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A comparison of eggs per gram (epg) from wet faecal smears, benchtop flotation and centrifugal techniques in determining the maximum number of GIN eggs in calf faeces - A case study

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Abstract
Parasitic infections of livestock pose a serious problem for farmers and a major obstacle for the development and expansion of the meat industry into a sustainable and profitable market. The purpose of this study was to determine the best technique to quantify gastrointestinal intestinal (GIN) eggs in calf faeces by comparing the wet faecal smear, faecal floatation and centrifugation floatation techniques of faeces of a moderately parasitized calf infested with an initial eggs per gram (epg) Mc master method, of nine hundred and fifty (950). The solutions used for the quantification were sodium chloride (NaCl), zinc sulphate (ZnSO4) and sucrose. Descriptive statistics showed that centrifugation floatation techniques showed the highest quantification of GIN eggs. Egg counts by centrifugation were highest using floatation solutions sodium chloride followed by sucrose then zinc sulphate. These findings corresponded with highly significant difference (P<0.0001) of the latter, found among techniques used. Therefore, we recommend that centrifugation techniques be used for quantifying GIN eggs and the order of floatation solutions in targeting follow up anthelmintic treatment in livestock affected by gastrointestinal parasites.

Keywords: GIN eggs, solutions, eggs per gram, faeces, centrifugation, floatation

1. Introduction
Parasitic infections of livestock pose a serious problem for farmers and a major obstacle for the development and expansion of the meat industry into a sustainable and profitable market (Palbergen and Nijsse, 2013) [16]. The direct losses caused by parasites are usually attributed to acute illness and death, premature slaughter and rejection of some parts at meat inspection. Indirect losses include the diminution of productive potential such as decreased growth rate, weight loss in young growing animals, inconsistent reproductive cycling, late maturity of slaughter stock and poor milk production (Bowan 2003; Pfkeni *et al.,* 2007;) [3, 18]. These infections can be either clinical or sub clinical, the latter being the most prevalent and of great economic significance. The economic losses of parasitic infections vary from loss of condition of animals to loss of production.

These GIT parasitic worms belong to the Phyla Platyhelminthes, Nemathelminthes or Nematoda, Acanthocephala and Annelida (Boomker *et al.,* 1989, 1994) [1, 2]. The Phylum Platyhelminthes contains three classes of worms namely; Turbellaria, Trematoda and Cestoda. All three classes are typically soft-bodied, flattened dorso-ventrally and hermaphroditic. Of major importance however, to grazing livestock are the Nematodes commonly called roundworms, from their appearance in cross-section. The nematode eggs however, differ greatly in size and shape, and the shell is of variable thickness. Note that, Calves under one year of age in tropical environments are more susceptible to nematodes than older animals (Marskole *et al.*; 2016) [13].

GIT parasites can be detected by examining faecal samples through various techniques. Faecal egg count or eggs per gram (epg) techniques are used to measure the prevalence and intensity of infections for epidemiological surveys, for recommendation of chemotherapies, and in detecting anthelmintic resistance.

There are several other well-established techniques for recovering GIT parasites eggs from faeces including direct smear, sedimentation, floatation and faecal ELISA techniques. The quantitative analysis method for estimating the number of eggs per sample is the McMaster test (David and Lindquist, 1982) [7].

The direct faecal smear is used to identify protozoan trophozoite (Giardia, trichimonads, amoebae) or other structures that float poorly or are readily distorted by floatation solutions. This method is suitable for a very rapid examination, but will usually fail to detect low-grade infections. The main limitation of direct smears is sample size, with the result that negative smears may not reveal light to moderate parasite levels (Boomker *et al.;* 1994 ; Fikru *et al.;* 2016) [2, 10]. Floatation solutions are made by adding a measured amount of Sodium chloride, Zinc Sulphate or Sucrose to a specific amount of water to produce a solution of desired specific gravity (SG), using a hydrometer. Floatation techniques can vary from the simplest to the complex, which relies on the differences in the specific gravity (SG), of the egg(s), faecal debris, and floatation solution. Floatation techniques using sucrose media is used for detecting (whipworms), Taenia species (Tapeworms), Toxocara species (roundworms), Eucoleus (Capillaria) species (roundworms), and Isospora (coccidian) species (David and Lindquist 1982; Fikru *et al.;* 2016) [7, 10]. The main limitation of floatation is their inability to float organism whose diagnostic stage has a specific gravity higher than of the floatation medium and can unintentionally omit up to 50% undetected eggs (Dryden *et al.*;2005) [9], most commonly being the heavy ova of trematodes. (Mir *et al.,* 2016) [14] Identified of particular species of GIN parasites in zoo animals by direct smear examination, standard sedimentation, and floatation techniques. Studies like the latter are sparsely reported in the literature

This purpose of this study was to quantify of GIN eggs from a selected moderately infested calf faecal sample, by comparing faecal floatation and centrifugation techniques using sodium chloride, zinc sulphate and sucrose solutions.

2. Materials and Techniques

2.1 Samplings

Faecal samples were taken using rectal sleeves from seven (7) calves at the Centeno Livestock Station. From the seven (7) calves examined, the faecal samples showed by the McMaster counting method that four (4) were negative for GIN eggs, two (2) were lightly infected (both samples contained 250 eggs per gram) and one (1) was heavily infected with nine hundred and fifty (950) eggs per gram. The sample with nine hundred and fifty (950) eggs per gram was selected as the test sample for the study.

3. Methodology

The sample was thoroughly mixed with a wooden palette to achieve a homogenous egg distribution. The McMaster method quantifying GIN eggs was carried out using seven techniques namely, the direct faecal smear, including the faecal floatation and centrifugation techniques with sodium chloride, zinc sulphate and sucrose solutions, respectively. Five (5) grams of fresh faeces was weighed initially and 30 mls of the chosen floatation solution (sodium chloride, zinc sulphate or sucrose) were combined to attain a specific gravity between 1.18 and 1.20 using a hydrometer. Immediately afterwards the chambers of the McMaster slide were filled with the mixture using a Pasteur pipette or syringe. If visible air bubbles were detected, the fluid was removed and refilled. The idea was to focus on the slide was on the top layer, which contained the very small (pinhead) air bubbles. At this layer, the lines of the grid also became in focus. Eggs were then counted including oocysts present in each lane of both chambers. The number of parasite eggs per gram, were added from the counts for both chambers. The results of both chambers were counted as the eggs in 0.3ml, which was1/200th of the total volume of 60ml. The number of eggs was then multiplied by 200. However, since the experiment began with 4 g of faeces, the resulting count was divided by 4 to yield eggs per gram of faeces. Multiplying by 200 and dividing by 4 was equivalent to multiplying the number of eggs counted by 50.

Faecal floatation was based on the principle that when a faecal sample was placed in a sugar or salt solution, parasites (and other objects) less dense than the floatation solution moved to the top of the solution and parasites more dense than the solution will eventually settle to the bottom.

The faeces were properly emulsified with a tongue depressor. The solution was then strained thru a tea strainer with mixing being done to squeeze out all the fluids.

For the centrifugation techniques – centrifugation was carried out for 10 minutes at 3000 rpm and tube was left undisturbed with its cover-slip for 5 minutes (Christopher and Bernadette, 2008; Christie *et al*.; 2011; Urquart *et al*.; 1996) [5, 6, 21]. The speed of the centrifuge was then gradually increased to the target 3000 rpm. When the rotor speed was increased gradually to the target speed, the centrifuge bucket moved slowly to a horizontal position and the cover-slip will stayed in stable position. The cover-slip was removed from the sample tube one deliberate upward motion, and placed on the microscope for observation. One side of the cover-slip was placed on the slide first and lowered gradually at an angle onto the glass slide as described previously to prevent entrapped air bubbles. An analysis of variance due to technique was carried out using Minitab 19, 2013.

4. Results and Discussion

Table 1 shows the Mean, Standard Error of the Mean (SEM), Standard Deviation (SD), Maximum, Median, and Minimum eggs counts of the seven detection techniques studied. Table 2 and Table 3 shows the analysis of variance pertaining to technique used for quantifying GIN eggs. Mean, and median eggs counts showed that centrifugation floatation techniques resulted in the highest quantification of GIN eggs. These findings corresponded with high significant differences found (P<0.0001) among techniques used (Table 2 and 3). Higher GIN Eggs retrieval by Zinc sulphate has been found in preschool children in Irac. High egg counts for the centrifugation Zinc Sulphate method compared with the benchtop method have been reported in other studies Zajac *et al.*; (2002); Santare'm *et al.* (2009); Gates and Nolan (2013) [22, 20, 11]. Our study demonstrated that higher egg counts by centrifugation were found using floatation solutions sodium chloride followed by sucrose then zinc sulphate (Table 1). Note however, that flotation techniques using sucrose result in higher GIN eggs quantification compared with standard flotation using sodium chloride (Pitman *et al*., 2010) [19]. By comparison centrifugation using sucrose solution yielded higher GIN eggs than flotation techniques (Table 1). Centrifugation methods yielded higher detection of parasitic infections than Macmaster floatation methods as reported in Lamas and Alpacas by Cebra and Stang (2008) including and in veterinary in practice diagnostics (Dryden *et al.,* 2005; Dryden and Payne, 2010) [4, 8, 9].

Therefore, we recommend centrifugation techniques using sodium chloride and Zinc sulphate solutions be used for quantifying GIN eggs in targeting follow up anthelmintic treatment in livestock affected by gastrointestinal parasites.

Table 1: Mean, Standard Error of the Mean (SEM), Standard Deviation (SD), Maximum, Median, and Minimum eggs counts of the seven GIN quantification methods

Variable	Method	N	Mean	SEM	SD	Minimum	Median	Maximum
Egg Counts	1	6	3.667	0.558	1.366	2.000	3.500	130.0
	2	6	95.5	11.1	27.2	67.0	88.5	42.00
	3	6	23.33	7.41	18.15	5.00	22.50	104.0
	4	6	50.5	11.9	29.1	20.0	45.5	1979
	5	6	1349	194	476	658	1430	966.0
	6	6	748.7	82.6	202.4	452.0	742.5	2063
	7	6	1260	279	683	371	1392	

1- Faecal wet smear; 2- Feacal flotation Sodium Chloride; 3- Faecal flotation Zinc Sulphate; 3- Faecal Flotation Sucrose 5- Centrifugation flotation Sodium Chloride; 6- Centrifugation; Centrifugation Zinc Sulphate; 7- Centrifugation Flotation Sucrose

Table 2: Two-way ANOVA: Egg counts versus reps, trt

Source	DF	SS	MS	F	P
Reps	5	614572	122914	1.20	0.331
Method	6	13191448	2198575	21.53	0.000
Error	30	3064102	102137		
Total	41	16870123			

Methods: 1- Faecal wet smear; 2- Feacal flotation Sodium Chloride; 3- Faecal flotation Zinc Sulphate; 3- Faecal Flotation Sucrose 5- Centrifugation flotation Sodium Chloride; 6- Centrifugation; Centrifugation Zinc Sulphate; 7- Centrifugation Flotation Sucrose

Table 3: Analysis of Variance for sqrt, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Reps	5	112.82	112.82	22.56	0.84	0.532
Method	6	7838.45	7838.45	1306.41	48.61	0.000
Error	30	806.18	806.18	26.87		
Total	41	8757.45				

Methods; 1- Faecal wet smear; 2- Feacal flotation Sodium Chloride; 3- Faecal flotation Zinc Sulphate; 3- Faecal Flotation Sucrose 5- Centrifugation flotation Sodium Chloride; 6- Centrifugation; Centrifugation Zinc Sulphate; 7- Centrifugation Flotation Sucrose

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