PREVALENCE AND ASSOCIATED RISK FACTORS OF INTESTINAL PARASITIC INFECTION AMONG COMMUNITY PEOPLE OF KUSHMA MUNICIPALITY, PARBAT DISTRICT NEPAL

Entry 37
M.Sc. Zoo Dept. Parasitology
Signature ...
Date: 2079-11-30
14 March, 2023

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A Thesis Submitted in Partial Fulfillment of the Requirements for the Award of the Degree of Master of Science in Zoology with Special Paper Parasitology

Submitted to

Central Department of Zoology

Institute of Science and Technology

Tribhuvan University

Kirtipur, Kathmandu Nepal

April, 2023

DECLARATION

I hereby declare that the work presented in this thesis entitled "Prevalence and associated risk factors of intestinal parasitic infection among community people of Kushma Municipality, Parbat District, 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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RECOMMENDATIONS

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LETTER OF APPROVAL

On the recommendation of supervisor Prof. Dr. Mahendra Maharjan, this thesis submitted by Miss Sarada Paudel entitled "Prevalence and associated risk factors of intestinal parasitic infection among community people of Kushma Municipality, Parbat District, Nepal" is approved for the examination and submitted to the Tribhuvan University in partial fulfilment of the requirements for Master's Degree of Science in Zoology with special paper Parasitology.

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CERTIFICATE OF ACCEPTANCE

This thesis work submitted by Miss Sarada Paudel entitled "Prevalence and associated risk factors of intestinal parasitic infection among community people of Kushma Municipality, Parbat District, Nepal" has been accepted as a partial fulfilment for the requirements of Master's Degree of Science in Zoology with special paper parasitology.

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ACKNOWLEDGEMENT

The success of this thesis work is indebted to my honorable supervisor, **Prof. Dr. Mahendra Maharjan** and for his patience, untiring guidance, encouragement, motivation, support enthusiasm, advice and immense knowledge provided throughout the time as his student. I am indebted to have such great supervisor who cared so much about my work and responded to my questions and queries so promptly. I could not have imagined having better advisor and mentor for my work.

I am also extremely grateful to **Prof. Dr. Tej Bahadur Thapa**, Head of Department, Central Department of Zoology, TU, for his co-operation and support.

I am thankful to both the wards office of my study area, Ward-01, Pang and Ward-08, Chuwa for giving me the permission to conduct the research work in the area. I sincerely acknowledge all the participants of my study for their help and active participation without whom my work would not have started. My special thanks goes to all the staff of the Central Department of Zoology for their help and support during the labwork for providing the needed materials and chemicals and waiting till the time.

I am very thankful to **University Grant Commission** for providing me the financial support to conduct this research work. I want to express my gratitude to my friend **Anish Pandey** and **Heena Maharjan** for their help and support throughout the work.

Finally, I am so happy to express my gratefulness to my parents, family members and almighty, for their moral support and inspiration in my whole academic career. Last but not the least I would like to acknowledge all those who have helped me directly or indirectly to complete this work.

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LIST OF ABBREVIATIONS

Abbreviated form	**Details of abbreviation**
IPIs	Intestinal Parasitic Infections
STHs	Soil Transmitted Helminths
NTDs	Neglected Tropical Diseases
GBD	Global Burden of Diseases
DALY	Disability Adjusted Life Years
ZN	Ziehl Neelsen
MDA	Mass Drug Administration
sp.	Species
P-value	Probability value
CBS	Central Bureau of Statisctics
CDC	Centers for Disease control and Prevention
CDZ	Central Department of Zoology
Et al.	and his associate
µm	Mu meter
No.	Number
SPSS	Statistical Package for Social Sciences
Sat.	Saturated
FEA	Formalin-ethyl Acetate
C.F	Calibration Factor

ABSTRACT

Intestinal parasitic infection caused by protozoan and helminth are endemic worldwide and have been known to constitute the greatest single worldwide cause of disease that continues to become the major public health issue in developing countries with poverty, illiteracy, lack of quality water supply, and poor hygiene and sanitation. The study aimed to estimate prevalence and identify the factors associated with intestinal parasitic infections among the community people of Kushma Municipality of Parbat district. A cross-sectional purposive study was carried out and collected a total of 208 stool samples irrespective of ages and sexes then preserved in 2.5% $K_2Cr_2O_7$. The study used structured questionnaire for assessing the risk factors. The fecal samples were examined both macroscopically and microscopically (saline wet mount, iodine mount, concentration method i.e. floatation and sedimentation for all parasites and Ziehl Neelsen Acid Fast staining for coccidian parasites). The association between parasitic infection and associated risk factor was evaluated using univariate and multivariate logistic regression method. The overall prevalence was 24.04% (50/208). The common parasites found were *Ascaris lumbricoides* (17.3%), *Cryptosporidium* sp. (3.9%), *Enterobius vermicularis* (1%), *Hymenolepis nana* (1%), hookworm (0.5%) and *Taenia* sp. (0.5%). Male (27.6%) and 51-60 years age-group (31.3%) were observed to be highly infected. Age, sex and occupation did not show the significant relationship with parasitic infection ($p>0.05$). Use of slipper (OR= 15.4, 95% CI=1.9-125.7, p=0.011), drug consumption (OR= 0.2, 95% CI= 0.1-0.5, p=0.001) and nail trimming habit (OR= 4.1, 95%CI= 1.7-10.2, p=0.002) were found to be significant factors for intestinal parasitic infection.

Intestinal parasites are highly prevalent within Kushma Municipality and personal hygiene maintenance and biannual mass deworming programme are strongly needed to prevent and control the spread of the intestinal parasitic infection.

1. INTRODUCTION

1.1 Background

Parasitism is the most successful modes of life displayed by living organism (Poulin and Morand, 2000) and infect any free living organism. Intestinal parasites reside in the intestinal tract of their hosts and cause infections that lead to a significant morbidity and mortality. They potentially influence host health, behavior and population size, food web and community structure (Raga et al. 2009). Intestinal parasitic infections (IPIs) are the most Neglected Tropical Diseases (NTDs) causing chronic infection among the poorest population of the globe. More than 72 species of intestinal parasites infect human (Pozio, 2003). Intestinal parasites are majorly protozoan and helminths that infects humans and animals (Suntaravitun and Dokmaikaw, 2018). Intestinal helminths are broadly categorized as nematodes and cestodes. The medically important nematodes are *Ascaris lumbricoides*, *Truchuris trichiura* and hoookworms which are also known as soil transmitted helminths (STHs). STHs are the most NTDs infecting 1000 million people (Loukas et al. 2021). *Taenia* sp., *Hymenolepis nana* are the major helminthic cestodes transmitted mainly from the consumption of uncooked meats. The major protozoan parasites are *Entamoeba histolytica*, *Giardia lamblia*, *Cyclospora cayetanensis* and *Cryptosporidium* sp. and transmitted mainly via contaminated foods and water.

IPIs deprive the poorest of the poor health leading to worm burden and excess morbidity and mortality (Mehraj et al. 2008). Poverty is the synonym of IPIs (Norhayati et al. 2003). Despite the advancement in sanitation and hygiene status and health intervention, IPIs are still globally endemic and constitute one of the greatest cause of illness mainly in developing countries with poor sanitation and low socio-economic conditions. About 24% (1.5 billion) people of the globe are infected with IPIs (WHO 2020). Globally *A. lumbricoides* is the most predominant helminthic nematode harboring in about 1.2 billion people followed by *T. trichiura* in 795 million and then hookworms in 740 million people (CDC 2021). Diarrheal diseases caused by intestinal protozoan parasites are a major food-borne public health problem across the world. *G. lamblia* is the most predominating protozoan parasites infecting 200 million people globally (Pillai and Kain 2003) and *E. histolytica* infecting 50 million people (Obala et al. 2013).

Illiteracy (Alula et al. 2021), animal contact, hand washing habit after toilet (Feleke et al. 2019), hot and humid tropical climate (Mehraj et al. 2008), unprotected drinking water services (Gizaw et al. 2018), low house hold income (Shrestha et al. 2018), poor personal hygiene (Singh 2022), overcrowding (Feleke et al. 2021) and inflexible employment (Rotheram et al. 2021) are the leading cause of IPIs. The helminthic eggs are transmitted through infected feces that contaminates the soil and enter via skin (WHO 2020).

Although water is must but also plays an important role for dissemination of parasites. Protozoans like *G. lamblia* and *Cryptosporidium* sp. are important waterborne pathogens (Baldursson and Karanis 2011). Food contamination can also occur directly by contamination of vegetables (Tigabu et al. 2019) and handling process and indirectly via contaminated water of irrigation (Dawson 2005). Age is associated with protozoan parasites and lacking electricity with *A. lumbricoides* (Morales-Espinoza et al. 2003). The prevalence of infection is associated with those living in house made of wood or bamboo and those children from mother with least formal education (Holland et al. 1988). Better surrounding is required by every organism for sound health. However poor sanitation suits parasitic transmission which is supported by a study where healthy sanitation and water quality were associated with lower risk of protozoans (Speich et al. 2016). IPIs are persistent in urban areas with overcrowding and promiscuity (Joint Research Commission of the European Union, 2008).

In Nepal the situation of IPIs are still afflictive according to various studies conducted. Mortality and morbidity caused by IPIs are still high (Shrestha et al. 2019). Poor sanitation, polluted water and land, contaminated foods, lack of education and awareness, poor socio-economic condition, overcrowding, unmanaged settlements, farming occupation and cultural and religious practices are the crucial factors for infections. *A. lumbricoides, H. nana,* and *T. trichiura* are the most common helminths, while *G. lamblia* and *E. histolytica* are the most common protozoans causing parasitosis in Nepal. The major urban areas of Nepal are also highly infected by intestinal parasitic infection. Major cities also reported significant prevalent cases of IPIs. 68% of people from Kathmandu (Subedi et al. 2021) and 66.67% from Dharan (Ghimire et al. 2021) were infected by intestinal parasites. Comparatively higher prevalence of IPIs were reported from rural areas of Nepal than the urban areas. The lagging factors may be illiteracy, ignorance, dependency on natural waters for drinking and occupation.

Bertoncello et al. (2021) reported rural areas (52.3%) to have higher prevalence rate of IPIs than the urban areas (32.4%). Pokhara is the nearest major urban city to Parbat district. Various studies have been carried out in Pokhara city. 21.3% people of the city were infected by IPIs as reported by Chandrashekhar et al. (2020). However, no information was available regarding the prevalence and risk factors of intestinal parasitic infections among the residents of Kushma Municipality. In rural areas of Kushma Municipality, some people still defecate openly while mostly have temporary types of latrine. People from rural community majorly do farming as living occupation and depends on natural water reservoir for drinking and irrigation and have muddy house. Commonly people work and walk barefoot which causes the soil transmitted diseases. Majority of people are non-veg consuming mostly buff which is risk for *Taenia saginata* or pork leading to *Taenia solium*. Despite the facility of health post, majority of people are illiterate so they ignore or show less participation in deworming program. Till now Kushma Municipality lacks the researches on IPIs among the community people, this current study was consequently aimed to find the prevalence rate and potential risk factors associated with the intestinal parasitic infection among the people living in Kushma Municipality of Parbat district.

1.2 Objectives

1.2.1 General Objective

i. To identify the intestinal parasitic infection in relation to socio-economic status of Ward No. 1 (Pang) and Ward No. 8 (Chuwa) of Kushma municipality.

1.2.2 Specific Objectives

i. To determine the prevalence of intestinal parasitic infection in Kushma municipality.

ii. To find out the socio-economic status and associated risk factors of intestinal parasitic infection.

1.3 Significance of the study

Intestinal parasitic infection caused by helminths and protozoans parasites are among the most prevalent infections in human in both developed and developing countries. In country like Nepal, where there is lack of safe and quality water supply, sanitation, improper sanitation and unmanaged sewerage disposal, the parasitic infection has become the major health burden and needs urgent consideration. Study of IPIs in Kushma Municipality of Parbat district has been done for the first time to find the prevalence of IPIs and the associated factors responsible for the infection and consequently to aware the people and recommend the mitigation for the problem. Moreover the present study will also help to fill the knowledge gap of intestinal parasitic infection in Kushma municipality and help the future investigators to advance their knowledge and enhance light on different problems faced by the people of Kushma Municipality.

2. LITERATURE REVIEW

2.1 Intestinal Parasite and Infection

Parasites are the opportunistic organisms which attack or infect the person with low immunity power and poor sanitary condition (Chatterjee, 1998). Parasites found their ecological niche living in distinct species known as host (Gonçalves et al. 2003). And human are host to nearly 300 species of worms and over 70 species of protozoan parasites (Cox 2002). Intestinal parasitic infections (IPIs) are major cause of morbidity and mortality (Nyarango et al. 2008) specially in countries with low socio-economic status (Kucik et al. 2004) and mainly situated in the tropical and sub-tropical regions like sub- Saharan Africa, North and South America, China and East Asia (Dudlová et al. 2016). Currently in developed and developing countries, IPIs are a concern of public health agencies (Gomes et al. 2004). 25% of the known human infectious disease is caused by parasites (Alum et al. 2010) and globally more than 24% people are infected with parasitic infection (Dudlová et al. 2016).

Parasitic disease are so complicated and hidden disease that it causes symptoms indicating for other diseases and being late to earlier diagnose may also lead to severity and even death. A case reported by Karuna and Khadanga (2013) from an 18 years old farmer of India, who had been evaluated by many physicians and dermatologists and many associated to food allergy but the stool test revealed *Hymenolepis diminuta* and the severity were controlled by treatment in the line of hymenolepiasis.

Parasites are also opportunistic pathogen causing infection among immunosuppressed people, malnourished, HIV and tuberculosis patient. A study on opportunistic parasites in HIV infected people was carried out by Amatya et al. (2011) and found coccidian to be the frequent opportunistic parasites with other parasites like *G. lamblia*, *Blastocystis hominis* and hookworm.

Parasites are mainly categorized as protozoans and helminths (Haque 2007). Helminths are complex metazoans (Gazzinelli-Guimaraes and Nutman 2018) that comprises nematode (roundworms), trematode (flukes) and cestode (tapeworms) that diverged 0.6 billion or more years ago (Maizels et al. 2004). Three hundred and forty species of helminths have been associated with human (Crompton 1999). Simply, helminths are worms such as tapeworm, pinworm and roundworm (Hotez et al. 2007). Helminthic infection are a major public health problem and recent estimates suggest that 1.5 billion are infected (WHO, 2020). The global burden of disease (GBD) amounts to over 3.3 million disability adjusted life years (DALY) (Turne 2021). The most common helminthic infections are ascariasis, hookworm, trichuriasis and schistosomiasis which are also known as geohelminths or soil transmitted helminths (STH) (Haque 2007) and the most Neglected Tropical Disease (WHO, 2018). Another important helminthic infection is strongyloidiasis (Azzopardi et al. 2021). Globally more than 1.4 billion people are infected by *Ascaris lumbricoides* (Gupta et al. 2012) producing 7,54,000 DALY (Pullan et al. 2014); 1.3 billion by hookworm (Anisuzzaman and Tsuji 2020) with more than four million DALY (Umbrello et al. 2021) and 465 million people are affected by *Trichuris trichiura* (Pullan et al. 2014) with 6,40,000 DALY (Vos et al. 2016). Helminths are known to modulate the directed host immune response to bystander the pathogens (Maizels, 2016). Helminths are related to diarrhoea, malnutrition (Taren, 1987) vitamin A deficiency, iron deficiency and anaemia (Stephenson, 1987) leading to mortality and morbidity.

Protozoans are the single celled microscopic organism that are able to multiply inside the host body (Haque 2007). Intestinal protozoans are major pathogens of opportunistic infection among immunocompromised (Nissapatorn 2008) protracted diarrhea among travelers (Ericsson et al. 2001). *Entaemoeba histolytica*/ *dispar*, *Giardia lamblia*/ *duodenalis* and *Cryptosporidium* sp. are the major protozoan parasites infecting globally. *Entaemoeba histolytica* /*dispar* is the most common protozoa affecting 0.5 billion people globally (Shirley et al. 2018) with 2.2 million DALYs (Lozano et al. 2012). *Giardia lamblia*/ *duodenalis* infects 280 million people (Laishram et al. 2012) and *Cryptosporidium* sp. comes to second to rotavirus for causing diarrhea and mortality mainly in children (Sow et al. 2016) and results in 8.37 million DALYs (Pisarski 2019).

2.2 Associated Risk Factors of Intestinal Parasitic Infections

Intestinal parasitic infections (IPIs) are caused by many factors including their habit, knowledge, social, economic, cultural and bioenvironmental condition. Education level of the subject (Çeliksöz et al. 2005), being married or divorced or widowed (Taye et al. 2014), children with less educated mother (Asrat et al. 2011), knowledge on intestinal parasites their biology and epidemiology (Zvinorova et al. 2016), not knowing the importance of hand wash (Tulu et al. 2014), low monthly income, (Taye et al. 2014), and are among socio-demographic characters associated with IPIs.

Drinking unboiled water (Kitvatanachai et al. 2008), open defecation (Nyarango et al. 2008), washing hands without soap after defecation (Mumtaz et al. 2009), sweet things and rotten fruits (Eu et al. 2010), thumb sucking (Sah et al. 2013), nail trimming (Dash et al. 2010, Mahmud et al. 2015), consumption of raw/ unwashed vegetables, the personal hygiene (Liao et al. 2017), irregular shoe wearing habit (Hailegebriel 2017), and consumption of raw/ uncooked meat (Sitotaw et al. 2019) are among the behavioral characters for IPIs.

More members in the house or overcrowding (Khosrow et al. 2011), types of latrine (Akinbo et al. 2013) and presence of animals (Gedle et al. 2017), source of drinking water (Abbaszadeh Afshar et al. 2020) and types of house (Younes et al. 2021) are the living condition characters for the parasitic infection.

Environmental sanitation (Mengistu et al. 2007), previous parasitic infection (Mohammad et al. 2012) and medical treatment like iron supplements (Kalenga et al. 2003), no early consultation for therapy (Mohammad et al. 2012) and consumption of drugs (Elmonir et al. 2021) are other important factors for intestinal parasitic infection.

2.3 Intestinal Parasitic Infection and Associated Risk Factor in Global Context

Many studies on intestinal parasites and their infection has been carried out in different parts of globe as it is found most common health problem (Kang et al., 1998). Faria et al. (2017) conducted a cross-sectional study in Brazil to find out the distribution of IPI and their association with social determinants and found 17.5% of parasitic prevalence and infection was influenced by socio-economic status where risk of infection increased with higher level of deprivation.

Suntaravitun and Dokmaikaw (2018) from Thailand reported significantly higher helminthic infection (14.3%) than the protozoan infection (1.8%) and found sanitation and personal hygiene to be associated with the infection. While a population based study carried out by Abbaszadeh Afshar et al. (2020) in Iran found significantly higher protozoan infection (32.3%) than the helminthic infection (3.2%). The multiple logistic regression indicated source of drinking water and residency as predictors of the parasitic infection.

Sitotaw et al. (2019) carried out a study in Ethiopia and found 19.95% *Giardia lamblia*, followed by 13.8% hookworm, 10.3% *Schistosoma mansoni*, 5.9% *Entamoeba histolytica*/ *dispar*, 4.2% *Hymenolepis nana*, 3% *Taenia sp.* and 0.73% *Ascaris lumbricoides*. In a multi country study conducted by (Krumkamp et al. 2021) in Sub-Saharan Africa and among children, 13% of children showed the presence of *Cryptosporidium* sp. Muñoz-Antoli et al. (2011) found higher prevalence of *Cryptosporidium* sp. (35.7%) among the school children in Nicaragua and highly prevalent among girls (59.56%) than in boys (40.44%) and Ayres Hutter et al. (2020) from Canada reported female (55%) as highly infected than males (45%). For molecular detection and genetic characterization of *Cryptosporidium* in China, Wang et al. (2022) collected 609 stool of school children and boys 2% were infected more than girls (0.6%). Kwaga et al. (1988) from Nigeria manifested adults (29%) were significantly affected than children (8%) and more females (27%) than males (17%). In Philippines among 3,456 diarrheic patients, 1.9% prevalence rate of *Cryptosporidium* sp. was recorded of which pediatric patients (0-4 yrs) (2.9%) were significantly infected than adults (0.2%) and higher in male than females (Natividad et al. 2008).

From Enugu, a capital state of Nigeria, 19.11% of Ascariasis infection was reported by Chijioke et al. (2011) recommending to mass deworming exercise as a crucial part for the control of the nematode. Out of 162 samples, 29% ascariasis was reported highly prevalent among other parasites from Eastern Cape Province of South Africa by Nxasana et al. (2013). Similarly, Iyevhobu et al. (2022) from America collected 100 samples of children and routine stool examination revealed *A. lumbricoides* were highly prevalent in males. In Osun state of Nigeria, Ojurongbe et al. (2014) conducted a cross sectional study in four communities and showed males were more affected by hookworm (7.4% versus 3.7%). Similarly for age wise, Avokpaho et al. (2021) assessed the prevalence by kato-katz method in Benin and reported children (4.4%) were highly infected than adults (1.7%). Traub et al. (2004) conducted a study in tea-growing communities of Assam, India and revealed adults (54%) were more infected than children (46%). Polluted water, soil and vegetables were shown to be the cause of the infection from a study done by Ulukanligil et al. (2001) from Turkey. The association of ascariasis with poorer general intelligence was suggested by (Jardim- Botelho et al.

2008) among children from Brazil while, the global warming and ecosystem changes are also the factor for the accelerated transmission of hookworm (Kim et al. 2012).

In a research conducted among 112 handicapped at an institute of Korea, the overall prevalence was indicated to be 35.7% among which 20.6% were *Enterobius vermicularis* infected (Lee et al. 2000). Similarly, from a kindergarten and preschool of Amol, North Iran, out of 462 samples 32 were positive accounting for 7.1% of *E. vermicularis* and females (7.9%) were highly infected than males (6.3%) (Afrakhteh et al. 2016). Bahrami et al. (2018) employed a research work in Iran and collected 1383 fecal samples of which 21.5% were infected by any parasites and accounting 0.07% for *E. vermicularis*. Four routes of transmission i.e. i) direct transmission by itching anal and perianal regions from which the eggs are trapped in the fingernails and cause of autoinfection, ii) exposure to eggs on contaminated environmental objects, iii) contaminated clothing and lastly iv) retro infection were explained for Enterobius infection by Cook (1994).

Out of 3,826 samples collected from school children of Nigeria 25% accounts for hookworm infection (Abah and Arene 2015). Wei et al. (2017) collected 424 samples and reported hookworm infection higher in females than in males. Similarly, Srinivasan et al. (1987) collected 1113 sample from rural area of South India and accessed kato-katz method to find out the load of hookworm ova. They found females had higher load than males. A cross sectional study was carried out among 299 school children and the samples were processed by routine examination and modified kato-katz technique which showed higher prevalence of hookworm (10.7%) than other parasites Children who practiced open defecation and playing on the ground had higher risk of infection. So hookworm not only transmit through the penetration of skin but also has fecal- oral mode (Punsawad et al. 2018). Hotez (2008) said that because of its health and educational effects, hookworm infection also promotes the poverty, not only existing among poor. Hygiene, water quality and sanitation must be improved for the control of hookworm and many other intestinal parasites (Loukas et al. 2016).

From rural areas and urban areas of Iran 1060 samples were collected and 28% were prevalent of infection having 1.1% of *Hymenolepis nana* (Mahni et al. 2016). And from a cross sectional study in Canada by Abbaszadeh Afshar et al. (2020), 34.2% were reported to be infected by intestinal parasites with *H. nana* of 2.4% infection. Hamid et al. (2015) from Sudan reported quality water supply and environmental hygiene are the control factor of *Hymenolepis*.

A community based cross-sectional study carried out in Northeast Thailand by Boonjaraspinyo et al. (2013) and reported 37.2% infection from a total of 253 samples of which 1.6% were reported for Taeniasis. Similarly, Nkenfou et al. (2013) reported 0.25% of Taeniasis from HIV/ AIDS patient in Cameroon. Accidental cause of Taeniasis occurs via faecal-oral mode of transmission (Ito et al. 2003). Infection from *T. solium* occurs by the consumption of raw or uncooked pork meat while *T. saginata* infection occurs from beef meat (Ito et al. 2016). Infection by *T. solium* results in the cysticerci (larval stage) when they are located in the central nervous system and called Neurocysticercosis (NCC) (Sotelo 2000).

2.4 Intestinal Parasitic Infection and Associated risk factors in National Context

In context of Nepal, different studies have been carried out to find the IPIs and their associated risk factor in different parts. As our country is developing and still under the margin of poverty, parasitic infection has been the major public health issue and poverty further intensifies the disease. Approximately 66.7% of Nepalese are infected with parasites (Rai et al. 2001) and it can be over 80 in the rural parts Sharma et al. (2004) said that intestinal parasitic infection were highly prevalent in all age groups and geographical regions of the country. Also, the burden of IPIs are increasing in children, adult and elderly people (Shrestha et al. 2006). The parasitic burden in a remote village of western area was found to be high among the population and the prevalence was 100% (Estevez et al. 1983). While studying the researches on different parts of the country IPIs are major problem. Different results have been presented by several researchers from the Eastern part of Nepal. Like, (Sah et al. 2013) from Itahari reported 18.5% of the infection with parasites like *A. lumbricoides*, *Trichuris trichiura*, hookworm, *Taenia* sp., *E. histolytica* and *G. lamblia*. A study carried out by Rai et al. (2017) from Lokhim VDC, the eastern part of Nepal showed the prevalence of 30.92% and female were more infected than male. Likewise, a study carried out by Baral et al. (2017) in the Eastern region and examined 11,791 samples and 5.72% were found positive for intestinal protozoans and 2.45% were positive for helminths. They observed highest infection among adults. Yadav and Mahato (2017) collected 3,000 stool from children of Morang and indicated 83.3% helminthic prevalence with higher prevalence of *A. lumbricoides* followed by hookworm, *T. trichiura*, *E. vermicularis* and *H. nana*. Similarly, Limbu et al. (2021) conducted a study among childrens of Dharan, Eastern Nepal, and made us to know that the parasitic prevalence in that area is 7.75%.

The central part as being developed part of the country did not remain untouched by IPIs. Pandey et al. (2015) collected 300 samples from school children of Kathmandu and manifested different parasites prevalent among the children like *E. histolytica*, *G. lamblia, A. lumbricoides*, *H. nana* and *Cyclospora*. Shrestha et al. (2019) reported six percent infection among prison inmates from Kathmandu. Another study was conducted by Kumari et al. (2019) in Bharatpur and revealed 20.6% infection from 1558 samples. And a study was reported by Adhikari et al. (2021) who studied Chepang community from Chitwan and revealed the highest of 97% of overall prevalence.

From western region, Ishiyama et al. (2003) conducted a small scale study in a remote hilly village of Western Nepal and found 27% of the people were infected. Similarly, Shrestha et al. (2012) reported 21.05% of IPIs among school children of Baglung. Likewise, among school children of Pokhara, Khadka et al. (2013) reported 15% of infection from 100 samples. Accounting Mid-western, a study was conducted in combatants and their families of regional police hospital of Mid-western by (Paudel et al. 2014) and found 46% of infection among 2005 subjects. Higher than this rate was indicated by Shrestha et al. (2020) of 51.1% infection from rural part of Karnali. For Far-western, a hospital based study was made among HIV patients and found 19.3% infection of *Cryptosporidium* sp.

From 305 samples selected from Sunsari, males (53%) were more infected compared to males (Das et al. 2019). Similarly, in a transversal study among 125 participants of Chitwan, 54% male were highly infected than females (Adhikari et al. 2020) while in a study from Baglung, by Thapa et al. (2021) females were more infected than males and a study from Kathmandu among also showed higher prevalence among females (18.4% versus 17%) (Dahal et al. 2022). Regarding age groups, several reports have been presented from different parts of Nepal. From a retrospective study at Deukhury Community Hospital Lab, Dang Khanal et al. (2011) found children of years less than 15 were highly infected than the senior adults of more than 60 years. Higher infection among those aged more than 20 years were shown to be highly infected than those below 20 years (Dhakal and Subedi 2019). On the other hand, (Shrestha et al. 2019) reported 21-40 yrs age group were highly infected than others. Also Adhikari et al. (2020) revealed higher infection among 21-40 years compared to elder people. Similarly, a study among the patients visiting sub regional hospital of Dadeldhura, revealed less than 15 years were infected in comparison to 16-30years and above 30 years (Sharma et al. 2021).

In Chepang community, elderly males were found highly infected than females (Majhi and Tharu, 2006). Shakya et al., (2006) also found males being highly infected during his examination of 325 samples of elderly people of Kathmandu. The overall prevalence was recorded to be 41.7%. Moreover the highest infection rate was manifested from government run elderly home followed by rural community and lastly private elderly homes. A study among school children of Kathmandu revealed higher infection among children females than males (Dahal et al. 2018). Dhakal and Subedi (2019) revealed higher prevalence among adult females than males. Also the higher prevalence among adult females was shown by Subedi et al. (2021). On the counterpart, a study done by Chaudhary and Subedi (2020) revealed higher infection among adult males than females.

The most common helminthic parasite in Nepal are *A. lumbricoides*, hookworm, *E. vermicularis*, *T. trichiura* and *Strongyloides stercoralis*. *A. lumbricoides* was shown to be predominant by many studies like that done by Kumari et al. (2019). Similarly, a study among the school children of Kathmandu was conducted by Gurung et al. (2019) and found *A. lumbricoides* as the dominant than other parasites and Shrestha et al. (2020) and Dahal et al. (2022) also revealed *A. lumbricoides* as predominant. For a retrospective study, Singh et al. (2013) collected 5524 samples from both the outpatient as well as inpatient from a Hospital of Biratnagar and found that hookworm was the most dominant helminth among others. A descriptive cross-sectional carried out by Sharma et al. (2020) in Bhaktapur and also revealed higher prevalence of hookworm. A total of 562 fecal samples were collected from student in northeastern part of Kathmandu, and *T. trichiura* was found to be the common helminthic parasite (Sharma et al. 2004). On the other hand, Khanal et al. (2011) conducted a study among the students of Kathmandu to estimate the prevalence of IPIs and found the most dominating was *T. trichiura* (32%) followed by *A. lumbricoides*, *H. nana* and hookworm. Shrestha et al. (2018) also revealed high infection of *T. trichiura* (30.9%)

followed by hookworm (30.2%). Sah and Bhadani (2006) set a study to assess the possible relation between the parasite and acute appendicitis, and found the prevalence of pinworm among inflamed and normal appendices indicating the possible cause of symptoms of acute appendicitis. Khadka and Maharjan (2018) conducted a study to find out the infection of *E. vermicularis* among school children of Lalitpur and from the study, 10.28% were infected by the pinworm. Among the cestodes, *H. nana* and *Taenia* sp. were common in Nepal. Out of total 530 samples from Dadeldhura, Tiwari et al. (2013) found high prevalence of *H. nana*. Similarly, in 2014 (Pradhan et al.) found *H. nana* as the most prevalent cestode from the school going children. Thapa (2021) conducted his study in the indigenous communities of Salyantar and he also found *H. nana* as the dominating cestode. A cross-sectional study was carried out in schools of Itahari by Sah et al. (2013) and the study revealed *Taenia* sp. (6.5%) to be dominating helminth followed by hookworm, *A. lumbricoides* and *T. trichiura*. While dominance of *Taenia* sp. from overall parasitic infection was indicated by Bhattachan et al. (2015) from Chitwan.

For protozoan the most common parasites are *E. histolytica*, *G. lamblia*, C*ryptosporidium* sp. and *Cyclospora*. A study conducted among school children of Lalitpur by Tandukar et al. (2013) indicated higher prevalence of *G. lamblia* (7.4%) followed by *E. histolytica* (3.4%). Tiwari et al. (2013) also presented the higher prevalence of *G. lamblia* from Dadeldhura. Similarly through a study Shrestha et al. (2019) also presented higher prevalence of *G. lamblia* (10 out of 24) among protozoan parasites from the inmates of Kathmandu. From a study among the patients in Dang, Khanal et al. (2011) revealed the dominance of *E. histolytica* (49%) from all other protozoan and helminthc parasite. Also among seven different parasites, *E. histolytica* maintained its dominancy in another study done by Yadav and Prakash (2016) among schoolchildren. Dahal et al. (2018) conducted a study among school children of Kathmandu and revealed *E. histolytica* as the dominant protozoan parasite (8 out of 24). Several methods of routine processing of stool was done by Subedi et al. (2021) and the study also found *E. histolytica* to be the dominating from all other protozoan and helminthic parasite.

To study the relation of *Cryptosporidium* and diarrhoea, Sherchand and Shrestha (1996) collected 354 samples from acute diarrhoea patients and 6.8% of the sample showed oocyst by the modified Ziehl Neelsen (ZN) laboratory technique and the infection was more common among children. Similarly, Sherchand et al. (1996) revealed highest incidence of *Cryptosporidium* sp. among children. Four hundred and sixty loose, soft and watery samples were collected for an observational study in Kanti Children Hospital, Kathmandu by Sherchand et al. (2004) and found *Cryptosporidium* sp. was highly prevalent among children. From the pediatric patients of Eastern Nepal, total 4.4% coccidian parasites were revealed of which 4.1% is occupied by *Cryptosporidium* sp. and two by *Cyclospora* (Amatya et al. 2011). To detect the prevalence of *Cryptosporidium* and *Cyclospora* in a slum area of Kathmandu, Bhattachan et al. (2017). Bhattachan et al. (2018) indicated *Cyclospora cayetanensis* as an infestation among diarrheal patients in Kathmandu.

Parasites as opportunistic pathogen causing infection among immunosuppressed people, malnourished, HIV and Tuberculosis patient were also described from Nepal by Ghimire et al. (2021) who conducted a study on HIV and Tuberculosis patient in Dharan by and indicated 67% of parasitic infection with parasites like *G. lamblia*, *E. histolytica*, *C. parvum*, *Isospora belli*, Microsporidium, *B. hominis* and *Taenia* sp. In Nepal, the highest annual burden, during 12 years of study from 2000-2012, was obtruded by neurocysticercosis with 14,268 DALY followed by toxoplasmosis with 9255 DALYs, and cystic echinococcosis with 251 DALYs. And Nepal is probably prone to endemicity to diphyllobothriosis, food borne-trematodosis and toxocarasis (Devleesschauwer et al. 2014).

The overall literacy rate is 67.9%, this means still more people cannot read and write and understand the messages in posters newspaper and have sprinkled knowledge on parasites and infection. A team of Nepalese and Japanese investigators in 1996 and 1997 presented a report on health and sanitary status in a remote hilly village and found *A. lumbricoides* as the prevalent parasitic infection. And more than 50% of the people consulted witch doctors (Rai et al. 1997). People's ignorance and superstition also promotes the IPIs. Educational level of the children were directly associated with the IPIs (Pandey et al. 2015). Educational attainment of the subject is most important factor for parasitic infection (Raut et al. 2021). Lack of mothers and children education are contributing factor for infection (Gyawali et al. 2010). Sah et al. (2013) assessed the mother's educational factor to the parasitic infection and found the parasitic infection to those children whose mother's education was below SLC was significantly high than to those from mother having education attainment of SLC or above.

Hand washing habit without using soap promotes the IPIs (Tandukar et al. 2013). A cross-sectional study done by Shrestha et al. (2018) in Dolakha and Ramechhap among children indicated higher risk of parasitic infection to those who did not use soap to wash their hands compared to those who used soap to wash hands before and after meal and after defecation. Children's WASH (Water, Sanitation and Hygiene) condition were shown directly related to their nutritional status and IPIs (Shrestha et al. 2020).

Nail biting and thumb sucking facilitates fecal-oral transmission of parasites (Sah et al. 2013). A study among the tea estates workers of Ilam carried out by Sah et al. (2013), finger sucking was found to be the strong predictor of IPIs. To study the association of IPUs to the socio-economic characters Dhakal (2018) conducted a cross sectional study in Meche community of Jhapa and she reported thumb sucking as significant to cause the IPIs. Shrestha et al. (2021) revealed nail biting as the major source of parasitic infection from his study among children of Dharan.

The IPIs are related to the shoe wearing habit of people. Not using shoes outside was considered as potential risk factor as shown using multiple logistic regression method by Parajuli et al. (2014). People with never or occasional use of slipper had higher risk of IPIs (Adhikari et al. 2021). As parasites penetrate in to the host body through the skin on barefoot (Shrestha et al. 2020)

In a study done among the prison inmates of Kathmandu, those who were dewormed had less parasitic prevalence than those who were not dewormed. The deworming might end the development inside the host and transmission inside the prison (Shrestha et al. 2019). Upama et al. (2019) conducted a study among 134 students of Kapan, Kathmandu and found that children who had not taken anti-parasitic drugs were more infected than those who had taken drugs within six months.

Water as an important route for transmission of IPIs was shown by Rai et al. (2005). In a study by Tiwari et al. (2013), children who used water directly from the source had higher prevalence of IPIs (32%). Chandrashekhar et al. (2020) conducted a study to estimate the prevalence of IPIs in Kaski and indicated 21.3% of prevalence suggesting the infection is via water (water-borne). Shrestha et al. (2021) indicated open defecation, unmanaged disposal of wastes and flood water are the factors that contaminate water and the supply of such water increases the risk of infection. Contaminated soil helps in the overdispersion of parasites as minimal dispersion of *T.*

trichiura was seen in lower rate of soil contamination. (Rai et al. 2000). Poor sewerage system harbors the parasites (Sharma et al. 2004)

In a study among children of squatter community, Chongbang et al. (2016) revealed that those who did not use water at toilet had higher prevalence of parasitic infection than those who used bucket water at toilet. People using simple pit latrine had higher risk of infection than those using pour flush toilet (Shrestha et al. 2020). According to Jimba and Joshi (2001) in urban areas, infection with cestodes are primarily associated with slaughtering facilities. Consumption of unwashed fruits and vegetables were associated with the infection among vegetarians from a study by Sah et al. (2013). The risk of cestode increases with the consumption raw vegetables grown in soil contaminated with cestode eggs and eating of raw or improperly cooked meat specially pork and beef (Yadav and Prakash 2016).

3. MATERIALS AND METHODS

3.1 Study Area

Kushma is a hilly area of Nepal that falls under Gandaki Province and Parbat District of Nepal covering an area of 494 km^2 with population of 146,590 (CBS, 2011). This city lies in the western part of Nepal just about 57 km from Pokhara. Kushma is the headquarters of Parbat district and in 18 May, 2014 changed into municipality merging eight existing Village Development Committee i.e. Pang, Khurkot, Durlung, Shivalaya, Chuwa, Katuwa Chaupari, Pipaltari and Pakuwa.

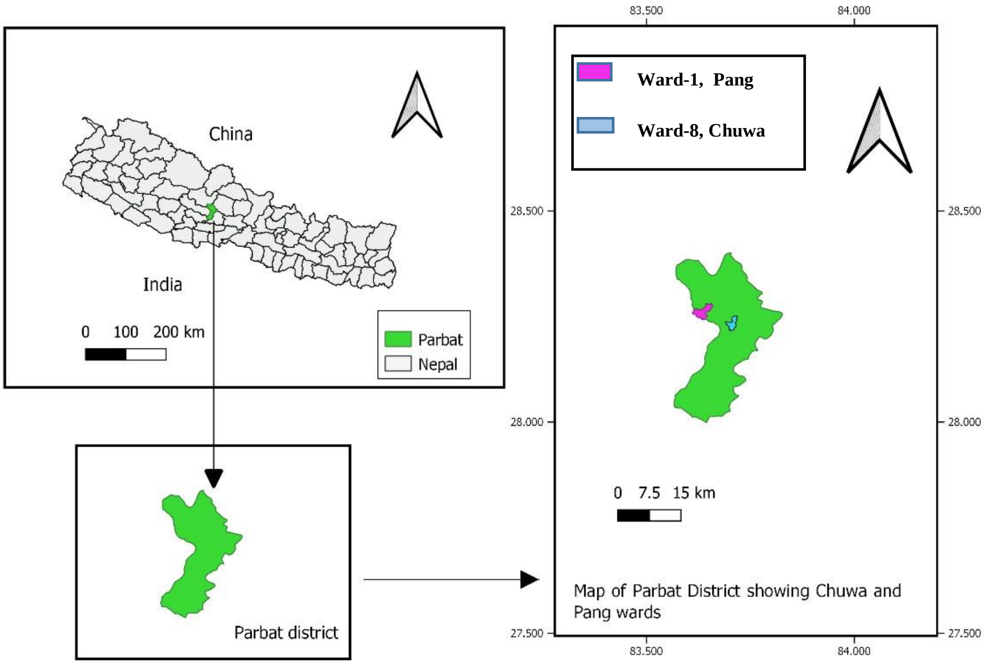

Fig. 1. Map of Nepal showing Kushma municipality with wrd-01, Pang and Ward- 08, Chuwa

According to the Kushma Municipality profile census handbook, 2075, for this study, Pang- 01and Chuwa-08 were selected purposively considering following given criterion:

- The two wards were selected comparatively as having high number of mud bonded bricks/ stones types of house foundation, dependency on reservoir as water source, use of firewood for cooking, houses without toilet or with ordinary toilet, illiteracy, poverty rate and agriculture as occupation according to CBS (2011).

3.1.1 Physiography and Climate

Two wards were selected i.e. Ward-01, Pang and ward-08, Chuwa from Kushma Municipality of Parbat for current study. Kushma is located at 28°13'06N 83°40'45E at an altitude of 1294 meters and 953 m above the sea level (http://www.kushmamun .gov.np/en/node/4). The annual rainfall is 2400 to 2600 mm. The normal maximum temperature in summer exceeds 32.3^0C and the normal winter temperature is about 7.5^0C. (Department of Hydrology and Meteorology). The soils are high to medium organic type (Department of Forest and Soil Conservation).

3.1.2 Population, Ethnicity and Occupation

Pang lies in the west of Kushma with an area of 8.76 km^2. The total population of this ward is 4566 with high number of female (2581). The major population includes Chhetri, Brahmin, Kami, Magar, Damai, Newar, Gurung, Dasnami, Thakuri, Majhi, Kumal and Rai.

Chuwa lies to the east with an area of 5.19 km2. The total population of this ward is 1759 with higher number of females (986). The major population of this ward includes Chhetri, Brahmin, Damai, Magar, Kami, Newar, Sanyasi and Thakuri. Agriculture, laborer, tailor and carpentry are the main occupation of the people of both wards. (Accessed on http://www.kushmamun.gov.np/en/ node/4).

3.2 Materials Required

3.2.1 Materials

Sterile vial and applicator, gloves, masks, strainer, Centrifuge and glass tubes, test tube stand, centrifuge machine, forceps, weighing machine, measuring cylinder, needle, glass slides, cover slip and compound microscope.

3.2.2 Chemicals

2.5% $K_2Cr_2O_7$ (Potassium dichromate), distilled water, normal saline (0.85%), Lugol’s iodine, 10% formalin, Ethyl acetate, sodium chloride, Ziehl-Neelsen (ZN) acid fast stain, immersion oil, sodium monophosphate and sodium bi-phosphate, Immersion oil and hand wash.

3.3 Methods

3.3.1 Preparation of 2.5% $K_2Cr_2O_7$

2.5 gm of $K_2Cr_2O_7$ was weighed accurately and dissolved in 100 ml of distilled water. This solution was used in the field to preserve the parasites present in the collected stool samples and also to maintain the integrity of the cyst or eggs present in the sample (Zajac and Conboy, 2012).

3.3.2 Preparation of Normal Saline

Normal saline was prepared by dissolving 8.5 gm of sodium chloride in 1000 ml of distilled water and used in unstained preparation (Zajac and Conboy, 2012).

3.3.3 Preparation on Lugol's Iodine

10 gm of potassium iodine was dissolved in 100 ml of distilled water and five gm of iodine crystal was added slowly in it. The solution was filtered and stored in a bottle. The iodine solution helps in studying the internal characters and identification of the protozoan species. (Zajac and Conboy, 2012).

3.3.4 Preparation of buffered 10% formalin solution

10 ml of 35-40% concentrated formaldehyde was added to 90 ml of distilled water. To this solution 0.08 gm of phosphate buffer was added. To prepare phosphate buffer, 6.1 gm of disodium phosphate and 0.15 gm of monosodium phosphate were weighed and mixed together to form a phosphate powder. Then 0.08 gm of buffered phosphate was weighed then added to the 10% formalin and mixed well (Garcia 2009).

3.4 Study Design

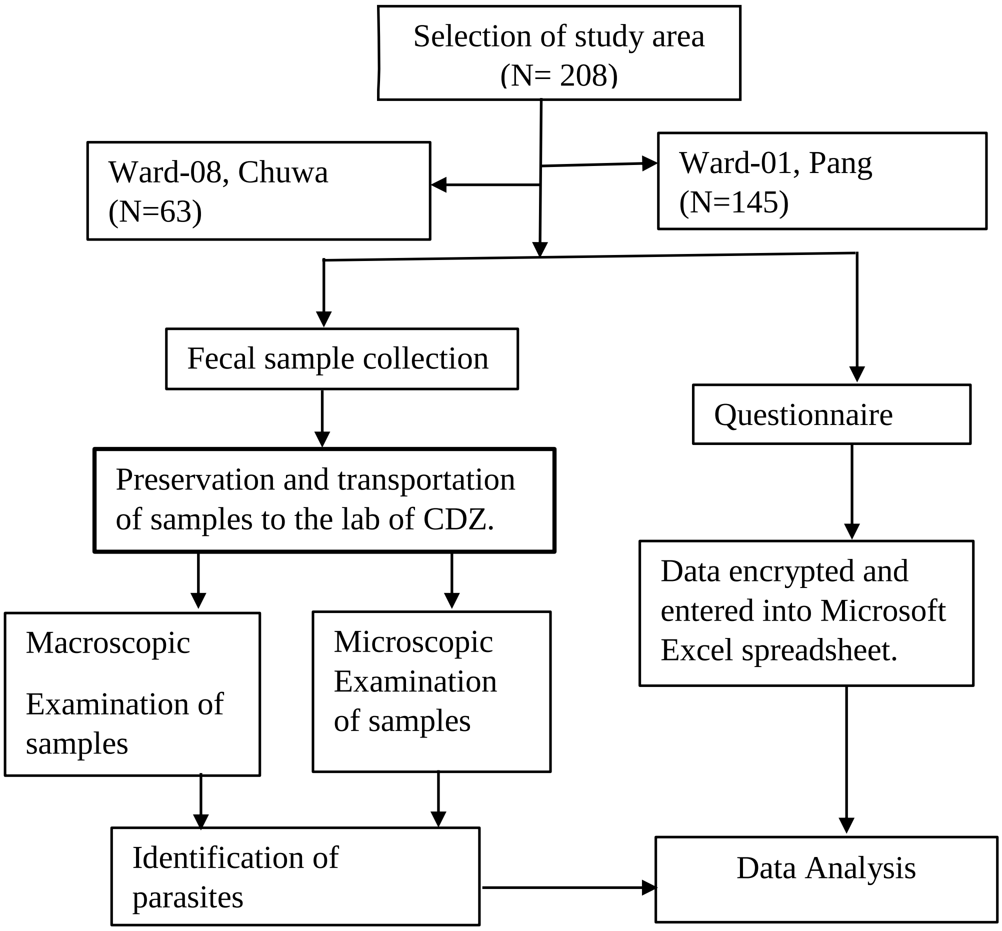

Fig. 2. Flow chart showing study design

3.5 Preliminary Survey

A preliminary survey was carried out in the study area in August to conceptualize the situation of the study area to form an effective research design.

3.6 Sample Size

Out of total population of each wards, approximately 10% population was taken. So calculated sample size was 160. In present study 208 samples were collected.

3.7 Survey Study

A purposive cross-sectional study was made from October 2021 to February 2022 to assess the prevalence of IPIs and associated risk factors among the community people of Kushma Municipality. Study subjects irrespective of ages and sexes were consecutively enrolled until the targeted sample of 208 was achieved. The study area was visited and different factors like educational attainment, personal hygiene, sanitation, latrine condition, drinking water supply and occupation were surveyed Different personal were met to gain the information about the study area and the tradition and activities of the people.

3.8 Questionnaire Survey

The precoded questionnaire elicited information on different demographic data (age, gender and educational attainment), socio-economic characters, behavioral (personal hygiene such as wearing slippers, washing hands before and after meal and after defecation with soap and water, thumb sucking, nail trimming and food consumption), medical treatment (whether the subject has taken drugs and iron supplements), environmental sanitation and living condition characteristics (source of drinking water, types of latrine and presence of animals) which were used to assess the potential risk factors. The questionnaire was first prepared in English and then translated into local language. In order to avoid the survey bias, response option was paid close attention. The data was gathered from pool of participants with varied features and demographics known as variables. This study contains multiple variables at the time of the data snapshot.

3.9 Sample Collection, Preservation and Transportation

Upon receipt of signed consent forms from both wards, a total of 208 samples were collected from October 2021 to February 2022 by convenience sampling technique. Prior to the collection of sample, people were properly instructed to collect the sample of the early morning and said to make sure not to contaminate with soil or urine. Sterile and leak proof vials were given along with wooden applicator. Then the samples were preserved immediately in 2.5% $K_2Cr_2O_7$ and uniquely coded on the submission of the sample then transported to the lab of Central Department of Zoology, TU, Kirtipur, Kathmandu. Seven samples while collecting from the participants were contaminated with urine and were discarded.

3.10 Laboratory Processing and Identification

The samples were processed for macroscopic and microscopic examination.

3.10.1 Macroscopic Examination

The samples were examined by naked eye for their color and presence of mucus, blood and adult nematodes or segments or proglottids of cestodes. This was done to obtain information on the type of parasitic infections that might be present. Consistency of the stool samples were also checked to determine whether there was any diarrheic stools or stools with unusual consistency were present.

3.10.2 Microscopic Examination

3.10.2.1 Saline wet mount examination

The preserved stool sample was first mixed thoroughly and one-two drops of sample were kept on a clean slide then gently covered with cover slip. The excess of fluid was removed with the help of filter paper and observed under microscope at a total magnification of 100 × and 400× power (Pradhan et al., 2005).

3.10.2.2 Stained preparation of stool smear

Two- three drops of sample was kept on a clean slide and a drop of iodine was added then covered with cover slip. Then the slide was observed under microscope at a total magnification of 100 × and 400× power. This method helps in the study of nuclear character of protozoan cysts and trophozoites (Malla et al., 2008).

3.10.2.3 Concentration method

Floatation and sedimentation are the two concentration method for routine examination of stool sample. Floatation mainly reveals the protozoan and sedimentation reveals helminthic parasites (Garcia and Procop 2016).

3.10.2.3.1 Floatation method

Saturated salt solution was used for this method. Floatation method is used to float the parasite with lighter density than the density of saturated salt (Garcia and Procop 2016).

2gm of sample was filtered and mixed with normal saline in a 15 ml centrifuge tube and then centrifuged at 1200 rpm for 5 minutes. The supernatant was discarded and few 4-5 ml of floatation solution was left in the tube. It was mixed well to resuspend the particles. Further concentrated Nacl solution was added and filled the tube up to 14 ml and centrifuged again at 1200 rpm for 5 minutes. The tube was further added with concentrated Nacl drop by drop until a convex surface was formed at the top. A clean coverslip was placed over the top of the tube avoiding any bubbles and was left undisturbed for at least 10 minutes. The coverslip was removed gently avoiding the dropping of the sample from cover slip and then placed over a clean glass slide. The slide was observed under compound microscope at a total magnification of 100 × and 400× power both with and without lugol's iodine.

3.10.2.3.2 Formalin Ethyl Acetate Sedimentation Method

Sedimentation method reveals the parasites with density higher than the density of the solution. It mainly detects trematode eggs, however some nematode eggs and larva (unfertilized Ascaris eggs) and some cestodes eggs (*Taenia* sp.) are also detected by this method as they do not float on concentration solution (Arora, 2012).

About 2 gm of sample was filtered thoroughly and mixed with normal saline in a 15ml centrifuge tube. The sample was centrifuged at 1200 rpm for five minutes. The supernatant was discarded and the sediment was mixed well. Then 10ml of 10% buffered formalin and 3 ml of ethyl acetate was added in the tube and again centrifuged. Four layers of ethyl acetate, plug of debris, 10% formalin and sediment were formed. The plug of debris were made free with wooden applicator stick then all of the supernatant fluid was decanted and discarded. Before bringing the tube to upright position ethyl acetate was made sure to be removed as it forms extensive bubbles under the microscope. In case if sediments were too dry one- two drops of 10%formalin were added and mixed well. A drop of sediment was placed on a clean slide, covered with cover slip and observed under microscope both with and without lugol's iodine (Garcia and Procop 2016).

3.10.2.4 ZN (Zeihl-Nelson) acid fast staining

The sediments obtained following the formalin ethyl acetate method were used to prepare thin smear on clear and dry glass slides. The smears were dried at room temperature and fixed with gentle heat. The smear was flooded with carbol Fuchsin stain (S005) and heated to steaming for 5 minutes with a low flame making sure the stain was not boiled and dried. Then the slide was allowed to stand for 5 minutes without further heating and washed in running tap water. The stain was decolorized with Acid Fast Decolorizer (S033) for 2 minutes or until no more stain came off in the washing. (If washing was not thorough, there was chance for false positive result). The slide was again washed with water and counterstained for 30 minutes with Methylene blue (S022). Finally the slide was washed with tap water, dried in air and then examined under oil immersion objective. (Henriksen and Pohlenz 1981).

3.10.2.5 Identification of egg, cyst and larva

All the above techniques were used to find the possible parasitic stages. However, coccidian were confirmed by the acid-fast staining methods. All the samples were observed under the optical microscope. The microscopic images were taken and morphometric analysis of cysts, and eggs was performed using different books (Arora and Arora, 2012; Chatterjee, 2009, Lima et al., 2018), internet sources, published and unpublished articles.

3.10.2.6 Calibration of egg, cyst and larva

The measurement of length, breadth and diameter of the parasite egg, cyst and larva was done by calibration of microscopic ocular and stage micrometer. They were measured with the calibration factors (C.F).

C.F= (No. of S.D/ No. of O.D) × 10 μm

C.F for 10x= 10 μm

C.F for 40x= 2.6 μm

3.11 Data analysis

The outcome variable was stool parasite status of the selected participants, whether positive or negative for any intestinal parasite, which was determined from the stool sample. Data on the independent variables, socio-demographic characteristics, behavioral characteristics and past medical history were collected by questionnaire. The collected data were encrypted and entered into Microsoft Excel spreadsheet. Data were analyzed using Pearson's Chi-squared ($\chi2$) and Fisher's exact test using SPSS software. Association of GI parasitic infections with respect to demographic, socioeconomic, occupational, and behavioral characteristics among the studied populations were analyzed. In all cases, (5% class interval CI value with $p<0.05$ was considered for the statistically significant difference). Odds ratios were computed to measure the strength of association. To determine independent risk factors for infection, unadjusted and adjusted logistic regression analysis at 95% CI was employed where appropriate.

3.12 Ethical issue

The required permission for the collection of the faecal samples was obtained from both the Ward offices of Pang and Chuwa. Participation was voluntary and the informed consent was obtained from each participants. In case of children, permission from child and parents was taken. Prior to the survey, the study's detailed purpose and procedures were explained verbally to the participants in the Nepali language. The aim of the study and benefits of participation was clearly pre-informed to the participants. Participants were told that they have right to withdraw at any time during the course of data collection. No experimental infection was established during this research work.

4. RESULTS

The present study was conducted in two wards of Kushma municipality, Parbat Nepal. Altogether 208 stool samples were collected and examined to determine the prevalence of intestinal parasites and their associated risk factor were assessed via structured questionnaire survey.

Table 1: Association between macroscopic properties of stool samples and intestinal parasitic infection.

Macroscopic properties **Sub-properties**		**Total (N=208)**	**Infected(n) (n/N)*100**	**%**
Color	Brown	106	41	38.7
	Black	4	0	0.0
	Green	11	1	9.1
	Red	9	1	11.1
	Grey	41	5	12.2
	White	1	0	0.0
	Yellow	37	2	5.4
Consistency	Formed	101	25	24.8
	Loose	78	18	23.1
	Soft	23	7	30.4
	Watery	6	0	0.0
Blood	Present	0	0	0.0
	Absent	208	50	24.0
Mucus	Present	3	1	33.3
	Absent	205	50	24.4
Worms	Present	0	0	0.0
	Absent	208	50	24.0

The samples collected were examined for their macroscopic characteristics like color, consistency and presence of mucus, blood and adult worms or segments of cestodes.

Out of 208 samples, higher prevalence was shown by brown colored stool (38.7%). While observing consistency, soft stool had higher prevalence (30.4%). Stool with the presence of mucus showed high rate of parasitic infection. Blood, adult worms and segments of cestodes were not seen in the sample of this study.

4.1 Prevalence of intestinal parasitic infection in Kushma Municipality, Parbat, Nepal

Out of 208 samples, 50 (24.04%) were found to be infected with one species of protozoan, three species of nematodes and two species of cestode parasites.

Table 2: Parasite-specific Prevalence in Kushma Municipality, Nepal.

Parasitic infection	Total (N=208)	
	Infected person (n)	Prevalence (%)
Protozoan infection		
Cryptosporidium sp.	8	3.9
Nematode infection		
Ascaris lumbricoides	36	17.3
Enterobius vermicularis	2	1
Hookworm	1	0.5
Cestode infection		
Taenia sp.	1	0.5
Hymenolepis nana	2	1

Only one species of protozoan parasite i.e *Cryptosporidium* sp. was found. Out of five total helminth parasites, *A. lumbricoides* was found to be predominating while prevalence of hookworm (0.5%) and *Taenia* sp. (0.5%) were found to be least (Table 2).

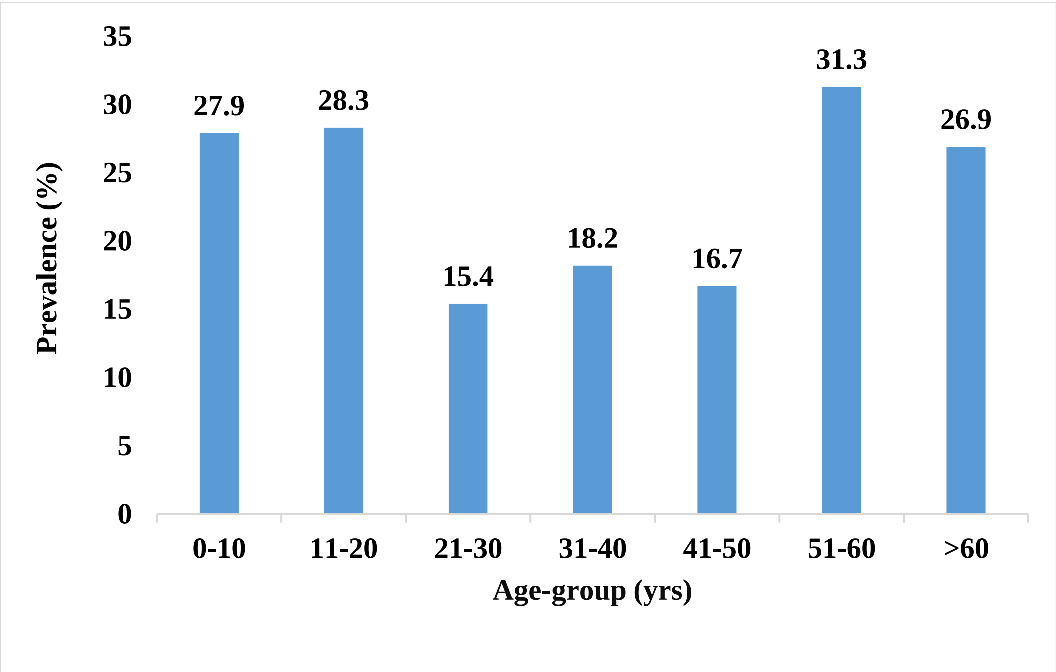

Fig. 3. Age-wise prevalence of Parasitic Infection in Kushma Municipality

The study subject were categorized into seven different age-groups with the gap of 10 years each and it was found that age groups of 51-60 were highly infected however there was no much difference in infection between the age groups of 0-10 and 11-20. Comparatively, participants of age group 21-30 were least infected. (χ^2= 3.598[a], P>0.05). (Figure 5).

Table 3: Parasite specific Prevalence among different Age-groups of Kushma Municipality

Parasites	Infected person among different age-group (%)						
	0-10	**11-20**	**21-30**	**31-40**	**41-50**	**51-60**	**>60**
Protozoan Infection							
Cryptosporidium spp.	3 (6.5)	1 (2.3)	1 (3.1)	0	1 (5.9)	0	2 (7.1)
Nematode Infection							
Ascaris lumbricoides	10 (21.7)	9 (20.9)	2 (6.3)	2 (11.8)	4 (23.5)	5 (20)	4 (14.3)
Enterobius vermicularis	1 (2.2)	0	0	0	1 (5.9)	0	0
Hookworm	0	0	1 (3.1)	0	0	0	0
Cestode Infection							
Taenia spp.	1 (2.2)	0	0	0	0	0	0
Hymenolepis nana	1 (2.2)	0	1 (3.1)	0	0	0	0

Analyzing Table 3, it was found that senior adults of age more than 60 years were highly (7.1%) infected by *Cryptosporidium* sp. *A.* lumbricoides (23.5%) was prevalent among the age groups of 41-50, *E.* vermicularis (5.9%) among participants of 41-50 years. While hookworm was prevalent among 21-30 years age group only and *Taenia* sp. Only among 0-10 years age group. On the other hand *Hymenolepis nana* was highly present among 11-20 years age group participants.

Table 4: Intestinal parasitic infection among Male and Female of Kushma Municipality

Parasite species	Sexes		p-value
	Male (N=87)	**Female (N= 121)**	
Protozoan			
Cryptosporidium sp.	5 (5.8%)	3 (2.5%)	0.283
Nematodes			
Ascaris lumbricoides	17 (19.5%)	19 (15.7%)	0.578
Enterobius vermicularis	1 (1.2%)	1 (0.8%)	
Hookworm	0	1 (0.8%)	
Cestodes			
Taenia sp.	0	1 (0.8%)	
Hymenolepis nana	1 (1.2%)	1 (0.8%)	
Total parasitic infection	24 (27.6%)	26 (21.5%)	0.31

Table 4 shows that, comparatively, both male (19.5%) and female (15.7%) were highly infected by *Ascaris* sp. hookworm and *Taenia* sp. were absent among male but were equally (0.8% each) present among female. There was no significant association of *Cryptosporidium* sp. and *Ascaris* sp. infection between male and female.

Also the prevalence of intestinal parasitic infection for male and female was found to be 27.6 % and 21.5%% respectively showing higher prevalence in male than in female. However the statistical association between the sex was insignificant (χ^2= 1.031[a], P>0.05). P-value was calculated using Fisher's exact test and chi-square test.

Table 5: Pattern of Parasitic Infection in Kushma Municipality

Parasitic infection	Infected No.	Overall prevalence (%)
Total Protozoan infection	8	3.9
Total Nematode infection	39	18.8
Total Cestode Infection	3	1.4
Double Infection (Hookworm+ *E. vermicularis*)	1	0.5
Quadruplet Infection (Hookworm+ A.*lumbricoides*+ *E. vermicularis* + *Taenia sp.*)	1	0.5

Considering the infection concurrency, both double and quadruplet infection were equally prevalent (Table 5). Among helminths, nematode infection (18.8%) has higher prevalence rate.

Table 6: Intestinal Parasitic Infection in two Wards of Kushma Municipality.

Parasite species	**Wards**		**p-value**
	Pang (N= 163)	**Chuwa (N= 45)**	
Protozoan			0.05
Cryptosporidium sp.	7 (4.3%)	1 (2.2%)	
Nematodes			
Ascaris lumbricoides	22 (13.5%)	14 (31.1%)	
Enterobius vermicularis	2 (1.2%)	0	
Hookworm	1 (0.6%)	0	
Cestodes			
Taenia sp.	1 (0.6%)	0	
Hymenolepis nana	1 (0.6%)	1 (2.2%)	
Total parasitic infection	34 (20.9%)	16 (35.6%)	0.041

Studying Table 6, it was found that *Ascaris* sp. was highly prevalent in Ward-08, Chuwa. *Cryptosporidium* sp. and *H. nana* were equally prevalent (2.2%) in Chuwa.

While, *E. vermicularis*, hookworm and *Taenia* sp. were prevalent only in ward 01-Pang. Similarly, *Cryptosporidium* sp. was highly prevalent in Pang than in Chuwa. Statistically, prevalence of species wise intestinal parasitic infection was significantly associated between the two wards ($P<0.05$).

While comparing the prevalence of IPIs in between two wards selected for the present study, parasitic prevalence was found comparatively high in ward-08, Chuwa (35.6%) than ward-01, Pang (20.9%). Also, statistically parasitic Infection was significantly prevalent in Chuwa than in Pang (χ^2=4.171[a], p=0.041). P-value was calculated using Fisher's exact test and chi-square test.

4.2 Assessment of the risk factors associated with intestinal parasitic infection in Kushma Municipality, Parbat, Nepal.

Several demographic, socioeconomic, occupational, and behavioral characteristics among people of Kushma Municipality were evaluated for their association with prevalence of intestinal parasitic infection. For the identification of risk factors, 17 different factors were assessed using univariate analysis and multivariate analysis.

Table 7: The unadjusted association between intestinal parasitic infection and all the risk factors.

Risk factor	**Sub groups**	**No. of examined**	**No. of infected (%)**	**Crude OR**	**p-value**
Sex	Female	121	26 (21.5)	1	
	Male	87	24 (27.6)	1.4 (0.7-2.6)	0.311
Age	0-10	43	12 (27.9)	2.1 (0.6-7.5)	0.239
	11-20	46	13 (28.3)	2.2 (0.6-7.5)	0.223
	21-30	26	4 (15.4)	1	
	31-40	33	6 (18.2)	1.2 (0.3-4.9)	0.776
	41-50	18	3 (16.7)	1.1 (0.2-5.6)	0.909
	51-60	16	5 (31.3)	2.5 (0.6-11.2)	0.231
	>60	26	7 (26.9)	2.0 (0.5-8.0)	0.313
Literate	Yes	128	27 (21.1)	1	
	No	80	23 (28.8)	1.5 (0.8-2.9)	0.21
Occupation	Farmer	28	8 (28.6)	1.9 (0.6-5.8)	0.307
	Housewife	56	13 (23.2)	1.4 (0.5-3.9)	0.537
	Students	85	22 (25.9)	1.6 (0.6-4.1)	0.334
	Others	39	7 (18)	1	
Use of slipper	Always	79	1 (1.3)	1	
	Occasional	129	49 (38)	47.8 (6.4-354.5)	0

Nail trimming	Yes	129	10 (7.8)	1	
	No	79	40 (50.6)	12.2 (5.6-26.7)	0
Nail biting	Yes	31	14 (45.2)	3.2 (1.5-7.2)	0.004
	No	177	36 (20.3)	1	
Thumb sucking	No	11	2 (18.2)	1	
	Yes	197	48 (24.4)	1.5 (0.3-6.9)	0.642
Drug Consumption	Within 6 months	85	5 (5.9)	1	
	Before 6 months	123	45 (36.6)	9.2 (3.5-24.5)	0
Source of drinking water	Tap water	160	38 (23.8)	1	
	Reservoir	48	12 (25)	0.9 (0.4-2.0)	0.859
Use of boiled/ filtered water	Yes	48	5 (10.4)	1	
	No	160	45 (28.1)	0.3 (0.1-0.8)	0.016
Use of soap	Yes	192	42 (21.9)	1	
	No	16	8 (50)	3.6 (1.3-10.1)	0.016
types of house	Concrete	78	16 (20.5)	1	
	Muddy	130	34 (26.2)	1.4 (0.7-2.7)	0.358
Types of Toilet	Permanent	169	37 (21.9)	1	
	Temporary	39	13 (33.3)	1.8 (0.8-3.8)	0.135
Family size	Less than 5	132	27 (20.5)	1	
	More than 5	76	23 (30.3)	1.7 (0.9-3.2)	0.113
Presence of animals	No	57	9 (15.8)	1	
	Yes	151	41 (27.2)	2.0 (0.9-4.4)	0.091
Frequency of meat consumption	Once in a week	102	22 (21.6)	1	
	Once in 2-3 weeks	103	27 (26.2)	0.6 (0.5-6.4)	0.632

OR: Odds Ratio, ** highly significant, * significant

From unadjusted odds ratio, age, sex, educational status and occupation showed no significant relation to intestinal parasitic infection. However use of slipper, nail trimming and drug consumption were found to be the strong predictors of IPIs. When compared with always use of slipper, occasional use of slipper were more likely to be infected with IPIs (COR= 47.8, 95%CI= 6.4- 354.5, p=0). Similarly, people with untrimmed nails were more likely to be infected than those with trimmed nails (COR= 12.2, 95% CI= 5.6- 26.7, p=0). Likewise, when compared with dewormed people, those with irregular or no consumption of drugs were found to be more infected with IPIs (COR= 9.2, 95% CI= 3.5- 24.5, p=0).

Other important predictors were nail biting (COR= 3.2, 95% CI= 1.5- 7.2, p=0.004), use of soap to wash hands (COR= 3.6, 95%CI= 1.3- 10.1, p= 0.016) and use of boiled or filtered water for drinking purpose (COR= 0.3, 95% CI= 0.1- 0.8, p= 0.016).

Table 8: The Adjusted association between intestinal parasitic infection and the risk factors.

Risk factor		Model I[a]		Model II[b]	
Sub groups		AOR	p-value	AOR	p-value
Use of slipper	Always	1			
	Occasional	17.8 (2.1-150.1)	0.008	15.4 (1.9-125.7)	0.011
Nail trimming	Yes	1			
	No	4.3 (1.7-11.2)	0.003	4.1 (1.7-10.2)	0.002
Nail biting	No	1			
	Yes	1.1 (0.4-2.9)	0.917	1.2 (0.5-3.1)	0.732
Drug Consumption	Within 6 months	1			
	Before 6 months	0.2 (0.1-0.5)	0.001	0.2 (0.6-0.5)	0.001
Use of boiled/ filtered water	Yes	1			
	No	2.1 (0.6-6.7)	0.253	2.0 (0.6-6.4)	0.246
Use of soap	Yes	1			
	No	3.8 (0.9-16.5)	0.074	2.5 (0.7-9.3)	0.178
Types of Toilet	Permanent	1			
	Temporary	0.5 (0.2-1.3)	0.16		
Family size	Less than 5	1			
	More than 5	1.6 (0.6-3.8)	0.319		
Presence of animals	No	1			
	Yes	2.3 (0.8-6.2)	0.115		

Model I[a]: adjusted for use of slipper, nail trimming, nail biting, drug consumption, use of filtered/ boiled water, soap to wash hands, type of toilet, family size and presence of animals, where the variables have their p-value <0.2 in univariate analysis.

Model II[b]: adjusted for use of slipper, nail trimming and drug consumption, where all the variables have p-value <0.05 in the univariate analysis.

Table 8 shows the adjusted association between IPIs and various risk factors. Use of slipper (AOR= 15.4, 95%CI= 1.9-125.7, p= 0.011)), nail trimming (AOR= 4.1, 95%CI= 1.7-10.2, p= 0.002) and drug consumption (AOR= 0.2, 95%CI= 0.1- 0.5, p=0.001) were found to be associated with the intestinal parasitic infection.

5. DISCUSSION

5.1 Intestinal Parasitic Infection

Intestinal parasitic infection is the significant cause of mortality and morbidity, particularly in developing countries like Nepal and especially in people with low socio-economic status with existing poverty, poor lifestyle and housing, illiteracy and ignorance and poor environmental conditions. Current study shows overall prevalence of 24.04% of intestinal parasitic infection. The finding was found to be exact to the result of Tandukar et al. (2018) who found 24.3% prevalence among 333 school children from five different public school of Kathmandu. Likewise, the result was almost similar to the findings from different parts of Nepal like, Dharan municipality (Gyawali et al. 2009) where 22.5% prevalence from 182 participants was manifested; Parsa (Shakya et al. 2009) where 20.7% among 2221 hospital visiting participants were infected by intestinal parasites; Banke (Rai et al. 2018) from where 25.96% prevalence was detected; Jhapa (Dhakal and Subedi 2019) where 27.3% was found while studying the prevalence of parasite in relation to socio-economic status of Meche community; Kathmandu (Acharya et al. 2021) from where 25.67% of prevalence was reported; Butwal and neighboring district Kaski (Chandrashekhar et al. 2020) from where 21.3% overall prevalence of IPIs was detected among 2091 samples. However this study showed higher prevalence of intestinal parasitic infection compared to those by Shrestha et al. (2021) who found 11.5% overall prevalence among 400 private school pupils of Dharan and similarly, Limbu et al. (2021) presented a report of 7.75% overall prevalence from 116 participants of Dharan. In contradiction, current result was less than from other parts of Nepal, like those from Dolakha and Ramechhap (Shrestha et al. 2018) where 39.7% among 708 participants were infected by intestinal parasites; rural Nepal (Bertoncello et al. 2021) where 37.6% of the rural people were found to be infected when variables were relates to dwelling area and sex; Karnali (Shrestha et al. 2020) (51.1%); Sunsari (Das et al. 2019) (50.5%); Bhairahawa (Raut et al. 2021) (46.5%); Kathmandu (Gurung et al. 2019) (40%); Makwanpur and Nawalparasi (Khadka et al. 2021) (36.6%); Chitwan (Adhikari et al. 2021) (97%) and neighboring district, Baglung (Thapa et al. 2021) where 47.5% among 498 participants from Sarki ethnic group were infected by intestinal parasitosis.

Comparing to our neighboring countries, the overall IPIs among community people of Kushma municipality was in agreement to the studies by Wali et al. (2019) who revealed 22.8% of prevalence of intestinal parasitic infection among 450 participant from Pakistan. However, our finding was lower than the findings by Mareeswaran et al. (2018) from India, who found the prevalence rate as 34.06% from the people of Kancheepuram district of Tamil Nadu India. Similarly, Chandi and Lakhani (2018) reported 31.2% of prevalence among school children from Chhatisgarh, India. From Pakistan many researcher presented their finding on IPIs. Like, Bhatti et al. (2018) reported prevalence of 73% among the people in a rural community from Pakistan; Afridi et al. (2021) showed 57.67% of prevalence of IPIs in relation to wasting among children; Rahman et al. (2021) manifested 71.7% prevalence among children of urban parts; Ulhaq et al. (2021) reported 82% prevalence from the rural areas and Irum et al. (2021) presented 78.3% of prevalence based on socio-economic determinants in Pakistan. On the other hand, our result was higher than the findings from India by Jayaram et al. (2021) who presented 13.4% of overall prevalence of IPIs, from Pakistan by Arshad et al. (2019) who reported 12.4% prevalence, from china by Li et al. (2014) who presented 14.9% of prevalence and Ying-dan et al. (2020) who reported 3.30% of IPIs from the national survey.

Comparing with global studies of intestinal parasitic infection in relation to socio-economic status and socio-environmental condition, the overall prevalence of IPIs of community people of Kushma municipality was in accordance to those reported from Southern Ethiopia as 26.14% (Seid et al. 2022), Minnesota as 25% (Lifson et al. 2016) and Brazil as 28.88% (Barcelos et al. 2018). However this result was higher than findings in Turkey where 8.8% prevalence of IPI was reported (Gündüz et al. 2008); Italy reported 1.3% of overall prevalence (Masucci et al. 2011); Accra reported 15% of prevalence (Forson et al. 2018); Jimma, Ethiopia as 20.6% (Belete et al. 2021) and Iran reported 22.26% of prevalence (Barati et al. 2021). While this result was lower than the findings from Eritrea (Kesete et al. 2020); South America where 85% of IPI prevalence was evidenced; Kenya, Africa (46.5%) (Kimosop et al. 2021); Argentina (92.7%) (Candela et al. 2021); Egypt (46.2%) (Elmonir et al. 2021); Bule Hora, Ethiopia (46.3%) (Hajare et al. 2021); Egypt (Elmonir et al. 2021) and Nicaragua, America (Muñoz-Antoli et al. 2022) where 97% of prevalence was evidenced. These differences may be due to different climatic conditions, geographical features, employment of differing diagnostic techniques or due to different demographic and socioeconomic patterns of the populations studied (age, presence of indigenous groups, rural versus urban areas)

In this study only one protozoan and five helminthic parasites were recorded. For protozoan species *Cryptosporidium* sp. was found with the prevalence rate of 3.9%. This result was higher than the studies carried out in different parts of Nepal by Ono et al. (2001), (Bhattachan et al. 2017) and other countries by, Ranjbar et al. (2016), Kiani et al. (2017), Xu et al. (2020). In contrast, current finding is lower than those reported from Nepal (Sherchand and Shrestha 1996), (Sherchand et al. 2016) and (Bhattachan et al. 2017) and other countries (Muñoz-Antoli et al. 2011), (Ayres Hutter et al. 2020), (Wang et al. 2018) and (Lebbad et al. 2021). However this result is supported by (Bhattacharya et al. 1997), (Taghipour et al. 2011), (Sharbatkhori et al. 2015) and (Yang et al. 2018). *Cryptosporidium* sp. are the main cause of diarrhea in immunodeficient (Hunter and Nichols 2002). It is transmitted from soil, food and water (Smith et al. 2006) and a major zoonotic disease (Feltus et al. 2006). Therefore muddy house type, occasional use of slipper and close contact with the animals might have been the cause for the prevalence of *Cryptosporidium* sp.

This study showed the higher prevalence of helminth (nematodes and cestodes) over protozoan. This finding of higher infection of helminth was in accordance with various findings from Nepal (Sharma et al. 2004), (Khadka et al. 2021) and (Mishra and Ghimire, 2005); India (Kumar et al. 2014); Nigeria (Okodua et al. 2003) and (Obiamiwe and Nmorsi 1991). However findings from Nepal (Shrestha et al. 2018) and (Yong et al. 2000) India (Sehgal et al. 2010) and (Dash et al. 2010) contrasted the current result. Poor housing and drinking water (Utzinger et al. 2010), poverty (Hotez et al. 2008), human-animal contact (Deplazes et al. 2011) and poor environmental hygiene (Brooker et al. 2003) are the main cause for helminthic infection. Hence, irregularity in biannual deworming, irregular use of slippers, nail biting and use of untreated water may be the reason for high prevalence of helminths. The high prevalence of helminthic infection may be partly due to the fact that the study was carried out in the rural communities that account for poverty, poor socio-economic development, and unhygienic environment that facilitate the transmission of parasites.

Among the helminth, *Ascaris* sp. was highly dominating (17.3%) which is also dominating in the finding from Nepal by Shakya et al. (2009) Subba and Singh (2020); from Pakistan by Ali et al. (2020); from Nigeria by Chijioke et al. (2011); from South Africa by Nxasana et al. (2013) and Madagascar by Kightlinger et al. (1995). However lower rate of prevalence of *Ascaris* sp. was reported by Yadav and Prakash (2016) from Nepal; Langbang et al. (2019) and Gopalakrishnan et al. (2018) from India; Yang et al. (2018) from China and Hunninghake et al. (2007) from Costa Rica. The presence of cats, dogs and livestock, occasional use of slipper causing direct contact to soil, habit of nail biting and thumb sucking and use of untreated drinking water might act as transmission route for *Ascaris* which is also discussed by (Shalaby et al. 2010), (Schulz and Kroeger 1992), (Ali et al. 2020) and (Wali et al. 2019) respectively.

E. vermicularis was the other nematode which had prevalence rate of 1% which was lower than those recorded from Nepal as 1.5% by Shrestha et al. (2021); 12.72% by Dahal and Maharjan (2015) and 4.11% by Dahal et al. (2022); 20.6% from Korea by Lee et al. (2000) and 7.1% from Iran by Afrakhteh et al. (2016). In contradiction, current result was higher than those from Nepal (0.4%) (Shrestha and Maharjan 2013), (0.05%) (Baral et al. 2017); from Nigeria (0.01%) (Abah and Arene 2015); From Iran 0.07% (Bahrami et al. 2018); from South Africa 0.26% (Meloni et al. 1993) and from Thailand (0.5%) (Kitvatanachai et al. 2008). The prevalence of *E. vermicularis* might be mainly due to thumb sucking and nail biting habit that aid in the ingestion of the infective pinworm eggs that were on the fingers or fingernails which is also suggested by (Fan et al. 2019). Hand wash by not using soap furthermore helps in the ingestion of the eggs which could prevent the infection if soap was used.

Hookworm, the nematode helminth was found in 0.5% of the study subject. The current prevalence was lower than the finding from Nepal (1%) (Baral et al. 2017); Thailand (1.4%) (Kitvatanachai et al. 2008); Southern Ethiopia (5.9%) (Nyantekyi et al. 2010) and Nigeria (25%) (Abah and Arene 2015). And it was higher than those from Northeast Thailand (0.4%) (Boonjaraspinyo et al. 2013). The infection from hookworm may be due to the habit of walking and working barefoot which facilitate the ease of penetrating the host skin as suggested by (Tadege et al. 2022) and (Hussein et al. 2022). Community based Mass Drug Administration (MDA) is the major factor for controlling Soil Transmitted Helminths specially hookworms (Avokpaho et al. 2021). So irregularity in biannual deworming might also be the reason.

Among cestode, current study shows higher prevalence of *H. nana* than *Taenia* sp. *H. nana* showed 1% of prevalence which is lower than reported by others from Nepal 1.37% Dahal et al. (2022); 16% (Khanal et al. 2011); 46.56% (Tiwari et al. 2013); India 9.9% (Mirdha and Samantray 2002); Pakistan 1.81% (Tasawar et al. 2004); Iran 1.1-2.4% (Abbaszadeh Afshar et al. 2020) and (Mahni et al. 2016) and higher than that reported from Nepal 0.44% 0.81% (Malla et al. 2004), (Pandey et al. 2015) and (Shrestha and Maharjan 2013); Iran 0.07- 0.16% (Bahrami et al. 2018) and (Memar et al. 2007). *Taenia* sp. was another cestode found in this study with prevalence rate of 0.48%. Comparing to this result, higher prevalence rate were reported from Nepal 1.01-21.0% (Shrestha and Maharjan 2013) and (Bhattachan et al. 2015); from Ethiopia 4.5% (Nyantekyi et al. 2010) and from Thailand 0.9-1.6% (Kitvatanachai et al. 2008) and (Boonjaraspinyo et al. 2013). Lower rate were reported from Iran 0.07% (Bahrami et al. 2018); West Cameroon 0.25% (Nkenfou et al. 2013), from Nigeria 0.89% (Abah and Arene 2015). The prevalence of infection from cestode might be due to the consumption of raw or improperly cooked pork (Rajshekhar et al. 2003).

Age-wise, from current study higher rate of infection was found among those aged 51-60 years (31.3%) and lower rate among those aged 21-30 years (15.4%) which is supported by (Khanal and Bhujel, 2018). In their study also 51-60 age group were highly infected (33.3%). Similarly, 100% of people aged 40-59 were infected in the study done by (Adhikari et al. 2020). Likewise, Hajare et al. (2021) from Ethiopia also reported low infection among the participants aged 21-30 years (44.8%) than other age groups. However this study is different from those of Thapa (2018) where maximum infection was found among 11-20 age-groups (80%) and lower rate was found among 41-50 age groups (38.46%). Also this study was different from that by Shah et al., (2010) in Kaghan valley, Pakistan where adults were highly infected than senior age groups. The higher IPI among senior age group may be due to their unhygienic habit like nail biting, irregular deworming and barefoot walking and even at home. Less education might also be the reason which is as discussed by (Tappeh et al. 2010). Personal hygiene and deworming of animals could result in less IPIs (Forson et al. 2018).

For species wise prevalence of IPI among different age-groups, current study revealed high protozoan infection i.e. *Cryptosporidium* sp. among senior adults aged above 60 years (7.1%) and among 0-10 age groups (6.5%) while those aged 31-40 years and 51-

60 years were not infected by the protozoan parasite. This result is in tune to other findings like, Xu et al. (2020) from China also found higher prevalence (40.3%) of *Cryptosporidium* sp. among age groups >69 years than other age groups; Qasem et al. (2022) collected high specimen from senior adults (22.29%) in Yemen. Similarly, no *Cryptosporidium* sp. was seen among the age group of 31-40 and 51-60 years and was seen in one patients if age group 41- 50 years. However, unlike our result, infection was not seen among senior adults in the study done among immunocompromised patients of Turkey in 2022 (Bayraktar et al.). However lower prevalence (0.54%) of *Cryptosporidium* sp. infection was revealed by Bern et al. (2000) among senior adults than other age groups of Central America; Ghanadi et al. (2022) also revealed higher prevalence among above 60 age groups in western Iran. Treatment of drinking water and handling of domestic animals might be the cause of infection as also discussed by Walter et al. (2021). The higher prevalence among senior adults aged more than 60 years might be also due to their low immune system

Ascaris sp. was highly prevalent among participants of age group 41-50 years and low among 21-30 age groups which is supported to the study done by Akinsanya et al. (2021) in Nigeria. In their study epidemiology and risk factors associated with Soil Transmitted Helminth (STH) were investigated and found participant aged more than 40 were highly infected by Ascariasis. Report on lower prevalence among 21-30 age groups was also added by Kurscheid et al. (2020) from Indonesia. In contrast, highest prevalence among 21-30 age groups (8%) and lowest prevalence among 41-50 age group (6.7%) was shown by Onyido et al. (2022) among the food vendors of Nigeria. The possible reason for high prevalence among 41-50 age group might be because the adults were engaged in agricultural works which might have facilitated in the ingestion of food by contaminated hands. This age categories are also more prone because as parents or elder family member they might have constant contact with the younger children and might get infected added by poor hygiene.

In current study, *E. vermicularis* was only present among 0-10 (2.2%) and 41-50 (5.9%) age groups while absent among all other age groups. Similarly. Highest prevalence among children was reported from Tanahun of Nepal (Dahal and Maharjan 2015).Likewise, presence among 0-10 (16.7%) and 21-30 (5.5%) while absent among other age groups was also documented in 2019 while investigating the relation of parasitic infection with appendicitis in Iraq (Al-Hamairy et al.). Highest prevalence among children than other age groups was also presented from Sudan (Abdalatee 2019). To rebut this argument, Sabry et al. (2021) showed higher prevalence among 0-10 age groups while absent among 41-50 age groups of Iraq. In 2022 (Sangani et al.) reported higher prevalence among 0-10 and 21-30 age groups while absence among other age groups including 41-50 age categories in a tertiary care hospital in India. Presence among 11-20 age category only and absence among other was also documented in 2017 by Badamasi and Liadi (2017). The highest prevalence might be because of poor health condition, nail biting (Wendt et al. 2019), anal to finger to mouth contamination (AL-kafaji and Alsaadi 2022)

In current study, hookworm was found only among adults of age 21-30 years and absent among all others age groups. Similarly, higher prevalence of hookworm among 21-30 age group (1.3%) and absent among 31-40 and above 50 years age group was also presented by Onyido et al. (2022) from Nigeria. Withal, (Sangani et al.) from India in 2020 also documented high prevalence among 21-30 age categories. From Nigeria high prevalence of hookworm among the age group of 21-30 years when studied among three rural villages was also made forward (Ugbomeh et al. 2018). Just opposite to our result, absence of hookworm only among 21-30 age groups while present among other age groups was indicated by Badamasi and Liadi (2017) from Nigeria. Highest prevalence among children (23.8%) was reported by Gebrezgabiher et al. (2022) from Ethiopia. As people of 21-30 age group are more engaged in agricultural works and having the habit of working and walking barefoot might have caused highest rate of infection. A campaign in Uganda for wearing shoes reduced the intensity of STH up to six months after deworming (Paige et al. 2017).

Taenia sp. was found only in children of 0-10 years and absent among all other age groups. Likewise, higher prevalence of taeniasis among children (14.7%) was also detected by Onyido et al. (2022) from Nigeria. Similarly, children were highly infected by taeniasis in Western China as found from a cross-sectional study (Li et al. 2019). Unlike our study, highest prevalence among 51-60 age group was detected in Ghana (Addo et al. 2021). Similarly, highest prevalence among adults of 31-40 years (19.8%) was reported when taeniasis and cysticercosis and their related factors were studied in Vietnam (Binh et al. 2021). Taeniasis might be due to the consumption of raw or improperly cooked pork and also the weak immunity of children. Taeniasis and cysticercosis can be related to poor sanitation and education which are indicator of low socio-economic status and disease (Carrique-Mas et al. 2001).

In this study, *H. nana* were present only among 21-30 (3.1%) and 0-10 (2.2%) age groups while absent among all other age groups. Exact to our finding, *H. nana* was also present only among 0-10 and 21-30 age groups and absent among other age groups in the study done among patients of Tertiary care of India by (Sangani et al.) in 2020. Similarly, absence of the parasite among age group more than 40 years and high prevalence among 10-40 years (2.37%) was also detected from a total of 2212 samples in Pakistan (Arshad et al. 2019). Likewise, high prevalence of the parasite among children was also shown among the patients visiting the sub regional hospital of Dadheldhura, Nepal (Jha 2019). Presence of the parasite only among 21-30 (14.29%) and absent among others was shown by among outpatients visiting general hospitals of Nigeria by Badamasi and Liadi (2017). But, *H. nana* infection among children only and absence among other age-group was also reported from Pakistan by (Tasawar et al. 2004). Other studies i.e. (Kaminsky 1991); (Meloni et al. 1993); (AL-Marsome 2012) from Iraq and (Haq et al. 2015) from Pakistan reported higher prevalence among children than other age groups. This could be related to poor health hygiene, crowding, low education and socio-economic status (Al- Shammari et al. 2001). Also as the age of host increases the immunity against the parasite also increases (Tasawar et al. 2004).

Regarding sex-wise, male (27.6%) were highly infected than female (21.5%) in this study. There was no significant association in the prevalence of the parasitic infection to the sex. This result resembles with Sharma et al. (2004) (male-68.6% and female-64.7%) and Khanal et al. (2011) (22% compared with 13.5%) from Nepal; Choubisa et al. (2012) (male- 56.52% and female- 46.78%) from India; Tian et al. (2012) (male-12.6% and female- 4.4%) from China and Shield et al. (2015) (47% compared with 30%) from Australia. While this current result disagrees with Yong et al. (2000) (female- 46.3% and male- 42.1%) from Nepal, Marothi and Singh (2011) (27.4% compared to 18.2%) from India; Gelaw et al. (2013) (35.9% and 32.1%) from Nigeria. However the sex predominance is still not confirmed. The possible reasons might be due to the more involvement of males in agriculture and other outdoor business via which they come in contact to various pathogens.

Discussing about species wise prevalence of IPIs in sex, Protozoan infection by *Cryptosporidium* sp. was significantly high in males (75%) than females (2.5%) which is parallel to the results by Painter et al. (2015) in America; Rahi and Khlaif (2021) from Iraq; Guy et al. (2021) in Canada and Karimi-Dehkordi et al. (2021) in Iran. While current result is opposite to that reported by Ghimire et al. (2021) from Nepal; Yoder et al. (2012) from America; Hailu et al. (2022) from Ethiopia and Dankwa et al. (2021) from Ghana. Differences in social speciesities that expose male to infection more than female could be the reason for the variation. For example, male are responsible for herding the cattle while female are involved in household chores; such social may cause increased contact with livestock with herding and milking. This assertion can be supported by Siwila et al. (2007) from Zambia, where farm workers are more infected by *Cryptosporidium* sp. than the household workers.

Comparatively *A. lumbricoides* was predominating in male (19.5%) than female (15.7%) which is supported by (Brooker et al. 2004) (Jardim- Botelho et al. 2008); (Abu-Madi et al. 2010); (Abu-Madi et al. 2016) and (Avokpaho et al. 2021). However it is opposed by that from Nepal (Rijal et al. 2001); Nigeria (Iyevhobu et al. 2022) and Cambodia (Colella et al. 2021). This might be due to the use of unboiled water and longtime gap for consumption of anthelminthic drugs which were also shown to be significantly associated to IPIs in current study. Relation between use of untreated water and irregularity in deworming drugs are also shown by (Ojurongbe et al. 2014) and (Bethony et al. 2006) respectively. The family number also is associated with the burden of *A. lumbricoides* (Haswell-Elkins et al. 1989).

Hookworm and *Taenia* sp. were only prevalent among females while *H. nana* among males only. High prevalence of *A. lumbricoides* in males and hookworm in female was also shown by (Ojurongbe et al. 2014). Hookworm predominating in females was supported by (Rai et al. 1997) and (Baral et al. 2017) from Nepal; (Srinivasan et al. 1987) from India; (Wei et al. 2017) and (Ying-dan et al. 2020) from China and (Stella et al. 2018) from Enugu. Opposed by (W.H.O., 1995); (De Clercq et al. 1997) from Mali, South Africa and (Geiger et al. 2002) from Brazil. The high prevalence of hookworm in female may be due to barefoot and long-term gap in deworming drug consumption. Use of footwear to protect from contact has been recognized as an important preventive measure against hookworm infection since the 1920s when the association between hookworm and plantation work documented (Stoltzfus et al. 1997).

Similarly, other studies also stated bare foot (Crompton 2000) and no consumption of anthelminthic drug (Hossain and Bhuiyan 2016) as potential risk factor for hookworm infection.

In between the two wards selected for present study ward -08, Chuwa has significantly high prevalence of IPI than ward-01, Pang. Discussing species wise prevalence of intestinal parasites, *Cryptosporidium* sp. was higher in Pang. For Intestinal helminths, *A. lumbricoides* was significantly high in Chuwa, *E.vermicularis*, hookworm and *Taenia sp.* were prevalent only in Pang while *H. nana* only in Chuwa. The high prevalence of parasites in Chuwa might be related to poor education and low socio-economic status.

5.2 Risk factor associated with Intestinal Parasitic Infection

From current study, the highest number of IPIs was found in those with the occasional use of slipper and less among using slipper always. Several studies carried out by (Rai 2002) in Nepal; (Kurup 2010) in South Saint Lucia and (Eyasu et al. 2022) in Ethiopia also reported that the rate of IPIs was significantly more among those who did not use slipper while working and walking. This might be the reason for high infection as parasites come in direct contact with skin which is also supported by (Pasaribu et al. 2019).

Untrimmed nails was also highly significant factor for IPIs as shown by this study which is in tune with the study done by (Yadav and Prakash 2016) from Nepal; (Alemu et al. 2019) and (Yeshanew et al. 2021) from Ethiopia and (Mascie-Taylor et al. 1999) from Bangladesh. Untrimmed nails may be site for parasites eggs residence which when come in contact to the mouth may be ingested and cause IPIs as discussed by (Omalu et al. 2013). The highest number of intestinal parasites was significantly seen in those who were not dewormed and less among those who were dewormed. This outcome was supported by Gabrie et al. (2014) from Honduras; Espinosa Aranzales et al. (2018) from Colombia and Elmonir et al. (2021) from Egypt. Deworming is necessary as the prevalence of parasite was significantly reduced after the implementation of biannual drug administration in Lao People's Democratic Republic (Nanthavong et al. 2017). From current study, nail biting habit was also significant predictor of IPIs which is in agreement with the studies conducted in Nepal by Sah et al. (2013), Brazil by Almeida et al. (2017) and in Taiwan by Sung et al. (2001). Nail biting may act as direct faecal-oral route for infection of parasites also said by Mehraj et al. (2008). Nail biting habit accounts for auto infection and long term parasitosis (Sofiana et al. 2011).

Use of unboiled/ unfiltered water for consumption was found to be significant explanatory factor of the intestinal parasites. This result was in accordance to the result from Nepal by Dhital et al. (2016) and Bertoncello et al. (2017); Malaysia by Ngui et al. (2011) and from Cambodia by Liao et al. (2017). This may be due to the contamination of water by human and animal wastes. This is in agreement with the finding from Ethiopia by Bolka and Gebremedhin (2019) and Ghana by Abaka-Yawson et al. (2020). Lastly, people who did not wash their hands by soap before and after meal and after defecation were significantly infected by intestinal parasites. This finding was in accordance with the studies conducted by Sah et al. (2013), Chongbang et al. (2016) in Nepal; Curtale et al. (1998) in Egypt and Feleke and Jember (2018) in Ethiopia. Hand washing without soap may facilitate the fecal-oral transmission of intestinal parasites (Vivas et al. 2010).

6. CONCLUSION AND RECOMMENDATION

6.1 Conclusion

Parasitosis is still a major public health problem in developing countries with low socio-economic status. The study was carried out to find out the prevalence of intestinal parasitic infection in relation to socio-economic status of Kusma Municipality, Parbat, Nepal and the associated risk factor. Out of 208 stool samples, the overall prevalence of intestinal parasitic infection was found in 50 (24.04%) samples in which helminthic infection 42 (20.2%) was higher than protozoan infection 8 (3.9%). Altogether one species of protozoan and five species of helminthic parasites were detected. Among helminths, the most common was *Ascaris lumbricoides* (17.3%) followed by *Enterobius vermicularis* and *Hymenolepis nana* (1% each) and hookworm and *Taenia* sp. (0.5% each). The study also showed single infection (23.1%) was higher than double and quadruplet infection (0.5% each).

Sex-wise prevalence showed parasitic infection higher in males (27.6%) than in females (24.0%). Parasite species wise infection in sex showed significantly higher infection of *Cryptosporidium* sp. in males. For helminthic infection, *A. lumbricoides* was found highly in males (19.5%). *E. vermicularis* (1.2%) and *H. nana* (1.2%) were prevalent in males only while hookworm (0.8%) and *Taenia* sp. (0.8%) were prevalent in females only.

Out of different age groups, the intestinal parasite was found to be highest among 51-60 age group (31.3%) and lowest among 21-30 age groups (15.4%). Parasite species wise prevalence among different age groups indicated highest rate of *Cryptosporidium* sp. infection among above 60 years age categories. *A. lumbricoides* (23.5%) *E. vermicularis* (5.9%) was found highly among 41-50 age groups, hookworm was prevalent only among 21-30 years age groups (3.1%). *Taenia* sp. was present only among 0-10 years age groups (2.2%) while *H. nana* was highly prevalent among 21-30 years age categories.

In between two wards, ward-08, Chuwa (35.6%) was found to have higher prevalence of intestinal parasites than ward- 01, Pang (20.9%). For parasite wise prevalence in between these two wards, *Cryptosporidium* sp. (4.3%) and *A. lumbricoides* (31.1%) was found highly in Pang. *E. vermicularis* (1.2%), hookworm (0.6%) and *Taenia* sp.

(0.6%) were prevalent in Pang only. While Chuwa was highly found to be have prevalence of *H. nana*.

For associated risk factor, occasional use of slipper was found to have 15.4 times high risk of intestinal parasitic infection than those of regular user of slippers (95% CI=1.9-125.7, p=0.011); people with untrimmed nails were 4.1 times at risk of acquiring intestinal parasitic infection than those with trimmed nails (95% CI= 1.7-10.2, p=0002); dewormed people were 0.2 times at lower risk of infection than those who were not dewormed (95% CI= 0.1- 0.5, p=0.001) and found to be highly significant predictor of intestinal parasites. While, other contributors to the acquisition of intestinal parasitic infection were nail biting, treatment of water and handwashing habit.

6.2 Recommendations

On the basis of present study, following recommendations have been suggested for the effective control of intestinal parasitic infection in Kushma Municipality:

- Biannual Mass Drug Administration should be implicated effectively ensuring each member of every family have participated.
- This study suggests the immediate need for control measures including improvement of sanitation practices, personal healthy hygiene and provision of safe drinking water.
- Knowledge on intestinal parasites and awareness to control the infection should disseminated.

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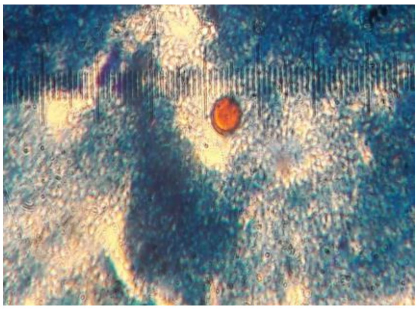
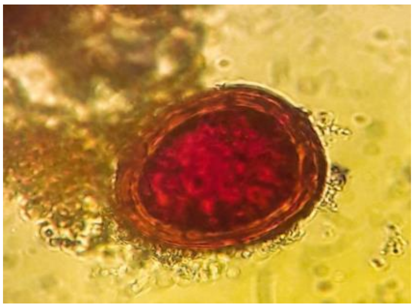
ANNEX- 01

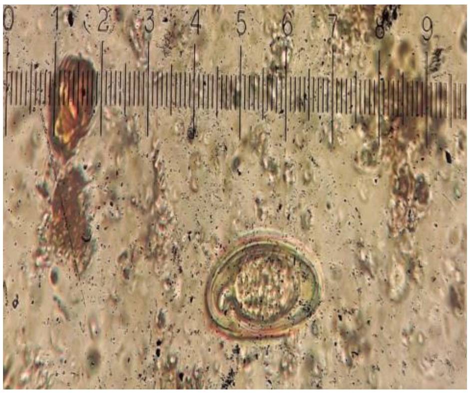
Photographs of Intestinal Parasites



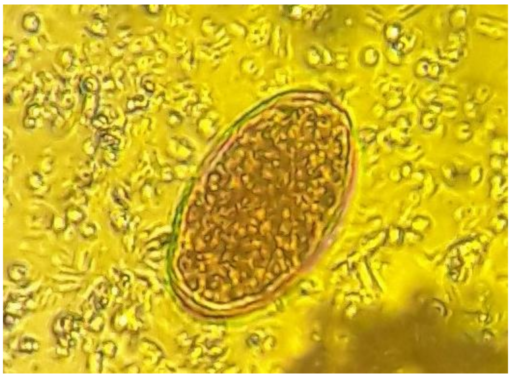
Photograph 1: Oocyst of *Cryptosporidium* sp. 4×4µm at 1000× after ZN stain under oil immersion.



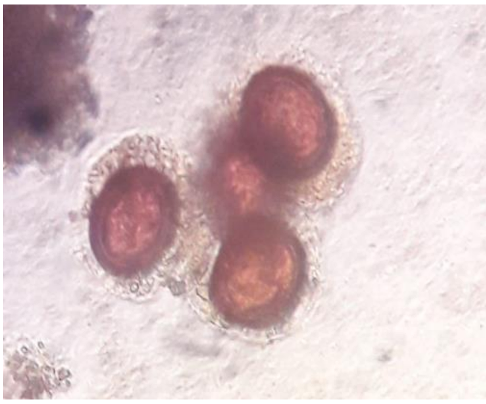
Photograph 2: Egg of *A. lumbricoides*. 62×50 µm at 400× after sat. salt floatation at lugol's iodine stain.



Photograph 3: Egg of *E. vermicularis*. 60×30µm at 400× after sat. salt floatation at normal saline.



Photograph 4: Egg of Hookworm. 65×40 µm at 400× after FEA sedimentation at lugol's iodine stain.



Photograph 5: Egg of *Taenia* sp. 34.14 µm at 400× after FEA sedimentation at lugol's iodine.

Photograph 6: Egg of *H. nana*. 44.92 µm at 400× after FEA sedimentation at lugol's iodine.

Photographs of Field and Lab

Photograph 7: Samples collected from the study area

Photograph 8: Eliciting information via Questionnaire

Photograph 9: Laboratory processing of the sample

Photograph 10: Microscopic examination of the processed sample.

ANNEX- 02

Questionnaire

Date:

Name of Interviewer: Name of Community:

Gender of Respondent: Profession of parents:

Age of Respondent: No. of family members:

Type of House: Muddy/ Concrete:

1. Are you literate? If yes, up to which level?

   ____

2. In which level does your child read?
   - Montessori
   - Lower secondary
   - Primary
   - Higher secondary
3. What type of drinking water do you use?
   - Tap water
   - Reservoir
4. Do you boil or filter drinking water?
   - Yes
   - No
5. Where do you and/or your child defecate?
   - Open area
   - Near water sources
   - Toilet
   - Pot toilet
6. Which type of toilet do you have in your house?
   - Temporary toilet
   - Permanent toilet
7. Do you/ your children wash hands before and after meal and after defecation?
   - Yes
   - No
8. How do you wash your hands?
   - With soap
   - Water only
   - With mud
9. Do you trim your nail on a regular basis?
   - Yes
   - No
10. Do you use slipper while working and walking?
    - Always
    - Occasional
11. Do you have habit of nail biting?
    - Yes
    - No

12. Does you or your child suck finger?

- Yes
- No

13. Have you taken deworming tablet before?

- Yes
- No

14. If yes, when was the last time you took your deworming tablet?

- A week before
- A month before
- Six months before
- Unknown history

15. Do you have pet in your house?

- Yes
- No

16. If yes, what are they?

- Dogs
- Cats
- Livestock
- All

17. Do you consume meat? If yes what types of meat?

- Veg
- Buff
- Pork
- Mutton
- All

18. How frequently do you eat meat?

- Frequently
- Once a week
- Once in 2-3 weeks
- Once in a month

19. How do you eat the meat?

- Boiled
- Air dried/ smoked
- Raw
- Properly cooked

20. Do you know the cause of worm infection? If yes, what are those?

____

21. Have you ever seen worms in your or your child stool?

- Yes
- No

22. If yes what do you do for treatment?

- Visit doctor
- Rely on dhami/jhakri
- Use herbal medicine available at home

23. Do you know the symptoms of worm infection? If yes, what are those?

____

24. Do you know how to control and prevent worm infection?

- Yes
- No