PREVALENCE OF GASTROINTESTINAL PARASITES IN BUFFALO (*Bubalus bubalis*) IN MALARANI RURAL MUNICIPALITY OF ARGHAKHANCHI, NEPAL

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Submitted to

Central Department of Zoology

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Kirtipur, Kathmandu, Nepal

May, 2023

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 source of information have been specifically acknowledged by reference to the author(s) or institutions(s).

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RECOMMENDATION

This is to recommend that the thesis entitled "**PREVALENCE OF GASTROINTESTINAL PARASITES IN BUFFALO (*Bubalus bubalis*) IN MALARANI RURAL MUNICIPALITY OF ARGHAKHANCHI, NEPAL**" has been carried out by Basanti Chhetri 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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This thesis work submitted by Basanti Chhetri entitled **"PREVALENCE OF GASTROINTESTINAL PARASITES IN BUFFALO (*Bubalus bubalis*) IN MALARANI RURAL MUNICIPALITY OF ARGHAKHANCHI, NEPAL"** 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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LIST OF ABBREVIATIONS

Abbreviated form	Details of abbreviations
GI	Gastrointestinal
gm	Gram
µm	Micrometer
km^2	Square Kilometer
FAO	Food and Agriculture Organization
MoAD	Ministry of Agriculture Development
P value	Probability value
SPSS	Statistical Package for the Social Sciences
IBM	International Business Machine
VDC	Village Development Committee
$\chi 2$	Chi square
df	Degree of freedom
rpm	Rotation per minute

ABSTRACT

The primary component of Nepal's agricultural system and a substantial contributor to the country's economy is livestock. Buffaloes are one of the main types of livestock grown in Nepal. Total 200 fecal samples were collected in order to determine the prevalence and distribution of gastrointestinal parasites in buffaloes of Arghakhanchi, Nepal. The samples were microscopically examined by direct wet mount, sedimentation, and floatation techniques. One or more intestinal parasites were detected in 125 (62.5%) of the samples. Female buffaloes (58.5%) had a higher frequency of parasitic infection than male buffaloes (4%). Among the 200 samples, 60 were collected from young buffaloes and 140 from adults. Age wise prevalence of parasitic infection indicated that 32% in young and 68% in adult buffaloes were found infected. Adult buffaloes had a significantly high parasitic infection compared to young one (($\chi 2=16.20$, df=1, $p<0.05$). Protozoan parasites *Eimeria* and *Entamoeba* were found equally common in young and adult buffaloes. Trematodes including *Fasciola, Paramphistomum*, and *Eurytrema*, which are helminths, were more abundant in adult buffaloes. Except for *Ascaris* and *Capillaria*, other nematodes had a higher prevalence in adult buffaloes. Only adult buffaloes were found to be infected with cestode parasite. Buffaloes of Malarani rural municipality were found to be highly infected with *Ascaris* sp and *Entamoeba* sp belonging to helminth and protozoan parasites respectively. Ward wise parasitic prevalence rate indicated that the distribution of parasitic prevalence was not significant ($\chi 2=1.216$, df=2, $p>0.05$). Overall parasitic prevalence showed protozoa (42.50%), nematodes (37%), trematodes (15.50%), and cestodes (1%) infection in buffaloes of study area. Overall, ten different parasitic infection was found in buffaloes of Malarani rural municipality with high prevalence indicating urgent need of intervention programms.

1. INTRODUCTION

1.1 Background

The domestic water buffalo (*Bubalus bubalis*) belong to the family Bovidae and sub-family bovinae. Swamp buffalo and water buffalo are two separate categories of buffaloes. The swamp buffaloes are frequently used as draught animal and are mostly found in Southeast Asia. In terms of morphology, swamp buffaloes resemble the wild buffaloes of species *arni*. Water buffaloes are the most prevalent breed in countries like India, Nepal, Pakistan, Bulgaria, Hungary, Turkey, Italy, Egypt, and Brazil (Cockrill, 1994,1997). They are large, usually with curled horns, and prefer to wade in clear rivers. More Water buffaloes have been chosen for their ability to produce milk. About 48.5% of households in the nation raise buffaloes, moving them from the plains to the Himalayan highlands' high alpine meadows for summer grazing, which may be the highest location on earth for buffalo rearing. In the developing countries like Nepal and other Asian country, livestock is a crucial part of small-scale crop-livestock mixed farming systems and a primary source of many essential dietary elements (Devendra *et al.*, 2002). The main limitations on the production of ruminants in Nepal and around the world are significantly influenced by parasite infestations.

Gastrointestinal parasitic infection generates significant global economic loss as a result of decreased weight gain, digestive disruption, decreased production, impaired reproductive performance, condemnation of affected organs, and mortality in infected animals (Raza *et al*., 2007). Gastrointestinal parasites such as coccidian, ascarid, strongyle, and amphistomes have been identified in nations with tropical and temperate climates, including India, Nepal, Bangladesh, South Africa, Sri Lanka, Italy, and Mongolia with a prevalence rate ranging from 20 to 96% (Sharma *et al*., 2014 and Gebeyehu., *et al*, 2013). Over the past ten years, anthelmintics and antiprotozoal medications have been utilized to control gastrointestinal parasite infections (Gordon, 1935) but no one has been able to completely diminish reinfestation (Sutherland and Scott, 2010). Therefore, to control and manage parasitic diseases at the earliest stages of infection in farm management methods, periodic monitoring of parasite species among livestock animals would be helpful.

1.2 Agricultural practices of buffaloes in Nepal

Buffalo farming is the integral component of rural economy. They are raised for milk, meat, draft power and manure. According to FAO (2012), there are 199 million buffalo globally, among them 96% of the population exist in Asia. There were 5,159,931 buffalo heads in Nepal contributing about 57.3% of Nepal's yearly milk production MoAD 2020/ 21. The three breeds of Hill buffalo are lime, parkote, and gaddi. Lime is more prevalent in the northern high mountain ranges, whereas parkote are more prevalent in the southern mid hills. Far-western Development Region is where Gaddi buffaloes can be found (Rasali, 1998). However, terai buffalo are considered as non-discript type. The main sources of food in Nepal include leaves from fodder trees, grasses, legumes, straw, stove d on the nutrients found in fodder tree leaves, grasses, and legumes (Osti et *al*., 2006). Due of the terraced hills in the highlands and mountains, buffaloes are not utilized as draft animals. Most homes raise at least one buffalo to produce milk and dung, and they sell the male calves or adult males to make money for their families.

Eighty six percent of Nepal's population live in rural areas. In Arghakhanchi most of people involve in farming. Livestock rearing play a significant role in agriculture and source of incomes for farmers. There are 98,517 population of buffalo reared in Arghakhanchi district producing 7.86% of milk in Lumbini province MoAD (2020/21). After Kavre, Arghakhanchi has also become an independent milk-producing district, with average annual milk sales of Rs 70 million by Rastriya Samachar Samiti 2017. According to Bishnu Poudel (2017), a veterinarian at the District Livestock Service Office (DLSO), most of the overall production is consumed in the district itself, with some being shipped to Palpa and Gulmi as outlying districts. In order to increase the farmers' production of dairy products, the DLSO has been offering technical support and subsidies to those engaged in commercial animal husbandry.

1.3 Diseases of buffaloes

Water buffalo are renowned for their ruggedness and high adaptation to various topographies, soils, and climatic conditions. They are nevertheless susceptible to contracting a number of infectious diseases, which have a significant negative impact on their capacity for production. Some of the diseases affecting buffaloes have been categorized as follows:- Bacterial diseases- Anthrax, Black Quarters, Hemorrhagic

Septicaemia, Bovine Brucellosis, Tuberculosis; Viral diseases- Foot and Mouth diseases, Infectious Bovine Rhinotracheitid, Bovine Viral diarrhea, Rabies; Parasitic diseases-Theilariasis, Babesiosis, Anaplasmosis, Trypanosomiasis, Strongylosis, Ascariasis, Coccidiasis, Fasciolosis, Schistosomiasis; Neonatal dirrheal diseases-Cryptosporidiosis; Fungal diseases- Deg Nala diseases (Fagiolo *et al*., 2004).

The virus damages the villi as soon as it enters the small intestine, essentially preventing the animal from absorbing nutrients, which causes dehydration. The infected calves typically don't have a fever during the virus's 12- to 24-hour incubation period (Jakobsson, 2013). Water buffalo are susceptible to the disease Deg Nala, which causes lameness, edema of the extremities, gangrenous ulcers or necrosis of the mouth, ears, hooves, and tail (sloughing of epidermis), general wasting, recumbency, and ultimately death (Reddy *et al.,* 2016). This causes a sizable reduction in milk production, which has not yet been quantified. Epithelial cells, dendritic cells, macrophages, and placental trophoblasts can all be invaded by *Brucella abortus*. According to reports, cattle and buffaloes were the main sources of *B. abortus* (Wareth *et al.,* 2014). Crytosporidiosis causes diarrhea in humans and animals, although it is self-limiting in hosts with a functioning immune system. Additionally, young, immune-competent hosts could pass away from life-threatening diarrhea (Chalmers *et al.,* 2010 and Xiao *et al.,* 2003). While anemia, wasting, submandibular edema, and decreased milk production are among the clinical manifestations of chronic fasciolosis, heavily infected cattle do not show any clinical signs despite having severe liver damage and a 7–10week survival rate in subacute fasciolosis cases (Kaplan *et al.,*1995).

1.4 Veterinary services

In Arghakhanchi, district veterinary hospital located in Gorusinghe road, Sandhikhankha. Services available in veterinary hospital are: Behavior counseling, Dental care, Health certificates, Lab services, Nutrition consultation and pet emergency services. Malarani rural municipality contains nine words, each of which have veterinary clinics with individual veterinarian personnel. This clinic provides free medicine like antiparasitic drug and vitamin, and home services to the people of this area.

1.5 Significance of the study

Buffalo (*Bubalus bubalis*) is one of the important livestock which play a significant role to increase the Nepalese economy. In Nepal, different species of GI parasites was reported in buffaloes. Till the dates parasitic studies among the buffalo has not been carried out in Malarani rural municipality. Similarly, the study conducted in Pokharathok VDC showed 68% of overall prevalence and gastrointestinal parasitic infection in ruminant livestock become the biggest problem for both small and large-scale farmers (Devi, 2012). Besides, Buffalo milk is used in the dairy industry to make mozzarella, cream, yogurt, and butter since it has more fat and protein than cow's milk (Coroian, 2012). The present study provides some baseline for the parasitic information on buffaloes and also help to formulate the strategies to reduce the GI parasitic problem in Malarani rural municipality.

1.6 Objectives of the study

1.6.1 General objectives

- To determine the prevalence of gastrointestinal parasites in buffalo (*Bubalus bubalis*) of Malarani rural municipality, Arghakhanchi, Nepal.

1.6.2 Specific objectives

- To find out the distribution of gastrointestinal parasites of buffalo in Malarani rural municipality, Arghakhanchi, Nepal.
- To investigate the intensity and concurrency of GIs parasites.
- To assess the knowledge, attitude and practices of buffalo's owner in relation to parasitic infection.

2 LITERATURE REVIEW

Endoparasitic infection among the large ruminant are most common in topical and sub-tropical countries. Parasitic infection causes direct and indirect effect on normal functioning of host body which ultimately induce the loss of economy for the farmers. The ruminant digestive tract is home to helminth infections, which are both common and seasonal and have a considerable negative influence on animal productivity (Charlier *et al.,* 2014).

2.1 Global distribution of gastrointestinal parasites

2.1.1 Protozoan parasites

Protozoan infections are a significant barrier to the development of dairy production worldwide, but especially in developing countries (Om *et al.,* 2010). Among the protozoan parasites, coccidiosis can be a serious disease in livestock. More than 11 species of *Eimeria* are commonly found in water buffalo (Dubey, 2018). Nalbantoglu *et al.,* (2008) had reported *Isospors* spp. for the first time in the province of Afyon in water buffaloes including *E. zuernii* (55.1*%), E. auburnensis* (44.9%), *E. bovis* (44.9%), *E. ellipsoidalis* (28.2%), *E. ankarensis* (16.7%), *E. subspherica (*16.7%), *E. alabamensis* (11.5%), *E. cylindrica* (10. 3%). Infection with *Eimeria* parasites has been reported in several parts of India such as Gujarat (Thakre *et al.*, 2019), Jabalpur (Marskole *et al.,* 2016). Similarly, *Eimeria* infection has been reported from Egypt (El-Alfy *et al.,* 2019), Romania (Bărburaș *et al.,* 2021), Pakistan (Khan *et al.,* 2023), Turkey (Nalbantoglu *et al*., 2008), Sri Lanka (Gunathilaka *et al.,* 2018), Indonesia (Nurhidayah *et al.*, 2019), Central Ethiopia (Terfa *et al*., 2023). Besides coccidian parasites, buffaloes have been found to be infected with *Buxtonella sulcate* in Andhra Pradesh, India, Romania(Bărburaș *et al.,* 2021). Likewise, *Entamoeba* spp. in Pakistan (Khan *et al*., 2023).

2.1.2 Helminths parasites

Helminth parasites can be very harmful to animal populations' health and result in significant economic losses. The prevalence of fascioliasis is global (Blood *et al.,* 1990). helminthiasis lead to slowed growth (Kochapakdee *et al.,* 1995), decreased productivity (Perry and Randolph,1999), death (Sykes, 1994), and significant economic losses (Iqbal *et al.* 1993), which have an impact on the revenue of small-scale dairy

farming groups. Rumen fluke is more common in tropical and subtropical regions because the climatic conditions are favorable for intermediate hosts like mud snails or freshwater snails to develop and finish the parasite life cycle (Gordon *et al*., 2013; Hajipour *et al*., 2021). Mixed infections with *Fasciola gigantica* and *Amphistomes* were common (Saha *et al.*, 2014). *Fasciola hepatica* has been reported in Romania (Bărburaș et al., 2021), Poland (Kobak & Pilarczyk, 2012) and Italy (Cringoli *et al.*, 2009). *Fasciola gigantica* has been reported in Bangladesh (Saha *et al.,* 2014) and India (Marskole *et al.*, 2016).

Similarly, *Paramphistomum* is the intestinal fluke which causes enteritis and anaemia in livestock. Since mud snails and freshwater snails are suitable intermediary hosts for the development and completion of the parasite life cycle, rumen fluke is more prevalent in tropical and subtropical regions (Gordon *et al.,* 2013; Hajipour *et al.,* 2021). *Paramphistomum* sp. has been recored in different Asian countries like India (Yadav, 2020), Bangladesh (Saha *et al.*, 2014), Europe, Italy (Cringoli *et al.*, 2009), Sri Lanka (Gunathilaka *et al.,* 2018). In addition, *Paramphistomum cervi* recorded in Poland (Kobak & Pilarczyk, 2012) and Romania (Bărburaș *et al.*, 2021).

Schiostosoma is the genus trematode, known as blood fluke and it cause nasal, and viceral diseases in animals. *Schistosoma bavis, S.indicum and S. spindale* have been repoted in Bangaldesh by shan (2014) and (Manum *et al.,* 2011). Likewise, *Dicrocoelium dentriticum*, the lancet liver fluke that dwells in bile duct, canaliculi and gall bladder of host animal. *Dicrocoelium dentriticum* has been reported in Italy (Cringoli *et al.*, 2009).

The most common nematode parasites among livestock are *Haemonchus* spp*, Taxocara* spp, *Strongyloids* spp*, Oesophagostomum* spp*, Bunostomum* spp*, Trichuris* spp*, Trichostrongylus* spp and *Capillaria* spp. Nemotodes infections are a significant contributor to economic loss on farms all over the world. Clinical illness or slower development rates in young animals as well as decreases in milk production in adult cows are the main causes of the losses. *Strongyloides* and strongyle has been recorded in India (Manzer, 2022) and (Marskole *et al*., 2016), Romania (Bărburaș *et al.,* 2021), Indonesia (Nurhidayah *et al.,* 2019), Italy (Cringoli *et al.,* 2009).

Similarly, *Trichostrongylus* also known as pseusdohookworm which exist in stomach or gut of ruminant worldwide. *Trichostrongylus* has been recorded in the buffalo of

India (Khadse *et al.*, 2022), Pakistan (Khan *et al.,* 2010). *Trichostrongylus axei* has also been reported in buffaloes of Bangladesh (Saha *et al.,* 2014) and India (Patel *et al*., 2015) and cattle of Nigeria (Ibrahim *et al.,* 2022).

Taxocara vitolorum is the round worm of Bubalus and boss species found mostly throughout the world (Starke-Buzetti, 2006). *T. vitolorum* is one of the most harmful parasites that affect calves, and when its larvae migrate, it severely damages several organs, most specially the liver and the gut. It can cause up to 50% of the mortality in calves of cattle and buffalo (Srivastava and Sharma, 1981). *Taxocara* spp. has been reported in buffaloes of India (Khadse *et al.,* 2022), Bangladesh (Kanu *et al*., 2021), Indonesia (Saukhan *et al*., 2022), Romania (Bărburaș *et al.,* 2021), Combodia (Dorny *et al.,* 2015) and America (Goossens *et al.,* 2007).

Trichuris sp. is commonly known as whipworm found in livestock worldwide (Matsubayashi *et al.,* 2009). Heavy infections with this parasite caused diarrhea, anorexia and weight loss (Jiménez *et al* 2010).Trichuris sp. has been recorded in India (Khadse *et al.,* 2022) (Chavhan *et al.,* 2008) (Manzer, 2022), Sri Lanka (Gunathilaka *et al.,* 2018), Indonesia (Nurhidayah *et al.,* 2019), Pakistan (Khan *et al.,* 2010). *Haemonchus* spp has been recorded in India (Khadse *et al.,* 2022), Pakistan (Khan *et al.,* 2021),Iran (Nouri *et al.,* 2022). *Oesophagostomum spp*. has been identified in India (Khadse *et al.,* 2022), Pakistan (Khan *et al*., 2010), Iran (Nouri *et al.*, 2022). *Bunostomum* sp has been found in Sri Lanka(Gunathilaka *et al.,* 2018), India (Khadse *et al.,* 2022). *Capillaris* spp has been recorded in Romania (Bărburaș *et al.,* 2021), Bangladesh (Biswas, 2012).

There are various cestode species, including *Moniezia* spp., *Taenia* spp. and *Echinococcus* spp. that live in the small intestines of both domestic and wild animals, including sheep, goats, cows, buffalo and other ruminants. Cestode has been recorded in buffalo of India (Thakre *et al*., 2019), (Marskole *et al.*, 2016),Romania (Bărburaș *et al.,* 2021), Pakistan (Khan *et al.,* 2021; Khan *et al.,* 2010), Italy (Cringoli *et al.,* 2009), Sri Lanka (Gunathilaka *et al.,* 2018), while some study has showed nematode and trematode only by (Kobak & Pilarczyk, 2012; Saha *et al.,* 2014).

2.2 Distribution of gastrointestinal parasites in Nepal

2.2.1 Protozoan

In the context of Nepal, limited study has been conducted on protozoan parasites rather than helminths. *Eimeria* sp. has been recorded in buffaloes of Chitwan (Adhikari & Dhakal, 2023),Satari and Udaypur district KTWR (Gupta, 2017), Chitwan Annapurna Landscape (Dhakal *et al.*, 2022). *Cryptosporidium* spp. has been recorded by Chalisa (2013) in KTWR and in Chitwan Annapurna Landscape(Dhakal *et al.,* 2022). *Entamoeba* sp. and *Balantidium coli* has also been recorded in captive buffalo Chitwan (Adhikari and Dhakal, 2023).

2.2.2 Helminths

Helminth parasites have a negative impact on farm animal breeding; they harm the host either directly or indirectly. It includes nematode, trematode and cestode which are found in liver, bile duct and digestive tract of host body. *Fasciola* spp. is one of the most important pathogen species which cause the diseases fascioliasis. *Fasciola* spp and *Paramphistomum* spp has been identified in buffalo of Kavreplanchok (Tamang and Sukupayo,2022), Chitwan (Adhikari and Dhakal, 2023),Chitwan Annapurna Landscape (Dhakal *et al*., 2022), Dharampur, Dhanusha district (Saha, 2015), Pokharathok, Arghakhanchi (Devi,2012),wild buffalo of Saptari and Udaypur district KTWR (Gupta, 2017), Satungal, Kathmandu (Mukhiya, 2007). Similarly, *Schistosoma* spp has been recorded in buffaloes of Chitwan (Adhikari and Dhakal, 2023), Arghakhanchi (Devi,2012), Kathmandu (Mukhiya ,2007).

Similarly, Nematodes are most important and prevalent parasites among the small and large ruminant. These parasites complete the life cycle within single host and transmitted by feacal contamination food, water and soil. *Taxocara* spp, *Haemonchus* spp and *Strongyloids* spp has been recorded in the buffaloes of Chitwan (Adhikari and Dhakal, 2023), in wild buffaloes of Saptari and Udaypur district KTWR (Gupta, 2017) , Chitwan Annapurna Landscape (Dhakal *et al*., 2022), Dhanusha district (Shah,2015), Arghakhanchi (Devi,2012), Kathmandu (Mukhiya, 2007). *Trichostrongylus* sp has been recorded in Kavreplanchok (Tamang and Sukupayo, 2022), KTWR (Gupta, 2017), Chitwan Annapurna Landscape (Dhakal *et al.,* 2022). Arghakhanchi (Devi,2012). Likewise, *Trichuris* spp and *Capillaria* spp has been identified in buffaloes of Chitwan

(Adhikari and Dhakal, 2023), Pokharathol, Arghakhanchi (Devi, 2012). Satungal Kathmandu (Mukhiya, 2007).

Cestode are the ribbon like flat worm which found in gut of ruminant. These parasites required intermediate host to complete the life cycle. *Monienzia* spp has been recorded in the buffaloes of Chitwan (Adhikari and Dhakal, 2023), Satungal, Kathamandu (Mukhiya ,2007), Pokharathok, Arghakhanchi (Devi, 2012). *Taenia* spp has also been reported in Kavreplanchok (Tamang and Sukupayo, 2022).

3. MATERIALS AND METHODS

3.1 Study Area

Arghakhanchi District, a part of Lumbini Zone, is one of the seventy-seven districts of Nepal. The district with Sandhikharka as its district headquarters, covers an area of 1,193 km² and has population 177,086 in 2021. The district is bound by Palpa in the east, Gulmi in the North, Kapilbastu and Rupandehi in the south and Dang and Pyuthan in the west. About 68% of the total area lies in the Mahabharat Range, and the remaining lies in the Siwalik Region. It is situated between 305m-2575m above sea level, about 40% of its total area is covered by forest.

Malarani is a Rural Municipality in the Lumbini Province of Nepal's Arghakhanchi District. According to 2021 census conducted in Nepal, the rural municipality has a population of 24,150. Most of the people in this municipality are engaged in agriculture sector and reared buffalo, cow goat, chicken, pigeon, pig as a livestock. Majority of households reared buffalo for personal and economic purpose. Buffaloes are reared under captive condition while other livestock are reared as free ranging animal. Out of nine wards three wards has been selected for the study to represent the whole ward.

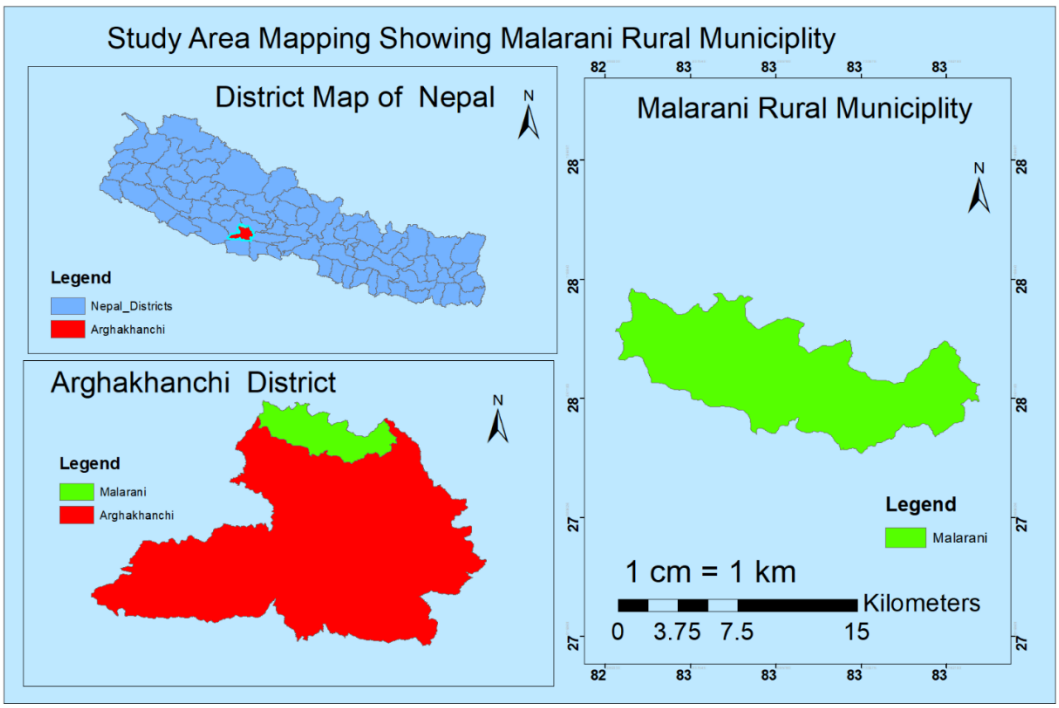

Figure 1: Showing Study Area

3.2 Materials

The materials used during research are listed below:

3.2.1 Materials for field

I. Sterial vials

II. Camera

III. Gloves

IV. Potassium dichromate ($K_2Cr_2O_7$)

3.2.2 Materials for laboratory

I. Electric microscope(SWIFT)

II. Ocular micrometer (Ubuy)

II. Stage micrometer (Ubuy)

IV. Centrifuge machine (REMI)

3.3.3 Chemicals

I. Potassium dichromate ($K_2Cr_2O_7$)

II. Distilled water (D/W)

III. Saturated NaCl solution

IV. Methylene blue

V. Lugol's iodine solution

3.3 Study design

3.3.1 Sample size

200 Feacal samples were collected from three wards i.e 66, 73, 61 samples from 3, 5 and 7 wards.

3.3.2 Sample collection/preservation

Sample were collected during winter (November/December,2022) from three wards of Malarani rural municipality. Fresh dung samples were carefully collected in sterial vials with the help of spatula and preserved in 2.5% potassium dichromate that helps in maintaining the morphology of protozoan parasites and preventing of some helminth eggs and larvae, mucus as well as parasitic segments. The collected sample were transported to laboratory of Central referral veterinary hospital.

The study was designed to determine the parasitic infection in buffaloes as fallows;

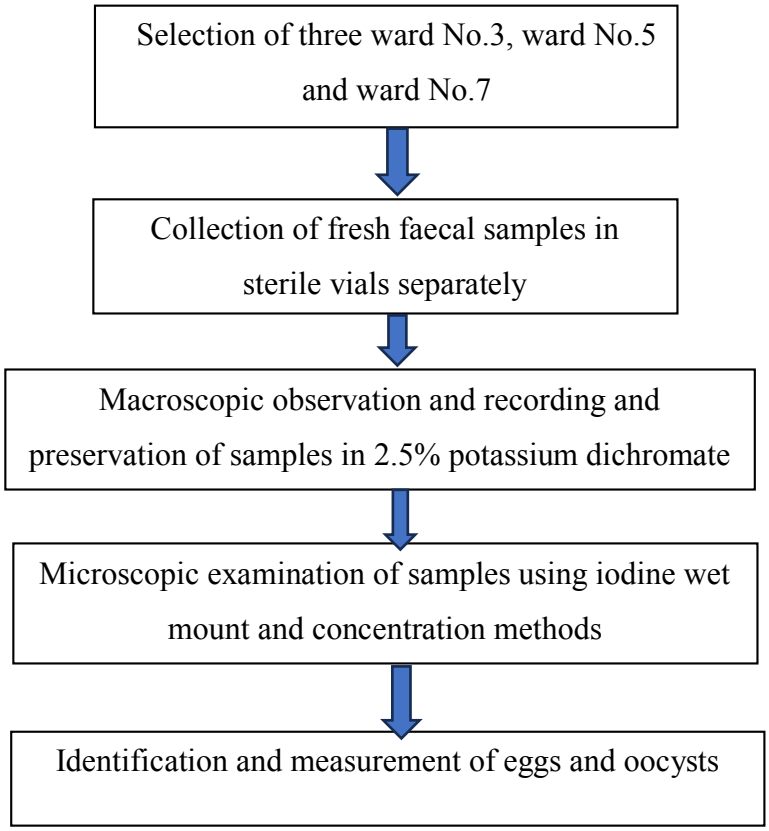

Figure 2: Flow chart showing research outline

3.4 Microscopic examination

All the collected samples were examined in laboratory of central referral veterinary hospital, Tripureshor, Kathmandu. For microscopic analysis, the samples were prepared. Further measurement of infected samples were done in Central Department of Zoology by using ocular and stage micrometer. The identification of eggs of parasites was performed by using previously published literature (Kaufmann, 1996; Zajac and Conboy, 2012; Hansen and Perry, 1994; Soulsby, 2012).

3.4.1 Iodine wet method

On a clean glass slide, a little amount of samples were emulsified in a drop of Lugol's Iodine solution before being covered with a clean cover slip. A 10X and 40X electric microscope was used to analyze the smear (Soulsby, 2012).

3.4.2 Concentration techniques

3.4.2.1 Floatation technique

This procedure is widely used for detecting the eggs of nematode and cestode. These eggs are lighter and can float on floatation liquid. About 3 gm of stool sample was taken in a beaker and added 42 ml of water with the help of motar and pestle, sample was grinded and clarified with tea stainer. The samples were filtered, placed in 15 ml plastic tubes and centrifuged for 5 minutes at 1000 rpm. The plastic tube was removed from the centrifuge, and the upper portion of water was poured using a pipette. The tube was once more filled with NaCl solution and centrifuged for 5-7 minutes at 1000 rpm. To fill the tube all the way to the tip, NaCl solution was added. In order to allow the Sodium chloride to trace the cover slip for a short period of time, the cover slip was placed on a slide and magnified 10X (Khan et al., 2018).

3.4.2.2 Sedimentation technique

This technique is used for detecting trematodes eggs. It provides better results as the eggs of trematodes are a bit heavier than any other eggs. Sediments of centrifuged content are taken for eggs detection. Saturated salt solution was removed gently from the test tube after examining the floatation portion and poured the sediment content into watch glass and stirred the content gently to mix it. 1-2 drop from mixture was taken to prepare another slide. The specimen was stained with iodine wet mounts solution. In this was two sides were prepared from one sample (one from floatation and one from sedimentation) were examined under 10X and 40X with objectives of microscopes to detect eggs of protozoan, helminths or cysts of gastrointestinal parasites (Soulsby, 2012).

3.4.3 Determination of concurrency and intensity

3.4.3.1 Concurrency

The host harbors one or more parasites with low numbers; in such circumstances, the host does not develop sickness yet might continue to be a source of infection for an extended length of time. Single, double, triple and multiple infections of gastrointestinal parasites were classified. A single infection was defined as the presence of only one egg/cyst/larva of the parasite per field, whereas double, triple and multiple infections

were characterized as the presence of two, three or more egg/cyst/larva of the parasite per field.

3.4.3.2 Intensity

The total amount of eggs/oocysts and larvae detected per field was used to calculate the intensity of parasite infection. The level of gastrointestinal parasite infection was divided into three categories: light infection, moderate infection, and heavy infection. The presence of less than two eggs/cysts/larva of the same species per field was used to assess light infection. In a similar manner the presence of 2-5 egg/cyst/larva, and 6 or more egg/cyst/larva of the same species per field characterized moderate, and heavy infections.

3.5 Eggs, cysts and larva size measurement

Eggs and cysts size were measured by using micrometer. The calibration factor was found to be 2.4 μm for 40x of objective lens.

3.6 Interview format

The semi-structured questionnaire was asked to the owners individually and the data were gathered.

3.7 Data analysis

All the data recorded after examination were entered into the Microsoft excel 2007 and statistical was performed using IBM SPSS statistics 27 software package. Data were statistically analyzed using the chi-square test. 95% confidence interval (CI) and $P \leq 0.05$ were always taken into consideration for statistically significant differences.

4. RESULTS

Out of 200 faecal samples examined, 125 (62.5%) were positive for one or more intestinal parasites of which 116(58.5%) female and 9(4%) males.

4.1 Age wise prevalence of gastrointestinal parasites of buffaloes

Out of 200 feacal samples, 60 young and 140 adults were examined. Among the young GIs parasitic infection was found 32% (40) and among the adult 68% (85). Parasitic infection in adult buffaloes showed significantly high ($\chi 2$= 16.20, df=1, $p<0.05$) (Fig.2).

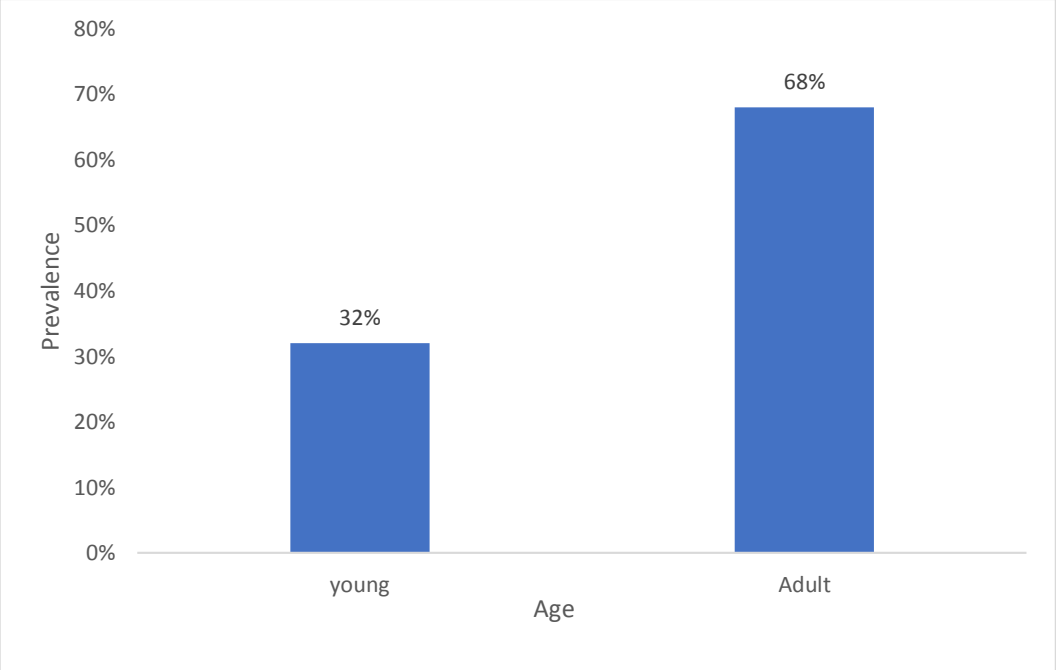

Figure 3: Age wise prevalence of gastrointestinal parasites of buffaloes

4.2 Species wise gastrointestinal parasitic prevalence in young and adult buffaloes.

In protozoa, both *Eimeria* and *Entamoeba* almost equally prevalent in young and adult buffaloes but comparatively *Entamoeba* infection found higher in buffaloes than *Eimeria*. Trematode i.e *Fasciola, Paramphistomum and Eurytrema* were higher in adults. Besides *Ascaris* and *Capillaria,* other nematode were found higher in adult buffaloes. Cestode were found only in adults. Overall gastrointestinal parasitic infection indicated highest prevalence of *Entamoeba* among protozoan parasites and *Ascaris* among helminths parasites (Table 1).

Table 1: Gastrointestinal parasitic prevalence in young and adult buffaloes

Class	Parasites	Young<3 (n = 60)	Adult>3(n = 140)	Overall Positive Prevalence
Protozoa	*Eimeria* spp	19 (31.67 %)	33(23.52 %)	52 (26%)
	Entamoeba spp	23 (38.33 %)	56(40%)	79(39.5%)
Trematode	*Fasciola* spp	2(3.33%)	12 (8.57 %)	14 (7 %)
	Paramphistomum spp	1(1.67 %)	7 (5 %)	8 (4 %)
	Eurytrema spp	1(1.67 %)	8 (5.71 %)	9 (4.5 %)
Nematode	Strongyle	4(6.67 %)	18 (12.85 %)	22 (11 %)
	Ascaris spp	27(45%)	22(15.71%)	49(24.5%)
	Nematodirus spp	1(1.67%)	3 (2.14 %)	4 (2 %)
	Capillaria spp	2(3.33 %)	2 (1.42 %)	4 (2 %)
Cestode	*Moniezia* spp	-	2 (1.42 %)	2 (1 %)

4.3 Overall ward wise prevalence of GIs parasites in buffaloes

Out of 66,73 and 61 sample of ward no. 3, 5 and 7 were examined which showed 41 (62.12%), 37(50.68%) and 47(77.04%) of prevalence with one or more parasitic infection. Among them ward 7 showed the highest prevalence rate while ward 5 showed the lowest prevalence rate. Although parasitic infection was found high in ward 7, the parasitic distribution among wards insignificant (($\chi 2$= 1.216, df=2, p>0.05).

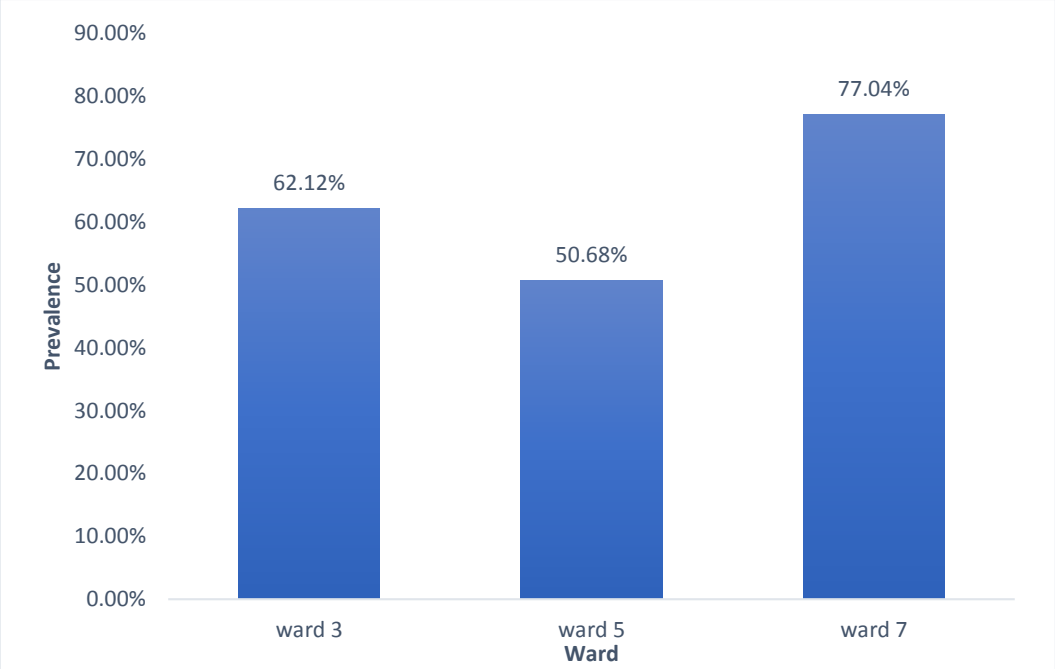

Figure 4: Ward wise prevalence of GIs parasitic infection in buffaloes

4.4 Ward wise prevalence of GIs parasites in buffaloes

Eimeria and *Entamoeba* were highest in ward 3 but *Eimeria* comparatively high in ward 5 and *Entamoeba* in ward 7. *Fasciola* and *Paramphistomum* were highly found in 7 but *Eurytrema* in ward 5. *Ascaris* were higher in ward 3 but strongyle in ward 7. However, cestode were only in ward 5.

Table 2: Ward wise prevalence of gastrointestinal parasite with specific species.

Class	**Species**	**Ward no. 3 (n=66)**	**Ward no. 5(n=73)**	**Ward no. 7(n=61)**	**Total prevalence**
Protozoa	*Eimeria* spp	25(31.87%)	17(23.28%)	10(16.39%)	52(26%)
	Entamoeba spp	33(50%)	22(30.13%)	24(39.34%)	79(39.5%)
Trematode	*Fasciola* spp	3(4.54%)	4(5.47%)	7(11.47%)	14(7%)
	Paraphistomum spp	1(1.51%)	3(4.10%)	4(6.55%)	8(4%)
	Eurytrema spp	2(3.03%)	5(6.84%)	2(3.27%)	9(4.5%)
Nematode	Strongyle	6(9.09%)	4(5.47%)	12(19.67%)	22(11%)
	Ascaris spp	26(39.39%)	14(19.17%)	9(14.75%)	49(24.5%)
	Nematodirus spp	-	1(1.36%)	2(3.27%)	3(1.5%)
	Capillaria spp	2(3.03%)	1(1.36%)	1(1.63%)	4(2%)
Cestode	*Moniezia* spp	-	2(2.73%)	-	2(1%)

4.5 Class wise distribution of GIs parasite in buffaloes

In the class wise distribution of parasites, protozoa (42.50%) showed the highest prevalence followed by nematode (37%), trematode 15.50% and cestode (1%) respectively.

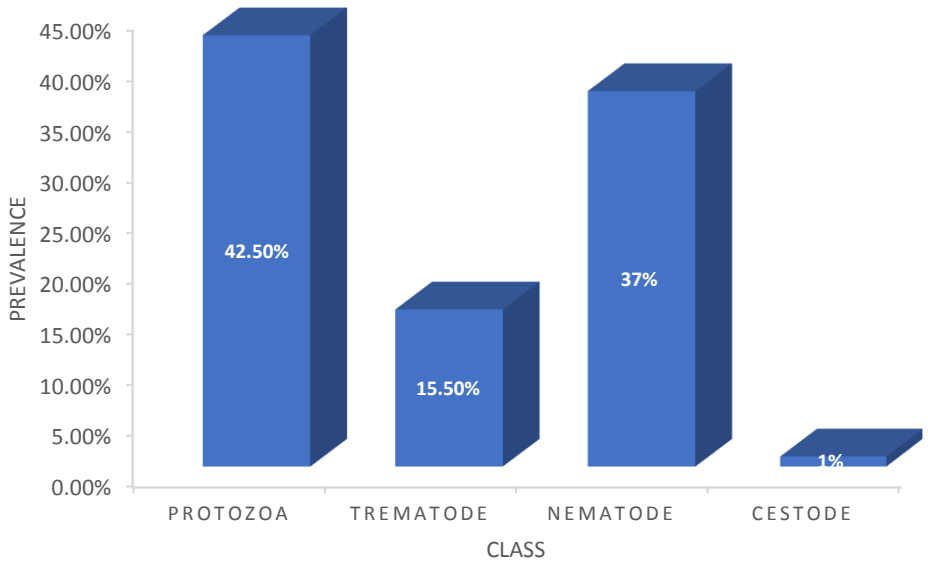

Figure 5: Class wise prevalence of GIs parasites in buffaloes

4.6 Concurrency of parasite

Among four type of infection i.e single, double, triple and multiple. Double infection showed highest rate 30.5% than other type of infection. Multiple infection (2.5%), triple infection (7%) and single infection (22.5%) was encountered in this study.

Table 3: Overall concurrency of parasites

Infection type	Positive	prevalence %
Single	45	22.5%
Double	61	30.5%
Triple	14	7%
Multiple	5	2.5%

4.7 Intensity of Parasites

Parasitic infections in buffaloes showed different intensity. Most of the buffaloes

revealed with light parasitic infection. Species like *Entamoeba, Ascaris* and *Eimeria* showed a sign of heavy infection in 9, 2 and 7 samples respectively.

Table 4: Overall intensity of specific parasites

Class	Name of genera	Light infection (<2)	Moderate infection (2-5)	Heavy infection (>6)
Protozoa	*Entamoeba* (n=79)	32(40.50%)	38 (48.10%)	9 (11.39%)
	Eimeria(n=52)	19((36.53%)	16 (30.76%)	7 (13.46%)
Trematode	*Fasciola* (n=14)	12 (85.71%)	2 (14.28%)	-
	Paramphistomum(n=8)	6 (75.0%)	2 (25%)	-
	Eurytrema(n=9)	7 (77.77%)	2 (22.22%)	-
Nematode	Strongyle(n=22)	17 (77.27%)	5 (22.72%)	-
	Nematodirus(n=4)	4 (100.0%)	-	-
	Ascaris(n=49)	18 (36.73%)	29 (59.18%)	2 (4.08%)
	Capillaria(n=4)	4 (100.0%)	-	-
Cestode	*Monienzia* (n=20	2 (100.0%)	-	-

4.8 Response of owner toward questionnaire

A total of 108 farmers were asked structured questionnaire to asses knowledge, attitude and practice. In case of knowledge, educated owner was found higher (18.9%) followed by uneducated and so on. Maximum 95.37% of owner did not observed parasites directly in fecal sample. 37.03% of the owner have knowledge about parasites. Most of the 75.92% of owner know the name of parasites. In case of practice field, 92.59% of owner did not use gloves, 65.745% have rear buffaloes for personal use. In case of attitude practice, 6.48% have superstitious believe. However, there was insignificant relation in parasitic knowledge and source of water ($p>0.005$) with prevalence of parasites, whereas significant relationship was found in education, parasite observed, parasite name, use of gloves, purpose of rearing, occupation, treatment, vet consultant, attitude and superstation belief ($p< 0.005$).

Table 5: Overall response of questionnaire

Knowledge			
		N=108 (%)	**Significance (P value)**
Education	Uneducated	28(11.5%)	0.001
	Educated	46 18.9%)	
	+2	22(20.37%)	
	Higher	12(11.11%)	
Parasite observed	Yes	5(4.62%)	0.001
	No	103(95.37%)	
Parasitic knowledge	Yes	40 (37.03%)	0.07
	No	68(62.96%)	
Parasite name	Yes	80(75.92%)	0.001
	No	28(25.92%)	
Effect of parasite	Yes	12(11.11%)	0.001
	No	96 (88.88%)	
Practice			
Use of gloves	Yes	8(7.40%)	0.001
	No	100(92.59%)	
Purpose of rearing	Personal	71(65.745)	0.001
	Commercial	37 (34.25%)	
Source of water	River	41(37.96%)	0.012
	Underground	67(62.03%)	
Occupation	Agriculture	74 (68.51%)	0.001
	Others	34(31.48%)	
Treatment	Yes	85 (78.70%)	0.001
	No	23((21.29%)	
Vet consultant	Yes	108 (100%)	0
	No	0	
Attitude			
Superstation belief	Yes	7(6.48%)	0.001
	No	101(93.51%)	

5. DISCUSSION

The current study aimed to investigate the prevalence of GIs parasites in buffaloes. A total of 200 samples were collected from Malarani rural municipality of Arghakhanchi and examined microscopically. Overall prevalence rate found to be 125 (62.5%) which was lower than the previous study conducted by Devi (2018), (68%) in Pokharathok VDC, Arghakhanchi. Similarly, the different study of Nepal recorded 90% (Adhikari and Dhakal, 2023), 62.7% (Dhakal *et al*., 2022) from Chitwan, 74% (Yadav, 2015)from Dhanusha, 47.5%(Gupta, 2017) from Sunsari, 57.14% from Bhaktapur (Tamang and Sukupayo, 2022) and 83.96% (Mukhia, 2007) from Kathmandu. Among these, the present study showed the hightest prevalence rate from two studies but lower than other studies of Nepal. Similarly, the prevalence rate was found to be lower than India 70.45% (Marskole *et al*., 2016), 85.24% (Manzer, 2022), Pakistan 63.55% (Khan *et al.,* 2023), Russia 71.42% (Hadree *et al.*, 2022),Bangladesh 84.90% (Hasan *et al.*, 2020), and higher than reported from Indonesia 37.65% (Nurhidayah *et al.*, 2019), Poland 44% (Kobak and Pilarczyk, 2012), Mexico 60%(Ojeda-Robertos *et al.,* 2017).

In the current study, parasitic infection showed higher in adult in comparison to young significantly higher ($p<0.05$) with both protozoan and helminths parasites. This findings is congruent with the results from Romania (Bărburaș *et al.*, 2021) and Indonesia (Saukhan *et al.*, 2022) while it is constrast with the result from India (AnandaRao *et al.*, 2020) and Sri Lanka (Gunathilaka *et al*., 2018).

The diversity of parasitic species was found slightly different in young and adult (9 species versus 10 species). In both age group protozoan was the most prominent parasitic which includes *Eimeria* and *Entamoeba* and helminths; *Fasciola*, *Paramphistomum*, *Eurytrema*, *Strongyle*, *Ascaris*, *Nematodirus*, *Capillaria* observed in both population except the *Monienzia* only in adult. Besides *Ascaris* and *Capillaria* other helminths prominently found in adult. The prevalence rate of helminths was higher in adult which close with the findings from Bangladesh (Saha *et al.,* 2014) and constrast with the result from Indonesia (Nurhidayah *et al.*, 2019). Among helminths overall prevalence of *Fasciola* was found 7% which was lower than recorded by (Ahmad, 2023) 20% from Pakistan.

During the study, samples was collected from three wards i.e., 3,5 and 7 of Malarani rural municipality, Arghakhanchi. Out of these the parasitic prevalence was found high

in ward 7 in comparasion to ward 3 and 5. Although the parasitic distribution among the wards found insignificant ($p>0.05$).(Saha *et al*., 2018) recorded the prevalence of *Fasciola* with three different location found insignificant which support the present study.

The present study recorded the prevalence of parasitic species with wards. Among the wards *Eimeria* and *entamoeba* found highest in ward 3. Comparatively *Eimeria* found higher in ward 5 but *Entamoeba* found higher in ward 7. *Fasciola* and *Paramphistomum* found higher in ward 7 but *Eurytrema* in ward 5. Likewise, Nematodes; *Strongyle*, *Ascaris*, *Nematodirus*, *Capillaria* was also recorded high in ward 3 in comparasion to others but strongyle in ward 7. However, cestode *Monienzia* were only conformed in ward 5.

Regarding the class wise distribution of parasites, the present study recorded the highest prevalence with protozoa 42.50% fallowed by nematode37%, trematode15.50% and cestode 1%. This findings is concordant with the findings of India (Thakre *et* al., 2019) and contradict with the result of Pakistan (Khan *et al.*, 2023) and Nepal protozoa 35%, nematode 38.75%, trematode 11.82% (Gupta, 2017).

Notably, the current investigation only found one cestode species in buffaloes, *Moniezia*. The prevalence of this tapeworm is 1%.was lower than Malaysia's 1.10%, (Zainalabidin., *et al,* 2019) and Mexico's 18.1% (Ojeda-Robertos., *et al* 2017) but similar to finding of Nepal 1% (Mukhya,2007.)

The present study discovered concurrency of parasites, most of the buffaloes showed double infection 30.5% followed by single 22.5%, triple 7% and multiple 2.5% similar with the finding of (Adhikari and Dhakal, 2023).

The examination of faecal samples showed that the intensity of parasites most abundantly found in the light infection subsequently followed by moderate infection and heavy infection. The parasites like *Entaomeb*a 32 (40.50%), *Eimeria* 19 (36.53%) and *Ascaris* 18 (36.73%) was found in case of light infection. Similarly, the parasites like *Entaomeba* 38 (48.10%), *Ascaris* 29 (59.18 %) and *Eimeria* 16 (30.76%) was found in the case of the moderate infection and the parasites like *Entaomeba* 9 (11.39%) *Eimeria* 7 (13.46%) and *Ascaris* 2 (4.08%) was found in case of heavy infection respectively. Since, the parasites species like *Entaomeba*, *Eimeria* and *Ascaris* are the most responsible intensifying agents to cause the infection.

6. CONCLUSION AND RECOMMENDATION

6.1 CONCLUSION

In the present study, the overall prevalence of gastrointestinal parasites of buffalo (*Bubalus bubalis*) was found to 62.5% with male 4% and female 58.5%. The parasitic infection of protozoa was found to be 42.5% followed by nematode 37%, trematode 15.50% cestode 1%. Ten different parasitic species were revealed in the buffalo of Malarani rural municipality such as *Eimeria* and *Entamoeba* among the protozoa; *Fasciola*, *Paramphistomum*, *Eurytrema* among trematode; Strongyle, *Ascaris*, *Nematodirus* and *Capillaria* among nematode and *Moniezia* among cestode. Out of all these identified GI parasites, *Eimeria* and *Entamoeba* showed the highest prevalent rate in buffalo. The parasites like *Entaomeba* 40.50%, *Eimeria* 36.53% and *Ascaris* 36.73% were found to be the most intensifying agent to cause the light infection and moderate and heavy infection. The study indicated that buffalo of Malarani rural municipality were highly infected with GI parasites. Therefore, it is urgently need to control the parasitic infection in buffalo of Malarani rural municipality.

6.2 RECOMMENDATIONS

- Anti-helminth medications program need to be conducted through the veterinary professionals.
- Instead of allowing the animal to drink from diverse water sources because water is the primary source of contamination for different GI parasites, purified water should be provided.

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APPENDIX I

Photo 1: ***Nematodirus*** **spp. (57.6/175.2µm)**

Photo 2: Strongyle (52.8/68.4µm)

Photo 3: ***Fasciola*** **spp. (148/67.2µm)**

Photo 4: ***Paramphistomum*** **spp. (144/72µm)**

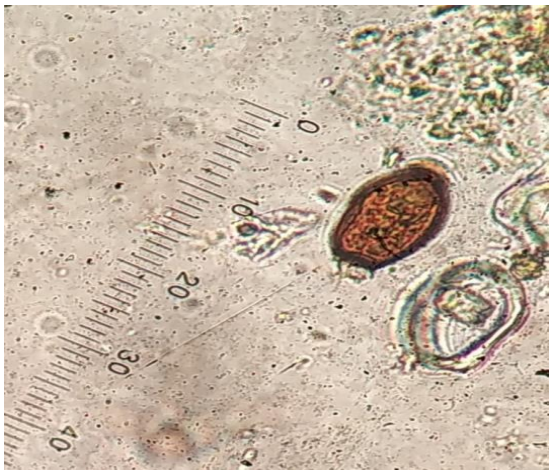

Photo 5: ***Capillaria*** **spp. (31.2/16.8µm)**

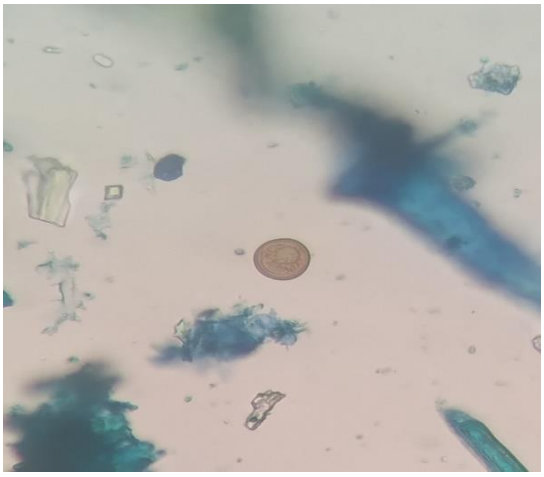

Photo 6: ***Entamoeba*** **spp. (60µm)**

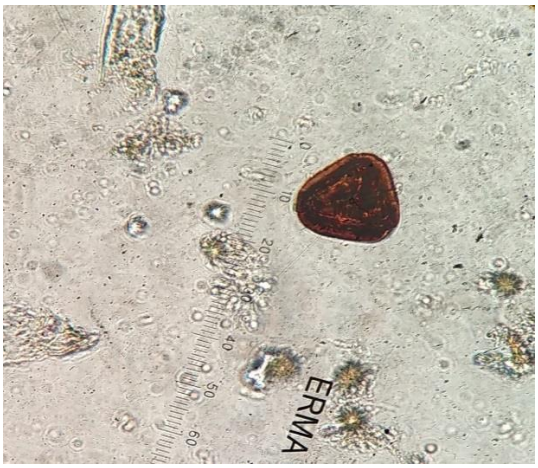

Photo 7: *Moniezia* spp. (43.2µm)

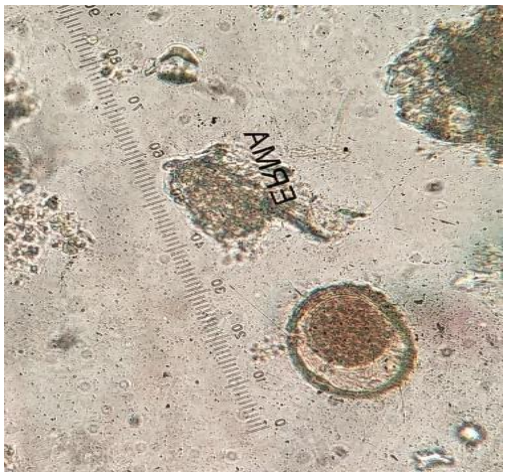

Photo 8: *Ascaris* spp. (60/48µm)

Photo 9: *Eimeria* spp. (50.4µm)

Photo 10: *Eurytrema* spp. (36/21.6µm)

APPENDIX II

Photo 11: Microscopic examination

Photo 12: Sample collection

Photo 13: Questionnare survey

Photo 14: Concentration method

APPENDIX III

Questionnaire Form

Gastrointestinal Parasite Surveillance in Domestic animal (buffaloes) in Malarani rural municipality, Arghakhanchi, Nepal

Name of owner: ……...

Sample Code: …….

Address: ……...

Date: ……...

Education level: …….

Major occupation: …….

1. How many buffaloes do you have?

……

2. Species/ages of buffaloes?

……

3. Which sources of water do you use for buffaloes?

……

4. Do you have any knowledge about parasite?

…….

5. If yes, mention the name and their effects?

……..

6. Do you see any parasites on dung?

…….

7. Do you call a veterinary doctor when buffaloes get sick?

……

8. Do you believe in dhami/jhakri?

……

9. Did you use any anti-parasitic medicines before 6 months?

……

10. Do you use the gloves while caring the buffaloes?

……

11. What is the purpose of rearing buffaloes?

……