

# Diagnosis of bovine gastrointestinal parasites: comparison of different techniques and different solutions

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## Research note

**Keywords:** Cattle, copro-analysis, Mini-FLOTAC®, parasite elements, Salt/Sugar

**Posted Date:** January 22nd, 2020

**DOI:** <https://doi.org/10.21203/rs.2.21588/v1>

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## Abstract

**OBJECTIVES :** Cattle are important livestock species in Nigeria. But disease agents, especially gastrointestinal (GI) parasites, hinder their productivity. Non availability of rapid and affordable tools for GI parasite diagnosis poses problem to practitioners thus, the use of rapid, highly sensitive diagnostic techniques is required. One hundred faecal samples from cattle were analyzed through Simple Faecal Flotation (SFF), Modified Centrifugal Faecal Flotation (MCFF) and Mini-FLOTAC® techniques (MFT) using Sodium Chloride, Brine, Sugar, Salt/Sugar and Zinc Sulphate at specific gravity of 1.2, 1.23, 1.25, 1.3 and 1.3 respectively as Flotation Solutions (FS).

**RESULTS :** 100% GI parasite prevalence of was recorded with the following distribution: Strongyle (99%), Strongyloides spp (97%), Neoscaris vitulorum (78%), Trichostrongylus (56%), Nematodirus (46%), Capillaria spp (14%) and Trichuris spp (6%), Moniezia benedini (24%), Moniezia expansa (16%), Taenia-like egg (3%), Schistosoma eggs (3%) and Eimeria oocyst (100%). Using Salt/Sugar the sensitivity of the techniques was 61.99%, 58.49% and 54.24%. for MFT, SFF and MCFF respectively. Considering the sensitivity of Salt/Sugar FS with availability and affordability its use as a routine FS is advocated. The ease and rapid use of Mini-FLOTAC in low economy country in addition to safety of diagnostic staff and the possibility of re-usage.

## Introduction

Livestock contribute to natural, financial, human, physical and social lives in different ways and to different degrees within small holder dairy, crop-livestock and livestock-dependent systems [1]

Cattle are the single most important livestock species in Nigeria and play a significant role in the socio-economic life of the people. In addition to the production of meat and milk, cattle provide draught power for cultivation of the agricultural lands of many peasants. Skins and hides are also important components of the livestock sector in generating foreign export earnings [2]

Gastrointestinal helminthes are ubiquitous parasitic agents of livestock especially ruminants and are known to limit cattle production in many climatic areas and countries [3] While most helminth parasites reside in the intestines, they can also be seen in the stomach, bile duct, lungs, liver and even gall bladder of ruminants [4].

Helminth infection of ruminants are mostly caused by nematodes (Haemonchus spp, Ostertagia spp, Trichostrongylus spp, Cooperia spp, Trichuris spp, Cappillaria spp, Strongyloides spp), trematodes (Fasciola hepatica, Fasciola gigantica, Dicrocoelium spp, Parahistomum spp) and cestodes (Monezia benedini and Monezia expansa, Avitellina spp, Thysaniezia spp, Taenia spp) [5].

There appears to be almost endless possibilities in the number of substances that could be used in the flotation technique. Indeed, everything from simple table salt and table sugar (sucrose) to other chemicals have been used in flotation procedures. Although solutions with specific gravities of 1.15 to 1.55 have been investigated, efforts have generally been focused on solutions with specific gravities between 1.18 and 1.30 [6, 7, 8, 9]

As polyparasitism is very common and in response to the recent trend toward integrated control of multiple parasitic diseases, there is a need for sensitive and accurate diagnostic tools that are simple to apply [10]. In this wise, Cringoli et al. [11] developed a multivalent technique, denominated FLOTAC®, for qualitative and quantitative copro-microscopic diagnosis of parasitic infections in animals and humans. The FLOTAC® technique proved to be more sensitive compared to the McMaster technique in a survey of anthelmintic resistance in cattle [12]. However, FLOTAC® is more time consuming and requires a centrifuge for plates which limits its adoption for use where a centrifuge is not available or electric power supply is irregular, as is the case with several developing countries like Nigeria. To overcome this constraint, a more user-friendly technique, mini-FLOTAC®, was developed by the same researchers in 2013 [13]. Mini-FLOTAC® does not require centrifugation and can detect as low as 10 eggs per gram of faeces.

We are not aware of studies regarding the evaluation of this technique in Nigeria. Hence this study on the feasibility of employing the technique for the detection and quantification of GIT parasite eggs in cattle from the study area.

## Material And Methods

One hundred faecal samples were collected from the rectum of cattle. Each sample was collected into a clean, labeled Universal sample bottle. All the samples were transported in an ice box to the Parasitology Laboratory College of Veterinary Medicine, Federal University of Agriculture, Abeokuta, for analysis.

Flotation solutions were prepared as follows: Brine [14], Saturated sugar solution, Salt/sugar solution, Zinc sulphate solution, Sodium chloride Solution [15]

Fourteen (14) different test analysis were performed on each faecal sample using three faecal examination techniques: Simple Faecal Flotation (SFF), Modified Centrifugal Faecal Flotation (MCFF) and Mini-FLOTAC (MFT). Five different flotation solutions were used for MCFF and MFT techniques while four out of the five were used for SFF. The flotation solutions with their specific gravity (SG) were: Sodium Chloride (NaCl; SG 1.20), Brine (SG1.23), Sugar (SG 1.25), Salt/Sugar (SG1.30) and Zinc Sulphate (ZnSO<sub>4</sub>; SG 1.30).

## Parasitological techniques

The techniques used were: Simple Faecal Flotation, Modified Centrifugal Faecal Flotation and Mini-FLOTAC® [16, 17, 13]

## Data Analysis

Data obtained were subjected to descriptive statistical analysis using percentages for prevalence rates across different breeds, age, body condition score and sex.

Results from this study were also applied by means of comparisons of positive data from faecal samples obtained by parasitological techniques, to determine, their sensitivity. Total positive from all the three techniques and the flotation solutions was taken to be the gold standard [18] to cater for the merits and demerits of each technique and floatation solutions.

## Results

Of the one hundred cattle sampled all were positive for at least one gastrointestinal parasite as helminth egg or protozoan oocysts (100%). Eleven genera of gastrointestinal parasites were recovered in this study. They include: seven nematodes- Strongyle (99%), *Trichostrongylus* (56%), *Nematodirus* (46%), *Neoascaris vitulorum* (78%), *Strongyloides* spp (97%), *Capillaria* spp (14%) and *Trichuris* spp (6%); two cestodes: *Moniezia benedini* (24%), *Moniezia expansa* (16%) and *Taenia* egg (3%); one trematode: *Schistosoma* spp(3%) and one protozoa: *Eimeria* (100%) (Table I).

While Salt MCFF and Brine MCFF, gave 89% prevalence, Mini-FLOTAC using Brine and Zinc sulphate, gave 99% and 98% respectively, Simple Faecal Flotation using Brine (Brine SFF) and Modified Centrifugal Faecal Flotation using Zinc Sulphate (Zinc Sulphate MCFF) had 94% and 91% respectively. However, the number of infections detected, was higher with Mini-FLOTAC using salt, sugar and salt/sugar as flotation solutions. The Mini-FLOTAC detected single, double, triple, quadruple, quintuple or sextuple infection in the study samples.

Eleven genera of parasite elements, ten of helminths and one of protozoa, were identified by fourteen faecal analytical techniques making a total of 1400 clinical analyses. Only the Mini-FLOTAC technique with salt/sugar flotation solution (Salt/sugar MFT) was able to detect all the eleven genera. It is therefore comparable to the Gold Standard; the sum of all diagnostic technique since it showed the highest accuracy in the identification of 336 positive analyses.

In terms of the diagnostic performance of the techniques, using sensitivity, the salt/sugar Mini-FLOTAC showed sensitivity of 61.99% compared to 58.49%, 57.80%, 54.24%, 52.77%, 50.0%, 48.71%, 47.60%, 45.76%, 45.57%, 42.80%, 38.93%, 38.38% and 36.53% of salt/sugar SFF, sugar MFT, salt/sugar MCFF, Znso4 MFT, sugar SFF, sugar MCFF, brine MFT, salt SFF, salt MFT, Znso4 MCFF, brine SFF, brine MCFF and salt MCFF respectively.

In terms of rapidity of the three techniques, simple flotation takes two minutes for processing a sample, twenty minutes required for floating and one minute for observation under the microscope (23 minutes all together). Modified Centrifugal Faecal Flotation takes seventeen minutes for processing a sample, minimum of ten minutes for floating of parasite elements and one minute for microscopic examination (28 minutes all together). Mini-FLOTAC technique takes three minutes to process a sample, ten minutes for parasite elements to float and one minute for microscopic examination of the reading disk (14 minutes all together).

## Discussion

The findings of this study showed that all the cattle screened had helminth infection, thus providing valuable information on the burden of helminths among cattle in sampled areas.

The overall prevalence of 100% of GI parasite infection obtained in this study was higher than those by [19] and [20] who reported a prevalence of 47.4% and 41.6% in South-South and South-West Nigeria respectively. It was also higher than 50.8% and 62.1% earlier reported in South-Eastern and South-South Nigeria by [4] and [21] respectively.

The difference in the prevalence obtained could be attributed to the existence of favourable environmental factors necessary for the prolonged survival and development of infective larval stage of most helminths [22], variation in sample size and management system. [23] reported that animals that are solely graze on pasture throughout the year are prone to the effect of seasonal variation of availability of forgeable feed and then difference in plane of nutrition.

*Strongyle* eggs, *Trichostrongylus*, *Nematodirus*, *Neoascaris vitulorum*, *Strongyloides* spp, *Capillaria* spp and *Trichuris* spp, *Moniezia benedini*, *Moniezia expansa*, *Taenia* egg, *Schistosoma* eggs and *Eimeria* oocyst were identified in this study using simple flotation, modified centrifugal faecal flotation and Mini-FLOTAC techniques

An important observation from this study is that the *Capillaria* eggs detected is from Muturu breed of cattle sampled in a rural Area. In this area, many people defecate on the farmland and these animals are grazed on the same farmland that had been contaminated with human faeces. Although bovine coprological examination carried out by various researchers in other countries reported *Capillaria* spp as one of the parasite elements detected [24], to date no such report has been made from cattle in Nigeria.

The sensitivity of Mini-FLOTAC using salt/sugar solution is higher compared to the salt/sugar modified centrifugal faecal flotation. This diagnostic assessment clearly indicated that the solution was the best under the Mini-FLOTAC technique.

In general, diagnostic assessment of the five flotation solutions clearly indicated that salt/sugar solution gave the optimum results under each faecal test for the three techniques compared to other solutions suggesting that the solution is highly efficient in the diagnosis of parasite elements, Salt/sugar is easy to prepare, the solute is readily available, does not grow mouldy unlike sugar solution, does not crystalize or form cast, does not require preservative, it is not sticky or messy to work with, non-toxic to the environment and finally it has excellent clarity under the microscope.

One plausible explanation for the superior performance of the salt/sugar solution under each technique is that salt helps in homogenization of faeces and sugar which is more viscous make it the Parasite elements appear clearer with increasing chance of detection [25].

For the diagnostic techniques, Mini-FLOTAC produced optimum results compared to the other two conventional techniques (Simple Faecal Flotation and Modified Centrifugal Faecal Flotation) especially with the use of salt/sugar as flotation solution. Also, Mini-FLOTAC apparatus is heat resistant, re-useable after thorough washing, has excellent clarity under the microscope, does not require centrifugation and is good for both qualitative and quantitative faecal analysis.

Findings from this study showed that only salt/sugar solution was highly efficient in detecting the eleven genera of bovine gastrointestinal parasites identified in this study using Mini-FLOTAC technique. This further support RCV/FAO recommendation that salt/sugar flotation solution is a general-purpose flotation solution.

This is the first-time salt/sugar and sugar flotation solutions were used with Mini-FLOTAC technique, just recently Salt/Glucose was used by the inventors [13] on a test running scheme, salt solution (Nacl) and zinc sulphate are the only two flotation solutions used for the Mini-FLOTAC technique [26]

The excellent performance of salt/sugar solution under Mini-FLOTAC technique from this study supported the report by [26] that the sensitivity of the Mini-FLOTAC technique is highly dependent on the flotation solution used.

In terms of diagnosis of co-infection, Mini-FLOTAC technique performed better than the other two techniques showing that Mini-FLOTAC is a more sensitive technique.

The only protozoa parasite detected by the three parasitological techniques is Eimeria oocyst which shows that it is the most prevalence protozoa parasite in the sampled area. This finding is like that of [13] which reported Eimeria oocyst as the only protozoan parasite detected using Mini-FLOTAC technique.

Based on the performance of salt/sugar flotation solution under each technique for this study, the use of salt/sugar as flotation solution in the laboratory is recommended.

For routine diagnosis, Mini-FLOTAC can be adopted because of the ease of usage.

The use of Mini-FLOTAC needs to be promoted in developing countries particularly in the laboratory with resource-limited settings.

#### LIMITATIONS

- Mini-FLOTAC to work with is quite expensive for routine use in Nigeria
- Paucity of funds to work with to get quality work done.
- Limited sample size due to cost implications.
- No support to do quality research

## Abbreviations

SFF

Simple Faecal Flotation

MCFE

Modified Centrifugal Faecal Flotation

MFT

Mini-FLOTAC® techniques

FS

Flotation Solution

GIP

Gastro Intestinal Parasite

## Declarations

- Ethics approval and consent to participate: Not applicable
- Consent to publish: not applicable
- Availability of data and materials: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.
- Competing interests: authors have no competing interest
- Funding: no funding was received for this work
- Authors' Contributions: FAA conceived the idea, FAA & AMP: designed the study, AMP: Collected samples, FAA & AMP did the lab work, analyzed the result, wrote and approve of the final manuscript
- Acknowledgements: All livestock farmers whose animals were used for collection of faecal sample.

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## Tables

Table I: The distribution of different bovine gastrointestinal parasites eggs and oocysts using three diagnostic techniques.

Parasite Elements	No examined	No positive (%)
<i>Strongyle</i>	100	99
<i>Strongyloides</i>	100	97
<i>Trichostrongylus</i>	100	56
<i>Nematodirus</i>	100	46
<i>N. vitulorum</i>	100	78
<i>Trichuris</i>	100	6
<i>Capillaria</i>	100	14
<i>M. benedini</i>	100	24
<i>M. expansa</i>	100	16
<i>Taenia</i>	100	3
<i>Schistosoma</i>	100	3
<i>Eimeria oocyst</i>	100	100

DIAGNOSTIC METHODS	FLOTATION SOLUTIONS	TYPES OF PARASITIC INFECTION						Total	% Positive
		Single	Double	Triple	Quadruple	Quintuple	Sextuple		
SFF	Salt SF	20	31	33	13	3	0	248	100
	Brine SF	22	39	23	7	3	0	211	94
	Sugar SF	10	31	43	13	2	1	271	100
	Salt/sugar SF	1	24	46	18	10	1	316	100
MCFF	Salt MCFF	31	28	14	12	4	0	198	89
	Brine MCFF	29	23	18	16	3	0	208	89
	Sugar MCFF	16	31	33	13	7	0	264	100
	Salt/sugar MCFF	14	24	26	27	9	0	294	100
	ZnSO <sub>4</sub> MCFF	12	33	32	12	2	0	232	91
MFT	Salt MFT	24	37	19	12	6	2	247	100
	Brine MFT	14	38	27	14	5	1	258	99
	Sugar MFT	11	30	29	15	12	3	297	100
	Salt/sugar MFT	3	17	33	33	12	2	336	100
	ZnSO <sub>4</sub> MFT	7	23	43	23	2	0	286	98

II: Prevalence and Number of parasitic infections found in the faeces

\*LEGEND

SFF: Simple faecal flotation MCFF: Modified centrifugal faecal flotation MFT: Mini-FLOTAC technique

Table III: Parasites detected Versus Gold standard

\*GS: GOLD STANDARD

Parasite	SIMPLE FAECAL FLOTATION				MODIFIED CENTRIFUGAL FAECAL FLOTATION					Mini-FLOTAC TECHNIQUE						GS*
	Salt	Brine	Sugar	Salt/sugar	Salt	Brine	Sugar	Salt/Sugar	Znso4	Salt	Brine	sugar	Salt/Sugar	ZnSO <sub>4</sub>		
<i>Strongyle</i>	90	86	94	96	78	79	84	73	76	91	86	88	79	82	99	
<i>Strongyloides</i>	45	36	62	88	32	41	55	74	70	24	30	41	74	85	97	
<i>Trichostrongylus</i>	22	19	05	15	13	20	05	05	02	17	19	10	03	03	56	
<i>Nematodirus</i>	22	13	03	07	04	03	03	02	01	20	16	03	07	06	46	
<i>N. vitulorum</i>	0	04	07	11	02	0	26	34	15	05	06	44	53	09	78	
<i>Capillaria</i>	0	0	0	02	03	0	01	04	01	03	01	06	03	02	14	
<i>Trichuris</i>	0	0	03	0	0	0	02	0	01	0	0	01	01	0	06	
<i>M.benedini</i>	09	08	11	12	08	11	08	09	09	10	15	10	11	09	24	
<i>M.expansa</i>	02	01	02	03	01	0	01	0	0	02	03	02	06	05	16	
<i>Taenia</i>	0	0	0	0	0	0	0	0	0	0	0	01	02	0	03	
<i>Schistosoma</i>	0	0	0	0	0	0	0	0	0	0	0	0	02	01	03	
<i>Eimeria</i>	58	44	84	83	57	54	79	93	57	75	82	91	95	84	100	
Total	248	211	271	317	198	208	264	294	232	247	258	297	336	286	542	