Characterization of Intestinal Parasitosis in Pregant Women at Ram Janaki Hospital, Janakpurdham

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ABSTRACT

Objectives: The objective of this study was to determine the prevalence, detection and identification of intestinal parasites and its associated factors among pregnant women.

Methods: Total 264 stool samples were collected in a labeled dry, clean disinfectant free wide mouthed plastic container during antenatal visits at Ram Janaki Hospital, Janakpurdham and were examined by macroscopically and microscopically. The detection and identification of protozoal cysts, oocysts, trophozoites and helminthic eggs or larva was done by wet preparation and formal-ether sedimentation concentration technique. The data was analysed using SPSS 20 version and Microsoft Excel 2007. A Chi-square test was performed to predict the parasite detection using predictor variables. The p-values <0.05 was considered as significant.

Results: The prevalence of intestinal parasitosis among pregnant women was 42%. There was positive association of symptoms of intestinal parasitosis among pregnant women ($p < 0.05$). The most predominant intestinal parasites among study participants were *E. histolytica* (20%) slightly dropped by *G. lamblia* (16%) followed by Hook worm (13%) and *A. lumbricoides* (11%). The correlation between all the variables with intestinal parasites presence and absence was statistically significant ($p<0.05$) but statistically insignificant for age and consumption of green leafy vegetables ($p>0.05$).

Conclusion: The overall prevalence of intestinal parasitosis was relatively moderate. Lack of awareness, low hygienic and sanitation habits regarding parasitic infections were the major determinant factors for higher prevalence. Improving sanitation, awareness creation and public health programes should be organized at regular interval in community.

Keywords: Helminths, Hygiene, Infestation, Intestinal parasites, Pregnancy, Sanitation

INTRODUCTION

Pregnant women are one of the most vulnerable groups and often experience more severe infections due to their immune suppression during their pregnancy (Yakasai and Umar, 2013). Intestinal parasitic infections caused by protozoa and soil helminths which are transmitted faeco-orally through contaminated sources (Yadav and Prakash 2016; Rai et al. 2002). Most common intestinal parasites reported from Nepal are *Ascaris lumbricoides*, *Hymenolepis nana, Hookworm, Trichuris trichiura, Giardia lamblia* and *Entamoeba histolytica*. Of the protozoal infections, amoebiasis and giardiasis are most frequently reported. *Ascaris lumbricoides, Trichuris trichiura* and Hookworms, collectively referred to as soil-transmitted helminths (STHs) which are the most common intestinal parasites (Yadav and Prakash 2016; Mehraj et al. 2006).

Globally, approximately, 4.5 billion people are at risk, more than 1 billion people become infected, and 450 million are ill from STHs (WHO, 2014). High prevalence of STHs is mainly related to poverty, poor living conditions, personal and environmental

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hygiene, sanitation, and water supply facilities (Ohaeri and Orji, 2013) low literacy rate, the habit of eating raw vegetables, walking barefoot, malnutrition and hot and humid tropical climate are some of the factors associated with the STH infections (Derso et al. 2016). Intestinal parasitic infections disturb pregnancy, directly or indirectly lead to a spectrum of adverse maternal and fetal/placental effects (Dotters-Katz et al. 2011). Infected pregnant women develop malnutrition; maternal anemia; total energy, protein, folate, and zinc loss (Stephenson et al. 2020); low pregnancy weight gain (Khor 2003) and increased vulnerability to other infections (Steketee, 2003). STH infections also show adverse outcomes on the offspring such as low birth weight, intrauterine fetal growth restriction, and perinatal mortality (Steketee 2003).

STHs is a significant community health problem, especially in developing countries of both Asia and Africa (De Silva et al. 2013). Soil transmitted helminthes infections are endemic in the communities where poor environmental sanitation and poor personal hygiene play an important role in transmission of STH infections. *A. lumbricoides* and Hookworms cause morbidity in humans in different ways by affecting nutritional equilibrium, inducing intestinal bleeding, inducing malabsorption of micronutrients, reducing growth, reducing food intake, causing complications such as obstruction rectal prolapsed, abscess and affecting congenital development (Mehraj et al. 2006).

Intestinal parasites (especially helminths) can be tissue dwelling or intestinal but all induce a dramatic expansion of the Th2 lymphocyte subset (Finkelman and Urban 2001). It remains unclear whether these Th2-derived responses, including IgE, eosinophilia, and mastocytosis are important in the protective immune response to the parasite, or are responsible for immune-mediated pathology, or both but at least is a paraclinical marker of infection (Finkelman and Urban 2001).

To the best of our knowledge, institution-based information revealed that infection with protozoa and geohelminths is the primary disease among pregnant women (Hailu et al. 2020) but the prevalence and factors associated with parasitic infections are still unknown in Nepal. Therefore, this study was carried out to find out the prevalence of intestinal parasites among pregnant women attending for antenatal check ups at a tertiary care hospital and its association with various socio-demographic factors which will provide an opportunity to recommend the prevention, control and treatment of the intestinal parasites.

MATERIAL AND METHODS

Study design and area

This cross-sectional study was conducted among the pregnant women attending for antenatal care checkup at Department of Obstetrics and Gynecology at Ram Janaki Hospital, Janakpurdham. All the laboratory procedures were carried out at Microbiology Department of Clinical Pathology and Laboratory Medicine at Ram Janaki Hospital, Janakpurdham located in Dhanusha district at Province No. 2 of Nepal from June 2018 to September 2019. The random sampling technique was applied on 264 pregnant women.

Data collection

During the process of specimen collection from study participants, a structured questionnaire accompanying the queries about their sociodemographic variables (age, residence, occupation and religion), clinical history, hygienic practice and nutritional behavior were collected by face-to-face interviews. The data was collected by trained midwifery health professionals.

Sample collection

The stool samples were collected from the pregnant women. The containers were labeled with name, code number, date and time of collection. A labeled dry, clean disinfectant free wide mouthed plastic container was distributed to all study participants during antenatal visits to bring about 10 gms stool sample. They were advised not to contaminate the stool with water and urine. The collected stool samples were immediately preserved with 10% formalin solution.

Inclusion and exclusion criteria

Asymptomatic pregnant woman were included after informed consent. Women refusing to give consent and those who received prior treatment with anti-parasitic drugs before two weeks, severely ill or with previous diagnosis of infectious diseases as HIV/AIDS, HBV infection, syphilis, or toxoplasmosis were not enrolled in the study. Also, those participants who came with stool samples contaminated with water and urine were excluded from study.

Laboratory investigation

The collected stool samples were examined by macroscopic and microscopic examination.

Macroscopic examination

The stool samples were observed for color, consistency, presence of blood and mucus, presence of adult worms and segments and other abnormalities.

Microscopic examination

The detection and identification of protozoal cysts, oocysts, trophozoites and helminthic eggs or larva by wet (normal saline and iodine) preparation and formal-ether sedimentation concentration technique (FECT) employed for all the stool specimens.

Formal ether concentration techniques (FECT)

About 0.5 gm stool sample was transferred into 10 ml of normal saline in a glass container and mixed thoroughly. Two layers of gauze were placed in a funnel and strained the contents into a 15 ml centrifuge tube. Then, 2.5 ml of 10% formaldehyde and 1 ml of ether were added to a test tube. The test tubes were mixed well and centrifuged at 1,000 rpm for 3 minutes. The supernatant was removed and sediment was further proceeded for wet mount preparation.

Normal saline and iodine mount techniques

The sediment was mixed well, prepared on two slides one with 2ml of normal saline and the other with 2ml of iodine solution, and covered with cover slide and detected under a microscope at 10X and 40X.

Ethical consideration

Informed verbal consent was obtained from the participants prior to the study before preceding the questionnaire and specimen collection. Work approval letter was taken from Ram Janaki Technical Institute and Ram Janaki Hospital, Janakpurdham, Nepal.

Statistical analysis

A descriptive analysis was done for the positivity among different age groups. The obtained data was analysed using SPSS 20 version and Microsoft Excel 2007. A Chi-square test was performed to predict the parasite detection using predictor variables for hand wash before eating, hand wash after defecation, green leafy vegetable consumption, use of latrine and footwear. The association of parasitic infection with sanitary practices and socio-demographic factors were also assessed by using the Chi-square test. The p-value < 0.05 was considered significant.

RESULTS

Macroscopic examination of stool sample

Table 1 shows 35.98% stool samples had normal color, 64.01% had abnormal color, 27.65% had normal consistency and 72.34% had abnormal consistency in pregnant women. Blood, mucus and worm were detected in 31.06%, 24.62% and 14.77% stool samples respectively whereas they were not detected in 68.93%, 75.37% and 85.22% stool samples of pregnant women respectively.

Table 1: Macroscopic examination of stool sample

Properties	Macroscopic examination				Total
	Normal (%)	Abnormal (%)	Presence (%)	Absence (%)	
Color	95 (35.98)	169 (64.01)	-	-	
Consistency	73 (27.65)	191 (72.34)	-	-	
Blood	-	-	82 (31.06)	182 (68.93)	264
Mucus	-	-	65 (24.62)	199 (75.37)	
Worm	-	-	39 (14.77)	225 (85.22)	

Prevalence of intestinal parasitosis among pregnant women

The prevalence of intestinal parasitosis among pregnant women was 42% as shown in figure 1.

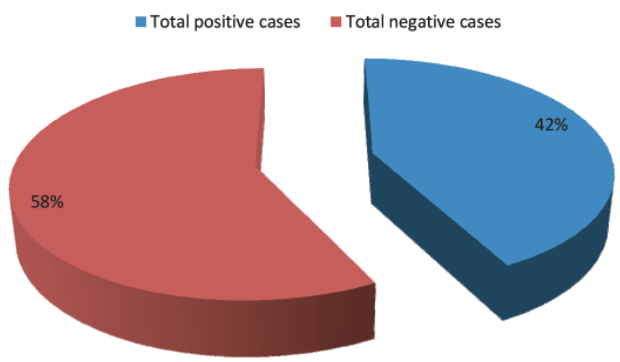

Figure 1. Prevalence of intestinal parasitosis among pregnant women

Sociodemographic characteristics of pregnant women and its association with intestinal parasites detected and not detected

Table 2 reflects the relationship of age among pregnant women with intestinal parasites detected and not detected which was statistically insignificant (p >0.05). Similarly, the relationship of religion, residence, occupation and education among pregnant women with intestinal parasites detected and not detected was statistically significant (p <0.05).

Table 2: Sociodemiographic characteristics of pregnant women and its association with intestinal parasites detected and not detected

Age (years)	Intestinal parasites detected (n=112) (%)	Intestinal parasites not detected (n=152) (%)	Total (N= 264)	Statistics
15-19	23 (20.53)	18 (11.84)	41 (15.53)	
20-24	32 (28.57)	36 (23.68)	68 (25.75)	
25-29	19 (16.95)	33 (21.71)	52 (19.69)	$x2 = 5.88$ p = 0.20
30-34	15 (13.39)	28 (18.42)	43 (16.28)	
>35	23 (20.53)	37 (24.34)	60 (22.72)	
Religion				
Hindu	54 (48.21)	143 (94.07)	197 (74.62)	$x2 = 71.62$ p= 0.00001
Muslim	58 (51.78)	9 (5.92)	67 (25.37)	
Residence				
Rural	97 (86.60)	86 (56.57)	183 (69.31)	$x2 = 27.33$ p= 0.00001
Urban	15 (13.39)	66 (43.42)	81 (30.68)	
Occupation				
Employed	13 (11.60)	59 (38.81)	72 (27.27)	$x2=24.06$ p= 0.00001
Unemployed	99 (88.39)	93 (61.18)	192 (72.72)	
Education				
Literate	52 (46.42)	45 (29.60)	97 (36.60)	$x2 = 7.85$ p =0.005
Illiterate	60 (53.57)	107 (70.39)	167 (63.25)	

Symptomwise distribution of positive cases and its association

The association of positive cases with the symptoms of intestinal parasites among pregnant women was statistically significant (p < 0.05) as shown in table 3.

Table 3: Symptomwise distribution of positive cases and its association

Parameter	Total no. (%)	Positive cases (%)	Statistics
Symptomatic	189 (71.59)	91 (81.25)	$x\ 2 = 3.85$ p = 0.04
Asymptomatic	75 (28.40)	21 (18.75)	
Total	**264**	**112**	

Trimester wise distribution of study population and its association with parasites detected

Table 4 shows the association of trimesters among pregnant women with positive cases was statistically difference (p > 0.05).

Table 4: Trimester wise distribution of study population and its association with parasites detected

Trimesters	Total no. (%)	Positive cases (%)	Statistics
1st	81 (30)	22 (19.64)	
2nd	86 (32.57)	39 (76.78)	$x2 = 5.16$ p = 0.75
3rd	97 (36.74)	51 (45.53)	
Total	**264**	**112**	

Obstetrics history of pregnant women and its association with positive cases

The association of gravida and parity of pregnant women with positive cases was found to be statistically insignificant (p >0.05) as shown in table 5.

Table 5: Obstetrics history of pregnant women and its association with positive cases

Gravida	Total No. (%)	Positive cases (%)	Statistics
1	66 (25)	26 (23.21)	
2	59 (22.26)	21 (18.75)	
3	61 (23.10)	28 (25)	**x2 = 1.01 p = 0.79**
4	78 (29.54)	37 (33.03)	
Total	**264**	**112**	
Parity			
0	19 (7.19)	5 (4.46)	
1	54 (20.54)	12 (10.71)	
2	55 (20.83)	35 (31.25)	**x2 = 9.05 p = 0.05**
3	71 (26.89)	29 (25.89)	
4	65 (24.62)	31 (27.67)	
Total	**264**	**112**	

Types of parasites detected from stool sample

Figure 2 depicts altogether 8 different types of parasites were detected from stool samples of study population. Among the parasite positive samples, 13% showed presence of Hook worm, followed by 11% *A. lumbricoides*, 2% *E. vermicularis*, 20% *E. histolytica*, 16% *G. lamblia*, 6% *Taenia spp.*, 7% *H. nana* and 4% *cyclospora spp.* Likewise, 3% samples contained both Hookworm and *Taenia spp.*, 4% had Hookworm and *A. lumbricoides*, 2% had *A. lumbricoides* and *Taenia spp.*, 4% had Hookworm and *E. histolytica*, 3% had Hookworm and *G. lamblia*, 4% had *G. lamblia* + *E. histolytica* and 1% had *T. trichura* and Hookworm.

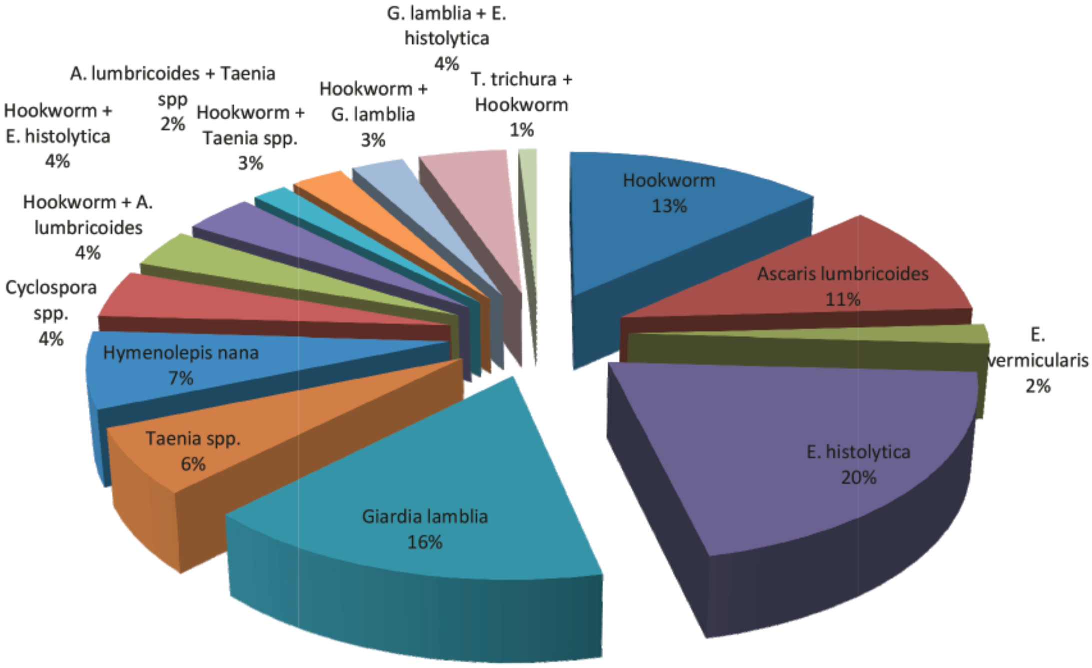

Figure 2: Types of parasites detected from stool sample

Pattern of infection

Figure 3 shows two different types of intestinal parasitic infection among study population. 80% single types of parasites were detected and 20% multiple types of parasites were detected which can cause single and multiple infection.

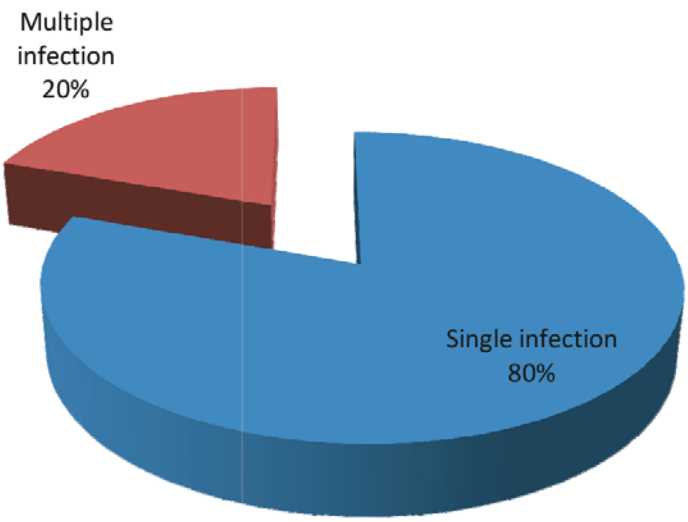

Figure 3: Pattern of infection

Association of trimesterwise study population with single and multiple parasitic infections

The association of trimesters of pregnant women with single and multiple infections was found to be statistically insignificant ($p > 0.05$) as shown in table 6.

Table 6: Association of trimesterwise study population with single and multiple parasitic infections

Trimesters	Single infection (%)	Multiple infections (%)	Total no. (%)	p-value
1st	15 (16.66)	7 (31.81)	22 (19.64)	
2nd	30 (33.33)	9 (40.90)	39 (34.82)	p = 0.11
3rd	45 (50)	6 (27.27)	51 (45.53)	
Total	**90**	**22**	112	

Association of agewise study population with single and multiple parasitic infections

Table 7 reveals the association of age of pregnant women with single and multiple infections which was found to be statistically insignificant ($p > 0.05$).

Table 7: Association of agewise study population with single and multiple parasitic infections

Age (years)	Single infection (%)	Multiple infections (%)	Total no. (%)	p- value
15-19	18 (20)	5 (22.72)	23 (20.53)	
20-24	29 (32.22)	3 (13.63)	32 (28.57)	
25-29	15 (16.66)	4 (18.18)	19 (16.96)	p = 0.51
30-34	13 (14.44)	2 (9.09)	15 (13.39)	
>35	15 (16.66)	8 (36.36)	23 (20.53)	
Total	**90**	**22**	**112**	

Correlation of variables with intestinal parasites in pregnant women

The correlation between all the variables with intestinal parasites detected and not detected was found to be statistically significant ($p < 0.05$) except consumption of green leafy vegetables was found statistically difference ($p > 0.05$) as shown in table 8.

Table 8: Correlation of variables with intestinal parasites in pregnant women

Variables	Intestinal parasites detected (n=112) (%)	Intestinal parasites not detected (n=152) (%)	Total (N= 264)	p-value
Hand washing before eating with soap and water				
Never	79 (70.53)	53 (34.86)	132 (50)	
Sometimes	23 (20.53)	47 (30.92)	70 (26.51)	**p < 0.001**
Most of the times	10 (8.92)	52 (34.21)	62 (23.48)	
Hand washing after defecation				
Never	51 (45.53)	12 (7.89)	63 (23.86)	
Sometimes	43 (38.39)	136 (89.47)	179 (67.80)	**p < 0.001**
Most of the times	18 (16.07)	4 (2.63)	22 (8.33)	
Consumption of green leafy vegetables				
Never	6 (5.35)	13 (8.55)	19 (7.19)	
Sometimes	20 (17.85)	39 (25.65)	59 (22.34)	**p =0.15**
Most of the times	86 (76.78)	100 (65.78)	186 (70.45)	
Use of dirty latrines				
Never	25 (22.31)	12 (7.89)	37 (14.01)	
Sometimes	39 (34.82)	33 (21.71)	72 (27.27)	**p < 0.001**
Most of the times	48 (42.85)	107 (70.39)	155 (58.71)	
Use of footwear outside home				
Never	47 (41.96)	9 (5.92)	56 (21.21)	
Sometimes	33 (29.46)	4 (2.63)	37 (14.01)	**p < 0.001**
Most of the times	32 (28.51)	139 (91.44)	171 (64.72)	

DISCUSSION

Intestinal parasitosis is one of the most prevalent infectious diseases in women of reproductive age and children in developing countries including Nepal (Nipurte et al. 2020). According to WHO, IPI is considered as a public health problem if its prevalence is greater than 20% (WHO, 2017). The prevalence of intestinal parasitosis among pregnant women in the present study was 42%. Previously, Yesuf et al. in 2019, Hailu et al. in 2020, Nipurte et al. in 2020 have reported 43.8%, 37.3% and 42.67% prevalence. These all findings are almost analogous to the present study. This could be due to the presence of intestinal parasites which is indicative of fecal pollution of soil and domestic water supply due to poor sanitation and improper sewage disposal. Also, it can be attributed to unhygienic practices and lack of awareness of transmission of these intestinal parasites (Nipurte et al. 2020).

In contrast, previous findings reported from Bogota, Colombia (1.2%) (Espinosa et al. 2018), Nepal (35%) (Sapkota and Maharjan, 2018), kwale district of Kenya (25.23%) (Hopkins et al. 2013), Bahirdar, North West Ethiopia (31.5%) (Derso et al. 2016), Gandhi memorial hospital (25.2%) (Gebre, 2012) and Debre Markos, North West Ethiopia (27.4%) (Kumera et al. 2018) have revealed lower prevalence than present study. These variations could be attributable to smaller sample size, the differences in - socio-demographic status, geographical area and cultural practices, implementation of various intervention strategies, study settings, time of the study, and the methods employed for stool examination. The another possible reason might be due to inappropriate handwashing practice, poor shoe wearing habit and difference in existing sanitation facilities and practices.

The result of this study depicts maximum number of parasites detected in age between 20-24 years (28.57%) followed by age group greater than 35 years (20.53%) and 25-29 years (16.95%). Nipurte et al. in 2020 observed the parasites were seen predominantly in the age group 34-40 years (50 %) followed by 26-33 years (44.7%) and 18-25 years (40.4%). Studies conducted by Alli et al. in 2011 and Usip et al. in 2017 also showed similar findings which is not in accordance with the present study. This might be due to the variation in study population's age group size, food habit behaviour and hygiene practice. The association of intestinal parasites detected in relation to age was found to be statistically difference (p=0.20).

The present study reports the higher incidence of intestinal parasites in pregnant women was found in Muslim compared to Hindu religion. Maximum participants were infected from the rural area and those who were unemployed. Also, other related studies in pregnant women carried out by Derso et al. in 2016 and Mahande et al. in 2016 had accounted predominance in rural populations which is in accord with the present study. The prospects might be due to low socioeconomic status, the surrounding environmental factors, level of sanitation and hygiene would have been similar to that of a rural set up which are the major confounding factors for the transmission of intestinal parasitic infection. But, Nipurte et al. in 2020 observed the prevalence of intestinal parasites was almost similar in both rural and urban women contrast to our study (Nipurte et al. 2020).

Education is considered as one of the most basic strategies for health improvement and promotion of quality of life (Mirzaee et al. 2013). The present study showed mostly illiterate participants were infected. Similar findings were also reported by Obiakor-Okeke et al. in 2014 and Hailu et al. in 2020. This might be due to the lack of knowledge, low educational level, unawareness about infections and non-hygienic practices of pregnant mothers which ultimately increase risk of infections. The association of intestinal parasites detected in relation to religion, residence, occupation and education was statistiscally significant ($p<0.05$).

This study reveals the highest number (71.59%) of intestinal parasites detected in those who have symptoms were statistically significant ($p=0.04$). But, the study conducted in Venezuela by Morales et al. in 2006 observed a high prevalence of intestinal parasitosis (more than 70%) those who had no symptoms which are not in harmony with the present study. This might be due to the transversal analysis of pregnant women attending to prenatal control outpatient health care centers in Venezuela which have the larger study population (Morales et al. 2006).

In this study, maximum study participants were infected in 3rd trimester of pregnancy followed by 2nd trimester of pregnancy which was statistically insignificant ($p=0.75$). Since, it is expected to have lower level of haemoglobin in 3rd trimester which is a physiological process in pregnancy. There is no evidence that the cause of infection in 3rd trimester of pregnancy established as to whether it is due to worm infestation or due to physiological cause with superimposed worm infestation (Raut et al. 2016).

The mothers of younger children are expected to come in close contact with their children during their daily activities leading to the more prevalence of infection. The findings of this study depict the maximum number of participants infected with parasites who had four gravida followed by the three, one and two. Higher numbers of respondents were infected those who were multiparous. Similar findngs were also attained in previous studies conducted by Nipurte et al. in 2020 and Alli et al. in 2011. The infection becomes more severe in women who are pregnant for the first time (primigravida) compared with other gravida as reported by Muhangi et al. in 2007. The association of gravida and parity of pregnant women with positive cases was found to be statistically insignificant ($p= 0.79$ and $p= 0.05$) respectively.

Women may even acquire parasitic infections in the process of growing the family’s food where insufficiently composted human faeces may be used as fertilizer on vegetable crops (Humphries et al. 1997). In the present study, altogether 8 different types of parasites were detected from stool samples. The most predominant parasites noted during pregnancy were *E. histolytica* (20%), followed by *G. lamblia* (16%) and chased by Hook worm (13%) and *A. lumbricoides* with 11%. Infection with *E. histolytica* is common inhabitants of developing countries and predominantly affects people with poor socio-economic conditions, non-hygienic practices and malnutrition (Braga et al. 2001).

Infections with *E. histolytica, G. lamblia,* hookworm, and *A. lumbricoids* parasites are the most common infection in rural areas, and their transmission is closely associated with socio-economic status, poor sanitation, and absence of adequate safe drinking water supplies (Merid et al. 2001). *G. lamblia* was found to be the second most common protozoan among study participants in current study. The highest prevalence of *Giardia* indicates the poor sanitary and personal hygienic condition of the respondents. Furthermore, the cyst of *G. lamblia* is resistant to the normal level of chlorination, and therefore, it can be easily transmitted through drinking water. The common causes of acute or persisting diarrhoea in people which interferes with intestinal absorption nutrients and growth rate of children (Yadav and Prakash, 2016).

The present study reflects the highest number of protozoa were detected whereas less number for helminths. A similar study conducted by Hailu et al. in 2020 in West Gojjam Zone, Northwest Ethiopia also observed the prevalence of intestinal protozoa was higher than helminths which are coexisting with the present study. The least number of helminths were detected which might be due to the differences in the distribution of helminths from place to place or from one geographical area to another. The temperature, soil type, rainfall, altitude, and humidity are also the major environmental factors that influence the pre-existence of helminthic infections in one geographical area (De Silva, 2003).

But, a study done in Gondar town, Northwest Ethiopia the presence of protozoa detected was fewer (Alem et al. 2013) which is distinct with this study. The differences might be due to the disparity in the detection method used to identify intestinal parasites. FECT which has higher sensitivity than direct microscopy was used as means of diagnosis in the present study.

The current study depicts Hookworm infection among pregnant women was 13%. Hailu et al. in 2020 reported 18.6% hookworm attacked to pregnant women slightly higher not in accord with this study. The difference might be due to the diversity in shoe-wearing habit and the level exposed to contaminated soil with hookworm larvae that penetrate the human skin. Working bare hands and walking barefoot are the major means of transmission for hookworm infection. But, a similar study conducted by Roberts et al. 2011 in Nepal reported the same trend parallel to our study (Roberts et al. 2011).

The present study depicts 11% *A. lumbricoids* infected the study participants. Nalini et al. 2017 reported the most prevalent ascariasis infestation was 76.5% during pregnancy period incongruent with this study which might be due to larger study population size. Larocque et al. 2005 also accounted ascariasis as the most common infestation in pregnant women. *A. lumbricoids* may cause intestinal obstruction, liver abscess, local irritation, and damage with malabsorption as main cellular related events associated with the infection (Fuseini et al. 2009). *A. lumbricoides* plays an important role in precipitating protein-energy malnutrition in undernourished children (Fuseini et al. 2009).

The other explanations are that the Ascaris ova are also spread by coprophilous animals and can be carried to areas away from defecation sites (Obiamiwe et al. 1991) their eggs resist drying and can survive for long periods in soil. Being coated with mucopolysaccharides ascaris eggs powerfully adheres to different surfaces (Awolaju et al. 2009).

This study also depicted attention to lower prevalence of *H. nana* and *Taenia spp*. which is worldwide parasitic disease with great importance. Lower prevalence was observed in this study which may be due to difficulty in identification of larva. Also, it might be due to rarely transmission occurred from the ingestion of food contaminated with fleas harbouring the cysticercoid larvae. The occurrence of *Taenia* may be due to risk factors associated with eating raw or insufficiently cooked pork, raw vegetables grown in field fertilized with human faeces contaminated with eggs of *T. solium* (Yadav and Prakash, 2016).

This study shows 80% single types of parasites and 20% multiple types of parasites were detected which can cause single and multiple infection. Similar findings were also obtained in the study conducted by Hailu et al. in 2020.

This study reveals maximum number of study participants was infected from single infection in 3rd trimester of pregnancy whereas multiple infections were found in 2nd trimester of pregnancy. The association of trimesters of pregnant women with single and multiple infections was statistically insignificant (p=0.11).This might be due to the fact that parasitic infection could occur at any stage of the three trimesters during pregnancy, but infection during the first trimester is associated with more severe fetal and placental consequences than those occurring later in pregnancy (Muhangi et al. 2007).

The present study established more number of single infections was in age between 20-24 years followed by 15-19 years whereas multiple infections was detected in greater than 35 years. The association of age of pregnant women with single and multiple infections was observed statistically insignificant (p= 0.51). The probable reason is that the health status of young women who are underweight or stunted, those with anemia through its multiple causes or chronic infection will start a pregnancy at great disadvantage of intestinal helminth infection, iron deficiency, and malaria are at increased risk of delivering low birth weight infants. (Steketee et al. 2001)

Hands are the main pathways of germ transmission. The present study shows the intestinal parasites detected in pregnant women who never washed their hands with soap and water before eating was 70.53%. But, women washing their hands most of the times with soap and water before eating were only 8.92%. The correlation between hand washing before eating with soap and water with intestinal parasites detected and not detected was found to be statistically significant (p= 0.00001). The result of this study is almost in accord with the study carried by Nipurte et al. 2020. This prospect may be due to the fact that washing hands before eating a meal is a simple and effective method of infection prevention and protection against germs and illness. In contrast, Raut et al. 2016 found 32.7% respondents had the habit of handwashing with soap and water frequently (Raut et al. 2016) which is distinctive with the present study.

This study showed the pregnant women who washed their hands regularly after defecation had lesser (16.07%) probability of intestinal parasites followed by women (38.39%) who sometimes washed hands and the probability was highest in women (45.53%) who never washed hands after defecation. The reason may be that critical hand washing is preferred as the best washing practices.Other possibility may be due to traditional practice and understanding the importance of cleaning and washing hands with after defecation. The correlation between hand washing after defecation with intestinal parasites detected and not detected was found to be statistically significant (p=0.00001). These findings are similar to the study conducted by Nipurte et al. (2020), Derso et al. (2016) and Mengist et al. (2017).

Pregnancy requires extra nutrients, especially iron, and produces a "physiological anemia" due to hemodilution (Derso et al. 2016). In the present study, the women with high intake of green leafy vegetable were more prone to intestinal parasitic infections which are in accord with the findings of Nipurte et al. in 2020 and Hailu et al. in 2020. Similar findings were also reported in Northwest Ethiopia (Derso et al. 2016) and East Wolega, Ethiopia (Shiferaw et al. 2015). The correlation between consumption of green leafy vegetables with intestinal parasites detected and not detected was statistically insignificant (p=0.15). This might be due to the lack of awareness and the absence of education. Also, it may be due to the pregnant women involved in agriculture and cultivation has limited knowledge about how and when intestinal parasites are transmitted. As a result, eating raw vegetables, open defecation, living in unclean environment, and food with soil during pregnancy are a common phenomenon of infection.

But, in a study conducted by Dutta et al. in 2013 showed that dietary practice of taking green leafy vegetables and fruits had protective effect during pregnancy which is not similar to this study. This depicts that the pregnant women should be advised to have plenty of green leafy vegetables but emphasis should be made on washing it thoroughly before consumption.

In the present study, the occurrence of parasitic infection in pregnant women who used dirty latrine repeatedly was more and reduces the occurrence of infection in women who never used dirty latrines and women who sometimes used dirty latrines. The correlation between use of dirty latrines with intestinal parasites detected and not detected was found to be statistically significant (p=0.00001). These findings are similar to the study conducted by Nipurte et al. 2020. This may be due to lack of cleanliness and poor hygiene practices.

Regarding to the mode of infection of hookworm, the present study evaluated the prevalence of hookworm infection was more in women who never wore footwares (41.96%) followed by those who wore footwares sometimes (29.46%) as compared to those who wore most of the time (28.51%). This might be due to avoiding the personal hygiene like barefoot walking which helps in provoking the infections with soil transmitted helminths like hookworm. In similar type of study, Nipurte et al. in 2020 reported the prevalence of hookworm infection was more in women who never used sandals (7.5%) as compared to those who used sandals frequently (1%). The correlation between use of footwears with intestinal parasites detected and not detected was found to be statistically significant (p=0.00001).

Tesfaye et al. in 2015 and Lorocque et al. in 2005 have also noted positive correlation between barefoot walking and prevalence of hookworm infection which is similar to the present study. This might be due to manipulating the irrigation activity, barefoot and bare hands which leads to parasites like hookworm to enter by skin penetration. Moreover, water for irrigation is not clean and individuals who have the habit of eating food after cleaning their hands with such water have a possibility to ingest the parasites.

CONCLUSION

This study concluded that the low hygiene and sanitation habits with lack of awareness about intestinal parasitic infections were the major determinant factors for the moderate prevalence of protozoan infection during pregnancy. *E. histolytica* followed by *G. lamblia* were predominant parasites. The highest number of single type of parasites was identified.

There was positive association of cases with the symptoms of intestinal parasites but negatively associated with trimesters. The relationship of age and trimesters of pregnant women with single and multiple infections was statistically insignificant. Further, the correlation between all the variables with intestinal parasites detected and not detected was statistically significant except for age and consumption of green leafy vegetables. Strengthening the existing water, sanitation and hygiene programs and public health measures like routine deworming of pregnant mothers should be encouraged in community setting to minimize the burden of intestinal parasitic infection.

LIMITATIONS

This study limits with the small sample size and uses only single stool specimen to assess infection status avoiding the assessment of the HIV-status and anemia among study participants. Further, more research is recommended to conduct with a large sample size in this region among pregnant women.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests regarding the publication of this paper.

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