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Evaluation of an Inhouse Rapid ELISA Test for Detection of *Giardia* in Domestic Sheep (*Ovis aries*)

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Sheep (*Ovis aries*) are increasingly used at our institution as models of human disease. Within the research environment, routine husbandry and handling of sheep has potential for transmission of zoonotic agents, including *Giardia*. The prevalence of *Giardia* in sheep may approach 68%. Classic diagnostic testing involves microscopic examination for fecal cysts or trophozoites; however, limitations of microscopy include time, labor, and potential false-negative results due to intermittent shedding. We wished to determine whether a commercial rapid ELISA used for *Giardia* detection in dogs and cats could be used in sheep. Fecal samples collected from sheep (*n* = 93) were tested with a combination of 6 methods: reference laboratory fecal flotation, reference laboratory ELISA, inhouse fecal flotation, and commercially available tests (enzyme immunoassay, direct fluorescence antibody assay, and rapid ELISA). Prevalence of *Giardia* infection in facility sheep was 11.8% (11 of 93 animals). Of the 11 samples considered positive, 3 were confirmed by multiple testing methods, and 5 were positive by microscopy alone. Inhouse fecal flotation for 8 samples was positive on only 1 of 2 consecutive testing days. The rapid ELISA test exhibited 0% sensitivity for sheep giardiasis. Overall, the examined methods had low sensitivities and low positive predictive values. Despite limitations, microscopic analysis of repeat fecal samples remained the most accurate diagnostic method for ovine giardiasis among the methods tested.

Abbreviations: DFA, direct fluorescent antibody assay; EIA, enzyme immunoassay; IFA, immunofluorescent assay.

Giardia duodenalis (also known as *G. lamblia* and *G. intestinalis*) is the most commonly diagnosed intestinal parasite of humans and livestock species worldwide. In the United States, it is estimated that as many as 2.5 million cases of giardiasis occur annually.[7] *Giardia* exists as 2 morphologic forms, trophozoites and cysts. The cysts are responsible for transmission and environmental survival of *Giardia*, with infected subjects shedding as many as 10 million cysts per gram of feces. Infection can occur after ingestion of as few as 10 cysts.[6] The majority of giardiasis cases can be traced to fecal–oral contact or to ingestion of food or water contaminated with *Giardia* cysts. There is increasing evidence that *Giardia* is zoonotic and can be transmitted from animals to humans.[2,6,10,15,19,24-26,28]

The prevalence of *G. duodenalis* infection in livestock may be as high as 38% in adult sheep and 68% in lambs.[2,3,20,24,25] Many of these animals are asymptomatic carriers but may shed large numbers of cysts into the environment.[3] *G. duodenalis* can be separated genetically into 7 distinct genotypes (also called 'assemblages'). Only assemblages A and B have been detected in humans and other mammalian hosts, whereas the other assemblages (C through G) appear to be host-specific. Although assemblages A and B are not the most common genotypes to infect sheep, they do occur in this frequently used laboratory species and therefore may pose a risk to human health.[1,3,24-26,28] Due to the nature of many chronic in vivo research studies, care of sheep requires continuous daily contact with members of veterinary, husbandry, and research staff. In this research environment, it is possible that animals can serve as reservoirs of zoonotic pathogens.

In the animal housing facility, sheep runs are sanitized at least once daily, with the entire room completely disinfected on a scheduled interval. Animal rooms typically are constructed of materials that are nonpermeable, resulting in a more readily sanitized environment than that of free-range sheep herds. *Giardia* cysts are inactivated effectively with quaternary ammonia and steam, but there have been mixed reports of the effectiveness of bleach and iodine and reports of resistance to alcohol-based products.[13] Due to biomedical research demand, there is often a dynamic population of sheep within research facilities, with high rates of animal turnover. There is a concomitant increase in sanitation effort, resulting in a damp environment (flooring, walls, and others), where *Giardia* cysts may persist. Environmental persistence of cysts can lead to infections that are undetected during the quarantine period or that develop after quarantine release, as well as potential reinfection of animals that had previously been diagnosed and treated. Within animal facilities, personal protective equipment (hairnet, face mask, gown, shoe covers, and gloves) is worn routinely to shield research animals from infectious agents carried by personnel, as well as to safeguard personnel from exposure to zoonotic agents and allergens carried by animals.

Historically, the most trusted diagnostic test for giardiasis has been visual examination of feces or intestinal tissue samples for cysts or trophozoites. Microscopic examination after fecal flotation for *Giardia* is most sensitive when multiple samples are assessed within a 3- to 5-d interval, because of the intermittency of cyst shedding.[16,17] However, because cyst excretion occurs irregularly, false-negative results may be common at the time of microscopic exam. Fecal flotation exams typically

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are time-consuming, in that they require appropriate methods of preparation, as well as sufficient operator experience or appropriate training, for differentiation of *Giardia* cysts from other protozoan cysts. Other available methods to diagnose giardiasis in both humans and animals include ELISA and enzyme immunoassays (EIA). These tests detect different soluble antigens dispersed in fecal matter rather than detecting cysts, trophozoites, or antigens on the surfaces of these morphologic forms. Direct fluorescence antibody assays (DFA) and PCR-based tests can also be used to diagnose infection. These methods also can be time-consuming and require training and experience to perfect, resulting in drawbacks similar to those for fecal flotation. The described testing methods can be performed by contract diagnostic laboratories; however, this option inherently creates additional time delays (at minimum, 2 to 3 d after sample submission) before results are made available and appropriate responses can be instituted.

The need for rapid and cost-effective methods for diagnosis of *Giardia* in domesticated animal species (for example, dogs and cats) has led to the development of ELISA tests that can be purchased commercially and performed on-site within a facility (inhouse). These tests are simple to perform and, by design, require minimal personnel training for proficiency. The rapid ELISA that we elected to study is reported by the manufacturer to be up to 92% sensitive and 99% specific when compared with a reference lab ELISA and 90% sensitive and 96% specific when compared with DFA tests.[11] Various EIA have been found to be more sensitive than microscopy in detecting *Giardia* when testing only a single fecal sample, although these assays detect a different antigen than that of the aforementioned rapid ELISA.[16] The time delay from sample collection to deliverable test results for the EIA, DFA, and PCR methods renders these testing options less desirable than the rapid (8 min) ELISA performed inhouse.

At our institution, sheep are quarantined from use in research studies until diagnostic test results are determined to be negative for a select panel of pathogens, including *Giardia* testing by both ELISA and fecal flotation at a reference laboratory. The ELISA used by the reference laboratory is unique to that site and is described as ensuring at least 90% accuracy; however, data are not available for test sensitivity or specificity.[22] Prior to the current study, within a designated period of 6 mo during which both aforementioned diagnostic methods were used, *Giardia* was identified in 13.5% of facility sheep by ELISA, with 70% of those concurrently positive for *Giardia* by using microscopy of fecal samples. This pilot analysis confirmed that there was a discrepancy between the ELISA and microscopy results.

With the knowledge that the rapid ELISA is specific for the genus *Giardia* in dogs and cats, we hypothesized that this test would be sensitive and specific for detecting this potentially zoonotic parasite in sheep. We wanted to compare this commercial test method with classic methods to determine whether the rapid ELISA was an effective and reliable *Giardia* test for sheep used in biomedical research. Our intent was to expedite diagnosis and initiation of appropriate treatment for *Giardia* and ultimately minimize further dissemination of infectious cysts into the research environment. This is the first study to evaluate efficacy of the rapid ELISA test for detection of ovine giardiasis in the biomedical research environment.

Materials and Methods

Humane care and use of animals. All fecal samples were collected from male Dorset-cross sheep ($n = 93$; age, approximately 4 mo; weight, approximately 30 kg) selected for enrollment into research protocols approved by the University of Pennsylvania Institutional Animal Care and Use Committee. Facilities housing these animals were AAALAC-accredited.

Housing and husbandry. Sheep were purchased from a commercial vendor, where they were group-housed on various types of contact bedding, such as pine shavings, straw, and hay. Prior to shipment from the vendor, all animals were treated prophylactically with ivermectin (30 mg SC, given once) and albendazole (1136 mg PO, given once). Selectively, prophylactic amprolium (96 mg/5 lb body weight PO for 5 consecutive days) and fenbendazole (1000 mg PO for 3 consecutive days) were given to 81% and 56% of the animals, respectively, based on parasites that were being observed in the vendor's colony at the time. On arrival at our facility, all animals were examined by the veterinary staff and deemed clinically healthy. Feces and blood (10 mL from the jugular vein) were collected from each animal to screen for intestinal parasites and *Coxiella burnetii*, respectively. The sheep were housed individually in raised floor runs with fiberglass slatted flooring (Britz and Company, Wheatland, WY) in rooms housing as many as 8 sheep. Holding runs were cleaned and floors scrubbed with water daily. Every 2 wk, the runs were disinfected with a quaternary ammonia product and power-washed. On a semiannual basis, the room, including runs and flooring, was foamed with a degreasing agent. Animals were fed approximately 750 g of pellets (Purina LabDiet 5508 Rumilab, PMI Nutrition International, St Louis, MO) twice daily, along with alfalfa cube supplementation and were provided water ad libitum by means of an automatic watering system.

Study design. Collections from each sheep were obtained on 2 consecutive days and were designated either day 1 or day 2. Fecal samples (approximately 16 g) were collected on day 1 manually from the rectum of all animals and divided for *Giardia* testing with up to 6 different methods: fecal flotation at a reference laboratory and *Giardia* ELISA developed for a reference laboratory (Antech Diagnostics, Lake Success, NY); fecal flotation with centrifugation performed inhouse; a commercially available EIA (ProSpecT *Giardia* Microplate Assay, Alexon, Sunnyvale, CA; performed by Marshfield Laboratories, Marshfield, WI), a DFA (Merifluor *Cryptosporidium–Giardia* Direct Immunofluorescent Assay, Meridian Diagnostics, Cincinnati, OH; performed by Marshfield Laboratories), and an inhouse rapid ELISA *Giardia* test (SNAP *Giardia*, IDEXX, Westbrook, ME). Day 2 samples consisted of an additional fecal sample (approximately 5 g) tested by inhouse fecal flotation with centrifugation only.

After testing of the initial cohort of animals ($n = 11$) by using the reference laboratory tests (fecal floatation and ELISA) as compared with the rapid ELISA, a discrepancy in agreement between test results was recognized. At that point in the experiments, we determined that inclusion of additional diagnostic tests would be useful to improve interpretation of results. Therefore, the commercial EIA and DFA tests were added to the panel of diagnostic tests, resulting in a variation in animal numbers among testing groups. During the experimental period, the availability of the rapid ELISA test became unexpectedly limited due to commercial demand, resulting in fewer sheep ($n = 62$) tested by this method.

Reference laboratory ELISA and fecal flotation (n = 93). Fecal samples (approximately 5 g) were submitted to a reference laboratory for *Giardia* ELISA and fecal flotation. The fecal flotation was performed by using $ZnSO_4$ solution with centrifugation. The ELISA test was developed specifically for the reference labo-

ratory. Both tests were performed within the same department, and the order in which the tests were performed varied.

Inhouse fecal flotation with centrifugation (n = 93). Fecal samples (approximately 5 g) were mixed with 33% $ZnSO_4$ solution (15 mL) and strained into 15-mL conical centrifuge tubes. Tubes then were spun in a swinging-bucket benchtop centrifuge (TRIAC Centrifuge, Clay Adams, Parsippany, NJ) at 880 × *g* for 5 min. Approximately 2 min after centrifugation, an inoculation loop was used to collect a small sample of the fecal mixture from the meniscus of the solution. Samples were placed on a microscope slide with Lugol iodine, and a cover slip was placed on the sample. The slide was examined at 40× power for the presence of *Giardia* cysts. This test was repeated in duplicate from 2 distinct fecal samples (per animal) collected on 2 consecutive days in an attempt to detect cysts during intermittent shedding. The same operator read all slides and was trained by a veterinary parasitologist (Ryan Veterinary Hospital, University of Pennsylvania).

EIA and DFA (n = 82 and n = 71, respectively). Fecal samples (approximately 5 g) were placed in 10% buffered formalin solution and shipped to Marshfield Laboratories for evaluation by using commercially available EIA (ProSpecT *Giardia* Microplate Assay, Alexon) and DFA (Merifluor *Cryptosporidium–Giardia* Direct Immunofluorescent Assay, Meridian Diagnostics). Each test was run in parallel with positive and negative control samples.

Inhouse rapid ELISA (n = 62). Fecal samples (approximately 1 g) were handled according to instructions provided with the rapid ELISA (SNAP *Giardia*, IDEXX), except that the fecal material was pulverized initially and mixed with a small amount of distilled water to create a slurry. The provided swab was then coated thoroughly with a thin layer of the fecal slurry. The swab was replaced in the provided tube, the stem within the reagent bulb was broken, and the conjugate was passed through the swab tip 3 times; subsequently, 5 drops of the conjugate–sample solution was placed into the sample well of the commercial device. The sample was allowed to flow across the result window and reach the activation circle, at which time the activator button was pressed. The tests were incubated at room temperature for 8 min before they were read. Samples were considered positive when the 'positive control' spot was present and the sample spot was a darker color than the 'negative control' spot, which serves as a safeguard against false positives. If the color on the negative-control spot was darker than that of the sample spot, the test was considered invalid. For purposes of this study, this type of test result was classified as 'unsatisfactory.'

Data analysis. Sensitivity, specificity, positive predictive values, and negative predictive values were calculated for each test. Sensitivity is the proportion of actual positives that were identified correctly as positive. Specificity is the proportion of actual negatives that were identified correctly as negative. The positive predictive value is the proportion of positive test results that were identified correctly, and the negative predictive value is the proportion of negative test results that were identified correctly. All of these values are determined by comparing results to those from a classically accepted test. In the present study, the accepted test used for comparison was that of inhouse fecal flotation.

Results

Sample results were considered true positives if at least one inhouse fecal flotation identified the presence of at least one *Giardia* cyst or trophozoite during microscopic examination. Therefore, according to these criteria for a true positive result, the prevalence of *Giardia* infection in sheep entering our facility was 11.8% (11 of 93 animals). The rapid ELISA test, labeled for *Giardia* detection in dogs and cats, was found to have a sensitivity of 0% for sheep giardiasis. Nine samples tested positive by this test, but all also showed a negative control spot that was darker than the sample spot and therefore were categorized as unsatisfactory tests. No single true-positive rapid ELISA test result was obtained during this study, even for those sheep deemed to yield true positive results by microscopy. Of those fecal samples considered true positives, only 3 of 11 were confirmed positive by multiple testing methods (all described tests, except the rapid ELISA), and 5 of 11 were positive only by microscopy (Table 1). Inhouse fecal flotation found 8 of 11 samples that were positive on only one of the day 1 and day 2 samples; 9 of 11 samples were positive by inhouse fecal flotation on day 1. Comparison of the 5 tests with inhouse fecal flotation, considered the classically accepted test for this study, led to test sensitivities ranging from 0% to 40% and test specificities ranging from 82.2% to 100% (Table 2). Positive predictive values ranged from 0% to 100%, and negative predictive values ranged from 90.4% to 92.8%.

Discussion

The goal of this study was to determine whether a commercially available rapid ELISA test, effective for detection of *Giardia* in dogs and cats, could diagnose the presence of *Giardia* in sheep used in biomedical research programs. The overall proposed outcome of this work ultimately was to expedite treatments and reduce the amount of time any sheep would be shedding potentially infectious cysts into the research housing environment. Unequivocally, the rapid ELISA test was not a reliable method for diagnosis of *Giardia* in sheep and did not confirm any single true-positive animal. If used in sheep, this rapid ELISA might result in an inaccurate assumption that all tested animals are negative and that research herds are free of *Giardia*. Overall, we were unable to identify any single test mechanism that best fit our study objectives for rapid diagnosis of *Giardia*.

The EIA used in this study detects the GSA65 antigen that is associated with the *Giardia* cyst wall and trophozoite. Reports of the success of this EIA for giardiasis detection in cats, dogs, cattle, and white-tailed deer have not included documented efficacy for sheep.[4,5,9,18,21,27] The DFA had previously been used to detect the prevalence of *Giardia* in lambs.[8] The rapid ELISA *Giardia* test detects a soluble or 'free-floating' antigen, not associated with the cyst wall or trophozoite. We presumed that the rapid ELISA test would be superior for *Giardia* detection in sheep treated prophylactically with antiparasitics, due to likely destruction of organisms and release of antigen; however this supposition was not found to be the case. In light of the overall results, the rapid ELISA test likely detects only antigen specific to the canine and feline assemblage of *Giardia* and does not detect assemblages from sheep. Recent work has indicated that a PCR-based assay is a highly sensitive test for *Giardia*, with detection of twice as many positive samples as compared with microscopy.[25] Although not evaluated in our current study, PCR also may be an option for detection of *Giardia* in sheep.[25] At our facility, PCR screening for animals entering the facility, in the absence of any overt clinical gastrointestinal disease, was viewed as an unjustifiable financial expenditure.

Overall, the examined diagnostic methods had low sensitivities and low positive predictive values, compared with the inhouse fecal float, when testing sheep feces for *Giardia*. As anticipated, testing likely was complicated by intermittent fecal shedding of the organism, leading to false negative test results if the organism was not shed on either of the 2 consecu-

Table 1. Comparison of test results

Inhouse fecal flotation	Reference laboratory														
	Fecal flotation			ELISA			Rapid ELISA			EIA			DFA		
	+	–	Total	+	–	Total	+	–	Total	+	–	Total	+	–	Total
Positive	4	7	11	4	7	11	0	5	5	4	6	10	3	5	8
Negative	1	81	82	1	81	82	9[a]	48	57	13	59	72	0	63	63
Total	5	88	93	5	88	93	9[a]	53	62	17	65	82	3	68	71

[a]Tests in which the negative control spot on the rapid ELISA was darker in color than the sample spot, thereby rendering the results unsatisfactory

Table 2. Characteristics of tests relative to the inhouse fecal flotation test

	Reference laboratory				
	Fecal flotation	ELISA	Rapid ELISA	EIA	DFA
Sensitivity (%)	36.4	36.4	0	40	37.5
Specificity (%)	98.8	98.8	84.2	81.9	100
Positive predictive value (%)	80.0	80.0	0	23.5	100
Negative predictive value (%)	92.0	92.0	90.5	90.7	92.6

tive days of testing. The inhouse fecal flotation was performed on 2 separate samples on 2 consecutive days, whereas all other tests were performed by using only the day 1 fecal sample, potentially introducing a variable in the study. However, the tests recognizing antigen should not have been affected by testing only a single sample, assuming that antigen should be present regardless of whole cyst detection; therefore, only the reference laboratory fecal floatation test comparison may have been biased by our study design. With the expectation that intermittent cyst shedding warrants testing of multiple fecal samples over multiple days to increase accuracy of detection, we decided that the inhouse fecal flotation should be performed on consecutive days in an attempt to overcome the limitations of intermittent shedding.[16,17] By testing animals on 2 consecutive days, we were able to diagnose giardiasis in 2 additional sheep that were not detectably shedding the organism on the first day of testing.

Testing samples by multiple methodologies may have led to false positives on the antigen tests compared with flotation with centrifugation, if any present cysts were destroyed, releasing the antigen and making whole organisms undetectable by microscopy. This situation could have occurred after the vendor prophylactically treated the sheep with ivermectin, albendazole, or fenbendazole, all of which have been used for the treatment of giardiasis.[12,14,23,29] Of the 11 sheep that were considered positive for *Giardia* by inhouse fecal flotation, only one was observed to have diarrhea, a clinical sign that could have been associated with protozoal infection. However, this sheep was found to be infected also with *Cryptosporidium* by DFA, and these clinical signs were unlikely to be due to giardiasis, which is most often subclinical in adult sheep.[3] In addition, another 2 sheep of the 93 tested were observed to have diarrhea and yet tested negative for all intestinal parasites. The observations in this study, in particular that most sheep positive for *Giardia* had no clinical gastrointestinal abnormalities and that certain animals with diarrhea did not test positive for any parasites, reinforce the inherent difficulty in clearly identifying true positive infections, regardless of diagnostic test used.

For the diagnostic *Giardia* tests that were compared in this study, microscopic examination, following fecal flotation and centrifugation, was the most consistent method for identification of true positive infections. Therefore, for the best likelihood of detecting *Giardia duodenalis* in sheep, we support the continued use of direct microscopy of multiple fecal samples collected over 2 d or more. Despite the low prevalence of *Giardia* infection in sheep at our facility, the requisite close contact between sheep and their human caregivers in the research environment warrants continued investment in diagnostics and treatment of *Giardia* to mitigate both spread to other animals and risk of zoonotic transmission to personnel.

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