Bioresearch Communications
Volume 02, Issue 02, July 2016.

Journal Homepage: www.bioresearchcommunications.com

Original Article

Presence of bacteria and parasites in cow dung and pit soil, a usual mean of bio-fertilizer in different area of Bangladesh

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ABSTRACT: The present study was performed to find out the prevalence of parasites and bacteria from cow dung and pit soil from 4 different areas of Bangladesh. Modified centrifugal flotation and conventional culture techniques were used to isolate parasites and bacteria respectively. In case of cow dung, Mymensing was provided with highest average for all studied bacteria. Total coliforms, feacal coliforms and *Escherichia coli* were 100% prevalent but enterococci were 97.5% prevalent. 40% cattle were found infected with one or more intestinal parasites. The highest prevalence of parasites was 32.5% for both *Trichostrongylus* and coccidians. Overall intensity was highest for coccidian cysts (127.17) and lowest for *Toxocara* (13.5). On the other hand, in pit soil total colioforms, faecal coliforms and *Escherichia coli* were at highest average $\log_{10}$ value in Hazaribagh, where enterococci in Dohar. 85% prevalence of total coliforms, feacal coliforms and *Escherichia coli* were found along with 82.5% prevalence of enterococci. 57.5% of pit samples were positive for one or more parasites where, the prevalence of *Ascaris lumbricoides* was highest (47.5%) and *Hymenolepis nana* was lowest (7.5%). *Ancylostoma duodenale* had the highest intensity (350.45) and lowest for *Enterobius vermicularis* (3.00). Without proper management such huge burden of pathogen can easily be transmitted.

KEYWORDS: prevalence, intensity, modified centrifugal flotation, conventional culture

CITATION: Das, P. K., Khanum, H., Mahmud, Z. H., Hossainey, M. R. H., Islam, M. S. 2016. Presence of bacteria and parasites in cow dung and pit soil, a usual mean of bio-fertilizer in different area of Bangladesh. *Biores Comm.* **2**(2),

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INTRODUCTION

Livestock dung and human pit have been playing an increasingly important role in agriculture of Bangladesh. About 80% people of our country are directly or indirectly depend on agriculture. With an annual growth rate of over 8% since 1993, the contribution of the livestock sub-sector to GDP is currently 3.2% (Department of Livestock Services, DLS, 2012). Gastro-intestinal parasites are a major constraint in small ruminant production in humid tropics of south-east Asia (Lebbie, *et al.*, 1994)[1]. Afazuddin (1985)[2] estimated an annual loss of taka 0.1 million due to parasite infection in Savar, Military farm, Dhaka. The most important and widely prevalent parasites are *Ostertagia* sp., *Trichostrongylus* sp., *Cooperia* sp., *Oesophagostomum* sp. *Moniezia* sp., *Taenia* sp. *Fasciola* sp., *Schistosoma* sp., *aramphistomum* sp. etc. *Toxocara* sp. and *Dictyocaulus* sp. have the worldwide distribution and the prevalence is higher in cattle. Trichostrongyliasis, schistosomiasis or bilharziasis can be fatal. General nutritional status of most of the cattle in Bangladesh is in subnormal level, which greatly increases susceptibility to parasitic diseases (Blood *et al.*, 1990)[3]. The temperature, humidity and rainfall of the country are highly favorable for parasites. Like other diseases, parasitic infections causes economic losses in terms of mortality, stunted growth, loss of body weight gain leading to poor quality of skin, decreased milk and meat production (Nooruddin *et al.* 1987; Ahmed *et al.*1994)[4,5]. Debnath *et al.*, (1995)[6]

suggested that 50% calves up to 1 year of age died due to gastrointestinal parasites. Different helminth infections are responsible for about 54.22% calf mortality in Bangladesh. In Bangladesh a limited number of studies on some epidemiological aspects of different gastro-intestinal parasites have been carried out (Rahman and Razzak, 1973; Rahman and Ahmed, 1991)[7,8] in indigenous breed of cattle.

On the other hand, today, it is estimated that over a third of the world's population are infected by one or more parasitic helminths (worms) and protozoans (de Silva *et al.*, 2003; Snow *et al.*, 2005)[9,10]. Intestinal protozoans and soil transmitted helminthes (STHs) are major problems in health worldwide, especially in the tropical and sub-tropical regions (Savioli, and Albonico, 2004; Bundy, 1997)[11,12]. Helminthiasis is particularly common in regions where poverty and poor sanitary conditions are dominant. Globally, it is estimated that two billion people are affected by intestinal parasites, of whom 300 million suffer from associated severe morbidity; however, it is difficult to estimate the actual burden due basically to underreporting (WHO, 2002; Kosek *et al.*, 2003)[13,14], whereas, STHs are a group of nematodes that infect more than a billion people worldwide (Bethony *et al.* 2006)[15].
The prevalence of intestinal parasites in Bangladesh, especially adolescents and children are at high risk (Khanum *et al.* 2008)[16]. The first five year plan of Bangladesh (1973-78) reported 64% of the children of Bangladesh suffered from intestinal parasitic infections. However, the intestinal parasites of human beings have received very little attention from research workers in Bangladesh. Geohelminth infection bears a large bulk of the problem and helminthiasis is common throughout the country (Huq *et al.* 1982)[17].

Coliform bacteria populate the intestinal tract, and are pervasive in faeces. Their presence in the environment is therefore used as an indicator of faecal contamination. *E. coli* is the target organism that has traditionally been used to identify faecal contamination in the environment (Feachem *et al.*, 1983)[18]. Pell (1997)[19] indicated that there are numerous pathogens in livestock manure which can infect humans. The principal ones are *Crytosporidium parvum*, *Giardia sp.*, *L. monocytogenes*, *E.coli* O157:H7, *Salmonella* sp. and *M. paratuberculosis*. Healthy cattle and sheep sporadically carry *E. coli* O157:H7 in their gastrointestinal tract and shed the bacteria in their faeces (Kudva *et al.*, 1997)[20].

It has been estimated that several hundred diseases may be transmitted from animal to animal and that more than one hundred and fifty may be transmitted from animal to man (Strauch, 1994)[21]. So the people handling those cow dung or human pit are at great risk. The purpose of the study is to provide sufficient epidemiological data, which can help the people to be aware of infectious agents.

MATERIALS AND METHODS

Study design

The study was conducted from 1st May, 2014 to 31st May, 2015. Cow dung was collected from Dohar, Keranigonj, Mymensing and Gopalgonj, and the pit samples were collected from Dohar, Hazaribagh, Keranigonj and Gopalgonj. A total of 40 cow-dung samples and 40 pit faecal samples were collected, 10 from each site. Approximately 200 to 250 grams of fecal sludge and fresh cow dung were collected and stored in sterile plastic containers. These were kept in an insulated foam box with ice packs and transported to the Environmental Microbiology Laboratory, icddr,b, Dhaka, maintaining temperature around 4-8°C. Collected samples were processed within 24 hours to avoid disintegration.

Isolation and identification of bacteria

To isolate *E. coli* 100 µl of serially diluted samples (10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4}) were dropped on mTEC agar plate (Difco, MD, USA). Plates were incubated at 37°C for the initial 2 hours, and then at 44.5°C for 18–24 hours. Purple colored colonies were counted as *E. coli*. Similarly for enterococci, samples were dropped on Slanetz and Bartley Agar (SBA) (Slanetz and Bartley, 1957)[22] plates. Plates were incubated at 37°C for 48 hours. Dark brown colored colonies were selected and suspected as enterococci and for further confirmation suspected colonies were sub cultured on enterococci agar plate and incubated at 44°C for 2 hours. Colonies with black hue were confirmed and enumerated as enterococci. For faecal coliforms and total coliforms isolation m-FC agar plates were used and incubated at 44°C for 22-24 hours and at 37°C for 22-24 hours respectively (Myers, *et al.*, 2007)[23].

Isolation and identification of parasites

Modified centrifugal floatation technique (Cheesbrough, 2005)[24] was performed for quantitative examination of parasites. To concentrate the helminth ova from the fecal sludge, one gram of the collected sample was suspended into 5 ml sterile normal saline and homogenized using a vortex machine. The large particulates were removed by filtering through a strainer (160 µm). The filtrate was then centrifuged for 3.5 minutes at 1800 rpm. The supernatant was discarded leaving a small amount of fluid just above the sediment. 5 ml salt ($ZnSO_4$) solution (S.G-1.20) was added and the sediment was re-suspended using a vortex machine. Homogenized sample was centrifuged for 1.5 minutes at 1500 rpm. The flotation procedure yields a surface layer that contains parasite eggs, larva and cysts. 1 ml of surface layer was transferred into a microcentrifuge tube and a drop of eosin methyline blue was added and homogenized to check the viability. The tube was then kept undisturbed for two minutes. After two minutes the sample was homogenized again and examined with light microscope. Eggs, larva and cysts as observed under the microscope were identified by following the descriptions and pictures published by Chatterjee (1980)[25] and

Cheesbrough (2005)[24]. The number of ova, larva and cysts retrieved was expressed in ova per gram.

According to recommendations of Public Health Agency of Canada (2011), safety and security measures were followed in the Environmental Microbiology Laboratory, icddr,b during the study period to ensure the biosafety and biosecurity.

RESULTS AND DISCUSSION

Average of Bacteria and Parasites

A total of 40% samples were found positive for one or more parasites. In cow dung, the $\log_{10}$ value of average of total coliforms and enterococci were highest in Mymensing (7.59) followed by Gopalgonj (7.19), Keranigonj (7.17) and Dohar (6.99). Faecal coliforms revealed highest value in Mymensing (7.48) followed by Gopalgonj (7.14), Keranigonj (7.4) and Dohar (6.88). *E. coli* revealed highest value in Mymensing (7.20) followed by Keranigonj (6.92), Gopalgonj (6.78), and Dohar (6.76). Enterococci also were at highest average in Mymensing (5.18) followed by Gopalgonj (4.59), Dohar (4.55) and Keranigonj (4.19) (Figure 1).

In pit soil, the average $\log_{10}$ value of enterococci were highest in Dohar (6.62) followed by Hazaribagh (3.38) Keranigonj (3.22) and Gopalgonj (2.92). *E. coli* was at highest average in Hazaribagh (4.84) followed by Keranigonj (4.74), Dohar (4.31) and Gopalgonj (3.94). Faecal coliforms and total coliforms revealed highest value in Hazaribagh (4.99, 5.12) followed by Keranigonj (4.88, 5.00), Dohar (4.07, 4.70) and Gopalgonj (3.98, 4.10) (Figure 2).

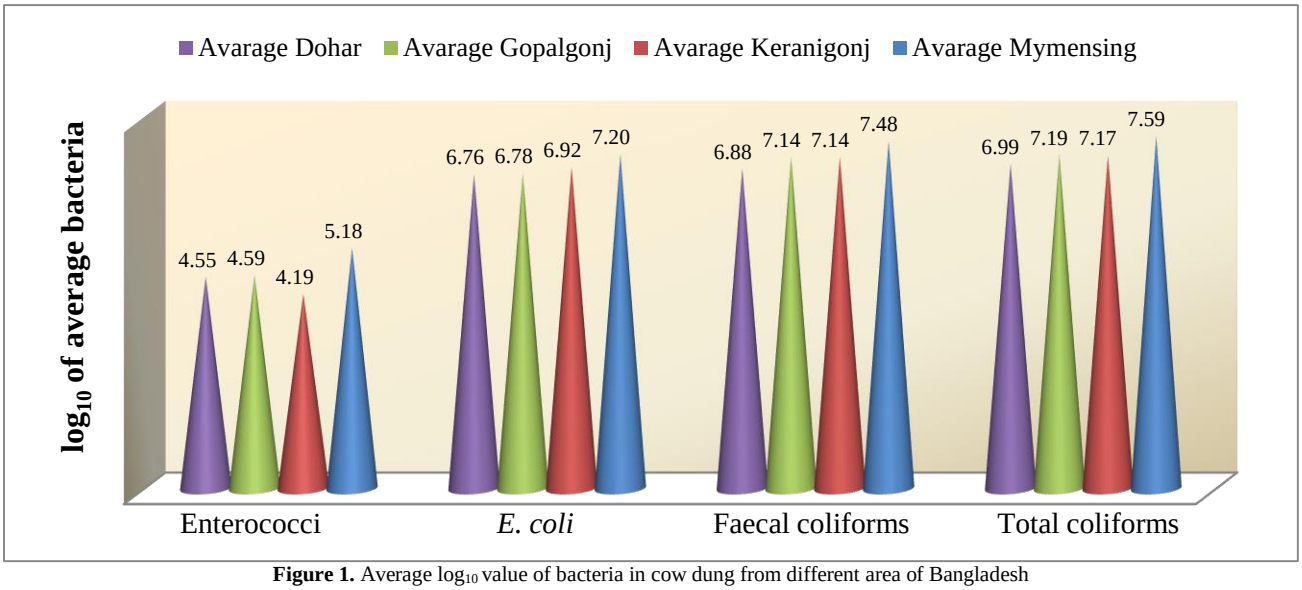

Figure 1. Average $\log_{10}$ value of bacteria in cow dung from different area of Bangladesh

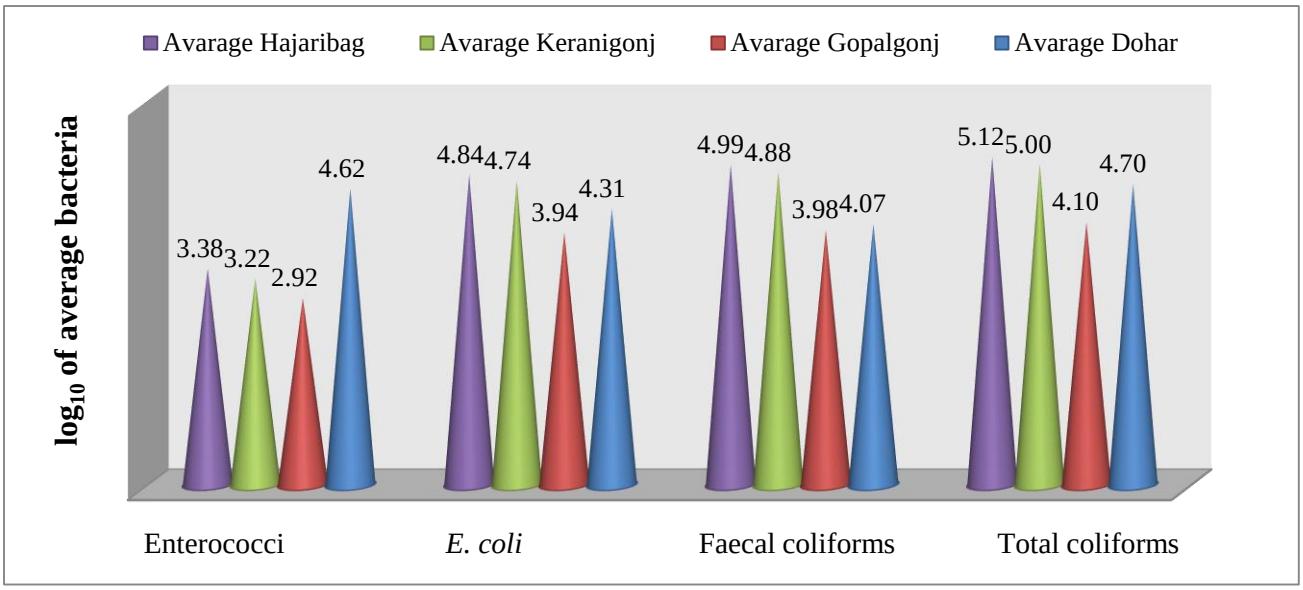

Figure 2. Average $\log_{10}$ value of bacteria in pit from different area of Bangladesh

Prevalence of Bacteria

Enterococci, *E. coli*, faecal coliforms and total coliforms had 100% prevalence in cow dung from all four study sites except enterococci from Keranigonj, which had 90% of prevalence. Overall prevalence of total coliforms, faecal coliforms and *E. coli* were 100% prevalent whereas, enterococci were 97.50% prevalent in cow dung. On the other hand, pit soil samples from Dohar had 100% prevalence for all four bacterial groups, where Gopalgonj, Keranigonj and Hazaribagh had 80% except for enterococci in Gopalgonj (70%). Enterococci had 82.50% of overall prevalence where *E. coli*, faecal coliforms and total coliforms had 85% in pit soil samples (Table 1 & Figure 3).

Table 1. Prevalence of bacteria in cow dung and pit soil

Sample types	Sampling sites	Prevalence (%)			
		Enterococci	*E.coli*	Faecal coliforms	Total Coliforms
Cow dung	Dohar	100	100	100	100
	Gopalgonj	100	100	100	100
	Keranigonj	90	100	100	100
	Mymensing	100	100	100	100
	Overall	97.50	100	100	100
Pit soil	Dohar	100	100	100	100
	Gopalgonj	70	80	80	80
	Keranigonj	80	80	80	80
	Hazaribagh	80	80	80	80
	Overall	82.50	85	85	85

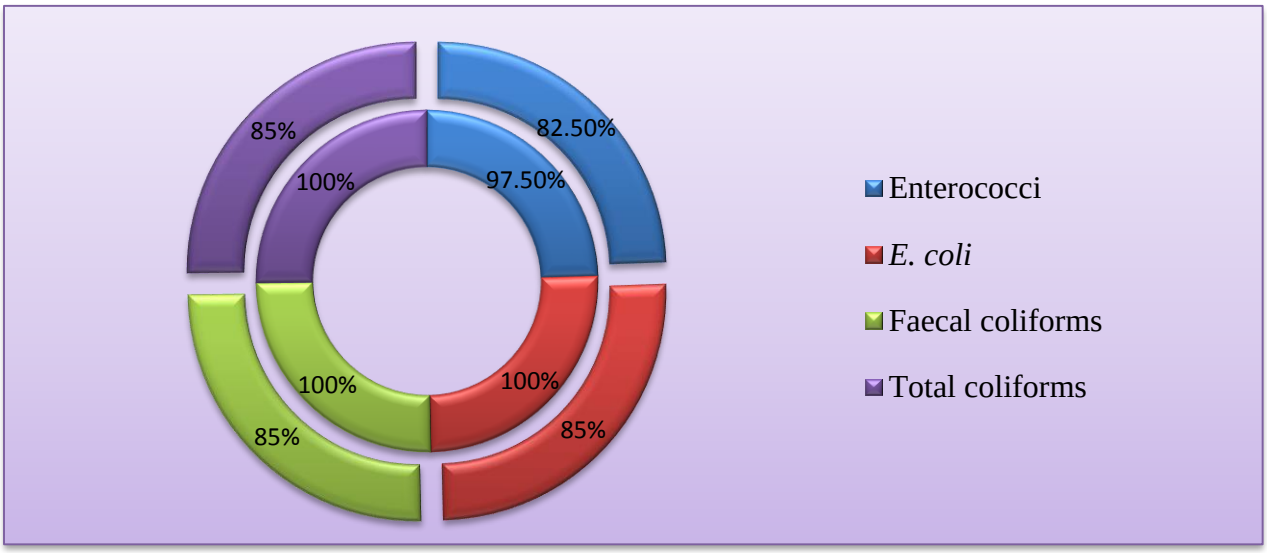

Figure 3. Pie chart of overall prevalence of bacteria in cow dung (inside) and pit soil (outside).

Prevalence of parasites

In cow dung, highest prevalence of *Trichostrongylus* found both in Gopalgonj and Keranigonj (40%) followed by Dohar (30%) and Mymensing (30%). *Strongyloides* had 10% prevalence for Dohar, Gopalgonj and Keranigonj, and 30% for Mymensing. *Paramphistomum* (10%) found only in Gopalgonj. *Haemonchus* was at 10% of prevalence in Gopalgonj and Mymensing whereas; *Toxocara* was at 10% in Dohar and Mymensing. Coccisians were at highest prevalence in Dohar (50%), followed by Mymensing (30%), Keranigonj (30) ang Gopalgonj (20). *Trichostrongylus* (32.5%) had highest overall prevalence where *Paramphistomum* had the lowest (2.5%).

In pit soil, all the seven parasite species were found in Dohar. Samples from Gopalgonj were positive for *A. lumbricoides*, *T. trichiura*, *S. stercoralis* and *A. duodenale*. . Samples from Keranigonj were positive for *A. lumbricoides*, *T. trichiura*, *S. stercoralis*, *A. duodenale* and *E. histolytica* whereas, samples from Hazaribagh were positive for all but *E. vermicularis.* Highest prevalence for *A. lumbricoides* found in Dohar (80%) followed by Hazaribagh (50%), Keranigonj (40%) and

Gopalgonj (30%). Highest prevalence for *T. trichiura* was found in Dohar (50%) followed by Hazaribagh (20%), Keranigonj (10%) and Gopalgonj (10%). Highest prevalence for *S. stercoralis* was found in Hazaribagh (50%) followed by Keranigonj (40%), Dohar (20%) and Gopalgonj (10%). *E. vermicularis* was found only in Dohar at 30% of prevalence. *E. histolytica* had 10% prevalence in Dohar, Keranigonj and Hazaribagh, and absent in Gopalgonj. *H. nana* had 20% prevalence in Dohar, 10% in Hazaribagh but absent in others. *A. lumbricoides* was at highest overall prevalence (47.5%) followed by *S. stercoralis* (30%), *A.duodenale* (27.5%), *T. trichiura* (22.5%), *E. histolytica* (10%), *E. vermicularis* (7.5%) *and H. nana* (7.5%) (Figure 4).

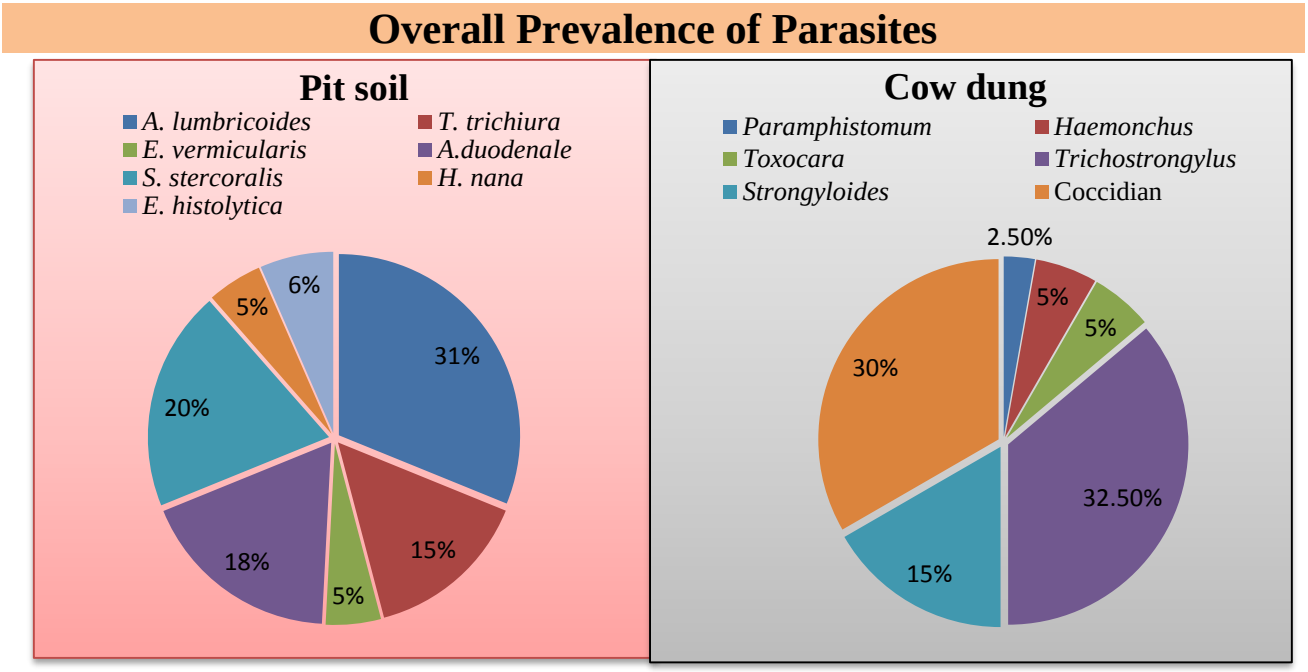

Figure 4. Pie chart of overall prevalence of parasites in cow dung and pit soil.

Intensity of Parasites

Overall intensity was highest for coccidian cysts (127.17) and lowest for *Toxocara* (13.5) in cow dung. In pit soil *A. duodenale* had the highest intensity (350.45) followed by *S. stercoralis* (302.42), *A. lumbricoides* (230.68), *T. trichiura* (27.33), *E. histolytica* (14.25), *H. nana* (10.33) and *E. vermicularis* (3.00) (Figure 5).

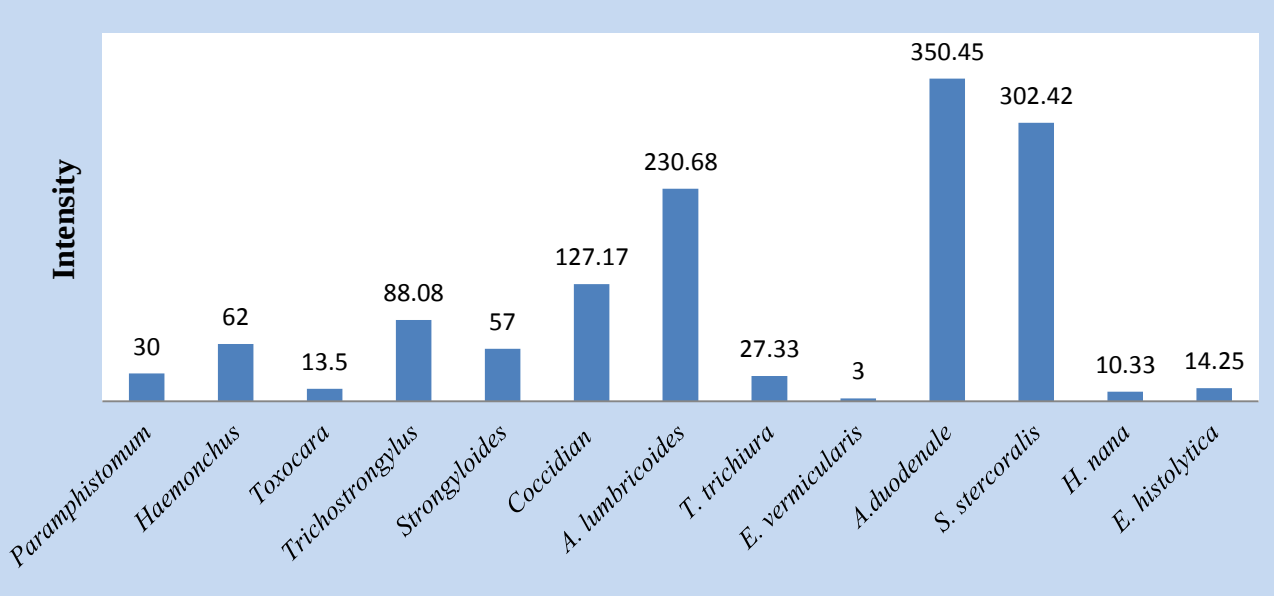

Figure 5. Overall intensity graph of parasites in cow dung and pit soil.

The current result of cattle parasites resembles the reports of a number of different authors such as Hirani *et al.* (2006)[26] in Gujarat, Afzal *et al.* (1984)[27] in Pakistan. But higher prevalence was reported by several authors, Keyyu *et al*. (2003)[28] in Tanzania and Leo Poldino *et al.* (1999)[29] in Brazil. Moreover, in Bangladesh, Muttalib (1976)[30] reported 47% of helminthiasis in buffalo calves. Begum and Rahman (1975)[31] and Khanum *et al.* (2010, 2013)[32, 33] reported similar result of parasites in human stool or pit. Muttalib and Islam (1976)[34] reported that the infestation of *Ascaris lumbricoides* as 40%, *A. duodenale* as 8% *and T. trichiura* as 18% cases. Hla-Myint (1970)[35] reported 87.7% infestation of *A. lumbricoides*, 87.4% of *T. trichiura*, 21.1% of *Giardia and* 5.7% infestation of *E. histolytica.* Dhar (2011)[36] found 15% prevalence of *Giardia* spp in soil sample as the highest and *Entamoeba* spp as the lowest (2.5%) with *E. vermicularis* and *S. stercoralis*, a prevalence of 2.5% each. In Joypur of Comilla, Jotirmoyee (2010)[37] observed highest percentage (23.33%) of *Ascaris lumbricoides* followed by *Entamoeba histolytica* (18.06%), *Taenia* Spp. (14.89%), *Strongyloides stercoralis* (10.35%), *Enterobius vermicularis* (9.24%), *Ancylostoma duodenale* (7.60%), *Giardia lamblia* (6.07%) and lowest for *Trichomonas hominis* (2.37%). The prevalence of all parasites in this study was higher than earlier studies except *E. vermicularis* (7.5%) and *E. histolytica* (10%). No *Giardia* was found among the samples examined. This variation among the present and previous study might be due to the differences in geographical niches, temperature, climatic conditions, topography, rearing and management of sheep, breeds of sheep, lack of control group of population and the variation in the sampling collection procedures.

Cow dung and human pit are valuable resource suitable for agricultural purposes. Many parts of Bangladesh use dried cow dung as fuel. Because of its availability people using it at random, that increases the risk of being infected. The present study provided bacteria and parasitological data of different area of Bangladesh. Further research should be carried out through out of the country along with awareness program.

ACKNOWLEDGEMENTS

We thank all the members of the Environmental Microbiology Laboratory at the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B). ICDDR,B is thankful to the Governments of Australia, Bangladesh, Canada, Sweden and the UK for providing core/unrestricted support.

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