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Production tool

Modified technique for collecting and processing fecal material for diagnosing intestinal parasites in swine

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Summary

Intestinal parasites of swine are still present under conditions of modern swine management and can have a significant impact on growth rate and feed efficiency. Diagnosis of intestinal parasites can be accomplished by demonstrating adult parasites in feces or at necropsy, and by demonstrating eggs in feces. This article details a protocol for sampling fecal material to diagnose intestinal parasites in swine populations.

Keywords: swine, parasites, fecal collection, fecal flotation, diagnostic test

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Resumen - Técnica modificada para la recolección y procesamiento de material fecal para el diagnóstico de parásitos intestinales en cerdos

Los parásitos intestinales del cerdo se presentan aún bajo condiciones modernas de manejo porcino y pueden tener un impacto significativo en el crecimiento y la eficiencia alimenticia. El diagnóstico de parásitos intestinales puede lograrse al demostrar la presencia de parásitos adultos en las heces fecales ó a la necropsia, y al demostrar la presencia de huevecillos en las heces fecales. Este artículo detalla un protocolo para la toma de muestras de materia fecal para diagnosticar parásitos intestinales en poblaciones porcinas.

Résumé - Technique modifiée pour le prélèvement et la manipulation de matériel fécal pour le diagnostic de parasites intestinaux chez le porc

Les parasites intestinaux du porc sont encore présents malgré les méthodes modernes de conduite d'élevage et peuvent avoir un impact significatif sur le taux de croissance et l'efficacité alimentaire. Le diagnostic des parasitoses intestinales peut être accompli par la démonstration de parasites adultes dans les fèces ou à la nécropsie, et en démontrant la présence d'œufs dans les fèces. Le présent article fait état d'un protocole pour échantillonner du matériel fécal et pour diagnostiquer les parasites intestinaux dans les populations porcines.

Intestinal parasites of swine are still a concern in modern swine production and cause ill thrift, reduced growth rates, decreased feed efficiency, respiratory distress, and condemnations of livers and carcasses at harvest.[1-3] Fecal-flotation methods for the diagnosis of intestinal parasites are commonly used in many areas of veterinary medicine and can be easily implemented for swine in any clinic or laboratory.[4] Detecting the presence of intestinal parasites in small animals using the Modified Wisconsin Sugar Centrifugal-Flotation Method is the most sensitive and accurate method of detecting parasite eggs.[5] While similar comparative work has not been done for the parasites of swine, the Modified Wisconsin Sugar Centrifugal-Flotation Method is a commonly used diagnostic protocol for swine fecal samples. The eggs of *Ascaris suum* (large roundworm), *Trichuris suis* (swine whipworm), *Strongyloides ransomi* (intestinal threadworm), *Oesophagostomum dentatum* (nodular worm), *Metastrongylus* spp (lung worm), *Stephanurus dentatus* (kidney worm, eggs in urine samples only), and *Hyostongylus rubidus* (red stomach worm), and the oocysts of *Isospora suis* (coccidosis), can be observed using this flotation method.[4,6] In addition, flotation methods can be used to monitor the effectiveness of deworming protocols used in swine populations.

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Purpose

In preparation for an extensive parasite survey within a large integrated swine-production system, the authors made adjustments to the Modified Wisconsin Sugar Centrifugal-Flotation Method protocol in order to streamline the collection, transport, processing, and disposal of a large volume of samples. Although the process was altered, the fundamentals of the process were maintained; therefore, no changes in the sensitivity or specificity of the test are expected. The amount of feces tested is the same; only the collection process and the physical location of sample collection was changed. Instead of collecting a large volume of feces from the farm and returning to the laboratory where the desired amount of sample would be partitioned, the desired sample size was taken at the farm. This reduced excess transport, storage, and disposal of unused portions of the feces. In addition, this made the sample size consistent across samplings. The concentrated sugar solution used for the process is of the same specific gravity as that indicated in the Modified Wisconsin Sugar Centrifugal-Flotation Method, and a consistent amount of solution was utilized for each of the sample

types. Centrifugation force and time are consistent with the originally described modified Wisconsin method, as are the times allowed for flotation. Lastly, the amount of feces, volume of solution, size of centrifuge tubes, and method of evaluating eggs on cover slips are all in accordance with the modified Wisconsin method. The authors herein detail the Waverly Modified Wisconsin Sugar Centrifugal-Flotation Method of collecting and processing large numbers of fecal samples for the detection of intestinal parasites in swine.

Sample collection

In order to effectively evaluate a population of pigs for intestinal parasites, a level of confidence and expected prevalence must be determined before sampling is initiated.

Sampling the breeding herd

A 95% confidence level for detecting a 10% or greater prevalence (95-10) sampling scheme was used.[7] In our system, to meet these criteria, 30 samples were collected regardless of sow-herd size, which ranged from 500 to 3600 sows. This number of samples (30) should be appropriate for most sow herds. Sows were sampled 7 to 14 days postpartum, which has been shown in sheep, cattle, and goats to be associated with a periparturient rise in egg counts, likely due to parturition or lactation stress.[8-10] Although this has not been extensively studied in swine, it is a commonly recommended and utilized sampling time. If necessary, samples may be collected from lactating sows outside this range to achieve 30 total samples. A fresh 2-cc fecal core sample is taken from manure from each sow, using a modified disposable syringe. A new syringe must be used for each sample because of the potentially high numbers of eggs in an individual sample and the "sticky" nature of parasite eggs which could contaminate subsequent samples. This is especially a concern with the eggs of *A suum*.

Sampling the growing pigs

For growing-pig populations, we utilized a composite sampling method whereby one fresh 1-cc sample was taken from each of five separate fecal piles, totaling 5 cc, within a single pen within a nursery or finisher barn. The number of composites was based on the number of animals in the barn. A minimum of 10 composite samples were taken from barns with ≤ 800 animals. Twelve (12) composites were taken from barns with > 800 to 1200 animals. Above 1200 animals in a barn, one composite per 80 animals was added, up to a maximum of 24 composites per barn. In our system, most pens house approximately 25 pigs. By pen, this sampling represents a 95% confidence and 22% prevalence method, while by animal it represents a 95% confidence and 6% prevalence method.[7] The true prevalence detection rate is somewhere between 6% and 22%.

Syringe method for collecting fecal sample

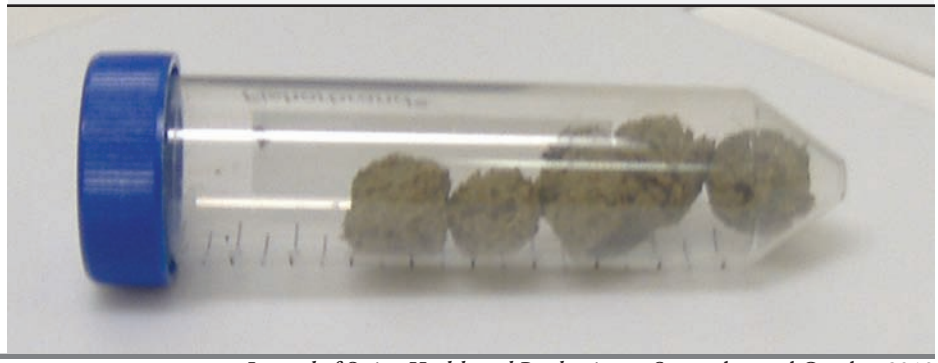
Common protocols describe collection of fecal samples by placing "golf ball" size or greater pieces in a plastic bag. This inevitably leads to variation in the amount of fecal material and the plastic container provided to the clinic or laboratory (eg, common field submissions are palpation sleeves filled with excessive feces). The authors developed a collection method that allowed a more consistent sample volume, provided a reduction in number of overall steps of the original protocol, and minimized the amount of material that needed to be handled, processed, and disposed of at the laboratory, which also reduced odor levels. To accomplish this, a 10-mL syringe was cut at the needle hub end using a knife, leaving a slight lip to prevent the plunger from pushing through the cut end (Figure 1). The modified syringe was then used to take a core sample of a determined volume from a fecal pile. Using this method, and depending on the density of the fecal material, each 1 cc of fecal material collected approximately equaled 1 gram.

50-mL conical centrifuge tube

Each core sample was then discharged into a 50-mL conical centrifuge tube and labeled with pertinent information (Figure 2). A single 2-cc core sample was taken from sows and five individual 1-cc core samples were used for the finisher composite. The 50-mL conical tube takes the place of the plastic bag commonly used for submission of fecal samples, providing only the required amount of fecal material for the flotation protocol. The tube also acts as the mixing container

Figure 1: Modified syringe collection device. Note the lip (arrow) that prevents the plunger from pushing through. Samples are collected by using the syringe to plunge down into a fecal pile, taking a core sample of various volumes determined by pre-setting the plunger to the volume setting. The flooring or underlying fecal material acts to push the sampled material into the syringe.

Figure 2: 50-mL conical centrifuge tube collection vessel with five 1-cc fecal plugs.



for the first laboratory step, thereby reducing additional steps and equipment.

Sample processing

The 50-mL tubes are returned to the laboratory, and the processing steps listed below are performed using the identified equipment (Figure 3).

Processing steps for fecal samples

1. A concentrated sugar solution (specific gravity 1.27) is added to the fecal samples directly into the 50-mL tubes.

   a. The concentrated sugar solution is prepared by slowly stirring 454 g of sugar into 355 mL of hot (but not boiling) water. This may be kept for several weeks in the refrigerator to prevent bacterial or fungal overgrowth.
   b. Fifteen mL of sugar solution is added to the 2-cc sow fecal samples and 20 mL to the 5-cc growing-pig fecal composites.

2. The fecal-sugar mixture is then homogenized by shaking the 50-mL tubes vigorously or by stirring with a wooden tongue depressor.

3. The entire fecal-sugar mixture is then strained through a tea strainer into a 5-oz waxed-paper cup.

4. The remaining liquid is pressed through the strainer using a wooden tongue depressor.

5. The strained contents are transferred into a 15-mL conical centrifuge tube.

6. The tube is centrifuged for 5 minutes at a *g* force of 145.4.

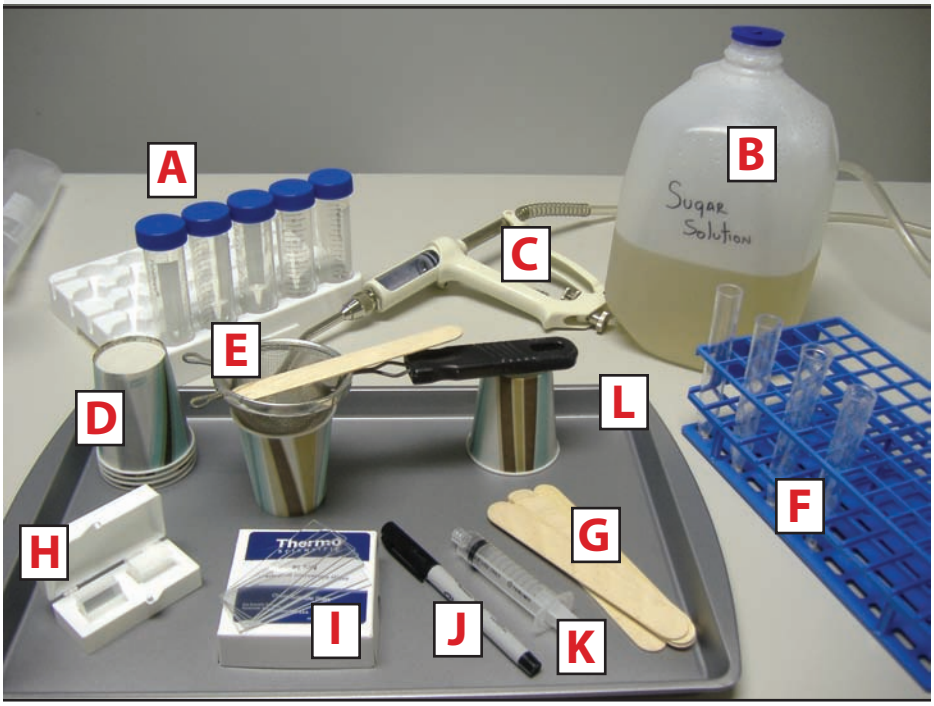
7. The tube is then removed from the centrifuge and placed in a test-tube rack. Sugar solution is again added to the tube until a slight meniscus forms above the top of the tube.

8. A 22 × 22-mm cover slip is placed on top of the meniscus, and allowed to sit in place for a minimum of 5 minutes.

9. The cover slip is removed and placed squarely on a microscope slide.

   a. Two cover slips can be placed on opposite ends of the same slide.
   b. Be sure that sugar solution is not transferred between cover slips, as eggs may be transferred.

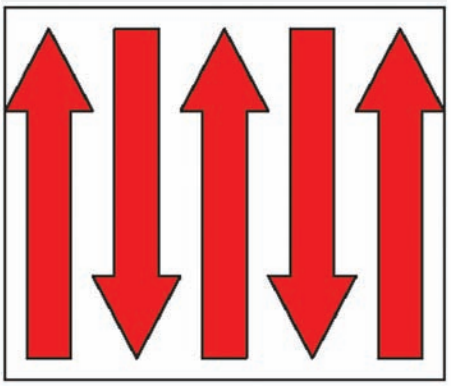
Figure 3: Supplies needed for fecal flotation (excluding centrifuge). A: 50-mL tubes with samples; B: sugar solution; C: drenching syringe for dispensing repeated large volumes of sugar solution; D: 5-oz paper cups; E: strainer; F: 15-mL centrifuge tubes with rack; G: wooden tongue depressors; H: 22 × 22-mm glass cover slips; I: microscope slides; J: permanent marking pen; K: syringe for dispensing small volumes of sugar solution; L: tray for holding completed slides.



Reading the slide

Once the cover slip has been placed on the microscope slide it can be read at any time, but be aware that the concentrated sugar solution may eventually crystallize. Slides should be read using a 10× eyepiece and a 4× objective (40× total magnification) on a binocular microscope with a movable stage, enabling easy evaluation of the entire cover slip. Start at one corner of the cover slip and move in a vertical plane, identifying and counting intestinal parasite eggs.[5] When the end of the first vertical plane is reached, move the field horizontally until you are in a new vertical plane. Continue this method until the entire cover slip has been read (Figure 4).The vertical motion of scanning causes less eye strain than horizontal motion. A total egg count is maintained (eg, with a hand counter) for each type of parasite egg seen on the slide. In addition, each cover slip is scored qualitatively on the basis of the total egg count: 0 eggs per cover slip = 0 (-); 1 to 10 eggs per cover slip = 1 (+); 11 to 49 eggs per cover slip = 2 (++); and ≥ 50 eggs per cover slip = 3 (+++). The score and total egg count can be recorded and written on the slide next to the evaluated cover slip with a permanent marking pen. These data can be used to estimate eggs per gram (EPG) of feces, knowing that each 1 cc of fecal material is approximately a gram, or actual measurement if fecal material is weighed prior to beginning the floatation process. Slides should be stored flat in a refrigerator to prevent bacterial and fungal growth, and can be kept in this fashion for several weeks before reading.

Figure 4: Diagram of cover-slip reading method for processing fecal flotation samples.



Diagnostic advantage

The described modified method should allow any veterinarian or veterinary clinic to perform in-house diagnostic testing for intestinal parasites. The modifications were made by the authors as part of a large-scale intestinal parasite survey, in an effort to standardize sample collection methods, minimize excess fecal material handled, and consolidate steps to allow for faster processing. The protocol provided clean, easy-to-read cover slips and allowed for accurate detection of *Ascaris suum* and *Trichuris suis* eggs in > 3000 samples tested to date.

Implications

- Intestinal parasites are still a concern for modern swine production.
- Fecal-flotation methods can be effectively used to diagnose swine intestinal parasites.
- The Modified Wisconsin Sugar Flotation Method (or the described "Waverly" modified method) is the most sensitive and accurate method available for diagnosis of intestinal parasites.
- Collecting and processing samples can be simplified by following the Waverly Modified Wisconsin Sugar Centrifugal-Flotation Method in a variety of settings.

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References

1. USDA:APHIS:VS. Use of Antimicrobials, Parasite Treatments, or Feed Additives in Weaned Pigs. Swine 2006 Part II: Reference of Swine Health and Health Management Practices in the United States, 2006. Fort Collins, Colorado: National Animal Health Monitoring System (NAHMS); 2007 August N471.1107. Available at: **http://www.aphis.usda.gov/vs/ceah/ncahs/nahms/swine.** Accessed 11 June 2010.

2. Roepstorff A, Nilsson O, O'Callaghan CJ, Oksanen A, Gjerde B, Richter SH, Örtenberg EO, Christensson D, Nansen P, Eriksen L, Medley GF. Intestinal parasites in swine in the Nordic countries: multilevel modeling of *Ascaris suum* infections in relation to production factors. *Parasitology.* 1999;119:521–534.

3. Stewart TB, Hoyt PG. Internal parasites. In: Straw BE, Zimmerman JJ, D'Allaire S, Taylor DJ, eds. *Diseases of Swine*. 9th ed. Ames, Iowa: Blackwell Publishing; 2006:901–914.

4. Bowman DD. Diagnostic parasitology. In: Bowman DD, ed. *Georgis' Parasitology for Veterinarians*. 9th ed. St Louis, Missouri: Saunders Elsevier; 2009:295–371.

5. Dryden MW, Payne PA, Ridley R, Smith V. Comparison of common fecal flotation techniques for the recovery of parasite eggs and oocysts. *Vet Ther.* 2005;6:15–28.

*6. Corwin RM. Diagnostic notes: Pig parasite diagnosis. *Swine Health Prod*. 1997;5:67–70.

7. Thrusfield M. *Veterinary Epidemiology*. 2nd ed. Cambridge, Massachusetts: Blackwell Science, Inc; 1995:187–188.

8. Courtney CH, Parker CF, McClure KE, Herd RP. A comparison of the periparturient rise in fecal egg counts of exotic and domestic ewes. *Int J Parasitol.* 1984;14:377–381.

9. Hammerberg B, Lamm WD. Changes in periparturient fecal egg counts in beef cows calving in the spring. *Am J Vet Res*. 1980;41:1686–1689.

10. Rahman WA, Collins GH. An association of faecal egg counts and prolactin concentrations in sera of periparturient Angora goats. *Vet Parasitol*. 1992;43:85–91.

* Non-refereed reference.