

Recovery rates of *Eimeria* oocysts from cattle feces by Wisconsin sugar flotation method

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Abstract

The purpose of this study was to know the recovery rates of *Eimeria* oocysts through the course of flotation in sugar solution by WSFM. A fecal sample was collected from a calf infected with *Eimeria bovis*. Stage one of this study involved recovering oocysts from surface of sugar solution in a test tube with cover slips replaced at time points of 10, 20, 30, 40, 50, 60, 90 min, 2, 3, 4, 5, 6, 24, 48 hr., and 5 days. Oocysts were continuously detected up to 48 hr, with a linear increase in recovery counts for the first 2 hr and the average total count was 953 per gram. Recovery rates of oocysts were 8.1%, 13.3% and 16.4% at 10, 20 and 30 min, respectively. Stage 2 involved a set of three tubes. Cover slips were put at intervals of 10, 20 and 30 min on the first tube, while one cover slip was kept for 20 min on the second tube and one for 30 min on the third tube. No significant differences were detected in oocyst counts at 20 min between the first and second tubes, and at 30 min between the first and third tubes. Stage 3 involved the comparison of recovery rates at 10 min between sugar solutions with specific gravities of 1.200 and 1.266. There was no significant difference in oocyst counts between the two solutions. These results indicate that it takes time to recover all *Eimeria* oocysts from cattle fecal samples by WSFM, but total oocyst counts per gram can be estimated by counting oocysts detected at any sampling time points, such as 10, 20 or 30 min using one cover slip. No improvement of recovery rates was seen by using a higher concentrated sugar solution.

Keywords: WSFM, Oocyst, Cattle

INTRODUCTION

Infections of the gastrointestinal tract by various helminths and protozoa may cause severe economic losses among cattle (Charlier et al., 2014; Rahimi Esboei et al., 2020). Although clinical parasitism has received considerable attention as a result of its obvious severity, the study of parasitism in herds without clinical sign of infection has been largely neglected (Bahrami & Alborzi, 2013). Subclinical parasitism in cattle may cause considerable economic losses (Sargison et al., 2016; Cruvinel et al., 2018). Coccidiosis, one of the most important protozoan parasitic diseases can infect many of livestock including human (Dauguschies & Najdrowski, 2005; Bruhn et al., 2011). Coccidiosis is caused by the protozoan parasite belong to the genus *Eimeria* spp (Sudharakara et al.,

2015). The diseases is uncommon in adult cattle but occasional cases and sometimes, epidemics of disease have been reported in dairy cows (Nain et al., 2017; Cardim et al., 2018; Gebeyehu et al., 2018; Sodha et al., 2021). This disease is responsible for major economic losses. An impact of US\$ 400 million on the American market has been estimated, due to clinical cases alone, while over US\$ 3.8 million are lost through treatments for bovine coccidiosis in Canada (Matjila & Penzhorn, 2002; Rehman et al., 2011).

Fecal techniques using centrifugal force are more efficient in recovering eggs than those using gravitational force (Parameshwarappa et al., 2012; Paras et al., 2018). Centrifugation consistently recover more eggs than other

methods (Dryden et al., 2005; Zajac & Conboy, 2012; Craig, 2018). Wisconsin Sugar Flotation Method (WSFM) is the most popular method for detection of parasite oocysts, cysts, larvae and eggs in fecal samples (Das et al., 2015). Ito, (1980) reported 90% nematode eggs by WSFM in 20 min. The result is a clean preparation for microscopic examination with a minimal amount of distracting fecal debris. However, there is no critical information on the numbers of oocysts that can be recovered by WSFM over the course of flotation period. The purpose of this study was therefore carried to determine the recovery rates of *Eimeria* oocysts through the course of flotation in sugar solution by WSFM.

MATERIAL AND METHODS

Fecal sample

The fecal sample was collected from 3 months old Holstein cattle (Infected with *Eimeria bovis*) which had diarrhea and the sample mixed with the fecal sample of healthy cattle. From the heavily infected calf, 355 oocysts were recovered from 1 g of feces in 20 min, making it difficult to count several times. Therefore, part of heavily infected feces was mixed with 3 parts of normal feces to give a specimen with lower numbers of oocysts for easy counting.

Wisconsin sugar flotation method

A sugar centrifugal-flotation technique was used to examine the fecal samples. A 1 g portion of feces was mixed with 10 ml of water and poured through a strainer with 16 meshes per inch into another beaker. The total fluid poured into the 15 ml conical tip centrifuge tubes. After centrifugation at 2000 rpm for ten min, the supernatant fluid was decanted and 2 ml sugar solution (sp. G. 1.2) added in each tube and mixed with a wooden applicator stick and mixed with vortex. Additional 8 ml of sugar solution added and stirred gently with a stick and centrifuged again for 10 min at 2000 rpm, additional sugar solution added until a convex meniscus was formed on top of the tube. An 18 mm x 18 mm square cover glass was then placed in contact with sugar solution on top of each tube and left to stand for different times at room temperature. The cover glass was lifted off and placed on a glass slide, and the entire area under each was examined and oocysts counted microscopically at a magnification of 100 x.

Preparation of sugar solution

Specific gravity = 1.200 solution: 600 g sugar with 700 ml distilled water, after the solution mixed with 5 ml fecal sample the SG was 1.189.

Specific gravity = 1.266 solution: 128 g sugar with 100ml distilled water, after the sample mixed with 5 ml fecal sample the SG was 1.253.

Direct count of oocyst

The direct examination techniques conducted to be sure about the number of oocysts in fecal sample. The sample contained 7.8 oocysts per 0.01 g.

This study was conducted in three stages:

- 1- **Total oocysts recovery over time:** To know the total number of oocysts contained in a given mass of feces, several cover slips placed at certain time intervals until no oocysts was detected. When this test is repeated several times and proportion of oocysts recovered is similar in the replicates, then measuring oocysts at a particular time point used to estimate the total number of oocysts in feces for same parasite species in a short time. This stage involved recovering oocysts from surface of sugar solution in a test tube (1.5 cm in diameter, 10.5 cm in length) with cover slips replaced at time intervals of 10 min, giving oocysts collection time points of 10, 20, 30, 40, 50, 60, 90 min, 2, 3, 4, 5, 6, 24, 48 hr, and 5 days.
- 2- **Comparison between changing cover slip and not changing:** This stage was conducted to know whether replacing cover slips every 10 min is better in recovering oocysts than holding it once for 20 or 30 min. the stage involved a set of three tubes. Fresh cover slips were put at intervals of 10 min on the first test tube, giving recovery time points of 10, 20 and 30 min, while one cover slip was kept for 20 min on the second tube and one cover slip for 30 min on the third tube, then the recovered oocysts were counted.
- 3- **Comparison between two sugar gravity:** The standard specific gravity of 1.200 takes a longer time to recover most oocysts from a given fecal sample. In this study, a higher specific gravity was used to know if more numbers of oocysts could be recovered in a shorter time. This stage involved the comparison of numbers of oocysts recovered from 2 different sugar solutions of specific gravities 1.200 and 1.266 with cover slips kept on each tube for 10 min.

RESULTS

Oocysts were continuously detected up to 48 hr, with a linear increase in recovery counts (Figure 1) for the first 2 hr, and the average total count was 953 per gram (by stage one method). Recovery rates of oocysts were 8.1%, 13.3% and 16.4% at 10, 20 and 30 min, respectively. Very few oocysts are detectable by this method after 48 hr, with only two oocysts recovered at five days in this study.

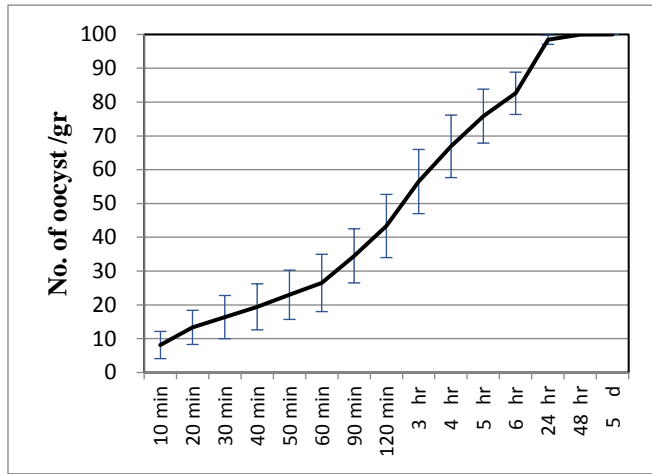


Figure 1. Percentage detection

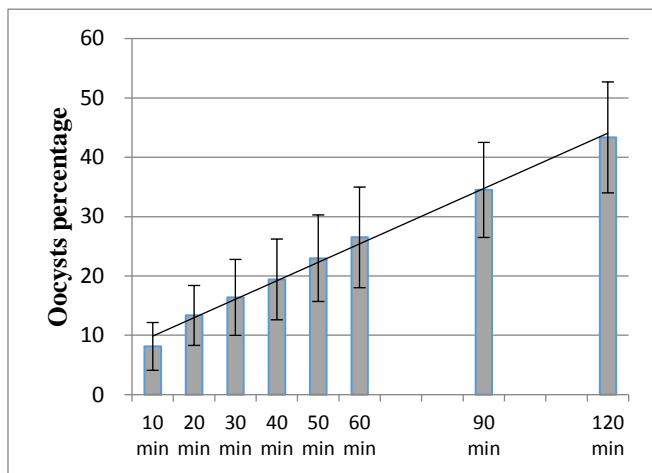


Figure 2. Oocyst counts/10 min

Figure 2 shows the number of oocysts detected every 10 min. The number detected in the first 10 min was high and reduced in the second 10 min (20 min) but recovery was stable from third 10 min (30 min) to 8 - 10 min (120 min) then reducing gradually up to five days.

DISCUSSION

Recovery of oocysts continuously for 48 hr to 5 days ensures total recovery of oocysts from a given gram of feces (Dryden et al., 2005). This method is tedious and time consuming making rapid diagnosis of parasitic diseases difficult. However, by replicating this method several times for about 5 days, over regular time intervals, an average proportion of oocysts contained in a given gram of feces can be estimated at a particular time and the total number of oocysts can be calculated with no need to wait for 5 days. The result indicated that total oocyst counts per gram can be estimated by counting

oocysts detected at any sampling time points, such as 10, 20 or 30 min using one cover slip. This finding is not similar to what Ito, (1980) reported with nematode egg counts in bovine feces. He reported the recovering of nematode eggs in WSFM to be 90% in 20 min. It should however be noted that the number of oocysts recovered is not directly proportional to the number of adult parasites in the host.

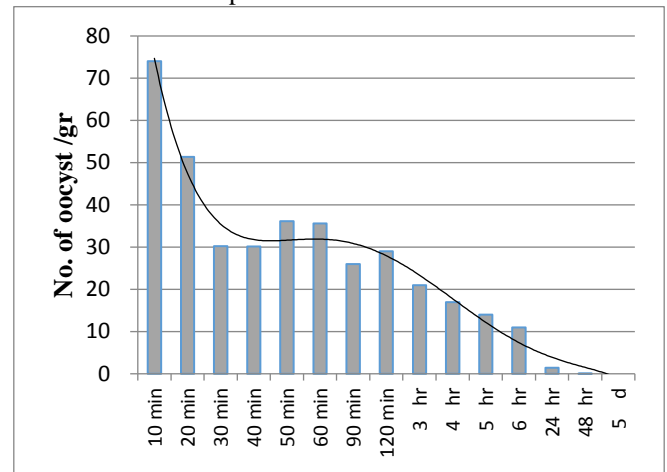


Figure 3. Percentage detected, up to 120 min
The graphs 3 showing the percentage detection up to 120 min, which is showing linear increase up to 120 min.

In stage 2 replicated 12 times, no significant differences were observed in oocyst counts at 20 min between the first and second tubes, and at 30 min between the first and third tubes. T-test between 10 and 20 min of first test tube and 20 min of second test tube was 0.845 and T-test between 10, 20, 30 min of first test tube and 30 min of third tube was 0.368.

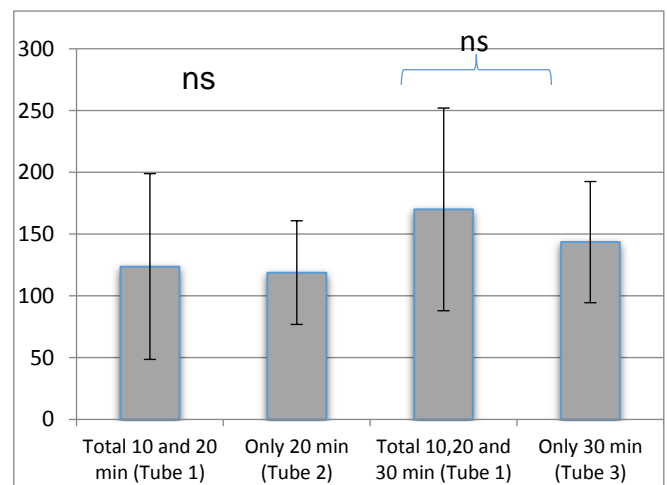


Figure 4. In stage 3, replicated eight times, no significant difference was observed in oocyst counts between the two solutions with specific gravities of 1.200 and 1.266 (t=0.127).

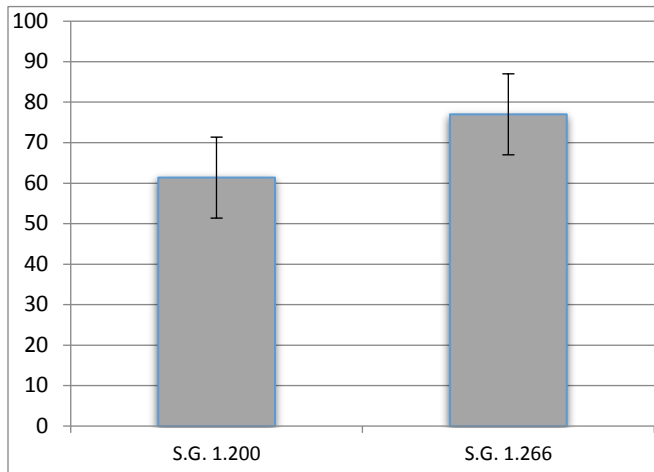


Figure 5. No of oocysts in two different SG

The graph shows that there is no significant difference observe in oocyst counts between the two sugars solutions with specific gravities of 1.200 and 1.266.

Putting cover slip once for 20 min is sufficient to estimate the total load of oocysts in a given gram of feces, thereby saving time. Ito 1980, found a similar result with nematode eggs in bovines.

In the present study, no improvement of recovery rates was seen by using a higher concentrated sugar solution. Grady and Slocombe in (1979), reported that Coccidial oocysts from sheep floated best in a narrow range of SpGr from 1.22 – 1.27. Also Ito, (1980) reported that nematode eggs are recovering better on sugar solution with S.G. 1.200.

CONCLUSION

In conclusion, these results indicated that it takes time to recover all *Eimeria* oocysts from cattle fecal samples by WFSM, but total oocyst counts per gram can be estimated by counting oocysts detected at any sampling time points, such as 10, 20 or 30 min using one cover slip. No improvement of recovery rates was seen by using a higher concentrated.

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REFERENCES

- Bahrami, S., & Alborzi, A. R. (2013). Prevalence of subclinical coccidiosis in river buffalo calves of southwest of Iran. *Acta Parasitologica*, 58(4), 527–530. <https://doi.org/10.2478/s11686-013-0167-1>
- Bruhn, F. R. P., Lopes, M. A., Demeu, F. A., Perazza, C. A., Pedrosa, M. F., Guimarães, A. M. (2011). Frequency of species of *Eimeria* in females of the Holstein-Friesian breed at the post-weaning stage during autumn and winter. *Rev Bras Parasitol Vet*, 20(4), 303-307.
- Cardim, S. T., Seixas, M., Tabacow, V. B. D., Taroda, A., Carneiro, P. G., Martins, T. A., de Barros, L. D., Minutti, A. F., Chryssafidis, A. L., Vidotto, O., & Garcia, J. L. (2018). Prevalence of *Eimeria* spp. in calves from dairy farms in northern Paraná state, Brazil. *Revista Brasileira de Parasitologia Veterinaria*, 27(1), 119–123.
- Charlier, J., Vercruyse, J., Morgan, E., Van Dijk, J., & Williams, D. (2014). Recent advances in the diagnosis, impact on production and prediction of *Fasciola hepatica* in cattle. *Parasitology*, 141(3), 326-335.
- Craig, T. M. (2018). Gastrointestinal nematodes, diagnosis and control. *Vet Clin North Am Food Anim Pract*, 34(1), 185–99.
- Cruvinel, L. B., Nicaretta, J. E., Bastos, T. D. S. A., Couto, L. F. M., Santos, J. B. D., Zapa, D. M. B., Cavalcante, A. S. D. A., Cruz, B. C., Borges, D. G. L., Borges, F. D. A., Soares, V. E. & Lopes, W. D. Z. (2018). *Eimeria* species in dairy and beef cattle of different ages in Goiás state, Brazil. *Brazil Journal of Veterinary Parasitology*, 27(2), 169-176.
- Das, M., Deka, D. K., Sarmah, P. C., Islam, S., & Sarma, S. (2015). Diversity of *eimeria* spp. In dairy cattle of guwahati, assam, india. *Veterinary World*, 8(8), 941–945. <https://doi.org/10.14202/vetworld>
- Dauguschies, A., & Najdrowski, M. (2005). Eimeriosis in cattle: current understanding. *Journal of Veterinary Medicine B, Infectious Disease and Veterinary Public Health*, 52(10), 417–427.
- Dryden, M. W., Payne, P. A., Ridley, R., & Smith V. (2005). Comparison of common fecal flotation techniques for the recovery of parasite eggs and oocysts. *Veterinary Spring*, 6(1), 15-28.
- Gebeyehu, B., Kebede, E., Kifleyohannes, T., Abebe, N., & Kumar, N. (2018). Prevalence of calf coccidiosis in

- Mekelle, northern Ethiopia. *Ethiopian Veterinary Journal*, 22(2), 1. <https://doi.org/10.4314/evj.v22i2.1>
- Grady, M. R., & Slocombe, J. O. (1980). An investigation of variables in a fecal flotation technique. *Canadian Journal of Comparative Medicine*, 44(2):148–157.
- Matjila, P. T., & Penzhorn, B. L. (2002). Occurrence and diversity of bovine coccidia at three localities in South Africa. *Vet Parasitol*, 104(2), 93-102.
- Nain, N., Gupta, S. K., Sangwan, A. K., & Gupta, S. (2017). Prevalence of Eimeria Species in Buffalo Calves of Haryana. *Pdfs.Semanticscholar.Org*, 56(1), 5–8.
- Parameshwarappa, K. D., Chandrakanth, C., & Sunil, B. (2012). The prevalence of intestinal parasitic infestations and the evaluation of different concentration techniques of the stool examination. *Journal of Clinical and Diagnostic Research*, 6(7 SUPPL.), 1188–1191.
- Paras, K. L., George, M. M., Vidyashankar, A. N. (2018). Comparison of fecal egg counting methods in four livestock species. *Veterinary Parasitology*, 15(257), 21–27. doi: 10.1016/j.vetpar.2018.05.015.
- Rehman, T. U., Khan, M. N., Sajid, M. S., Abbas, R. Z., Arshad, M., & Iqbal, Z. (2011). Epidemiology of Eimeria and associated risk factors in cattle of district Toba Tek Singh, Pakistan. *Parasitol Res*, 108(5), 1171-1177.
- Sodha, D. B., Chouhan, A. K., & Pilia, P. K. (2021). Prevalence and risk assessment of coccidiosis in dairy animals of transitional plain in luni basin of Rajasthan. *Haryana Vet*, 9(1), 706–710.
- Sueharu, Ito. (1980). Modified Wisconsin sugar centrifugal-flotation technique for nematode eggs in bovine feces. *Journal of the Japan Veterinary Medical Association*, 33(9), 424-429.
- Sudhakar Reddy, B., Sivajothi, S., & Rayulu, V. C. (2015). Clinical Coccidiosis in Cattle. *Journal of Parasite Diseases*, 39(3): 557–559.
- Zajac, A. M., & Conboy, G. (2012). *Veterinary clinical parasitology*. 8th edition. West Sussex (UK): Wiley-Blackwell.