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IP International Journal of Medical Microbiology and Tropical Diseases

Journal homepage: https://www.ijmmtd.org/

Original Research Article

A study on prevalence of Intestinal worm infestation and a comparative analysis between two floatation methods, for demonstrating intestinal parasites in stool (dung) of cattle

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ARTICLE INFO

Article history:
Received 13-03-2023
Accepted 26-03-2023
Available online 19-04-2023

Keywords:
Hookworm
sugar alcohol
Balantidium coli

ABSTRACT

Background: Parasitic infections of the gut are difficult to detect. It depends on microscopy and also different concentration techniques. All the concentration techniques may not be good and may not give reproducible results. Though there are some standard solutions giving good results but they are not always affordable in all geographical areas and there are very less solutions able to detect parasitic ova in both soil and faeces. We here report a new concentration technique i.e. sugar-ethanol solution for gut parasites.
Materials and Methods: In this study we compared our novel solution (sugar ethanol) with saturated salt solution by flotation technique in the dung sample of cattle and soil samples around them in rural area of West Bengal.
Results: Results were conformable and new sugar-akcohol based solution was superior to Saturated saline.
Conclusion: This solution can be used for concentration technique in stool samples.

1. Introduction

India is the largest producer of milk in the world and produced around 194,800 thousand tonnes of milk, that accounted for 40.41% of the world's production of milk in 2020.[1] India could achieve this feat due to its cattle population (305,500 thousand heads), which is the largest in the world.[2] Along with the numerous benefits which is brought about by this huge cattle population, there also arises a risk of numerous zoonotic infections which can adversely affect the cattle as well as their handlers. Amongst the major infections that can affect the cattle are Intestinal worm infestations.

Intestinal worm infestations are amongst a major constraint in running a profitable dairy industry. Intestinal worm infestations are more common in tropical and sub-tropical countries which includes India.[3] Gastrointestinal (GI) parasites can cause considerable global and domestic economic losses as a consequence of impaired weight gain, digestive tract disturbances, decreased production, impaired reproductive performance, condemnation of affected organs and mortality in infected animals.[4] To counter this menace we need to plan and implement effective deworming programs which can be planned only after having a proper base line data and handy techniques for detection of the parasites which can be implemented in the field. Many if the cattle parasites are also transmission to man.

The current study was done with the objective of determining the prevalence of Intestinal worm infestation

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https://doi.org/10.18231/j.ijmmtd.2023.006

amongst the cattle population in a village of the sunderban area of West Bengal and to determine the utility of a novel solution (454 gm of Sucrose to 300 ml of very hot distilled water, along with 45 ml 95% ethanol) vis-à-vis standard salt solution.

2. Materials and Methods

2.1. Ethical approval

Ethical approval from the institutional ethical committee was taken before commencement of the study. Permission was also duly taken from Krishi Vikas Kendra, Nimpith, South 24 Parganas as the village Kaikhali, in which the study was being conducted came under their ambit. Owner of each cattle head was properly explained about the procedure being conducted and only after due consent, dung sample was collected. The results of the test were conveyed to the cattle owners as well as the Krishi Vikas Kendra, Nimpith.

2.2. Study area

The study was conducted in Kaikhali village, Nimpith. It was under the jurisdiction of South 24 Parganas district of West Bengal. The village lies adjacent to the Matla River and is geographically located at the peripheral area of sunderbans. The area receives plenty of rainfall and has typical tropical climate.

2.3. Study population

Sample size calculation for number of cattle heads was calculated using the Cochrane formula, $n = \left[(Z_{1-\alpha})^2 \times p(1-p)\right]/L^2$ (prevalence of Intestinal worm infestation, p=0.75(3), 95% confidence interval, 20% relative error (e), Design Effect 1.5 Minimum sample size = 48. Finally, the study was conducted among 50 heads of cattle. Eight number of soil specimen were also collected for examination, in order to determine the local microbial flora.

Cattle with acute illnesses, pregnant cattle and those which were untagged were left out of study. Cattle were selected randomly from households as well as small farms (maximum herd size 8).

2.4. Study technique

Fresh faecal sample was collected directly from the rectum of the cattle after properly restraining the animal. Around 20 grams of faecal sample was collected from each cattle head. All aseptic precautions like usage of gloves and sterile containers were done for the procedure. The study was conducted in the month of August 2022. The sample were transported back to the laboratory and examined on the very same day of collection. The sample was processed using two types of floatation technique, one using the conventional Salt floatation and other using a Novel Solution (454 gm of Sucrose to 300 ml of very hot distilled water, along with 45 ml 95% ethanol). If any of the two techniques yielded a parasite ova or egg, it was considered positive.

2.5. Statistical analysis

It was done by Microsoft Excel-2010 & SPSS version 16.0. Predictor variables were seen by test of significance at 95% confidence interval in a Logistic Regression model.

3. Results

Amongst the 50 heads of cattle studied, majority were females (82%) and >2 years of age (76%). Most cattle were of native varieties (76%) and only a minority were cross breeds (24%), no pure foreign breed cattle were observed. The cattle were mostly owned by Individual households (60%) and two farms in the region owned the rest (40%) of the cattle heads. Of all the cattle examined, 29 specimen were found to be positive (As per the working definition in the study, dung sample which showed parasitic specimen in either of the floatation technique were counted as positive).

Table 1: Distribution of Cattle according to the their Intestinal Parasitic Prevalence and associated variables.

	Intestinal worm infestation (n=50)		Total
Sex of the Cattle	**Positive (n=29)**	**Negative (n=21)**	
Male	5(55%)	4(45%)	9
Female	24(58%)	17(42%)	41
Age of the Cattle			
<2 years	9(75%)	3(25%)	12
>2 years	20(53%)	18(47%)	38
Breed of the Cattle			
Native	22(58%)	16(42%)	38
Mixed	7(58%)	5(42%)	12
Ownership Status			
Individual Household	16(53%)	14(47%)	30
Farm	13(65%)	7(35%)	20

Along with cattle dung, 8 number of soil specimen were collected from random areas in the village, near the vicinity of the cattle sheds. The soil specimens too were checked for the presence of parasitic specimen. Of the 8 specimens examined, none showed any parasitic presence in Salt Floatation while 3 showed the presence of *Strongyloides* spp. in floatation done through Novel Solution.

On further analysis through Regression Analysis, we found that there is no significant association between presence of Intestinal Parasitic infestation and any of the other variables.

Of the 50 total samples observed, we found out that 29 samples (58%) are positive for intestinal worm infestation i.e., in at least one of the two floatation technique we

Table 2: Regression analysis of Presence of Intestinal parasitic infestation along with other variables.

Variables	p-Value	Odds Ratio (C.I)
Sex of the Cattle		
Female	0.87	1.12 (0.26-4.83)
Male		1
Age of the Cattle		
<2 years	0.18	2.7 (0.63-11.51)
>2 years		1
Breed of the Cattle		
Native	0.97	0.98 (0.26-3.66)
Mixed		1
Ownership Status		
Farm	0.41	1.62 (0.507-5.21)
Individual Household		1

could identify a parasitic specimen. The occurrence rate of Individual species was – *Strongyloides* spp. -16 (32%), *Balantidium coli*- 12 (24%), *Entamoeba* spp. – 2 (4%) and Amphistome – 1 (2%). Upon conducting microscopy after floatation with Standard Salt Solution we found that the prevalence as:- *Strongyloides* spp. - 8 (16%), *Balantidium coli*- 6 (12%), *Entamoeba* Sp. – 1 (2%) and Amphistome – 1 (2%). In contrast to it, more isolates could be identified through the Novel solution, *Strongyloides* - 10 (20%), *Balantidium Coli*- 9 (18%), *Entamoeba* Sp. – 1 (2%) and Amphistome – 0 (0%). The description of solution wise isolate is further elaborated in Table 1.

Table 3: Flotation Solution wise species isolated

Parasitic Species	Salt Floatation	NovelSolution Floatation
Strongyloides spp.	8	10
Balantidium coli	6	9
Entamoeba Sp.	1	1
Amphistome spp.	1	0
Total Positive	16	20

There was a difference observed upon comparison of positivity rate between the two floatation techniques. Floatation done through Novel Solution showed a higher yield as compared to standard Salt floatation technique. On further analysis to check for the agreement between the two techniques, we found out that Cohen's k is 0.0517 which indicates moderate agreement.

New sugar alcohol mixture was found to be very good for parasite-infested samples containing *Balantidium coli*, Hookworm and others.

4. Discussion

Most commonly collected samples for studying parasites spreading from cattle to man, are faecal and soil samples. So far there are very few solutions which show positive

Table 4: Comparison betweenyield of Novel Solution vs. Concentrated salt solution

Technique	Parasite Detected	Parasite Not Detected	Positivity
Standard Salt Floatation	16	34	32%
Novel Solution Floatation	20	30	40%

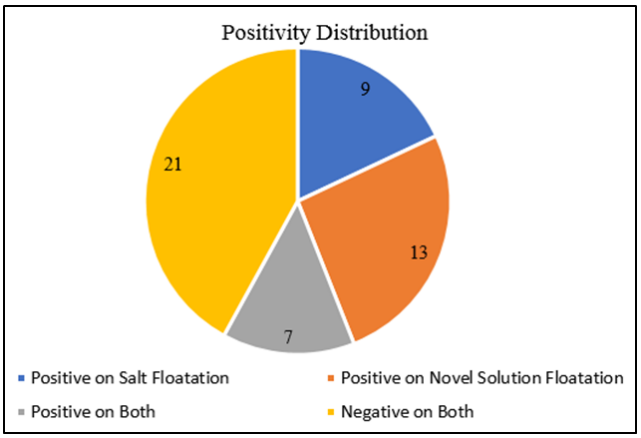

Fig. 1: Pie chart showing distribution of samples according to the positivity

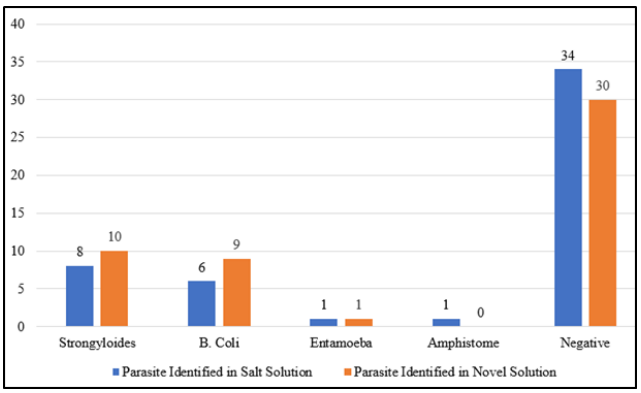

Fig. 2: Parasitic species identified through salt floatation and novel solution

results in both faecal and soil samples. In this study we collected dung samples of cattle from the rectum and also soil samples around the cattle holding area. Concentration methods like floatation and sedimentation are necessary for detection of ova and cysts in stool samples because of poor sensitivity of direct microscopy of stool samples. Proper concentration technique is further necessitated due to intermittent shedding of parasitic cysts and ova in stool.[5] There are many standard solutions and methods used in concentration technique for the detection of parasitic ova

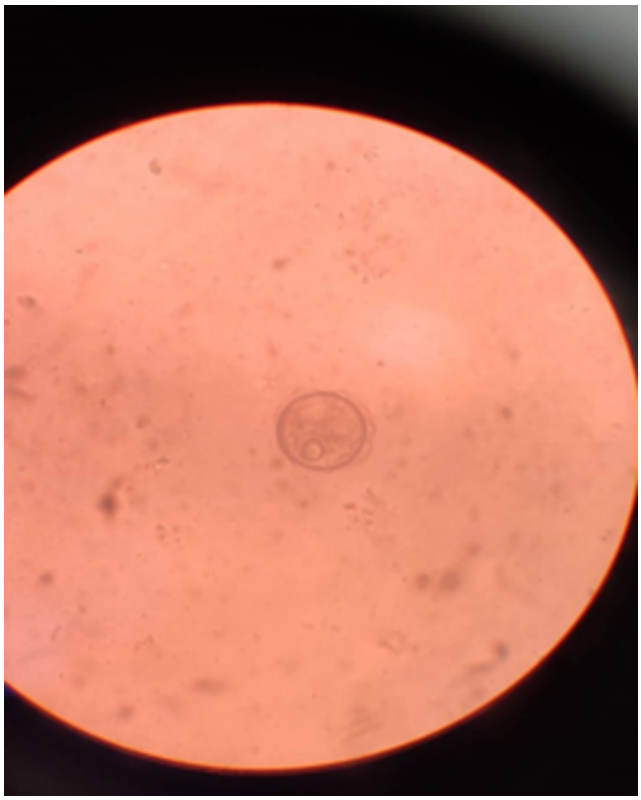

Fig. 3: *Balantidium coli* cyst in saturated normal saline

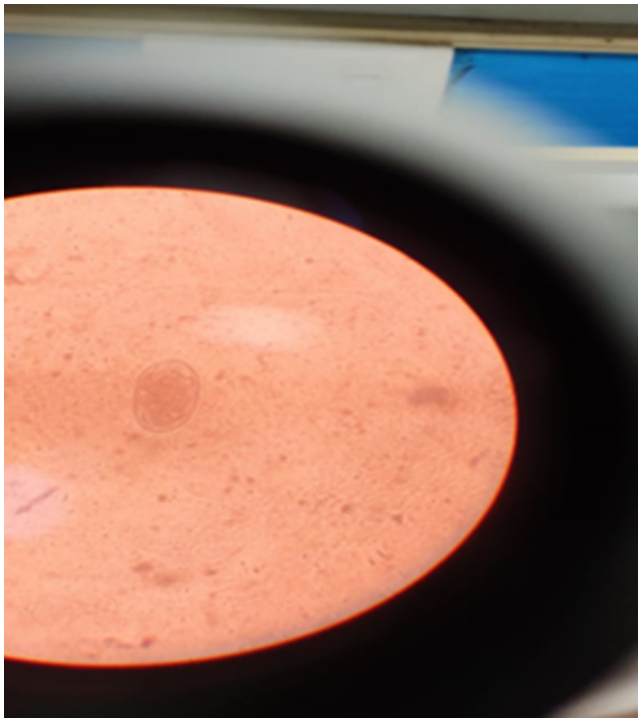

Fig. 4: Hookworm eggs seen in new sugar alcohol concentration technique

from the stool samples, like Saturated saline and Formol-ether sedimentation.[6] A major disadvantage of standard floatation methods like Saturated Saline floatation is that cyst and ova walls often collapse.[7] This was not observed in samples processed through our novel solution.

Faeces of domestic animals should be disposed properly due to the risk of transmission of parasites like *Cryptosporidium spp*., soil transmitted helminths and others.[8] However, as most of the domestic animal owners do not take proper precautions during collection and disposal of dung, there may be chances of transmission of these parasites directly and also indirectly through soil, causing infestation in humans. Dog faeces can transmit many parasitic infections to man, like roundworms and thread worms.[9] Studies have found high prevalence of parasites like coccidian cysts and *Trichostrongylus* spp. in cow dung.[10] Researchers from Ghana have also found high burden of roundworms like Ascaris spp. in faecal matter of cattle which can infect man, especially children.[11] A study from Kolkata, India had also found high burden of protozoa like *Entamoeba histolytica* in cow dung.[12] So both dung and soil samples were examined for parasitic ova and simultaneous comparison was made between our sugar-ethanol and saturated salt solution. Our results have shown positive results in both methods. There are many concentration methods, each with their own advantages and disadvantages. For example, formol-ether sedimentation method is better for recovery of *Schistosoma* eggs.[13] The disadvantage of saturated salt solution floatation is that delay in examination can lead to distortion.[14] However floatation methods are overall better than sedimentation due to formation of a cleaner material for examination.[15]

New floatation and concentration methods which preserve all ova and cysts equally well, are the need of the hour for stool samples. Our new method was very effective and gave good results for hookworm eggs. Compared to other standard solutions it is economical and can also be prepared easily. Results were good and reproducible for *Balantidium coli,* Hookworm and other parasites with the novel solution. Thus, it can be recommended to be used in human stool samples also since many of these parasites can cause both human and animal infections. The novel preparation is cheap and effective and sometimes more sensitive as compared to Saturated saline. As far as we know, nobody has tried this method for concentration method for parasitic ova and yeasts. Sucrose will help in maintaining hyperosmolarity facilitating floatation and alcohol will help in preservation. So, these findings are important from microbiological angle, public health viewpoint as well as from one health and zoonotic perspectives.

5. Conclusion

The new sugar alcohol method of concentration can be used safely and successfully for concentration techniques in all stool samples for detecting parasitic ova and cysts.

6. Source of Funding

None.

7. Conflicts of interest

There are no conflicts of interest.

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Cite this article: Triparagiri SKV, Chakraborty S, Bhattacharyya S, Banik A, Raj A. A study on prevalence of Intestinal worm infestation and a comparative analysis between two floatation methods, for demonstrating intestinal parasites in stool (dung) of cattle. *IP Int J Med Microbiol Trop Dis* 2023;9(1):31-35.