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Original Research Article

Prevalence of intestinal parasites in pregnant women

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ABSTRACT

Background: Parasitic infections affect tens of millions of pregnant women worldwide, and directly or indirectly lead to a spectrum of adverse maternal and fetal/placental effects.
Objective: To determine the prevalence of intestinal parasites in pregnant women and its association with various factors.
Materials and Methods: Stool specimens were collected from pregnant women in a clean, wide mouth, leak-proof screw capped container. Formol ether Concentration technique was performed to increase the yield of the eggs and larvae. Modified Acid Fast staining was done for opportunistic parasitic infections.
Result: Out of the 300 pregnant women screened for presence of intestinal parasites, the prevalence of intestinal parasites was 42.67%. Protozoa (88.65%) were predominant than the helminths (11.34%). The prevalence of intestinal parasites was more in the second and the third trimester as compared to the first trimester. Primigravida women had greater positivity of intestinal parasites than the multigravida. Out of 220 anemic females, 115 (52.27%) had presence of intestinal parasites showing an association between anemia and intestinal parasites. Intestinal parasitosis showed a significant correlation with eosinophilia. Two independently collected stool specimens for routine stool examination should be sufficient to ensure adequate diagnostic sensitivity.
Conclusion: Routine screening of stool samples for intestinal parasites, especially in anemic, malnourished and women with eosinophilia should be considered as a part of the routine antenatal care. Considering the fact that sanitation and hygiene is suboptimal in most parts of the country, there should be a strong emphasis on the recommendations in the national guidelines regarding deworming in pregnancy.

1. Introduction

Intestinal parasitic infections are one of the major health problems in several developing countries including India.[1] It is reported that those sectors of populations which are in a period of intense physical and intellectual growth exhibit a more susceptible disposition to acquiring these infections. Thus, such a category includes schoolchildren, women of childbearing age, adolescent girls and pregnant women.[2] Pregnancy drains the body physically, physiologically and immunologically. This burden is aggravated when combined with parasite infection.[3] Intestinal parasitic infections in pregnancy are associated with serious adverse outcomes, both for the mother and the unborn baby. Many cases of unexplained pregnancy loss are due to undiagnosed tropical diseases. Malnutrition or anemia caused by intestinal worms may be worsened by pregnancy and make the pregnancy difficult. In India, studies have been carried out on prevalence of intestinal parasites in general population but studies on prevalence of intestinal parasites in pregnant women are lacking. The present study was carried out to find out the prevalence of intestinal parasites in pregnant women and its relation with various factors

2. Materials and Methods

A cross-sectional study was conducted in a tertiary care multispecialty teaching hospital in Mumbai, India after

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obtaining institutional ethics committee permission. The Sample size was calculated based on the prevalence of intestinal parasites of 25%[4] and precision of 5%. 300 pregnant women were recruited in the study. After obtaining an informed written consent, the clinical as well as demographic history was taken as per the case record form. Three stool specimens were collected from patients on three consecutive visits in a clean, wide mouth, leak-proof screw capped container. Gross examination was performed with respect to its colour, consistency, presence of blood, mucus, visible parasites. A saline and Iodine mount was prepared and examined microscopically to demonstrate helminthic eggs, motility of protozoan trophozoites and larvae of Strongyloides stercoralis. Saline and Formol ether Concentration technique was performed to increase the yield of the eggs and larvae. Modified Acid Fast staining was done for opportunistic parasitic infections. All the pregnant women diagnosed with intestinal parasitic infections were referred to the OBGY OPD for further medical management.

2.1. Statistical analysis

Prevalence of intestinal parasitic infection in the recruited study population was calculated. A descriptive analysis was done on the distribution of protozoal and helminthic infections and hemoglobin. A multiple logistic regression was performed to predict the odds of parasite detection using predictor variables for trimester and gravida. Eosinophilia and intestinal parasites was assessed and analysed using chi square test. P<0.05 was considered to be significant.

3. Results

Table 1: Prevalence of intestinal parasites in pregnancy

Intestinal parasites detected	Intestinal parasites not detected	Total
128(42.66%)	172 (57.34%)	300

Table 2: Distribution of intestinal parasites inpregnancy

	Intestinal parasites	No. (%)
Protozoa	**E.histolytica* / E.dispar	73 (51.77%)
	**Giardia lamblia*	28 (19.85%)
	**Blastocystis hominis*	24 (17.02%)
	Total protozoa	125 (88.65%)
Helminths	Hookworm	10 (7.09%)
	Ascaris lumbricoides	06 (4.2%)
	Total helminths	16 (11.34%)
Total		141 (47%)

*Co-infections - Eh + Gl = 03, Eh + Bh = 06, Eh +Bh + Gl = 02

1. The highest prevalence of intestinal parasites was found in 2^{nd} trimester 59.33%, followed by 3^{rd} trimester 54.54%. First trimester had the lowest prevalence 9.47%.
2. 2^{nd} trimester increases odds of intestinal parasitic infection as compared to 1^{st} trimester and the result is highly significant ($p = 1.13 \times 10^{-06}$).
3. 3^{rd} trimester increases odds of intestinal parasitic infection as compared to 1^{st} trimester and the result is significant (p= 0.003).

Table 3: Correlation of trimester with intestinal parasites in pregnancy

Trimester	Intestinal Parasites detected	Intestinal Parasites not detected	Total no. of samples examined
1st	9 (9.47%)	86 (90.52%)	95
2nd	89 (59.33%)	61 (40.66%)	150
3rd	30 (54.54%)	25 (45.45%)	55
Total	128(42.66%)	172 (57.33%)	300

Table 4: Correlation of gravida with intestinal parasites during pregnancy

Gravida	Intestinal parasites detected	Intestinal parasites not detected	Total no. ofsamples examined
Primigravida	75 (62.5%)	45 (37.5%)	120
Bigravida	40 (51.28%)	38 (48.87%)	78
Multigravida	13 (12.7%)	89 (87.25%)	102
Total	128(42.66%)	172 (57.33%)	300

1. The highest prevalence of intestinal parasites was found in primigravida 62.5% followed by Bigravida (51.28%). Multigravida had lowest prevalence of parasites 12.7%.
2. Bigravida decreases odds of intestinal parasitic infections as compared to primigravida and the result is significant (p= 0.016).
3. Multigravida decreases odds of intestinal parasitic infections as compared to primigravida and the result is highly significant ($p = 6.84 \times 10^{-05}$).

Table 5: Correlation of haemoglobin and intestinal parasites in pregnancy

Intestinal parasites	< 11gm/dl	> 11gm/dl	Total
Present	115 (52.27%)	13 (16.25%)	128
Absent	105 (47.72%)	67 (83.75%)	172
Total	220 (73.33%)	80 (26.66%)	300

1. Out of 300 pregnant women, 220 had anemia.
2. Out of 220, 115 (52.27%) had presence of intestinal parasites and 105(47.72%) did not had intestinal parasites.

Table 6: Correlation of haemoglobin levels and parasites species in pregnancy

Hb level	Hookworm	*A.lumbrico -ides*	*B.ho*minis	*E.histolytica*	*G.lamblia*	Mixed	Total
>11 gm%	0 (0.0%)	0 (0.0%)	1 (7.7%)	3 (23.1%)	9 (69.2%)	0 (0.0%)	13
<11 gm%	10 (8.7%)	6 (5.2%)	15 (13.0%)	59 (51.3%)	14 (12.2%)	11 (9.6%)	115
Total	10 (7.8%)	6 (4.7%)	16 (12.5%)	62 (48.4%)	23 (18%)	11 (8.6%)	128

E.histolytica, *B.hominis*, Hookworm, *A.lumbricoides* and mixed infections were associated with anemia

Table 7: Correlation of presence of eosinophilia and Intestinal parasites in pregnancy

Eosinophils	Intestinal parasites detected	Intestinal parasites not detected	Total no. ofsamples examined
Normal eosinophils (0.0 – 6%)	103 (37.72%)	170 (62.27%)	273
Raised eosinophils (> 6%)	25 (92.59%)	02 (7.40%)	27
Total	128	172	300

Chi square statistic – 30.33, Df – 1, p 0.001

1. Out of 300, 27 had raised eosinophils. Out of these 27, 25 had presence of intestinal parasites.
2. There was significant correlation between eosinophils and presence of intestinal parasites (p value = 0.001).

1. Out of 128, 25 pregnant women had raised eosinophils. Out of these 25, helminths had 100% association with eosinophilia.
2. Significant correlation was seen between raised eosinophils and helminths (p value =0.001).

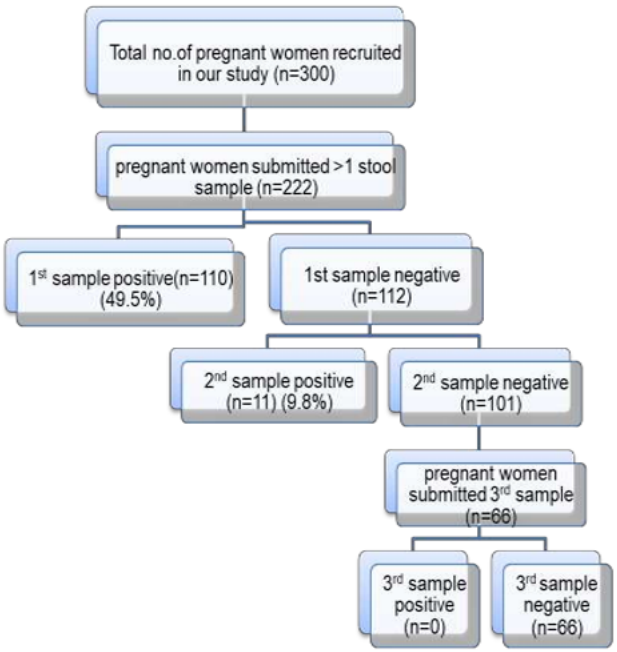

Fig. 1: No of stool samples taken and detection rate of intestinal parasites

4. Discussion

Intestinal parasitic infections constitute a global health burden causing clinical morbidity in 450 million people, many of these are women of reproductive age and children in developing countries. Elevated intestinal parasitic infections have been seen in developing countries because of poverty, low literacy rate, lack of safe drinking water, poor hygiene, malnutrition and hot and humid tropical climate.[5] The natural immune response to pregnancy causes women to be more susceptible to parasitic infections when pregnant than in non-pregnant state. Moreover intestinal parasitic infections disturb pregnancy at the maternal and fetal level.[6] In India, studies have been carried out on prevalence of intestinal parasites in general population but studies on prevalence of intestinal parasites in pregnant women are lacking. The present study was carried out to find out the prevalence of intestinal parasites in pregnant women and its relation with various factors.

The prevalence of intestinal parasites in the present study was 42.66% [Table 1]. A similar finding is reported in the study by Alli et al[7] A lower prevalence is reported in the study by Derso et al., Sehgal R et al., Sinjita et al[4,5,8] and a high prevalence by Rodriguez et al[9] and Guelzim et al[10] as compared to the present study. Differences in findings among various studies could be explained by variations in geography, socio-economic conditions, the environmental sanitation levels and cultural practices.

In the present study, prevalence of protozoans predominated (88.65%) followed by helminths (11.34%) [Table 2]. Other studies conducted in pregnant women also have reported protozoan infections to be significantly higher as compared to helminthic infections.[5,8,11] The high prevalence of protozoa is an indicator of inadequate sanitation.[12] The decrease in prevalence of soil transmitted helminths can be explained by efforts taken up by WHO in deworming the population at risk, particularly school age children to meet the Millennium Development Goals.[13] The

Table 8: Correlation of presence of eosinophilia with helminths and protozoa in pregnancy

Parasites	Raised eosinophils (> 6%)	Normal eosinophils (0 – 6%)	Total
Helminths	16 (100%)	0 (0%)	16
Protozoa	9 (8.0%)	103 (91.96%)	112
Total	25	103	128

Chisquare statistic = 30.233, Df = 1, p-value = 0.001

higher prevalence of protozoal parasites suggests that there is a need for interventional measures such as provision of safe drinking water, proper waste disposal and improvement in sanitation. Also emphasis should be made on counseling of the pregnant women regarding personal hygiene.

Among the protozoal parasites detected in our study Entamoeba histolytica/ Entamoeba dispar was the most predominant 51.77% followed by Giardia lamblia (19.85%) and *Blastocystis hominis* (17.02%). In the present study, the prevalence of Entamoeba histolytica (51.77%) was higher as compared to other studies.[5,11] The higher prevalence of E. histolytica / E.dispar in our study may be explained by the fact that there is favorable climatic condition for survival of cysts outside the human host and also for its transmission. There is difficulty in determining the clinical significance of Entamoeba histolytica in stool as its cyst is similar to Entamoeba dispar morphologically and hence cannot be differentiated. As per the literature, about 90% of Entamoeba histolytica reported is actually Entamoeba dispar which is known to be non-pathogenic. Hence to differentiate the two, molecular analysis or ELISA test needs to be performed on stool specimens. However, these tests are not feasible in routine parasitology laboratories, hence careful history of the patient needs to be taken and if stool specimen is found to have Entamoeba histolytica/dispar, treatment for the same needs to be initiated and patient should be observed for relief of symptoms.[14] The limitation of the present study was that the differentiation between pathogenic *E.histolytica* and non-pathogenic E.dispar was not done. Majority of the patients with Entamoeba histolytica (95%) were asymptomatic but presented with anemia. Repeated exposure to *E.histolytica* with the development of partial immunity to this parasite could explain the low rate of symptoms despite a high rate of infection by *E.histolytica*. Other possibility is that there may be restricted invasiveness of some strains of E.histolytica.[15] Lopez et al[16] in their study have reported that *E.histolytica* requires a high concentration of iron to survive which might cause the decreasing iron load in the host. This could have been the reason for the presentation of anemia in pregnant women with *E.histolytica* infection in the present study. Protozoan infections, particularly *E.histolytica,* increase the risk for IUGR among women of short stature, as do G.lamblia among underweight mothers. They can aggravate poor maternal nutritional and health status by producing colitis, diarrhoea, lactose intolerance, malabsorption and dehydration. These illness episodes during pregnancy can reduce caloric intake and increase metabolic cost (fever and host defense), thus reducing nutrient availability to the fetus, particularly among already malnourished mothers.[17] Infection with *E.histolytica* has also been associated with poorer maternal iron status and reduced fetal growth.[18] No medical treatment is generally recommended for asymptomatic or minimally symptomatic patients during pregnancy.[19,20] However, Villar et al[17] in their study have suggested that chronically malnourished mothers infected with parasites, yet asymptomatic could be selected as a high risk group for nutritional and prenatal care interventions during pregnancy.

Blastocystis hominis was considered a commensal of the gastrointestinal tract for a number of years.[21–23] Recently *B.hominis* has been considered as potential pathogen.[24–26] In the present study all pregnant women with *B.hominis* were asymptomatic but there was an association with anemia. El Deeb et al[27] in their study have reported that *B.hominis* infection contributes to the development of iron deficiency anemia in pregnant women. In the present study out of 24 women with *B. hominis* infection 23 presented with anemia. So even though *B. hominis* might be asymptomatic, it might add to the burden of anemia and its related outcome. This finding suggest that treatment for *B.hominis* could be considered for pregnant women with anemia and also women with *B.hominis* should be checked for anemia and vice versa.

Among the helminthic parasites detected, prevalence of Hookworm (7.09%) was predominant followed by *Ascaris lumbricoides* (4.2%). The greatest concern from hookworm infection is blood loss. Aided by an organic anticoagulant, a hookworm consumes about 0.25 mL of host blood per day. The blood loss caused by hookworms can produce microcytic hypochromic anemia. Compensatory volume expansion contributes to hypoproteinemia, edema, pica, and wasting.[15] Pregnancy requires additional nutrients especially iron, and produces a physiologic anemia due to hemodilution.[10] The presence of intestinal parasites like hookworm in pregnant women produce double burden for the women. This may result in both decreased appetite and lowered aerobic and physical work capacity in women affecting her daily activity.[28] Hookworm infection has been established as a strong predictor of iron deficiency and anemia in other population and few studies have examined these relationships in pregnant women.[29–31] Hookworm infection during pregnancy could result in vertical transmission to neonates, possibly through ingestion

of hookworm third-stage larvae in milk and colostrum.[32] Hence diagnosing it early will help in the treatment and will reduce the morbidity in the pregnant women and the newborn. Hookworm infection is usually acquired by walking barefoot on fecally contaminated soil.[33]

The prevalence of Hookworm suggests that the pregnant women should be counseled during their routine antenatal checkup about their personal hygiene including, avoiding walking barefoot to prevent infections with soil transmitted helminths like hookworm.

A.lumbricoides can cause malnutrition by consumption of nutrients which are needed by the host; interference with intestinal absorption due to mucosal damage by the parasite; it can cause protein energy malnutrition and night blindness due to vitamin A deficiency.[33] Pregnancy requires extra nutrients and infestation with *A.lumbricoides* will deplete the extra nutreints which might be the cause of low birth weight babies.[28] Malla et al[34] have shown that extracts from *A.lumbricoides* increase the clotting time as well as the partial thromboplastin time. Zapardiel et al[35] reported a case of postpartum hemorrhage unresponsive to uterotonics and blood products, where the patient was noted to have an international normalized ratio (INR) of 1.5 and was later found to have *Ascaris* infection. These coagulopathic properties coupled with the fact that *Ascaris* infection occurs in as many as 25% of the world's population suggest that Ascaris infection may play a role in postpartum hemorrhage worldwide. Most human infections are asymptomatic.[6] Even in the present study all six women with *Ascaris* infection were asymptomatic suggesting that a routine screening of stool samples can be done to detect the infection so as to prevent the complications of *Ascaris* infection.

Prevalence of geophagy increases the transmission of *A.lumbricoides* as studied by Kawai et al.[36] But in the present study there was no correlation between geophagy & *Ascaris* infection which was similar to the findings by Young SL et al.[37]

Out of 128 pregnant women co-infection was seen in 8.5%. As most of the intestinal parasites cause nutritional deficiencies and anemia, the presence of more than one parasite will enhance this deficiency and increase the morbidity in the pregnant women.

Prevalence of parasites was seen predominantly in 2^{nd} trimester (59.33%) followed by 3^{rd} (54.54%) and 1^{st} (9.47%) trimester [Table 3]. Similar findings were reported in the study conducted by Alli et al[7] and Derso et al.[5] As pregnancy increases there are higher anti-inflammatory responses and lower proinflammatory responses. Progesterone is typically regarded as anti-inflammatory. Elevated concentrations of progesterone during 2^{nd} trimester correlated with reduced activity of regulatory Th1 cells increases susceptibility to infections. By 3^{rd} trimester anti-inflammatory responses are more elevated.[38] This could be the reason for the greater prevalence of infections in the second and third trimester as compared to the first trimester.

Intestinal parasites were predominantly seen in primigravida (62.5%) followed by bigravida (51.28%) and multigravida (12.7%) which was statistically significant [Table 4]. Similar findings were seen in study conducted by Derso et al[5] and Adedjo et al.[39] This could be due to poor knowledge on the effect of exposure to parasites during their pregnancy period in contrast to the multigravida who are likely more familiar with health management of preventive methods and control measures

The mean minimum value for haemoglobin accepted by the World Health Organization (WHO) is 11.0g/dl. A woman with haemoglobin levels below this value occurring in pregnancy has definition anaemia in pregnancy.[40] Anemia in pregnancy is a major public health problem affecting >56 million pregnant women worldwide. It is an important cause of maternal morbidity and mortality, pre-term birth, Intra Uterine Growth Retardation (IUGR), Low Birth Weight (LBW) and poor iron status in the infant.[41] In the present study the proportion of anemia was 73.3%. Similar prevalence has been reported in a study by Sanjitha et al.[4] The NFHS 3 survey have shown 57.9% proportion of pregnant women with anemia.[42] Agarwal et al[43] in an interstate study in India have shown a prevalence of anemia in pregnant women ranging from 68% to 93%.

A high rate of anemia among Indian women reflect their social and biological vulnerability both within the society and household. Certain customs and cultural taboos often aggravate the anemic status of the women. Certain practices like eating last in family, open air defecation, walking barefoot, early age at marriage and teenage pregnancy can be attributed to anemia in these women. Also factors like poverty, illiteracy and unemployment take a heavy toll on anemic pregnant women.[4] Out of 220 anemic females, 115 (52.27%) had presence of intestinal parasites showing an association between anemia and intestinal parasites [Table 5]. Other studies also have shown a similar finding.[9,44,45] So, intestinal parasites can be one of the cause of anemia in pregnant women.

In the present study out of the 115 anemic pregnant women, *E.histolytica* was predominant (51.3%) followed by *B.hominis* (13%), hookworm (8.7%) and *A.lumbricoides* (5.2%). [Table 6] This finding was in contrast with other studies which have reported helminthic infections predominant than protozoal infections.[9,46]

The association between intestinal parasites, especially hookworm, and the development of anemia is well known, however, infection with other parasites such as *E.histolytica* can also lead to anemia through blood loss in diarrhea.[47] *E.histolytica* requires a high concentration of iron to survive. This parasitic protozoan is able to obtain iron from the host proteins[16] so heavy infection with *E.histolytica* may

cause decrease in iron level in the host.[48] Infection with *E.histolytica* in pregnant women has also been associated with poorer maternal iron status and reduced fetal growth.[18] Women and young children have the lowest iron stores and are therefore most vulnerable to chronic blood loss as the result of hookworm infection.[31,49] Iron is absorbed through the intestinal wall in the duodenum and jejunum and it is believed that iron absorption could be impaired by the presence of *A. lumbricoides* in this part of the intestine.[50]

The World Health Organization estimates that because of increased physiological demands for iron during pregnancy combined with malnutrition, more than half of the pregnant women in developing countries have problems related to iron-deficiency anemia. Severe iron-deficiency anemia during pregnancy has been linked to increased maternal mortality, impaired lactation, and prematurity and low birth weight.[51] Although oral Iron and Folic Acid (IFA) supplementation is a part of the Anemia Control Programme for pregnant women since the last three decades, the desired reduction in anemia has not been achieved so far by this single intervention. There is, thus, a need to address the contributing factors leading to anemia especially among pregnant women. Considering the demonstrated benefits, variance in the use of de-worming, and the fact that sanitation and hygiene is suboptimal in most parts of the country, there was a felt need for framing clear guidelines for the use of antihelminthic drugs during pregnancy.[41] Considering the evidence around safety, efficacy, and tolerance, it is recommended that Bezimidazoles are the most suitable for deworming during pregnancy. However, Albendazole being a single dose drug(400 mg) is more cost effective and has better potential for compliance, and as such, is being recommended as the drug of choice under this programme after the first trimester.[41]

A variety of infectious agents, especially helminth parasites, are responsible for presence of eosinophilia. Infections caused by protozoa, fungi and ectoparasites to a lesser extent have also been associated with eosinophilia.[52] Among helminthic parasitic infections, strongyloidiasis, fascioliasis, filariasis, trichinellosis, toxocariasis, and hookworms which undergo a tissue migration during their life cycles have been reported to be associated with persistent increase in peripheral eosinophils.[53,54] The degree of eosinophilia in parasitic infections depends on the level of contact of parasite with immune cells in host tissues. Therefore, presence of eosinophilia is the highest among those parasites with a phase of development that involves tissue migration.[52] 0-6% is taken as normal eeosinophil count whereas above 6 is considered eosinophilia.[55]

In the present study, intestinal parasitosis showed a significant correlation with eosinophila (p 0.001) [Table 7]. Helminths showed 100% correlation with eosinophilia (p 0.001) [Table 8]. In a study by Rodriguez et al.[9] presence of eosinophilia was used as a marker to diagnose intestinal parasitosis during pregnancy. Thus, eosinophilia can be used as a marker for suspecting intestinal parasitosis during pregnancy and the stool samples in such women should be screened for the intestinal parasite.

The historical recommendation is that examination of at least 3 stool samples should be done to evaluate a patient for infection with enteric parasites.[56,57] In the present study, 222 pregnant women submitted more than two samples, out of which, 110 (49.5%) were positive and out of the 112 negative samples, 11 (9.8%) were positive in the second sample. 66 women who submitted the third sample were negative. [Figure 1] These findings suggest that two samples should be sufficient for adequate detection of parasites. This finding is concurrent with Cartwright PC et al[58] who have reported that in populations with a high prevalence of intestinal parasitic infections, two independently collected stool specimens should be subjected to routine stool examination to ensure adequate diagnostic sensitivity.

5. Conclusion

In developing countries as anemia and malnourishment preexist, the presence of intestinal parasites is a double burden in pregnancy which may affect the pregnancy and its outcome Routine screening of stool samples for intestinal parasites, especially in anemic and malnourished women should be considered as a part of the routine antenatal care. Considering the fact that sanitation and hygiene is suboptimal in most parts of the country, there should be a strong emphasis on the recommendations in the national guidelines regarding deworming in pregnancy. Eosinophilia can be considered as a marker of the intestinal parasites and the pregnant women with eosinophilia should be screened for the same.

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None.

7. Conflict of Interest

The author(s) declare(s) that there is no conflict of interest regarding the publication of this article.

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