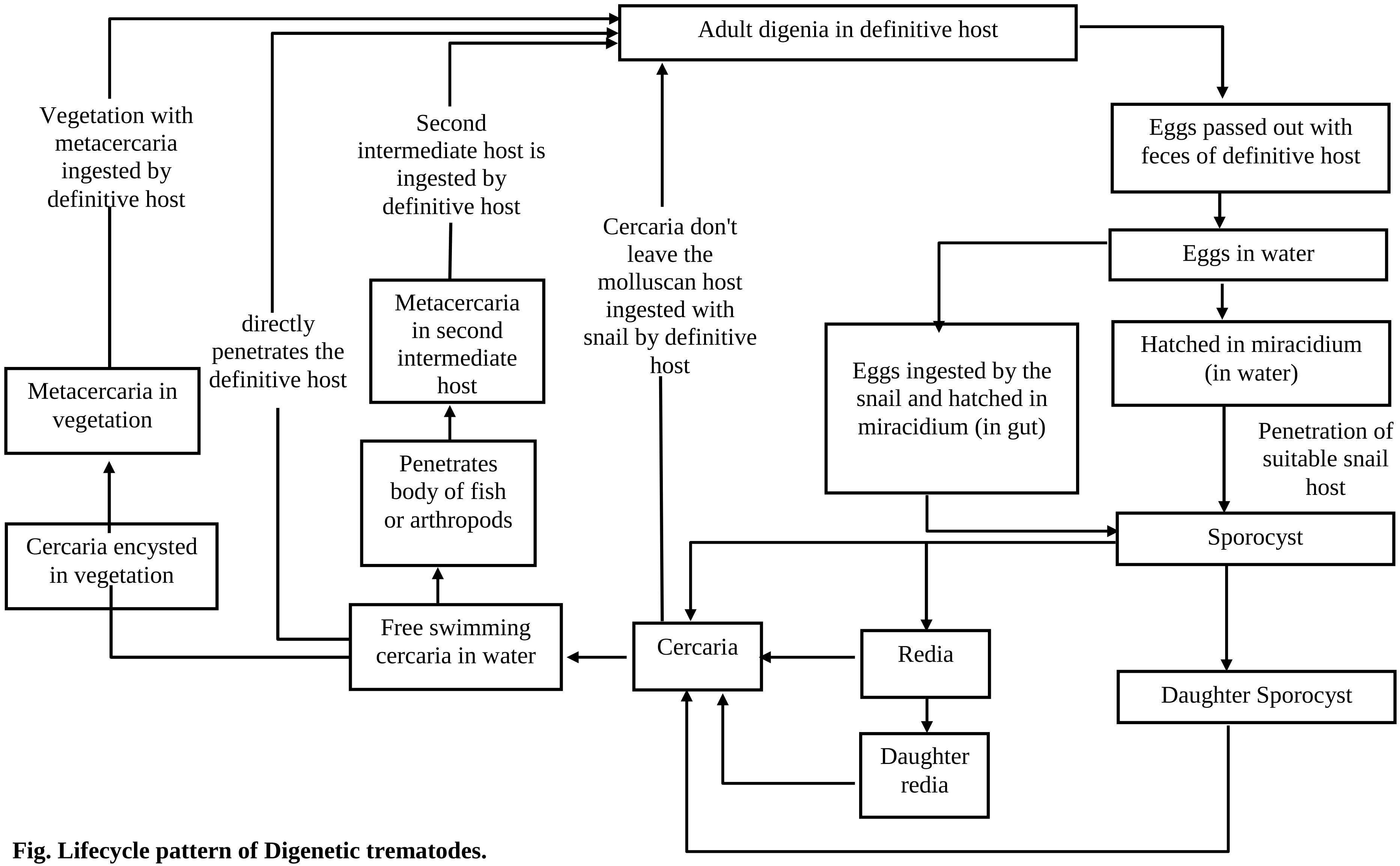

Fig. Lifecycle pattern of Digenetic trematodes.
Cheng, 2006; Chandler and Clark, 1961; Chatterjee, 1998; Hyman, 1993 and Smyth, 1996

1.3 Factors Influencing the Emergence of Cercaria

Different larval stages of trematodes may co-exist in a single snail. It follows that all the cercariae do not mature at the same time and as a consequence they are likely to leave the snail over an extended period.

Although the emergence of cercariae from any individual snail occupies a considerable period of time, it is not a continuous process, groups of parasites leaving the snail at intervals. The pattern of emergence seems to be partly determined by an innate rhythm and is greatly influenced by external factors. At normal temperature the most important stimulus is provided by light, while temperature itself has a secondary importance (McClelland, 1967).

Kendall and McCullough (1951) investigated the factors such as change in temperature, depletion of oxygen, an increase in the concentration of carbon-dioxide and a change in the hydrogen ion concentration of the water have great influence in the emergence and survival of cercariae.

The cercariae of *Paramphistomum microbothrium* are stimulated to emerge by exposing the infected snails to light, and greater the light intensity the more cercariae will emerge (Lengy, 1960 cited in Horak, 1971). The majority of cercariae are shed within 4 hrs. of exposure to light and the infected snails continue to shed cercariae for about a year (Dinnik and Dinnik, 1954 cited in Horak, 1971).

The minimum temperature for cercarial emergence in *F. hepatica* is 9^0C, however, no evidence of emergence is inhibited at high temperatures (Kendall and McCullough, 1951). It appeared that cercariae emerged equally at 26^0c. Kendall and McCullough (1951) also suggested that light do not appear to be an important factor for the cercarial emergence of *F. hepatica* from *Lymnaea truncatula*. Emergence of cercariae found to be occurring equally by day and night. Furthermore, no relationship between the emergence of cercariae from the snails recorded at any particular period of the day or night.

1.4 Significance of the Study

Many freshwater snails act as an intermediate host of different digenean trematodes of birds and mammals including human beings. But the knowledge on particular host snail and parasites are poorly known in Nepal. Due to lack of trained medical malacologists in the country, host-parasite relationship of these both important taxa are virtually ignored by parasitologists as well.

Studies on larval trematode infections in freshwater snails in Nepal are rare in spite of the fact that the documentation of snail species and their larval trematode fauna help in our understanding of snail-borne diseases. Larval trematodes may act as regulators of snail populations, if prevalence of infection in natural populations are high (May, 1983; Brown et al; 1988). It is claimed that certain trematodes may in some cases be responsible for the elimination of snail populations (Loker et al., 1981). In order to assess the regulatory effect of larval trematodes on snail populations, the natural prevalence of trematode infections must be determined. Studies on larval trematodes can also reveal the possible existence of certain trematode species that could be manipulated to achieve biological control of diseases transmitted by snails (Combes, 1982; Davis, 1998). Boray (1967) established that an unidentified echinostome is dominant over *F. hepatica* in *Lymnaea tomentosa*. Larval trematode infections can also be used as bio-indicators of environmental quality (Kuris and Lafferty, 1994; Keas and Blankespoor, 1997).

However, information on the abundance, diversity and intermediate host relationship of trematode species is virtually at nix. The aim of the present study is to fill the gap of information by documenting freshwater snails and parasitic infection in them in Chitwan and Nawalparasi districts.

1.5 Limitations of the Study

- At the beginning of this study, the good microscope was not available, so some of the cercariae might be missed or overlooked during the preliminary study period.
- The study was primarily done on trematode infection in freshwater snails however few elephant dung samples were also analysed. Human beings and other domestic animals were not included.
- The snails collected were not crushed or dissected for pre-patent and patent infection observations. But the snails were kept alive and monitored the emergence of the mature cercariae usually termed as 'patent infection'. (The cercariae like Gymnocephalous: cercaria of *Fasciola* spp.) cannot be detected easily in 'patent infection').
- The identification was based on only morphology and not at molecular level.

1.6 Objectives

The general objective of the study was to determine the trematode cercariae infection in freshwater snails in Chitwan and Nawalparasi district. The specific objectives of the study are to;

i. identify trematode cercariae recovered from freshwater snails of Chitwan and Nawalparasi districts,

ii. find intermediate host snails of digenean trematode parasites,

iii. evaluate prevalence rate of trematode cercariae in different habitat types,

iv. ensure if domestic elephants of Chitwan were infected from any digenean trematode parasites.

2. LITERATURE REVIEW

The famous Ebers papyrus (1550 B.C) contained some of the earliest records of the presence of parasites in man. He described four worms, evidently *Ascaris lumbricoides*, *Taenia saginata*, *Dracunculus medinensis* and possibly *Schistosoma haematobium*. Important advances in helminthology including clinical description of urinary schistosomiasis and filarial elephantiasis were made by Arabic and Persian Physicians upto 1200 A.D. (Belding, 1953).

The knowledge of trematode begins with Gabucinus in 1547, who described the occurrence of the liver fluke in sheep, which was, however, referred to by Jehan de Brie as early as 1379 (Benham, 1901). Zeder (1800) classified the parasitic worms or "Helminths" into five families to which he gave German equivalents of (a) Round worms (b) Hooked worms (c) Sucking worms (d) Tape worms and (e) Bladder worms. The term "Trematoda" was invented by Rudolphi (1808) for Zeder's "Sucking worms" (Benham, 1901. In 1842, Steenstrup recognized cercariae as larval flukes which previously considered being adult animals and disclosed that the life cycle of the digenea involves an alternation of hosts (Belding, 1953).

2.1 Brief Review of digenean trematodes and their cercariae at global context

Montgomery (1906) described *Schistosoma bomfordi* and *S. spindalis* from the portal veins of cattle and *S. indicum* from that of horse, donkey and sheep. He also recorded *S. bovis* from sheep in Bareilly and Lahore districts and pointed that eggs of *S. haematobium* reported by Bomford might be that of *S. indicum* as no infection of *S. haematobium* in cattle was observed since.

In 1910, Ruffer demonstrated microscopically the presence of calcified blood fluke ova in the nuclei of urinary calculi and in the cortex of kidneys of mummies (1200-1090 BC).

Leiper (1915) discovered that *S. haematobium* and *S. mansoni* had similar life cycle and proved that *Bulinus* sp. served as intermediate host for the former and *Planorbis boissyi* for the latter.

Liston and Soparker (1918) worked out the life history of *S. spindalis* by artificially infecting a kid with furcocercous cercaria liberated by the snail *Indoplanorbis exustus* and by infecting the snails with the miracidia hatched out of spindle shaped eggs obtained form a goat.

Sewell (1919) recorded the occurrence of a cercaria, resembling that of *S. japonicum*, in *I. exustus* and *Lymnaea amygdalum* in Calcutta. He also (1922) recovered cercariae indicae xxx, from these two snails, which was proved to be the larval stage of *S. nasalis* by Rao (1934).

Malkani (1932) published a short note on a form of nasal schistosomiasis in which Cauliflower like growths develop on nasal septum of cattle. Datt in same year published a detailed account of the symptoms and histopathology of this disease. Rao in 1934 named this parasite as *S. nasalis*.

Mudalier and Ramanujachari (1945) described a new blood fluke, *S. nairi* from elephants, which was transferred to the genus *Ornithobilharzia* by Bhalerao (1947) and subsequently to *Bivitellobilharzia* by Dutt and Srivastava (1955).

Benglapedia (1988) reported the prevalence of *S. spindalis* and *S. indicum* among cattle all over Bangladesh. He also pointed out that the adult cattle above 3 years of age were severely affected upto 25 percent incidence.

He further suggested that the *S. nasalis* was widespread among cattle and buffaloes all over the country.

Agatsuma et al. (2004) performed the phylogenetic analysis using the nuclear DNA of the elephant schistosome, *Bivitellobilharzia nairi*, discovered in Sri Lanka from a domestic elephant and showed that *B. nairi* is basal to all of species of the genus *Schistosoma*.

Varma (1961 cited in Horak, 1971) explained that the miracidia of *Cotylophoron cotylophorum* seemed to be attracted to the intermediate snail host *I. exustus* and after penetration develop into sporocysts. The first free rediae were found after 6 days. The first cercariae were shaded after 26 days.

Chakraborty and Chaudhary (1992) studied on the pathology of *F. jacksoni* infestation in elephants. They discovered infection in 2 out of 3 elephants autopsied at Assam State Zoo, India, during 1985 to 1989.

Hurley et al. (1994) studied the larval trematodes in freshwater snails with emphasis on *Trichobilharzia* sp (p); causative agents of Swimmer's itch. They reported three Pulmonate snails a lymnaeid, *Austropeplea lessoni*, and two planorbids *Gyraulus gilberti* and *Amerianna carinata* and one prosobranch snail, *Thiara balonnensis* were common in the reservoir which were infected with trematode cercariae representing six different families. Authors suggested both *A. lessoni* (4.5%) and *G. gilberti* (1.8%) act as intermediate hosts of *Trichobilharzia* sp. and trematode infection levels increased as snails size increased.

Saidul (1997) studied on some aspects of fascioliasis in wild and captive Asian elephants (*Elephas maximus*) in Assam, India. He recorded the prevalence rate of 33.78 percent in wild elephants and 42.50, 62.28 and 18.18 percent in captive elephants according to locality. He also recorded a diurnal fluctuation in fecal egg count, with average counts of 4.89, 2.47 and 2.76 during the morning, noon and evening respectively. His study also showed that young animals were most affected by the parasite.

Chingwena et al. (2002) collected a total of 13,789 freshwater snails representing ten species from 21 sites from the highveld and lowveld areas of Zimbabwe to determine the occurrence of larval trematodes and recorded that 916 (6.6%) harboured patent trematode infections representing eight morphologically distinguishable types of cercariae. They reported that *Bulinus tropicus* had the highest overall prevalence of infection (13.1%) and the Echinostome was the most common type of cercariae recovered, contributing 38.2 percent of all infections. They recovered schistosoma cercariae mainly from the highveld and comprised 8.0 percent of all infections. In their study Amphistome cercariae contributed 37.6 percent of all infections and were recorded from both the highveld and lowveld areas with a peak prevalence occurring during the post-rainy period (March-May).

Chingwena et al. (2002) examined the susceptibility of *B. tropicus, B. globosus, Biomphalaria pfeifferi, Lymnaea natalensis* and *Melanoides tuberculata* to *Calicophoron microbothrium*. They reported that *B. tropicus* had the highest prevalence (65.0%) followed by *B. pfeifferi* (37.5%), *B. globosus* (6.8%) and *Melanoides tuberculata* (5.9%). Their study also suggested that *B. tropicus* snails infected with *C. microbothrium* alone or co-infected with either *S. haematobium* or *S. mattheei.*

de Kock and Wolmarans (2004) studied the distribution and habitats of *Gyraulus connollyi* snail intermediate host of intestinal flukes of the family Echinostomatidae, in South Africa and reported the presence of this species from 13 different types of water bodies, however, the highest percentage by far was collected in streams and rivers and in habitats of the water was described as perennial, clear and fresh.

Kirinoki et al. (2005), performed the comparative studies on susceptibilities of two different Japanese isolates on *Oncomelania nosophora* to three strains of *S. japonicum* originating from Japan, China and Philippines. They reported that both isolates of *Oncomelania nosophora* showed high susceptibility to the Japanese strain of *S. japonicum* (74.0% - 82.2% for the Nirasaki isolate and 58.0 - 56.0% for the Kisarazu isolate) and low susceptibility to the Chinese stain (0.0% - 1.3% and 1.4% - 79% respectively).

Miura and Chiba (2007) studied the effects of trematode double infection on the shell size and distribution of snail hosts. They reported that of the eight trematode species found in *Batillaria attramentaria* in Japan, *Cercaria batillariae* (40.2%) and the renicolid cercaria I (13.0%) were the most prevalent species in the study. In addition, 7 percent snails in their study were simultaneously parasitized with both trematode species *(Cercaria batillariae* and the renicolid) and other species contributed a small proportion to the total trematodes recovered (only 2.2%).

Gerard et al. (2007) studied 13280 gastropods belonging to 17 species (mostly Pulmonates with Planorbidae as the dominant family) from the lake over 10 years of time series. They determined the trematode larvae belonging to 11 morphotypes of cercariae in 15 of the 17 species of gastropods and had a total prevalence of 2.9 percent.

Phiri et al. (2007) studied the trematode infections in freshwater snails and cattle from the Kafue wetlands of Zambia during a period of highest cattle-water contact. They collected a total 984 snails, comprising nine species from six areas in the Kafue wetlands between August and October 2003 and found 135 (13.7%) were positive. Authors recorded the most trematode infections form *Lymnaea natalensis* (42.8%), which harboured four of the five morphologically different cercariae but no trematodes were recovered from *Bellamya capillata, Biomphalaria pfeifferi, Melanoides tuberculatus, Physa acuta* and *Cleopatra nswendweensis*. In their study they found one individual (0.2%) of 416 *Bulinus* shed brevifurcate-apharyngeate distome cercariae, while three (0.7%) shed amphistomes. Gymnocephalous and longifurcate-pharyngeate distome were the commonest types of cercariae they recorded while Xiphidioceraria was the least common.

2.2 Review of Literature in Nepalese Context

Ghimire (1987) conducted a study on incidence of common diseases of cattle and buffaloes in Surkhet district and found that Fascioliasis, Toxocariasis, Paramphistompasis, internal Schistosomiasis and Monieziasis were the indo-parasitic infections.

Mahato (1993) explained that the parasitism in snails with Xiphidiocercariae and *Chaetogaster* spp. occurred more commonly in the hills than in the terai. In his study mixed infections with *F. gigantica* and *Chaetogaster* spp. were rarely seen while infection with *F. gigantica* and Xiphidiocercariae was never recorded. He reported the prevalence of immature infection (redial stage) in the snails varied from 0.00 to 0.48 percent with the peaks in August and December in the hills and the highest prevalence (0.67%) of immature infection in June in the Terai region of Nepal. He recorded the mature infections every month with a gradual rise in prevalence from April to July.

Sherchand et al. (1999) reported eggs of *S. mansoni* in Dhanusha district, southern Nepal from human stool.

Pandey (2001) studied that prevalence of Fascioliasis in buffaloes in relation to *Fasciola* larvae infection in *Lymnaea* snails in Devbhumi Baluwa VDC of Kavre district. He examined 2040 individuals of *L. auricularia* from rice fields, springs, irrigation channels and streams. He reported the highest increased prevalence rate of *Fasciola* larvae in November (5.35%) and no larvae ware found in March, April and May.

Adhikari et al. (2003) performed a study on the prevalence and diversity of *Fasciola* sp. in cattle and buffaloes in areas of Kathmandu valley from 23 April to 30 June 2003. They reported the prevalence of 36 percent, and 61 percent of *Fasciola* sp. in cattle and buffaloes respectively. They also found *Paramphistomum* sp. (11 %) and strongyloides (10%) from cattle and buffaloes.

CVL (2004/2005) collected a total of 1633 fecal samples of cattle and examined and found that the *Fasciola* sp. was the highest positive cases (430), followed by Ascarids + strongyles (387) and the paramphistomes (168).

3. MATERIALS AND METHODS

3.1 Study Area

Chitwan and Nawalparasi districts were selected for this study. Chitwan district is situated in inner terai. It lies between 27^0 21' N and 27^0 53' N latitude and 83^0 55' E and 84^0 48'E longitude. It has the area of 2218 km^2. The topography is described as low hills steeply dissected by numerous permanent and seasonal drainages. The altitude gradually raised from 150m from the floodplain to 2000m from sea level. The flood plain consists of a series of ascending alluvial terraces laid down by the rivers. Narayani, Rapti, Khegeri, etc. are some major rivers.

The climate is subtropical monsoon, which is characterized by high temperature and extreme variability in precipitation. The temperature reaches up to 40^0 Celsius in the very summer time and decreases up to 5^0 Celsius in the winter time. In average the temperature remains between 15^0c to 34^0c. Climatically three seasons prevail, Spring (February to May), Summer (June to September) and winter (October to January).

Nawalparasi district is situated in Lumbini zone. This district has an area of 2162 km^2. It lies between 27^0 21' and 27^0 52' N latitude to 83^0 35' and 84^0 26'E longitude. This district is surrounded by Chitwan district in the east, Rupandehi district in the west and Palpa and Tanahu districts in the north. Its southern boundary marks the Nepal-India border.

The climate is subtropical, monsoon rainfall occurs between June and September delivers and average of 85 percent of the total annual rainfall.

3.2 Data Collection

The primary data were collected by visiting the study sites from August to November, 2007. The secondary data were collected from internets, books, journals, articles, dissertation, theses and other related reports of government, INGOs and NGOs.

3.3 Collection of freshwater snails.

The freshwater snails were collected from different habitats such as rivers, paddy fields, grazing swampy areas, lakes, temporary pond and forest swamp with the help of tri-angular scoop mounted on a long bamboo handle. The collected snails were kept in a black dark bottles and containers with wet pieces of papers. The samples were immediately brought to room for screening.

3.4 Observation of Infected Snails

Collected snails were rinsed in chlorine free tap water to remove mud and plants. Each snails were kept in well - plates with small amount of clean water. The well-plates containing fresh snail samples were covered and kept near window with enough light or under tube-light.

A well-plate is a plastic device with different number of cavities in it. In this study, the well plates with six (diameter = 3.5cm), twelve (diameter= 2.2cm) and twenty four (diameter=1.6cm) cavities were used. The length and breadth of the all types of well plates and the depth of the cavities were same (length = 12.8 cm, breadth = 8.5 cm and depth of the cavity = 1.8cm).

All snails kept in well-plates were observed under stereomicroscope for about an hour later. As the snails found to be infected, the cercariae were clearly seen swimming in a well. A detail morphology of a cercaria was observed under high magnification compound scope on a slide. The cercaria was photographed by using a camera fitted in a microscope and identified by using an introductory guide to the identification of cercariae from African freshwater snails with special reference to cercariae of trematode species of medical and veterinary importance (Frandsen and Christensen, 1984).

If a snail was not shedding cercarie, it was kept back to its proper well again and observed at certain interval for 24 hours. The method used in the present study is to keep the snails alive, monitoring the emergence of the mature cercariae, usually termed 'patent infections' (Ito, 1978).

3.5 Examination of elephant dung samples

The dung samples of four different private elephants in Sauraha, Chitwan were collected, sieved in different mesh sizes from coarse to very fine. The remains of the fine sieve was kept on the petridish in small volume, and observed under the microscope to locate the presence of trematode eggs. The eggs were identified in the field by E.S. Loker, Professor, The university of New Mexico, USA.

3.6 Data Analysis

The data obtained by the research were edited, coded, classified, tabulated and analyzed. Analysis was done by representing with the table, bar diagrams, pie-charts etc. on the basis of findings like percentage infection morphotype of cercariae etc. The significant value of r_{xy} was calculated by t-test and $\Re^2$ - test and it was considered significant positive correlation if the value of r_{xy} was positive and the value of t-calculated was greater than the value of t-tabulated at 1% level of significant. Same way if the value of $\Re^2$ calculated was greater than the value of $\Re^2$ tabulated at 1 percent level of significant it was considered significant.

4. RESULTS

A total of 2525 individual snails of ten different species belonging to families Viviparidae, Ampullariidae, Bithyniidae, Thiaridae, Pleuroceridae and Planorbidae were collected and examined from August 2007 to November 2007 in Chitwan and Nawalparasi district.

Six different species of snails were found to harbour patent trematode infections. A five morphotype of the cercariae were recovered from 89 individuals of freshwater snails (3.52 percent of total sampled individuals).

4.1 Freshwater Snails and Prevalence of Larval Trematodes Cercariae

The highest percent of infection was recorded in *Gabbia orcula* (6.5%) followed by the *Indoplanorbis exustus* (4.5%). The overall infection rate was recorded 3.52 percent. The *Bellamya bengalensis*, *Brotia costula*, *Pila globosa* and *Segmentina* sp. were devoid of any trematodes. There was significant positive correlation between the total number of particular snail species examined and the total number of snail infected ($r_{xy} = 0.96$, $t_{cal} = 9.7$, d.f. = 8, $p < 0.01$) (Table 1).

Table 1. Freshwater Snails and Prevalence of Trematodes

Type of Snail	Total No. Examined	Total No. Infected (%)
Bellamya bengalensis	128	0
Brotia costula	78	0
Gabbia orcula	385	25 (6.5)
Gyraulus sp.	290	9 (3.1)
Indoplanorbis exustus	890	40 (4.5)
Lymnaea spp.	377	11 (2.92)
Melanoides tuberculatus	165	3 (1.82)
Pila globosa	20	0
Segmentina sp.	32	0
Thiara spp.	160	1 (0.63)
Total	2525	89 (3.52)

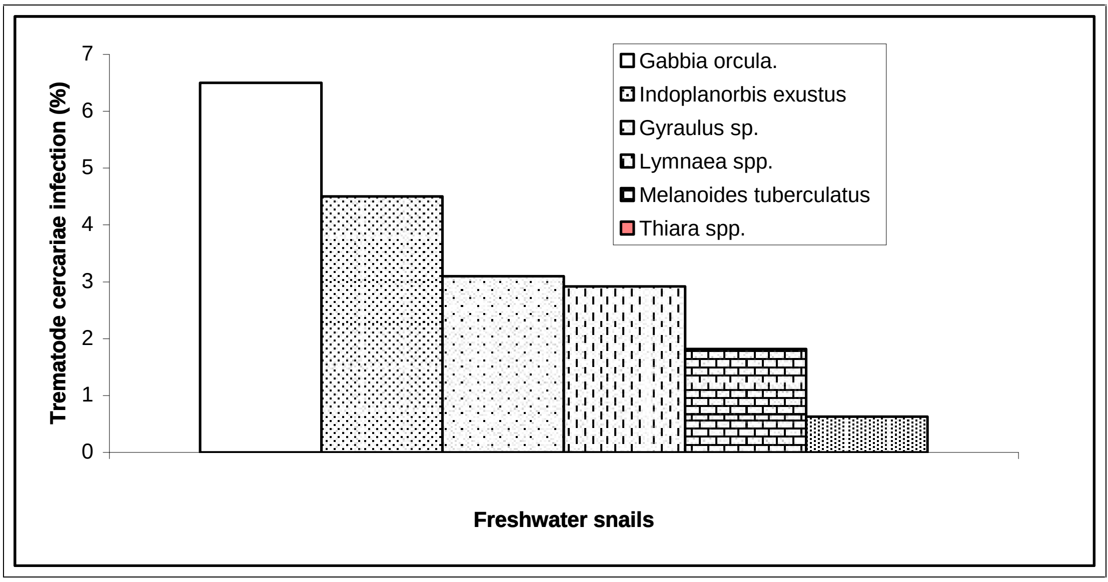

Figure 2. Freshwater Snails and Prevalence of Trematodes

4.2 Distribution of Trematode Cercariae in Freshwater Snails in Different Habitat Types

The highest prevalence of trematode cercaria was recorded in the temporary small ponds (6.23%) followed by grazing swamp (5.73%), lakes (5%), forest swamp (4.01%), rivers (1.85%) and paddy field (1.51%). Chi-square test indicated that the difference in the prevalence of larval trematode in different habitats was significant ($\Re^2$ = 29.59, p = 0.01) even at one percent level of significance. It means that the prevalence of larval trematode was not uniform in different habitats (Table 2).

Table 2. Distribution of trematode cercariae in freshwater snails in different habitat types

S.N.	Habitat Type	**Number of snails**				% Infection
		Species Examined	Individual Examined	Species infected	Individual infected	
1	Forest swamp	7	122	2	5	4.01
2	Grazing swamp	7	716	3	41	5.73
3	Lakes	5	80	2	4	5.00
4	Paddy field	7	794	3	12	1.51
5	River	7	540	3	10	1.85
6	Temp. small ponds	5	273	4	17	6.23
	Total	11	2525	6	89	3.52

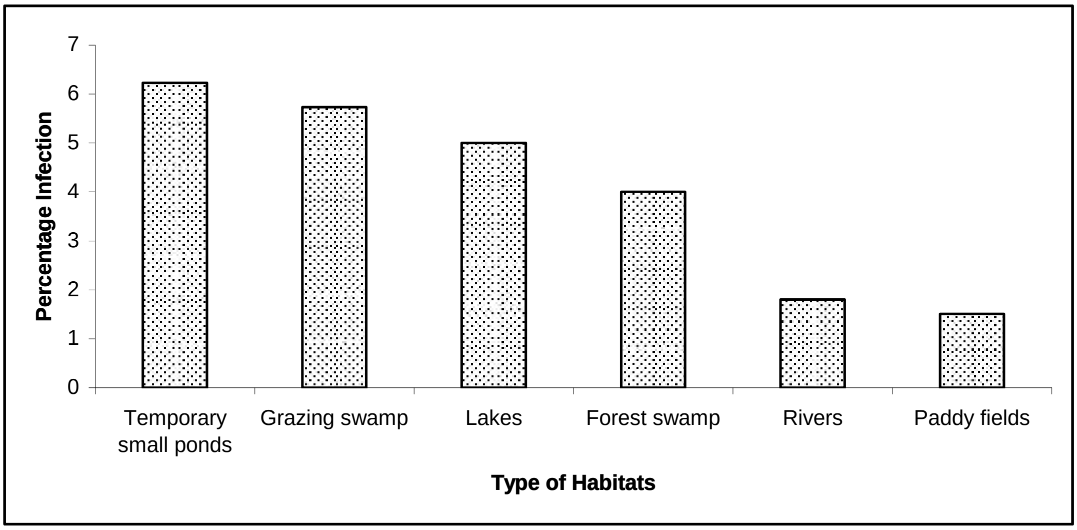

Figure 3. Distribution of trematode cercariae in freshwater snails in different habitat types

4.3 Prevalence of Trematode Cercariae during the Study Period

The present survey was done in August, September and November 2007. The highest prevalence of trematode cercariae was reported in September (4.16%) followed by August (2.1605%) and November (2.03%). The percentage of infected snail individuals were 50 percent in September following August (26%) and November (24%) (Figure 4).

Table 3. Prevalence of Larval Trematode During the Study Period

Months	Snail individuals		% Infection
	Examined	Infected	
August	648	14	2.1605
September	1729	72	4.16
November	148	3	2.03
Total	2525	89	3.52

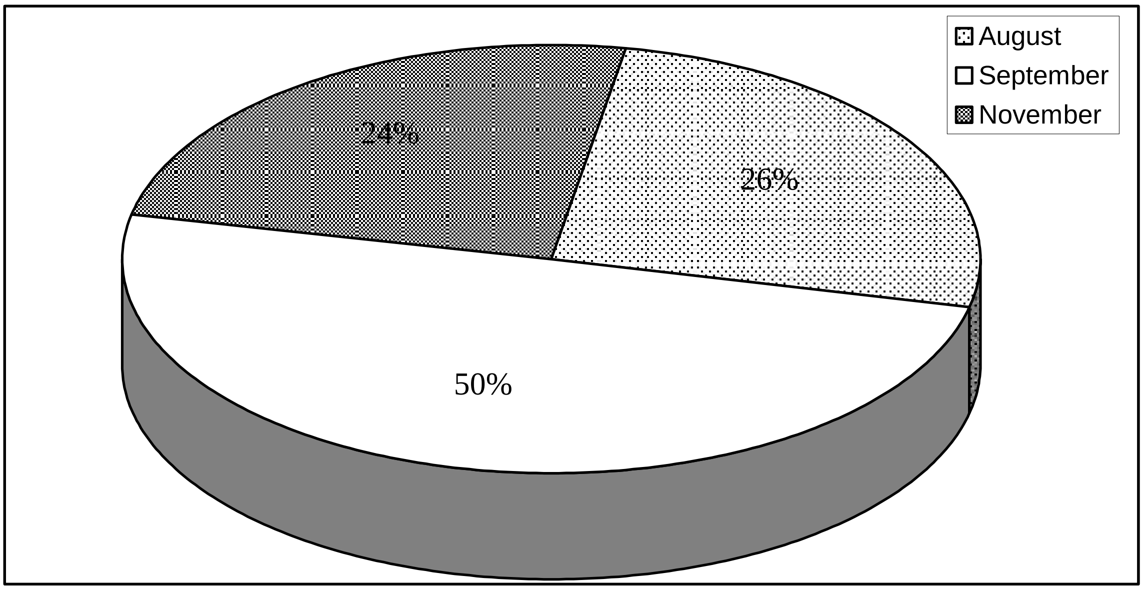

Figure 4. Prevalence of Larval Trematode During the Study Period

4.4 Freshwater Host Snails and Their Trematode Cercariae

Altogether five different morphotypes of cercariae were reported during the study period. They were Longifurcate-pharyngeate (LP), Clinostomoid cercaria (CC), Brevifurcate-apharyngeate (BA), Amphistome cercaria (AC) and Xiphidiocercaria (XC). The AC was the most commonly found (43.82%) followed by XC (42.7%). The LP was found in a single host snail *Indoplanorbis exustus* (1.12%). The XC showed the highest host range and found in *Melanoides tuberculatus*, *Gabbia orcula*, *Lymnaea* spp. and Thiara spp. Similarly AC was also reported from *I. exustus*, *Gyraulus* sp. and *G. orcula*. The CC and BA cercariae were found only from *I. exustus.* The *I. exustus* was found infected with four different types of trematode cercariae (LP, CC, BA and AC) including double infection with AC and BA (Table 4).

Table 4. Freshwater Host Snails and Their Trematode Cercariae

Host Snail	Morphotypes of Cercaria					
	LPC	CC	BA	AC	XC	Remarks
Indoplanorbis exustus	0.11 (1.12)	0.34 (3.37)	0.79 (7.87)	3.15 (31.46)		One individual of *I. exustus* with AC + BA
Gabbia orcula				0.52 (10.11)	5.97 (25.84)	
Gyraulus sp.				3.10 (2.25)		
Melanoides tuberculatus					1.82 (3.37)	
Lymnaea spp.					2.92 (12.36)	
Thiara spp.					0.63 (1.12)	
Total	0.11 (1.12)	0.34 (3.37)	0.79 (7.87)	2.49 (43.82)	3.49 (42.7)	0.11 (1.12)

Note: Number without bracket indicates prevalence rate and number in bracket indicate percentage of total positive cases. LPC: Longifurcate-pharyngeate cercaria; CC: Clinostomoid cercaria; BA: Brevifurcate-apharyngeate cercaria; AC: Amphistome cercaria; XC: Xiphidiocercaria.

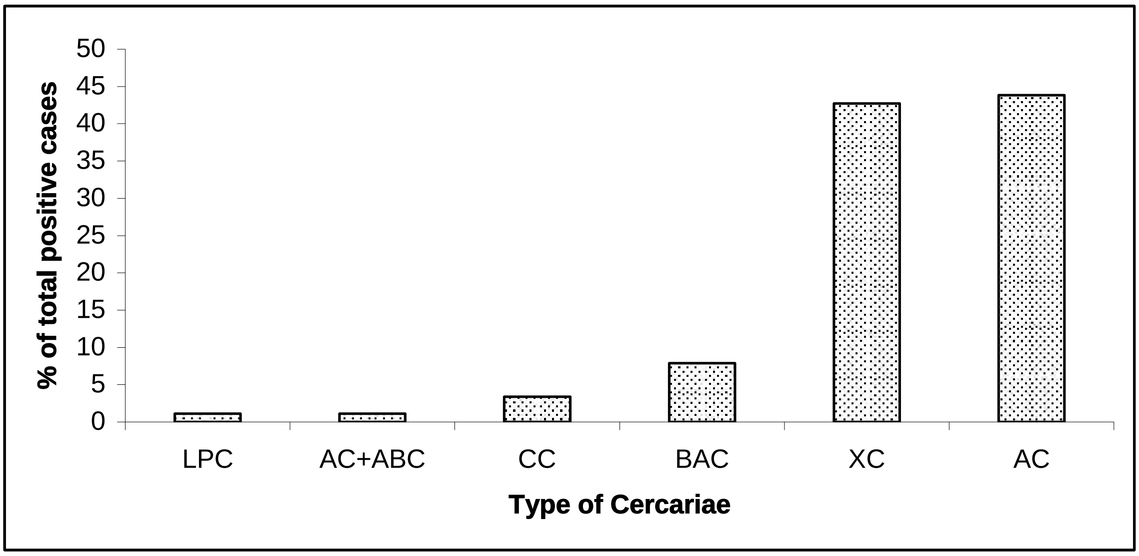

Figure 5. **Freshwater Host Snails and Their Trematode Cercariae**

4.5 Trematode Infections in Domestic Elephants

Four fecal samples of domestic elephants in Sauraha, Chitwan were examined during the study period. The study showed that 75 percent of fecal samples were positive for trematode eggs (Table 5). Two samples were found to be positive for *Schistosoma* sp. The eggs resembled with *Bivitellobilharzia nairi* (Photoplates: 13, 14, 15). They were elongated oval, flattened on one side with rugose shell, having a blunt spine at one end.

Same way the two fecal samples (50%) were found to be positive for eggs of *Fasciola* sp. (Photoplates 11, 12). The eggs were operculated and golden brown in colour, which can be easily distinguished from the eggs of amphistomes as they are clear, and contained large granules.

Table 5. Trematode infection in Domestic elephants

Elephant dung sample	*Fasciola* sp.	*Schistosoma* sp.
1. Private Elephant at Sauraha	++	++
2. Private Elephant at Sauraha	-	++
3. Sample Picked from the road	-	-
4. Sample Picked from the road	++	-

5. DISCUSSION

Trematode infection is a common health problem in livestock, wildlife, birds and human beings. So, biology, ecology and transmission of trematode parasites in definitive and intermediate hosts are an interesting field of investigations. The present study reveals the diversity of larval trematode parasites in snails in the Chitwan and Nawalparasi districts and the presence of eggs of *Schistosoma* sp. and *Fasciola* sp. in domestic elephants of Chitwan district.

Altogether 2525 individual snails from different localities were examined. A total of 89 (3.52%) snails were found infected by trematode cercariae. An infection level of 3.52 percent is comparable to the previous studies. Chingwena et al. (2002) found 6.6 percent of infection in snails in highveld and lowveld areas of Zimbabwa and 13.7 percent in Kafu wetlands of Zambia (Phiri et al., 2007); 1.7 percent *Fasciola* larvae infection in *Lymnaea* snails in Devbhumi Baluwa Village Development Committee (VDC) of Kavre district (Pandey, 2001). Similarly, dynamics and community structure of freshwater gastropods showed 2.9 percent parasitism and other environmental stressors (Gerard et al., 2007).

Such low infection rate in natural snail populations might be due to the direct consequence of high rates of parasite-induced mortality. Begon et al. (1990) argued that as a result of host-parasite co-evolution, hosts usually develop acquired resistance to infection and thus the observed low levels of prevalence. The low prevalence of infection could also be due to low parasite pressure, simply making contact between miracidia and snails a rare event.

The present study showed that *Gabbia orcula* was the most commonly infected snail host (6.5%) followed by the *I. exustus* (4.5%), *Gyraulus* sp. (3.1%), *Lymnaea* spp. (2.92%), *M. tuberculatus* (1.82%) and *Thiara* spp. (0.63%). But no infection was recorded in *B. bengalensis, B. costula, P. globosa* and *Segmentina* sp. This finding can be compared with the following findings: 6.4-50percent in *Gyraulus euphraticus,* 5.0-71.1

percent in *M. tuberculatus*, 0.5-44.2 percent in *I. exustus* in Calcutta (Sewell, 1922). Trematode infection in *I. exustus* was recorded upto 50.0 percent in Wynaad, Madras, India (Sewell, 1922). Chingwena et al. (2002) reported *Bulinus tropicus* (13.1%) the highest prevalence of infection. But no termatodes were recorded from *B. capillata, Biomphalaria pfeifferi*, *M. tuberculata, Physa acuta* and *Cleopatra nswendweensis* in the wetlands of Zambia (Phiri et al; 2007). Likewise, no trematodes were recorded from *B. capillata*, *Lanistes ovum* or *Gyraulus costulatus* (Chingwena et al; 2002). The possible reason could have been snails were resistant to trematode infection. Resistance of snails to trematode infection has been reported to play a role in determining infection rates (Bayne and Yoshino, 1989).

Caution must be applied when comparing the levels of infection of intermediate host snails from different studies. The present study was based on the "Patent infection". The infection levels of this study might have been considerably higher if the presence of "pre-patent infections" has been established.

Snails do not become infected with cercariae till they have reached maturity, that is about third month" (Manson-Bahr and Fairley, 1920). Twenty large examples of *M. tuberculatus* taken from the basin in the fern house, Indian Museum, Calcutta, showed a percentage infection upto 35.0, while in fifty small examples, not a single case of infection was met with (Kemp and Gravely, 1919). The percentage infection in present study may also be influenced by these factors. Large snails are more likely to be infected with parasite since large (therefore old) snails have had most chronic exposure to infective miracidia (Miura and Chiba, 2007).

The degree of infection varies greatly with different mollusks. The examination of comparatively few snails from only one or two sources is liable, however, to give one of false impression of the susceptibility or otherwise of the particular species under review.

In this study, the highest prevalence of trematode cercariae was recorded from the small temporary ponds (6.23%) followed by grazing swamp (5.73%), lakes (5%), forest swamps (4.01%), rivers (1.85%) and paddy fields (1.51%). This can be compared with the rice field (1.67%) and stream (0%) as the study on the prevalence of fasciolasis in buffaloes in relation to *Fasciola* larvae infection in *Lymnaea* snails in Kavre district, Nepal (Pandey, 2001). Infection rate in different stream types in Zimbabwe ranged from 1.6 percentage to 3.52 percentage (Chingwena et al; 2002).

The highest prevalence rates in small temporary ponds and grazing swamps obviously due to continuous cattle grazing areas that livestock frequently come to water contact. The high prevalence rates in lakes and forest swamps was due to presence of migratory and residential bird species, and varieties of fishes and reptiles. The degree of infection may vary so enormously in snails of the same species which have been taken from different areas of water, even through these areas may be slightly quite close to each other and their general conditions appear to be exactly similar. The results obtained did match with Sewell (1922).

The study was conducted in August, September and November of 2007 and recorded the following infection level; August (2.16%), September (4.16%) and November (2.03%). Pandey (2001) examined several individuals of *Lymnaea* sp. and found *Fasciola* larvae infection (1.49%), (3.03%) and (5.35%) in August, September and November respectively in middle hills of Nepal. The variation may be due to the abundance of snail population, level of parasite pressure, the number and the species of snails observed.

Five morphologically different types of larval trematodes were recorded infecting freshwater snails in the present study which showed similar trends in Zimbabwe, where Chingwena et al. (2002) found eight trematode cercariae. Likewise, the infection rate varies in different studies in different countries were reported; six different families in Northern Australia (Hurley et al., 1994), eight trematode species in *Batillaria attramentaria* in Japan (Miura and Chiba, 2007) and five morphologically different types of larval trematodes infecting freshwater snails in Kafue wetlands of Zambia (Phiri et al., 2007).

The present study revealed that the apparently capable of acting as primary host to large number of trematode species was *I. exustus* with four of the five morphologically different forms of cercariae, *Gabbia orcula* with two forms of cercariae and other positive cases consisted of single form of cercaria. This finding can be compared with the following findings: *M. tuberculatus* with seventeen forms of cercariae, *I. exustus* with fifteen forms and *Amnicola travencorica* with twelve forms (Sewell, 1922); *Lymnaea natalensis* harboured four of the five morphologically different cercariae in wetlands of Zambia (Phiri et al., 2007). Loker et al. (1981) recorded *L. natalensis* as the most important intermediate snail host for transmitting a wide variety of trematode species. Chingwena et al. (2002) suggest that *Bulinus tropicus* may play a major role as a host for a variety of larval trematodes.

The Amphistome cercaria was found to be most commonly occurring cercariae (43.82%) of the positive cases followed by Xiphidiocercaria (42.7%), Brevifurcate-apharyngeate (Schistosoma) cercaria (7.87%), Clinostomoid cercaria (3.37%), Longifurcate-pharyngeate (strigea) cercaria (1.12%) and double infection (Amphistome and Brevifurcate-apharyngeate (1.12%). This study follows the other studies as; the

Echinostome cercaria (38.2%), Amphistome cercariae (37.6%) and Schistosoma cercariae (8.0%) of all infections (Chingwena et al., 2002); The cercaria of family Echinostomidae (37.81%), immature infections (those that were not identifiable, including rediae and sporocysts 26.20%), Strigeidea (16.86%), Schistosomatidae (11.39%), Clinostomidae (3.64%), Notocotylidae (2.05%) and Xiphidiocercariae types (2.05%) were recorded in freshwater snails at Australia (Hurely et al., 1994).

Double infection

Although as a general rule only one form of trematode parasite is found undergoing actual development in any single mollusk individual. But rare cases had been noticed as a double infection (two forms of trematodes developing simultaneously in the same snail). Concurrent infections with more than one parasite species are comparatively uncommon (Kendall, 1964).

The present study reported a single individual of *I. exustus* with double infection (1.12%). Brevifurcate apharyngeate cercaria and Amphistome cercaria were observed in the same individual snail. This finding can be compared with a true double infection with trematode parasites in two species of snail host namely *M. tuberculatus* and *I. exustus* (Sewell, 1922). The presence of Echinostome larvae within their snail hosts has been shown to inhibit or preclude development of larvae of other trematode species in the study of Intramolluscan, Inter-trematode Antagonism (Lim and Heyneman, 1972). This finding also supports that the multiple infection is usually very rare. Miura and Chiba (2007) reported 7 percent of snails were simultaneously parasitized with double infection but this type of infection depends on the shell size and distribution of snail hosts.

It seems probable that a double infection in any molluscs individual is merely accidental and in consequence occurs most frequently in those snails which can act as primary host to the greatest number of trematode species and is the result of infection with those trematodes which posses the greatest power of developing in several mollusks; but the infrequency of these cases of double infection, in less than 0.5 percent of cases (Sewell, 1922). No double infections were recorded in the study made by Chingwena et al. (2002) and Phiri et al. (2007). The double infections could be more pathogenic as compared to single species infections and as a result, snails with multiple infections suffer higher mortality (Sousa, 1992), and may therefore be under represented in snails collections. Temporal and spatial variations in the abundance of eggs and miracidia of different trematode species in relation to how often a snail can be simultaneously infected with two or more species are other reasons (Williams and Esch, 1991; Sousa, 1993).

The study also recorded overall prevalence rate of trematode in the fecal examination of captive elephants was very high in Sauraha, Chitwan. Out of four samples ¾ of elephant samples were identified as infected from *Fasciola* and *Schistosoma* sp. Among the positive cases 25 percent samples were positive with both *Fasciola* sp. and *Schistosoma* sp. and 25 percent with *Schistosoma* sp. and 25 percent with *Fasciola* sp. infection only.

This finding can be compared with the overall prevalence rate of *F. jacksoni* (18.18-62.28%) in the captive elephants of Assam, India, (Saidul, 1997). *F. Jacksoni* infection in 2 out of 3 elephants autopsied as Assam state Zoo, India (Chakraborty and Chaudhary, 1992). Accurate detection by fecal parasitological sampling has been difficult because of the voluminous feces of the elephants.

6. CONCLUSION

* Altogether 2525 individual snails representing eleven species from six habitats were collected and studied for the presence of trematode cercariae.
* Among them 89 (3.52%) individual snails harboured patent trematode infections.
* The highest overall prevalence infection (6.5%) was recorded in *Gabbia orcula.*
* No trematode infection was recorded in *Bellamya bengalensis, Brotia costula*, *Pila globosa* and *Segmentina* sp.
* The highest percentage of trematode infection (6.23%) was found in temporary ponds.
* Five morphologically distinguishable types of cercariae were identified during the study period.
* The Amphistome cercaria was the most common (43.82%) cercaria.
* The Xiphidiocercaria (42.7%) was recorded from four different snail species.
* A *Indoplanorbis exustus* with double infection (Amphistome + Schistosoma) was also reported.
* 25 percent elephant dung samples was found to be positive for *Fasciola* sp. and *Schistosoma* sp. and 25 percent with *Schistosoma sp.* and 25 percent with *Fasciola sp.* infection only.

7. RECOMMENDATIONS

Awareness

- People should be made aware to graze their domestic animals in clean and dry areas not in swampy areas.
- People should be made aware about the harms caused by swimming in the water polluted by human and other animal activities.
- People should be provided with knowledge about feeding behaviour like use of boiled water for drinking purpose and consuming properly cooked fish, crab, snails etc; which are used as meat.

Regular monitoring

- To break the life cycle of trematode parasites, water snails should be controlled by using molluscicides periodically.
- The domestic animals should be regularly treated by periodic anthelmintic drugs.

Further research

- The further work, on the patent and prepatent infections on the snail hosts, larval behaviour and morphology, geographical distribution of trematodes infection in human beings, livestock and wildlife and clinical manifestations in Nepal are inevitable.

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Annex 1

T-test for significance of an observed sample correlation coefficient

S.N.	Total No. Examined (X)	Total No. Infected (Y)	X^2	Y^2	XY
1	128	0	16384	0	0
2	78	0	6084	0	0
3	385	25	148225	625	9625
4	290	9	84100	81	2610
5	890	40	792100	1600	35600
6	377	11	142129	121	4147
7	165	3	27225	9	495
8	20	0	400	0	0
9	32	0	1024	0	0
10	160	1	25600	1	160
Total	$\Sigma X=2525$	$\Sigma Y=89$	$\Sigma X^2=1243271$	$\Sigma Y^2=2437$	$\Sigma XY=52637$

Correlation (r_{xy}) = $\dfrac{n\Sigma xy - \Sigma x\,\Sigma y}{\sqrt{\left(n\Sigma x^2 - (\Sigma x)^2\right)\left(n\Sigma y^2 - (\Sigma y)^2\right)}}$

$$= \frac{10 \text{ x } 52637 - 2525 \text{ x } 89}{\sqrt{[10\text{x}1243271 - (2525)^2]\,[10\text{x}2437 - (89)^2]}}$$

$$= \frac{526370 - 224725}{\sqrt{(12432710 - 6375625)\,(24370 - 7921)}} \text{ X } \frac{301645}{\sqrt{6057085\text{x}16449}} \text{ X } \frac{301645}{315646.94}$$

= 0.96

$\therefore r_{xy} = 0.96$

This means, that the number of individual snails examined and the number of individual snails infected are positively co-related with each other.

Test of significant of the value of r_{xy}

$H_0 : r_{xy} = 0$ (value of r_{xy} is not significant)

$H_a : r_{xy} > 0$ (Value of r_{xy} is significant)

$r_{xy} = 0.96$

n = 10

Test statistic

Under H_o, the test statistic is

$$t = \frac{r\sqrt{n\ Z 2}}{\sqrt{1\ Z r^2}} \qquad \text{with n-2 d.f.}$$

$$= \frac{0.96\sqrt{10\ Z 2}}{\sqrt{1\ Z (0.96)^2}} \qquad \text{with (10-2) d.f.}$$

$$= \frac{2.7153}{\sqrt{0.0784}} \qquad \text{with 8 d.f.}$$

$$= \frac{2.7153}{0.28} \qquad \text{with 8 d.f.}$$

…t = 9.7

The calculated value of t is 9.7 for 8 d.f. the table value of t at 0.01 p is 3.355 at 8 d.f. the calculated value of 't' is more than the tabulated value at 0.01p. So, the value of 't' is significant, i.e. H_a; $r_{xy} > 0$ is accepted.

$\Re^2$ - test for Goodness of fit.

Null hypothesis Ho: there is no significant different between observed and expected frequencies.

Alternative hypothesis Ha: there is significant observed and expected frequencies.

S.N.	Observed Value (O)	Expected (E)	(O-E)	$(O-E)^2$	$\frac{(O-E)^2}{E}$
1	10	19.0	-9	81.0	4.26
2	12	27.95	-15.95	254.4	9.102
3	41	25.2	15.8	249.64	9.906
4	4	9.6	1.18	1.4	0.5
5	17	9.6	7.4	54.76	5.704
6	5	4.3	0.7	0.49	0.114
					$\phi \frac{(O-E)^2}{E}$ = 29.53

$$\Re^2 = \frac{(O-E)^2}{E} = 29.59$$

$\Re^2 = 29.59$

The calculated value of $\Re^2$ is 29.59 at 5 d.f. where as tabulated value is 15.086 at 0.01 p and 5d.f. the calculated value of $\Re^2$ > the tabulated $\Re^2$, it is significant and H_o is rejected.

Annex - 2

Habitat Description of collected freshwater snails

1. *Bellamya bengalensis* : This species is found nearly in all types of lowland water bodies like rivers, streams, lakes, ponds, wetlands, marshes and paddy fields. This study recorded it from all of the habitat types (paddy field, small water pits, forest swamps, grazing swamps, lake and rivers).

2. *Brotia costula* : It is mainly found in fast running streams and rivers of lowlands. It is reported in muddy water and on muddy bottom of rivers and stagnant water. This species was mainly recorded from the river/stream (Elevation 255m, N 27^0 37.774'; E 084^0 29.361')

3. *Gabbi orcula* : *Gabbia orcula* is found in stagnant water (except lakes) with dense vegetation. They are found in paddy fields, forest swamps or wetlands, small water pits etc. They are absent in large rivers. In this study, this species was specially collected from water logged small ponds (Elevation 255m, N 27^0 37.774', E 084^0 29.362') and rice fields (Elevation : 210m, N 27^0 34.909', E. 84^0 01.293', Elevation 171m, N 27^0 39.203' E 84^0 10.410') . No samples of this species was found from lake and rivers.

4. *Gyraulus* sp. : It is common species occurring in lakes, ditches, ponds and rice fields. In this research, this species was collected mainly from rice fields and small water pits with full of vegetation where they were found to attached with them.

5. *Indoplanorbis exustus* : This species is found in various types of stagnant water with dense vegetation. In this research most of the *Indoplanorbis exustus* were collected from the rice fields. Some of them were collected from small water pits near swamps and wetlands.

6. *Lymnaea* spp. : *Lymnaea* spp. are found in slowly running rivers, streams, ponds, lakes and wet lands with abundant vegetation. I collected them from rice fields, forest swamps, grazing swamps, small water pits and lake with full of aquatic vegetation but not from river/stream.

7. *Melanoides tuberculatus* : This is found in streams, rivers, irrigation canals and stagnant water ponds. It is dominating in the plains, being nearly absents from the hills. In this study, this species was mainly collected from the river sides (Elevation 255m, N 27^0 37.774', E 084^0 29.361'; Elevation 211m, N 27^0 35.523', E 84^0 30.011').

8. *Pila globosa* : This species prefers the stagnant water bodies (specially wetlands) which are dry for at least one time per year. In this study this species was collected from forest swamps (Elevation 161m, N 27^0 37.306' E 84^0 27.661'; Elevation 189m, N 27^0 36.92', E 84^0 26.193') and grazing swamps (Elevation 211m, N 27^0 35.523' E 84^0 30.011'; Elevation 255m, N 27^0 37.774', E 084^0 29.361')

9. *Segmentina* sp. : It is found in stagnant waters, lakes, ponds in submerged dense vegetation. I collected this species from small temporary pond with good vegetation (collected only from two sites Elevation 255m, N 27^0 37.774' E 084^0 29.361; Elevation 211m, N 27^0 35.523' E 84^0 30.011')

10. *Thiara* spp. : These species are found in slowly running lowland rivers ad streams with rich invertebrate fauna. During field work they were collected from only one habitat type i.e. river (Budhi Rapti).