I

INTRODUCTION

1.1 Background

Nepal is an agricultural country. Agriculture contributes 38% of the nations GDP and livestock contributing almost 11% to GDP (World Bank, 2002). Livestock population in Nepal for 2004/2005 was estimated to be 6.99 M cattle, 4.08 M buffaloes, 7.15 M goats and 0.86 M sheep (www.moacwto.gov.np.statistics/livestock/pdf). Roughly 70% of households keep some type of livestocks, including cows, buffaloes, pigs and chickens (FAO, 2005). Buffalo is one of the major livestock species for milk and meat production, which constitutes about two thirds of the meat consumption in Nepal (TLDP, 2002).

Meat is an indispensable food consumed by most of the people of this universe from very beginning of human civilization till now. The word meat comes from the old English word mete which referred to food in general. Meat, in its broadest definition, is animal tissue used as food (www.wikipedia.org/meat). Meat is widely used to define flesh and offal including their natural associates' skin and gristle, derived from carcass of any animal and bird normally used for human consumption. Because of essential nutrients it contains, meat eaten in moderate amounts can be considered a valuable component of complete diet and because of its special flavor and taste, meat helps to make eating a joyful experience.

There is a shortage of meat, especially in the cities of Nepal, which is filled by the importation of animals from neighboring countries (TLDP, 2002). About 0.3 millions meat buffaloes are annually imported from India alone (DLS, 2002). Kathmandu has the country's largest per capita consumption of meat. Each resident consumes 18kg of meat a year, whereas the average Nepali eats only 10kg-way below the worldwide average of 40kg per person per year. 140 truckloads of live buffalos are transported to the Valley from the Terai and India every week, and each truck is packed with up to 20 buffaloes. A truck can carry up to 200 goats, and 42 tonnes of goat meat are consumed in Kathmandu every day (Himal Magazine, 2004).

Meat consumption varies widely while comparing different countries as it depends on factors like socio-economic condition, religious beliefs, cultural practices, etc. In a resource poor and developing country like Nepal, natives have included meat as important part of their diet to supplement nutritional requirements. In Nepal buffaloes, contribute about 64% of meat consumed, followed by goat meat 20%, pork 7%, chicken 6% and sheep 3% (Joshi *et al*., 2001).

A slaughter house, also called an abattoir, is a facility where animals are killed and processed into meat products. The design, process and location of slaughter house respond to a variety of concerns. Slaughtering animals on a large scale possess significant logistical problems and public health concerns (www.wikipedia.org/slaughterhouse).

Due to its rich nutrient content, meat is an excellent media for the growth of pathogenic organisms. Meat and other meat products contribute significantly to high incidence of food borne diseases and zoonotic diseases. In Nepal, sub-tropical climate, poor sanitary condition, improper facilities, improper handling, poor food hygienic practices and lack of prevention against diseases has caused a number of diseases to erupt from the meat source. Bryan (1973) listed approximately 200 diseases that can be transmitted to man by food. The list of pathogens which can be transmitted from animals to human by food contain about 16 kinds of bacteria, 3 groups of viruses, 22 parasites and 5 protozoa (Singh and Koulikovski, 1995).

In Nepal, lack of appropriate slaughtering facilities and unsatisfactory slaughtering techniques are causing unnecessary losses in meat as well as valuable byproducts. Animals particularly buffaloes are slaughtered in Kathmandu in slaughtering places which are frequently polluted with street dust, garbage, human excreta, animal blood, intestinal contents and dirty effluents and which are not protected against dogs, rodents and insects. Meat products under such conditions are generally deteriorated due to bacterial infections and which cause food poisoning time to time. Due to lack of meat inspection, meats from the unhealthy and parasitic infected animals are acting as a source for infection to human as well as to animals. Besides, meat quality is adversely affected by careless handling condition in the slaughtering places as well as in the meat markets or shops (Joshi, 1991).

Buffalo is susceptible to most diseases and parasites that afflict cattle, although the effects of disease on the buffalo and its productivity are sometimes less evident (Fagiolo *et al*., 2005). The parasites may cause disease and infection to human if the meat of such infected animals is consumed.

"Those diseases and infections which are naturally transmitted between vertebrate animals and man" have been defined as Zoonotic diseases by World Health Organization. Some of the important parasites that infect buffalo and cause zoonoses to man are

1/ *Fasciola* spp. (*F. hepatica* and *F. gigantica*) causing Fascioliasis

2/ *Echinococcus granulosus* causing Echinococcosis

3/ *Taenia saginata* causing Taeniasis

1.2 Fascioliasis

Fascioliasis is infection caused by one of two liver flukes, either *Fasciola hepatica* or *Fasciola gigantica* (Mas-coma, 2007). It is a zoonotic disease for which humans act as an accidental host during the life cycle of the parasite (Price *et al*., 1993).

Fasciola is a well known parasite of herbivorous animals. It has a worldwide distribution in the animal reservoir host. A large variety of animals, such as sheep, goats, cattle, buffalo, horses and rabbits show infection rates that may reach 90% in some areas. Infection of the human host was very sporadic until the last two decades when clinical cases and outbreaks were reported. It is now taking an important emerging foodborne trematode infection of increasing concern (Cheng *et al*., 1990). The different local names of these diseases, such as Namle, Matey, Lew etc. in different regions are proof of its continued existence for many yrs. in the animal population of the country.). *F. hepatica* is widespread in Europe and in the higher altitude districts of India. *Lymnea truncatula*, a mud snail, is involved as the intermediate host for this species in these areas. *F. gigantica* is widely prevalent in the Indian sub-continent and Southeast Asia. *Lymnea rufescens*, an aquatic snail, acts as an intermediate host in the Indian sub continent (FAO, 1977).

Infection of domestic ruminants with *Fasciola hepatica* and *F. gigantica* causes significant loss estimated at over US$ 2000 million per year to the agriculture sector worldwide with over 600 million animals affected (Hansen, 1994).The economic loss due to fascioliasis in Nepal was estimated to be Rs. 14.2 crore (Lohani & Rasaili, 1995). The prevalence of fascioliasis ranging between 50% to 90% has been reported in cattle. In addition fascioliasis is now recognized as an emerging human disease.

The estimated number of people infected is 2.4 million in 61 countries. The number at risk is more than 180 million throughout the world. The largest numbers of infected people have been reported from Bolivia, China, Ecuador, Egypt, France, Islamic Republic of Iran, Peru and Portugal (WHO, 1995).

1.2.1 Morphology

Fasciola hepatica

Fasciola hepatica is large, broad fluke 20 to 50 mm by 6 to 13mm. Its anterior end is cone-shaped and the posterior end is bluntly rounded. The tegument contains spines. Oral and ventral suckers are adjacent in the short, cone-shaped area. There is a prominent pharynx, a short esophagus and 2 highly branched ceca that extend to the posterior end of the fluke. Two highly branched testis lie to in the second and third quarters of the body. The single branched ovary is on the right side and pretesticular. Vitellaria are in the midbody below the acetabulum (Meyers *et al*., 2000).

<u>Description of Eggs</u>:

Eggs are 130 – 197 by 63 – 104 um in size, oval shaped, yellowish in colour, consists of embryonic mass and shell, operculum usually indistinct.

Fasciola gigantica

Adult *Fasciola gigantica* are larger than adult *Fasciola hepatica* (24 to 76mm by 5 to 13mm). The average length/width ratio of *F. gigantica* is 4.39 to 5.20, while that of *F. hepatica* is 1.88 to 2.32. *F. gigantica* also differs from *F. hepatica* in having a shorter cephalic cone, a larger acetabulum and more anteriorly oriented testis. The average distance between the posterior border of the body and the posterior testis is longer in *F. gigantica* (14.9mm; range: 6to 19mm) than *F. hepatica* (7.78mm; range: 3 to 13 mm) and the ovary and ceca are more branched (Sahba *et al*., 1972).

1.2.2 Different between *Fascoila hepatica* and *Fascoila gigantica*

The *Fasciola* species collected from different animals were separately analyzed to differentiate into respective species i.e. they were differentiated into *F. hepatica* and *F. gigantica*. Careful naked eye observation and microscopic observations were carried out for this. Differentiation was made according to difference they have got in their shape and size as shown in the table below.

Fasciola hepatica	***Fasciola gigantica***
1. Body size: 20 to 50mm by 6 to 13mm.The average length/width ratio is 1.88 to 2.32.	1. Body size: 24 to 76mm by 5 to 13mm.The average length/width ratio is 4.39 to 5.20.
2. The cephalic cone is large.	2. The cephalic cone is small.
3. The shoulders are well developed.	3. The shoulders are less developed.
4. Smaller acetabulum.	4. Larger acetabulum.
5. The ovary and ceca are less branched.	5. The ovary and ceca are more branched.
6. The average distance between the posterior border of the body and the posterior testis is shorter (7.78mm; range: 3 to 13 mm).	6. The average distance between the posterior border of the body and the posterior testis is longer (14.9mm; range: 6 to 19 mm).

Source: Sahba *et al*., 1972

1.3 Life cycle and transmission

Fasciola hepatica and *F. gigantica* have similar aquatic diheteroxenous life cycles. Humans are infected by ingesting encysted metacercariae on raw vegetation and probably also floating infective metacercariae in contaminated water (Smithers, 1982).

Sheep, goats, cattle, horses donkeys, mules, camels, buffalo, deer, wild sheep, pigs, marsupials, rabbit hare and other rodents and monkeys serve as definitive hosts for both *F. hepatica* and *F. gigantica* (Boray, 1982). Adult *Fasciola* spp. deposit immature eggs that pass in feces. Eggs become embryonated in water. Eggs release miracidia, which invade a suitable snail intermediate host, including many species of the genus *Lymnae.* After penetrating a snail host, miracidia transform into elliptical secular sporocysts. Sporocysts produce mother rediae, which produce daughter rediae, and finally cercariae in about 6 to 7 weeks. Snail release motile cercariae into fresh water, where some find water plant to land on. These cercariae shed their tails, encyst and transform into metacercariae, and become infective within 24 hours (Mas Coma *et al.*, 2000). Mammals acquire the infection by eating vegetation containing metacercariae. Humans can become infected by ingesting metacercariae-containing freshwater plants especially watercress. After ingestion, the metacercariae excyst in the duodenum and migrate through the intestinal wall, the peritoneal cavity, and the liver parenchyma into the biliary ducts, where they develop into adults (http://www.dpd.cdc.gov/DPDX/ Fascioliasis). The entire life cycle takes 14 to 23 weeks (Lapage, 1968).

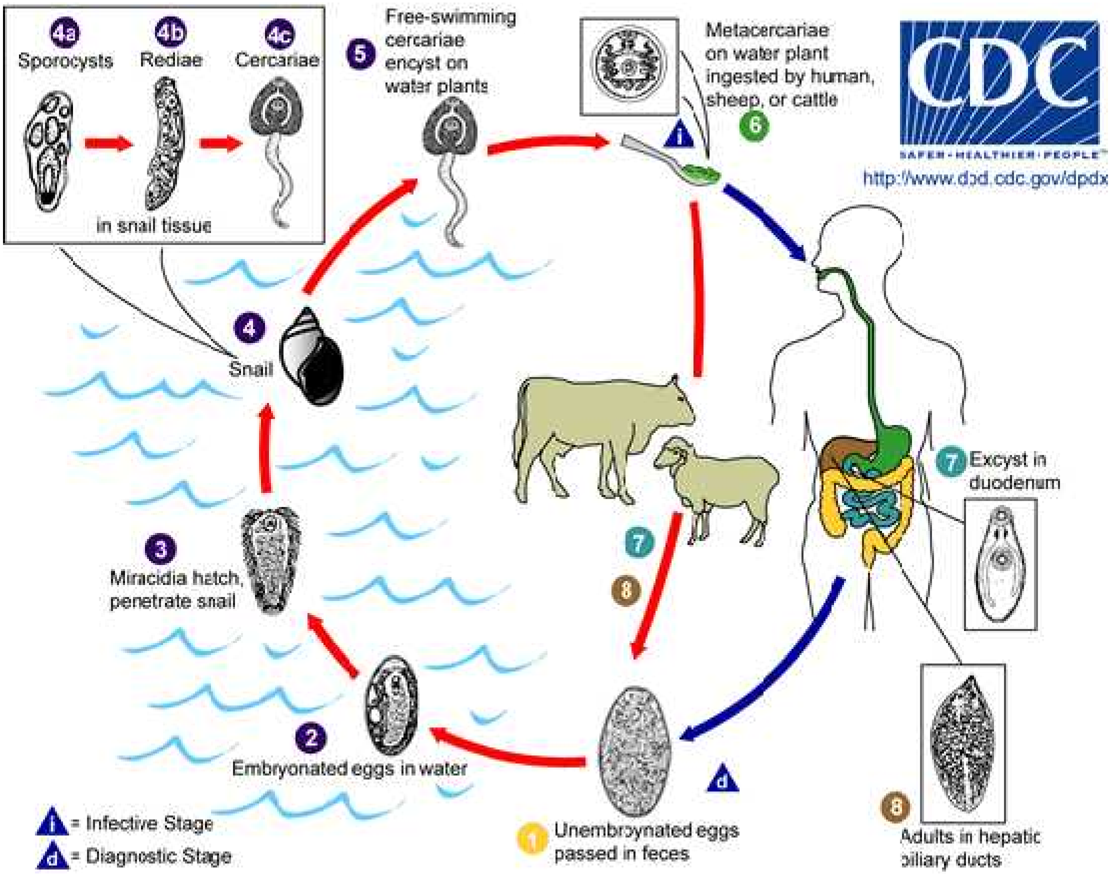


Image Source:

http://www.dpd.cdc.gov/dpdx/images/ParasiteImages/Fascioliasis/*Fasciola*_LifeCycle

Life cycle of *Fasciola*

1.4 Significance

Of all the body's organs, Fascioliasis does the most damage to the liver, hence the term "common liverfluke infection". Following ingestion of the larvae, a symptomless incubation phase starts, which lasts for a few days up to a few months. When symptoms do appear, they occur in the following patterns:

Acute Phase The acute phase of infection is rarely seen in humans and occurs only when a large number of metacercariae are ingested at once. Fever, tender hepatomegaly, and abdominal pain are the most frequent symptoms of this stage of infection although vomiting, diarrhoea, urticaria (hives), anemia, etc may also be present.

The above characteristics of the acute phase are caused by the migration of the *F. hepatica* larvae throughout the liver parenchyma. The larvae penetrate the liver capsule and begin to produce the above symptoms 4-7 days after ingestion. Migration and thus the acute phase continue for 6-8 weeks until the larvae mature and settle in the bile ducts.

Chronic Phase The symptoms of chronic infection are much more common in human populations and include biliary cholic, abdominal pain, tender hepatomegaly, and jaundice. In children, severe anemia is a common result of infection and is the greatest source of disability from infection in this age group. These symptoms reflect the biliary obstruction and inflammation caused by the presence of the large adult worms and their metabolic waste in the bile ducts. Inflammation of the bile ducts eventually leads to fibrosis and a condition called "pipestem liver", a term describing the white appearance of the biliary ducts after fibrosis. The final outcome of severe infections is portal cirrhosis and even death (www.stanford.edu/class/humbio103/parasites2001/fascioliasis/*Fasciola*.htm#epidemiology)

It is of interest to note that if raw bovine liver harboring *Fasciola* is eaten by humans, young flukes may become attached to the buccal or pharyngeal membranes causing pain, irritation, hoarseness and coughing. This condition is known as 'halzoun' (Cheng, 2006).

1.5 Public Health and Prevention Strategies:

The presence of infection in a population is dependent upon and exacerbated by four factors:

- the presence of a substantial reservoir
- the presence of the intermediate host, the *Lymanaea* snails

- the opportunity for water source contamination by human and non-human hosts
- dietary practices that include the consumption of raw, untreated aquatic vegetation

Therefore, the most effective public health measures need to address several, if not all, of these factors.

Education: Behaviour changes have the potential to be the most effective and cost efficient approaches to disease control and thus, education is an essential aspect of any public health effort. The primary message of *Fasciola hepatica* campaigns is to keep domestic animal herds separate from the growing sites of aquatic. This limits the risk of contaminating the vegetation and thus decreases both human infection and the animal reservoir.

Teaching the washing of vegetables in either 6% vinegar or potassium permanganate for 5 to 10 minutes, which destroys the encysted metacercariae, is another useful educational effort. This approach has proven more acceptable to communities than past attempts to entirely halt the consumption of raw vegetables.

Molluscicides: The most frequently used public health intervention is the application of molluscicides to decrease the population of *Lymnaea* snails, the intermediate hosts of *Fasciola hepatica.*

Chemotherapy: Chemotherapy has been used for years in animal populations to decrease the animal reservoir and reduce agricultural losses. Until recently, however, bithionol was the only treatment available for fascioliasis and its cost, high doses, and the extended length of treatment effectively prohibited its use for large-scale campaigns.

Treatment

Treatment for fascioliasis has an 80-100% success rate. In many countries, a 5-10 day course of oral bithionol at 30 mg/kg body weight is not only the preferred treatment but the only one available. It is highly effective. Triclabendazole, an antihelmintic agent, has recently been proven to provide substantial human benefit at much lower doses than bithionol. In 1990, the WHO and pharmaceutical company, Ciba-Geigy, partnered to conduct clinical trials of triclabendazole and found that 1-2 oral doses at 10 mg/kg body weight administered in a single 24 hour period results in virtually no side effects and has a success rate approaching 100%. Along with pharmaceutical therapy, surgery may be necessary in very extreme cases to clear the biliary tract.

1.6 Significance of the study

People are unaware about the pathogenicity of helminthes to the cattle and results decrease in dairy products. In the study "an effort has been made to identify the prevalence of different helminth parasites. Scanty work has been done previously in Nepal.

Moreover the present study may help to the future investigator to advance their knowledge. The present study throws light on different problems found by public and butcher notifying the burden of infection of the trematode. The next step to be considered are the most practical economic way by which goal can be achieved. Continuous research is vitally important.

1.7 Limitation of the study

The present study bears following limitations which are mainly due to time and cost limitations.

- Data of only two seasons were accumulated.
- Since the butchers have to deliver the carcass of the slaughtered animals to the meat shops at very early morning, time spent on observing the carcass was limited and if not always, sometimes, rapid observations of the carcass have to be made.
- fascioliasis is known as zoonotic diseases but during research, they have only been studied on the slaughtered buffaloes, their impacts on human have been includeded only from few hospitals.

II

OBJECTIVES

2.1 General objective

- Prevalence of fascioliasis present in buffaloes of slaughter house of kirtipur Municipality.

2.2 Specific objectives

- To determine the prevalence of fascioliasis in slaughtered buffaloes.
- To observe the distribution of slaughtering places and type of meat shops occurring in Kirtipur.
- To observe the hygienic condition in and around the slaughtering area and meat shops.
- To observe the feces of the animals to search for the eggs of *fasciola*.
- To create a background for further studies regarding fascioliasis in different parts of country.
- To create awareness among butchers, meat sellers and consumer

III

LITERATURE REVIEW

Upto the middle of the 17th century ,knowledge of parasitology was limited to a few external parasites such as lice and flies, and some internal parasites as roundwarms, pinwarms, tapewarms, but they were considered as natural products bodies.

Linnaeus gave another view about these internal parasites that they originated from accidentally swallowed free living organisms. However, this belief was erased in the half of the 17th century by Francisco Redi, the grandfather of parasitology. He demonstrated development of maggots from eggs of flies. He also proved that Ascariasis had males and females and produced eggs. At the same time, Leeuwenhoek perfected microscope and discovered protozoan parasites.

3.1 LITERATURE REVIEW IN CONTEXT OF THE WORLD

In 1379, Brie de was the first to describe trematod *Fascoila.*

Price (1953) examined *Fasciola* species from various parts of the United States and Found that some areas had species identical to *F. gigantica* from old world whereas other area had *F. gigantica.* An intermediate form between *F. hepatica* and *F.gigantica* also. Occurred

Varma (1953) described a new species of liver fluke following his study of. *Fasciola* infection in cattle, buffaloes and goats in Bihar, India. He named it *F. indica,* which was differentiated from *F. gigantica* on the basis of their cuticular armature, gut branching and testes of the adult, shape and size of the eggs.

Boray (1963) studied the effect of temperature on the development of *F. hepatica* in *Lymnaea tomentosa* in Australia. He showed that the increase of temperature decreased the development time of *F. hepatica.* He (1969) estimated daily faecal out in light sub clinical *F. hepatica* infection in sheep to be 0.5 million. Animals with moderate infections shed 2.5 to 3 million eggs daily in their faeces.

Kendall (1965) studied considered *F. hepatica* and *F. gigantica* to be only two valid species. It has been suggested that geographical isolation is responsible for the appearance of various morphological strains and physiological races of *Fasciola* species.

Blamire *et al*.,(1970) studied the condemnation rate for fascoiliasis in adult cattle in England the Wales ranged between 22% and 35%. It then fell steadily from 20% in 1970 to 6% in 1978 (Blamire *et al.*,1980). In 1978, the rate for fascoiliasis in England and Wales was 5.6% (MAFF,1979) and in 1980 6.5%(MAFF,1980)

Bindernagel 1972 studied the liver fluke *fasciola gigantica* in african buffalo and antelopes in Uganda, east Africa. The liver fluke *Fasciola gigantica* was recovered from 58 percent of 82 African buffalo (*Syncerus caffer*), 47 percent of 103 Uganda kob (*Adenota (Kobus) kob*), and 47 percent of 47 Jackson's hartebeest (*Alcelaphus buselaphus jacksoni*) examined in the West Acholi District of Uganda in 1965-67. None of 22 oribi (*Ourebia ourebi*) was infected. There was no significant difference in prevalence between males and females or between host age groups except in Jackson's hartebeest. In this host the prevalence was higher in older animals.In 5 percent of the infected hartebeest, 17 percent of the infected kob, and 69 percent of the infected buffalo, flukes were recovered only from the gall bladder. The importance of examining the gall bladder in addition to the bile ducts to detect *F. gigantica* is emphasized.

Dumag *et al*., 1975 conducted survey of 522 water buffaloes in endemic province an of 94.2% was found positive for *Fascoila* egg. In some areas the incidence of liverfluke infection varied from 95%-100% was found positive.

Kennet 1981 studied the 330 cattle, *Fasciola* spp was found in 3.64%. *F. gigantica* was recovered in 3.03% of the 330 cattles. *F. hepatica* was found only in mixed infection with *F. gigantica* in 0.60% of cattle.

Molif. *et al.*,(1989) found 42.07%, 35% and 35% buffaloes infected with *Fascoila gigantica* at Bareilly, Ludhiana and Patna respectively.

Pfukenyi *et al*., 1990-1999 studied a retrospective study of the prevalence and seasonal variation of *Fasciola gifantica* in cattle slaughtered in the major abattoirs of Zimbabwe between 1990 and 1999. A retrospective study covering a period of 10 years (1990–1999) was conducted using post mortem meat inspection records of the Veterinary Department Information Management Unit at Harare to determine the prevalence and seasonal variation of bovine fasciolosis in Zimbabwe. Records of monthly and annual returns from five major abattoirs were examined in regard to total cattle slaughtered and the corresponding number of livers condemned due to *Fasciola gigantica* infection. Prevalence of fasciolosis was calculated as the number of cattle slaughtered. Seasonal variations in the prevalence were examined by pooling respective monthly condemnation data over a 10-year (1990-1999) period. A total of 2 474 232 cattle were slaughtered during this period and 197 565 (37.1%) of these cattle were infected with *F. gigantica*. The pattern of distribution of *F. gigantica* was significantly higher in cattle originating from catchment during the wet season than those slaughtered during the dry season ($P < 0.05$). Based on the study findings a control programme for the disease in Zimbabwe is suggested.

Srihakim *et al*., (1991) studied the Problem of fascioliasis in animal husbandry in Thailand. One of the most important parasitic diseases in adult cattle and buffalo in Thailand is fascioliasis, caused mainly by *Fasciola gigantica*. The economic loss from fascioliasis in cattle and buffalo throughout Thailand had been assessed at not less than 100 million Baht. Recent investigations have been shown that the average prevalence of *F. gigantica* in cattle and buffalo in Thailand was 11.8%. However, the prevalence varies considerably between villages, ranging from 0 to 85%. The prevalence is high in areas surrounding dams or large ponds in which *Lymnaea auricularia rubiginosa*, the intermediate host of *F. gigantica* is found. An epidemiological study revealed that the disease has a seasonal pattern. Strategic liver fluke treatment of all cattle and buffalo which were older than 8 months were carried out once a year in September. In addition, animals in poor condition were treated in April to prevent severe losses, especially in high prevalence areas or where strategic treatment was missed. Problems of liver fluke control include the lack of knowledge about the parasite on the part of the farmers and the lack of availability of drug supplies at the village level, both of which were important to allow strategic treatment of animals. To approach these problems, the government had developed "Farmer Self-Help Worm Control Program" in seven provinces in Northeast Thailand which was operated by village farmers trained as program "keymen". This program was very effective and was extended throughout Thailand in the next Seventh Social and Economic Development plan.

Singh and Sah (1992,1993) conducted a survey in the low lands of IAAS, Rampur, to investigate the relationship between snail population and liver fluke infection in ruminants. The liver fluke infection was 48.57%, 28.57%, 25% and 21.28% in buffaloes, cattle, sheep and and goats respectively. The highest infection rate of liver fluke was observed in September (50%), followed by the month October (43.75%) and January (35%). The snail population was negatively associated to rainfall: the highest snail population being in February and March (22 snails/13sq.m). None of the metrological factors had significant positive correlation with the incidence of liver fluke in different species of animals. There was also non-significant, negative correlation between liver fluke infection and snail population.

Roberts and Suhardono (1996) approached to the control of fasciolosis in ruminants were compared for developing countries with particular reference to regions growing irrigated rice. Strategic and control fasciolosis in these developing countries existing families possess small numbers of animals, feed and water sources were shared by many families, and the products were mainly draft power, fertilizer and meat for local consumption. Consequently the agricultural cycle, and the life cycle of parasite and intermediate hosts, was closely interrelated and there were some scope for controlling infection by modifying husbandry practices. Anthelmintics were not affordable. Recent observation of a major *Fasciola* resistance gene with substantial dominance, in Indonesia thin tail sheep infected with *F. gigantica,* suggest that parasite control by breed substitution, of cross– breeding and selection were teasible. Such control would be in expensive to implement and sustainable.

Rai *et al.*, (1996) conducted a survey on epidemiological study of bovine fascioliasis in Andaman and Nicobar Islands. The incidences were higher from September to April. The *Lymnaea* snails in pastures and water logging areas were in higher number between June to December. Preliminary field trial conducted showed that ducks in open range system could be an effective biological control of lymnaeid snails and achieve a blance in the infection. Deworming with Albendozole twice a year i.e. September/Octorber followed by February/March reduced the incidence of *F. gigantica* infection.

EI-Bahy (1997) collected and examined faecal sample from June 1995 to June 1996 in Egypt. Faecal sample positive for *Fasciola* were 14.5% of 1225 buffaloes, 26.6% of 3500 sheep, 12.3% of 708 donkeys and 6% of 1800 human. *Fasciola* infection rates were highest during the summer. The highest prevalence being buffaloes over three years old.

Marques and Seroferneker (1999) conducted a study for *Fascoila hepatica* infection in buffaloes in the state of Rio Grande Do Sul Brazil. They examined 105 slaughtered buffaoes at a meat packing plant between April 1999 and November 1999, in Viamao in Rio Grand Do Sul in Southern Brazil. The occurrence rate of *Fascoila hepatica* was 20% for buffaloes.

Mehra *et al*., (1999) investigated the effects of *Fasciola gigantica* infection on bodyweight gain, dry matter intake, digestibility of nutrients and feed conversion efficiency in buffalo calves. Nine male buffalo calves of the Murrah breed, aged 12 to 15 months with a mean bodyweight of 166 kg, were randomly assigned to groups of five (group 1) and four (group 2). The animals in group 1 were given 1000 viable, mature metacercariae of *F. gigantica* orally, while the animals in group 2 served as uninfected controls. They were stall fed on diets containing a concentrate mixture and ad libitum wheat straw and were maintained by standard management practices for a period of 165 days after infection. The average daily live weight gain of the infected animals was 110.6 g, compared with 439.4 g in the uninfected controls, and was associated with the appearance and establishment of immature flukes in hepatic bile ducts. The feed conversion efficiency declined significantly ($P<0.01$) from 41 days after infection and was lowest at the end of the experiment. *F gigantica* infection did not influence the digestibility of the nutrients. The impaired feed conversion efficiency was mainly due to a reduction in dry matter intake due to in appetence.

Nguyen *et al*., (1999)carried out a survey of 955 swamp buffaloes livers in Vietnam from January to July 1998. Out of 955 buffaloes studied, 46.67% were found infected with *Fasciola* spp. The buffaloes infected with *Fasciola gigantica* were 14.14% and both *Fasciola hepatica* and *Fasciola gigantica* were 32.04%. The mean numbers of flukes in the infected organ were 78.

Yadav *et al*., (1999) studied the Primary experimental infection of riverine buffaloes with *Fasciola gigantica* in India. The clinical course of the primary experimental *Fasciola gigantica* infection was investigated in riverine buffalo calves of the Murrah breed. Nine male calves aged 12–15 months were randomly assigned to two groups of five (Group I) and four (Group II) animals. Each animal in Group I, was orally infected with 1000 metacercariae (mc) of *F. gigantica*, whereas Group II animals did not receive any infection dose and served as uninfected controls. No clinical signs of fasciolosis were observed until the sixth week post-infection (PI). Group I animals, however, developed recognised symptoms of acute fasciolosis, comprising apyrexic inappetance, anemia, poor weight gain, diarrhoea and sub-mandibular and facial oedema, respectively, from 5, 6, 8, 16 and 17 weeks PI. The signs were intermittent in nature and of variable duration. The prepatent period was of 92–97 days (mean 95.2 ± 3.1). One of the five infected animals died on Day 147 PI. At necropsy, 36.8 ± 11.0% of the infection dose was recovered as adult fluke population. The gross lesions were primarily biliary in nature. Group II, the uninfected controls, throughout the study period of 165 days PI, did not show any symptom and were negative for *F. gigantica*. The study demonstrated that the onset of adverse effects of *F. gigantica* on the growth and health of the infected host was mainly noted during late prepatency much before coprological prediction and diagnosis. The significance of preventive therapy against fasciolosis during prepatency has been stressed in endemic areas.

Rangel-Ruiz (1999) found the seasonal trend in infection and maturity of *Fasciola hepatica* in cattle in relation to macro-climatic factors, 2730 condemned lives were examined from March 1989 to February 1992. The maturation stages detected in the cattle showed (a) *Fasciola hepatica* mature throughout the year (b) Persistence of mature, gravid *F. hepatica* indicated that parasite eggs were shed throughout the year. Recuitment of *F. hepatica* occurred throughout the year with two major periods of infection. The first and mean period dry season (from February to June) and second minor infection pattern in snails, as well as fluctuation in the snail population according to rainfall and temperature.

Iassan et al.,(2000) conducted a study, on prevalence of fascoiliasis in buffaloes in Giza Governorate. They collected 1,042 buffaloes faecal samples. Their coprological examination that 16.46% of the examined buffaloes were harbouring *Fascoila* spp.

Harrison and Pearson (2000) established chronic *Fasciola gigantica* infection in lambs maintained on diets differing in their protein and nitrogen content. It was suggested that diet containing approximately 14% protein help alleviate the negative production effect associated with fasciolosis. Similarly diets with too high protein content (>19%) should be avoided in areas endemic for fasciolosis.

Okewole (2001) studied the clinical evaluation of three chemoprophylactic regimes against ovine helminthosis in a *Fasciola*-endemic farm in Ibadan, Nigeria. Fasciolosis is an economically important disease of ruminants world-wide, and especially in Europe, North America, Asia and Africa, where it causes significant morbidity and grave economic loss (1-5). Acute infections result from the immature flukes tunneling through the liver parenchyma with extensive tissue damage and haemorrhage that culminate in severe clinical disease with high mortality in grazing sheep in Africa (3,6,-10). The chronic disease practically results from the adult flukes, often in pairs, lodging within the bile duct, causing duct wall hyperplasia, progressive occlusion and ultimate calcification of the duct wall with characteristic chronic wasting syndromes and various hepatopathies (6,7,9,10,11). Other economic production parameters observable in chronic disease include reduced conception/pregnancy rate (2,9,12-14), delayed onset of puberty (15), reduced lambing rate (13,14), reduced birth weight (14,15) and reduced multiple birth rates which were reported to be sequela to the low mineral and protein supply to the bone marrow as a result of extensive liver damage characteristic of chronic fascioliasis (2,13,16,17).

Lezeriuc *et al*., (2002) studied 28,878 slaughtered cattle between 1995 and 2001 in the abattoir at Bacau, Romania. During this period, 2,220 cattle were diagnosed with fascioliasis. The prevalence of bovine fascioliasis was found higher in 2001 (14%) as compared in 1995 (4%).

Maqbool (2002) undertook an epidemiological study at slaughter house, livestock farms, and veterinary hospitals and on household buffaloes under the different climatic conditions existing in Punjab province. Infection rate was found 25.99% at slaughter house, 20.10% at livestock farms, 13.70% at veterinary hospitals and 10.50% at household buffaloes. Overall highest 24% seasonal prevalence in all types of buffaloes was recorded during autumn, spring and winter. It was found that a higher infection rate was recorded in older buffaloes than in youngsters (below 2 yrs of age). Buffaloes of either sex were equally affected.

Oliveria (2002) carried out survey on hepatic fascioliasis. Among 130 slaughtered buffaloes studied at the Cajati abattoir of Vale Do Riberia, Sao Paulo, Brazil from Sept. 2002 to Dec. 2002, the incidence of livers infected by *Fasciola hepatica* was 75%.

Haridy *et al*., (2002) Fascioliasis studied in Dakahlia centres based on parasitological examination of buffaloes and the rate of infection was 9.73%. The mean egg per gram stool was 13.6 for buffaloes. The mean number of *Fascoila* works was 62.7. The highly infected buffaloes was in Manzalla (19.29%) and the lowest was in Mit Ghamr (4.93%).

Theodoropoulos *et al*., (2002) carried out a long year survey of 10, 277 slaughtered farm animals in the region of Trikala, Greece to estimate the prevalence of parasitic infections responsible for the condemnation of carcasses and viscera during meat inspection, and their economic implication. The organs examined for the presence of parasitic lesions during meat inspection were: liver and lungs of all animals, rumen of cattle, small intestine of lambs and kids, and muscles of cattle and swine. The parasitic lesions observed in the lungs of cattle, sheep and goats were caused only by hydatid cysts. No hydatid cysts were observed in the lungs of swine. The parasitic lesions observed in the liver of cattle, sheep and goats were as a result of hydatid cysts and flukes of *Fasciola hepatica* and *Dicrocoelium dendriticum,* while those of swine were due to milk spots only. The prevalence of parasites responsible for the condemnation of marketable organs was low (0.26%). Parasites were responsible for 22% of the total of condemned organs, and their annual cost was 99,500 GDR.

Azhar Maqbool (2002) conducted the Epidemiology of fascoilasis in buffaloes under different managemental conditions. Epidemiological studies were undertaken at slaughter houses, livestock farms and veterinary hospitals and on household buffaloes under the different climatic conditions existing in Punjab province. Infections rate was 25.59, 26.16, 13.7 and 10.5 per cent, respectively in slaughtered buffaloes, buffaloes at livestock farms, veterinary hospitals and in household buffaloes. Overall highest (24.0%) seasonal prevalence in all types of buffaloes was recorded during autumn, followed by spring (20.0%), winter (13.0%). While the lowest (9.0%) was recorded during summer. It was noticed that a higher infection rate was recorded in older buffaloes than in youngsters (below 2 years of age) where as sex showed no significant difference. Buffaloes of either sex are equally affected.

El-Shazly (2002) studied the Fascioliasis among live and slaughtered animals in nine centers of Dakahilia Governarate, Egypt.. The overall rates of infection were 12.31%, 9.73%, 17.84% and 5.40% in cows, buffaloes, sheep and goats respectively. The mean eggs per gram stool were 22, 13.6, 148.3 and 8.6 for cows, buffaloes, sheep and goats. The mean numbers of Fasciola worms/liver/animal were 69.1, 62.7 and 208.1 for cows, buffaloes and sheep respectively. The highly infected sheep was in Manzalla (23.07%), the lowest was in Mataria (6.35%). The highly infected cows was in Manzalla (20.9%), the lowest was in Sherbeen (9.43%). The highly infected buffaloes was in Manzalla (19.29%), the lowest was in Mit Ghamr (4.93%). The relatively highly infected goats was in Manzalla (12.5%) and the lowest was zero in Mit Ghamr. Overall partial condemnation of liver was 3.81% (1997), 3.24% (1998), 2.66% (1999) and 2.64% (2000). Regarding the type of animal, it was 6.38% in cows, 1.74% in buffaloes and 1.0% in sheep.

Maqbool *et.al*., (2002) studied the epidemiology of fasciolosis in buffaloes under different managemental conditions. Epidemiological studies were undertaken at salaughter houses, livestock farms, veterinary hospitals and on household buffaloes under the different climatic conditions existing in Punjab province. Infection rate was 25.59, 26.16, 13.7 and 10.5 per cent, respectively in slaughtered buffaloes, buffaloes at livestock farms, veterinary hospitals and in household buffaloes. Overall highest (24.0%) seasonal prevalence in all types of buffaloes was recorded during autumn, followed by spring (20.0%), winter (13.0%). While the lowest (9.0%) was recorded during summer. It was noticed that a higher infection rate was recorded in older buffaloes than in youngsters (below 2 years of age) where as sex showed no significant difference. Buffaloes of either sex are equally affected.

Akhtar *et al*., (2003) conducted a study on prevalence of helminthiasis on buffaloes in colony, Hyderabad. The prevalence of helminthiasis was found to be 15.2% in buffaloes. Out of 500 samples examined, 9.2% were infected with nematodes and 5.4% were infected with trematodes respectively. The chief trematodes identified were *Fasciola gigantica* 3.2% and *F. hepatica* 2.2%.

Basu *et al.*, (2003) reported *Fasciola hepatica* and *Fasciola gigantica* be the major parasites involved in causing economic losses to livestock in East Africa. *Fasciola hepatica* has shown to be the most important fluke species in Ethiopian livestocks with distribution over 3 quarters of the nation except in the arid Northeast and East of the country. The distribution of *Fasciola gigantica* was mainly localized in the Western humid zone of the country. The prevalence of bovine fascioliasis has shown range from 11.5% to 87%. A rough estimate of the economic loss due to fascioliasis in bovine is about 350 million birr per year.

Marques *et al.*, (2003) examined 482 livers, 377 from cattle and 105 from buffaloes slaughtered at a meat packing plant between April 1999 and November 1999, in Viamao, a town in the state of Rio Grande do Sul, in southern Brazil. The cattle slaughtered at the meat packing plant belonged to eleven towns. The occurrence rate of *Fasciola hepatica* was 10.34% for cattle in seven of the eleven towns included in the study (63.6%) and 20% for buffaloes in four of the five towns (80%). In terms of age, the occurrence rate was 81% for buffaloes with up to two years of life and 19% for buffaloes older than two years.

Bory (2003) observed prevalence of liver fluke in buffaloes and beef cattle slaughtered at VISSAN. The infection in buffaloes and beet cattle were 14.83% and 22.92% respectively. *Fasciola gigantica* infected both buffaloes and cattle where *Fasciola hepatica* infected only buffaloes.

Asharaf *et al.*, (2004) carried out the study to investigate the distribution and natural infections of local lymnaeids, environmental characteristics related to the disease transmission and determining the most prevalent fasciolids and definitive hosts in human endemic areas of Gilan province, Iran. According to the data obtained from slaughterhouse observations in Bandar-Anzali and Rasht, the main fasciolid in local cattle is *F. gigantica*. Of 928 adult liver flukes collected from 13 infected livers of cattle, in Rasht and Bandar-Anzali slaughterhouses, 91.1% were diagnosed as *F. gigantica* and 8.9% as *F. hepatica*. *L. gedrosiana* and *L. palustris* were the most prevalent lymnaeid snails in this endemic zone.

Irwin *et al.*,(2004) studied Glycosidase activity in the excretory–secretory products of the liver fluke, *Fasciola hepatica*. *Fasciola hepatica* secretes proteolytic enzymes and other molecules that are essential for host penetration and migration. This mixture may include enzymes required for the degradation of supramucosal gels, which defend epithelial surfaces against pathogen entry. These contain hydrated mucins that are heavily glycosylated, Exvretory–secretory products(ES) from *F. hepatica* ware examined for a range of glycosidase activites, using syntetic 4–methylumbelliferyl glycoside as substrates. The ESproduct contained at least 8 different glycosidase activities, the most abundant of which were *β*-N-acetylhexosaminidase, *β*-glucuronidase, ℑ–mannosidase and neuraminidase were also present. *β*-glucosidase. Alpha–fucosidase, *β*-N–acetylhexosaminidase and *β*-galactosidase were present in multiple isoforms (at least 4), whereas *β*-glucosidase appeared to exist as one isoenzyme with a pl <3.8, All three enzymes had acidic pH optima (45-50). Ovine small intestinal mucin was degraded by ES. The ability of *F. hepatica* ES to degrade mucin in the presence or absence of active cathepsin L Suggests that cathepsin L is not essential for mucin degradation. The abundance of *β*-galactosidase and *β*-hexosaminidase in ES supports a role for these enzymes in mucin degradation.

Yosihara *et al*., (2004) studied ingestion of *Fasciola gigantica* metacercarias by the intermediate host snail, *Lymnaea ollula*, and infectivity of discharged metacercariae. The rate of ingestion of Fasciola normal metacercariae (NMc) encysted on plants by Lymnaea ollula was examined, and the infectivity of the ingested metacercariae (IMc) in the feces of the host snail to mice was studied. As a result of ingestion by snails, the metacercarial outer cyst disappeared in about 50% of IMc in feces. There was no significant difference in the liver juvenile recovery at autopsy between mice inoculated with NMc and IMc kinds of metacercariae. Compared with NMc, the number of IMc could more easily be counted, because the separation of IMc from fecal contents under a microscope was not laborious.

Phiri *et al*., (2005) carried out a study at selected major abattoirs in Zambia to determine the prevalence and some factors influencing occurrence of fascioliasis in cattle. Of 841 cattle livers inspected and 677 faecal samples analyzed, prevalence rates of 53.9% and 48.9%, respectively, were found. Cumulative prevalence of 60.9% (n = 677) was recorded. According to age, no significant difference of infection was found. Female cattle on liver inspection (59.3%) and coprological examination (65.2%) had significantly higher ($P < 0.001$) rates than males (44.5% and 36.3% respectively). Origin of cattle had a significant influence ($P < 0.001$) on the prevalence rate. According to fluke egg count classification, 68.5% of cattle had light infection, 20.9% moderate, 4.4% heavy and 6.2% severe. These results indicate that *Fasciola gigantica* infection is an important condition that leads to high liver condemnations and/or trimmings in cattle tendered for slaughter.

Cruz *et al*.,(2005) studied seasonal transmission of *Fasciola hepatica* in cattle and Lymnaea (Fossaria) humilis in central Mexico. A 19–month study on the prevalence of fasciolosis in 30 naturally infected cows, the presence of infected and non–infected Lymnaea (Fossaria) humilis snails, and variation in soil temperature and humidity is reported. The prevalence of fasciolosis in cattle declined from around 50% in March to 30% in July, then, it increased from August, reaching a plateau of 100% in November in June and July, respectively, which peaked between August and November was observed. In July, L. (F) humilis snails appeared, but the infection could only be found in these in August and November. The number of infected snails did not reflect the infestation rate in cows, even though the infestation kinetics in both hosts behaved as predicted from the life cycle of the parasite.

Legocki *et al.,* (2005). studied Immunoproteetive properties of transgenic plants expressing E2 glycoprotein from CSFV and cysteine protease from *Fasciola hepatica*. Immune responses were elicited in laboratory animals after oral vaccination by transgenic plants (lettuce and alfalfa) expressing the E2 glycoprotein on Classical Swine Fever Virus (CSFV) or cysteine protease from Fasciala hepatica. ELISA analyses demonstrated that the oral route is effective in inducing a specific antibody response against these antigens in mice.

Alcala *et al.*., (2005) studied *Fasciola hepatica* proteolytic activity in liver revealed by in situ zymography. *Fasciola hepatica* secretes cysteine proteases that play a role in facilitating parasite migration. The aim of this study was to detect the inhibition of the proteolytic activity of *F. hepatica* cysteine proteases in the liver of C57BL/6 cathepsing B knockout mice (cat B–/–) and wild–type contrls (cat B+/+) by intraperitoneal administration of N-[N-(L-3-trans-carboxyoxirane-2- carbony1)-L-leucyl]-agmatine, (E-64) using the film in situ zymorgraphy (FIZ) technique and image analysis. The FIZ technique revealed that intraperitoneal administration of E-64 dramatically reduced (85%) *F. hepatica* proteolytic activity in the liver of experimentally infected mice with no discernable side effects. These results suggest the usefulness of the FIZ for determining in vivo activity of *F. hepatica* proteases, as well as their inhibition by intraperitoneal administration of E-64 in hepatic tissue of infected mice.

Shirai *et al.*, (2006) investigated 117 livers with Fascioliasis in buffaloes. The study was focused on the number of *Fasciola*, the number and intrahepatic localization of affected hepatic ducts and bile ducts, and the degree of fibrosis in the hepatic segments and bile ducts. The degree of pathological changes in bile ducts caused by fascioliasis was classified into five levels. The site of *Fasciola* habitation was most often the hepatic ducts of the porta hepatis: it was the left hepatic duct in 101 livers and the right hepatic duct in 88 livers.

Maher *et al*.,(2006) studied the purified hydatid glycoprotein fraction effectively diagnoses human hydatidosis in Egypt. Diagnosis and quantification of *Echinococcus*. *Granulosus* infection in man and animal hosts are centralized to feasible control. This study included 93 serum samples, 25 sure positive hydatid cases confirmed surgically, 7 suspected cases diagnosed by indirect haemgglutination IHA and 41 cases other parasitic infections (15 S. *mansoni*, 8 *Fasciola*, 7 *Ascaris*, 5 *H. nona* & 6 Ancylostoma) diagnosed by microscopic examination and were negative by ELISA and/or M for anti–hydatid antibody. Twenty negative serum samples served as healthy controls. Six types of hydatid fluid antigens (crude, host-free -& Con-A purified) of human and camel origin were subjected to electrophoretic separation (SDS–PAGE) and immunoblotting (EITB). The anti–hydatid lgG was detected in sera of the different groups for evaluation of sensitivity, specificity and diagnostic efficacy of each type of antigens. Detection of circulating hydatid antigen (CAg) was performed using anti rabbit hyperimmune sera raised against Con-A purified either human or camel hydatid antigen. SDS-PAGE revealed several bands ranging from 55-185 kDa with 10 kDa band shared by all antigens. The specific bands revealed by EITB for Con–A purified camel and human antigens were at 80, 110 & 55, 110 kDa respectively. ELISA highes sensitivity (96.9%) was by using host-free Con–A purified glycoprotein fraction of human hydatid antigen Highest specificity (98.4%) was recorded upon use of either Con–A purified camel or human antigen with 94.5% & 97.7% diagnostic efficacy respectively. Detection of circulating antigen by polyclonal antibodies against Con–A purified human hydatid antigen revealed 91.8% specificity.

Bakry*et et al.,*(2006) studied effect of non target snails on some biological of *lymnaea* natalensis snails and their infection to *Fasciola gigantica* in Egypt. The influence of non–target freshwater snails (Melanoides tuberculata and Planorbis planorbis) on the capacity of *Fasciola* egg production *F, gigantica* miracidia to infect *Lymnaea* natalensis and their effect on mortality and growth rotes showed that the snails exhibited a competitive ability against *L, natalensis*. The mortality rate existed in mixed cultures with snails was greatly increased, and increased with increase of snails number. The egg production and growth rate were negatively affected by the presence of M. tuberculate and P. planorbis which was more pronounced when snails were at higher ratio IL; 10D. Also, the snails showed significant degree of reduction in infection rate of L, natalensis with F. *gigantica* miracidia.

EI Shazly *et al.*, (2006) studied intestinal parasites in Dakahlia governorate, with different techniques in diagnosing protozoa. A total of 3180 partients attending Mansoura. University Hospitals' Clinics, were subjected to stool examination by direct wet smear, formol–ether concentration, original formol–tween concentration, modified formol–tween concentration, modified sheather's sugar floatation, Potassium hydroxide concentration and Gomori's Trichrome stain, and modified kinyoun's acid–fast stain, and Ryan's Trichrome blue stain for Microsporidia. The intestinal helminthes in a descending order of abundance were: *S mansoni* (5.3%). *Fasciola sp.* (4.8%), *H. heterophyes* (4.2%), *Hymenolepis nana* (3.9%), Trichostrongylus sp. (2.6%), A lumbricoides (1.8%), *Strongyloides stercoralis* (1.5%), *H diminuta* (1.4%), *Taenia saginata* (1.1%), *E vermicularis* (by smear; 1.1 %), T. trichura (0.7%) and lastly A. duodenate (0.1%). The intestinal protozoa in a descending order of abundance were Blastocystis hominis (22.4%), Giardia lamblia (19.6%), *Entamoeba histolytica/E.dispar* (19%), *lodamoeba butschhii* (16%), *Cryptosporidium parvum* (143%), *E coli* (9.7%), *Lsospora homins* (7.7%), *Endolimax nana* (6.9%), *E. hartmani* (5.9%), *Dientamoeba fragilis* (5.1%), *Chilomastix mesnili* (5.1%), *Trichomonas horminis* (4.2%), *Cyclospora cayetanensis* (4.2%), *Microsporidia spores* (3.2%), *Enteromonas hominis* (1.9%) and *Embodomonas intestinalis* (13%). The sesults were discussed.

Aroonroch *et al.*, (2006) conducted Hepatic fascioliasis due to *Fasciola hepatica*: A two-case report. Two cases of *hepatica* fascioliasis due to *Fasciola hepatica* were retrived from our surgical-pathology file since the hospital foundation in 1969 up to 2005. The diagnosis of hepatic fascioliasis was based on detection of one live fluke in a large cystic lesion in the lobectomized liver specemen in one case and of deposited eggs in the liver specimen obtained from open biopsy in the other *Hepatica* jascioloasis is rather rare and almost worldwide in distribution including Thailand The diagnosis should be considered in the patient from endemic areas consisting of the nortern, northeastern and upper-central regions of the country, with a history of ingesting fresh water plants or drinking untreated water and having fever right-upper-quadrant pain or intrahepatic cystic lesion(s) together with absolute peripheral blood eosinophilia.

Alcala *et al*., (2007) studied effect of a cysteine protease inhibitor on *Fasciola hepatica* (liver fluke) fecundity, egg vibility, parasite burden, and size in experimentally infected sheep in Mexico. *Fasciola hepatica* secretes proteolytic enzymes during liver invasion. The present study examined the effects of the cysteine protease inhibitor Ep-475 on sheep considering the following variables: serum levels of aspartate aminotransferase, < sep > 1 < sep >–lactate dehydrogenase, and γ–glutamyltransferase, liver fluke fecundity, egg viability, parasite burden, and size. Twenty–four male sheep were randomly allocated in four groups of six animals each as follows: group A was infected with *F. hepatica* metacercariae and treated with 50 mg/kg of Ep–475, group B was infected and untreated, group C was uninfected and treated, and group D was uninfected and untreated. All animals were euthanized 10 were significantly lower in Ep–475 treated sheep than in untreaed controls, although liver damage was produced. No significant reduction in total worm burden was observed between treated and untreated sheep. However, there was a significant difference on the average size, structure development, ova counts, and egg viability of liver flukes from these two groups, Results showed that Ep–475 reduces liver damage due to fasciolosis and induces an impairment of liver fluke growth and fecundity. These effects pinpoint liver fluke proteases as potential targets for pharmacological intervention.

Weiming *et.al*., (2007) studied an evaluation of a recombinant excretory secretory Haemonchus contortus protein for use in a diagnostic enzyme-linked immunosorbent assay in China. The nematode Haemonchus contortus (H. contortus) is one of the most pathogenic and economically important parasites of sheep. A 24 kDa protein is one of the important components in H. contortus excretory/ secretory (ES), which was shown to have important biological function. In our research, the cDNA of its open reading frame (ES24) was obtained and analyzed. Then the ES24 was subcloned into pET–30a expression vector. The recombinant vector that codes hexahistidyl peptide fusion protein (His–ES24) was transformed into Escherichia coli BL21 (DE3) strain. After induction, a high expression level of His–ES24 was found at 6 h taking about 26% of the total bacterial protein analyzed by gel thin–layer scanning. The expressed His–ES24 was purified and then used in an enzyme–linked immunosorbent assay (ELISA) to detect specific antibodies in serum samples. The ELISA was able to differentiate between H. contotus–infected sheep serum and *Fasciola hepatica*–infected sheep serum or non–infected sheep serum. No cross–reaction was observed in sheep sera that have been experimentally infected with F hepatica. A total of 153 field sheep serum samples conserved in our laboratory were examined using the His–ES24 ELISA, and 82 (53.6%) of them were found seropositive to H. contortus. Our results demonstrate that the prokaryotic–expressed His–ES24 might be a useful diagnostic reagent for epidemiological studies of H. contortus in sheep.

Inoue *et al.,* (2007) studied a case of human faseiolosis: discrepancy between egg size and genotype of Fasciola sp in Japan. In human fasciolosis, differential diagnosis of the eausative flukes, *Fasciola hepatica*l and *Fasciola gigantica*, is problematic. We report a rare case of human fascilosis in which an adult worn was recovered from the bile duct of a Japanese man. Morphometric data of the worm were consistent with those of F. hepatica, whereas the size of eggs in the stool indicated infection with *F. gigantica*. Nucleotide sequences of ITS–1 and –2 and COI genes of the DNA extracted from the eggs revealed that the genotype was that of *F. hepatica*. These findings suggest that the size of eggs is not a suitable marker for species identification in human fasciolosis, especially in settings such as the East Asian region where different karyotypes and hybrid genotypes of F. hepatica and F. *gigantica* have been found

Kleiman *et al*., (2007) studied Dynamics of *Fasciola hepatica* transmission in the Andean. *hepatica* at its southern distribution range. Studies of prevalence and egg output in cattle and population dynamics and infection in snails were preformed in a farm in the Andean Patagonian valleys, Argentina, between December 1998 and February 2002. Snail surveys were herd at the beginning and end of the study, and in a cohort of heifers at the beginning and end of 2001. A twice-a-year anthelmintic treatment was implemented in 1999.. Overall prevalence in L. viatrix was 0.67% (range: 0.9–14%) and infection was detected in summer and autumn At the beginning of the study, calves were the least infected age group (15%). Prevalences and median egg counts in grazing animals were similar at the beginning (heifers: 81%, 3.3 epg; cows 60%, 1.3 epg) and end of the study (heifers and cows: around 51%, 1 epg). Likewise, the prevalence in the cohort of heifers remained similar (around 40%) between surveys. Transmission to cattle was highly effective despite of the short activity period and the low infection rate of snails, and the regular anthelminthic treatment. There would be two seasonal transmission peaks, one in summer–autumn, when infected snails were present, and the other in early spring due to overwintering metacercariae.

Jhannes *et al*.,(2007) studied associations between anti-*Fasciola hepatica* antibody levels in bulk–tank milk samples and production parameters in dairy herds. Our primary objective was to determine the relationships between *Fasciola*–specific antibody levels in bulk-tank milk and measures of productivity to estimate economic losses that are associated with *Fasciola* infections. A bulk–tank milk–ample was collected in March 2004 from 1105 dairy herds in Flanders and the antibody levels against *Fasciola hepatica* (ODRf) and Ostertagia ostertagi (ODRo) were determined. The association of ODRf with four production parameters (milk yield, milk–protein

%, milk–fat % and inter–calving interval) was assessed by multivariable linear–regression models, Production data were available for 463 out of the 1105 herds sampled. An increase in ODRf from the 25% quanfile (1.064) was associated with a decrease in the annual average milk yield of 0.7 kg/(cow day) (P=0.002), with a decrease in the average milk–fat % of 0.06% (P < 0.001) and with an increase of the mean inter–calving interval of 4.7 days (P = 0.03). No significant relationship was found with the average milk–protein %.

Almeida *et al*., (2007) studied preliminary antigenic characterization of an adult worm vomit preparation of *Fasciola hepatica* by infected human sera**.** Fascioliasis is an emerging/re–emerging vector-borne disease with the widest known distribution, Approximately 17 million people are infected around the world, being the Andean region the most affected area. There is an important necessity to develop sensitive and specific diagnostic tools to treat patients early and to avoid complications. In this paper we evaluated the immune response of infected humans against two antigenic preparations: the total soluble extract (FhTSE) and the adult worm vomit (FhAWV) in orther to identify antigenic fractions specific for *Fasciola hepatica*. Both preparations were processed by SDS–PAGE and Western blot with human sera with fascioliasis (F), other parasitosis and healthy individuals. In the immunoblot of FhTSE, sera F recognized nine bands with MW from eight to 95 kDa, from which those of 8, 12, 15 and 24 kDa were specific. Some bands of cross-reaction were evident with sera from patients with other parasitoses, more frequent with the FhTSE. Bands within the MW mentioned, Particularly that of eight kDa, have been shown to be specific by other, and deserve additional characterization for their potential use in immunodiagnosis.

Gurung (2007) studied prevalence of eggs of three trematode genera (*Fasciola* Spp. *Dicrocoelium* Spp and *Schistosoma* spp) in buffaloes of Satungal slaughter house, kathmandu. A study on the prevalence of trematodes in buffalo was conducted in Satungal, Kathmandu during the period of December 2006-January 2007. A total of 210 stool samples were collected during the study period and examined employing sedimentation method. The overall prevalence of helminth parasite was found 61.90%. Significant difference was found in the prevalence of there genera of trematode infection among buffaloes. The parasitic infections of *Fasciola* spp was 38.57%, Dicrocoelium spp one species) were observed among 8.57%. Mixed infections of different genera of trematodes (*Fasciola* spp, *Dicrocoelium* spp and *Schistosoma* spp) were also observed and was found in 14.76%. It was noticed that a higher infection rate was recorded in buffaloes aboce 2 years (71.65%) than buffaloes below 2 years (46.99%). Most of the buffaloes examined during the present survey had low to moderate *Fasciola*, *Schistosoma* and *Dicrocoelium* egg counts suggesting that the infections were usually Subclinical. Pseudoparasites were also observed among 23 (10.9*5%) positive samples. No work regarding these pseudo parasites was found.

Le *et al.*, (2008) examined a large number of *Fasciola* spp. collected from domestic stock (cattle and buffalo) at slaughter and also from human patients and reported the presence of hybrid and/or introgressed liver flukes, hybrid between *Fasciola hepatica* and *Fasciola gigantica* in Vietnam. This hybrid form was reported to have serious public health importance.

Thanh *et al*., (2008) studied Human fascioliasis and the presence of hybrid/introgressed forms of *Fasciola hepatica* and *Fasciola gigantica* in Vietnam.The two species common of liver fluke, *Fasciola hepatica* and *Fasciola gigantica*, cause human fascioliasis. Hybrids between these species, and introgressed forms of *Fasciola*, are known from temperate and subtropical regions of eastern Asia. Here, we report the presence of hybrid and/or introgressed liver flukes in Vietnam where it has recently been recognised that human fascioliasis is an important zoonotic disease. Specimens examined came from domestic stock (cattle and buffalo) at slaughter and also from human patients. DNA sequences were obtained from the nuclear ribosomal second internal transcribed spacer (ITS-2) and from portions of two mitochondrial protein-coding genes. Mitochondrial sequences in every case were similar to those of *Fasciola gigantica*. Nuclear ITS-2 sequences belonged to one or other of the *Fasciola* species, or, sequences from both were found in the same individual worm. This study extends the known range of hybrids or introgressed forms of *Fasciola* into tropical regions of Asia.

3.2 Literature review in context of Nepal :-

Nepal is loved all over the world for its colourful festivals. Each and all feativals are followed by delicious diets. In each festival meat varities are served as indespensible dishes. All ethenic groups consume meat with delicacy. Along with nutrients meat also transmit many zoonotic diseases which are unknown to most of the remote places of our country. Very less survey about fascioliasis have been done in our country.

Lolani and Jaeckle (1981) conducted a study to identify *Fasciola* spp in Palpa. Livestocks specimens were collected from five slaughtering places of Tansen in the last week of July and beginning of August 1981. identification was done by Hoerning Institute of Parasitology, University of Berna and results were mixed infections with predominance of *Fasciola gigantica.*

Lohani and Jaeckele (1981/82) identified the *Fasciola* species in Palpa. Out of 24 animals, four animals were infected with *Fasciola gigantica,* five animals with both *F. hepatica and F. gigantica,* and fifteen animals infected with *F. hepatica F gigantica* and intermediate from between them. The animals infected with *F. hepatica* were not found. Out of 181 *Fasciola* collected from total animal, 16% were *F. hepatica, 69.6%* were *F. gigantica* and 14.4% were intermediate from between them.

Morel and Mahato (1987) determined the epidemiological cycle of *Fasciola hepatica* in the intermediate and definitive hosts. *Lymnaea auricularia* race rufescens and *L. luteola* were responsible for the transmission of *F. gigantica* in the koshi hills of Nepal Redial and cercarial infection in the snails was detected from May to August and again in the November. The prevalence of the disease in cattle was the highest during the monsoon (June–September) and again during January to February.

Ghimire (1987) conducted a study on incidence of common diseases of cattle and buffaloes in Surkhet district. The endoparasitic infections recorded were fascioliasis.

Parajuli (1992) studied the prevalence of *Fasciola* in cattle and buffaloes in Surkhet. 60.00% of cattle and 83.00% of buffaloes were found to be positive for the flukes.

Shrestha *et al*., (1992) studied the prevalence of fascioliasis in cattle and buffaloes in Dhankuta. The prevalence rate was found range from 15.4% to 31.7% in the cattle and 20% to 87.5% in buffaloes depending upon different age groups of the animals and the ecological condition of the area. The higher prevalence rate was recorded in older animals and in areas, which were ecologically suitable for snail habitats.

Singh *et al*., (1992-1993) conducted a survey in lowlands of IAAS, Rampur to investigate the relationship between snail population and liverfluke infection in ruminants. The liverfluke infection was found 48.57%, 28.57, 25.00% and 21.28% in buffaloes, cattle, sheep and goats respectively. The highest rate of infection was observed in Sept. (50.00%), followed by the month Oct. (43.57%) and Jan. (35.00%). The snail population was negatively associated with population.

Mahato (1993) reported *Fasciola* prevalences of 57.9% in buffalo, 44.8% in cattle, 22.4% in goats and 18.2% in sheep in the hills of Nepal. In the Terai area, prevalences were 51.4% in cattle, 4.3% in buffalo and 13.3% in goats.

Mahato (1993) identified four *Lymnaea* species in Nepal. He reported *L. auricularia* race rufescens, *L. auricularia sensu stricto*. *L. viridis* and *I. luteola* in Nepal. The high snail population density was found during dry period and declined with the onset of monsoon. *Fosciola* larvae were found in snails from May to February in the hilly regions. The higher prevalence of fasciolosis was observed in stall-fed buffaloes than out grazed ones.

Acharya (1996) conducted a study on efficiency of Trichobendazole and Oxyclozanide against Fascioliasis of lactating buffaloes and cattle. The study was conducted between January 1996 and March 1996. Of the 317 lactating cow and buffaloes examined 21.6% cow and 30% buffaloes were positive for *Fasciola* infection. However, buffaloes did not response in either treatment groups as effectively as cows.

Shrestha (1996) studied the occurrence of helminth parasites in ruminants of Kathmandu valley for 2 years. The study showed that the prevalence rate is more than 75% both in large and small ruminants. For the first year, the positive percentages of helminths parasites were 82.50%, 83.89% and 75.91% respectively for buffaloes, small ruminants and cattle. And for the second year the positive case in percentage were 82.26%, 77.22% and 75.07% respectively for buffaloes, small ruminants and cattle. Buffaloes were more infected with helminths parasites followed by small ruminants and cattle. Paramphistomes infestation was higher in cattle in comparison to *Fasciola* whereas in case of buffaloes it was opposite.

Ghimire and Karki (1996) studied the prevalence of fasciolosis and efficacy of various anthelminties in buffaloes of rural Kathmandu. The faecal examination revealed a high prevalence of fasciolosis in adult buffaloes of rice belt area. An elevation showed that the efficacy of available anthelmintics were in the order of rafoxanide (67%), oxycloznide (65%), triclabendazole (52%) and albendazole (48%) against fasciolosis in buffaloes.

Joshi *et al.*, (1997) reported more than 50% *Fasciola* spp in cattle and buffaloes in different parts of Nepal.

Mahato *et al*., (1997, 2000) reported an epidemiological basis of the contron of Fasioliasis in Nepal. Despite increased awareness of the disease and massive increase in the last two decades. They found no impact on the prevalence of the disease here mainly due to lack of information about its epidemiology in country.

Shrestha and Joshi (1997) carried out study to evaluate the effectiveness of strategic drenching against Fascioliasis in cattle in the western hills of Nepal. Faecal samples were collected at monthly intervals and were examined by standard sedimentation method for the presence of *fasciola* eggs. The strategic reduced the overall infection in treated animals.

Sharma (1998-99) conducted a study to on parasitic infection in animals of panchthar district. Fasciolaiasis was found in 40.12%.

Regmi *et al.,*(1999) conducted a study to know the fascioliasis prevalence in Thuladihi VDC of Syanja district. Coprological examination revealed that 67.66% buffaloes and 62.10% cattle were affected with fascioliasis.

Ghimire and Karki (1999) conducted a coprological study and it revealed a higher prevalence of fascoiliasis in the adult buffaloes population of rice between area of Nagleblure and Lapsiphedi VDCs of Kathmandu district.

Pandey (2001) studied the fecal samples of 182 buffaloes and found 50% sample positive for fascioliasis. The prevalence of fascioliasis gradually increased from the month of June (40.00%) to Dec. (58.62%) and again increased from the month of Feb. (48.48%) to April (52.77%). The prevalence of fascioliasis was highest in old buffaloes (65.30%) followed by 56.80%, 48.78%, 35.29% and 14.25% in the age group of 7-8, 5-6, 3-4 and 0-2 years respectively.

CVL (2002/2003) conducted a study on the prevalence and diversity of *Fasciola* spp in buffaloes in area of Kathmandu valley. 92 faecal samples of buffaloes were analyzed , were found positive for *Fascoila* spp.

Adhikari *et al*., (2003) conducted a study on the prevalence and diversity of *Fasciola* spp. in buffaloes and cattle in areas of Kathmandu Valley from April 2003 to 30 June 2003. The prevalence of *Fasciola* spp. was found to be 36% in cattle and 61% in buffaloes respectively.

Yadav *et al*., (2003) reported the prevalence rate of Fascioliasis is high in Terai region of India followed by hills and plains respectively. Buffaloes were the most susceptible hosts followed by cattle and sheep.

Mukhia, G.(2007) conducted a study and reported 90.90 percent sample positive for trematodes in buffaloes of Satungal VDC, Kathmandu. *Schistosoma* spp was found in 46.94 percent followed by *Fasciola* spp 32.6 percent and *Dicrocoelium* spp 20.61 percent.

Gurung, B .(2007) studied Prevalence of eggs of three trematode genera (*Fasciola* Spp. *Dicrocoelium* Spp and *Schistosoma* spp) in buffaloes of Satungal slaughter house, kathmandu. A study on the prevalence of trematodes in buffalo was conducted in Satungal, Kathmandu during the period of December 2006-January 2007. A total of 210 stool samples were collected during the study period and examined employing sedimentation method. The overall prevalence of helminth parasite was found 61.90%. Significant difference was found in the prevalence of there genera of trematode infection among buffaloes. The parasitic infections of *Fasciola* spp was 38.57%, Dicrocoelium spp one species) were observed among 8.57%. Mixed infections of different genera of trematodes (*Fasciola* spp, *Dicrocoelium* spp and *Schistosoma* spp) were also observed and was found in 14.76%. It was noticed that a higher infection rate was recorded in buffaloes aboce 2 years (71.65%) than buffaloes below 2 years (46.99%). Most of the buffaloes examined during the present survey had low to moderate *Fasciola*, *Schistosoma* and *Dicrocoelium* egg counts suggesting that the infections were usually Subclinical. Pseudoparasites were also observed among 23 (10.9*5%) positive samples. No work regarding these pseudo parasites was found.

IV

MATERIALS AND METHODS

4.1 STUDY AREA

The proposed study area for the research is Kirtipur Municipality of the Kathmandu district. Kirtipur is one of the recently urbanized city of Kathmandu valley located some 8 kms. South-west of the central Kathmandu. It is declared as municipality in 2053 and is divided into 19 wards. It extends from 27° 41´36˝ – 27° 38´37˝ N to 85˚18´00˝ – 85˚14´64˝ E. It is surrounded by the Bagmati River in the east, Tinthana and Machchhegaon VDC in the west, ward no. 14 of the Kathmandu Metropolitan in the north and Chalnakhel VDC and Shesnarayan VDC in the south. The shape of the municipality resembles almost a square, the area being 14.76 sq.km (Nepal Gazettes part III 2053) and the study area covers an all over area of the municipality.

The total population of the municipality is 46,670 (WHO, 2006). Its population is increasingly continuously. People of various castes live in the municipality however Newars are in majority (67.37%) followed by Chhetris (17.37%), Brahmans (10.00%), Magars (2.10%) and others (3.16%) (CBS 2001). Nayabazar is the central market and busy area of the municipality.

In the municipality, a total of 12 buffalo slaughtering places and a total of 50 meat shops were surveyed.

4.2 STUDY POPULATION AND SAMPLING

During the study period, 200 slaughtered water buffaloes were examined thoroughly. 100 were observed during the winter; from November 27, 2007 to February 10, 2008 and 100 were observed during the summer; from June 18, 2008 to July 26, 2008.

Out of them, 93 (46.50%) were males and 107 (53.50%) were females. 54 (27%) of them were calves, 51 (25.50%) were adults and 95 (47.50%) were olds.

Samples were collected by visiting the different slaughtering places. The frequency of visit to the slaughtering places and the corresponding number of slaughtered animals observed in them are shown in the table below.

Table 1: NUMBER OF SLAUGHTERED ANIMALS OBSERVED IN DIFFERENT SLAUGHTERING PLACE

	No. of slaughtering place*	Name of the owner/main butcher of the slaughtering place	During winter		During summer	
			Frequency of slaughtering place visit	No. of animals slaughtered observed	Frequency of slaughtering place visit	No. of animals slaughtered observed
1	1	Bishnu Shahi	8	19	6	17
2	2	Shanta Shahi	8	9	6	9
3	3	Shyam Shahi	8	10	7	10
4	4	Bhim Bahadur Shahi	8	14	6	17
5	5	Dinesh/ Ramesh Shahi	7	7	2	3
6	6	Mani Raj Shahi	7	8	3	4
7	7	Nanda Bahadur Shahi	7	15	4	8
8	8	Raju Shahi	4	4	3	3
9	9	Bhai Raja Shahi	3	3	5	9
10	10	Rajendra Shahi	3	3	5	5
11	11	Ashok Shahi	4	8	5	12
12	12	Tari Babu Khadgi	-	-	3	3
	Total			100		100

*Numbering of slaughtering places was done for the convenience of studying the results and for comparing the data

4.3 MATERIALS

4.3.1 Equipments

i. Gloves
ii. Forceps
iii. Scissors
iv. Refrigerator
v. Sampling bottles
vi. Petridishes
vii. Tray
viii. Measuring scale
ix. Microscope
x. Slides
xi. Coverslips
xii. Beaker
xiii. Small flat tap tubes
xiv. Eye-droper
xv. Cotton

4.3.2 Chemicals

i. Formalin
ii. 70% ethanol
iii. Soap

4.4 METHOD

From November 2007 to July 2008, a sample of 200 water buffaloes slaughtered for meat in the whole Kirtipur Municipality was examined for the presence of different parasites.

The incidence of *fasciola* sps were studied. Sanitary condition prevailing around the butchering area and meat shops, the type of water used, the place of waste disposal, dog problems around them were studied. The knowledge and awareness of butchers, meat sellers and consumers towards meat and meat borne diseases were also comprehensively studied both by the field observation and through questionnaires.

The *fascoila* occurring in the meat and sometimes the meat of the water buffalo itself were then collected and suitably analyzed in the laboratory.

4.4.1 DATA COLLECTION

Field observations were the source of most of the data collected. Besides data were also collected through surveillance study among butchers, meat sellers and meat consumers. Similarly data also come from the laboratory work.

4.4.1.1 DATA COLLECTION THOROUGH FIELD OBSERVATIONS

The field observations were directly responsible for the data regarding age, sex of slaughtered animals, and prevalence of different parasites. It also helps for the data regarding sanitation around the slaughtering area and meat shops.

4.4.1.2 DATA COLLECTION THROUGH SURVEILLANCE STUDY

During the research work, the surveillance study was carried out among butchers, meat sellers and meat consumers. Different questions were put on to them and formal and informal interviews were carried out to get true and relevant data as much as possible. Separate questions for butchers, meat sellers and consumers were made and were asked orally to each of the participants to answer.

4.4.2 BUTCHERS SURVEILLANCE STUDY

During the survey, 28 butchers in 12 slaughtering places were asked various questions and their corresponding replies were collected. 23 of them were local residents of the area whereas 5 were outsiders. All the slaughtering places were carefully observed and necessary photographs were also taken.

Data obtained from the survey of butchers gave the status of:

a) slaughtering conditions

b) way of slaughtering the animal

c) animals being slaughtered each day

d) waste disposal

e) facilities present in the slaughtering places

f) dog visit and feed of the meat

g) knowledge about the meat borne diseases

h) knowledge about the environment pollution

4.4.3 MEAT SELLERS SURVEILLANCE STUDY

50 meat sellers of meat 50 shops were put to various questions to know about the facilities of meat shops and the practices of meat selling. The meat shops and their environment were thoroughly observed and relevant photographs were taken too.

Data obtained from the survey of meat sellers gave the status of:

a) the type of meat sold

b) facilities present in the meat shops

c) knowledge about the meat borne diseases

4.4.4 CONSUMERS SURVEILLANCE STUDY

300 meat consumers were questioned during the survey to know about their meat eating habit and so on. Among them 250 were local residents of Kirtipur and 50 were outsiders.

Data obtained from the survey of consumers gave the status of:

a) type of meat they consume

b) frequency of meat eating

c) knowledge about the meat borne diseases

d) their health status (getting sick) after consuming meat

e) satisfaction and complains about meat hygiene and sanitation

4.4.5 DATA OBTAINED FROM THE LABORATORY

Fasciola spp. collected form each of the infected animals were kept in the separate plastic bags and were brought to the lab. for further work and analysis.

FOR *Fasciola* SPECIES

The *Fasciola* species collected from different animals were separately analyzed to differentiate into respective species i.e. they were differentiated into *F. hepatica* and *F. gigantica*. Careful naked eye observation and microscopic observations were carried out for this. Differentiation was made according to difference they have got in their shape and size as shown in the table below.

Fasciola hepatica	*Fasciola gigantica*
7. Body size: 20 to 50mm by 6 to 13mm.The average length/width ratio is 1.88 to 2.32.	7. Body size: 24 to 76mm by 5 to 13mm.The average length/width ratio is 4.39 to 5.20.
8. The cephalic cone is large.	8. The cephalic cone is small.
9. The shoulders are well developed.	9. The shoulders are less developed.
10. Smaller acetabulum.	10. Larger acetabulum.
11. The ovary and ceca are less branched.	11. The ovary and ceca are more branched.
12. The average distance between the posterior border of the body and the posterior testis is shorter (7.78mm; range: 3 to 13 mm).	12. The average distance between the posterior border of the body and the posterior testis is longer (14.9mm; range: 6 to 19 mm).

Source: Sahba *et al*., 1972

The data obtained were then calculated, classified, tabulated and statistically tested using λ^2 with the help of supervisor, co-supervisor and other expertises.

4.5. FAECAL SAMPLING

200 faecal samples were collected from Kirtipur.

- Fresh faecal samples for parasitlogical examination were collected.
- 10% formalin was added into the faeces to preserve the parasite eggs.
- Collected samples were dispatched as soon as possible to a laboratory in plastic bags.
- Samples were immediately stored in the refrigerator (4⌈c) until they were processed.

4.5.1 STOOL EXAMINATION

The stool samples were examined by sedimentation technique.

4.5.1.1 SEDIMENTATION TECHNIQUE

The sedimentation technique is a qualitative method for deteting trematodes eggs in the faeces. Most trematodes eggs are relatively large and heavy. This technique concentrates them in sediment.

METHOD

3 gm of stool sample was taken in a beaker and 50ml of water was added. With the help of motor and pistle, the sample was grinded highly and filtered with a tea strainer. The filtered sample was poured into plastic tube of 15ml and centrifuged

at 1000 rmp for 15 minutes. The tube was taken out and removed the water with the help of pipette. A drop of sediment was taken with the pipette and placed on the slides, added drop of methylene blue into it and was covered with a cover a cover slip.

MICROSCOPIOCAL EXAMINATION OF PREPARED SAMPLE

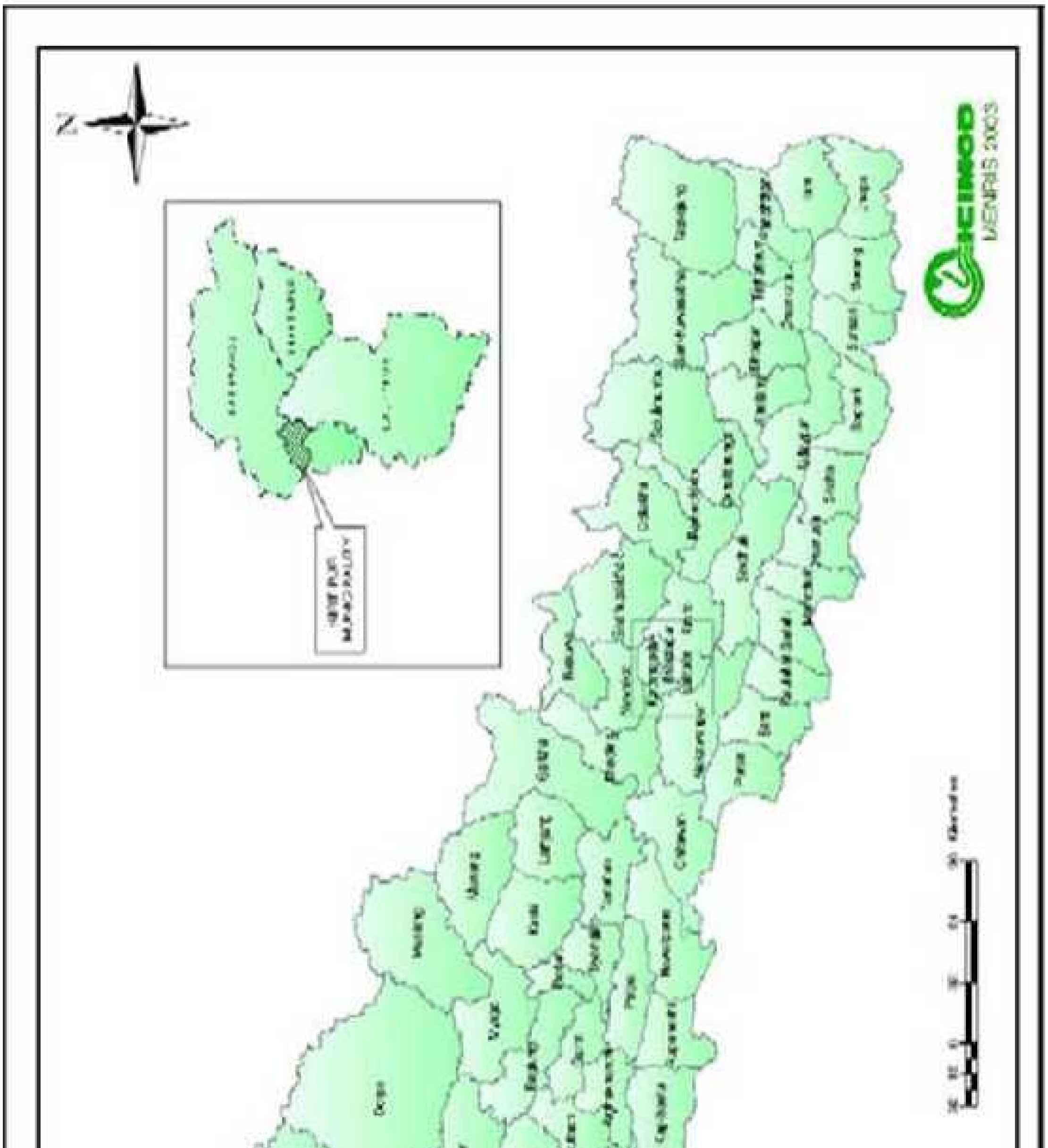
The prepared samples on microslides from the sedimentation method were examined under a microscope at the magnification livel of 4x and 10x.

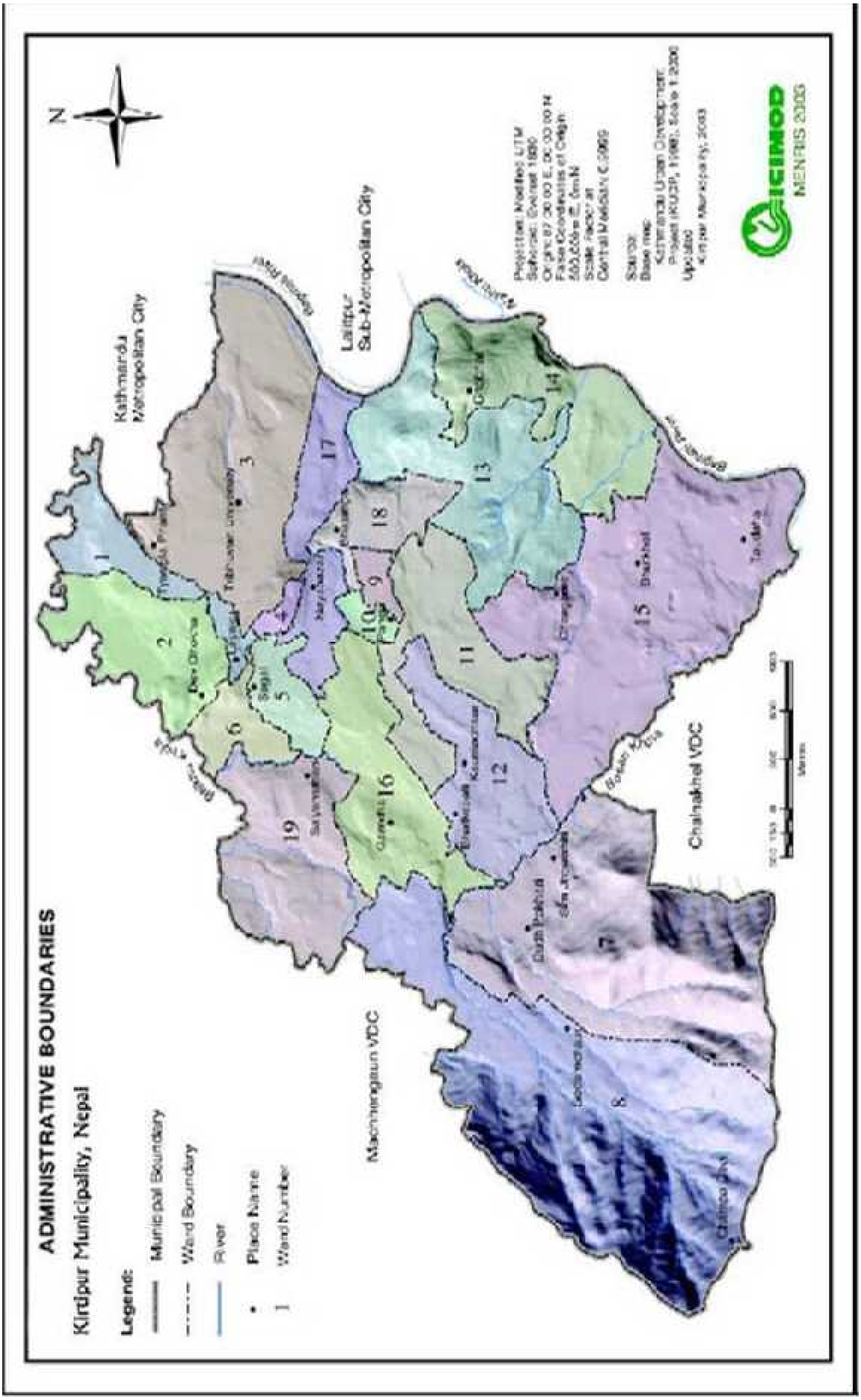
4.5.1.2 FAECAL EXAMINATION USING THE STOOL EGG COUNTING METHOD:

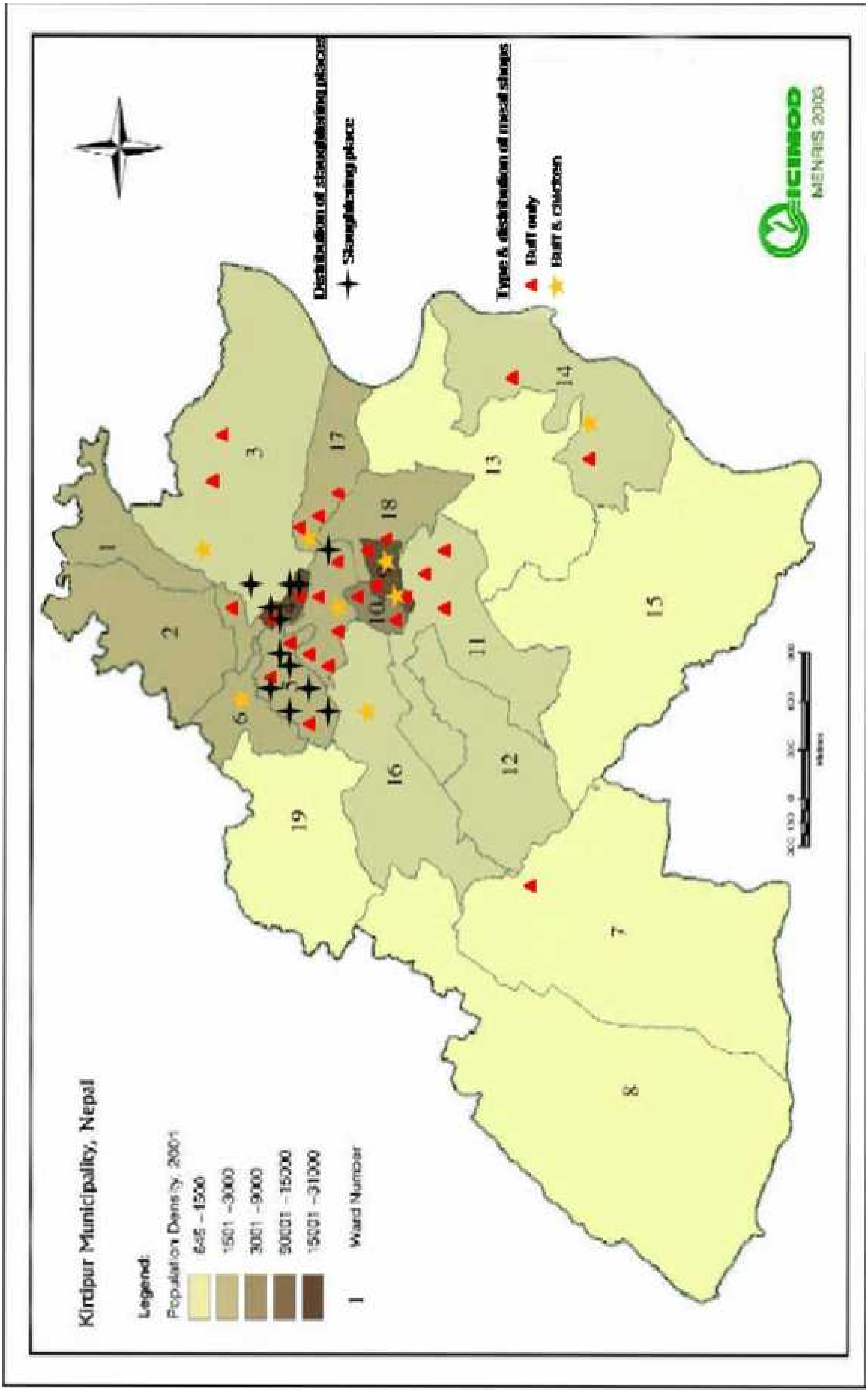
According to Dr. Tom Nolan, University of Pennsylvania (2004) it is the easiest quantitative method to count the number of eggs present in the field without the hlp of Mcmaster.

3 gm of faeces was taken in a beaker and 42ml of water added. Using a tongue depressor, 3gm of faeces was pushed through a sieve into the water. Then the sieve was lift and was hold over the dish. Then the remaining water was push out from the faeces. After stirring the water-faeces mixture 0.15 of the suspension was taken and spread over 2 slides. Each slide was cover with a long coverslip. Then both slips were examined for eggs. The total amount of eggs counted X 100 represents the number of eggs per gram of faeces.

The data obtained were then calculated, classified, tabulated and statistically tested using λ^2 with the help of supervisor, co-supervisor and other expertises.


LOCATION MAP
Kirtipur Municipality, Nepal
Source:
Base map of Nepal
Topographical Zonal Map, Scale 1:250000
Department of Survey, 1986
ICIMOD
MENRIS 2003


ADMINISTRATIVE BOUNDARIES
Kirtipur Municipality, Nepal
Legend:
Municipal Boundary
Ward Boundary
River
Place Name
Ward Number
N
Kathmandu Metropolitan City
Lalitpur Sub-Metropolitan City
Machhegaun VDC
Chalnakhel VDC
Meters
ICIMOD
MENRIS 2003


Kirtipur Municipality, Nepal
Legend:
Population Density 2001
Ward Number
Distribution of slaughtering places
Slaughtering place
Type & distribution of meat shops
Buff only
Buff & chicken
ICIMOD

V

RESULTS

The results have been presented under following headings. Headings are again divided into sub headings.

5.1 DISTRIBUTION OF SLAUGHTERING PLACES IN KIRTIPUR MUNICIPALITY

In Kirtipur area, a total of 12 slaughtering places were found. Most of them were located in Khasibazzar and Nayabazzar sites, the two main market areas. Kirtipur Municipality is divided into 19 wards and the ward-wise distribution of slaughtering places is shown in the Table 2.

Table 2: DISTRIBUTION OF SLAUGHTERING PLACES IN KIRTIPUR MUNICIPALITY

S.N	Wards	Number of slaughtering places
1	1	0
2	2	0
3	3	1
4	4	4
5	5	6
6	6	0
7	7	0
8	8	0
9	9	0
10	10	0
11	11	0
12	12	0
13	13	0
14	14	0
15	15	0
16	16	0
17	17	1
18	18	0
19	19	0
	Total	**12**

5.2 NUMBER OF BUFFALO/ES SLAUGHTERED PER DAY IN DIFFERENT SLAUGHTERING PLACES

In Kirtipur, each day, an average of 25 buffaloes were found slaughtered. However the number were found increased upto 30 to 35 during Saturdays and during festivals due to more demand of meat. Similarly during Ekadashi and Aaunshi, the number was found reduced as the tradition prohibits the consumption of meat in these days. No. of buffalo/es slaughtered per day in different slaughtering places is shown in Fig.1.

Fig. 1: No. OF BUFFALO/ES SLAUGHTERED PER DAY IN DIFFERENT SLAUGHTERING PLACES

5.3 BUTCHERS' SURVEY RESULT

Following facts were obtained after surveying and questioning the butchers of these 12 slaughtering places in the Municipality.

The survey was conducted among the butchers and workers at the various slaughter houses through questionnaires and direct observation. Almost all butchers were from Khadgi and Shahi cast group. Workers were from other cast but all were from socio-economically weak house hold category. 12 slaughter houses located in different wards of Kirtipur were visited for survey.

5.3.1 PLACES FROM WHERE THE BUFFALOES ARE BROUGHT

The buffaloes, for slaughtering, were all brought from outside the valley. They were mostly imported from India to Jitpur, Nawalparasi, Chitwan, Hetauda, Birgunj and Nepalgunj and the butchers bought them and brought them to their place in trucks. Some butchers indirectly brought the buffaloes from Thankot which had been brought from the above mentioned places as well. Nobody had tamed buffaloes for milk or agricultural purpose. They had imported the buffaloes only for meat.

5.3.2: ANIMALS KEPT PRIOR TO SLAUGHTERING

Prior to slaughtering, these buffaloes were either kept in slaughter shed, or in the ground floor of the butcher's house. In fact 50 % of the slaughtering places had separate slaughter shed and in rest 50% of the slaughtering places, buffaloes were just kept in the ground floor of the butcher's house. The Fig.2 shows the place where the animals are kept prior to slaughtering.

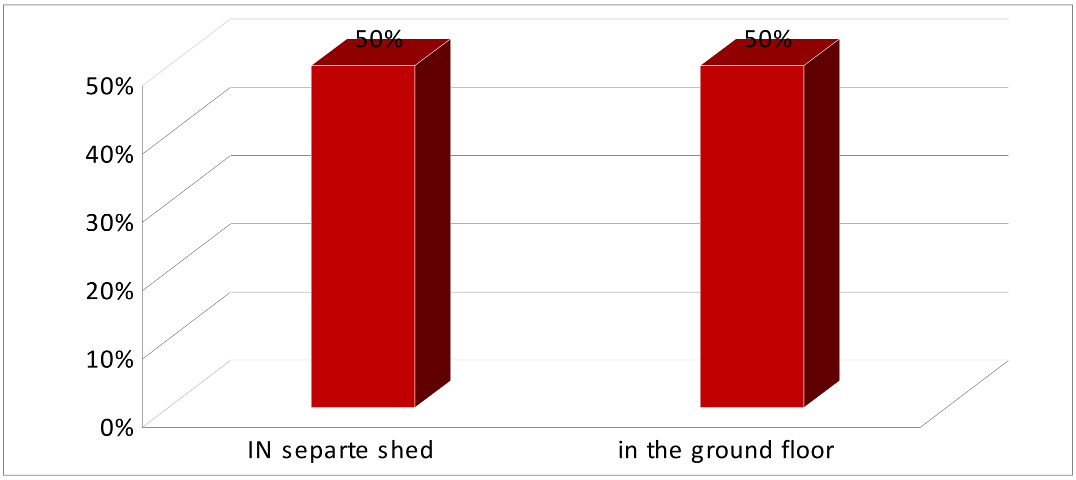


Fig 2: ANIMALS KEPT PRIOR TO SLAUGHTERING

5.3.3: PRACTICES OF SLAUGHTERING

The buffaloes were slaughtered at early morning. The slaughtering practices were found traditional and inhumane. The animals were killed by striking on their head with hammer after their legs had been tied with thick rope to prevent from escaping. Their mouth was also tied with the rope to prevent from screaming. They kept striking till the buffalo fell down and got unconscious or die. Then their head was cut off.

5.3.4: PLACES OF BUFFALO SLAUGHTERING

In most of the slaughtering places, in fact in 66.66%, buffaloes were slaughtered along the road side and they were always found visited by dogs, while in 33.33%, they were slaughtered in the slaughter house. Dogs' movement was not found in these places as they had walls and doors. Places of buffalo slaughtering is shown in Fig.3.

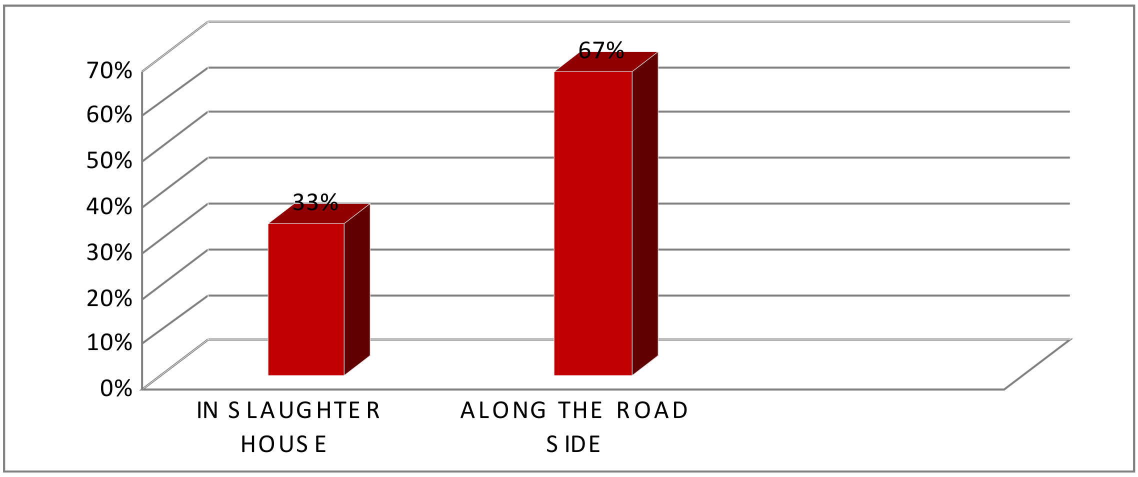


Fig 3: PLACES OF BUFFALO SLAUGHTERING

5.3.5: DOGS' MOVEMENT AROUND THE SLAUGHTERING PLACE

66.66% of the slaughtering areas were easily accessible to dogs while in 33.33% of the slaughtering places, dogs' movement was not found which is shown in Fig.4.

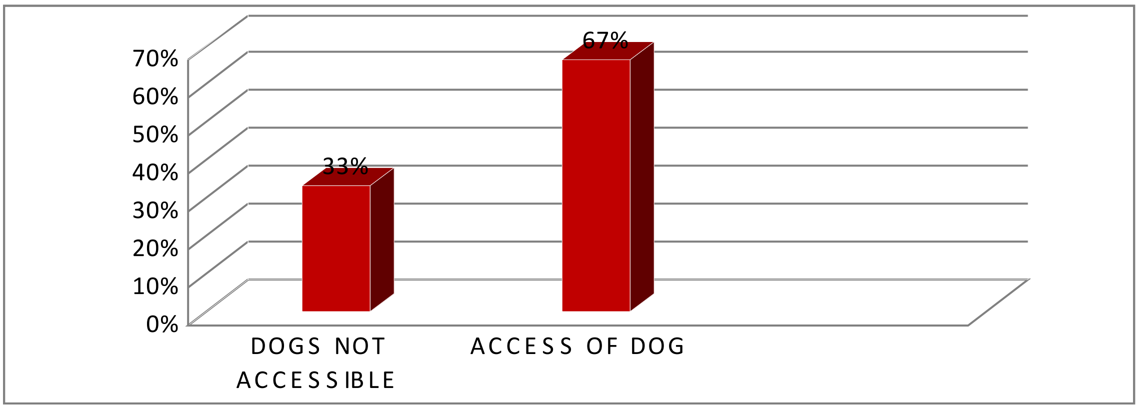


Fig. 4: DOGS' MOVEMENT AROUND THE SLAUGHTERING PLACE

The slaughtered animals were then skinned and internal organs were separated. But most of the calves slaughtered were burnt and their skin also served as a part of delicacy.

5.3.6: TYPE OF WATER USED IN THE SLAUGHTERING PLACES

For cleaning the burnt calves, cleaning the intestine and stomach of the slaughtered animals, butchers used either tap water (16.66%) or well water (66.66) or both tap and well water (16.66%) according to the access they were with which is shown in Fig.5.

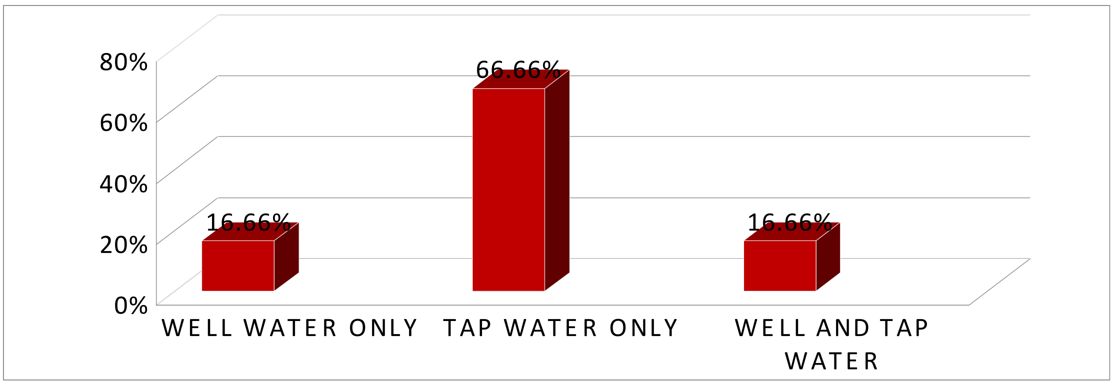


Fig. 5: TYPE OF WATER USED IN THE SLAUGHTERING PLACE

5.3.7: DISPOSAL OF WASTES FROM SLAUGHTERING PLACES

During slaughtering of the animal, large amount of wastes and offal are produced. The wastes mainly constitute the stomach and intestinal content of the animal while offal includes horns, bones, etc. Through observations and questioning of the butchers, it was known that 33.33% of the butchers threw the wastes in the nearby drainage while 66.66% of butchers said that they deposited them along the street side to be used as fertilizer. Regarding the offal, all the butchers or slaughtering places (100%) had container or separate place to store them as they have commercial use. The place, where the wastes and offal from the slaughtering places were disposed is shown in Fig.6.

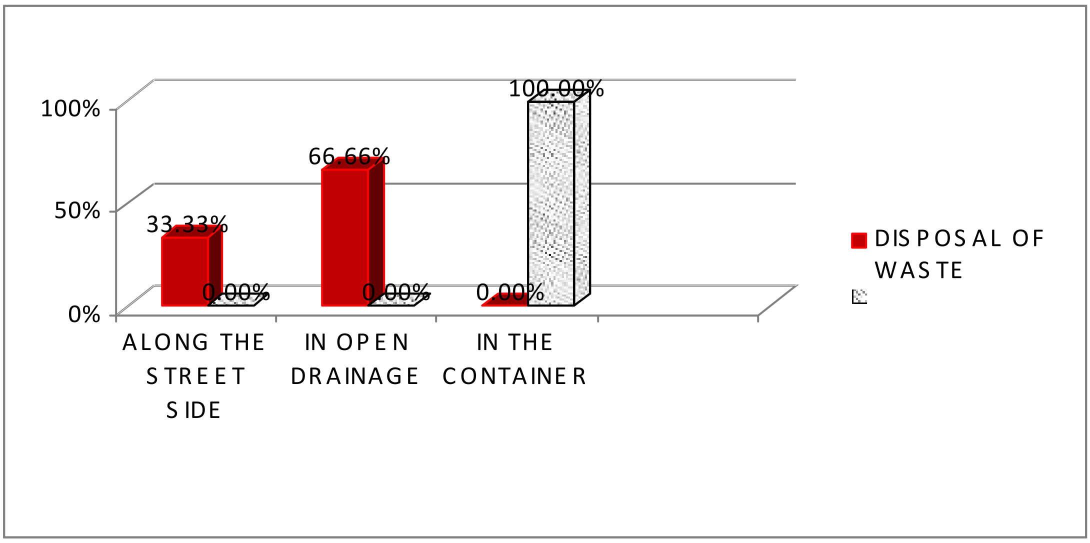


Fig. 6: DISPOSAL OF WASTES AND OFFAL BY BUTCHERS

5.3.8: FACILITIES PRESENT IN THE SLAUGHTER HOUSES

As mentioned earlier, in Kirtipur, 12 slaughtering places were found. Among them only 4 have boundary walls and they have been categorized as slaughter house. They had one or more facilities like facilities of roof, concrete floor, water and electricity supply, space or container for offal disposal drainage, etc. For rest, they had just been called as slaughtering place as they lacked separate boundaries and area too. The butchers used the street nearby their house for slaughtering the animals. They also lacked any of the above mentioned facilities except for the drainage and offal disposing facilities. The floors of them were dirty and dusty during dry seasons and muddy when rain poured on. As they lacked roof, during rainy days, slaughtering activities were found carried out inside the ground floor of their own house. The floors of them were made up of bricks and mud. The water and electricity were found supplied from the nearby source.

All the slaughtering places including slaughter houses were found devoid of meat storing facilities. So, the butchers used to sell the meat to the wholesale meat sellers on the spot. All the butchers, in fact, were found to have one or more meat shops of their own. Meat was usually found carried to those shops in the bamboo basket and mostly without any cover. The meat from the slaughtering places was also found being transported outside of the Kirtipur, to the places like Kalimati, Balaju, Koteswor, etc.

The butchers were found to have no separate clothes, boots or apron for slaughtering. They used to wear the same usual clothing during slaughtering.

Facilities present or observed in the slaughter house are given in Fig. 7.

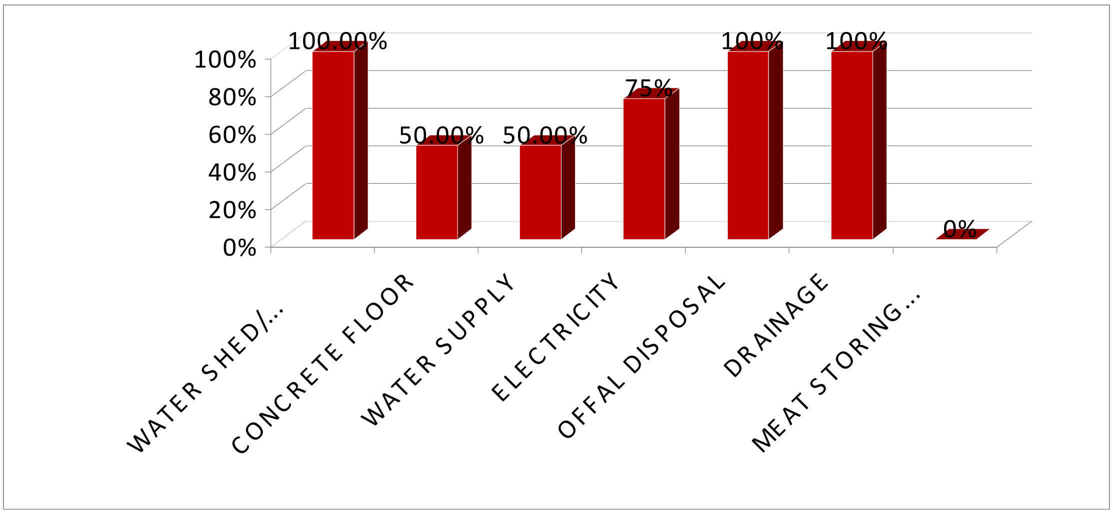


Fig. 7: FACILITIES PRESENT IN THE SLAUGHTER HOUSE

5.3.9: BUTCHERS' KNOWLEDGE ABOUT ENVIRONMENT PROBLEM

Among 28 butchers questioned, 10 (38.71%) answered that they had knowledge regarding environmental effect due to slaughtering places such as pollution of environment due to disposal of wastes and offal, pollution from the water that had been used to clean the slaughtered animals, etc.

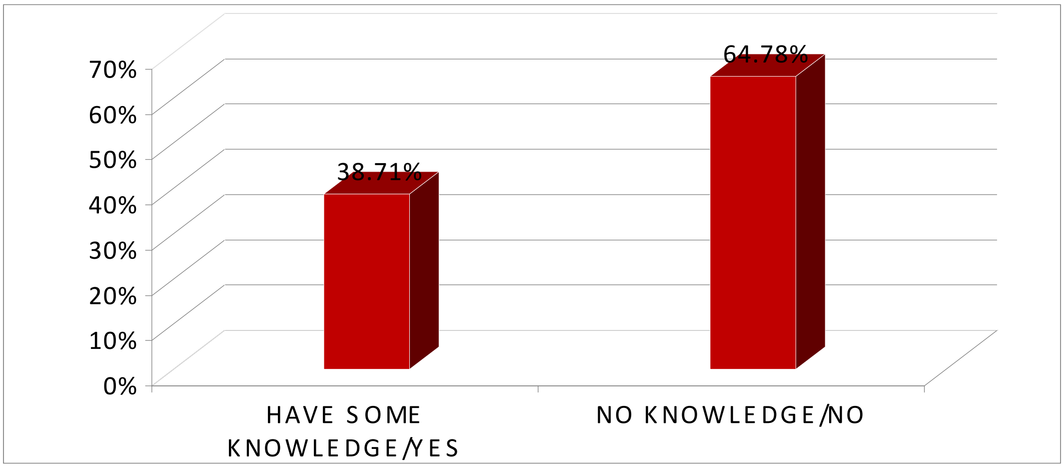


Fig. 8: BUTCHERS' KNOWLEDGE ABOUT ENVIRONMENT PROBLEM

5.3.10: INSPECTION OF SLAUGHTERING PLACES

When the butchers were asked about visiting of any meat inspection team, they denied of coming anyone for meat inspection. They themselves, however, revealed and accepted that the slaughtering practices and the condition of slaughtering places could have been much better had there been regular meat inspection and had they been aware.

5. 4 MEAT SELLERS' SURVEY RESULT

Through the survey, number of meat shops over the entire Kirtipur municipality were found out. A total of 36 buff selling meat shops were found which are shown in the Table 3. Buff selling shops were found to be 36.

Table 3: TYPE OF MEAT SHOPS FOUND AND THEIR DISTRIBUTION IN KIRTIPUR

S.No.	Ward No.	Buff Meat Shop	Buff and chicken Shop	Total
1	1	1		
2	2			
3	3	**2**	**1**	**3**
4	4	**2**		**2**
5	5	**3**		**3**
6	6		**1**	**1**
7	7	**1**		**1**
8	8			
9	9	**4**	**2**	**6**
10	10	**2**		**2**
11	11	**3**		**3**
12	12			
13	13			
14	14	**2**	**1**	**3**
15	15			
16	16		**1**	**1**
17	17	**8**	**2**	**10**
18	18			
19	19			
	total	**28**	**8**	**36**

During the research, 36 meat sellers i.e. all the buff meat selling shops were surveyed. The shops were surveyed mainly to generate information regarding facilities in the shops and hygienic status of the meat they are selling.

During survey, 14 (38.88%) of the meat sellers replied that they had got their own slaughtering places and they got meat from them. The rest 22 (61.11%) answered that they got meat from the butchers.

11 (30.55%) meat sellers said that they got the meat brought to their shops from the slaughtering places on vehicle (on bike or cycle) while 25 (69.44%) replied that they had men to carry the meat in the bamboo basket to their shops.

5.4.1: HOUSING CONDITION OF MEAT SHOPS

It was found that 32 (88.88%) of the meat sellers had permanent housing with a roof. 4 (11.11%) of the meat sellers were found selling meat openly on the streets. Fig.10 shows the housing condition of the meat shops. The unsold meat was carried to their home and kept in refrigerator.

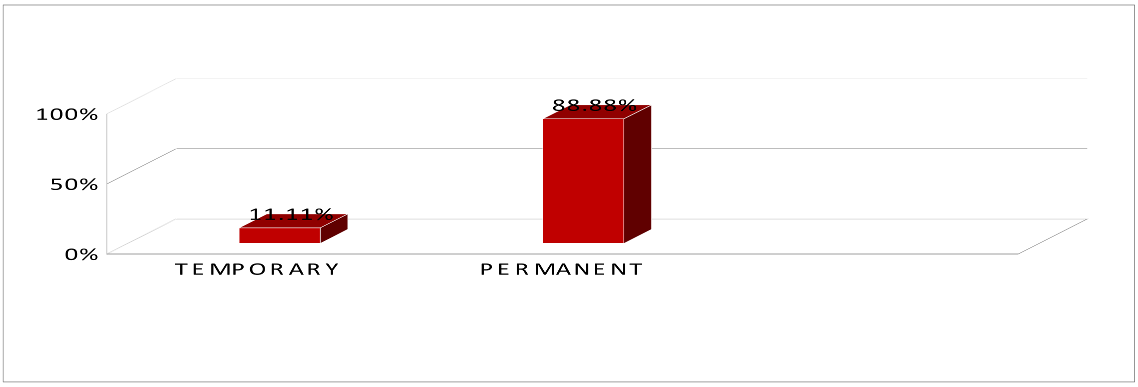


Fig. 9: HOUSING CONDITION OF MEAT SHOPS

5.4.2: DISPOSAL OF WASTES AND OFFAL BY MEAT SELLERS

When the meat sellers were questioned about the waste disposal, 13 (36.11%) of the meat sellers replied that they threw the wastes into the drainage system, 16 (44.44%) of them replied that they threw the wastes in the container and 7 (19.44%) answered that they just casted away the wastes along the road side. Such thrown away wastes and offal materials were very prone to be eaten by dogs as dogs were found roaming around the areas.

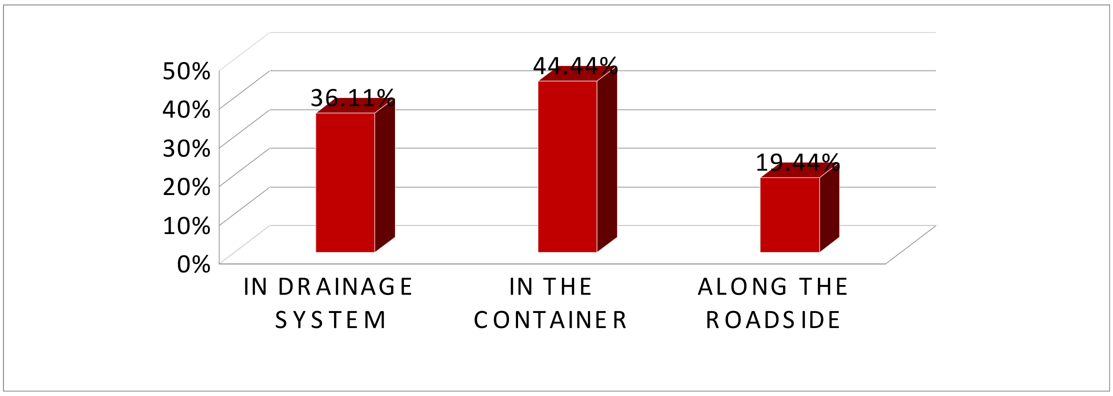


Fig. 10: DISPOSAL OF WASTES AND OFFAL BY MEAT SELLERS

D.3: SANITARY CONDITION AND FACILITIES PRESENT IN THE MEAT SHOPS

Regarding sanitation and facilities in the meat shops, all the butchers answered that they used tap water to wash their meat and hands if required. 35 (97.22%) meat sellers didn't have separate clothing or apron to wear on during meat selling. They were found wearing the same usual clothes which they used to wear when they were not selling meat. 34 (94.44%) of the meat sellers replied that their shops were fly proof as they used fly nets. Nets were often found being used. Regarding access to dogs, it was found that all (100.00%) the meat shops were surrounded by dog/s. The meat sellers also used to give bones, hides, etc not bought by the consumers. Regarding electricity and cold storage, 32 (88.88%) meat shops were found to have electricity and 27 (75.00%) meat sellers replied that they had their shops accessed with the facility of cold storage. The sanitary condition and facilities present in the meat shops is shown in Fig.11.

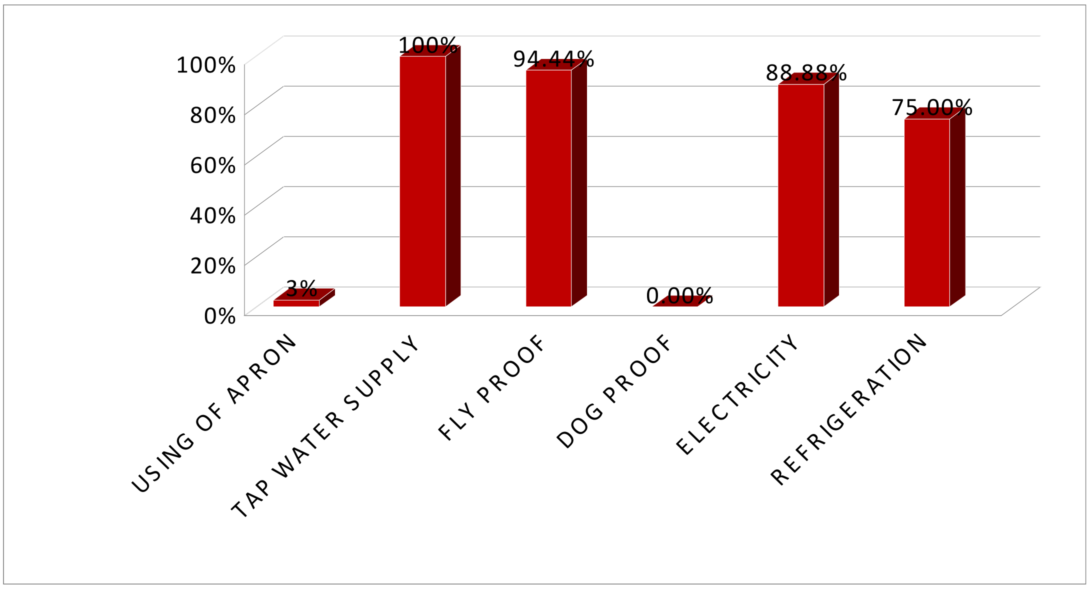


Fig 11: SANITARY CONDITION AND FACILITIES PRESENT IN THE MEAT SHOPS

.5.5 CONSUMERS' SURVEY RESULT

Kirtipur is one of the old villages/town of Newars who have lots of feasts and festivals to celebrate throughout the year and in all these occasions various dishes prepared from meat like 'Daykula' (cooked meat), 'Chhoyala' (burned meat), 'Bhutan' (fried meat), 'Sekuwa' (baked meat), 'Kachila' (raw meat), 'Chhengu' (raw skin attached with flesh), etc are a must. One can't imagine Newari festivals without meat. 'Kachila' and 'Chhengu' are raw meats, so have got much parasitic importance.

5.5.1: TYPE OF MEAT CONSUMED

300 meat consumers were surveyed during the research. Among them 250 were local residents of Kirtipur and 50 were outsiders. During the survey, 250 consumers replied that that preferred buff over other meat whereas 50 replied that they had never consumed buff and rather they ate mutton or chicken or pork or any. The type of meat consumed by the respondents is given in Fig.15.

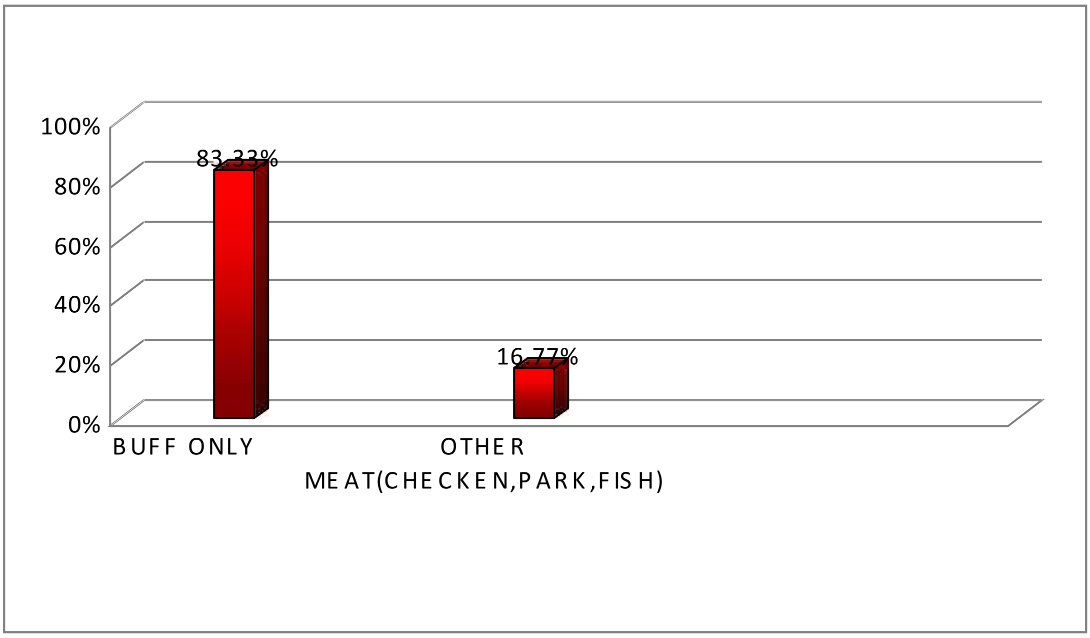


Fig. 12: TYPE OF MEAT CONSUMED

5.5.2: FREQUENCY OF BUFF CONSUMPTION

During the research, the meat consuming frequency of buff consumers were also surveyed. 133 (53.20%) among 250 buff consumers replied that they consumed meat once a week. 100 (40.00%) of them said that they ate meat much frequently round about twice a week. 4 (1.6%) said they ate meat once a month while 13 (5.20%) consumers answered; they consumed meat in a long gap only during the occasions.

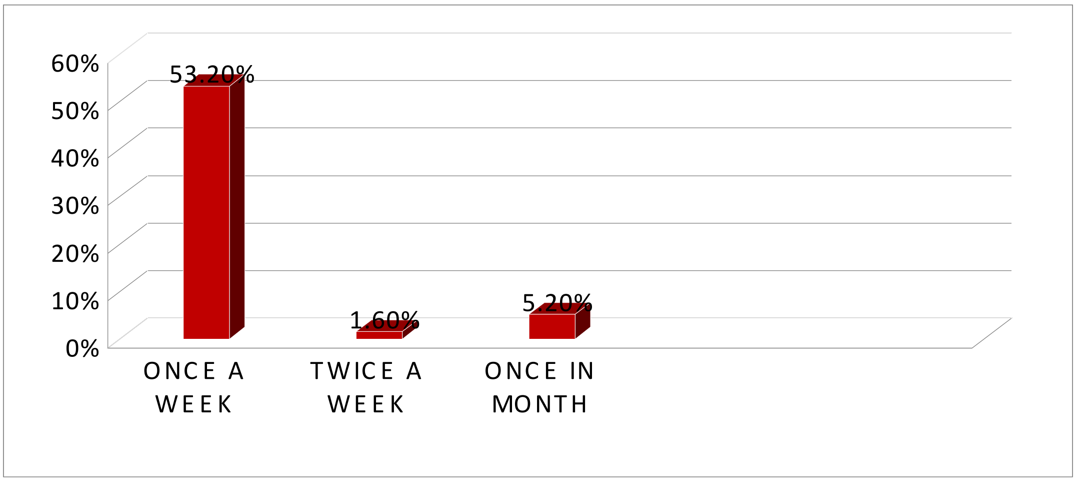


Fig. 13: FREQUENCY OF BUFF CONSUMPTION

5.5.3: FASCOILA OBSERVED IN THE MEAT BY CONSUMERS

183 (73.20%) buff consumers replied that they had never seen parasites in the meat they had bought while 67 (26.80%) said they had sometimes seen the parasites in liver, rumen intestine, etc.

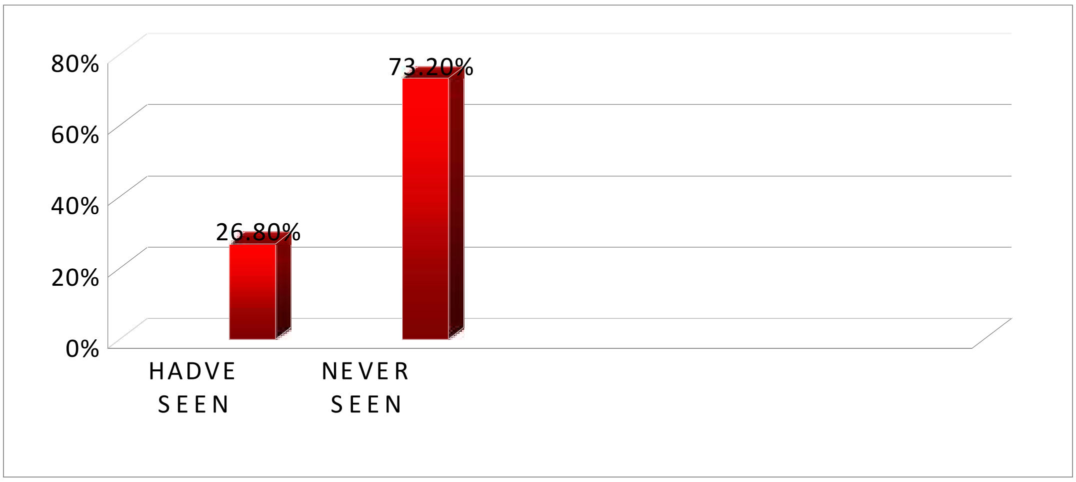


Fig. 14: FASCOILA OBSERVED IN THE MEAT BY CONSUMERS

5.5.4: CONSUMERS' KNOWLEDGE ABOUT FASCIOLIASIS

167 (66.80%) consumers seemed to be satisfied with the hygienic and sanitary conditions of the meat shops while 83 (33.20%) of them said that the shops should be made fly and dog proof. 90 (36.00%) of the consumers said, they had some idea about Fascoiliasis while rest 160 (64.00%) simply replied that they had got no idea which is graphically shown in Fig.15.

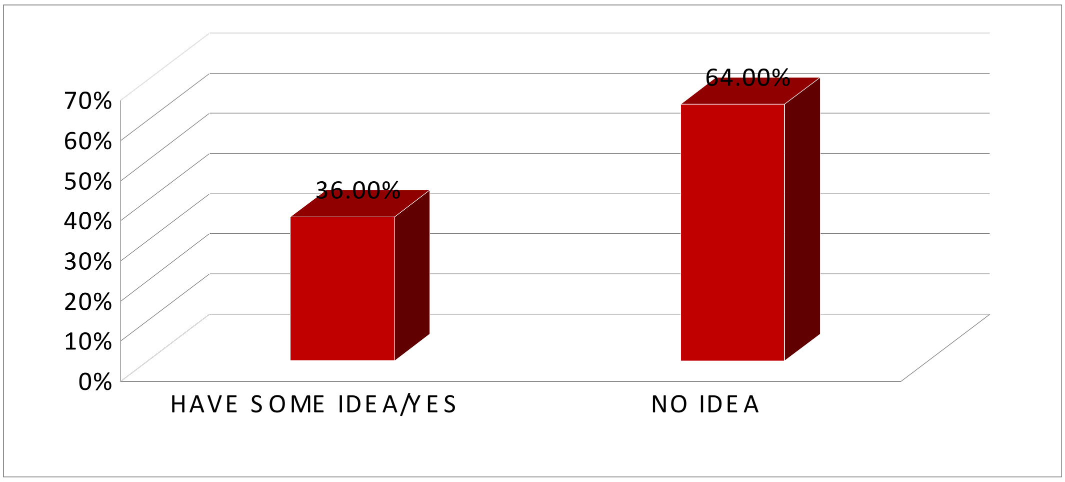


Fig. 15: CONSUMERS' KNOWLEDGE ABOUT FASCIOLIASIS

5.6. OBSERVATION OF SLAUGHTERED BUFFALOES

During the field visits of the slaughtering places, the carcasses of 200 slaughtered buffaloes, 100 each during winter and summer seasons were carefully observed to find out the comparative prevalence of Fascoiliasis. The *Fasciola* species (a trematode) which were observed and studied included *Fascoila gigantica*, and *Fasciola hepatica*.

5.6.1 Sex-wise observation of slaughtered buffaloes

Out of 100 slaughtered buffaloes observed during winter, 40 were males and 60 were females while during summer, out of again 100 slaughtered animals observed, 47 were males and 53 were females i.e. a total of 87 males and 113 females were observed during the research. The sexes of the animals were recorded as accordingly said by the butchers.

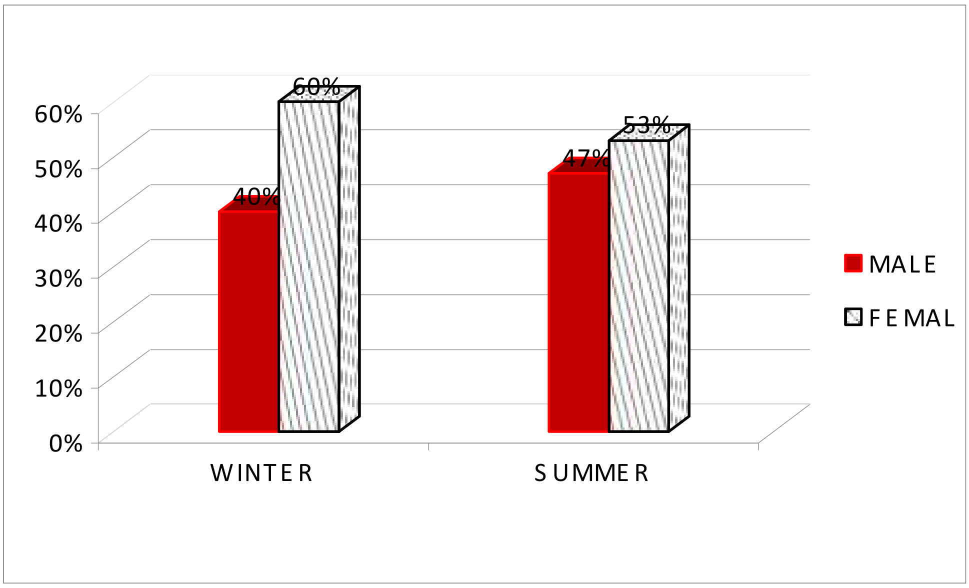


Fig. 16: SEX-WISE OBSERVATIONS OF SLAUGHTERED BUFFALOES

5.6.2 Age-wise observation of slaughtered buffaloes

Out of 100 carcasses observed during winter, 20 belonged to calves, 30 belonged to adults and 50 were that of older ones. Similarly out of 100 slaughtered buffaloes observed during summer, 34 were calves, 21 were adults and 45 were olds. So a total of 54 calves, 51 adults and 95 old buffaloes were studied during the research. Age separation was made consulting with the butchers plus by observing the slaughtered animal.

Following things were considered to separate the age of the slaughtered animal:

	YOUNG	ADULT	OLD
Size	Small	Large	Large
Separation of hides/skin	The animal is usually burnt after killed and skin is not separated from flesh.	The animal is not burnt and hides are separated. Not used for eating	The animal is not burnt and hides are separated. Not used for eating
Type of body	Usually shining skin.	Usually smooth and shining skin. Incase of female the breast is much swollen and not shrunken.	Skin wrinkled or oldish. Males have clear rough mark on the upper part of their neck as they had been used once for ploughing. Females have shrunken breasts.

The age-wise observation of slaughtered buffaloes during the winter and summer is shown in Fig.17.

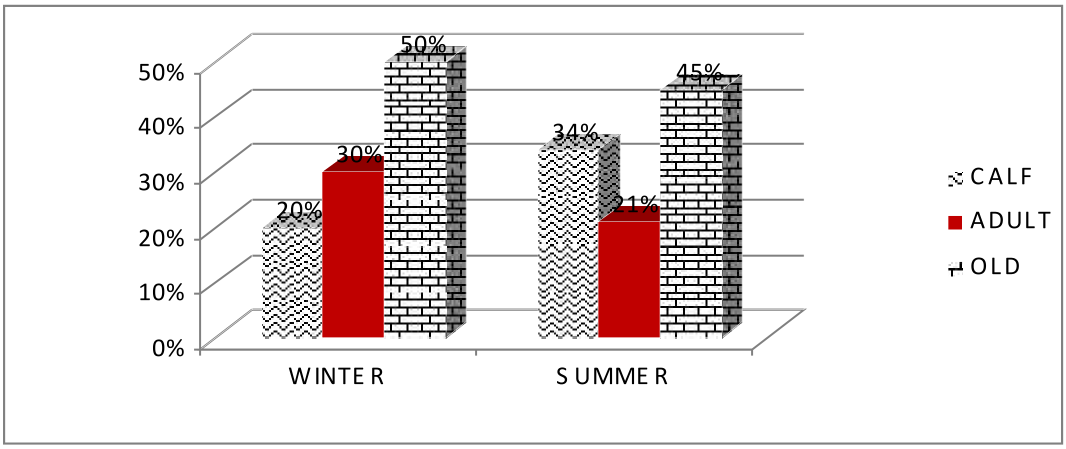


Fig. 17: AGE-WISE OBSERVATIONS OF SLAUGHTERED BUFFALOES

5.7 PREVALENCE OF *Fasciola* INFECTION

5.7.1 General prevalence

57 (28.50%) buffaloes were found positive for fascioliasis, out of 200 slaughtered buffaloes observed.

5.7.2 Season-wise prevalence

35 and 22 positive cases of fascioliasis were observed out of 200 slaughtered animals observed, 100 each during winter and summer season, which is shown in Fig. 18. The season-wise prevalence of fascioliasis was found significant as indicated by Chi-square test ($\chi^2_{0.05}$, 1d.f. =4.14).

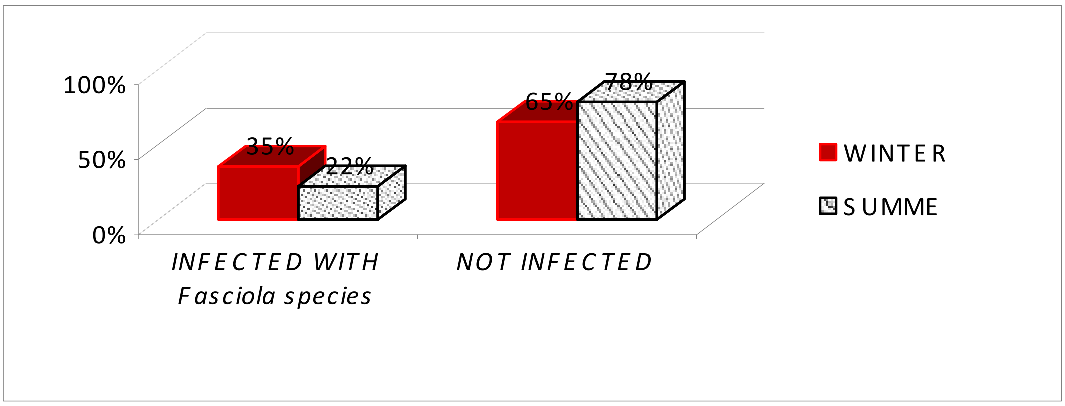


Fig. 18: SEASON-WISE PREVALENCE OF *Fasciola* species

5.7.3 Sex-wise prevalence of *Fasciola*

Females were found more infected with fascioliasis. 38.05% of the females had fascioliasis as compared to 16.09% of males. The difference in sex-wise prevalence of fascioliasis was found significant ($\chi^2_{0.05}$, 1d.f. =11.63).

Table 4: SEX-WISE PREVALENCE OF *Fasciola*

MONTH	WINTER			SUMMER			TOTAL		
SEX	Observed	Infected	Prevalence	Observed	Infected	Prevalence	Observed	Infected	Prevalence
MALE	40	8	20.00%	47	6	12.76%	87	14	16.09%
FEMALE	60	27	45.00%	53	16	30.18%	113	43	38.05%
TOTAL	**100**	**35**	**35.00%**	**100**	**22**	**22.00%**	**200**	**57**	**28.50%**

5.7.4 Age-wise prevalence of *Fasciola*

Old animals (35.78%) were infected with *Fasciola* more often than calves (14.81%) and (29.41%) adults which is shown in Table 5. Difference in age-wise prevalence of *Fasciola* was significant ($\chi^2_{0.05}$, 2d.f. =7.45).

Table 5: AGE-WISE PREVALENCE OF *Fasciola*

MONTH	WINTER			SUMMER			TOTAL		
AGE	Observed	Infected	Prevalence	Observed	Infected	Prevalence	Observed	Infected	Prevalence
CALF	20	4	20.00%	34	4	11.76%	54	8	14.81%
ADULT	30	8	26.66%	21	7	33.33%	51	15	29.41%
OLD	50	23	46.00%	45	11	24.44%	95	34	35.78%
TOTAL	**100**	**35**	**35.00%**	**100**	**22**	**22.00%**	**200**	**57**	**28.50%**

5.7.5 Species-wise Prevalence of *Fasciola*

Out of 35 positive cases of *Fasciola* infection observed during winter, 20 (57.14%) buffaloes had only *Fasciola hepatica* while 11 (31.42%) had only *F. gigantica* and 4 (11.42%) had both *F. hepatica* and *F gigantica.* Similarly during summer, out of 22 animals that had fascioliasis, *F. hepatica* was found in 7 (31.81%) of them while *F. gigantica* was observed in 12 (54.54%) of them and 3 (13.63%) of them had both *F. hepatica* and *F. gigantica.* Infection with *Fasciola hepatica* (59.65%) was found slightly higher than *Fasciola gigantica* (52.63%).

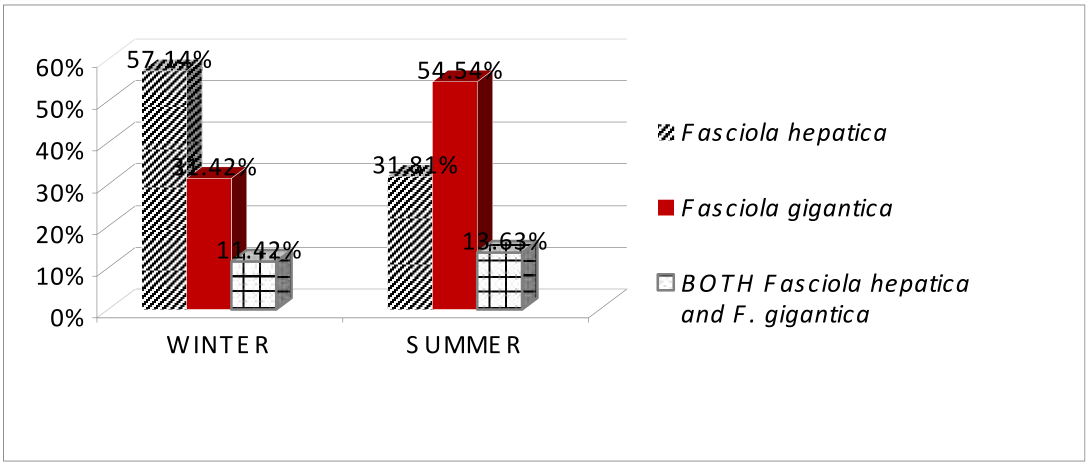


Fig. 19: SPECIES-WISE PREVALENCE OF *Fasciola*

5.8 .PREVALENCE OF *Fasciola* INFECTION (according to faecal samples)

5.8.1General prevalence

55 (27.50%) buffaloes were found positive for fascioliasis, out of 200 slaughtered buffaloes observed.

5.8.2 Season-wise prevalence

33 and 22 positive cases of fascioliasis were observed out of 200 slaughtered animals observed, 100 each during winter and summer season, which is shown in Fig. 20. The season-wise prevalence of fascioliasis was found significant as indicated by Chi-square test ($\chi^2_{0.05}$, 1d.f. =4.14).

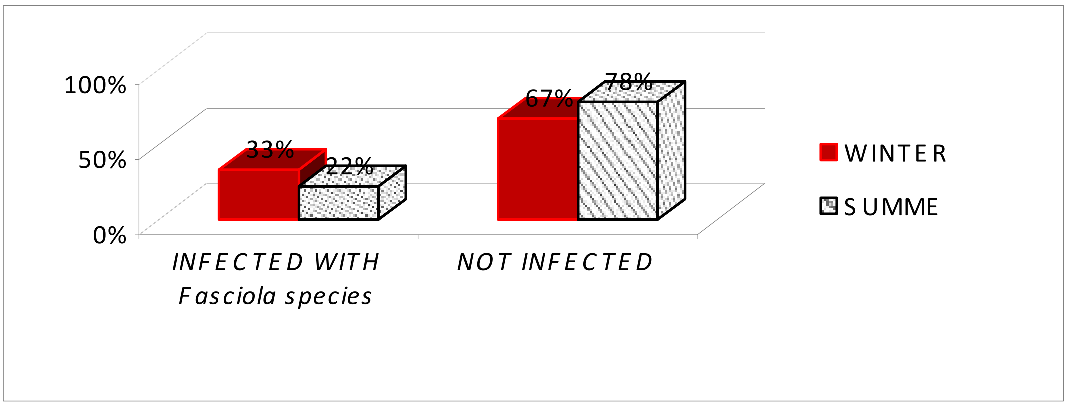


Fig. 20: SEASON-WISE PREVALENCE OF *Fasciola* species

5.8.3 Sex-wise prevalence of *Fasciola*

Females were found more infected with fascioliasis. 37.04% of the females had fascioliasis as compared to 16.84% of males.

Table 6: SEX-WISE PREVALENCE OF *Fasciola* (In faecal)

MONTH	WINTER			SUMMER			TOTAL		
SEX	Observed	Infected	Prevalence	Observed	Infected	Prevalence	Observed	Infected	Prevalence
MALE	50	10	20.00%	45	6	13.33%	95	16	16.84%
FEMALE	50	23	46.00%	55	16	29.09%	105	39	37.14%
TOTAL	**100**	**33**	**33.00%**	**100**	**22**	**22.00%**	**200**	**55**	**27.50%**

5.8.4 Age-wise prevalence of *Fasciola*

Old animals (33.68%) were infected with *Fasciola* more often than calves (14.81%) and (29.41%) adults which is shown in Table 7.

Table 7: AGE-WISE PREVALENCE OF *Fasciola* (In faecal)

MONTH	WINTER			SUMMER			TOTAL		
AGE	Observed	Infected	Prevalence	Observed	Infected	Prevalence	Observed	Infected	Prevalence
CALF	20	4	20.00%	34	4	11.76%	54	8	14.81%
ADULT	30	8	26.66%	21	7	33.33%	51	15	29.41%
OLD	50	21	42.00%	45	11	24.44%	95	32	33.68%
TOTAL	**100**	**33**	**33.00%**	**100**	**22**	**22.00%**	**200**	**55**	**27.50%**

5.9 MEASUREMENT OF ECOLOGICAL FACTORS

Buffaloes for the slaughtering purposes are mainly brought from the Terai of Nepal and Utter Pradesh and Bihar of India. Rice is a dominant crop in these areas so rice husks are the important part of buffaloes' diet. The prevalences of different diseases in buffalo could be related to the food it feeds on. The intermediate host of *Fasciola* i.e. lymnaeid snails are usually found in the moist region. They are often found in the paddy fields. Cercaria of *Fasciola* after being liberated from the snails' body, encyst on the nearby vegetations which therefore mainly includes the paddy. So there is every possibilities of hay containing the infective metacercariae which when ingested by the buffalos, they become infected.

5.10 MEASUREMENT OF SOCIO-CULTURAL AND RELIGIOUS FACTORS

Majority of people in Kirtipur are Newars. The ethnic group is renowned for its culture and tradition. They have lots of feasts and festivals to celebrate and in each of these occasions, varieties of dishes prepared from the meat serve as an important part. Kachila' (raw meat), and 'Chhengu' (raw skin attached with flesh), are among the varieties of meat dishes. They are raw meats, so have got zoonotic significances. The survey found that, in Kirtipur, each day, an average of 25 buffaloes is slaughtered. However the number was found increased upto 30 to 35 during festivals due to more demand of meat. Similarly during Ekadashi and Aaunshi, the number was found reduced as the tradition prohibits the consumption of meat in these days. More calves were found slaughtered during Saturdays and holidays. The reason for this is that during holidays Newars, the ethnic people of the study area prefer to eat the cuisine prepared from buffaloes' skin called 'Chhengu'. As the calves' skin is softer than the adults and olds, they are slaughtered more often to meet the demand of 'Chhengu'.

5.11 MEASUREMENT OF LEGAL FACTORS

Though 'The Slaughter House and Meat Inspection Act 1998' has come to legislation, not a single example of its implementation has been found during the study. The butches and meat sellers said that no one has ever come for meat inspection. They themselves also revealed that the condition of slaughtering places and meat shops could have been much better had there been regular meat inspection. The law strictly demands the requirement of license for carrying out the slaughtering and meat selling activities. It demands certain basic requirements in the slaughtering places and meat shops. It prohibits the slaughter of diseased animals, females, etc but due to absence of ante-mortem inspection of animals, inspection of slaughter place and meat shops, and post-mortem inspection of carcass, all above mentioned activities which are supposed be illegal have been carried out without any threats. The urgent requirement of the implementation of 'The Slaughter House and Meat Inspection Act 1998' is the foremost and uttermost demand of the study.

VI

DISCUSSION AND CONCLUSION

Meat, one of the delicious and nutritious dishes, is an indispensable food consumed by most of the people. It is rich in protein, containing all of the essential amino acids, and in most cases, is a good source of zinc, vitamin B12, selenium, phosphorus, niacin, vitamin B6, iron and riboflavin (http://www.beef.org/uDocs/whatyoumisswithoutmeat638.pdf).

As the demand of meat is ever-increasing, the importance and significance of it in meat borne diseases can't be ignored. Meat and other meat products contribute significantly to high incidence of food borne diseases and zoonotic diseases. Meat, like any food, can also transmit certain diseases, but undercooked meat is especially susceptible.

Through the questionnaires and observations it was found that, among the slaughtered animals, females outnumbered the males. Out of the 200 slaughtered buffaloes observed during the survey.113 (56.50%) were females and 87 (43.5%) were males. This is against the law and fact that, slaughtering of females is prohibited in Nepal. So most of the butchers used to lie the consumers about the slaughtered animal as consumers prefer not to buy the meat of female buffaloes.

The study also found out that the old animals are slaughtered more often than the adults and the calves. Among 200 slaughtered animals observed, 95 (47.50%) were the old animals. 51 (25.50%) were adults and the rest 54 (27.00%) were calves. Loss of productivity in old animals makes them cheaper to buy, as revealed by the butchers and this seemed to be the main reason behind their greater slaughtering. The study found an interesting thing that more calves were slaughtered during Saturdays and holidays. The reason for this is that during holidays Newars, the ethnic people of the study area prefer to eat the cuisine prepared from buffaloes' skin called 'Chhengu'. As the calves' skin is softer than the adults and olds, they are slaughtered more often to meet the demand of 'Chhengu'.

The study found fascioliasis, the overall prevalence in slaughtered buffaloes was found to be 28.50%. The prevalence of fascioliasis during winter (35.00%) was higher in compare to summer (22.00%) which was significant ($\chi^2_{0.05}$, 1d.f. =4.14). Females were found more infected with fascioliasis. 38.05% of the females had fascioliasis as compared to 16.09% males. The difference in sex-wise prevalence of fascioliasis was also found significant ($\chi^2_{0.05,}$ 1d.f. =11.63). Also old animals (35.78%) were found more infected with fascioliasis than calves (14.81%) and adults (29.41%). This difference was found significant as well ($\chi^2_{0.05}$, 2d.f. =7.45).

The prevalence of fascioliasis in buffaloes was also observed by Parajuli (1996), 83.00% in Surkhet; Shrestha *et al*., (1992), 20% to 87.5% in Dhankuta; Singh *et al*., (1992-1993), 48.57% in Chitwan; Mahato (1993), 57.9% in the hills and 4.3% in the Terai of Nepal; Adhikari *et al*., (2003), 61% in Kathmandu; Nguyen *et al* (1999), 46.67% in Vietnam; Oliveria (2002), 75% at the Cajati abattoir of Vale Do Riberia, Sao Paulo, Brazil; Marques *et al*., (2003), 20% in Viamao, southern Brazil.

The prevalence to fascioliasis in buffaloes was found more during winter than in summer. Seasonal or month wise prevalence of fascioliasis in buffaloes is also studied by Singh and Mahato (1992) who showed that the higher incidence of liverfluke occurred during the month of Sept. through Nov. and again Jan. Similarly, Pandey (2001) found increase in *Fasciola* infection from June to December, decrease in Feb., and again rise in April, the prevalence being highest during December.

The age-wise prevalence showed that old animals (35.78%) were infected more than the calves (14.81%) and (29.41%) adults. This finding is in agreement with Mahato (1993), Ghimire and Karki (1996), Regmi *et al*., (1999) and Pandey (2001). Ghimire and Karki (1996) found higher prevalence of fascioliasis in old animals (94.30%) compared to heifers (54.16%) and calves (34.60%) in rural Kathmandu. Mahato (1993) found increase in prevalence of fascioliasis with increase age group i.e. < l, 1-2, 3-4, 5-6, 7-8, 9-10 and 11 > was 17.6%, 43.1%, 57.9%, 55.6%, 67.8% and 70.3% respectively in eastern Nepal. Regmi *et al*., (1999) found 63.83% prevalence of fascioliasis below age group three and 69.16% prevalence above age group three in Syangja. Pandey (2001) found the highest prevalence of fascioliasis in old buffaloes (65.30%) followed by 56.80%, 48.78%, 35.29% and 14.25% in the age group of 7-8, 5-6, 3-4 and 0-2 years respectively. Maqbool (2002) who undertook an epidemiological study at slaughter house, livestock farms, and veterinary hospitals and on household buffaloes under the different climatic conditions existing in Punjab province also recorded a higher infection rate in older buffaloes than in youngsters (below 2 yrs of age).

Out of 35 positive cases of *Fasciola* infection observed during winter, 20 (57.14%) buffaloes had only *Fasciola hepatica* while 11 (31.42%) had only *F. gigantica* and 4 (11.42%) had both *F. hepatica* and *F. gigantica.* Similarly during summer out of 22 animals that had fascioliasis. *F. hepatica* was found in 7 (31.81%) of them while *F. gigantica* was observed in 12 (54.54%) of them and 3 (13.63%) of them had both *F. hepatica* and *F. gigantica.*

During research a total of 57 positive cases of fascioliasis were reported, 35 (61.40%) during winter and 22 (38.59%) during summer. 27 (47.36%) slaughtered animals were infected with only *Fasciola hepatica*, 23 (40.35%) with only *Fasciola gigantica* and 7 (12.28%) animals with both *F. hepatica* and *F. gigantica.* It showed that prevalence of *Fasciola hepatica* (59.65%) was slightly greater when compared to *Fasciola gigantica* (52.63%) among the slaughtered animals.

Nguyen *et al*., (1999) studied livers of 955 buffaloes. The buffaloes infected with *Fasciola gigantica* were found 14.14% and both *Fasciola hepatica* and *Fasciola gigantica* were found 32.04%. The mean numbers of flukes in the infected organ were 78. Asharaf *et al*., (2004) collected 928 adult liver flukes from 13 infected livers of cattle, in Rasht and Bandar-Anzali slaughterhouses in Iran, 91.1% were diagnosed as *F. gigantica* and 8.9% as *F. hepatica*.

From the result obtained it was found that the prevalence of diseases were more among females in comparison to males and again more in old animals in comparison to the adult or young.

Higher prevalence in old animals might be due to loss in resistance associated with old age and also might be due to carrying residual infection from previous years. Swarup and Pachauri (1987) in India suggested that the higher prevalence in older female animals is due to relaxation of resistance at parturition or during lactation.

The study found higher prevalence of fascioliasis during winter (35.00%) as compared to summer (22.00%). Reason for this may again be associated with the type of food buffaloes are provided with during the winter and summer. During winter, buffaloes are mainly feed with rice straw as rice are harvested mainly during winter and grass from the rice fields; and during summer, their food mainly consists of maize stover, maize husks, millet etc. Encysted cercariae i.e. metacercariae which is the infective stage of *Fasciola* spp. to buffaloes (Definitive host) are plentiful in the paddy fields as Lymnaeid snails their intermediate host and source of cercaria are found in them. When these rice straws or grass from the paddy fields containing the infective metacercariae is ingested, buffaloes become infective.

The findings of the field research reveal the prevalence of fascioliasis in the slaughtered buffaloes and the situation has been aggravated by the unhygienic condition of the slaughtering places and meat shops, lack of infrastructure and facilities in them, lack of meat inspection programme and unawareness of the meat borne diseases. The study demands an urgent need of meat inspection.

VII

RECOMMENDATIONS

From the outcome of the study performed, following recommendations have been drawn.

1. Slaughter house, at least a mini abattoir should be established in each ward depending the consumption of the meat.
2. Slaughtering practices should be made humane.
3. Slaughter shed should be constructed.
4. Infrastructure to facilitate the slaughter and marketing of meat should be established.
5. Meat marketing system is unhygienic and lacks quality. It should be improved.
6. The butchers and meat sellers should be trained and should be made aware about fascioliasis.
7. Information regarding meat borne diseases and zoonotic diseases should be introduced in the text book of primary and secondary level as a compulsory subject.
8. The Slaughter House and Meat Inspection Law which has been approved in 1998 by the then HMG should be strictly considered and implemented.

VIII

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ANNEX

Questionnaires

Questionnaire for butchers

1. Name:

2. Address:

3. What type of animal do you slaughter?
   a) buffalo b) goat c) pig d) chicken e) fish

4. How do you slaughter the animal?
   a) stunning b) electric shock c) euthanasia d) other

5. What is the total number of animal you slaughter each day?
   a) 1 b) 2 c) 3 d) 4 e) more than 4

6. Where do you bring the animal from?
   a) Local place (name of the place) b) outside the valley

7. How do you transport the animal?
   a) on foot b) by truck c) other if any

8. Do you have shed for the animal (buffalo) before slaughter?
   a) yes b) no
   if no, where do you keep them? ……………………………………

9. What do you do with …..?
   a) hide and skin b) bones c) blood d) gall bladder

10. Where do you dispose the waste of the slaughtered animal?
    a) street site b) river site c) drainage system d) container e) other

11. Do you sell the meat yourself or give to others?
    a) oneself b) give to others

    if others, give it inside or outside the Kirtipur?
    a) inside b) outside

12. Where do you bring the water from?
    a) well b) tap c) river d) other

13. Do you have slaughter house?

a) yes b) no

if yes,

i) slaughter place with shed

a) yes b) no

ii) slaughter place with concrete floor

a) yes b) no

iii) slaughter place with water supply

a) yes b) no

iv) slaughter place with electricity supply

a) yes b) no

v) slaughter place with offal disposal

a) yes b) no

vi) slaughter place with drainage

a) yes b) no

vii) slaughter place with meat storing facilities

a) yes b) no

if no,

i) why didn't you make the slaughter house?

a) no money b) no place c) other

ii) if somebody makes slaughter house do you join with him?

a) yes b) no

14. Have you got any training on meat business?

a) yes b) no

15. Has municipality or other ever come to check your meat?

a) yes b) no

16. Do you have any idea about *fasciola* in meat?

a) yes b) no

17. if *fascoila* are found, what do you do?

a) yes b) no

18. in which part in *fascoila* mostly found ?
    a) liver b) lung c) whole body

19. Do you have knowledge of environment pollution due to the wastes from your slaughtering place?
    a) yes b) no

Questionnaire for meat sellers

1. Name:

2. Address:

3. What type of meat do you sell?
   a) buffalo meat(buff) b) mutton c) pork d) chicken
   e) fish

4. Do you have adequate facilities at your shop for meat marketing?
   a) yes b) no

if yes,
what type of facilities do you have?
i) housing a) permanent b) temporary shed c) road side d) hut

ii) water supply provision
a) yes b) no

if yes,
what type of water do you use?
a) well b) tap c) river d) other

iii) electricity
a) yes b) no

iv) fly proof
a) yes b) no

v) cold storage facilities
a) yes b) no

vi) dog proof
a) yes b) no

vii) offal waste disposal container
a) yes b) no

if no, where do you dispose them?
a) street side b) road side c) drainage system d) other

viii) clothing of meat seller
a) appron b) cap c) gloves d) gumboot e) none

5. Where do you get the meat from?
   a) from the butcher b) slaughter oneself

6. How do you get the meat?
   a) on vehicle b) via manpower

7. Have you seen any cysts (water pockets)) parasites on the meat?
   a) yes b) no

8. Do you have any idea about meat borne diseases?
   a) yes b) no

9. Have you seen *fascoila* in liver of meat?
   a) yes b) no

10. do you sell meat with *fascoila*?
   a) yes b) no

11. If *fascoila* are found , what do you do?
   a) yes b) no

Questionnaire for consumers

1. Name:

2. Address:

3. What type of meat you most buy?
   a) buffalo meat(buff) b) mutton c) pork d) chicken e) fish

4. How often do you eat meat?
   a) once a week b) twice a week c) once a month d) twice a month e) in a long gap f) other

5. Have you seen any cysts (water pockets) in the meat you buy?
   a) yes b) no

6. Have you your family member ever been sick after having meat?
   a) yes b) no

7. Are you satisfied with the meat hygiene and sanitation?
   a) yes b) no

8. Do you have any idea about meat borne diseases?
   a) yes b) no

9. have you seen fascoila on the meat?
   a) yes b) no

ANNEX

Animal Slaughterhouse and

Meat Inspection Act 2055' (A translation)

Preamble

To safeguard the health and welfare of public and to prohibit adulteration in meat and meat products, to prevent any fraud which lowers the wholesomeness quality and adequacy of meat, it is imperative to establish slaughterhouse and arrangement for meat inspection.

Therefore, on the 26th year of accession **of His Majesty the King Birendra Bir Bikram Shah Dev**, the Parliament has enacted this Act.

1. Short title and commencement

1. This Act shall be called "Slaughterhouse and Meat Inspection Act, 2055".
2. This Act shall come into force in prescribed area on a prescribed date which His Majesty Government may notify it in the Nepal Gazette, to specify it.

2. Definition

1. "Animal" means castrated or un-castrated goat, sheep, pig, wild boar, chyangra goat, buffalo, bull, rabbit or the females above which are fit for meat other than cow and ox and this word also includes poultry, ducks, pigeon or other species kept for meat purpose.
2. "Meat" means meat of the animals which is fit for human consumption.
3. "Meat inspector" means persons appointed or as prescribed under Article 6.
4. "Meat supervisors" mean persons as prescribed under Article 7.
5. "Meat sellers" mean persons who sell the meat professionally.
6. "Prescribed or as prescribed" means prescribed or as prescribed on the regulation made under this Act.

3. Prohibit to established slaughterhouse and selling of meat without license

Nobody shall establish slaughterhouse or sell the meat without obtaining license under this Act.

4. Establishment of slaughterhouse

1. His Majesty Government may establish the slaughterhouse in any area of the country by the notification in Nepal Gazette.
2. What so ever is written in Sub-Article (1) His Majesty's Government may give permission to non-government sector as well to establish the slaughterhouse.

3. The term and conditions and specification to establish the slaughterhouse under Article (1) or (2) shall be as prescribed.

5. Application for license

1. Persons interested to establish a slaughterhouse or selling of meat shall obtain license as prescribed.
2. To obtain the license under Sub-Article (1) , a fee is to be paid as prescribed.

6. Appointment of meat inspector

1. His Majesty's Government may nominate) appoint a person as a meat inspector to examine animals of meat, who is at least graduate in veterinary science.
2. Other rights and duties of meat inspector will be as prescribed, in addition to those mentioned in this Act.

7. Nomination of meat supervisor

1. His Majesty's Government may nominate a meat supervisor to be any civil servant who is at least graduate in veterinary science for supervision of slaughterhouse management and as meat supervisor.
2. His Majesty's Government may nominate the meat supervisor as meat inspector in case if there is no meat inspector.
3. Other functions, duties and rights of meat supervisor shall be as prescribed.

8. Inspections of animals before slaughtering (anti-mortem inspection)

1. Animals to be slaughtered should be produced for inspection and anti-mortem exanimation before meat supervisor, at slaughterhouse, and in the area where slaughterhouse is not yet constructed such animals should be inspected at the site prescribed by the meat supervisor. Procedure for examination of animals will be as prescribed.
2. After inspection under Sub- Article (1) animal found suitable for slaughter may be permitted for slaughter with marking the animal by meat inspector.
3. Upon inspection under Sub- Article (1) if animal is found diseased, such animal should be prohibited for slaughter.

9. Slaughtering of animals in slaughterhouse

1. Animal fit for slaughtering under Article (8) shall be slaughtered in the slaughterhouse.
2. Whatever is written in Sub-Article (1) if there is no slaughterhouse in those areas, animal shall be slaughter at the place and time as prescribed by the meat supervisor.

10. Meat examination of the slaughtered animal

1. Meat inspector should inspect the meat of the slaughtered animal under Article 9, as prescribed.
2. If meat inspectors find any disease in meat of slaughtered animal upon inspection under Sub-Article (1) such meat may be prohibited for selling partially or completely by meat inspector.

11. Prohibition on sale of carcass

1. Sale of meat of animal other than mentioned in section (A) of Article 2 shall not be allowed.
2. Meat of dead animal due to disease or any other cause shall not be allowed for sale.
3. Sale of meat with skin shall not be allowed.

But this article shall not be applied for selling meat of birds, pigs, wild pigs and in other species identifying organs or portions like heat and legs with skins.

12. Marketing of stamping on carcass

1. Meat inspector shall affix clearly visible marking of stamp as prescribed during the certification of meat for sale.
2. No meat shall be sold without the marks or stamps retired under Sub-Article (1).

13. Adulterated meat shall not allowed to sale

1. Adulteration of carcass with another species of animal or deceiving the species of animal while selling meat shall not be allowed.
2. Adulteration of meat with any substance that alters the normal quality or taste or weight shall not be permitted for sale.

14. Fee to be paid

Inspection of animal or mat under this Act., fee shall be levied as prescribed.

15. Authority to enter

Meat inspector or meat supervisor may enter in slaughterhouse or butcher's shop and may take sample of meat or inspection of animal or carcass. To help on this work will be the duty of all concerned.

16. No objection

Whatever is contained in this Act, there will be no objection on slaughter of animal for household purposes the relevant Articles of this Act shall not be effective.

17. Penalty and punishment

1. Person who contrivance Sub-Article (1) or (3) of Article 8, Article 9, Sub-Article (2) of Article 10, Sub-Article (3) of Article 11 or Sub-Article (2) of Article 12 shall be punished with a fine of maximum of Rupees one thousand.
2. Persons who contrivance Article 3, Sub-Article (1) or (2) of Article 11 or Article 13 shall be punished with a maximum fine of Rupees of five hundred.

18. Delegation of authority

Authority vested on His Majesty's Government under this Act may be delegated with limitation to other bodies as required.

19. His Majesty's Government shall be plaintiff

His Majesty's Government shall be plaintiff on case under this Act.

20. Investigation of crime and defence

1. Meat investigator shall investigate the case under this Act and register the case after completing the investigation; he shall file the case to judiciary powered officer.
2. During the registration of the case under Sub- Article (1) investigation personnel may take advice with government lawyer.

21. Judicial authority

The judicial authority for cases and hearing appeal related to this Act should be vested on prescribed officer.

22. Shall be according to the prevailing law

Whatever mentioned in this Act shall be decided accordingly, while in other matter it will be as per the prevailing law.

23. Authority to frame rules

For the implementation of purpose of this Act. His Majesty's Government shall have authority to make the necessary rules.

24. Repeal

Whatever is written in Muliki Ain (Act) under Chaupaya (four footed animal) on paragraph number 3 and 16 has been repealed.