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Date:	2022-1-12
	2025-4-25

**Gastro-Intestinal Parasitic Infections and Risk Factors in
Buffaloes Under Different Farming Systems in Butwal, Nepal**

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A dissertation submitted

**In partial fulfilment of the requirements for the award of the degree
of Master of Science in Zoology with special paper Parasitology**

April 2025



Gastro-Intestinal Parasitic Infections and Risk Factors in Buffaloes Under Different Farming Systems in Butwal, Nepal

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April, 2025

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April 2025

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Citation: Neupane, A. (2025). *Gastro-Intestinal Parasitic Infections and Risk Factors in Buffaloes Under Different Farming systems in Butwal, Nepal* (MSc dissertation). Central Department of Zoology, Tribhuvan University.

Declaration

I hereby declare that the work presented in this dissertation "Gastro-Intestinal Parasitic Infections and Risk Factors in Buffaloes Under Different Farming Systems in Butwal, 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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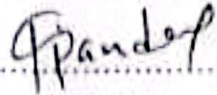
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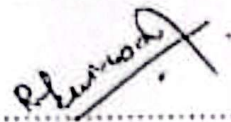
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This dissertation work submitted by Arti Neupane entitled "Gastro-Intestinal Parasitic Infections and Risk Factors in Buffaloes Under Different Farming Systems in Butwal, Nepal" has been accepted as a partial fulfillment for the requirements of a Master's Degree of Science in Zoology with special paper Parasitology.

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Acknowledgments

I would like to express my heartfelt gratitude and respect to my Supervisor, Dr. Kishor Pandey, Associate Professor, Central Department of Zoology, TU, Kirtipur, Kathmandu, and co-supervisor Dr Rajendra Parajuli, UCSD, California, USA for their expert guidance, encouragement, and helpful recommendations for the completion of my thesis work. I am grateful to respected Professor Dr. Kumar Sapkota, Head of the Central Department of Zoology, for providing the facilities needed for this research project, and the Nepal Veterinary Council (NVC) for providing ethical approval to proceed the thesis work. It is my pleasure to thank my friend Shristi Bhandari for her support throughout the field study.

I would like to express my gratitude to Dr. Akio Hakamata for the establishment of microscopic unit in the Department. My special thanks to all teaching and non-teaching staff of the Central Department of Zoology, TU, Kirtipur, Kathmandu. I am grateful to Mr. Hari Subedi and my honorable parents for their continuous support and encouragement throughout my academic career.

Abstract

Infections caused by gastro-intestinal parasites (GIPs) are a major hurdle in livestock farming, compromising animal performance and contributing to financial strain for farmers. This study investigates GIPs prevalence and associated risk factors in buffaloes under commercial and subsistence farming systems in Butwal Sub-Metropolitan City, Nepal, a region witnessing rapid growth in dairy production and where livestock contributes significantly to livelihoods and the economy. From July to September, 2023, a total of 224 fecal samples (112 from each farming systems) were collected from female buffaloes aged ≥ 3 years and analyzed microscopically for parasite identification. Associated factors were obtained all together using structured questionnaires and field observations. Results revealed an overall GIP prevalence of 58.9%, with protozoan (*Entamoeba* spp., *Balantidium* spp., coccidian), nematode (*Strongyloides* spp., *Toxocara* spp., *Strongyle* spp.), and trematode (*Fasciola* spp., *Paramphistomum* spp., *Schistosoma* sp.) infections observed. *Fasciola* spp. (30.8%) and *Entamoeba* spp. (26.3%) were the most prevalent parasites. Commercial farms exhibited significantly higher nematode infections (10.7% vs. 2.7%, $p=0.029$), while subsistence farms had marginally higher protozoan infections (33.9% vs. 25.9%). Risk factors such as free-ranging rearing style [adjusted odd ratio (aOR) =3.11, 95% CI:1.15–8.43] and irregular health checkups (aOR=2.92, 95% CI:1.05–8.12) were strongly associated with infections. Farm management practices, including bedding type, water sources, and treatment protocols, also influenced infection rates. The findings highlight critical gaps in parasite management across both systems. Targeted interventions such as rotational grazing, structured deworming protocols, and farmer education on zoonotic risks are urgently needed to enhance productivity and reduce economic losses. This study provides actionable insights for policymakers and farmers to optimize buffalo health in Nepal's evolving dairy sector.

Keywords: Buffalo, gastrointestinal parasites, prevalence, risk factors, farming systems, Butwal, Nepal

शोध सारांश

आन्द्रामा बस्ने परजीवीहरू (Gastro-intestinal parasites, GIPs) बाट हुने सङ्क्रमण पशुपालनमा ठूलो चुनौती बनेको छ, जसले जनावरको स्वास्थ्य र उत्पादन क्षमतामा असर पार्छ र किसानलाई आर्थिक रूपमा हानि गर्दछ। यो अध्ययनले दुग्ध उत्पादनमा तीव्र वृद्धि हुँदै गएको र जनजीवन तथा अर्थतन्त्रमा पशुपालनको महत्वपूर्ण योगदान रहेको बुटवल उप-महानगरपालिका क्षेत्रका व्यवसायिक (commercial) तथा पारिवारिक स्तरका (subsistence) भैंसी फार्महरूमा GIPs को सङ्क्रमण दर र त्यससँग सम्बन्धित जोखिम कारकहरूबारे अनुसन्धान गरेको छ। सन् २०२३ को जुलाई देखि सेप्टेम्बर सम्म, ३ वर्ष वा सोभन्दा बढी उमेरका पोथी भैंसीहरूबाट २२४ वटा दिसाको नमुना सङ्कलन गरिएको थियो (commercial र subsistence फर्मबाट समान रूपमा ११२/११२) र परजीवी पहिचानका लागि सूक्ष्मदर्शीको प्रयोग गरेर परीक्षण गरिएको थियो। सम्बन्धित जोखिम कारकहरू व्यवस्थित प्रश्नावली र फिल्ड अवलोकनको माध्यम ले सङ्कलन गरिएको थियो। अध्ययनमा कुल प्रकोप ५८.९% रहेको देखियो, जसमा प्रोटोजोआ (*Entamoeba* spp., *Balantidium* spp., coccidian), नेमाटोड (*Strongyloides* spp., *Toxocara* spp., *Strongyles* spp.) र ट्रेमाटोड (*Fasciola* spp., *Paramphistomum* spp., *Schistosoma* sp.) संक्रमणहरू पाइयो। *Fasciola* spp. (३०.८%) र *Entamoeba* spp. (२६.३%) सबैभन्दा बढी देखिएका परजीवीहरू थिए। commercial फर्ममा नेमाटोडको संक्रमण उल्लेखनीय रूपमा बढी देखियो (१०.७% विरुद्ध २.७% $p = 0.029$), भने subsistence फर्ममा प्रोटोजोआ संक्रमण थोरै बढी देखियो (३३.९% विरुद्ध २५.९%) फर्ममा भैंसीलाई खुला चराउने अभ्यास (aOR=3.11, 95% CI: 1.15-8.43) र अनियमित स्वास्थ्य परीक्षण जस्ता जोखिम कारकहरू (aOR=2.292, 95% CI: 1.05-8.43) संक्रमणसँग बलियो रूपमा सम्बन्धित भएको पाइयो। फर्ममा प्रयोग हुने ओछ्यानको प्रकार, पानीको स्रोत र उपचार गर्ने तरिका जस्ता व्यवस्थापन प्रणालीहरूले पनि सङ्क्रमणको दरमा प्रभाव पार्ने देखिएको छ। अनुसन्धानको निष्कर्षले commercial र subsistence दुवै प्रणालीमा परजीवी नियन्त्रणमा गम्भीर कमजोरीहरू रहेको देखाएको छ। उत्पादन वृद्धि गर्न र आर्थिक नोक्सानी घटाउनका लागि चरणबद्ध चरन, व्यवस्थित जुकानाशक (antihelmintic) को प्रयोग, र किसानहरूलाई जनावरबाट मानिसमा सर्ने रोगहरूको जोखिमबारे चेतना दिने जस्ता लक्षित उपायहरू तत्काल आवश्यक छन्। यो अध्ययनले नेपालमा विकासोन्मुख दुग्ध उत्पादन क्षेत्रमा भैंसीको स्वास्थ्यमा सुधार गर्न नीतिनिर्माताहरू र किसानहरूका लागि उपयोगी सिफारिसहरू पनि प्रस्तुत गरेको छ।

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List of abbreviations

Abbreviated form	Details of abbreviations
AGDP	Agricultural gross domestic product
aORs	Adjusted Odd Ratios
CI	Confidence Interval
GDP	Gross domestic product
GI	Gastrointestinal
GIPs	Gastro-intestinal Parasites
IOST	Institute of Science and Technology
IPIs	Intestinal Parasitic Infections
ml	Milliliter
NaCl	Sodium Chloride
NHRC	Nepal Health Research Council
NS	Not Significant
NVC	Nepal Veterinary Council
ORs	Odd Ratios
SPSS	Statistical Package for Social Sciences

1. Introduction

1.1 Background

Buffalo has been domesticated in Nepal and India for milk and meat, but now buffalo farming is almost a worldwide phenomenon. In Nepal, with the demand for buffalo meat and dairy products, commercial buffalo farming is increasing rapidly, and more entrepreneurs are involved in the dairy industry. The population of Buffaloes in Nepal is about 5.16 million, of which 23.95% is in Lumbini province with the highest population distribution in Nepal (Ministry of Agriculture and Livestock Development, 2023). Although Butwal is a Sub metropolitan city and a growing commercial hub, buffalo has always been an important component of livestock production for rural inhabitants of inner Butwal. Buffaloes are major contributors of milk, meat and compost manure in Nepal (NAFS, 2010). In Nepal, agriculture contributes around 24.12% to Nepal's total (Agricultural gross domestic product) AGDP while the livestock sector accounts for approximately 24.01% of AGDP, of which livestock contributed about 24.01% (Ministry of Agriculture and Livestock Development, 2023). Buffalo milk production contributes 7.28% whereas buffalo meat accounts for 4.23% of Nepal's total AGDP (Ministry of Agriculture and Livestock Development, 2023). With this rising demand for dairy products and meat many farmers are encouraged towards livestock farming in both small-scale subsistence and larger commercial farms. Smallholder farmers, basically in rural areas, often rear one to three buffaloes and feeding them with locally available resources like grass and crop residues also allowing them to graze freely in open areas such as fields, forests and riverbanks. This traditional approach supports the daily needs of subsistence farming families and requires minimal external inputs. On contrary, some farmers, including a few smallholders and larger commercial operators, rear buffaloes in confined spaces where they provide all their feed and fodder without allowing them to graze openly. This method is less common but is often chosen by commercial farmers aiming for higher productivity and efficiency.

Gastro-intestinal parasites (GIPs) are organisms that inhabit the gut, body cavity, liver and other internal organs of the host's body which includes nematodes, cestodes, trematodes and protozoan (Soulsby, 1982). GIPs in buffalo can cause inflammation of the intestinal wall leading to indigestion, malabsorption, diarrhea, and nutritional competition (Thapa Shrestha et al., 2020) causing retarded growth and loss of milk productivity (Harizt et al.,

2021) causing economic loss to the farming communities (Jyoti et al., 2012). Globally, parasites like *Schistosoma* spp., *Dictyocaulus viviparus*, *Strongylidae*, *Haemonchus* spp., *Cooperia* spp., *Ostertagia* spp, *Eimeria* spp, *Buxtonella sulcata*, *Oesophagostomum*., *Trichostrongylus* spp., *Fasciola* spp, *Paramphistomum* spp, *Entamoeba* spp., *Balantidium* spp. and *Cryptosporidium* spp. are found to be the common GI parasites infecting the Buffaloes (Thapa Shrestha et al., 2020). Previous studies reported a wide range of prevalence ranging from 35% (Rast et al., 2012) to 100% (Raza et al., 2007, Akande et al., 2021) of GIPs in different countries like Malaysia (Harizt et al., 2021) , China (Liu et al., 2009). Frequent practices of grazing buffaloes in open areas (Khan et al., 2010), anthelmintics administration frequency (Elelu et al., 2018), rearing styles. (Raza et al., 2007; Roberts et al., 1990) and not undergoing periodic health check up of buffalo leads to more gastro-intestinal infections (Raza et al., 2007).

The Climate (tropical temperatures, humidity) in the district and poor sanitation exacerbate parasite proliferation, with zoonotic species like *Cryptosporidium* threatening public health (Titcomb et al., 2021). The parasites' life cycle starts when it consumes infected grass, feed, and fodder. Furthermore, several animal organs go through distinct developmental phases (Soulsby 1982). Subsequently, the organism exhales parasite eggs or other forms, leading to a new infection or infestation. The parasite invades different cells and tissues inside the animal's body, eventually lowering the cattle's productivity (Regmi et al., 2021). The prevalence of GIPs can largely be determined by some ecological conditions such as the river nearby the community (Khan et al., 2021), temperature (Alam et al., 2016) humidity, and precipitation (Harizt et al., 2021), altitude and existence of the open sewage near pastureland (Shaw et al., 1997). In addition, within an environment, the microenvironment of the farm (i.e., source of grass, water, sanitation of shed, etc.) was suggested to be associated with the prevalence of GIPs (Roy et al., 2016). Earlier studies reported Fasciolosis and Paramphistomosis in adults and Ascariasis in buffalo calves (Joshi et al., 2013).

1.2 Statement of the problem

Parasitic infections may cause serious issues in cow and buffalo calves (60%) (Yousaf et al., 2021). This issue causes economic burden to the farm holder. The infection with the gastro-intestinal parasite is also associated with the proper growth and health of the buffaloes. The intestinal parasite-related illness is an important barrier to the production of

live animals. It raises mortality, which has an impact on rural communities' income. It's important to regularly track the prevalence of intestinal parasite infections in a particular setting and how they relate to risk factors that influence the spread of parasites. An estimated 0.72 to 84.1% of people worldwide are thought to be infected with gastrointestinal parasites (Khan et al., 2021). Yet, we are unaware of any report that evaluated the GIPs burden in Buffaloes of commercial farms and subsistence farms of Rupandehi district. This is the first attempt to evaluate the GIPs burden in buffaloes and the association of different farming systems (i.e., commercial farms and subsistence farms) and associated risk factors in Butwal Sub-Metropolitan City.

1.2.1 Research Question

- What is the prevalence of GIPs in buffaloes under commercial versus subsistence farming systems in Butwal?
- Which demographic, farm management, and health management factors are associated with GIP infection in these systems?

1.3 Objective of the study

1.3.1 General objective

- To compare the prevalence and associated factors of GIPs in buffaloes from subsistence and commercial farms of Butwal Sub-metropolitan city, Rupandehi, Nepal.

1.3.2 Specific objectives

- To compare the prevalence of GIPs in Buffaloes of commercial and subsistence farms.
- To identify the associated factors (demographics, farm management and health management) affecting the GIPs in Buffaloes in commercial and subsistence farms.

1.4 Hypothesis

- As subsistence farms may have poor sanitation of sheds, lack of regular check-ups, and lack of nutritious diet, the prevalence of GIPs in subsistence farms can

be relatively higher compared to commercial farms.

- Further, buffaloes from subsistence farms might be fed with grass from a nearby river or stream with snails (i.e., intermediate host of trematodes) causing more trematode infection compared to buffalos from commercial farms.
- On the contrary, commercial farms may have elevated GIPs because of cross-transmission because a large number of buffalos are kept in the same shed.

1.5 Significance of the study

With the increased popularity and selling price of buffalo milk compared to cow's milk, recently the commercial farms of buffalo are rapidly increasing in Butwal Sub-metropolitan city. Further, compared to cows, buffalos can be sold for meat after milk purposes or when they get old. This study conducted comparative studies of GIPs burden and factors associated in two different types of rearing system. This study will evaluate the feeding pattern, management of the shed and farms, status of medications and its implications in disease transmission among them. The research data will also be helpful for commercial entrepreneurs and farmers in controlling the factors affecting parasitic infections. The research data will also be submitted to the local government to facilitate the formulation of a strategy for preventive measures of diseases in buffaloes and if any GIPs of zoonotic importance, their possible transmission to the human community.

1.6 Limitations

This study has a few limitations. First, we could only collect samples from 224 buffaloes due to limited accessibility. Secondly, since the study focused only on Butwal, the findings might not apply to other parts of Nepal where farming practices or environments differ. Third, the lab methods we used (like fecal floatation) are good for detecting common parasites, but they might miss some that are present in very small amounts. Finally, when farmers told us about their deworming routines or farm practices, they might not have remembered everything perfectly, which could slightly affect the accuracy of the risk factors we identified.

2. Literature review

Parasites are living entities that depend on host for shelter, food and their metabolic activities. Parasites on the other hand originated from their free-living ancestors and they evolved along with their hosts. The association between parasites and their host is known as parasitism which poses major threat to ruminants. Parasites can directly influence host survival and reproductive success by causing pathological damage, such as blood loss, tissue injury, spontaneous abortions, congenital abnormalities, and even mortality. Additionally, they may weaken the host's immune system by compromising overall physiological health (Thawait et al., 2014).

Among ruminants, diseases in buffaloes have been identified as one of the major problems that cause the downfall of the dairy industry causing substantial economic loss to the farmers in the developing countries (Jyoti et al., 2014). Endoparasites are organisms that inhabit the gut, body cavity, liver and other internal organs of the host's body. Nematodes, cestodes, trematodes and protozoan all include endoparasites invading buffalo hosts (Soulsby, 1982). This section reviews key published studies relevant to gastrointestinal parasitic infections and their associated risk factors in buffaloes.

2.1 Prevalence of gastro-intestinal parasites in commercial and subsistence farms

Globally, gastro-intestinal parasite (GIP) prevalence in buffaloes reveals significant disparities between subsistence and commercial farming systems, particularly in developing countries with tropical and sub-tropical regions where climatic conditions favor parasite proliferation. A study in Punjab of India reported 73.58% GIP prevalence in buffalo calves, dominated by *Eimeria* spp. (54.55%) (Jyoti et al., 2012). In Sarawak, Malaysia, 75.2% *Paramphistomum* sp., 52.7% strongyles, and 48.1% coccidia were identified in buffaloes (Harizt et al., 2021). Studies from Sri Lanka (Gunathilaka et al., 2018) reported 31.25% GIP prevalence in buffaloes, contrasting with 100% prevalence in Nepal's hilly regions (Magar et al., 2023), where Multi parasitism (2–7 species) was statistically dominant.

Subsistence farms notably reported higher parasitic burdens with prevalence ranging from 65% - 100% in many countries like India, Nepal, Tanzania, Bangladesh, Pakistan, Kenya, Nigeria, Poland, and Mexico. A study among small holder buffaloes in India (Jyoti et al.,

2014) and Tanzania (Swai et al., 2013) reported 68% of *Paramphistomum* and 70% prevalence of helminthiasis with *Cooperia* sp. dominating respectively. In Terai region of Nepal, 62.7% prevalence in buffaloes lagged behind cattle (68.8%) (Patel et al., 2025) though semi-captive systems reported 100% infection rates with 30 parasite species prevalence of *Eimeria* from Chitwan, Nepal (Adhikari et al., 2022). According to study from Egypt only 28% of *Eimeria* was found prevalent (El-Alfy et al., 2019).

African and Asian countries reported higher trematode infections in subsistence farms due to wetland grazing. On the contrary, Brazil reported 22% prevalence of *Fasciola* due to strategic anti-helminthic use. Large dairy farms in Punjab, Pakistan showed 38% GIP prevalence (Yousaf, 2021). Commercial farms from Italy, Canada and Bulgaria reported <10%, <5% cryptosporidium and 12% prevalence of *Eimeria* respectively. The earlier literature has shown that cestode infections in buffaloes are not as common as nematode and trematode infections. Some researchers from Bangladesh indicated its presence in buffaloes (Roy et al., 2016) while the absence of cestode by others (Cringoli et al., 2009; Mamun et al., 2011; Multan et al., 2021; Saha et al., 2014; Sreedevi, 2014) Commercial farms shows lower prevalence comparatively due to rotational grazing, routine antihelminthic administration and improved housing systems.

2.2 Risk factors of gastro- intestinal parasitic infection

Several factors influence the prevalence and intensity of GIPs in buffaloes. These factors can be categorized into demographics, farm management practices, and health management strategies. The most common associated risk factors of gastrointestinal parasites in buffaloes are shared grazing lands and poor hygiene according to Swai et al., (2013) from Tanzania. The prevalence of GIPs may vary based on the age, sex and physiological status of buffaloes. Studies indicate that younger animals are more susceptible to parasitic infections due to underdeveloped immunity (Sharma & Joshi, 2020). Pregnant and lactating buffaloes are also at higher risk due to physiological stress and increased nutritional demands. Farms with smaller grazing areas exhibited a higher prevalence of *Strongyle* (70.5%), and *Paramphistome* (88.6%) compared to farms with a larger grazing area (43.5% and 68.2%), respectively (Harizt et al., 2021). Prevalence of *Strongyle* was lower (45.6%) in farms that did not implement a cut-and-carry system compared to those that did (64%) while the prevalence of *Paramphistome* was higher on farms with more than 40 animals (80.6%) compared to farms with fewer animals (Harizt et al., 2021).

Few earlier studies suggested that “not the nutritional status of buffalos” but the farm management, sex, age and watering systems as significant contributors of GIPs in buffalos. (Khan et al., 2010; Raza et al., 2007; Rehman et al., 2016; Thapa Shrestha et al., 2020).

Farm hygiene, housing conditions, feeding strategies, and grazing systems play a significant role in GIP prevalence. Buffaloes raised under extensive grazing systems are more prone to parasite infections compared to those in intensive or semi-intensive systems. Studies have shown that buffaloes kept in confined spaces with proper waste disposal and controlled feeding have lower GIP infections than those grazing in contaminated pastures (Shrestha et al., 2019). Regular deworming, veterinary check-ups, and proper medication significantly reduce the prevalence of GIPs. Research indicates that farmers who follow scheduled deworming programs report lower GIP burdens compared to those who do not (Harizt et al., 2021). Additionally, the use of ethnoveterinary medicines has been explored as an alternative approach in rural farming communities where access to veterinary services is limited.

Climate, temperature, humidity, and rainfall significantly influence the survival and transmission of GIPs. Warmer and more humid conditions provide an ideal environment for parasite eggs and larvae to thrive, increasing infection risks in grazing buffaloes (Tiwari et al., 2021; Gupta et al., 2023). Inadequate waste disposal and unhygienic living conditions create a favourable environment for parasite transmission. Farms with poor drainage and excessive manure build up often report higher GIP burdens (Singh et al., 2022). Free-range grazing increases exposure to contaminated pastures, while improper feed storage can lead to ingestion of parasite-infested fodder. Overcrowding also elevates transmission risks among buffaloes (Rai et al., 2021). Farms without routine deworming programs show a significantly higher prevalence of parasitic infections. Irregular or ineffective anthelmintic use contributes to resistance development in parasites, further complicating control efforts (Maharjan et al., 2020). Calves and older buffaloes with weaker immune systems are more susceptible to infections. Immunosuppressive conditions such as malnutrition and concurrent diseases intensify the susceptibility (Thapa Shrestha et al., 2020), limited access to veterinary services and lack of farmer awareness about GIP control strategies contribute to increased infection rates, especially in rural and subsistence farming systems (Dhakal et al., 2022).

3. Materials and methods

3.1 Study area

The study was conducted in Butwal Sub-Metropolitan city located within Rupandehi district, Lumbini province in the Mid-western part of Nepal (Figure 1) covering almost 101.61km² land and is divided into a total of 19 wards with overall population of 195054 (CBS, 2022) The city stands beside the bank of Tinau River and at the northern edge of the Terai plain below the Siwalik Hills where the landform is plane. It ranges in the altitudes 130-150 meters and lies between the coordinates 27.70°N., 83.46°E. Butwal lies at the intersection of Nepal's two National Highways, Mahendra Highway and Siddhartha Highway and 31.1 km north of India. It is surrounded by Devdaha Municipality in the east, Tilottama Municipality and Sudhodhan VDC in the south, Sainamaina Municipality in the west and Tinau VDC in the north. (Figure 1). Butwal is mostly a commercial city, but agriculture and animal rearing are also an accepted occupation by the people living there. Few people rear buffaloes and cows for commercial purposes while some of the farmers from the village area also rear buffaloes in their small local farms for their livelihood. Butwal was selected due to its mixed farming systems (commercial and subsistence) also its proximity to wetlands (Tinau River), and climatic suitability for parasites.

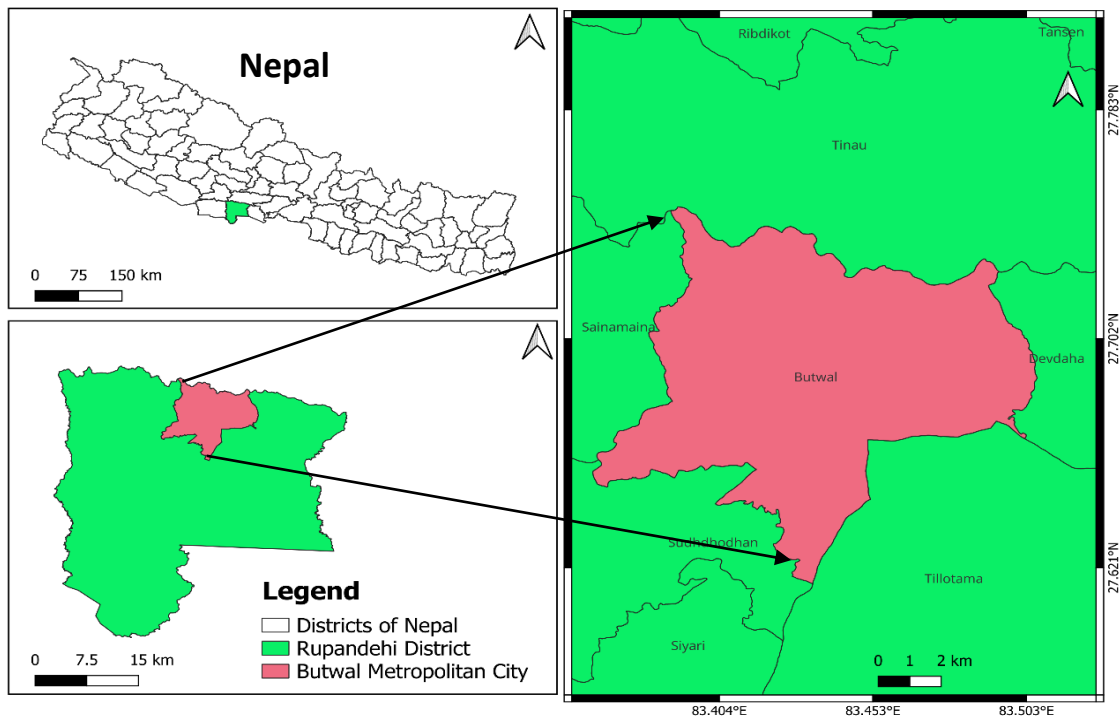


Figure I. Map representing Butwal sub-metropolitan city as a study area.

3.2 Study design

The farms were purposively selected collecting a total of 224 samples which were brought to Central Department of Zoology for microscopic analysis and later statistical analysis using SPSS (Figure 2).

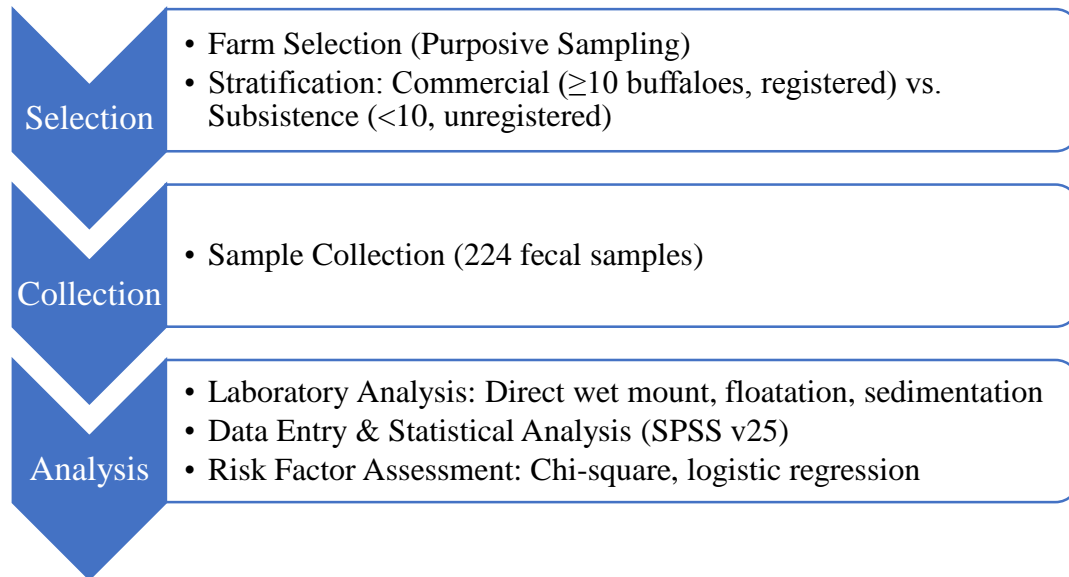


Figure 2. Flowchart representing methodology

3.2.1 Sampling method

A purposive sampling method was used, with farms having more than 10 buffaloes, who trade buffaloes commercially and registered in the municipality taken as commercial farms. While farms with less than 10 buffaloes and not registered in the municipality, only used for family livelihood, were taken as subsistence farms.

3.2.2 Selection criteria

Only female buffaloes aged above three years that have not been given antihelminth prior 3 months of the sample collection were selected. Also farms that keep up to date records on buffaloes' health were only selected for commercial farms.

Buffaloes less than three years old, males, recent deworming (< 3 months), and farms lacking health records (commercial only). were excluded.

3.2.3 Sample size

Since we focused on comparative study, a total of 224 samples were collected from nine

wards (Ward 11 to Ward 19) of the municipality with 112 from commercial farms and 112 from subsistence farms. Two commercial farms were selected from each of the seven wards. Only one commercial farm was taken for the study from ward 11 and 17 because of availability, making a total of 16 farms. From each farm, only seven freshly (i.e., 7 samples from each 16 farms producing 112 samples) voided dung samples were collected.

For the subsistence farms, seven households located near each of the selected 16 commercial farms were approached, and one freshly voided buffalo dung sample was collected from each household. This approach ensured an equal number of samples (112) from subsistence farms for ease of data analysis. The sample size was calculated using the formula for proportions:

$$n = \frac{Z^2 p(1 - p)}{E^2}$$

Assuming a 95% confidence level ($Z=1.96$), 50% prevalence ($p=0.5$, conservative estimate), and 10% margin of error ($E=0.1$), the minimum sample size was 97 per group. Adjusted to 112 per group (224 total) to account for non-response.

3.2.4 Ethical considerations

This study was approved by the Nepal Veterinary Council Ethics Committee (Approval No: 82/2080/81). All research activities were conducted in adherence to the approved protocols (Appendix 2). Informed consent was obtained from all farm owners before their involvement in the study. Confidentiality and anonymity were ensured, and participants had the right to withdraw at any stage without consequences.

3.2.5 Collection and preservation of sample

During July and September of 2023, farms were visited. Each farm owner was informed about the study objective and process. The farm owners were asked for their consent and requested to sign the informed consent once agreed. Around 10-15 gm of fresh fecal samples were collected from the ground without contaminating using gloves and spatula in screw-capped vials. The collected samples were kept in 2.5% potassium dichromate solution to preserve the parasites providing sample code. Other demographic criteria like farm management systems and health management systems were also recorded in the questionnaire (Appendix 1). Each questionnaire was given the same code as the sample code for the vial. The samples were then brought to the Central Department of Zoology,

TU, Kirtipur for further analysis.

3.3 Microscopic examination

The samples were examined in the laboratory of the Central Department of Zoology, Tribhuvan University, Kirtipur, Nepal. The ova/cyst/oocyst and larva of different parasites were examined and identified according to shape, size and color as seen in the iodine wet mount and concentration methods (Floatation and sedimentation). (Soulsby, 1982). All the samples were examined using Olympus CX40 optical microscope.

3.3.1 Direct wet mount

About 2 spatulas of fecal samples were stirred carefully. A drop of sample without Lugol's iodine and with Lugol's iodine was placed on a glass slide and covered with a cover slip. The samples were then examined using a microscope under 10X and 40X magnification (Soulsby, 1982).

3.3.2 Concentration

Eggs, cysts, and trophozoites were often in such low numbers in faces, that they are difficult to be detected in direct smears or mounts. So, these methods were performed which comprise floatation and sedimentation techniques (Soulsby, 1982).

3.3.2.1 Saturated salt floatation

To find nematode and cestode eggs this method is used. The eggs due to their small size and light weight float in the floatation liquid.

About two spatulas of fecal samples were filtered and strained into a 15 ml centrifuge tube and thoroughly mixed in 12 ml of 0.9% w/v sodium chloride (NaCl). After centrifuging the samples (1200 rpm for 5 minutes), the supernatant was discarded. The tube was again filled with 45% w/v NaCl and centrifuged at 1200 rpm for 5 minutes. The tube was removed from the centrifuge filled with saturated NaCl and left undisturbed for 15 minutes covering the rim of the tube with coverslip. The coverslip was finally removed kept on a glass slide and examined under the microscope (Adhikari & Ghimire, 2021).

3.3.2.2 Formalin-ether (FE) Sedimentation

As trematode eggs are slightly denser than other eggs and do not float in the floatation liquid, this approach primarily identifies and separates them using sedimentation.

In a 15 ml centrifuge tube, 2 spatulas of fecal samples were completely mixed in 12 ml of 0.9% w/v NaCl and centrifuged (1200 rpm for 5 minutes). The supernatant was then discarded and the tube was filled with 10 ml of 10% formalin and 3 ml of ether for centrifugation (1200 rpm for 5 minutes). After discarding the supernatant, a drop of the sediments was placed on a clean slide using a pipette and observed under microscope. (Beaver, 2017)

3.3.3 Identification

After analyzing both stained and unstained fecal slides under 10X and 40X magnification, the eggs and cyst size were measured using Olympus CX40 Trinocular Microscope. The eggs, oocysts and larvae of parasites were identified by using laboratory manual, supervisor and from their morphological characteristics like shape, size, external features and color (Beaver, 2017).

3.4 Data analysis

The findings from the questionnaire and sample analysis were compiled in Microsoft Excel 2016 for analysis. The characteristics and prevalence among buffaloes of different rearing systems were analyzed based on sociodemographic, farm management and hygiene factors. Differences between the groups (categorical data) were compared using chi-square tests /Fisher's exact tests, while the mean values were compared using an independent t-test. To investigate the association between the prevalence of overall GIPs and possible risk factors (sociodemographic, farm management and hygiene factors), bivariate and multivariate logistic regression was conducted. Factors that showed significant association in the bivariate analysis, were included in the multivariate model for mutual adjustment. Odds ratios (ORs) and adjusted ORs (aORs) with 95% confidence intervals (CIs) were calculated. The level of significance was set at $p < 0.05$. Statistical analyses were conducted using SPSS statistical software Version 25.

4. Results

The study was carried out in the commercial and subsistence buffalo farms of Butwal Sub-metropolitan city. From July 2023 to September 2023, 112 fecal samples from commercial farms and 112 fecal samples from subsistence farms were collected and analyzed.

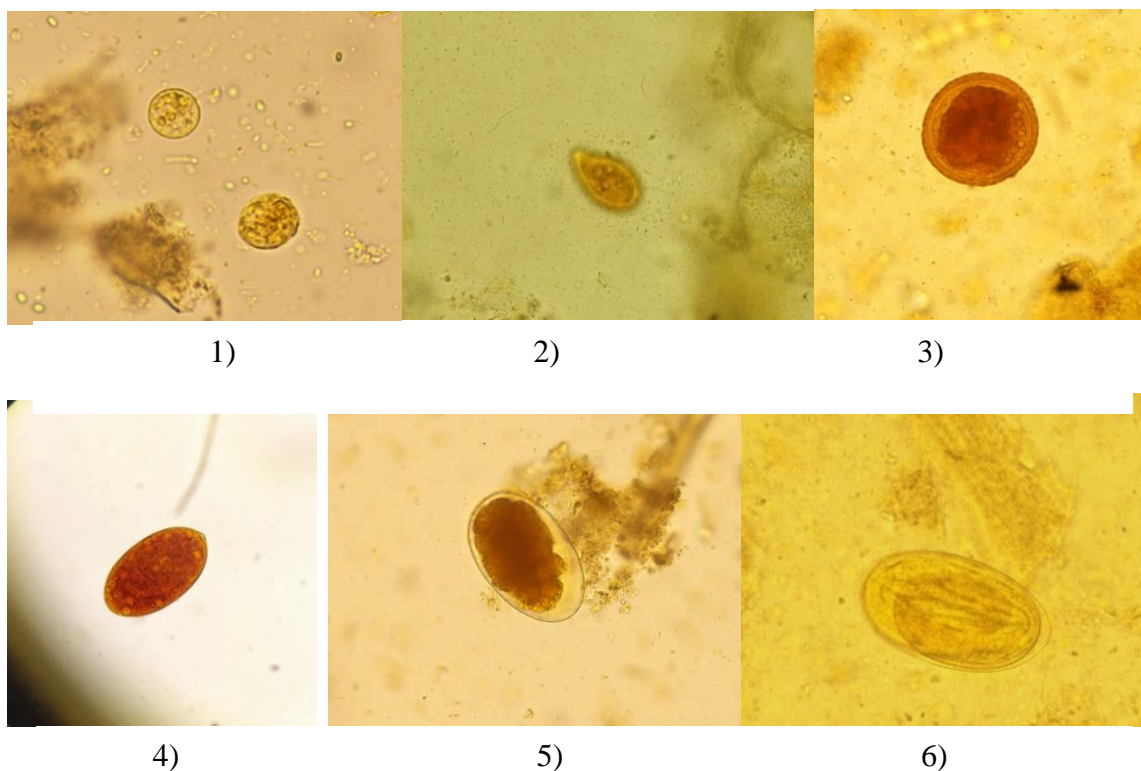
4.1 Prevalence of Gastro Intestinal Parasites (GIPs)

A total of 224 fecal samples were evaluated under the microscope with different methods as described in method. More than a half sample (i.e., 132 (58.9%) found shedding one or more species of GIPs (Table 1). Of the 9 species of parasites identified, 3 (i.e., *Entamoeba sp.*, *Balantidium sp.* and Coccidian) species belong to protozoa, 3 species to trematodes (i.e., *Fasciola sp.*, *Schistosoma sp.*, *Paramphistomum sp.*) and 3 species belongs to nematodes (i.e., *Strongyloides sp.*, *Toxocara sp.*, *Strongyle sp.*). Overall, *Fasciola sp.* has higher prevalence (30.8 %) followed by *Entamoeba sp.* (26.3%), and *Paramphistomum sp.* (12.1%). Buffalos at commercial farms indicated significantly high prevalence of nematode parasites compared to subsistence farms (Fisher exact test $p=0.029$) (Table 1). More than one third samples (i.e., 38.8%) indicated trematode parasites and while 29.9% samples indicated protozoan parasites. Four buffalo (1.8%) indicated multiple infections where one sixth samples (i.e., 17%) indicated double infections. Except for overall nematode infections, none of parasite or categories indicated any statistical difference between farm types. Overall, subsistence farms indicated more protozoan parasites and triple infections while commercial farms indicated more helminth parasites and double infections. Yet, none of such differences were statistically significant.

Table 1. Prevalence of intestinal parasites in female buffalo at commercial and subsistence farms (n = 224)

Parasite species	Commercial (n=112)	Subsistence (n=112)	Chi-Square P value	Total n (%)
Protozoan Parasites				
<i>Entamoeba</i> sp.	25 (22.3)	34(30.4)	NS*	59 (26.3)
<i>Balantidium</i> sp.	2 (1.8)	4(3.6)	NS#	6 (2.7)
Coccidian	2 (1.8)	1 (0.9)	NS#	3 (1.3)
Any protozoan	29(25.9)	38 (33.9)	NS*	67 (29.9)
Helminth Parasites				
Nematodes				
<i>Strongyloides</i> sp.	7 (6.3)	2 (1.8)	NS#	9 (4.0)
<i>Toxocara</i> sp.	3 (2.7)	1 (0.9)	NS#	4 (1.8)
Stongyle sp.	2 (1.8)	0 (0)	NS#	2 (0.9)
Any Nematode	12 (10.7)	3 (2.7)	0.029#	15 (6.7)
Trematodes				
<i>Fasciola</i> sp.	38(33.9)	31 (27.7)	NS*	69 (30.8)
<i>Schistosoma</i> sp.	0 (0)	1 (0.9)	NS#	1 (0.4)
<i>Paramphistomum</i> sp.	16 (14.3)	11 (9.8)	NS*	27 (12.1)
Any Trematode	48 (42.9)	39 (34.8)	NS*	87 (38.8)
Any infection	70 (62.5)	62 (55.4)	NS*	132 (58.9)
Single Infection	46 (41.1)	46 (41.1)	NS#	92 (41.1)
Double Infection	23 (20.5)	15 (13.4)	NS#	38 (17)
Triple Infection	1 (0.9)	3 (2.7)	NS#	4 (1.8)

* Chi-square test, # Fisher Exact test while any cell has count less than 5





7)



8)

Figure 3. i). Oocyst of *Entamoeba* sp. (25 μ m) ii). Trophozoite of *Balantidium* sp. (65 \times 35 μ m) iii). Egg of *Toxocara* sp. (80 \times 65 μ m) iv). Egg of *Fasciola* sp. (130 \times 65 μ m) (v). Egg of *Strongyle* spp. (95 \times 54 μ m) (vi). Egg of *Strongyloides* spp. (55 \times 30 μ m) vii). Egg of *Schistosoma* sp. (185 \times 45 μ m) viii). Egg of *Paramphistomum* sp. (142 \times 72 μ m)

4.2 Characteristics of buffaloes and buffalo farm management

In the study it was found that commercial farms keep mostly older buffalos than subsistence farms. Murrah breed of buffalo mostly from traders are common in commercial farms while cross breed and self-raised buffalos are common in subsistence farms. Concrete floor and tin roof with non-edible bedding are common in commercial farms while edible beddings are common in subsistence farms. Yet, status of buffalos (i.e., milking vs pregnant) and wallowing practices were not significantly different between commercial farms and subsistence farms. Commercial farms only indicated tap water as a source of water supply but subsistence farms indicated others sources too as a source of water supply. More subsistence farms reported free range practices than commercial farms as shown in table 2.

Table 2. Characteristic of buffaloes and buffalo farm management features at commercial and subsistence farms (n = 224).

Characteristics	Commercial (n=112)	Subsistence (n=112)	P-value	Total
Demographic Characteristics	Mean (SD)/n (%)	Mean (SD)/n (%)		Mean (SD)/n (%)
Age (in years)	7.38 (1.82)	6.48 (2.01)	0.000[§]	6.93 (1.97)
Other Characteristics				
Breed of buffalo				
Murrah	16 (14.3)	0 (0)	0.000[#]	16 (7.1)
Crossbred	96 (85.7)	112 (100)		208 (92.9)
Source of livestock				
Self-raised	10(8.9)	72(64.3)	0.000[*]	81 (36.3)
Brought from Traders	102(91.1)	40(35.7)		142 (63.4)
Type of Shed				
Stall barn	105 (93.8)	112 (100)	0.014[#]	217 (96.9)
Loose housing	7 (6.3)	0 (0)		7 (3.1)
Flooring and roofing of shed				
Concrete floor and tin roof	105 (100)	105 (93.8)	0.014[#]	210 (96.8)
Earth floor and tin roof	0 (0)	7 (6.3)		7 (3.2)
Use of bedding				
Non-Edible bedding	112 (100)	35 (31.3)	0.000[#]	147(65.6)
Edible bedding	0(0)	77 (68.8)		77(34.4)
Present condition of buffalo				
Pregnant	63(56.3)	57 (50.9)	NS [*]	120(53.6)
Milking	49(43.8)	55 (49.1)		104 (46.4)
Free Range				
No	84(75)	70 (62.5)	NS [*]	154(68.8)
Yes/Sometimes	28 (25)	42 (7.5)		70 (31.3)
Wallowing				
No	91 (81.3)	81 (72.3)	NS [*]	172 (76.8)
Yes	21(18.8)	31 (27.7)		52 (23.2)
Source of water				
Tap water	105(93.8)	112(100)	0.014[#]	217(96.9)
Other sources	7(6.3)	0(0)		7(3.1)

§: Independent T-test, * Chi-square test, # Fisher Exact test while any cell has count less than 5

4.3 Health management

The table below shows the health management features of female buffalo at commercial and subsistence farms. There was no significant difference between commercial and subsistence farms in health checkup frequency, antihelminth administration practices, having other free-range animals. Yet, most of the owners of commercial farms reported

knowledge about helminths, immediate response to buffalo sickness. However, commercial farms mostly depend on local pharmacist for treatment while subsistence farm mostly consulted veterinarian doctor or local method for treatment of their buffalos.

Table 3. Health Management features of female buffalo at commercial and subsistence farms (n = 224).

Practices/ Features	Commercial (n=112) n (%)	Subsistence(n=112) n (%)	P-value p <0.05	Total n (%)
Health checkup frequency				
Regularly	14(12.5)	7(6.3)	NS*	21(9.4)
When needed	98(87.5)	105(93.71)		202(90.6)
Feed antihelminth within 6 months				
Yes	10(8.9)	16(14.3)	NS*	26(11.6)
No	102(91.1)	96(85.7)		198(88.4)
Other free range animals				
Yes	56(50)	68(60.7)	NS*	124(55.4)
No	56(50)	44(39.3)		100(44.6)
Aware of worm infestation				
Yes	112(100)	100(89.3)	0.000#	212(94.6)
No	0(0)	12(10.7)		12(5.4)
Deworming frequency				
Every 6 months	21(20)	4(3.6)	0.000#	25(11.5)
When needed	84(80)	108(96.4)		192(88.5)
Use of local treatment				
Yes	2(1.8)	29(25.9)	0.000#	31(13.8)
No	110(98.2)	83(74.1)		193(86.2)
Response to sick animals				
Immediate	112(100)	49(43.8)	0.000#	161(71.9)
Wait for few days	0(0)	63(56.3)		63(28.1)
Treatment Procedure				
Local Pharmacist	28(25)	73(65.2)	0.000#	10(45.1)
Veterinarian Doctor	84(75)	39(34.8)		123(54.9)

§: Independent T-test, * Chi-square test, # Fisher Exact test while any cell has count less than 5

4.4 Parasitic infections and associated factors

Table 4 shows the association between parasitic infections and associated factors. The presence of any GIPs was higher among the free-range buffalos with reported wallowing, if owner have other free-range animal and the owner did not report regular health checks of buffalos compared to their counterparts (p <0.001) in univariate model. When all the associated factors in the univariate model were forcibly entered into the multivariate model for mutual adjustment, only free-range rearing style and lack of regular checkup practice remained still significant contributing factors for any GIPs.

Table 4. Prevalence and odds ratio of IPIs with farm practices, buffalos' characteristics using logistic regression analysis (n=224).

		Any IPIs (n=224)	
	%	Univariate OR (95% CI)	Multivariate* AOR (95% CI)
Socioeconomic (SES) Characteristics			
Farm Type			
Commercial	62.5	ref	
Subsistence	55.4	0.74 (0.43 to 1.27)	
Breed of buffalo			
Murrah	68.8	ref	
Crossbred	58.2	0.63 (0.21 to 1.89)	
Source of livestock			
Self-raised	59.8	ref	
Brought from Traders	58.5	0.95 (0.55 to 1.65)	
Type of Shed			
Stall barn	58.5	ref	
Loose housing	71.4	1.77 (0.34 to 9.34)	
Flooring and roofing of shed			
Concrete floor and tin roof	59.0	ref	
Earth floor and tin roof	42.9	0.52 (0.11 to 2.38)	
Use of bedding			
Non-Edible bedding	60.5	ref	
Edible bedding	55.8	0.82 (0.47 to 1.44)	
Present condition of buffalo			
Pregnant	57.5	ref	
Milking	60.6	1.14 (0.67 to 1.94)	
Free Range			
No	50.6	ref	Ref
Yes	77.1	3.29 (1.73 to 6.24)	3.11 (1.15 to 8.43)
Wallowing			
No	54.1	ref	Ref
Yes	75.0	2.55 (1.27 to 5.11)	0.87 (0.29 to 2.59)
Source of water			
Tap water	59.4	ref	
Other sources	42.9	0.51 (0.11 to 2.34)	
Health checkup frequency			
Regularly	28.6	ref	Ref
When needed	62.1	4.09 (1.52 to 10.99)	2.92 (1.05 to 8.12)
Feed antihelminth within 6 months			
Yes	50.0	ref	
No	60.1	1.51 (0.66 to 3.42)	
Other free range animals			
Yes	51.0	ref	Ref
No	65.3	1.81 (1.06 to 3.10)	1.54 (0.87 to 2.71)
Aware of worm infestation			
Yes	60.4	ref	
No	33.3	0.33 (0.10 to 1.12)	
Deworming frequency			
Every 6 months	52.0	ref	
When needed	59.4	1.35 (0.59 to 3.11)	
Use of local treatment			
Yes	64.5	ref	
No	58.0	0.76 (0.35 to 1.68)	
Response to sick animals			
Immediate	60.9	ref	
Wait for few days	54.0	0.75 (0.42 to 1.36)	
Treatment Procedure			
Local Pharmacist		ref	
Veterinarian Doctor		0.89 (0.52 to 1.53)	

: Odds Ratio, AOR: Adjusted Odds Ratio, 95% CI: 95% confidence interval, %: prevalence percentage, ref: reference,

*Model adjusted for all variables, Model Fit p <0.05, Significant, Nagelkerke R square: 0.11

5. Discussion

This study investigated the prevalence of gastro-intestinal parasites (GIPs) in the subsistence and commercial farms in Butwal Sub-Metropolitan city, Nepal. It was revealed in the study that the 58.9% of buffaloes were affected by GIPs which aligns with 60% prevalence reported in Mahottari and Dhanusha, Nepal (Yadav et al., 2015) and the 58.59% prevalence in cows and buffaloes in Pakistan (Khan et al., 2023). However, the findings were lower than the 90% prevalence reported from Chitwan, Nepal (Adhikari et al., 2022), 100% in Bangladesh (Roy et al., 2016) and 100% in Nigeria (Akande et al., 2021). Conversely, the prevalence was higher than the 39.9% reported in Eastern Nepal (Sah et al., 2018), 48.31% in Bangladesh (Mamun et al., 2011) and 44% in Poland (Kobak & Pilarczyk, 2012). This observed discrepancy in the prevalence might be due to poor farmer awareness and lack of anti-helminthic use (Khan et al., 2023), differences in climate, grazing practices, water sources, hygiene measures, and management techniques (Harizt et al., 2021). Seasonal fluctuations play a significant role, as monsoon periods create ideal conditions for parasite development and transmission (Marskole et al., 2016). However grazing practices (near about one third free range) at the bank of stream and rivers can also be a reason for relatively higher prevalence in this study as they provide easy access to the intermediate hosts (snails) as Trematodes were found more prevalent in the study area (Harizt et al., 2021). Farm management practices like using edible beddings could also be a contributing factors (Kobak et al., 2012; Mamun et al., 2011).

The prevalence in commercial farms (62.5%) was lower than that reported in Pakistan (68.13%) and India (70.45%) (T. Khan et al., 2023; Renwal et al., 2017) but higher than in Italy (33.1%) and Pakistan (29.04%) (Condoleo et al., 2016; M. N. Khan et al., 2010). Similarly, the prevalence in subsistence farms (55.4%) was higher than in Poland (44%) and Mexico (42%) (Kobak et al., 2012; Ojeda-Robertos et al., 2017) but lower than in Nepal (86%) and Greece (92.73%) (Founta et al., 2018; Yadav, 2015). *Fasciola* spp. (30.8%) and *Entamoeba* spp. (26.3%) followed by *Paramphistomum* spp. (12.1%) were the most prevalent parasites in both commercial and subsistence farms. These findings align with previous studies that have reported high prevalence rates of *Fasciola* spp. in buffaloes across different regions, including Nepal (Joshi et al., 2013), China (Zhang et al., 2019) and Bangladesh (Saha et al., 2014). Similarly, high rates of *Entamoeba* spp. and *Balantidium* spp. infections in Buffaloes have been reported in Chitwan, Nepal (Adhikari et al., 2022),

Mexico (Ojeda-Robertos et al., 2017) and Greece (Founta et al., 2018). As shown in Table 4 Buffaloes that were more exposed to free range were more likely to become infected with gastro-intestinal parasites. Interestingly, commercial farms show higher *Fasciola* spp. infections (33.9%) compared to subsistence (27.7%). This difference contrast with similar studies reported from Chitwan, Nepal (Adhikari et al., 2022) and southern Nepal (Patel et al., 2025) where captive farms and commercial farms respectively shows higher *Fasciola* spp. infections which could be due allowing of free ranging the buffaloes from commercial farms and also wallowing. As seen during the study, some commercial farm holders prepare a small pond around the farms so that the buffaloes can wallow during hot weather. Additionally, the high prevalence in some areas may be linked to a lack of deworming programs and poor farm sanitation, as evidenced in studies from Bangladesh (Mamun et al., 2011) and Sri Lanka (Gunathilaka et al., 2018). The relatively lower prevalence in some regions may be due to improved farm management practices, structured deworming programs, and access to veterinary healthcare (Jyoti et al., 2012) Also, one commercial farm was found using loose housing method for shed, where buffaloes can move freely in semi covered areas. A key objective of this study was to compare the GIP burden between commercial and subsistence farms. While there was no statistically significant difference in overall prevalence, commercial farms exhibited a significantly higher prevalence of nematodes ($p = 0.029$), whereas subsistence farms had a slightly higher prevalence of protozoan infections. The higher nematode burden in commercial farms may be attributed to the close confinement of buffaloes, facilitating parasite transmission (Gunathilaka et al., 2018). Conversely, higher protozoan infections in subsistence farms could be linked to water contamination and grazing on communal land (Rashid et al., 2022).

This study also indicates a higher prevalence of GIPs in the buffaloes who are allowed to free range either it be in closed confined areas or open river banks and fields along with other free ranging domestic animals. This study agreed with the study from southern Nepal (Patel et al., 2025). A study conducted in Uttar Pradesh, India found a higher prevalence of *Fasciola* spp. infections in buffaloes which is likely due to the buffaloes exposure to intermediate hosts, *Lymnaea* snails (Gupta et al., 2008). Buffaloes in commercial farms are more confined but also the grass fed were cut and carried from riverbanks and fields where buffaloes from subsistence farms are grazed as seen during the study. This could contribute to the higher trematode infections. Buffaloes while grazing near water bodies on riverbanks are more susceptible to ingesting parasites, including *Fasciola* spp., due to the presence of

infected *Lymnaea* snails in these areas. Moreover, these female buffaloes can contribute to the parasite's life cycle by shedding eggs in their dung along the riverbanks during grazing.

Multivariate logistic regression analysis identified buffaloes' health checkup frequency also as another significant risk factors associated with GIP infections. It shows a critical risk factor for GIP infections in buffaloes, as irregular or infrequent health monitoring and treatment significantly increase parasite prevalence and burden. Studies indicate that untreated or poorly managed buffaloes exhibit notably higher rates of GIP infections compared to regularly checked and treated animals (Khan et al., 2023). The lack of routine health checkups often correlates with farmers' unawareness of recommended parasite control measures and calf care practices, leading to high worm infestation and associated health issues such as diarrhea and poor growth (Yousaf et al., 2021). Routine health checks enable early detection and timely intervention, reducing parasite loads and preventing severe infections. Regular monitoring allows for strategic deworming, pasture management, and improved nutritional support, which collectively lower the risk and severity of GIP infections (Akande et al., 2021). The findings emphasize the need for improved farm management practices to reduce GIP prevalence. and strategic deworming programs should be promoted to control infections effectively (Adhikari et al., 2022). As suggested by studies in Malaysia (Harizt et al., 2021) and India (Marskole et al., 2016) restricting free-ranging and ensuring proper sanitation of water sources may reduce parasite transmission.

6. Conclusion and Recommendations

6.1 Conclusion

The study demonstrated a high prevalence of gastro-intestinal parasitic infections (58.9 %) in buffaloes across both commercial and subsistence farms in Butwal, Nepal. Trematodes, particularly *Fasciola* sp., dominated infections, followed by protozoans like *Entamoeba* sp. Commercial farms showed higher nematode prevalence, likely due to confined rearing conditions, while subsistence farms faced protozoan challenges linked to contaminated water and edible bedding. Multivariate logistic regression analysis shows that free-ranging practices and irregular veterinary care emerged as significant risk factors, increasing parasite transmission. These findings agree with global trends but local variations driven by ecological and management factors. The hypothesis that subsistence farms harbor higher infections due to poor sanitation was partially validated, though commercial farms' dense populations also posed risks. Addressing these issues is vital for enhancing buffalo health, productivity, and economic outcomes for farmers.

6.2 Recommendations

Maintain clean sheds with concrete floors, non-edible bedding, and avoid high-risk grazing areas. Implement routine deworming every six months with broad-spectrum anthelmintics. Improve commercial farm spacing to reduce animal density and cross-infection. Educate farmers on parasite life cycles and transmission pathways to avoid zoonotic risks. Collaborate with local authorities to subsidize treatments and organize mass deworming campaigns. Implementation of control measures may face several challenges. Many farmers have limited awareness about parasitic infections, which can be addressed through targeted training and awareness programs in local languages. Financial conditions may hinder deworming and shed improvements, so government or NGO subsidies can support these efforts. Limited access to veterinary services, especially in rural areas, can overcome by training local animal health workers. Resistance to changing traditional practices like open grazing can be managed by using model farms to demonstrate benefits. Lastly, improper use of deworming drugs can lead to resistance, which can be prevented through proper dosing, drug rotation, and veterinary supervision.

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8. Photographs



9) Wallowing area in commercial farm (ward-17)



10) Loose housing method in commercial farms (ward-15)



11). Farm owner bathing buffaloes in commercial farm (ward-13)



12). Buffaloes from subsistence farms Free Raging (ward-16)



13). Subsistence farms with edible bedding (ward-11)



14). Questionairre surveying from farm owners (ward-19)



15) Buffaloes from commercial farms Free Raging (ward-12)

9. Appendices

Appendix 1. Baseline Questionnaires

Section A (Socio-Demographic Questions)

Participant Code/

1. Name:
2. Gender: Male Female
3. Age:
4. Occupation:
5. Farm type: Subsistence farm Commercial farm
6. Breed of buffalo: Murrah Kathe Dogla (Cross of Murrah)

Section B (Farm Characteristic questions)

7. What is the source of your livestock?
Self-raised Traders
8. How many buffaloes are present in the herd/pen?
0-5 6-10 11- 20 21 and above
9. Shed type
 Loose housing system Stall barn
10. Shed floor and roof made of
Concrete and Tin Earth and tin Both concrete
11. Bedding made for buffalo
Edible Non edible None
12. Milking utensils
 Yes No
13. Feeding
Grazing in field Cut and carry system other specify
14. Additional feed
 Salt block Others/coarse/pellet None
15. Do you let them free rage?
 Yes No (Captive) Sometimes
16. Wallowing area
Pond/lake Stream None Others

17. What is the source of their drinking water?
 Rainwater Stream water Tap water Others

Section C (Health management questions)

18. At what interval do you check their health?
 Regularly Yearly When needed
19. Did you feed any medication for intestinal helminths parasites in the last 6 months?
 Yes No
20. Do you have other free-ranging animals in the house?
 Yes No
21. Are you aware about the worm infestation in buffaloes
 Yes No
22. How often do you deworm?
 Every 6 month When needed
23. What drugs do you use?
 Ivermectin Albendazole Fenbendazole Others
24. Do you use local treatment?
 Yes No
25. What types of infections signs do you see to identify worm infection?
 Diarrhoea Thin Lack of appetite Death Bad fur coating All Milk amount less Not eat grass but hay
26. Response to sick animals?
 Immediate Wait for few days
27. Primary treatment type
 Local herbal Local Pharmacist Veterinary
28. Diagnosis.
 Local Herbal Local Pharmacist Veterinarians
29. Budget for health check-up?
 Yes No
30. Vaccinations in last 1 year.

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Appendix 2. Participation informed consent form

सर्भैक्षणमा भागलिने सहमति पत्र

म यस सर्भैक्षणमा अनुसन्धानकर्ताले (आरती न्यौपाने) देहाय बमोजिम व्याख्या गरेको सर्भैक्षणमा भाग लिन मन्जुर छु ।

तपसिल

- १) यो सर्भैक्षणमा गरिने विधि र लिने नमुना बारे ।
- २) संकलित व्यक्तिगत सूचना र नमुनाको गोपनियताको बारे ।

अनुसन्धानको शिर्षक- **GASTRO-INTESTINAL PARASITIC INFECTIONS AND ASSOCIATED RISK FACTORS IN BUFFALOES REARED UNDER DIFFERENT FARMING STYLES IN BUTWAL**

सहभागि न : _____

अनुसन्धानकर्ताको नाम : _____

हस्ताक्षर _____

मिति : _____

सहभागिको नाम : _____

ठेगाना : _____

उमेर : _____

लिङ्ग : _____

हस्ताक्षर : _____

मिति : _____

Appendix 3: Ethical Approval of the study taken from NVC, Kathmandu, Nepal



Ref. no. ३१ /2080/81

Date: 7th September, 2023

Subject: Ethical Clearance for study on " Comparative GI parasitic infections and associated risk factors in Buffaloes reared under different farming styles in Butwal "

To
Ms. Arti Neupane
MSc Student, Central Department of Zoology, TU

Dear Ms. Neupane ,

With reference to your letter dated August 16th , 2023 regarding the ethical approval of a research study on the area mentioned below, I have the pleasure to inform you that the Council has approved ethical clearance for the specified research study with the following terms and conditions.

1. Study detail:
 - a. Title: " **Comparative GI parasitic infections and associated risk factors in Buffaloes reared under different farming styles in Butwal** "
 - b. Nature of study: A Cross sectional study with fecal collection of buffalo.
 - c. Principal researcher: Ms. Arti Neupane, MSc Student, Central Department of Zoology, TU
2. Supervisor / Co-supervisor: Dr. Tikaram Khanal, N.V.C Regd No. 1064, Dr. Kishor Pandey/ Dr.Rajendra Prasad Parajuli
3. Research laboratory: Lab of Central Department of Zoology, TU
4. The study described above shall be carried out according to standard protocol in compliance with the Code of Conduct, 2058 prescribed by Nepal Veterinary Council (NVC)
5. Care must be taken to ensure animal welfare. Any activity posing threat or cruelty to animal shall be punishable in accordance with the prevailing law.
6. The NVC shall retain the right to
 - a. withdraw or amend this Ethical Approval, if
 - i. any unethical principal or practices are revealed or suspected
 - ii. relevant information has been withheld or misrepresented
 - iii. regulatory changes of whatsoever nature so require
 - iv. the conditions contained in the directive have not been adhered to
 - b. access to any information or data or experimentation site at any time during the course or after completion of the study.
7. NVC shall have the right to stop the study any time in case of :
 - a. violation of animal welfare or
 - b. detection of activities intended cruelty to animal.
 - c. any other matter related to animal welfare or cruelty shall be dealt in accordance with the decision of NVC.
8. The Principal Researcher shall report to NVC in the prescribed format, where applicable,
 - a. six-monthly progress report regarding the ethical compliance, status of animal and
 - b. the completion report at the end of the project
 - c. however, NVC must be informed immediately if
 - i. any material change in the conditions or undertakings mentioned in the document.
 - ii. any material breaches of ethical undertakings or events that impact upon the ethical conduct of the research.
 - iii. any change or revision in protocol during the course of the study, and
9. NVC may carry out monitoring of the study as and when required. However, it is the responsibility of researcher to organize periodic monitoring of study by NVC.
10. The validity of this ethical clearance is one year effective from the 7th September 2023 to 7th December 2023. You will be required to apply for renewal of ethical clearance on an annual basis till the study is not completed.
11. Permission from NVC is mandatory before publication of the finding of study.
Wishing you well in your research

Copy to: Chairperson, Nepal Veterinary Council
Copy to: Vice Chairperson, Professional Standard and Complaint Assessment Committee,
Nepal Veterinary Council

Dr. Manoj Kumar Shahi

Registrar
Nepal Veterinary Council

Veterinary Complex, Tripureswor, P.O. Box No.: 21655, Kathmandu, Nepal
Tel.: +977 1 5359144, 5361210; Fax: +977 1 5359144, Email: info@vcn.gov.np, Website: www.vcn.gov.np

Appendix 4. Materials and chemicals used

Materials

- Compound microscope
- Ocular and Stage micrometer
- Samples vials
- Centrifuge machine
- Beakers
- Glass rod
- Centrifuge tubes
- Slides
- Dropper
- Coverslips
- Tea strainer
- Spatula
- Toothpicks
- Gloves
- Masks
- Cotton
- Volumetric Flasks

Chemicals

- Potassium dichromate (2.5%).
- Iodine solution
- Saturated Sodium chloride (NaCl) solution
- Formalin (40%)
- Alcohol series (30% - 100%)
- Ether
- Normal Saline
- Methylene blue
- Distilled water
- Hand-wash

Appendix 5. Data Analysis (Logistic regression)

Logistic Regression

Health_Check_R0_W1 * IPI_0NY1

Crosstab

		IPI_0NY1		Total	
		0	1		
Health_Check_R0_W1	0	Count	15	6	21
		% within Health_Check_R0_W1	71.4%	28.6%	100.0%
	1	Count	77	126	203
		% within Health_Check_R0_W1	37.9%	62.1%	100.0%
Total	Count	92	132	224	
	% within Health_Check_R0_W1	41.1%	58.9%	100.0%	

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	8.823 ^a	1	.003		
Continuity Correction ^b	7.493	1	.006		
Likelihood Ratio	8.749	1	.003		
Fisher's Exact Test				.004	.003
Linear-by-Linear Association	8.784	1	.003		
N of Valid Cases	224				

- a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 8.63.
- b. Computed only for a 2x2 table

Variables in the Equation

	B	S.E.	Wald	df	Sig.	Exp(B)
Step 1 ^a Health_Check_R0_W1	1.409	.504	7.806	1	.005	4.091
Constant	-.916	.483	3.598	1	.058	.400

Variables in the Equation

		95% C.I. for EXP(B)	
		Lower	Upper
Step 1 ^a Health_Check_R0_W1		1.523	10.991
Constant			

- a. Variable(s) entered on step 1: Health_Check_R0_W1.

Free_Range_N0_Y1 * IPI_0NY1

Crosstab

		IPI_0NY1		Total	
		0	1		
Free_Range_N0_Y1	0	Count	76	78	154
		% within Free_Range_N0_Y1	49.4%	50.6%	100.0%
	1	Count	16	54	70
		% within Free_Range_N0_Y1	22.9%	77.1%	100.0%
Total	Count	92	132	224	
	% within Free_Range_N0_Y1	41.1%	58.9%	100.0%	

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	13.957 ^a	1	<.001		
Continuity Correction ^b	12.884	1	<.001		
Likelihood Ratio	14.629	1	<.001		
Fisher's Exact Test				<.001	<.001
Linear-by-Linear Association	13.894	1	<.001		
N of Valid Cases	224				

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 28.75.

b. Computed only for a 2x2 table

Variables in the Equation

		B	S.E.	Wald	df	Sig.	Exp(B)
Step 1 ^a	Free_Range_N0_Y1	1.190	.327	13.244	1	<.001	3.288
	Constant	.026	.161	.026	1	.872	1.026

Variables in the Equation

		95% C.I. for EXP(B)	
		Lower	Upper
Step 1 ^a	Free_Range_N0_Y1	1.732	6.243
	Constant		

a. Variable(s) entered on step 1: Free_Range_N0_Y1.

Multivariate Testing

Variables in the Equation

		B	S.E.	Wald	df	Sig.
Step 1 ^a	Free_Range_N0_Y1	1.133	.509	4.951	1	.026
	Wallowing_N0_Y1	-.145	.559	.067	1	.795
	Health_Check_R0_W1	1.072	.522	4.227	1	.040
	Other_FreeRange_Animals_N0_Y1	.431	.288	2.230	1	.135
	Constant	-1.134	.504	5.073	1	.024

Variables in the Equation

		Exp(B)	95% C.I. for EXP(B)	
			Lower	Upper
Step 1 ^a	Free_Range_N0_Y1	3.106	1.145	8.427
	Wallowing_N0_Y1	.865	.289	2.586
	Health_Check_R0_W1	2.922	1.051	8.121
	Other_FreeRange_Animals_N0_Y1	1.538	.874	2.708
	Constant	.322		

a. Variable(s) entered on step 1: Free_Range_N0_Y1, Wallowing_N0_Y1, Health_Check_R0_W1, Other_FreeRange_Animals_N0