

**PREVALENCE OF GASTROINTESTINAL PARASITES OF
DOMESTIC PIG (*Sus scrofa domesticus* Carl Linnaeus, 1758) IN TWO
FARMS OF POKHARA VALLEY**



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RECOMMENDATION

This is to recommend that the thesis entitled "**Prevalence of Gastrointestinal Parasites of Domestic Pig (*Sus scrofa domesticus* Carl Linnaeus, 1758) in Two Farms of Pokhara Valley**" has been carried out by Sarala Poudel for the partial fulfillment of Master's Degree of Science in Zoology with special paper Parasitology. This is her original work and has been carried out under my supervision. To the best of my knowledge, this thesis work has not been submitted for any other degree in any institutions.

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LETTER OF APPROVAL

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CERTIFICATE OF ACCEPTANCE

This thesis work submitted by Sarala Poudel entitled "**Prevalence of Gastrointestinal Parasites of Domestic Pig (*Sus scrofa domestica*, Carl Linnaeus, 1758) in Two Farms of Pokhara Valley**" has been approved as a partial fulfillment for the requirements of Master's Degree of Science in Zoology with special paper Parasitology.

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DECLARATION

I hereby declare that the work presented in this thesis has been done by myself, and has not been submitted elsewhere for the award of any degree. All sources of information have been specifically acknowledged by reference to the author(s) or institution(s).

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ABSTRACT

Parasitic infection in pig is a common global burden causing loss of production and several parasitic diseases to them. The present study was conducted to determine the prevalence of gastrointestinal parasites (GI) of domestic pigs in two farms viz. Phursekhola pig farm and Saaru pig farm of Pokhara. A total of 120 faecal samples were collected by opportunistic random faecal sampling method. Iodine wet mount and different concentration technique (floatation and sedimentation) were used for faecal qualitative tests and verbally administered questionnaires for interview. Out of 120 faecal samples examined, 73 faecal samples were positive with 60.83% prevalence of parasitic infection. Total of eight GI parasites that includes protozoan: *Isospora* sp. (8.33%), *Eimeria* sp. (5.33%) and *B.coli* (25%) and helminths: *Ascaris* sp. (10.83%), *Trichuris* sp. (20.83%), *Strongyloides* sp. (8.33%), *Trichostrongylus* sp. (10.83%), and *Fasciolopsis* sp. (1.67%) were identified. Statistically, the difference in GI parasitic infection in specific parasites were found to be insignificant ($\chi^2=38.083$, $P>0.05$). The prevalence rate of protozoan parasites (39.17%) were higher than helminthes parasites (21.67%). Statistically, the difference in prevalence of GI parasitic infection among Protozoans and Helminthes parasites were found to be insignificant ($\chi^2= 3.505$, $P>0.05$). The higher prevalence of GI parasites was in Saaru pig farm (71.67%) and the lowest was in Phursekhola pig farm (50%). Statistically, the difference in prevalence of GI parasitic infection among study area was found to be insignificant ($\chi^2= 1.11$, $P>0.05$). Whereas the difference in prevalence of single infection (64.38%) and mixed infections (35.61%) were insignificant ($\chi^2=3.50$, $P>0.05$).

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LIST OF ABBREVIATIONS

µm	- Micrometre
CI	- confidence interval
RVL	- Regional Veterinay Laboratory
EPG	- Egg per gram
GI	- Gastro Intestine
Nacl	- Sodium Chloride
Viz.	- Namely
Conc.	- Concentrated

1. INTRODUCTION

1.1 Background

The domestic pig (*Sus scrofa domesticus* or *Sus domesticus*), often called swine or hog is a large even-toed ungulate. It is most often considered to be a sub-species of the wild boar, which was given the name *Sus scrofa* by Carl Linnaeus in 1758; following from this the formal name of domestic pig is *Sus scrofa domesticus* (Gentry *et al.*, 2004). Domestic pigs are farmed primarily for the consumption of their meat, called pork. The animal's bones, hide, and bristles are also used in commercial product. Gastrointestinal parasites are responsible for substantial loss of productivity in swine and other livestock industry. pig can harbour a range of parasites and diseases that can be transmitted to humans. These includes trichinosis, cysticercosis and fasciolopisis. Thirty nine percent (39%) of children have been found to be infected with *Fasciolopsis buski* in India and Bangladesh (Taposi *et al.*, 2014). They constitute a major impediment to efficient and profitable livestock production (Boes *et al.*, 2000; Joachim *et al.*, 2001). Gastrointestinal parasitism in swine affects swine's performance in terms of efficient feed conversion, poor growth rate, reduced weight gain and the condemnation of affected organs after slaughter (Nsoso *et al.*, 2000). Clinical signs of diarrhoea and emaciation noted on these pig farms at the time of sampling may have been caused by *Coccidia*, *Oesophagostomum* sp., *Trichuris suis* and *Strongyloides* sp. since these parasites have been reported to cause such clinical signs (Soulsby, 1982). In the tropical and sub-tropical areas, parasitic infections in pigs are estimated to be second to African swine fever (Permin *et al.*, 1999). Infection with parasites is associated with significant economic losses evidenced by decreased litter size, poor growth rate, reduced weight gain, organ condemnation at slaughter and death (Nsoso *et al.*, 2000).

The prevalence rate of GI parasites are influenced by various socio-economic and cultural factors, religious beliefs, cultural practices, etc (Joshi *et al.*, 2003). Intestinal infection, caused GI parasites of pig is much more prevalent in developing countries than in industrialized ones due to differences in environmental and personal hygiene standards (Joshi *et al.*, 2003). It is also associated with primitive pig-raising condition. The close relationship between man and pigs in some rural areas, where the animals live practically at the house of the owner and feed on kitchen wastes and excreta, explains the high prevalence of GI parasites infection.

1.2 Pig, human and parasites in nature

Pigs are kept in close contact with families. Human risk of infection with pig zoonoses arises from direct contact and consumption of unsafe pig products. Pigs are an important source of food and income and are kept by many rural residents (Holt *et al.*, 2016). Diseases that are transmitted between pigs and humans (zoonoses), namely hepatitis E, Japanese encephalitis, trichinellosis, cysticercosis and taeniasis. *Taenia solium* causes human and porcine cysticercosis and is considered one of the most important diseases in Southeast Asia, and a neglected zoonotic disease (Willingham *et al.*, 2010). Human taeniasis describes infection by the adult tapeworm following consumption of raw or undercooked pork contaminated with the larval stage of *T. solium* (or *T. saginata* in beef)

(Ito *et al.*, 2003). Cysticercosis in pigs and humans is caused by ingestion of *T. solium* eggs expelled from infected humans via food, water, or environmental faecal contamination. In humans, this can lead to the development of mature cysts in various organs including muscles, eyes, subcutaneous tissues and the central nervous system. Cysticercosis causes significant morbidity and mortality in humans and can lead to neurocysticercosis; the leading cause of epilepsy in the region (Willingham *et al.*, 2016). Although, asymptomatic in pigs, losses occur due to the development of metacestodes leading to carcass condemnation. Foodborne zoonoses have been estimated to annually affect 10% of the global population, among which zoonotic parasites constitute an important class of aetiological agents. The major meat borne parasites include the protozoa *Toxoplasma gondii* and *Sarcocystis* sp., and the helminths *Trichinella* sp. and *Taenia* sp., all of which may be transmitted by pork. The significance of zoonotic parasites transmitted by pork consumption is emphasized by the prediction by the Food and Agriculture Organization of an 18.5% increase in world pork production over the next 10 years (Schlundt, 2004).

1.3 Parasitic Infections

Parasites may be responsible for a number of serious health problems among pigs either directly or indirectly. Endoparasites are ever present in pigs and must be considered in the economic production of pork (Corwin *et al.*, 1999). Loss of appetite, reduction in daily weight gain, poor feed utilization and the potentiation of other pathogens that may be present are the more common results of parasitism. The damage caused by the presence or migration of parasites results in the condemnation of internal organs or carcasses unfit for human consumption. Pigs parasites commonly seen include protozoa (one-celled animals), helminths (worms) and arthropods (insects and mites). The effects vary from benign to acute death (Ritchie *et al.*, 1997). The parasites are biologically and ecologically associated with host. The effect of parasites on host is not constant but depend upon various factors.

There are more than 2,00,000 named species of protozoa of which nearly 10,000 are parasitic in invertebrates and in almost every species of vertebrate (Assafa *et al.*, 1998). Protozoans are single celled organisms that can affect the body at a cellular level, causing problems especially in the circulatory, endocrine and gastrointestinal system. As a general rule, protozoa multiply by asexual reproduction. This is not to say that sexual processes are absent in the protozoa. Some parasitic forms may have an asexual phase in one host and a sexual phase in another host. In most parasitic protozoa, the developmental stages are often transmitted from one host to another within a cyst. The reproduction process is also related to the formation of the cyst. Asexual reproduction of some ciliates and flagellates is associated with cyst formation, and sexual reproduction of sporozoa invariably results in a cyst. Protozoa of medical importance are classified on the base of their morphology and locomotive system (Assafa *et al.*, 2006).

Several helminth have been implicated in causing morbidity and mortality in pigs (Soulsby, 1982) as well as considered as the greatest impediments to profitable pig production (Galloway, 1972). The most important helminth species, classified into three major groups. They enter the body through different routes including mouth, skin and the respiratory tract (Assafa *et al.*, 2006). They are: a. Cestodes (Tape worms) b. Nematodes (Round worms) and c. Trematodes (Flukes). The prevalence and intensity of parasite may be influenced by several factors, such as climatic conditions (temperature and humidity) that alter the population dynamics of the parasites, resulting in dramatic changes in the

prevalence and intensity of helminths infections (Magwisha., 2002). The life cycle may be direct or indirect including an intermediate host. Some species require a second intermediate host or even a third (Permin and Jorgen, 1998).

A. Nematodes:

Roundworms, also called Nematodes are a group of worms which more than 28000 species have been described. More than 16000 roundworms species are parasitic of plants and animals including cattle, sheep, goats, pig, poultry, horses, dogs, cats as well as many other wild and domestic animals, humans and also plants. Roundworm of veterinary importance are all obligate parasites. The most common parasites of the pigs are; *Hyostrogylus* (redstomach worm), *Gnathostoma*, *Ascaris* (large roundworm), *Strongyloides* (threadworm), *Globocephalus* (hookworm), *Trichostrongylus*, *Oesophagostomum* (nodular worm), *Trichuris* (whipworm), *Metastrongylus* (lungworm), *Stephanurus* (kidney worm), *Trichinella* etc (Taylor *et al.*, 2007).

B. Trematodes:

All the trematode species are obligate parasites of either mollusks or vertebrates including livestock and other domestic animals as well as humans. Flukes are parasitic worms belonging to the group of the trematodes. Trematodes live in the bile duct and can affect lungs also. Some of the infective stages of the trematode are ingested and some others penetrate the skins of host for entrance. The eggs are passed out through faeces of hosts. The major trematode parasites of pigs are; *Fasciolopsis* (intestinal fluke), *Gastrodiscus*, *Fasciola* (liver fluke) *Schistosoma* (bloodfluke) etc (Taylor *et al.*, 2007).

C. Cestodes:

Cestodes are a class of parasitic worms (helminthes) that have a flat ribbon-shaped body and live in the digestive system of the host. All tapeworms are obligate parasites i.e. they cannot complete development without parasitizing their hosts. The major cestode parasites of pig are *Taenia solium*, *Cysticercus cellulosae*, *Cysticercus tenuicollis* etc (Taylor *et al.*, 2007).

The sources of the parasites are different. Exposure of parasites may occur in one of the following ways:

- a. Contaminated soil (Geo-helminths), water and food
- b. Blood sucking insects or arthropods
- c. Domestic or wild animals harboring the parasite
- d. Oneself (auto-infection)
- e. Sexual intercourse.

1.4 Objectives

1.4.1 General objective

- ❖ To determine the prevalence of gastrointestinal parasites of domestic pig (*Sus scrofa domestica* Carl Linnaeus, 1758) in two Farms viz. Phursekhola pig farm and Saaru pig farm of Pokhara valley.

1.4.2 Specific objectives

- ❖ To identify the gastrointestinal parasites of pig.
- ❖ To compare area-wise and infection-wise prevalence of gastrointestinal parasites of pig.

1.5 Justification of the study

Nepal is an agricultural country; about 80% of the people are involved in agriculture for their livelihood. In this context most of these people who are involved in agriculture are also in connection with the livestock farming. There are numerous people in our country who are involved in pig farming as their source of income. These farmers are mostly unknown about the disease and are suffered most as a result they have to bear a loss in their income. The work will certainly be a great boon to Nepalese farmers in term of controlling GI parasites achieving maximum progress in pig's productivity and ultimately promoting sustainable farming.

This study will help to determine the general prevalence, identification, compare area wise as well as infection wise prevalence and find out activities on health care regarding the GI parasites of pig in two farms of Pokhara valley. This study will also help to generate some awareness about the GI parasites of pigs in the farmers/peoples of the community and village who are unknown about the diseases caused by them. The study also informs the people suffered from the diseases caused by GI parasites of pig and provides some preventive and control measure on it.

1.6 Limitation of the study

This research has been carried out for the partial fulfillment of the requirements for the master's degree in Zoology at Tribhuvan University, Kathmandu, Nepal. The study was only limited to certain parameters related to the topic due to resources, cost and time constraints. The identification of parasite's eggs and cyst was limited to morphological basis with light microscopy so identification was not possible up to species level.

1.7 Hypothesis

The null and alternative hypothesis of this research work is:

H₀=There were no significant differences of gastrointestinal parasites and risk factors.

H₁=There were significant differences of gastrointestinal parasites and risk factors.

2. LITERATURE REVIEW

Among GIT parasites, helminthes are major health problem to those swine grazing on pasture. Helminthes importance on swine is chiefly economical with its sub-clinical infection delay's the achievement of market weight by being responsible for poor feed conversion rates (Borthakur *et al.*, 2007). The common helminth parasites of swine are *Ascaris*, *Trichuris*, *Oesophagostomum*, *Trichinella* and Strongyles species (Nganga *et al.*, 2008).while Fasciolopsis caused by *F. hepatica* and *F. gigantica* , is also one of the most prevalent helminthes infection of ruminants(also pseudoruminants, pig) in different parts of the world. It causes significant morbidity and mortality (Nsoso *et al.*, 2000). Among protozoan diseases, *Eimeria* and *Isospora* sp. are very common (Davis and Moon , 1990). The most known external parasite of swine is *Sarcoptic mange*, although in some condition swine may be infested by *Demodex mange* and lice (Abdu and Gashaw *et al.*, 2010). Gastrointestinal parasitism in swine affects swine's performance in terms of efficient feed conversion, poor growth rate, reduced weight gain and the condemnation of affected organs after slaughter (Nsoso *et al.*, 2000). In swine industry, the sustainable development of this sector is faced with a number of constraints, prominent among which is the disease is caused by intestinal parasites. Gastrointestinal parasites are responsible for substantial loss of productivity in swine and other livestock industry. They constitute a major impediment to efficient and profitable livestock production (Boes *et al.*, 2000; Joachim *et al.*, 2001).Parasite incidence can determine common health problems at all stages of production, and is therefore a limiting factor in pig breeding establishments (Carraro, 2002). The main negative effects on animals are reflected in economic losses for producers, such as: reduced feed conversion, reduced fertility, low number of piglets born and weaned, low weight piglets at birth and at weaning, as well as losses relating to viscera of high condemnation rate in slaughterhouses (Roepstorff *et al.*, 1998). In addition, pigs are considered the main reservoir of *Balantidium coli* and *Entamoeba polecki*, which can infect humans, especially farm laborers (Solaymani and Petri, 2006).

2.1 In global context

There are several research work have been carried out about intestinal parasitic infection on pigs in different place of the world. The prevalence of intestinal parasites in pigs had been reported in Northern England (Pattison *et al.*, 1980) which showed *Oesophagostomum dentatum* (85%) was the most prevalent followed by *H. rubidus* (28.5%), *A. suum*(16%), *T. suis* (23%). And protozoans *Isospora*, *Eimeria* and *Balantidium coli* was found to be (10%), (5%), and (3%) respectively. Similarly, *Oesophagostomum dentatum* (32.59%) has been reported as a highly prevalent intestinal helminth parasites followed by *Balantidium coli* (31.85%), *Trichuris suis* (11.11%), *Isospora suis* (1.48%), *strongyloides ransomi* (0.74%) and *Globocephalus urosubulatus* (0.74%) among the pigs in slaughter house of Mumbai (Dadas *et al.*, 2016). Another research showed *Oesophagostomum* sp. (86.7%) has been reported as a highly prevalent intestinal parasites followed by *Ascaris suum* (36.7%), *Metastrongylus* sp. (25.8%), *Strongyloides* sp. (25.8%), *Trichuris suis* (15.8%), *Globocephalus* sp. (6.7%), *Gnathostoma* sp. (4.2%), *Schistosoma japonicum* (5.0%) and *Fasciola* sp. (1.3%). Post mortem examination revealed the presence of *Oesophagostomum dentatum*, *O. quadrispinulatum*, *A. suum*, *Metastrongylus apri*, *M. pudendotectus*, *T. suis*, *G. hispidum* and *Ascarops dentate* among the pigs in Dongting Lake Region of China (Boes *et al.*, 2000).

The prevalence of intestinal parasites in pigs has been reported in industrial farms in different Nordic countries (Roepstorff *et al.*, 1998) which showed *Acaris suum* (35%) was the most prevalent followed by *Hyostrogylus rubidus* (22%), *Oesophagostomum* sp. (26%), *Isospora suis* (17%) and *Eimeria* sp. (12%) respectively. They examined that overall prevalence of positive samples, 40% were found to be mixed infection and 20% were found to be single infection. Similarly, *A.suum* (67.4%) has been reported as highly prevalent intestinal parasites among age group of 0-3 months which is followed by *Trichuris suis* (55.9%) in 3 to 6 and (58.4%) in 6 to 12 months age groups. *Strongyloides* sp. was mostly found in pigs up to 3 months of age (73.9%) whereas *Oesophagostomum* sp. (49.4%) and *Metastrongylus* sp. (44.2%) were highest in 6 to 12 months old pigs in Nagaland (Rajkhowa *et al.*, 2003). In another research *A.suum* (12.18%) was found to be highly prevalent parasites followed by *T.suis* (10.13%), *Oesophagostomum* sp. (10.13%), *Eimeria* sp.(9.53%), *Cystoisospora* sp. (5.02%), *Cryptosporidium* sp. 6.60%) and cyst of *B.coli* (22.7%) among pigs at pig breeding centre in Chongqing, China (Lai *et al.*, 2011). Similarly, *A.suum* (18.5%) was found to be higher prevalent rate which was followed by *Balantidium coli* (13.0%) & *Schistosoma japonicum* (13%) while *Strongyloides* sp. recorded the lowest prevalence (1.9%) in Pankshin Urban (Agumah *et al.*, 2015). Similarly, *A.suum* (12.55) has been reported as highly prevalent intestinal parasites followed by *Oesophagostomum* sp.(12%), *Strongyloid* sp. (3%), *Eimeria* oocysts (3%)& *Strongyle* sp. (2.5%), there was higher prevalence in female (58%) than males (42.%) and higher prevalence in adults (35.7%) than young pigs (20%) which was significantly different ($P<0.05$) in Plateau State Nigeria (Akannio *et al.*, 2010).

Several research works has been carried out in intestinal parasitic infection in pigs in Kabale District in Uganda (Nissen *et al.*, 2011) which showed that higher prevalence of *Strongyle* sp. (89%) followed by *Ascaris suum* (40%), *Trichuris suis* (17%) and *spiruroid* eggs (48%). Fifteen (15) pigs were selected for post-mortem examination and (93%) pigs were infected with *Oesophagostomum* sp., (73%) with *A. suum* , (67%) with *T. suis* , and (20%) with *Hyostrogylus rubidus*. Similarly, another research work found that *Strongyle* sp. (17.3%) which was followed by *A. suum* eggs 17 (11.3 %) harbored *B. coli* cyst, 10 (6.6 %) found positive for *Trichuris* eggs and 08 (5.0 %) harbored coccidian oocysts, 6 (4.0 %) samples showed *Schistosoma* sp. eggs and the remaining 19 (12.0 %) had mixed infection with *Strongyle* eggs, *B. colicyst*, and *Trichuris* sp. eggs respectively in America (Krishna *et al.*, 2016). Another research work showed that *Strongyloides* sp. (16.2%) has higher prevalence rate which was followed by *Ascaris suum* (*A. suum*), *Trichuris suis* (*T. suis*), *Oesophagostomum* sp., *Coccidia* sp. and *Fasciola hepatica* (*F. hepatica*) ova/oocyst, at 12.6% (49/390), 6.9% (27/390), 3.9% (15/390), 11.8% (46/390), 10.5% (41/390) respectively in Ethiopia (Geresu *et al.*, 2015).

In some research studies protozoan's parasites showed the higher prevalence rate and helminthes shows the lowest. Such type of research has been done in Camaragibe, Pernambuco (D'Alenar *et al.*, 2006) and obtained 0.27% to 1.6% for the incidence of helminthes and protozoa, respectively. Similarly, *Cryptosporidium* sp. (8.8%) showed the higher prevalence rate which was followed by *Giardia lamblia* (3.7%) and *B.coli* (1.6%) in Istanbul, Turkey (Uysal *et al.*, 2009). Similarly, another research found that *Isospora suis* (26.9%) and *Cryptosporidium parvum* (1.4%) among pigs in Southern Germany

(Wieler *et al.*, 2001). Similarly, Prevalence of Protozoans parasitic infection found to be (38.6%) and (39.7%) in Minas Gerais and Sao Paulo respectively. In another research Coccidian (39.81%) has higher prevalence which was followed by *oesophagostomum* sp. 33.93%, *strongyloides* sp. 19.90%, *T. suis* 17.19% in China (Keshaw *et al.* 2009). Similarly, Coccidian sp. (47.2%) has higher prevalence rate which was followed by *Trichuris suis* (5.2%), *Ascaris suum* (2.5%), *Oesophagostomum* sp. (24.9%) in China (Weng *et al.*, 2005). Similarly another research showed that the Coccidian sp. (14.5%) has higher prevalent rate which was followed by *Strongyle* sp., (11%), *Ascaris sum* (2%) and *Trichuris* sp. (0.5%) in Ejisu Municipality of Ghana (Atawalna *et al.*, 2016).

Some research found that predominant of nematodes parasitic infection which showed prevalence of *Trichuris suis* (23%) followed by *strongyles Hyostrongylus*, *Globocephalus* sp., *Trichostrongylus*, *Ascaris*, (20%, 10%, 9%, 5.5%, and 4%) respectively in Guangdong Province (Beloeil *et al.* 2003). However another research of (Pilarczyk *et al.*, 2004) found *Emeria* sp. (58.5%) has most prevalent which was followed by *Oesophagostomum dentatum*, *Metastrongylus* sp., *Trichuris suis* and *Ascaris suum* (25%, 30%, 10%, 8%, 10%) respectively in North-west Poland. Similarly, another research included *Trichuris suis* having a highest prevalence (12.2%) followed by *Ascaris suum* (11.1%), human hookworm (5.9%). Others were *S. dendatus* (1.1%) with the lowest prevalence and *Isospora suis* (6.3%) the only protozoan in Ibadan, south west Nigeria (Sowemimo *et al.*, 2012).

However in another research *B.coli* (47.5%) found to be highly prevalent followed by *T. suis* (5.7%), *Ascaris suum* (5.2%), strongyles (2.5%) and coccidia (24.9%) in China (Weng *et al.*, 2005). Similarly, *B.coli* (77.6%) has been reported as a highly prevalent intestinal parasites followed by coccidia (71.1%), *Strongyles* (45.3%), *Strongyloides ransomi* (36.9%), *A. suum* (27.6%), *T. suis* (14.3%) and *Metastrongylus* sp. (3.7%) in Federa District (Aguiar *et al.*, 2009). Similarly, the results revealed the presence of *Balantidium coli* (53.3%) has been found higher prevalence, which was followed by *Trichuris suis* (17.5%), *Ascaris suum* (15.8%), coccidia (11.6%), *strongyles* (8.3%), *Entamoeba suis* (2.5%) and *Echinostoma malayanum* (1.7%) in Malaysia (Edmund *et al.*, 2005). On the other hand in one research *Isospora suis* (20.79%) has higher prevalent rate which was followed by *Oesophagostomum dentatum* (9.90%), *Trichuris suis* (0.99%) and *Metastrongylus salini* (0.99%) respectively in Ibadan (Okorafor *et al.*, 2014).

Different research work have been carried out by many researchers in different place of the world. Another research showed that *Hyostrongylus rubidus* (55%) has been found higher prevalence rate which was followed by *A.suum* (23%), *T.suis* (17%), and other unidentified eggs (5%) in Bangladesh (Julius and Oladapo, 2016).

2.2. In context of Nepal

No literature regarding the topic of the present study of the Pokhara valley was found. However, similar such type of works had been performed by different authors at different parts of Nepal like, sero-prevalence of brucellosis in pig, farming and biodiversity of indigenous pig, assessment of pork handlers knowledge and hygienic status of pig meat shops, focusing campylobacteriosis risk factors have found doing research in pigs. In this connection some relevant literatures are given below.

Khanal (2068) surveyed about 105 pigs faecal samples in different location of Chandragiri municipality in Kathmandu district. From the study, overall prevalence of 88.57% GI parasite was observed. Out of 105 faecal sample examined, 9.52% (10/105), 4.76% (5/105), 38.09% (40/105), 14.28% (15/105), 7.61% (8/105), 23.80% (25/105), 42.8% (45/105), and 7.61% (8/105) were identified as *Fasciolopsis buski*, *Schistosoma suis*, *Ascaris suum*, *Strongyloides* sp., *Trichuris suis*, *Balantidium coli*, *Eimeria* spp., and *Isospora suis* ova/oocyst, respectively. The study had also revealed that about 15.23% and 73.33% pigs had harbored mixed and single infection, respectively.

3. MATERIALS AND METHODS

3.1 Study area

Pokhara is metro municipality and second largest city of Nepal. It is the head quarter of both the western development region and the Kaski district. It lies on the geographical coordinates of 28.27° North latitude and 83.97° East longitude. It covers an area of 55.66 sq. km i.e 2.7% area of the district and 0.04% area of the nation. The temperature usually ranges between 2°C to 33°C with an average annual rainfall of 3880 mm whereas the elevation ranges between 827m to 1740m above sea level. There is exclusively great floral and faunal diversity in Pokhara valley due to the prevalence of a wide range of climatic and topographical variations.

Pig farming in Pokhara is not new, it has been accepted socially and culturally by certain ethnic groups and its being a main occupation in Farmers of Pokhara. Pig farming trend is changing gradually due to urbanization some commercial and modern pig farming practice recently started in Pokhara. Pigs on the farm are raise in clean, healthy, natural environment with plenty of moving vegetable fields and sustainable pasture. Pork has a distinctive flavor good for nature, good for animal welfare, good for the economy and ultimately safer for every family to eat. The native pig breeds of Pokhara are Chwanche, Hurrah, Bampudke, Pakhribas black and Dharane kalo banggur etc. Exotic breeds of pigs are imported in Nepal since 1957 A.D like Landrace, Hampshire, Duroc and Yorkshire etc. The main area of my study are;

- A. Phursekhola Pig Farm in Birauta and
- B. Saaru Pig Farm in Mahatgauda

A. Phursekhola Pig farm:

This farm is located at Birauta, of Pokhara valley. The place is located latitude of 28.19 N and longitude of 83.97E. Pokhara airport with a distance of 1.4 km north-east of the city centre of Birauta. About 100 *Sus scrofa* kept here in this farm, it includes some piglets, some are adults. The defecation of pigs are swept in morning with water and thrown to the damping sites. The food mainly comprises kitchen wastages, meat, bones, remaining of foods, etc. The foods are provided by farmers, they collect foods from hotels, houses, hostels etc.

B. Saaru Pig Farm:

This farm is located at Mahatgauda of Pokhara valley. The place is located latitude of 28.18N and longitude of 83.99E. Pokhara airport with a distance of 2.4 km North of the city centre of Mahatgauda. Around total of 90 domestic pigs the faecal sample taken from only 60 pigs. The food mainly comprises kitchen wastages, meat, bones, remaining of foods, etc provided by farmers, they collect food for their livestock from hotels, hostels, houses, etc. The defecation of pigs are also swept in morning with the help of water and dispose on the damping sites.

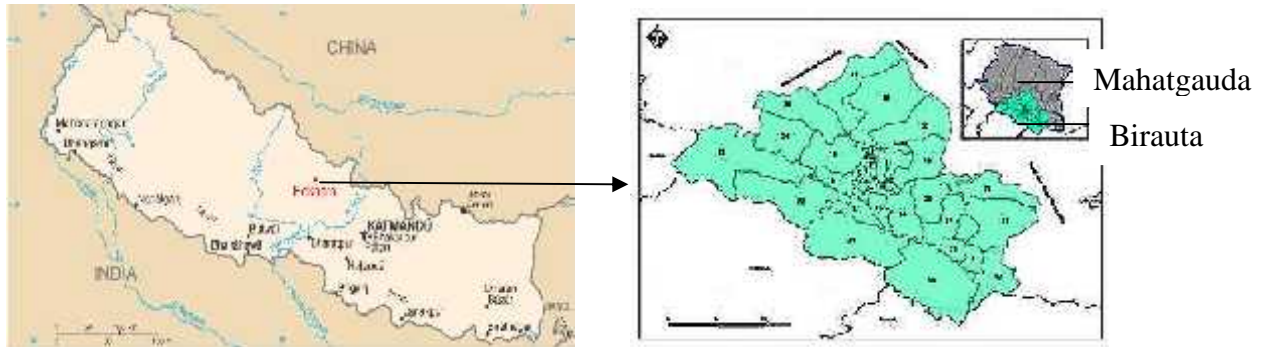


Photo 1: Map of Pokhara valley showing study area (source: LGCDP)

3.2 Materials used

3.2.1 Equipments

- | | |
|------------------------|-----------------------|
| I. Electric microscope | II. Ocular micrometer |
| III. Stage micrometer | IV. Volumetric flask |
| V. Centrifuge machine | VI. Centrifuge tubes |
| VII. Gloves | VIII. Beakers |
| IX. Cover slips | X. Slides |
| XI. Cotton | XII. Tea strainer |
| XIII. Glass rod | XIV. Cavity slide |
| XV. Watch glass | XVI. Rack |
| XVII. Dropper | XVIII. Tooth picks |
| XIX. Mask | XX. Glass vials |
| XXI. Dropper | XXII. Camera |
| XXIII. Gloves | XXIV. Tooth picks |

3.2.2 Chemicals

- | | |
|--------------------------------|---------------------------------|
| I. Potassium dichromate (2.5%) | II. Iodine solution |
| III. Nacl solution | IV. Methylene blue |
| V. Distilled water | VI. Conc.Zinc sulphate solution |

3.3 Study design

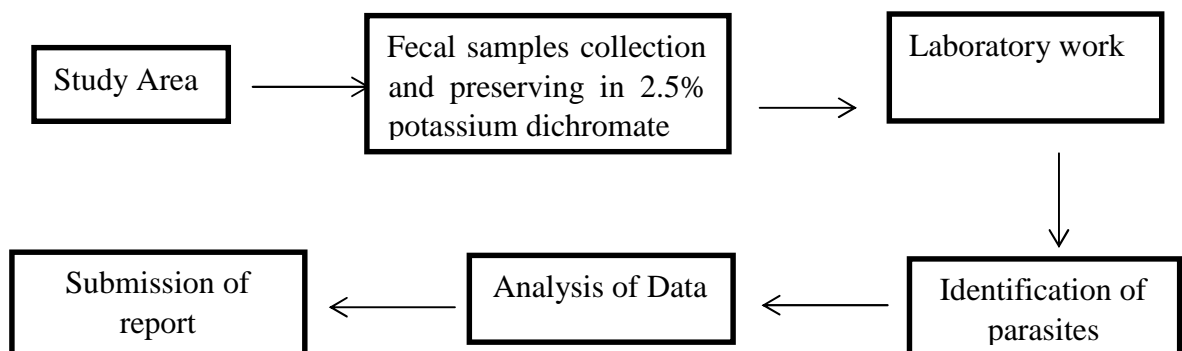


Fig 2. Frame work of Research Design.

The present study was designed to assess the gastrointestinal parasitic infection in *Sus scrofa* of two farms in Pokhara valley. The study comprises: a) Selection of farms with abundant *Sus scrofa*.

- A) Collection of fresh faecal samples in sterile glass vials by opportunistic random sampling.
- B) Preservation of faecal samples in 2.5% of Potassium dichromate solution.
- C) Examination of faecal samples by using iodine wet mount, floatation and sedimentation techniques.
- D) Identification and measurement of eggs and cysts of parasites.

3.3.1 Sample collection method

All the fresh faecal samples of *Sus scrofa*. were collected by opportunistic random sampling method in early hours of morning. It took each morning for sample collection on each site (four morning). About 5 gm of faecal sample was collected in clean, sterile vial with tooth-pick wearing gloves and mask. It was then preserved with 2.5% potassium dichromate. All the samples collected were labeled properly. The same collection process was repeated for all collected faecal samples.

3.3.2 Preservation of faecal samples

After sample collections, it was preserved in 2.5% Potassium dichromate solution (2.5 gm potassium dichromate powder dissolved in one liter of distilled water). It helps in maintaining morphology of protozoan parasites and preventing further development of helminth eggs and larva.

3.3.3 Sample size

There was around 200 *Sus scrofa* in whole study area. Out of these 120 faecal samples (60 from each) of domestic pigs were collected from the pocket of present study area. The sample size occupies about 60% of whole population.

3.4 Laboratory examination

The collected faecal samples in glass vials were brought from collected site to the Regional Veterinary Laboratory of Pokhara, for test. The faecal samples were subjected to coprological examination by different concentration technique (floatation and sedimentation) and iodine wet method.

3.4.1 Iodine wet mount

One tooth pick of faecal samples were emulsified in a drop of Lugol's Iodine solution on a clean glass slide and then covered with a clean cover-slip. The smear was examined under electric microscope at 10X and 40X (Soulsby, 1965).

3.4.2 Concentration techniques

Eggs, cysts and trophozoite are often in such low number in faeces, that they are difficult to be detected in direct smears or mounts. Therefore, these procedures were performed

which includes floatation and sedimentation techniques (Soulsby, 1982; Zajac and Conboy, 2012).

3.4.2.1 Floatation technique

This technique ensures the eggs float in the floatation liquid, which helps to identify the nematode and cestode eggs as well as protozoan cyst present in pig faeces. Approximately 3 gram of faecal samples was put in a beaker and 42 ml of water was added. The sample was grinded lightly with the help of rod or pistle and the solution was filtered by tea strainer. The filtrate solution was poured into a centrifuge tube of 15 ml and centrifuged at 1000 rpm for five minutes. The tube's water was replaced with super saturated Nacl solution and again centrifuged.

After centrifuged, more saturated Nacl solution was added to develop convex meniscus at the top of the tube and one drop of Methylene blue (to stained) was also added. A coverslip was placed for a five minutes. It was then removed from tube, placed on glass slide and examined microscopically at 10X and 40X. The photographs of eggs and cysts of parasites were taken and identified on the base of shape, shell and size (Soulsby, 1982; Zajac and Conboy, 2012).

Floatation technique also done by using conc. zinc sulphate solution. The procedures is same as that of Nacl solution. This technique is more effective to find the eggs of parasites.

3.4.2.2 Sedimentation technique

This technique is used for detection of trematode eggs. It provides a better result as the eggs of trematode are bit heavier than the other. Sediments of centrifuged contents were taken for eggs detection.

Saturated Nacl solution was removed gently from the centrifuge tube after examination of the floatation portion and the sediment content was poured into the watch glass and the content was stirred gently to mix it. One drop of faecal from the mixture was taken to prepare a second slide. The specimen was stained with Iodine wet mount's solution and examined microscopically at 10X and 40X (Soulsby, 1982; Zajac and Conboy, 2012). In this way, two slides were prepared from one sample (one from floatation and one from sedimentation).

3.4.3 Eggs and cysts size measurement

Eggs and cysts size were measured by using micrometry. The calibration factor was found to be 2.88 μm .

3.5 Data analysis

On the basis of laboratory experiment, the data was recorded. The recorded data were coded and entered into Microsoft Excel 2010. Statistical analysis was performed using "R", version 3.3.1 software packages. Chi-square test was used for statistical analysis of data. In all cases 95% confidence interval (CI) and $P < 0.05$ was considered for statistically significant difference. Percentage was used to calculate prevalence.

4. RESULTS

4.1 General prevalence of GI parasites

Out of 120 faecal samples examined, 73 faecal samples were positive for one or more specific GI parasites, showing 60.83% prevalence of parasitic infection whereas 47 (39.17%) faecal samples were negative. Statistically, the difference in prevalence of GI parasites in positive and negative cases were found to be insignificant ($\chi^2=3.35$, $P>0.05$).

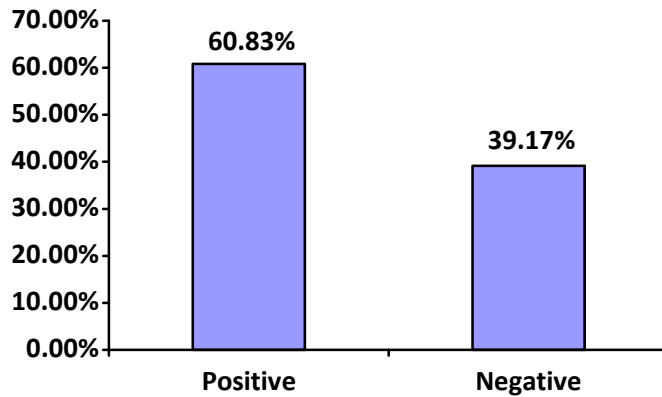


Figure 1: General prevalence of GI parasites

4.2 Prevalence of specific GI parasites

Out of 120 total samples, eight parasites have been identified which are all reported first time in Pokhara, Nepal. The prevalence rate 30 (25%) of *B.coli* and *Trichuris* sp. 25 (20.83%) showed highest prevalence. *Isospora* sp. 10 (8.33%), *Eimeria* sp. 7(5.83%) *Ascaris* sp. 12 (10%), *Strongyloides* sp. 10 (8.33%), *Trichostrongylus* sp. 14 (11.67%) and *Fasciolopsis* sp. 2 (1.67%) were recorded. Here Protozoans (*B.coli*, *Isospora* sp. and *Eimeria* sp.) and Helminths (*Trichuris* sp., *Ascaris* sp., *Strongyloides* sp., *Trichostrongylus* sp., and *Fasciolopsis* sp.) were recorded. Statistically, the difference in GI parasitic infection in specific parasites were found to be insignificant ($\chi^2=38.083$, $P>0.05$).

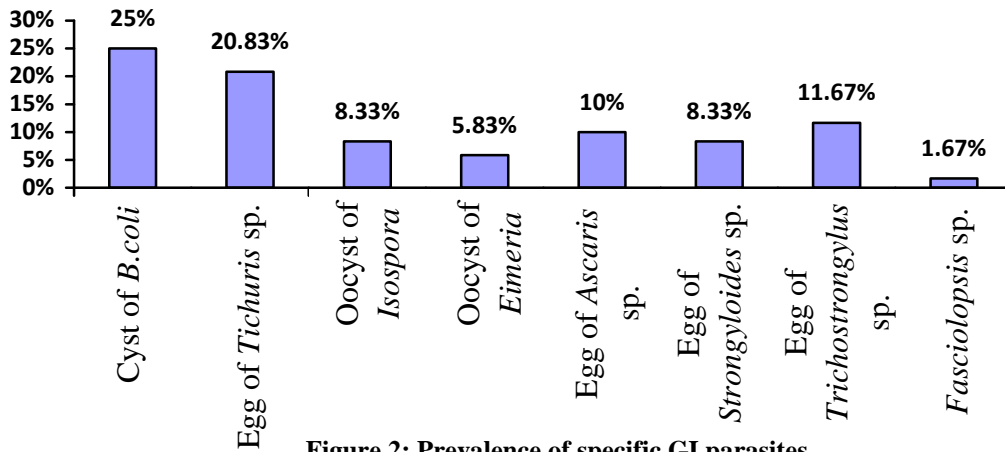


Figure 2: Prevalence of specific GI parasites

4.3 Prevalence of protozoan and helminth parasites

Out of 73 total positive samples, 47 (64.38%) were positive with protozoans whereas 26 (35.62%) were seen positive with helminthes parasites.

Statistically, the difference in prevalence of GI parasitic infection among Protozoans and Helminthes were found to be insignificant ($\chi^2 = 3.505, P > 0.05$).

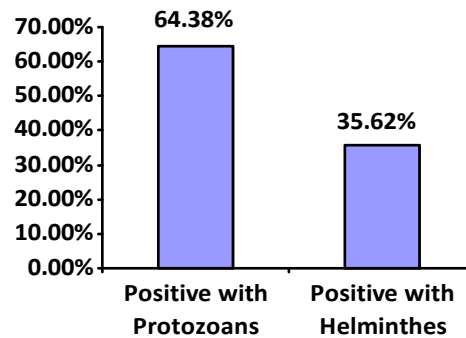


Figure 3: Prevalence of protozoan and helminth parasites

4.4 Area-wise prevalence

Out of two study area, sixty samples from each area (Saaru pig farm and Phursekhola pig farm) were taken for examination. The area with highest prevalence of GI parasites was in Saaru pig farm 43 (71.67%) and the lowest was in Phursekhola pig farm 30 (50%).

Statistically, the difference in prevalence of GI parasitic infection among study area was found to be insignificant ($\chi^2 = 1.11, P > 0.05$).

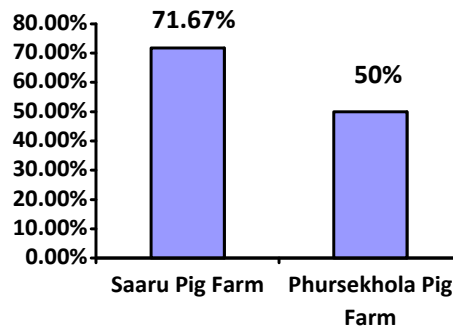


Figure 4: Prevalence of GI parasitic infection among study area

4.5 Infection-wise prevalence

Out of 73 positive samples, the highest prevalence was of single infection 47 (64.38%) than mixed infection 26 (35.61%). Statistically, the differences in the prevalence of single and mixed infections were found to be insignificant ($\chi^2 = 3.50, P > 0.05$).

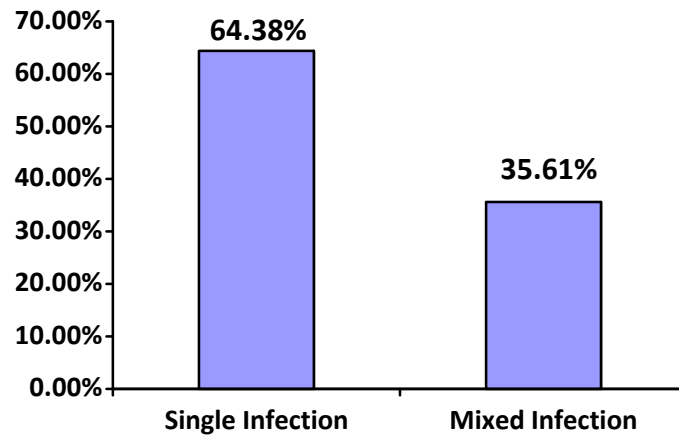


Figure 5: Prevalence of single and mixed infections



Photo1: Collecting sample at Saaru pig farm



Photo2: Collecting sample at Saaru pig farm



Photo3, 4: Collecting sample at Phursekhola pig farm



Photo4: Faecal samples and laboratory equipments



Photo5: Examination by using microscope



Photo 6, 7: Doing laboratory activities

Eggs and cysts of GI parasites in pig under 10X / 40X electronic microscope

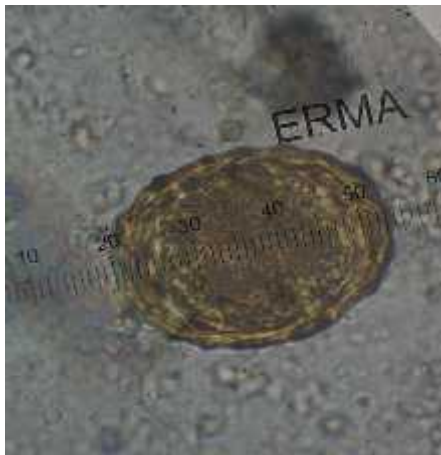


Photo8: Egg of *Ascaris* sp.
(53/ 40) Annex 1

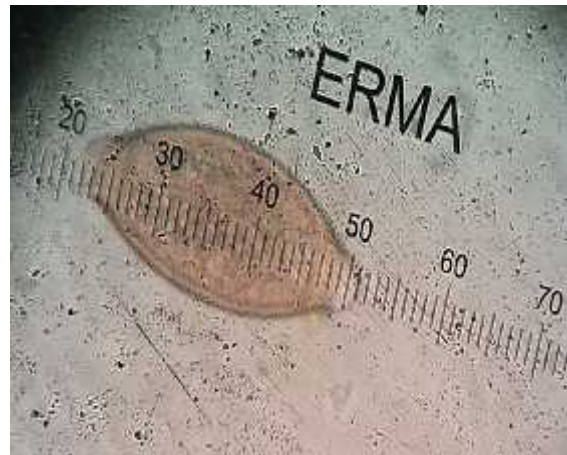


Photo9: Egg of *Trichuris* sp.
(40 / 17) Annex 1



Photo10: Egg of *Trichostrongylus* sp.
(90 / 33) Annex 1



Photo11: Egg of *Strongyloides* sp.
(55 / 30) Annex 1



Photo12: Egg of *Fasciolopsis* sp.
(120/ 100) Annex 1



Photo13: Egg of *Emerica* sp.
(12 / 11) Annex 1



Photo14: Egg of *Isospora* sp.
(25) Annex 1



Photo15: Cyst of *B.coli*
(55) Annex 1

5. DISCUSSION

Parasitic diseases are a major obstacle to the growth of the pig industry and are considered to be next in importance after African swine fever (Sangeeta *et al.*, 2002). Gastrointestinal parasites belong to three classes of parasites, trematodes, cestodes and nematodes respectively. These parasites are collectively called helminths. Helminthiasis in pigs is often associated with subclinical infections; poor feed conversion and delayed achievement of market weight. Information on the epidemiology of parasites of animals is very important in assisting farmers to develop preventive measures. Several studies have been conducted into the prevalence and economic importance of gastrointestinal parasites in pigs.

Intestinal parasites can contribute significantly to poor performance of piggery enterprises. Prevalence studies on intestinal parasites affecting pigs have been undertaken worldwide. The objective of this study was to estimate the prevalence of intestinal parasites in pigs by examination of faecal sample. Gastrointestinal parasites are responsible for substantial loss of productivity in swine and other livestock industry. They constitute a major impediment to efficient and profitable livestock production (Boes *et al.*, 2000; Joachim *et al.*, 2001). Gastrointestinal parasitism in swine affects swine's performance in terms of efficient feed conversion, poor growth rate, reduced weight gain and the condemnation of affected organs after slaughter (Nsoo *et al.*, 2000).

Poor environmental hygiene coupled with extensive management is reported as risk factors of infection of pigs with gastrointestinal parasites. Gastrointestinal helminthes including *Oesophagostomum dentatum*, *Trichuris suis*, *Ascaris suum*, *Oesophagostomum quadrispinulatum*, *Trichostrongylus axei*, *Strongyloides ransomi*, *Hyostrongylus rubidus* and *Physcocephalus sexalutus* and protozoans *Isospora*, *Eimeria*, *Balantidium coli* etc have been identified in pigs.

Three different types of faecal qualitative tests; namely iodine wet mount, floatation and sedimentation techniques were used to determine the prevalence of GI parasites of pig in two farms of Pokhara valley and verbally administered questionnaires to find out activities on health care of pig regarding the GI parasites.

In the present study, the prevalence of gastrointestinal parasites of pigs have been carried out from two different farms viz. Phursekhola pig farm in Birauta and Saaru pig farm in Mahatganda of Pokhara valley. Overall prevalence rate was found to be 60.83%. The total number of genera observed during faecal examination were eight in numbers. Among identified parasites *Fasciolopsis* sp.(1.67%) showed the lowest prevalence and *Balantidium coli* (25%) showed the highest prevalence . It could be due to the ability of the eggs to survive for long in the environment . Two parasites *Strongyloides* sp. and *Isospora suis* showed the same prevalence of 8.33 %. The remaining parasites showed *Ascaris* sp.(10%), *Eimeria* sp.(5.83%), *Trichuris* sp.(20.83%) and *Trichostrongylus* sp.(11.67%) prevalence respectively. Three different types of faecal qualitative tests; namely direct smear, floatation and sedimentation techniques that were used in present study was also used by them.

The general prevalence rate (60.83%) of GI parasites in the present study showed similar prevalence rates reported by Keshaw *et al.*, (2009) in Grenada, West Indies (68.78%), Kristina *et al.*, (2017) in Eastern Uganda (61.4%) , Julius *et al.*, (2016) in North central state of Nigeria (55%), Garesu *et al.*, (2015) in Ethiopian (61.8%) and Mandeep *et al.*, (2017) in West Indies (56.5%). The present result is higher than the previous rates of 35.8%, 28%, and 31% by (Sowemimo *et al.*, 2012; Atawalna *et al.*, 2016; Akanni *et al.*,

2017) respectively. The pig farmers included in this study was using either ivermectin or fenbendazole for internal parasite control. Despite use of anthelmintics, parasites were still a big problem on pig farms in Pokhara. However, the majority of farms were unsanitary, thus contributing to the parasite burden. Extension programmes to educate farmers on the correct measures for preventing and controlling intestinal parasites in pigs are therefore recommended.

The prevalence of 10.83% recorded for *Ascaridia* sp. in this study is nearly similar with earlier findings of Salifu *et al.*, (1990) in River states, Nigeria (10.4%), Kristina *et al.*, (2017) in Eastern Uganda (7.8%), Akannio *et al.*, (2010) in National veterinary institute vom, Nigeria (12.5%), Ajayi *et al.*, (2015) in plateau state, Nigeria (9.77%) and highly differ from Salifu *et al.*, (1990) in River state Nigeria (53.1%), Mandeep *et al.*, (2015) in England (30%), Tambour *et al.*, (2006) in Eastern centre provenance, Burkina Faso (40%), Tidi *et al.*, (2011) in Nigeria (68%). This variation in prevalence is due to the difference pig husbandry systems in these farms.

The present result shows the *Trichuris* sp. (20.83%) which was similar to Edmund *et al.*, (2005) in Malaysia (17.5%), Salifu *et al.*, (1990) in River state Nigeria (15%). It is due to the rearing , sanitary, and feeding methods become similar among these farms and higher than the result Kristina *et al.*, (2017) in Eastern Uganda (3.45), Mandeep *et al.*, (2015) in England (1%), Ajay *et al.*, (2015) in Plateau state, Nigeria (7.52%), Garesu *et al.*, (2015) in Ethiopia (6.9%), Okorafor *et al.*, (2014) in Iband , Oyo State (0.99%), Gueye *et al.*, (2017) in Nigeria (6.25%), Karaye *et al.*, (2016) in Nigeria (2.5%) and lower than Tidis *et al.*, (2011) in Nigeria (59.64%), Manga *et al.*, (1990) in River State Nigeria (47.25%).

The present study shows the prevalence of *Strongyloides* sp. (8.33%) which was found to be similar with Gueye *et al.*, (2017) in Nigeria (8.33%), Karaye *et al.*, (2016) in Nigeria (7.5%), Akannio *et al.*, (2010) in National Institute, Nigeria (6.5%), and Mandeep *et al.*, (2015) in England (6%). Where there was a great difference of present finding Salifu *et al.*, (1990) in Nigeria (87.7%), Tambour *et al.*, (2006) in Eastern Centre Province, Burkina Faso (21%), Garesu *et al.*, (2015) in Ethiopian (17%). The present result was found to be higher than Ajay *et al.*, (2015) in Nigeria (3%), Agumah *et al.*, (2015) in Nigeria (1.9%).

The prevalence of *Eimeria* sp. (5.83%) Recorded in this study is similar with arlier findings of Salifu *et al.*, (1990)in Rivers states, Migeria (3.6%), Akannio *et al.*, (2010) in Migeria (4%). The present report is higher than Gueye *et al.*, (2017) in Nigeria (2.08%), Delia *et al.*, (2017) in Eastern Uganda (2%) and this study is lower than Ella *et al.*, (2011) in Nigeria (68.80%), Garesu *et al.*, (2015) in Ethiopia (10.5%).The lower prevalence of intestinal parasites (*Eimeria*) recorded in this study could be as a result of effective management at the source farm(s), such as daily cleaning and disinfection of pens, giving high quality commercial feed and the use of effective anthelmintic drugs at the right time.

The present study shows the prevalence of *Isoospora* sp. 8.33%, which revealed lower prevalence of okorafor *et al.*, (2014). In Ibadan, oyo state (20.79%) and higher prevalence of Abayomi *et al.*, (2017) in Nigeria (2.08%). The high prevalence of *Isoospora suis* could probably be due to the ability of the cysts/oocysts to survive for long in the environment.

The present results found in the case of *Trichostrongylus* sp. Was 10.83%. Which was little bit similar with earlier finding of Gerusa *et al.*,(2015) in Ethiopia and Marufu *et al.*, (2008) in Zimbabwe which was 9.2% and 11 % respectively. Whereas there was a great difference of present finding Obonoyo *et al.*, (2012) in Kenya which was 28.9 %. The differences in prevalence of *Trichostrongylus* sp. was due to differences in climatic conditions, management systems and local circulatory parasites in the locality.

Among eight different Gastrointestinal parasites identified in present study,The prevalence rate of *B. coli* (25%) were higher. The prevalence rate of this study is higher than gueye *et al.*, (2017) in Nigeria (2.08), and lower than Edmund *et al.*, (2005) in Malaysia (53.3%),

In present study shows the prevalence rate of *Fasciolopsis* sp. (1.67%) were similar with 1% of kristina *et al.*, (2017) in Eastern Uganda.

6. CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The general prevalence of GI parasites of pigs (*Sus scrofa domesticus*) was found to be 60.83% with higher prevalence rates of *B.coli* 30 (25%). Total of eight GI parasites that includes three subclass of protozoan: *Isoospora* sp. 10 (8.33%), *Eimeria* sp. 7(5.83) and *B.coli* 30(25%), five genera of helminths: *Ascaris* sp. 13 (10.83%), *Trichuris* sp. 25 (20.83%), *Strongyloides* sp. 10 (8.33%), *Trichostrongylus* sp. 13 (10.83%), and two *Fasciolopsis* sp. (1.67%) were identified and reported first time in Pokhara, Nepal. Statistically, the difference in GI parasitic infection in specific parasites were found to be insignificant ($\chi^2=38.083$, $P>0.05$).

The higher prevalence of GI parasites was in Saaru pig farm 43 (71.67%) and the lowest was in Phursekhola pig farm 30 (50%). Statistically, the difference in prevalence of GI parasitic infection among study area was found to be insignificant ($\chi^2=1.11$, $P>0.05$). The prevalence of protozoans 47 (64.38%) were higher than helminths parasites 26 (35.62%). Statistically, the difference in prevalence of Protozoans and helminthes parasites was found to be insignificant ($\chi^2=3.505$, $P>0.05$). whereas the difference in prevalence of single infection 47 (64.38%) and mixed infections 26 (35.61%) were insignificant ($\chi^2=3.50$, $P>0.05$). Vaccination and other sanitary activities on health care of pig regarding the GI parasites were found.

This is the first study on GI parasites of pigs in two farms of Pokhara valley. The study indicated that pigs in two farms of Pokhara valley were highly susceptible to GI parasites. Therefore sustainable ways for controlling the parasitic infection and further studies need to be designed for the health and conservation of pigs.

6.2 Recommendations

- ❖ Health care programmes of pig such as routinely faecal examination and deworming should be done by its concerns for effective control of GI parasites.
- ❖ Further identification on species level of parasites is recommended.
- ❖ Farmer should be trained and should be made aware about the infection caused by GI parasites of pigs.
- ❖ Hosts including snail, ants, other insects carry parasites intermediately, called intermediate host should be controlled during life cycle of helminthes parasite to avoid transmission.

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ANNEX 1

Table 1: Identification characters of egg and cyst of parasites

S. N.	Parasites	Photo No.	Size (µm) Length / Breath	Morphological character
Helminths				
1.	<i>Ascaris</i> sp.	8	73-92 / 45-57	Eggs are yellowish brown, spherical and have smooth and thick membrane.
2.	<i>Trichuris</i> sp.	9	53-65 / 20-35	Eggs are yellowish brown, ovoid with bipolar plugs.
3.	<i>Strongyloides</i> sp.	11	53-57 / 30-34	Eggs are thin shelled having poles rounded, slightly to strongly barrel shaped and blastomers present .
4.	<i>Trichostrongylus</i> sp.	10	70-125 / 30-55	Eggs are thin shelled having transparent and smooth surface.
5.	<i>Fasciolopsis</i> sp.	12	130 / 155	Eggs were oval in shape, thin shell with an operculum.
Protozoan				
6.	<i>Eimeria</i> sp.	13	13-32 / 12-23	Cysts were small, spherical or oval in shape, translucent to brownish , with or without micropylar cap depending on species.
7.	<i>Isospora</i> sp.	14	20 – 17dia.	Oocyst is oval, smooth shelled without cap, two sporoblast in older speciemens.
8.	<i>B.coli</i>	15	50-60 dia.	Cysts were spherical of oval in shape but trophozoite were kidney or bean shaped

On the basis of size, shape and shell of published literature journals and books (Davis *et al.*, 1971; Soulsby, 1982; Carney, 1991; Ritchie, 1997; Gibbons *et al.*, 2005; Assafa *et al.*, 2006; Schantz, 2006; Cuomo *et al.*, 2009) eggs and cysts were identified (Table. 1:).