PREVALENCE AND RISK FACTORS OF GASTROINTESTINAL PARASITES IN PIGS IN TIKAPUR MUNICIPALITY, KAILALI, NEPAL

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LETTER OF APPROVAL

On the recommendation of supervisor Mr. Pitambar Dhakal, this thesis entitled **"Prevalence and risk factors of gastrointestinal parasites in pigs in Tikapur Municipality, Kailali Nepal"** submitted by Mr. Barshat Chaudhary is approved for the examination and submitted to the Tribhuvan University in partial fulfillment of the requirements for Master's Degree of Science in Zoology with special paper Parasitology.

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CERTIFICATE OF ACCEPTANCE

This thesis work entitled **"Prevalence and risk factors of gastrointestinal parasites in pigs in Tikapur Municipality, Kailali Nepal"** submitted by Mr. Barshat Chaudhary has been accepted 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 research work entitles **"Prevalence and risk factors of gastrointestinal parasites in pigs in Tikapur Municipality, Kailali Nepal"** 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

Pig *(Sus scrofa domesticus),* an important species of domestic livestock, is generally affected by helminth and protozoan parasites. This study was conducted to find out the prevalence of gastrointestinal in pigs reared in commercial and smallholder farms in Tikapur Municipality, Kailali, Nepal. A total 200 fecal samples were collected, among them 100 samples were from commercial farm and 100 samples were smallholder farms. The collected samples were examined to detect the presence of gastrointestinal parasite by direct wet mount, flotation and sedimentation techniques, and acid-fast stain was used for the identification of coccidian parasites. The parasites were identified morphologically under microscope. The result demonstrated the overall 86.5% samples were infected with one or more species of parasite. Three species of protozoan were recorded, namely *Eimeria* spp. (26%), *Entamoeba coli* (25.5%) and Coccidia (29%). Also, five species of helminthes parasite were identified like, Strongyles (66%), *Ascaris suum* (32.5%), *Trichusris suis* (30%), *Fasciola* spp. (17.5%), *Strongyloides* (17.5%).

Male farmers were mostly engaged towards pig rearing. There was significant difference between owner's education ($P<0.05$) and annual income ($p<0.05$) in prevalence of intestinal parasite in pigs of commercial farm and smallholder farms. The prevalence of gastrointestinal parasite in relation to style of rearing and gender of pigs were analyzed. Data revealed that there was no significant ($P>0.05$) difference in parasitic infection among commercial and smallholder farms. Similarly, no significant ($P>0.05$) different observed in infection rate between male and female pigs.

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LIST OF ABBREVIATION

GIPs	=	Gastrointestinal parasites
k.g.	=	Kilogram
k.m.	=	Kilometer
masl	=	Meter above sea level
NRs	=	Nepali rupees

1. INTRODUCTION

1.1 Background

Pigs are commonly called hogs or swine, which have been reared for source of food, leather and similar products since ancient time. More recently they have been used in biochemical research and treatment (Pam et al. 2013). The domestic pig (*Sus scrofa domesticus*), is reared in most parts of the world for the provision of biomedical raw material and pork. Globally, about 44% meat consumption in contributed by pork (FAO 2015). In most cases, the reasons for keeping pigs include the provision of protein/meat and manure for fertilizing the soil, particularly for farmers. However, swine could carry many intestinal pathogens which would hinder growth of pigs, leading to significant economic loss to the livestock industries (Joachim et al. 2001). Pigs have short generation interval, high fecundity rate and grow faster resulting in quick generation of cash for farmers. Pig farming does not require as much physical labor in handling, and pig keeping needs less land, hence women prefer to rear pigs highly (ILRI 2011). In addition, compared to ruminant productions, pig farming has the advantage of economy of space. Moreover, the ease in the marketability of pork in most parts of the world seems to guarantee speedy returns on any investment in the swine industry (Nwanta et al. 2011). Most of the pigs are produced under wasteful condition due to traditional smallholder systems. Due to poor final product, poor feed conversion and high mortality rate it is not as profitable as intensive production system (Lekule & Kyvsgaard 2003). However, for resource-poor farmers, the traditional pig production system is attractive, because it requires much less space (Phiri et al. 2003). Infection of pig with gastrointestinal parasite shown to be influenced by the type of pig management under practice and widely reported from all corners of the world and (Nansen & Roepstorff 1999) and their effect depends on the parasite burden and host response (Kipper et al. 2011). The gastrointestinal parasite prevalence is higher in adult pigs than in young pigs and higher in female pigs than in male pigs (Sah 2018). Pigs are a reservoirs of *Taenia solium*, and almost 50% peoples who rear pigs are infected by taeniosis, in Nepal (Rajshekhar et al. 2003). The clinical manifestations or symptomatology of helmithiasis may be similar, in both humans and animals. Mild or even heavy infections may give rise to malnutrition, diarrhea, dysentery, abdominal pain, emesis, in-appetence, thriftiness, general malaise,

tiredness, impaired cognitive development and growth retardation in pig. However, low gastrointestinal infection may not raise any type of clinical sign or symptoms (Ajibo et al. 2020). Chronic hookworm infections result in hemorrhage and decreased intestinal iron absorption due to the attachment sites of the parasite (Cross 1996).

Nepal is an agricultural country where most of the people (56%) are engaged in farming and animal husbandry. Thus, pig farming has a great potentiality in Nepal and exists as an additive source of income and uplifts the economic status of the farmers (Nansen & Roepstorff 1999, Chand et al. 2017). Nepal has about 1.1 million pigs, distributed throughout the country such as in the eastern (935,075), central (495,371), western (108,449), mid-western (126,172) and far western (47,853) (DLS 2005). Some of the ethnic (*Tharu*) in the plains (Taerai) region keep pigs as their cash crop/ property that could be sold in times of need, usually during festival (Joshi et al. 2007). Pig farming trend is increasing in Nepal due to reduced cultural biases against pig and new government programs to support pig farming as a low cost (Sah et al.,2018). The native or indigenous pig breeds of rural Nepal are;- Hurrah, Chwanche, Bampudke, synthetic pig breeds are;- Pakhribas Black, Nagpuri, and introduce breeds are;- Landrace, Hampshire, Saddle back (Gurung et al. 2014). Their feed mainly includes kitchen waste, green forages, taro plant (*Colocasia esculenta*), garbage, roots and locally available grains like rice bran, maize, husks, and others (Nidup et al. 2010).

Pork meat demand is increasing day by day in urban areas but pig farming practice is very poor, unhygienic and unscientific. But the poor social environment and unhygienic condition, affect their health (Sharma M. 2006). In Nepal, every year approximately 20,135 metric ton pork meat is produced (CBS 2016). However, GI parasitism induced by various species of protozoa and helminths parasites exists as a major hindrance regarding the pig industry and quantitative and qualitative meat production. GI parasitism may induce many health problems in the reared animals at their stages of production and growth (Roepstorff et al. 1998). The consumption of raw or under cooked pork can be a source of various zoonotic diseases including parasitic zoonosis such as trichinellosis, taeniosis, and giardiasis (Dorny et al. 2009). Regarding meat consumption in the non-vegetarian group, *Taenia* infection was found higher (6.5%) among pork eating than non-pork eating (4.7%) (Sah RB et al. 2012).

The disease in human and pig in developing countries are emerging as a major problem of global dimension (Sciutto et al. 2000). Infection is common in low socio-economic and poor sanitary areas of central Mexico and central and southern America. The infection is also present in India, Pakistan, North China, Thailand and Nepal (Schantz et al. 1992). Gastrointestinal parasites are common in pig and previous studies have shown that the coccidia (*Cystoisospora suis* and *Eimeria* spp.) as well as the nematodes (*Ascaris suum*, *Oesophagostomum* spp., and *Trichuris suis*) are the most common parasites to be found in pigs (Raue et al. 2017). However, many factors influence the parasite prevalence in pig, such as the housing facilities , type of flooring, and the use of bedding materials (Kochanowski et al. 2017). Pigs are generally slaughtered on slaughter slabs. According to the animal slaughterhouse and meat Inspection Acts, all slaughtered animals and meat should be examined by a meat inspector (Joshi et al.2004). However, the government of Nepal has so far been unable to implement these regulations (Joshi et al.2004).

1.2 Gastrointestinal parasite and disease in pigs

Parasites are organisms that live in hosts for survive. Some parasites don't noticeably affect their hosts. Others reproduce growth or invade organ systems that make their hosts sick, resulting in a parasitic infection (Marcin J. 2018).

In pigs, GI helminthes are always present, and their main effects are loss of appetite, reduction in daily gain, and poor feed utilization and potentiation of other pathogens. However, they rarely cause death. Adequate nutrition helps reduce the adverse effects of parasitism on feed efficiency and average daily gain (Ballweber L.R. 2016).

A. suum is the most common intestinal nematode of pig (Burrough 2021). Heavy infection causes emaciation. Adult nematodes in the intestine reduce feeding efficiency and impair absorption of vitamins A.

Eimeria spp. is common in pigs. Heavy infections may cause appreciable enterocolitis in young growing pigs. *Cyctoisospora suis* is a common and important causes of coccidiosis in piglets of 3-6 weeks old (Burrough 2021). Infection causes necrosis which leads to secondary bacterial infection of the injured intestinal mucosa. Mortality is of 20**%**-25%. *Trichuris suis* (whip worm) penetrate the mucosa of the

cecum and colon. It causes multifocal inflammation. Heavy infection causes diarrhea and emaciation, and become hemorrhagic. *Strongyloids ransomi* (thread worm) larvae can be transmitted via colostrum. Heavy infection in piglets may develop severe diarrhea when 10–14 days old, with high mortality rate (Burrough 2021).

The gastrointestinal parasitic infections are responsible for competing directly for nutrients required for optimum growth and reproduction, by poor feed conversion, loss of appetite, enterocolitis, multifocal inflammation, causing tissue injuries (lesions), diarrhoea and dehydration or even death of the animals, which may results the substantial loss on the efficiency of pig production.

In addition, zoonotic infections are also caused by some parasites. These include *Trichinella spiralis, Sarcocystis suihominis, Toxoplasma gondi, Ascaris suum, Taenia solium, Entamoeba polecki,* and *Fasciolopsis buski*. There is a wide range of transmission routes through oral, colostral, and percutaneous. The oral transmission can occur through ingesting embryonated eggs (*T. suis, A. suum*), cysts (*Entamoeba* spp.), or sporulated oocytes (coccidia), infective larva (Strongyle-type worms), items (aquatic plant) contaminated with metacercaria (*Fasciolopsis* spp.) (Roepstorff & Nansen 1998). The transmission of *Strongyloides ransomi* involves two pathways, either the suckling or ingestion of items or colostrum contaminated with infective larvae, or penetration of the host skin. Different factors can influence the prevalence of swine parasites including on farm hygiene, geographical location, system of management (intensive, semi-intensive, and extensive and anti-parasitic prophylactic treatment.

1.3 Gastrointestinal parasitic diseases

Most of the intestinal parasitic diseases are related to hygiene and sanitation of the community people. Most of them are transmitted through contaminated foods and drinks and some are transmitted through skin penetration mainly from contaminated soil.

Strongyloidiasis

Strongyloidiasis is a parasitic disease caused by a nematode parasite *Strongyliodes* spp. At the worldwide level, there is high uncertainty about the strongyloidiasis burden (Buonfrate et al. 2020). *Strongyloides* spp. affect approximately 100-370 million people globally (Eslahi et al. 2021). The infective stage of *Strongyliodes* spp. is L3 larva. The developmental cycle of this nematode consist of two pathways, i.e. direct and indirect.

In direct development, the infected L3 larva penetrates the skin and mucosa of the oral cavity (after ingestion) and migrates to the small intestine via lungs. Larva develops to parthenogenic female in the small intestine at 7-9 days. Larvae are arrested in until farrowing. Where after they, via colostrums, are transferred to the piglets in which is developing to parthenogenesis female in the small intestine in 4-5 days. Embryonated eggs are excreting along with feaces of pig, or lay by the free living females. Eggs are hatches in to 24-48 hour in a room temperature. After hatching the L1 larvae scape out from the egg and develop in to L1–L3 larvae (Thamsborg et al. 2016).Similarly in indirect development, the L3 larva develops in to free living larvae. Those larvae differentiate into the male and female parasite and start copulation. After mating, the female parasite laid eggs which develop in to L1 larva and finally develop in to L3 (Thamsborg et al. 2016).

Hyper infections also occur in which, eggs are hatched in the lumen of the bowel and develop in to L1 larvae. The L1 larvae penetrate bowel mucosa or perineal and perianal skin. These L1 larvae carried by circulation back to the intestine, where they changes into infective L3 larvae (Chatterjee K.D. 2019).

Ascariasis

Ascariasis is an infection caused by a parasitic worm *Ascaris suum* in pigs worldwide. *A. suum*, are mainly presents in the small intestine and transitorily in the large intestine during expulsion of the worms. The male and female make copulation and gravid female lays egg. The eggs are passed in faeces of pig and contaminate the soil. Transmission occurs by oral route through contaminated water, food and soil. In temperate region, the eggs stay dormant in winter ($<15^0$C) and resume development

when temperature rises in the spring. The eggs are highly resistant to chemical agents, but conditions with low humidity, heat or direct sunlight reduce their survival significantly. Under optimal conditions, egg may survive for 5-11 years (CDC 2019).

When the eggs are ingested, the larvae hatches in the intestine, penetrate the wall, and enter the portal circulation. After the short period in the liver, they are carried via circulation, where they pass through the capillaries into the alveolar. Approximately 9-10 days after ingestion, the larvae pass up the bronchial and are swallowed, and then return to the small intestine approximately 10-15 days after infection, where they mature into adult worms (CDC 2019).

About 53% pigs were infected by the *A. suum* (Boes et al. 1999). Infections of *A. suum* causes weight loss, unthrifty, difficulty breathing and slow weight gain. Which may causes the loss of production in pork.

Trichuriasis

Trichuriasis is a disease caused by the *Trichusis suis* in pigs. The lifecycle of *T. suis* does not require any intermediate host. J1 stage larvae are the infective stage of *T. suis*. The infective J1 stage within the egg is highly resistant and can remain in this form for several years in favorable conditions. They are mostly located in the cecum and proximal colon (Ballwweber L.R. 2015). Eggs are passed in feces from infected animals. Depending on the environmental temperature, infective J1 stage larvae develop within the shell in 3 weeks to 2 months. Once the infective J1 egg is ingested, the bipolar plugs are digested and the J1 larvae hatch in the small intestine and cecum. The J1 larvae penetrate the mucosa via the pits between villi in the distal ileum, cecum, and colon. During the next 5 weeks, the larvae undergo four molts (J2, J3, J4, J5) to the adult stage within the mucosal layers (Pittman et al. 2010).

T. suis, colonizes the small intestine and cecum resulting anorexia, muscoid to hemorrhagic diarrhea, dehydration and mortality in severe infection (Laber et al. 2002). It is transmitted through fecal-oral route, so the people and other vertebrates animal who make contact with infected animals are at high risk from the parasitic infection (Djurković-Djaković et al. 2013).

Fascioliasis

This fascioliasis shows a worldwide distribution due to the introduction of livestock almost everywhere. Fascioliasis is a zoonotic disease caused by *Fasciola hepatica* and *Fasciola gigantica* (Mas-Coma et al. 2009). The adults can live for 5–10 years in a mammalian host. They are commonly found in the bile duct of host. *Fasciola* pass through five phases in their Lifecycle which comprises of; - eggs, miracidium, cercaria, metacercaria, and adult fluke. Metacercariae is the infective stage of *Fasciola* spp (CDC 2019).

The eggs are passed through the feces of infected hosts. If the eggs are entering into freshwater, the eggs hatch into miracidia. The free swimming miracidia are free-swimming. The miracidia then infect the gastropod intermediate hosts and develop into cercariae, which released from the body of infected snail host and attach to aquatic plants. The cercariae then develop into metacercarial cysts. When these cysts are ingested by the host along with the aquatic plants by a host, they mature into adult flukes (CDC 2019). *Fascioal* spp. has two intermediate hosts i.e. mollusca and aquatic plant. When the aquatic plant with metacercariae are consumed by the animal. The metacercariae penetrates into the liver lobes, causing numerous circular greyish white lesions are situated in the liver parenchyma cell (Ross et al. 1967). Through the lesion in liver, the parasite enters into the bile duct can leads to anemia, inflammation, obstruction and cholangitis. So, this trematode may causes severe damage in liver and bile duct, which increase the mortality rate of infected animal (Lalor et al. 2021).

Ancylostomiasis

Ancylostomiasis is by species of hookworm throughout the globe. Adults attach to the jejunal mucosa by means of a large buccal capsule that lacks teeth or cutting plates. Young pigs are more likely to become anemic than older. However, clinical disease attributed to hookworms in pigs is rare (Holland & Kennedy 2002). Adults are about 7 mm in length and lay strongyle type eggs (52–56 × 25–35 μm). Infective third stage larvae (L3) develop in the environment and infect other pigs by ingestion or skin penetration (Holland & Kennedy 2002).

The life cycle of the hookworm is relatively simple, eggs are expelled throughout the faeces, hatch, resulting first stage larvae (L1), which then moult to become second stage larvae (L2), followed by third stage larvae (L3). The L3 larvae penetrate via the skin of the host. Then the larvae enter into the bloodstream and migrate through heart to the lungs. After that the L3 larva creep up the trachea and are swallowed, eventually residing in the small intestine as immature adult worms. In the gut they become mature (Gaze et al. 2012).

Coccidiosis

Coccidiosis is one of the economically important diseases for pigs, cattle, goat and sheep. It causes an acute invasion and destruction of intestinal mucosa. The major coccodian parasites are *Eimeria* spp., *Isospora* spp., *Cryptosporidium* sp., *Cyclospora* sp. Sporulated oocysts are infective stage for susceptible animals. When the sporulated oocysts are ingested by the host, the sporozoites escape out from the oocysts, invade the intestinal mucosa or epithelial cells in other locations, and develop schizonts. Each nucleus develops into merozoite (infective body) which; enters new cells and repeats the process known as reinfection (Constable 2016).

Some merozoites develop into either macrogametocytes (females) or microgametocytes (males). After being fertilized by a microgamete, the macrogamete develops into an oocyst. The oocysts have resistant walls and are discharged unsporulated in the feces. Oocysts do not survive well at temperatures below ~30°C or above 40°C (Constable 2016).Coccidian parasite may causes the diarrhea, fever, inappetence, weight loss, emaciation, and in extreme cases, death also. Coccidiosis causes the destruction of intestinal epithelium; this may be accompanied by hemorrhage into the lumen of the intestine, catarrhal inflammation (Gajadhar et al. 2015). Coccidian parasites are transmitted via fecal-oral route, so the animal or human at high risk of coccidian infection, whose make more with infected animal without proper precaution (Djurković-Djaković et al. 2013).

1.4 Objectives

General Objectives

To determine the prevalence and risk factors of gastrointestinal parasites in pigs in Tikapur Municipality, Kailali, Nepal.

Specific objectives

i. To determine the prevalence of intestinal protozoan and helminthes parasites of pig.
ii. To determine the associated risk factors of gastrointestinal parasites.

1.5 Rationale of the study

Pig rearing and slaughtering is one of the very common agricultural practices in Nepal. Pork consumers are at high risk with parasitic diseases. The gastrointestinal parasites are cause tissue lesion, diarrhea, dehydration, reduced weight, loss of appetite, weakness, lack of reproduction due to hormonal imbalance or even causes death of the animal, which may results a substantial loss on the efficiency of pig production (Adhikari et al. 2021). Cryptosporidiosis, acariasis, toxoplasmosis, trichinellosis, trichurissuis, giardiasis, taeniasis, ancylostomiasis, strongyloidiasis are the most important disease transmitted by infected pigs (CFSPH 2021). Pigs harbor various parasites such as *Trichinella* spp., *Taenia solium* and *Toxoplasma gondii* (Devleesschauwer et al. 2013) (Solaymani-Mohammadi & Petri 2006) which have been reported form Kathmandu valley. *Balantidium coli*, *Cryptosporidium* spp., *Giardia* spp., *Ascaris suum* and *Trichusris suis* are the parasites transmitting via faeco-oral pathway. Hence, the persons engaged in rearing pigs are also at high risk because they expose with the pigs and their faecal matter (Djurković-Djaković et al. 2013).

Tikapur Municipality is one of the potential animal rearing places. But the research carried in the field of parasitic infection related to pig is very scanty in Tikapur Municipality, Kailali. Researches carried out by Adhikari et al. (2021) in south-central Nepal, Subedi & Khanal (2020) in Chandraragiri Municipality Kathmandu, Adhikari & Ghimire (2019) in Shaktikhor Area of Nepal, Poudel et al. (2019) in Banke district,

Sah (2018) in Dhankuta and Sunsari district of Nepal, Joshi et al. (2007) in Kathmandu and Dharan of Nepal have contributed in documentation of parasitic diseases in pigs. Majorly, this type of research has been carried in eastern and mid region of Nepal. Earlier researcher mainly focused on the helminthes pig parasite and only little bit describe about the protozoan parasite. Therefore a careful attention has been laid to identify the coccidian parasite in this study along with the hemniths of zoonotic importance. The pig farmers and pork consumers are at high risk of parasitic infection through pigs. So the focus also needs to be underlined in the mode of transmission pathway of parasite from pig to human. Hence, selection of this research in Kailali, is associated to the lack of previous information regarding parasitic infection in pig and current scenario of pig rearing and management of slaughterhouse. Recently peoples are highly engaged towards pigs farming but in commercial level the large farms are not well managed. In village areas, many farmers' rear pigs in small scale but the management system is very poor. The owners are not aware about parasitic infection in pigs. So that it may pose zoonotic threats to the human community.

2. LITERATURE REVIEW

2.1 Intestinal parasite in pigs at global perspective

Permin et al. (1999) examined 259 fecal samples of pigs and reported that 93.4% of them excreted either oocysts or helminth eggs. The collected faecal samples were diagnosed through flotation method (normal saline) and sedimentation technique (formalin-ether). The parasitic infections differed due to management system of piggery, flooring methods, age of pigs, sex of pigs, climatic condition, and weight of pigs, pig's breeds, and feeding habitat. In case of protozoan parasites, three species were identified where *Eimeria* spp. (77.2%) contributed the highest prevalence followed by *Isospora suis* (27%) and *Balantidium coli* (19.3%). However, seven helminth parasites *Oesophagostomum* spp. (60.6%), *Metastrongylus salmi* (19.3%), *Physocephalus sexalatus* (17.4%), *Ascari ssuum* (12.7%), *Trichuris suis* (4.6%), *Paragonimussuis* (0.8%) and *Schistosomasuis* (0.4%) were reported.

In another study accomplished in Ibadan, Southwest Nigeria, out of the 271 pigs examined for intestinal parasitic infections, 97 were found infected with one or more parasite species, resulting an overall prevalence of 35.8%. The lower prevalence of gastrointestinal parasites in pigs recorded in this study because of effective management practices in the farms, daily cleaning and disinfectants of pens, giving high quality commercial feed and the use of effective antihelminthic drugs at the right time. Mainly five species of parasites were identified, and among them four were helminthes (*T. suis*, *A. suum*, hookworm, *S. dentetus*) and one was protozoan (*I. suis*) (Sowemimo et al. 2012). In this study, the parasitic infection was reported higher in piglets (<6 month) (37.2%) followed by adults (>12 month) (35.7%) and youngs (7-12 month) (32.4%). The parasitic load was recorded to be higher in males (45.0%) than in females (30.4%). The double infection (80.4%) was more prevalent than single infection (35.8%) and triple infection (2.1%) (Sowemimo et al. 2012).

Nonga and Paul (2015) recorded that 83% pigs were infected by gastrointestinal parasites in Tanzania. From 100 slaughter pigs, 300 intestinal content as samples were collected from different part of the intestine (i.e. small intestine, caecum and large intestine). The gastrointestinal helminthes infection rate was higher due to differences in environmental conditions, abundance of infected definitive hosts, stocking rate,

nature of the feed and feeding patterns of animals and inherent characteristics such as host immunity. Helminth (79.0%) and coccidian (19.0%) were common type of parasites. About 64% were infected with helminthes while only 4% infected with coccidian parasites, and 15% pigs were infected with both helminth and coccidian. According to gender wise prevalence in this study showed that, the parasitic prevalence was higher in male (56%) than female (44%), similarly adults (86%) were highly infected by gastrointestinal parasite than young (14%). Piglets were not slaughtered, so the samples of piglets were not included in this study.

Nur-E-Azam et al. (2015) in Bangladesh found that the overall prevalence of gastrointestinal parasitic infections (either single or mixed infection) was 65%. The parasitic occurrence was found to be depending on certain factors like geo-climatic conditions, sample size, breed, age, sex, plane of nutrition, stress, availability of intermediate host, vegetation, grazing pattern, rearing and husbandry measures, anthelmintic therapy, genetic resistance etc. Among different soil transmitted helmintha (STHs), the prevalence of *A. suum* was the highest (38%), followed by *S. ransomni* (20%). Among helminth parasitic infections, *A. suum* was highly prevalent in 6-12 months of old pig (45.24%) followed by pigs of less than 6 months ages (35.71%). *S. ransomni* infection was more common in all the age groups but more frequent in the age group less than 6 months and older pigs. Although, presence of protozoan parasites in all the age groups were less frequent but 7.14% coccidian infection was observed in 6-12 months of age. The same study shows that, female pigs were more susceptible to different species of gastrointestinal parasitic infection than male. However, prevalence of *A. suum* infection was the highest in female pigs (40.48%) followed by male pigs (36.21%). Because of female pigs are kept for longer periods due to their reproductive potential while the males are sold off. Prolonged rearing periods coupled with decreased immunity during gestation period may contribute to higher parasitic infection in females than male pigs.

Out of 390 pigs examined, the overall prevalence of gastrointestinal parasites was 54.97% and 45.19% in Addis Ababa area and Bishoftu area of Ethiopia respectively as, documented by Geresu et al. (2015). Similarly, the prevalence of gastrointestinal parasites in grower (65.85%) was higher, followed by adults (49.17%) and piglets (33.33%) recorded in same study. In Addis Ababa area, *A. suum* (18.5%) was more

prevalent followed by *F. hepatica* (15.2%), *Strongyloides* spp. (14.6%) and coccidian (13.9%). In Bishoftu area, *Strongyloides* spp. (17.2%) was more prevalent followed by *F. hepatica* (9.6%), *A. suum* (8.8%) and *T. suis* (8.4%). Among total gastrointestinal parasitic infection 11.28% and 37.69% pigs were found to harbor mixed and single infections respectively. In case of coccidian species which was highly prevalent in male pigs than in females but in case of helminth gastrointestinal parasite, female pigs were highly infected by GIPs identified in this study.

According to Dadas et al. (2016) in Mumbai, 135 pigs were examined, among which 69 were found to be infected with gastrointestinal parasite and the overall prevalence was 51.11%. With respect to species wise prevalence *A. suum* (32.59%) was found to be the most prevalent parasite followed by *B. coli* (31.85%), *T. suis* (11.11%), *I. suis* (1.48%) and lower prevalent species were *S. ransomi* and *Globocephalus urosubulatus* about 0.74%. The eggs of *A. suum* are thick-shelled for resistant to adverse environmental factors as well as chemicals and can maintain infectivity for long periods of time. On the basis of gender, the GIPs were found to be 56.71% prevalence in male and 45.58% in female. Prevalence of gastrointestinal parasites based on gender was found to be statistically non-significant. This may be because both male and female feed voraciously on garbage and have equal opportunity of getting exposed to parasitic infections.

In Madhya Pradesh, India, out of 455 faecal samples, 290 (63.74%) were positive for different gastrointestinal parasitic infections. *A. suum* were predominant (37.14%) followed by strongyles (27.47%), coccidia (22.86%), *Schistosoma incognitum* (19.12%), *B. coli* (15.60%), *Fasciolopsis buski* (15.16%), *Trichuris* sp. (5.05%), *Amphistome* (3.30%) and *Strongyloides* sp. (1.98%). The contribution of agro ecology and climatic condition plays an important role for the development and survivility of infective stage of gastrointestinal nematode. In this study, season wise prevalence of gastrointestinal parasitic infections in pigs were shown that the parasitic prevalence was higher in monsoon season (47.62%) followed by winter (18.84%) and summer (17.06%). This might be related that in monsoon season the parasite get the suitable macro and micro environment for development than in winter and summer seasons (Singh et al. 2017).

Kaur et al. (2017) examined that the total parasitic infection was 49.4% of total samples. The study conducted into 3 districts of Panjab and the parasitic infection was recorded higher in Hoshiarpur (64%) followed by Jalandhar (48%) and Ludhiana (43.9%). Due to varied climate factors at different geographical locations, unhygienic rearing practices, lack of proper garbage disposal systems and management practices could be attributed to the parasitic burden. On the basis of management system, the parasite load was found higher in scavenging pigs (50%) than in farm pigs 46.8% which indicated that the farm had better management system in comparison of scavenging. However, the piglets (<1month) get the better nourishment than young (>1month), which shows that the young had higher parasitic infection (56.5%) than piglets (39.6%). Same study shows that overall prevalence of *A. suum* was higher (27.5%) than that of *strongyloides* spp. (4.5%) and *T. suis* (1.8%).

In Abia state of Nigeria, out of 150 pigs from two farms and three slaughterhouses examined using formalin-ether sedimentation process, 42.7 % of them were infected as documented by Amadi et al. (2018). In this study, 93 samples were collected from 2 farms and 57 samples were collected from slaughter house in Abia state of Nigeria. The parasitic infection was found to be 24% and 18.66% in farm and slaughter house respectively. *A. suum* was most prevalent sp. (14.7%) parasitic infection followed by *Globocephalus* sp. (11.3%), *B. coli* (9.3%) and *Oesophagostomum* (2.7%). This study shows that the female (24.0%) pigs had higher parasitic infection than male (18.7%) pigs. Higher infection in the older pigs recorded in this study could be because of the older animals picking up more infection over time. In case of age related prevalence, the parasitic infection was recorded higher in adult (26%) followed by grower (10%) and piglets (6.7%).

To study about the influence of intestinal parasitic infection, stools were collected from 77 pigs owned by 16 household and diagnosed via using flotation technique and McMaster counting technique. The overall infection was 79.2%. The highest parasitic load was recorded in Botshabelo (91%), followed by ThabaNchu (72.7%) and Mangaung (66.7%). Botshabelo recorded the highest prevalence of *T. suis* (72.7%), while *Ascaris suum* was most prevalent in Mangaung (61.1%) and *Ascaris suum* was least prevalent in ThabaNchu (36.4%). The prevalence of coccidian parasites was high in all three farming locations. The parasitic prevalence differ because of various

factor like geographical and climatic conditions, pig breeds, farm management practices, the nutritional and health status of the pigs, method of sample collection and analysis. This study indicates that, the highest prevalence of intestinal parasites in female (86.7%) than male pigs (68.8%). However piglets recorded higher (88%) parasitic infection than adult pigs (68.8%) (Fourie et al. 2019).

Sharma et al. (2020) analyzed 839 faecal samples of pigs and among which 28.4% were found positive for one or more GIPs. *A. suum* was the most predominant species with a prevalence of 11.1%. The other parasites were recorded, coccidia (9.41%), *T. suis* (6.43 %), *B. coli* (4.5%), *Amphistome* (3.33%), strongyles (2.14%) and *Ascarops strongylina* (1.78%). The low weight of pigs or piglets had weak immune system in comparison to adults and growers, which may increase the chance of parasitic infection rate. The piglets were more infected (29.6%) than growers (28.9%) and adults (26.4%). There were no significant difference in parasitic infection between male (28.4%) and female (28.3%) which might be due to same type of food and treatment provided to both male and female. This study was recorded slightly different results in case of seasonality infection where the parasite prevalence was predominant in winter (46.3%) than in summer (28.4%) and rainy season (26.2%).

According to Okita et al. (2021), about 56.3% pigs were infected by GIPs in Makurdi area of Nigeria. Author, collected the 550 faecal samples were collected from Makurdi area on the basis of age and sex. The collected samples were diagnosed by using flotation method. Piggery management systems are often semi-intensive and this encourages their exposure to parasitic infections. Seven species of gastrointestinal parasites such as *A. suum* (24.3%), *S. ransomi* (10.2%), *Oesophagostomum* sp. (5.4%), *T. suis* (4.6%), *T. solium* (2.0%), *F. buski* (0.3%) and *G. lamblia* (0.2%).

2.2 Intestinal parasite of pig in context to Nepal

Owing to illiteracy, unhygienic living habits, poor socio-economic conditions, and conservative types of treatment done by traditional healers (Dhami and Jhankri) many pigs in study areas were the victims of different types of disease. Postmortem survey at slaughter houses in Kathmandu and Dharan municipality showed that 14% (34/250) of pigs were positive for cysticercosis of *T. solium* while in Syangja district lingual examination and serological tests respectively identified 32% (136/419) and 24%

(48/201) pigs positive for cycsticercosis of *T. solium* (Joshi et al. 2004). In another study, (Joshi et al. 2007) examined 250 slaughtered pigs, of which 13.6% were found positive for cysticercosis. The results showed that, sex-wise prevalence rate of infection was higher in female (24.05%) pigs than in male pigs (8.77%). The occurrence of cysticertosis among pigs slaughtered in Kathmandu is mainly because of supply of already infected pigs from rural areas of Nepal and India to Kathmandu. In another study conducted in Kathmandu valley out of 742 pigs examined serologically, 13.8% were infected by *T. solium* cysticerci which was higher followed by *Toxoplasma gondii* (11.7%) and *Trichinella* spp. (0.1%). This study found that people were at high risk of infection consuming raw or undercooked pork. There were mainly three most common parasites like *Trichinella* spp., *T. solium* cysticerci and *T. gondii* transmitted through pork consumption (Devleesschauwer et al. 2013).

Sah et al. (2018) recorded that pigs were suffered from different type of parasites in Dhankuta and Terhathum area. The pigs were kept in smallholder conditions. Dairrhoea was the most common disease recorded from that area. Almost 85% pigs were suffering from diarrhea followed by other diseases like gastroenteritis (81%), piglet's morbidity (76.5%), parasitic infestation (60%) and abortion (34%). This study concluded that, the major risk factors were housing system, materials and the feeding system of the pigs.

Overall parasitic prevalence was of 51.4% as documented by Sah (2018) form Dhankuta (28.51%), Murtidhunga (23.67%) and Inaruwa (6.16%). The prevalence of strongyle nematodes (27.3%) was more predominant than *A. suum* (19.8%) and *T. suis* (4.3%). There was higher prevalence of gastrointestinal parasites in female (56.3%) pigs than male (46.8%). Regarding age, the parasitic load was higher in adult (55.7%) than young (41.5%). The pasture infectivity could be higher in the monsoon season when the temperature and moisture levels are the most favorable for nematode development stages and farmers feed these infective grasses to pigs. Hence, parasitic infection was dominant in winter season (60.4%) followed by rainy season (49.8%) and summer season (40.1%) reported by this study.

Adhikari & Ghimire (2019) collected 10 fresh fecal samples of adult pigs from Shaktikhhor area of central Nepal, diagnosed via direct wet mount, sedimentation,

flotation, and acid fast techniques. Overall result shows that, 100% pigs were positive for one or more parasites. Regarding protozoan parasite, *Entamoeba* spp. (90%) was more prevalent followed by *Eimeria* spp. (70%), *B. coli* (50%) and *Cryptosporidium* spp. (40%) and regarding helminthes parasite, Strongyle (70%) was predominant species followed by S*strongyloides* spp. (60%), *Trichuris* spp. (40%) and *Trichostongylus* spp. (30%). The wet season provides a suitable environmental condition, for the survival and multiplications of parasites and difference in geography, management pattern and the sample size might be the causes of variation in parasitic burden. Total 50 pigs of different age groups were considered for examination of gastrointestinal parasite from Khumaltar, Lalitpur. The result of that study showed that a high prevalence of *A. suum* (60%) and strongyle (40%). The female and young groups of animals showed remarkable symptoms of parasitic infections compared to the male ones (Baskota & Shrestha 2019).

In another study from Kathmandu valley shows that overall 88.57% samples had positive results with parasitic infection. Among them, three protozoan and five helminths parasites were recorded. Protozoan parasites include *Eimeria* spp. (42.8%)had higher prevalence rate than *B. coli* (23.80%) and *Isospora suis* (7.61%) and whereas, helminths parasites include *A. suum* (38.09%) had higher prevalence followed by, *Strongyloide* sp. (14.28%), *Fascilopsis buski* (9.52%), *T. suis* (7.61%), and *Schistosoma suis* (4.76%). The differences in parasitic prevalence include different factors such as presence of reservoir hosts, presence of different vector as an intermediate host, husbandry practice and environmental factors. This study was conduct in three areas Kathmandu valley, out of that Kanchan Basti (95.55%) had higher prevalence of gastrointestinal parasite followed by Bishnu Devi (91.42%) and Balambu (72%). There were two type of infection, in which single infection (73.33%) had dominant than mixed infection (15.23%). Regarding, sex-wise prevalence, due to alternation of physiological conditions of the female during pregnancy, lactation and parturition causes the hormonal imbalance in female, which results that the parasitic infection in females (91.66%) had higher than males (87.65%) (Subedi & Khanal 2020).

Adhikari et al. (2021) recorded that, total 91% pigs had gastrointestinal parasitic infection in south-central area of Nepal. Many factors such as age, sex and breeds of

the pigs and their immune system, the diversity in the sampling season and husbandry practices, variation in sample size and the laboratory techniques for faecal analysis, causes the variation in parasitic burden in pigs. Protozoan parasites were highly (89%) prevalent in the pigs than helminthes (75%). Eight species of porotozoans *Entamoeba* spp.(61%), *Eimeria* spp. (47%), *B. coli* (28%), *Cystoisospora* sp. (21%), *Entamoeba coli* (11%), *Cryptosporidium* sp. (10%), *Idoamoeba butschlii* (8%), and *Giardia* sp. (7%) and six species of helminthes *Ascarid* spp. (45%), strongyles (32%), *Trichuris* spp. (30%), *Strongyloide* ssp. (23%), hookworm (20%) and *Fasciola* sp. (9%)] were recorded. In this study the overall prevalence of gastrointestinal parasites was higher in male pigs (95.7%) than the females (87%).

3. MATERIALS AND METHODS

3.1. Study area

This study was carried out in Tikapur Municipality located in Kailali distric, Sudurpachim Province. It lies in Tarai region at 205masl and extends within 28.50995^0 N, 81.1086^0 E with an area of 122.12 km^2. It is situated 14km south from Mahendra highway and 14km North from India. Divided in to nine wards this municipality is surrounded by Karnali River in East, Janaki Rural Municipality in North, India in South and Bhajani Municipality in West. Agriculture is the main occupation of the inhabitants of this region and it includes animal rearing (pig, goat, and buffalo), floriculture, and apiculture.

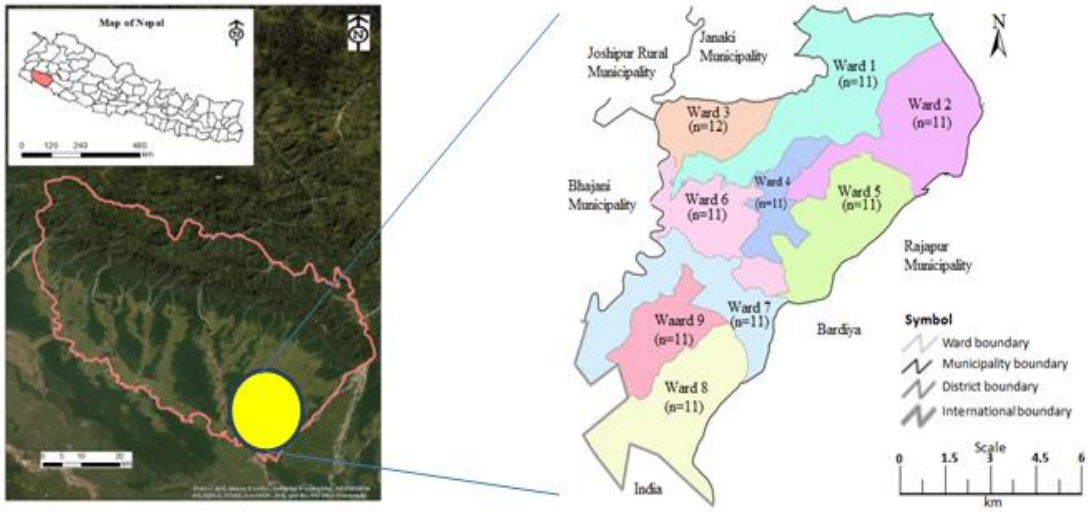

Figure 1. Nine wards of Tikapur Municipality, Kailali. Here, (n) represents the number of samples collected from the study wards.

3.2 Methods

Duration of the study in the pig intestinal parasitic infection was of one year started from April 2021 to March 2022 from sample collection to analysis. The prevalence rate of intestinal parasite in pig was studied in terms of their socio-economic, cultural and educational status. It was studied with the help of questionnaires and by direct field observations. The rate of parasitic infections was determined by the examinations of the fecal samples under compound microscope followed by

Morphometric analysis. The photographs of helminth larvae, eggs and trophozoids, cysts and oocysts of protozoan were taken.

3.3 Study population

As witnessed in the filed survey and discussion to the local farmers, the total numbers of pig populations was estimated to be 900 in Tikapur Municipality. A total of 200 faecal samples were obtained for the copromicroscopic study of intestinal parasites in pigs. Of the 100 samples from smallholder farms, 11 samples were collected from each ward and one extra sample was collected from ward number three while other 100 faecal samples were collected from commercial 10 farms. In these study 123 female and 77 males pigs were included. The lowest age of pigs was 1 month whereas the highest age of the pigs was 30 months. Personal hygiene, sanitary system, waste disposal system of pigs in both smallholder and commercial farms were directly observed and assessed. Risk factors were measured via questionnaire with farm owner and other workers.

3.4 Data analysis

Data analysis was done by using IBM SPSS version 20 software. According to the nature of data, multiple regression and T-test was used for analysis.

3.5 Sample size

Following formula was used for calculating a sample for proportions:

For populations that are large, Cochran developed the formula to yield a representative sample for proportions (Israel 1992, Pourhoseingholi et al. 2013).

$$n_o = \frac{Z^2 P (1-P)}{d^2}$$

Where, Z = the statistic corresponding to level of confidence.

P = Expected prevalence

d = Precision

n_o = Sample size

Therefore, confidence level = 90%

So, the corresponding value of Z = 1.64

P = 50% = 0.5

d = 5% = 0.05

According to formula,

$$n_o = \frac{Z^2\, P\,(1-P)}{d^2} = \frac{(1.64)^2\,.\,(0.5)\,.\,(1-0.5)}{(0.05)^2} = \frac{(1.64)^2\,.\,(0.5)\,.\,(0.5)}{(0.05)^2} = 269$$

Finite population correction for proportions

If the population is small then the sample size can be reduced slightly. The sample size (n0) can be adjusted using equation.

$$n = \frac{n_o}{1+\frac{n_o-1}{N}}$$

Where, n = sample size

N = Population size

For pilot survey,

N = 900

So, $n = \frac{n_o}{1+\frac{n_o-1}{N}} = \frac{269}{1+\frac{269-1}{900}} = 207$

There for, the sample size is 207 ≈ 200

3.6 Fecal sampling collection

Opportunistic sampling method was used for collection of stool samples from pigs during October to December, 2021. A prior written consent was taken from each farm owners and they were informed in detail regarding the aims and importance of the study. They were also informed that they had rights to either support for sample collection or withdraw their consent for the same. The body weight of the individual pig was recorded with the help of digital weighing machine and waited until defecation. Fresh fecal samples were collected from the ground immediately after defecation in the sterile containers with the help of sterile wooden spatula and preserved in 2.5% potassium dichromate solution until laboratory analysis. All the fecal samples were macroscopically examined for their consistency and if any adult worms were present. The samples were collected in the morning generally from 8 to 10 am when the farm owners and other farm workers were cleaning the animal farms. None of the animals were harmed in the process of sampling. While visiting the pig

farms, questionnaire survey was, conducted with all the pig-farm owner and workers involved in animal care.

3.7 Laboratory examination of fecal samples

After the collection of samples, they were safely carried out to parasitology laboratory at Central Department of Zoology (CDZ), Kritipur for microscopic examination. The parasite cysts, oocysts, egg and larva were identified on the basis of morphometric characteristics using Olympus MD-06 (M4000D) series of biological compound microscope.

3.7.1 Direct wet mount

In this method, small portion of stool sample was taken on two clean glass slides; one was mounted with normal saline and another with 1% Lugol's iodine solution and observes under the microscope. Iodine mount was basically used to determine the nuclear structure of protozoan parasite (Mengist et al. 2018).

3.7.2 Floatation technique

Saturated solutions of sodium chloride (specific gravity 1.20) was used as a floatation medium. This method was mainly applied for the detection of helminthes egg and protozoan cysts. Almost 3 ml of well-mixed fecal suspension was strained into a test-tube and each test-tube was filled with the saturated salt (NaCl) solution to developed convex surface at the top of the test-tube. The clean glass slide was placed at the top of the test-tube just to touch the convex layer for 1-2 hours and then transferred to microscope for observation (Scott & Stockham 2013, Prakash et al. 2018).

3.7.3 Sedimentation technique

The formalin-ether sedimentation technique was widely used for concentrating eggs, larvae, and cysts in fecal specimens. Approximately 10 ml of the well-mixed fecal suspension was strained into a 15-ml conical centrifuge tube and centrifuged for 1-2 minutes at 1000 rpm. Decanted supernatant, added 10ml fresh water and centrifuged again for 1-2 minutes at 1000 rpm. A 10 ml amount of 10% Formalin was added to the tube and thoroughly mixed with the sediment. After that, 3-ml of diethyl ether was

added in above mixture. Over all mixture, shaken vigorously, and then centrifuged for 2 minutes at 1000 rpm. Usual four layers resulted: solvent, a plug of debris, Formalin, and sediment. The debris was removed with an applicator stick, and decanted the rest of fluid. The settled sediment collected with the help of pipette and place on clean glass slide and then observed under microscope (Young et al. 1979, Foreyt 2013).

3.7.4. Acid-fast staining

This technique was introduced by Franz Ziehl and Friedrich Neelsen and it is also known as Ziehl-Neelsen technique. A thin smear was prepared on clean glass slide, allowed it to air dry and fixed by gentle heating over a burning flame. The smear was flooded with carbolfuction stain and heated to steaming for five minutes with low flame. It was then left for 5 minutes without heating and washed in running tap water. The smear was subjected to acid-fast decolourizer for 2 minutes (i.e. until no more stain comes off in the washing). After this the stained smear was washed with water, gently covered with counter stain methylene blue for 30 second, washed with tap water, dried in air and examined under the microscope at 100x objective lens (HML 2021).

4. RESULTS

The result shows that most of the owners were male (56.5%) with the mean age of 34.64 years, and their education level ranges from illiterate to high school graduates. The mean annual income of owner was about 40000 Nepali rupees. In this study most of the pigs were female (61.5%). The age of the pigs under study ranged from 1 to 30 months with mean weight of pig is 47 kg.

Table 1. Characteristic features of the pig owners and pigs (n = 200).

Feature	Number (%)	Range
Farming style		
Commercial farm	100 (50%)	
Smallholder farm	100 (50%)	
Gender of pigs		
Male	77 (38.5%)	
Female	123 (61.5%)	
	Mean (SD)	Range
Age of pigs (in month)	7.91 (5.35)	1 – 30
Weight of pigs (in kg)	47 (29.3)	5 – 120
Gender of owners		
Male	113 (56.5%)	
Female	87 (43.5%)	
	Mean (SD)	Range
Age of owners (in year)	34.64 (7.84)	19 – 56
Owner's education owners	5.49 (5.03)	0 – 13
Annual income owners (NRs)	38735 (22293.04)	12,000 – 1,00,000

This result shows differences in socioeconomic, socio-demographic characteristics. There was no difference in age and gender of pig or their owner between different styles of pig rearing. Yet, owner of commercial pig farms was more educated with higher annual income than the owner of home farm.

Table 2. Difference in characteristic between Farm and Home (n = 200)

Characteristics	**Commercial farms (n=100)**	**Smallholder farms (n= 100)**	**P-value**
Gender of owners			
Male	60 (60%)	53 (53%)	0.318
Female	40(40%)	47 (47%)	
Gender of pigs			
Male	42 (42%)	35 (35%)	0.309
Female	58 (58%)	65 (65%)	
	Mean (SD)	Mean (SD)	
Age of owner (in year)	34.1 (6.31)	35.18 (9.12)	0.332
Owner's education	**6.9 (4.97)**	**4.07 (4.70)**	**0.000**
Annual income	**565000 (18535.79)**	**20970 (4210.26)**	**0.000**
Age of pigs (in month)	7.8 (5.47)	8.03 (5.25)	0.757

The shows prevalence of intestinal parasites in pig from farm and home setting of rearing. A total of 173 faecal samples (86.5%) were found shedding one or more species of gastrointestinal parasites (Table 3). Of the twelve species of parasites identified, three species belong to protozoa, eight species to nematodes and one species to trematodes. Overall, Strongyle spp. (66%) has higher prevalence followed by *Ascaris suum* (32.5%) *Trichusris suis* (30%), *Coccidia* (29%), *Eimeria* spp. (26%), *Entamoeba coli* (25.5%), *Fasciola* spp. (17.5%), *Strongyloides* (17.5%). Style of rearing like commercial farm or home farm contributed significantly to prevalence of intestinal parasites. For example, in home farm pigs exhibited slightly higher prevalence of *E. coli* than commercial farm (Chi square $p<0.05$).

Table 3. Prevalence of gastrointestinal parasites in pigs by style of rearing (n = 200)

Parasite groups	**Parasite species**	**Commercial farm (%) n = 100**	**Smallholder farm (%) n = 100**	**P value**
Amoeba	***Entamoeba coli***	**18**	**33**	**0.015**
Coccidia	*Eimeria*	20	32	0.053
	Coccidia (in stained smear)	28	30	0.755
Nematodes	Strongyle	68	64	0.55
	Strongyloides	17	18	0.852
	Ascaris suum	31	34	0.651
	Trichuris suis	28	32	0.573
Trematodes	*Fasciola*	17	28	0.063
Total infection*		**2.27**	**2.71**	**0.034**
Any infection		84	89	0.301
Any protozoan		51	64	0.063
Number of protozoans*		**0.66**	**0.95**	**0.013**
Any helminth		83	89	0.753
Number of helminths*		1.61	1.76	0.275

*T-test

Prevalence of gastrointestinal parasites in pigs in commercial farm and smallholder farms setting based on gender of pigs has been depicted in Table 4. There was no difference in prevalence of most of intestinal parasites between styles of rearing like commercial farm or smallholder farms. Only statistical significance difference has been recorded in any protozoan infection between male and female (p = 0.032).

Table 4. Prevalence of gastrointestinal parasites in pigs by style of rearing and gender of pigs (n = 200).

Parasite groups	**Parasite species**	**Commercial farms (%) n = 100**			**Smallholder farms (%) n = 100**			**Total (%) n = 200**		
		M	**F**	**P-value**	**M**	**F**	**P-value**	**M**	**F**	**P-value**
Amoeba	*Entamoeba coli*	6 (6%)	12 (12%)	0.41	10 (10%)	23 (23%)	0.48	16 (8%)	35 (17.5%)	0.22
Coccidia	*Eimeria*	8 (8%)	12 (12%)	0.83	9 (9%)	23 (23%)	0.32	17 (8.5%)	35 (17.5%)	0.31
	Coccidia (In stained smear)	14 (14%)	14 (14%)	0.31	10 (10%)	20 (20%)	0.81	24 (12%)	34 (17%)	0.59
Nematodes	Strongyle	26 (26%)	42 (42%)	0.26	24 (24%)	40 (40%)	0.48	50 (25%)	82 (41%)	0.8
	Strongyloides	8 (8%)	9 (9%)	0.64	7 (7%)	11 (11%)	0.70	15 (7.5%)	20 (10%)	0.56
	Ascaris suum	14 (14%)	17 (17%)	0.66	10 (10%)	24 (24%)	0.40	24 (12%)	41 (20.5%)	0.50
	Trichuris suis	11 (11%)	17 (17%)	0.73	14 (14%)	18 (18%)	0.20	25 (12.5%)	35 (17.5%)	0.54
Trematodes	*Fasciola*	6 (6%)	11 (11%)	0.53	11 (11%)	17 (17%)	0.57	17 (8.5%)	28 (14%)	0.91
Total infection*		35	49	0.49	31	58	0.107	2.44	2.52	0.713
Any infection		35	49	0.877	31	58	0.920	66	107	0.797
Any protozoan		19	32	0.327	18	46	0.055	**37**	**78**	**0.032**
Number of protozoans*		19	32	0.106	18	46	0.185	0.74	0.58	0.41
Any helminth		34	49	0.643	31	58	0.920	65	107	0.609
Number of helminths*		34	49	0.91	31	58	0.290	1.7	1.67	0.85

*T-test

Parasite prevalence is always related to certain factors. Presence of any protozoan infection was higher in female pigs then in male pigs. Results indicate that the prevalence of *Ascaris suum* was significantly high among pigs reared by male owners ($p < 0.05$) but prevalence was lower among family with higher annual income ($p < 0.05$). Further, the prevalence of *Ascaris suum* in pig was significantly associated with weight of pig ($p<0.001$). However, the prevalence of *Fasciola* spp. was higher among pigs raised by female owners ($p = 0.002$) and not associated with income or weight of pig. Regarding overall infection, presence or burden (number of helminth) of parasitic infections or helminthiasis was negatively associated with weight of pigs after adjusting age of owners, gender of owners, owner's education, and family annual income, age of pigs, gender of pigs, rearing practice. As expected, presence or burden of intestinal parasites associated with reduced weight of pigs after adjustment with contextual factors and covariates like owner's education, family annual income, age of pigs, gender of pigs, and rearing practice.

Table 5. Factors associated with gastrointestinal parasites (n = 200). Only significantly associated factors are shown in the table.

Categories		Model		ß	95% CI	p-value
		Adjusted R^2	P-value			
Total infection	Weight of pigs	0.013	0.000	-0.439	-0.039 to -0.005	0.013
Any infection	Weight of pigs	0.106	0.000	-0.576	-0.011 to -0.002	0.002
Any protozoan	Gender of pigs	0.038	0.044	0.174	0.029 to 0.325	0.02
Any Helminth	Weight of pigs	0.102	0.000	-0.55	-0.011 to -0.002	0.003
No. of helminthes	Weight of pigs	0.257	0.000	-0.533	-0.029 to -0.007	0.002
Ascaris suum	Gender of owner	0.388	0.000	-0.134	-0.245 to -0.009	0.034
	Annual income			-0.219	0.000 to 0.000	0.046
	Weight of pigs			-0.692	-0.015 to -0.006	0.000
Fasciola spp.	Gender of owner	0.083	0.001	0.231	0.066 to 0.322	0.003

*Model adjusted for age of owners, gender of owners, owner's education, and family annual income, age of pigs, gender of pigs, rearing practice and weight of pigs as indicator of nutritional status.

Similarly the weight of pigs was significantly associated with overall prevalence and burden of parasitic infection and helminth infection. For example, total number of infections, any infection, any helminth infection, number of helminthes infection, *Ascaris suum* infection were associated with weight of the pigs after adjusting age of pigs, gender of pigs and rearing practices in the model.

Table 6. Factors associated with the weight of pigs. Only significantly associated result (n = 200).

Categories	Model*		ß	95% CI	p-value
	Adjusted R^2	P-value			
Total number of parasites in pigs	0.87	0.000	-0.072	-2.54 to -0.328	0.011
Any infection presents in pigs	0.872	0.000	-0.084	-11.652 to -2.681	0.002
Any helminths present in pigs	0.872	0.000	-0.080	-11.166 to -2.336	0.003
Number of helminth parasites in pigs	0.873	0.000	-0.093	-4.510 to -1.085	0.001
Prevalence of *Ascaris suum*	0.877	0.000	-0.129	-11.77 to -4.29	0.000

*Model adjusted for age of pigs, gender of pigs and rearing practices. All other parasites were evaluated for the association and were not significantly associated with weight of pig in adjusted model.

5. DISCUSSION

This study investigated the prevalence GIPs among pigs reared in smallholder and commercial farms in Tikapur Municipality, Kailali, Nepal. Prevalence of GIPs in our study (i.e., 86.5%) is comparable with prevalence of GIPs in the pigs reported form different study of Nepal such as that of 88.57% reported by (Subedi & Khanal 2020), (91%) in Central Nepal repotted by (Adhikari et al. 2021) and studies reported from other part of world like 83% in Tanzania reported by Nonga & Paul (2015). Yet, prevalence reported in our study is slightly lower than reported prevalence of 100% by Adhikari & Ghimire (2019) from Shaktikhhor Area, Chitwan and 99% prevalence reported by Widisuputri et al. (2020) from Indonesia. In contrast, prevalence observed in our study (i.e., 86.5%) is higher than the reports from Karnataka (64.6%); (Murthy et al. 2016), Punjab (49.4%); (Kaur et al. 2017), Brazil (51.86%); (Pradella et al. 2020), and Rajasthan (56.65%); (Yadav et al. 2021). Such a discrepancy in the prevalence of GIPs might be due to the difference in the climatic conditions, breeds (Subedi & Khanal 2020), rearing condition, different level of awareness (Adhikari et al. 2021). High prevalence of GIPs in our study subject may be partially explained by practice of open defecation with easy access of pig, lack of clean water, and poorly managed pig housing (Based on PI BC personal observation) like rearing pigs of different ages together, infrequent removal of dungs, non-disinfection of equipment, lack of routine deworming program. The pattern and severity of parasitic infection can be attributed to the environment and conditions in which animals are kept (Maganga et al. 2019), and poor routine treatment (Omoruyi & Agbinone 2020).

Most of the GIPs found to be almost equal among males and females pig overall or while with different rearing styles (Table 3) which is similar to other reports. (Njoga 2017; Amadi et al. 2018; Okoli et al. 2018; Patra et al. 2019; Subedi & Khanal 2020). Yet females pig indicated higher protozoan infection than male pigs. In contrary, rearing style did not alter such parasitic burden. Several factors such as hormonal imbalance, parturition, lactation and stress usually alter the physiologic state of female pigs leading to suppressed immunity (Fourie et al. 2019). In most of the farms female pigs are kept much longer for breeding purposes (Sah 2018) which may dwindle their immunity during gestation period (Okita et al. 2021). This may explain

such discrepancy in infection, but further study needs to be conducted to confirm this association.

Parasitic prevalence in our study was associated with rearing style pig (Table 4). Interestingly, *E. coli* and number protozoans were higher in home farm compared to commercial farm. Differences in educational status and annual income between owner of home farm and commercial farm may explain such discrepancy. Mostly pigs from commercial farm remained in captivity while pigs from home farm are semi free range. Further, pigs from commercial farms were better managed in term of water, food periodic treatment while lower financial ability with lower education/ awareness, home farm were provided with mostly kitchen waste and garbage and lack of proper water supply which many contribute higher *E. coli* as well as number of protozoans. Although difference in prevalence did not achieve statistical significance, most of the studied parasites were higher in home farm compared to commercial farm.

Regarding helminths, *Ascaris suum* was reported among 32.5% of the samples under investigation. The prevalence rate was lower than documented by Adhikari et al. (2021) from Nepal (45%), and Rajasthan, India (59.7%) reported by Yadav et al. (2021). Yet, prevalence rate in this study is higher than those from Nigeria (0.7%) (Abonyi & Njoga 2020), Indonesia (20%) (Widisuputri et al. 2020) and Greek (3.7%) (Symeonidou et al. 2020). In our findings shows that, the prevalence rate of *A. suum* was lower in case of female owners, it might be due to females were sensitive towards the sanitation of pigsties. They were usually engaged in cleaning of pigsty and try to improve feeding of pigs than male. Further, as shown by multiple regression adjusted for age of owners, gender of owners, owner's education, age of pigs, gender of pigs, rearing practice, as expected family annual income, weight of pigs as indicator of nutritional status were negatively associated with prevalence of *A. suum* which is obvious as better income (resource) or nutritional (immunity) status can downplay possibilities of helminth infections.

T. suis (30%) was second most prevalent parasite among the pigs which was similar to the finding from Nepal (30%) (Adhikari et al. 2021). Our prevalence report of this parasite was lower than the finding from Africa (50.6%) (Fourie et al. 2019) and higher than those reported from Greek (2.5%) (Symeonidou et al. 2020), Nepal (7.61%) (Subedi & Khanal 2020) and Indonesia (20%) (Widisuputri et al. 2020).

Strongyloides spp. was recorded in 17.5% of our samples. This rate was lower than the findings from Indonesia (18%) (Widisuputri et al. 2020), Bangladesh (20%) (Nur-E-Azam et al. 2015) and Hriyana (28%) (Vohra 2021) and higher than the results from Nepal (14.28%) (Subedi & Khanal 2020), Mumbai (0.74%) (Dadas et al. 2016) and Ethopia (16.2%) (Geresu et al. 2015). Similarly, the 26% prevalence of hookworm, observed in our study was higher than the documented by (Adhikari et al. 2021) from Nepal (20%), and (Sowemimo et al. 2012) from Nigeria (8%). *Oesophagostomum* (5.5%) was the least reported nematode. However, the rate of *Oesophagostomum* was lower than the findings from Indonesia (8%) (Widisuputri et al. 2020), Africa (26%) (Fourie et al. 2019) and Nigeria (11%) (Nathaniel et al. 2017) and higher than the results from Greek (1.4%) (Symeonidou et al. 2020) and Nigeria (2.7%) (Amadi et al. 2018).

Our result indicated that, more males engaged towards the pig rearing than females in Tikapur, Municipality though there is no statistical significance ($p = 0.318$). In commercial and home farms, the role of gender was seen in terms of the ownership, were dominated by man. Similar finding was also reported by (Ropa 2001). Woman was taking part in particular works, such as cleaning pigsty and feeding as illustrated by Saragih & Iyai (2015). In addition, the age of owner was not associated with pig farming system in line with observation by Saragih & Iyai (2015). Compared to home farm, commercial farm indicated better medication practice. At least pigs were treated when any disease symptoms occurred in pigs. Generally, owners called the veterinarian and treat the only those pigs which occurred symptoms which is less common in home farm. Difference in annual income and educational status may explain such discrepancy.

The weight and gender of pigs as well as gender and annual income of pig owners were significantly associated with the prevalence of GIPs in pigs. Wight of pig (proxy indicator of nutritional as well as better immunity) was consistently associated with our most of the outcome variables (different or as a whole parasitic prevalence or burden) considered in multivariate model even adjusted for age and gender of pigs, rearing practices. It may be due to weak immune system of pig associated with low weight as indicated by Sharma et al. (2020). Another possible explanation: piglets mostly left free ranging in home as well as commercial farm. Such free range of

young piglets increase the chance of infection via exposure with sewage, poor environmental sanitation observed around the study area or other infected animal and fomites (Conteh & Gogra 2019, Maganga et al. 2019).

Only trematode examined in this work i.e., *Fasciola* spp., indicated 17.5% prevalence which is higher than those from Nepal (9%) (Adhikari et al. 2021) or Ethiopia (11.18%) (Geresu et al. 2015) but lower than Bangladesh (66.27%) (Sarker et al. 2016) and North-Eastern region of India (20.55%) (Patra et al. 2019). The aquatic plant consuming pigs can more acquire by Fasciola infection (Nansen et al. 1972). In our finding showed that, the infection of *Fasciola* spp. was higher among female owners. Authors observed consistent practice in the study area. Most of the time, if farm owned/ managed by female owners, they mostly provide the aquatic plant and grass as a food of pigs compared to male owner which may harbor aquatic intermediate host like snails for trematode infections. However, future research should explore such possibilities.

In case of protozoan parasite, coccidian spp. was reported 29% of our total sample. The prevalence rate was lower than reported Africa (72.7%) (Fourie et al. 2019) and higher than reported from (Greek 6%) (Symeonidou et al. 2020), and Hariyana (14%) (Vohra 2021). Similarly, 26% of samples were infected from *Eimeria* spp. which was lower than finding from Nepal (42.8%) (Subedi & Khanal 2020) and Indonesia (78%) (Widisuputri et al. 2020) and higher than finding from Nigeria (3%) (Sowemimo et al. 2012). *Entamoeba coli* (25.5%) were least prevalent parasite in our study. Yet the prevalence rate was higher than prevalence rate (0.7%-3%) reported from Nigeria (Nathaniel et al. 2017, Amadi et al. 2018). Study by Adhikari et al. (2021) from Nepal (47%) and (Widisuputri et al. (2020)) from Indonesia (99%) reported much higher prevalence rate than observed in our study. Such variations in infections rate might be due to water supply system, climatic condition, different husbandry management practices, general health status of the sampled pigs and pig breeds.

Pig being infected with parasites or burden (total number of infections, any infection, any helminth infection and number of helminthes infection) consistently explained weight of pig even after adjustment with age of pigs, gender of pigs and rearing practices. *Ascaris suum* infection was also associated with weight of the pigs after adjusting age of pigs, gender of pigs and rearing practices in the model. Other parasite

did not indicate significant association; may be due to small sample size or prevalence. Hence, pig being reared for meat product to sale, all farmers is losing money because of parasitic infections and burden hampering weight gain by pigs. Though this finding may seem obvious, this study documented importance of rearing practices and associated risk factors to be considered for economic growth of society.

Cross sectional design and smaller sample size limit us to generalize our findings. Yet, this is the first study of this kind to evaluated associated factors with GIPS in different rearing system and documented effect of GIPs in weight (i.e., economic loss) of pig which the final product to sale in market (after considering effect of other factors) to link helminthiasis causing economic loss.

6. CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

Socio-economic and socio-demographic factors together with existing pig rearing practices were detrimental to parasitic burden in pig farming system in the study area. High prevalence of parasite observed in study area might have contributed poor productivity of pigs leading to economic loss to the pig farmers. Farmers need to be involved in awareness program related to parasitic disease and concomitant impact in the pig industry either it be smaller or larger. Regular monitoring of parasites in pigs is warranted as most of the intestinal parasites detected in this study are of zoonotic important.

6.2 Recommendations

- As the parasitic burden was found to be higher on the basis of socio-economic and socio-demographic factor, there he housing and management of pigs.
- Regular research should be carried out to updates the presence of parasites.

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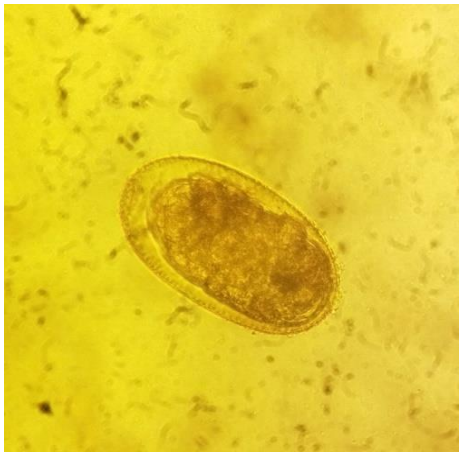
APPENDICES - 1

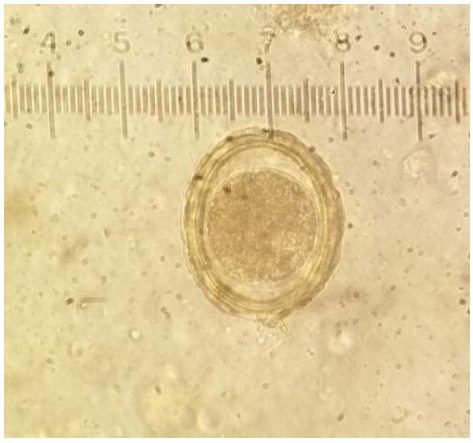
PHOTOGRAPHS

Nematodes



1. Strongyle type egg
(101.2μm × 46.2μm, 400x)

2. Egg of strongyle
(63.8μm× 39.6μm, 400x)



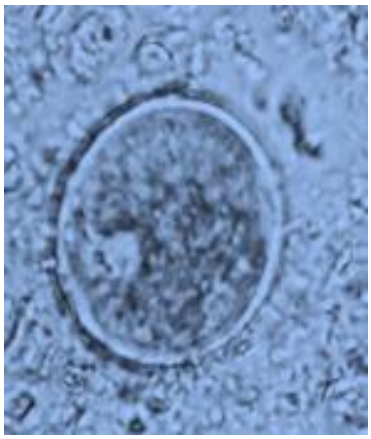
3. Egg of *Ascaris suum*
(corticated)
(48.8μm × 37.4μm, 400x)

4. Egg of *Trichuris suis*
(46.2μm × 24.2μm, 400x)

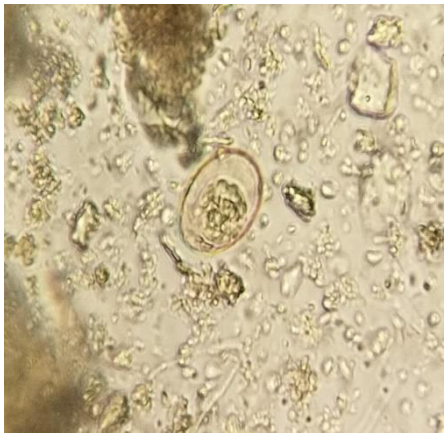
Trematode

5. Egg of *Fasciola*
(134.2μm × 46.2μm, 400x)

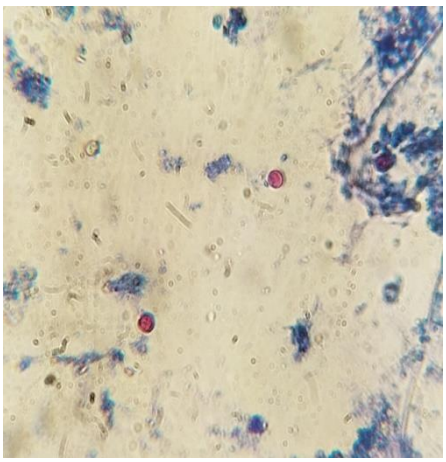
Protozoans



6. Cyst of *Entamoeba coli*
(46.2μm × 35.2μm, 400x)



7. Cyst of *Eimeria*
(28.6μm × 17.6μm, 400x)



8. Coccidian oocyst in stained film
(Modified ZN stain)
(5.22μm, 1000x)

APPENDICES - 2

QUESTIONNAIRES

S.N:

Name:

Address:

Age: Sex: M/F Education:

Number of family: Number of worker in farm:

1. What is your main occupation?
2. What is your main source of income in your family?
3. How much your annual income by pig farming?
4. Have you taken any training about pig's rearing practice?
5. Have you involved in any awareness program regarding disease transmission via pigs?
6. Have you know about management practice of pigs rearing?
7. How about your pigsty?
8. Is there proper housing and management system in your pigsty?
9. What is your pig's breed?
10. What is a pig's sex?
11. Where do you keep your pigs?
12. Are you keeping your pigs together or singly?
13. Are you keeping your pigs with other vertebrate animals?
14. How are you providing water to your pigs?
15. Which types of food you provide to pigs?
16. Have you seen any disease symptoms in yours pigs?
17. Have you seen any adult parasite in your pig's stool?
18. Have you ever treated your pigs?
19. Have you called the veterinarian for regular checkup of your pigs?