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EVALUATING HELMINTH INFECTIONS IN ANIMALS: A COMPARISON OF PARASEP® AND MCMASTER METHOD FOR ROUTINE LABORATORY DIAGNOSIS USING GOAT FAECES

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ABSTRACT. The worm ova estimation method is important to assess the degree of worm infestation in domestic animals. Currently, the method used in many veterinary laboratories is the McMaster method which can enumerate the number of eggs per gram of faeces. Due to emerging and re-emerging diseases currently being diagnosed in Veterinary Research Institute, Ipoh, it is important to seek new, less risky methods for diagnosis of faecal samples. In view of increasing risk to the laboratory personnel conducting tests on faecal samples, the Parasep® method was assessed to indicate its suitability as a routine test method. The results indicate that there was no significant differences between the worm egg counts enumerated by conventional McMaster method and Parasep® method ($Z = -1.111$, $P = 0.267$). It is however, critical that assessment based on costs, time and ease of conducting the

tests for lab staff be done before adopting this method in diagnostic laboratories.

INTRODUCTION

Cattle, goats and sheep are important sources of animal protein in many countries of the world. They are the sources for daily meat and dairy products in cities and villages. Infection caused by worm parasites is known to be a major problem through the world. Infection can cause a lot of economic losses in many ways such as reduction in food intake, lower weight gains, milk and meat production, lower fertility, reduce work capacity, involuntary culling, high treatment cost and mortality in heavily parasitized animals (Carmichael, 1972; Akerejola *et al.*, 1979). The major losses due to parasites were observed mainly in animal production rather than animal mortality (Anon.,

1991; McLeod, 1995). Parasite infection can cause economic losses ranging from 20% to 25% of the production rate (Gupta, 2006). It was found that the humid tropical environment such as in Malaysia is very favourable for the year round development and survival of the pre-parasitic stages of trichostrongyles on pastures (Ikeme *et al.*, 1987; Daud Ahmad, 1991).

In Malaysia, worm infection has been recognised as the most important infection as it cause a high morbidity in small ruminants and is known to be the second cause of mortality in sheep (Fatimah *et al.*, 1985; Sani and Rajamanickam, 1990; Sani *et al.*, 2004). Generally, the most prevalent and pathogenic nematode species of small ruminants in Malaysia is *Haemonchus contortus* (Dorny *et al.*, 2005, Chandrawathani *et al.*, 1999; 2003; 2004; Khadijah *et al.*, 2006a, b). The adults of this worm are present in large numbers in the abomasum of sheep and goats in this country, and their infection is associated with clinical signs such as anaemia and hypoproteinaemia (Sheikh Omar and Chulan, 1980). *Oesophagostomum columbianum* is another serious pathogen in sheep and goats which can cause diarrhoea with mucus and blood (Soulsby, 1982). Only 2000 adult strongyle worms were reportedly necessary to produce marked clinical signs in a one year old sheep (Soulsby, 1982). As for sheep and goats, strongyle infections can be acquired at an early age. The faecal worm egg counts reduce when the animal is eight months and above in sheep, and reduce from twelve to

eighteen month onwards in goats (Dorny *et al.*, 1995). As a result of this, young ruminants fail to develop an effective immunity to gastrointestinal nematodes and are susceptible to infection and to pathogenic effects of strongyles (Soulsby, 1985). In young animals, haemonchosis is associated with high faecal eggs count, low haematocrit count, ill thrift and high mortality (Soulsby, 1985).

Due to the severity of the worm infection, early diagnosis is important in order to determine on appropriate treatment and management approaches to control the infection. Veterinary laboratories are often requested to diagnose worm infection and estimate worm burdens quickly and accurately. The McMaster method which was developed and improved at the McMaster laboratory of the University of Sydney (Gordon and Whitlock, 1939; Whitlock, 1948) is the most universally used method for estimating the number of worm eggs in faeces (Rossanigo and Gruner, 1991; Nicholls and Obendorf, 1994). The McMaster method is widely used, and has been modified by different laboratories worldwide to suit the convenience and availability of reagents and equipment. However, common salt (sodium chloride), McMaster slide and dilutions are still maintained. The modification is related to the weight of the faecal sample, the specific gravity, the density of liquid used and also whether one or two chambers of McMaster slide is used for enumeration of eggs. The ratio of the weight of faecal sample and volume

of saturated salt solution should be 1:15 and the egg counting must be done within the ruled area of the McMaster chamber (Cringoli *et al.*, 2004). The advantages of the McMaster method are that, it can be done quickly, with no centrifugation needed (Rinaldi *et al.*, 2011), eggs easily float up for counting and low interference of faecal material during worm egg observation. This method is not suitable for all fluke eggs and is most likely to miss in a light infestation (Ministry of Agriculture Fisheries and Food, 1978).

Veterinary Parasep[®] Faecal Filters (Parasep[®]), is a commercial kit developed by Apacor which offers an improved method for determining worm egg counts of cattle, sheep, and horses. This kit is disposable and uses an enclosed system. Thus, chances of cross-contamination between samples is less and the technician works in a safe environment, as there will be minimum exposure to the faecal samples when using this kit. In order to use this kit in a veterinary diagnostic laboratory, the reliability of this kit needs to be evaluated.

Thus, the objective of this study is to compare the conventional method for modified McMaster method with the Parasep[®], based on the results of worm egg counts from the faecal same sample. This information will provide a safe alternative for evaluating faecal egg counts from livestock and is especially important for diagnostic laboratories involved in handling faecal samples from livestock with various other concurrent emerging or zoonotic diseases.

MATERIALS AND METHODS

Sample collection/Study sites and animals

This study was conducted at Parasitology and Haematology Laboratory in Veterinary Research Institute (VRI), Ipoh. A total of thirty female goat faecal samples were received on 3rd July 2014, of which 29 samples were from a government farm located at Sungai Siput, Perak and one sample from VRI. Twenty-five of them were Boer goats, four were Jamnapari and one Saanen crossbreed. Based on visual observations, the goats were thin and had soft, putrid smelling faeces, probably indicative of worm and coccidia infections.

McMaster Method (Christopher *et al.*, 1992).

Three grams of faeces was weighed into a jar and 45 ml of saturated salt solution was added (1:15 dilution). The sample was ground and mixed by using mortar and pestle. Then the sample was filtered through 85 mesh screen and the filtrate was collected. The filtrate was mixed well and the counting chamber of McMaster's slide (Chalex, USA) was filled up with the filtrate. The filtrate was observed under the microscope by using 10× objective lens and the eggs that have been seen in the grid area were counted. The sensitivity of this method is one egg equivalent to 100 eggs.

Veterinary Parasep® Faecal Filter (Apacor)

This method was conducted based on the protocols by Apacor, (Couturier *et al.*, 2015) provided with the kit. The Parasep® tube is a vertical filtration design that uses two separate vertical filtration steps (housed within the stool “spoon”) to concentrate stool specimens without the need for volatile solvents such as ether or ethyl acetate.

For sample preparation, one level scoop of plastic granules, two drops of antifoam, 3 grams of faecal sample, and 42 ml of saturated salt were mixed into the mixing tube. The tube was capped and shaken until the faecal matter was broken down. Then the samples were emulsified using glass beads provided in the kit. The 15 ml tube was screwed on the green filter and this assembly was screwed onto the mixing tube which has the sample that has been suspended. Then, the tube was shaken well in a horizontal position, and immediately inverted six times so the thinner 15 ml tube is pointing downwards. After that, the thinner tube was shaken vertically until it filled up to the 5 ml level. The mixing tube and filter assembly was discarded and the thinner tube was capped and inverted six times and the sample was pipetted immediately into the McMaster slide. This inversion process was repeated and the second sample was pipette into the second chamber of McMaster slide. For calculation, the worm egg count from the first chamber was added to the second egg

counts and divided by two for an average value. The average was multiplied with 100 to give eggs per gram (EPG) of faeces.

Statistical analysis

The arithmetic mean eggs per gram of faeces (EPG), standard deviation (SD), variation and Coefficient of Variation (CV) of EPG values were calculated for McMaster and Parasep® methods. The data were analysed by using SPSS software version 20 (IBM Corporation). The non-parametric test, Mann-Whitney U test was used to compare the worm egg count results between conventional McMaster method and Parasep® as the data of worm egg counts was not normally distributed.

RESULTS

The results of the evaluation of using both techniques encompass the quality of eggs seen and the quantity or number of eggs observed in the McMaster slide.

In terms of clarity of eggs seen, it was found that strongyle eggs processed using the Parasep® method (Figure 1a) were much clearer than the those processed using conventional McMaster method (Figure 1b). There was less debris in the slide which was processed via the Parasep® kit and the image of strongyle eggs was much clearer ensuring more accurate identification of worm eggs. However, in using the conventional McMaster method, more debris in the background was noted, making it more difficult, especially for

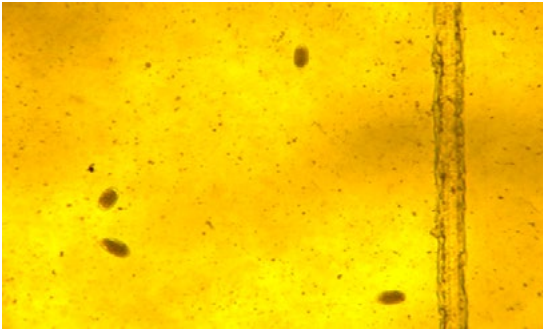


Figure 1a. Strongyle eggs observed in conventional Parasep® method with less debris

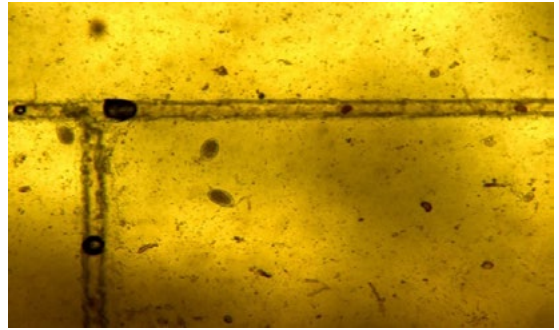


Figure 1b. Strongyle eggs observed in conventional McMaster method with more debris

inexperienced eyes, to locate and identify the eggs. This factor is important as this will have an impact on the identification of worm eggs and subsequently the accuracy of results.

However, there was no significant difference in the worm egg count done on each single faecal sample as observed between the Parasep® and McMaster methods ($Z=-1.11$, $P=0.267$). Thus, both techniques are equally efficient in floating up worm eggs and both techniques give similar results in terms of worm burdens for the animal. This indicates that both techniques can be used to evaluate worm burdens without compromising the number of eggs harvested in each technique.

DISCUSSION AND CONCLUSION

This study is to evaluate the efficiency of ova estimation of Veterinary Parasep® Faecal Filter method for the laboratory diagnosis of worm egg count by comparing it with the conventional McMaster method (Ministry

of Agriculture, Fisheries and Food, 1978) which is routinely used in some diagnostic laboratories. From the results, it is obvious that there is no significant difference in the faecal worm ova counts between the two methods used. This shows that Veterinary Parasep® Faecal Filter method could be used as an alternative to the conventional McMaster method. Based on observation, the main advantage of using Parasep® for worm egg count estimation is the minimal debris which may interfere with egg identification, while counting the worm eggs. The minimal background debris allows a clearer view of strongyle eggs under the microscope, is most probably due to the vertical filter that was used in Parasep® (425 micro pored) compared to the conventional McMaster method which uses only 85 μ mesh screen to filter the sample. The presence of debris in excess could mask the presence of small parasites especially oocysts, thereby producing inaccurate results leading to misdiagnosis of parasitic infections.

One of the advantages of the Parasep[®] method is that it is less laborious. The conventional McMaster method uses several apparatus such as mortar, pastel, small beaker and 85 μ mesh sieve which are labour intensive in terms of disinfecting and cleaning. The use of Parasep[®] will reduce labour, especially when diagnostic laboratories receive a large number of samples for worm egg counts.

In terms of laboratory safety, each Parasep[®] tube is equipped with air and liquid seals, as well as a safety lock. The seal prevents the release of aerosols and other hazardous materials while the lock ensures that the mixing chamber and filter are removed together for safe disposal after diagnosis is completed. It is also a single use device, thus minimising the risk of sample contamination by preventing any possibility of sample carryover if not cleaned correctly.

However, in terms of time consumption, conducting the conventional McMaster method is faster when compared to the Parasep[®] method. This is because a mortar and pestle is used to grind the faeces manually. In comparison, the Veterinary Parasep[®] Faecal Filter, requires several shaking steps to break down the faeces, especially if it is hard. In spite of the slightly longer time taken, the Parasep[®] method is a more convenient apparatus for use in a mobile lab or when dealing with hazardous material such as faeces from animals infected with other concurrent zoonotic or emerging diseases as there will be no laborious clean-up of sink, small

glass beakers and sieves, as the filtration, mixing and shaking will be performed inside the Parasep[®] kit, thus minimising the number of pieces of apparatus used.

In terms of cost, the Parasep[®] kit is more expensive than the conventional McMaster method. Conventional McMaster method is five to twelve times cheaper than the Parasep[®] (Couturier *et al.*, 2015), depending on the country it is used as it basically depends on the cost of salt as a reagent. Other items are one-off purchase of mortar, pestle, slides, beakers and microscope, which are used for both techniques. For developing countries, farmers may not be able to afford the cost of helminth diagnosis using the Parasep[®] method especially if they need to screen many animals. In Malaysia, however, the cost for livestock diagnosis is subsidised by the Department of Veterinary Services as part of the overall plan to increase and encourage livestock farming.

In conclusion, the Parasep[®] method can be used for worm egg count as an alternative to the conventional McMaster method for helminth infection diagnosis in livestock animals. Although the cost and technical expertise in identification of worm eggs may be a limiting factor, the Parasep[®] tool is a safe alternative when dealing with high risk samples such as faecal samples from animals with highly contagious or zoonotic diseases such as avian influenza, anthrax, colibacillosis, Q fever and others which can be inhaled via aerosol while preparation using the conventional mortar and pestle method.

Laboratories worldwide are now faced with diagnosis of several unknown diseases and this in turn will expose diagnostic staff to various pathogens. It is important that these factors are taken in consideration when choosing the appropriate technique to be utilised for the safety of staff and environment.

REFERENCES

- Akerejola O.O., Schillhorn Van Veen T.W. and Njoku C.O. (1979). Ovine and Caprine diseases in Nigeria: a review of economic losses. *Bulletin Animal Health Production Africa*, **27**: 65-70.
- Anonymous. (1991). *Report of the FAO Expert Consultation of the Worm Infections of Livestock in Developing Countries*. Rome Italy 23-27 September 1991. Food and Agriculture Organisation of the United Nations, Rome. AGA 815. pp 32.
- Couturier B.A., Jensen R., Arias N., Heffron M., Gubler E., Case K., Gowans J. and Couturier M. (2015). Clinical and analytical evaluation of a single vial stool collection device with formalin-free fixative for improved processing and comprehensive detection of gastrointestinal parasites. *J. Clin. Microbiol.* **53**(8):2539-2548 doi:10.1128/JCM.00838-15
- Carmichael I.H. (1972). Helminthiasis in domestic and wild ruminants in Botswana – preliminary investigations. *Tropical Animal Health Production*, **4**(3): 175-181.
- Charles T.P. (1989). Seasonal prevalence of gastrointestinal nematodes of goats in Pernambuco State, Brazil. *Vet. Parasitol.*, **30**: 335-343.
- Christopher R., Chandrawathani P. and Cheah Tong Soon (1992). *Manual on Parasitology. First edition*. Department of Veterinary Services Ministry of Agriculture Malaysia. p. 12
- Connor R.J., Munyuku A.P., Mackyao E. and Halliwell R.W. (1990). Wormosis in goats in Southern Tanzania: Investigations on epidemiology and control. *Trop. Anim. Health Prod.*, **22**: 1-6.
- Cringoli G., Rinaldi L., Veneziano V., Capelli G. and Scala A. (2004). The influence of flotation solution, sample dilution and the choice of McMaster area (volume) on the reliability of the McMaster method in estimating the faecal egg counts of gastrointestinal strongyles and *Dicrocoelium dendriticum* in sheep. *Vet. Parasitol.* **123**:121-131.
- Cringoli G., Rinaldi L., Maurelli M.P. and Utzinger J. (2010). FLOTAC: new multivalent methods for qualitative and quantitative copromicroscopic diagnosis of parasites in animals and humans. *Nat. Protoc.* **5**: 503–515.
- Colley D.G., LoVerde P.T., and Savioli L. (2001). Infectious disease: medical helminthology in the 21st century, *Science*, **293**(5534):1437–1438.
- Daud Ahmad (1991). *Gastrointestinal parasitism in goats – Bionomics of the suprapopulation and incidence in young goats*. M.Sc. Thesis, Universiti Pertanian Malaysia.
- Dorny P., Symoens C., Jalila A., Vercruyse J. and Sani R., (1995). Strongyle infections in sheep and goats under the traditional husbandry system in peninsular Malaysia. *Veterinary Parasitology*, **56**: 121-136.
- Duthaler U., Rinaldi L., Maurelli M.P., Vargas M., Utzinger J., Cringoli G. and Keiser J. (2010). *Fasciola hepatica*: comparison of the sedimentation and FLOTAC techniques for the detection and quantification of faecal egg counts in rats. *Exp. Parasitol.* **126**(2):161-166
- Gordon H.M. (1980). The contribution of McMaster. *Mod. Vet. Pract.* **91**: 97-100.
- Gordon H.M. and Whitlock H.V. (1939). A new technique for counting nematode eggs in sheep faeces. *J. Counc. Sci. Ind. Res.* **12**(1):50-52.
- Gupta J.L. (2006). *Sheep production and management*. New Delhi: CBS Publishers and Distributors, pp. 1-239.
- Ikeme M.M., Fatimah I. and Lee C.C. (1987). Seasonal changes in the prevalence of *Haemonchus* and *Trichostrongylus* hypobiotic larvae in tracer goats in Malaysia. *Trop. Animal Health Prod.*, **19**(3):184-190.
- McLeod R.S. (1995). Costs of major parasites to the Australian livestock industries. *International Journal of Parasitology* **25**: 1363-1367.
- Ministry of Agriculture, Fisheries and Food (1986). *Manual of veterinary parasitological laboratory methods (Reference Book; 418), 3rd ed.* London: Her Majesty's Stationery Office (HMSO). 160 p.
- Nicholls J. and Obendorf D.L. (1994). Application of a composite faecal egg count procedure in diagnostic parasitology. *Vet. Parasitol.* **52**:337-342.
- Rinaldi L., Coles G.C., Maurelli M.P., Musella V. and Cringoli G. (2011). Calibration and diagnostic accuracy of simple flotation, McMaster and FLOTAC for parasite egg counts in sheep. *Veterinary Parasitology* **177**(3-4):345-352
- Rinaldi L., Maurelli M.P., Musella V., Santaniello A., Coles G.C. and Cringoli G. (2010). FLOTAC: an improved method for diagnosis of lungworm infections in sheep. *Vet. Parasitol.* **169**:395-398.
- Rossanigo C.E. and Gruner L. (1991). Accuracy of two methods for counting eggs of sheep nematode parasites. *Vet. Parasitol.* **39**:115–121.

24. Sani R.A. and Rajamanickam C. (1990). Gastrointestinal parasitism in small ruminants. In: *Proc. Integrated Tree Cropping and Small Ruminant Production System workshop*. Medan, Indonesia, 4-9 September 1990, pp. 197-201.
25. Sheikh-Omar A.R. and Chulan U. (1980). Cases submitted for post-mortem examination to Universiti Pertanian Malaysia in 1977. *Pertanika* **3**:155-158.
26. Soulsby E.J.L. (1982). *Worms, Arthropods and Protozoa of Domesticated Animals*. Bailliere Tindall, London, UK.
27. Soulsby E.J.L. (1985). Advances in immunoparasitology. *Vet. Parasitol.*, **18**:303-319.
28. Symoens C., Dorny P., Alimon R., Jalila A., Hardouin J. and Vercurysse J. (1993). Productivity of goats in smallholdings of Peninsular Malaysia. In: *Advances in Sustainable Small Ruminant-Tree Cropping Integrated Systems*, Sivaraj S., Agamuthu P. and Mukherjee T.K. (Editors). IPT/IDRC, Kuala Lumpur, Malaysia, pp. 129-136.
29. Vercurysse J. (1983). A survey of seasonal changes in nematode faecal egg count levels of sheep and goats in Senegal. *Vet. Parasitol.*, **13**:239-244.
30. Whitlock H.V. (1948). Some modifications of the McMaster worm egg-counting method and apparatus. *J. Counc. Sci. Ind. Res.* **21**:177-180.

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