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Source: Journal of Wildlife Diseases, 44(4) : 992-998

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-44.4.992

Journal of Wildlife Diseases, 44(4), 2008, pp. 992–998

Detection of *Lawsonia intracellularis* by Real-time PCR in the Feces of Free-living Animals from Equine Farms with Documented Occurrence of Equine Proliferative Enteropathy

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ABSTRACT: The objective of this study was to determine whether *Lawsonia intracellularis* was present in the feces of free-living animals collected on two equine premises with documented occurrence of equine proliferative enteropathy (EPE). Fresh feces from black-tailed jackrabbits (*Lepus californicus*, $n=100$), striped skunks (*Mephitis mephitis*, $n=22$), feral cats (*Felis catus*, $n=14$), Brewer's Blackbirds (*Euphagus cyanocephalus*, $n=10$), Virginian opossums (*Didelphis virginiana*, $n=9$), raccoons (*Procyon lotor*, $n=4$), California ground squirrels (*Spermophilus beecheyi*, $n=3$), and coyotes (*Canis latrans*, $n=2$) were collected from August 2006 to January 2007 either from the ground while walking the premises or after trapping the animals using live traps. Nucleic acid purified from feces was directly processed for polymerase chain reaction (PCR) analysis using a real-time PCR assay targeting the aspartate ammonia lyase gene of *L. intracellularis*. Purified DNA samples were also precipitated, preamplified for *L. intracellularis*, and analyzed using the same real-time PCR assay, to increase the detection limit to one *L. intracellularis* organism per extracted sample. Feces from jackrabbits, striped skunks, Virginian opossums, and coyotes tested PCR positive for *L. intracellularis*, whereas all feces from feral cats, Brewer's Blackbirds, raccoons, and ground squirrels tested PCR negative for *L. intracellularis*. PCR testing on DNA extracted directly from feces was positive for *L. intracellularis* in six of 164 fecal samples. When DNA purification from feces was followed by a precipitation and preamplification step, five additional fecal samples tested PCR positive for *L. intracellularis* (11/164). The largest number of PCR positive *L. intracellularis* fecal samples was observed in striped skunks, followed by Virginian opossums, jackrabbits, and coyotes. This is the first description of *L. intracellularis* in these four species. Because the fecal samples were collected at equine farms with confirmed cases of EPE, striped skunks, Virginian opossums, jackrabbits, and coyotes may act as potential sources of infection to susceptible weanlings.

Key words: Equine proliferative enteropathy, feces, free-living animals, *Lawsonia intracellularis*, PCR, real-time polymerase chain reaction.

Lawsonia intracellularis is an obligate intracellular bacterium that causes proliferative enteropathy in a variety of domestic and wild animals (Lawson and Gebhart, 2000). In horses, the second most commonly affected domestic animal after pigs, the disease is known as equine proliferative enteropathy (EPE; Lavoie and Drolet, 2007). Equine cases of EPE have been reported from North America (United States and Canada), Europe (Great Britain, Belgium, and Switzerland), and Australia. The occurrence of EPE is often sporadic, and the age of affected foals is generally 4 to 7 mo. Affected weanlings commonly show rapid weight loss, lethargy, fever, subcutaneous edema due to hypoproteinemia, diarrhea, and colic (Lavoie and Drolet, 2007). The epidemiology of EPE has remained poorly investigated, and the transmission of infection in foals may occur through the ingestion of feed or water contaminated with *L. intracellularis*-infected feces from feral or domestic animals.

Due to the inability to culture *L. intracellularis* from fecal material, documentation of infection or exposure to *L. intracellularis* in a susceptible animal relies on the detection of *L. intracellularis*-specific antibodies in peripheral blood or on the detection of *L. intracellularis* DNA in feces (Lawson and Gebhart,

2000). The latest approach has been successfully used for the epidemiologic study of fecal shedding of *L. intracellularis* in domestic and wild pigs, calves, dogs, mice, wolves, foxes, red deer, fallow deer, white-tailed deer, and hedgehogs (Drolet et al., 1996; Herbst et al., 2003; Tomanová et al., 2003; Stege et al., 2004; van der Heijden et al., 2004; Bednar, 2006; Dezorzova-Tomanová et al., 2006; Feary et al., 2006). To determine the potential source of infection in foals, we evaluated feces collected from free-living animals for the detection of *L. intracellularis* by real-time polymerase chain reaction (PCR).

Study material was collected from two equine premises located in California. The premises were selected based on documented clinical cases of EPE (presence of clinical and clinicopathologic findings compatible with EPE, positive antibody titer against *L. intracellularis* by immunoperoxidase monolayer antigen assay, and molecular detection of *L. intracellularis* in feces by real-time PCR). The first premises (farm 1: 38°9′N, 121°41′W), a Percheron breeding farm with 190 resident horses housed on 8.1 ha, was visited several times over a period of 6 mo (August 2006 to January 2007) following the diagnosis of one weanling with EPE. The second premises (farm 2: 34°36′N, 120°4′W), a thoroughbred breeding farm with 300 resident horses housed on 16.2 ha, was visited twice (October and December 2006) after the diagnosis of six weanlings with EPE. The objective of each visit was to collect fecal samples from feral animals for the detection of *L. intracellularis*.

Fresh feces from free-living animals were collected either on the ground while walking the premises or after trapping the animals using live traps (Tomahawk Live Traps, Tomahawk, Wisconsin, USA). The traps were baited with dried fruits and left open overnight for 12 hr (from 6:00 PM to 6:00 AM). The trapping protocol was in compliance with the regulation for scientific trapping from the California Department of Fish and Game. After determining its species, the trapped animals were released unharmed, and feces dropped in the cage during the confinement period were collected. Specific characteristics such as size, weight, color, and markings were recorded to individually characterize captured animals (i.e., document single or multiple samples from the same animal). Additional fresh feces from free-living animals were collected from paddocks and pastures where horses were kept, and the species was determined according to the characteristic appearance of the feces. To increase individual fecal samples for a specific species and avoid sampling the same animal, collected feces had to be at least 46 m apart. All fecal samples were collected in separate cups, with the exception of bird samples, which were pooled to 10 single droppings per collection site. To prevent potential cross-contamination between fecal samples, separate disposable gloves were worn for each collected sample. In total, 164 samples (Table 1) were collected from farms 1 and 2, including 100 samples from black-tailed jackrabbits (*Lepus californicus*), 22 samples from striped skunks (*Mephitis mephitis*), 14 samples from feral cats (*Felis catus*), 10 pooled samples from Brewer's Blackbirds (*Euphagus cyanocephalus*), nine samples from Virginian opossums (*Didelphis virginiana*), four samples from raccoons (*Procyon lotor*), three samples from California ground squirrels (*Spermophilus beecheyi*), and two samples from coyotes (*Canis latrans*).

Fecal samples collected in the field were kept refrigerated at 4 C until processed for nucleic acid purification within 48 hr of collection. Two milliliters of phosphate-buffered saline (PBS) were added to 1 g of feces in a conical tube. Thereafter, each sample was vortexed for 10 sec and centrifuged at 13,000 × G for 1 min. To minimize contamination, all pipetting steps were performed in a laminar flow cabinet. Nucleic acid purification from 180 μl of supernatant fluid

TABLE 1. Fecal samples collected from free-living animals from two equine premises with sporadic occurrence of equine proliferative enteropathy.

Animal species	Collection method	No. of samples farm 1/farm 2
Black-tailed jackrabbit (*Lepus californicus*)	Ground collection	100/0
Striped skunk (*Mephitis mephitis*)	Trapping	22/0
Feral cat (*Felis catus*)	Ground collection	14/0
Brewer's Blackbird (*Euphagus cyanocephalus*)	Ground collection	0/10[a]
Virginian opossum (*Didelphis virginiana*)	Trapping	9/0
Raccoon (*Procyon lotor*)	Trapping	4/0
Ground squirrel (*Spermophilus beecheyi*)	Ground collection	0/3
Coyote (*Canis latrans*)	Ground collection	1/1
Total		150/14

[a] Each sample represents a pool of 10 bird droppings.

was performed using an automated nucleic acid extraction system (CAS-1820 Xtractor Gene, Corbett Life Science, Sydney, Australia) according to the manufacturer's recommendations.

To detect as few as one *L. intracellularis* organism from the processed fecal samples, each purified DNA sample underwent an additional precipitation and PCR preamplification step. DNA was precipitated using 100 µl of purified sample, 6 µl of 5 M NaCl (Applied Biosystems/Ambion, Foster City, California, USA), 10 µl of 5 mg/ml glycogen (MBI Fermentas Inc., Glen Burnie, Maryland, USA), and 300 µl of absolute ethanol (Glod Shield Chemical Co., Hayward, California, USA) as described previously (Baumgarth et al., 2004). The mixture was inverted in a 1.5-ml microcentrifuge tube several times and stored at −20 C overnight. The tubes were then centrifuged at 4 C at 16,000 × G for 15 min. The supernatant was removed, and 200 µl of 70% ethanol was added to the pellet and resuspended. The last step was repeated, followed by drying the pellet at room temperature for 5–10 min. Finally, the pellet was resuspended in 20 µl of water and stored at −20 C until analysis. Precautions taken to minimize contamination during the precipitation and preamplification steps included performing all pipetting steps in a laminar flow cabinet and including positive (DNA from cell grown *L. intracellularis*) and negative (*L. intracellularis*-free DNA from fecal samples) DNA controls. Furthermore, swabs were taken from centrifuges, laminar flow cabinets, and countertops and assayed for the aspartate ammonia lyase gene of *L. intracellularis* by real-time PCR to assess potential contamination.

Preamplification was done using the Advantage 2 Polymerase Mix (Clontech Laboratories, Inc., Mountain View, California, USA) containing the target primers (forward primer AATTTGTTGTGGATTGTATTCAAGGA and reverse primer CTTTCTCATGTCCCATAAGCTCAA) for the *L. intracellularis* aspartate ammonia lyase gene in a 50-µl total volume as follows: each reaction contained 5 µl of 10× buffer, 1 µl of 10 mM dNTPs, 1 µl of Advantage 2 Polymerase, 1 µl of target primers at 10 pmol, 20 µl of DNA, and 21 µl of water. The primer mix was amplified in a DNA Engine Dyad thermocycler (Peltier Thermal Cycler, Bio-Rad Laboratories, Hercules, California, USA), with the following conditions: 1 min at 94 C, 25 cycles of 15 sec at 94 C, 15 sec at 55 C and 40 sec at 70 C, followed by 5 min at 70 C.

All samples (primary DNA extraction and precipitated and preamplified DNA) were assayed for the presence of the aspartate ammonia lyase gene of *L. intracellularis* by real-time TaqMan PCR. Briefly, the real-time TaqMan PCR assay

used is based on the detection of a specific 104-base pair product of the aspartate ammonia lyase gene of *L. intracellularis* (GenBank accession no. AM180252; oligonucleotides: forward primer AATTTGTTGTGGATTGTATTCAAGGA, reverse primer CTTTCTCATGTCCCATAAGCTCAA, and probe 6FAM-CAGGGACAAGTACAAATATGAATGCTAATGAAGCAA). The samples were amplified in a combined thermocycler/fluorometer (7900 HTA, Applied Biosystems), with the following standard thermal cycling protocol: 2 min at 50 C, 10 min at 95 C, and 40 cycles of 15 sec at 95 and 60 sec at 60 C. Amplification efficiency of the aspartate ammonia lyase gene gene assay was calculated from the slope of a standard curve generated on 10-fold diluted *L. intracellularis*-positive DNA sample. High-amplification efficiency for the target gene of 95% indicated a high analytical sensitivity. Detection limit of the assay was determined by using 10-fold dilutions of *L. intracellularis* derived from cell culture in McCoy cells (mouse fibroblast cells) added to PBS or to *L. intracellularis*-free equine feces, respectively. Bacterial numbers were assessed by direct counting microscopically after indirect immunoperoxidase staining using *L. intracellularis*-specific antibody. The samples were then prepared as described previously. Extraction and amplification of the dilutions were run in triplicate. The detection limit for the assay was four and 40 *L. intracellularis* organisms, when the dilution samples were purified from PBS and feces, respectively. Analytical sensitivity was one *L. intracellularis* organism after DNA precipitation and PCR preamplification independently of the substrate used (PBS versus feces). Analytical specificity was verified by sequencing TaqMan PCR products for the target gene and using positive (DNA from cell grown *L. intracellularis*) and negative controls (*L. intracellularis*-free DNA from fecal samples) with each run. Prevalence of *L. intracellularis* PCR-positive feces was determined after both PCR protocols. Exact prevalence was determined for animal species with individual animal identification or when only one fecal sample tested PCR positive, whereas prevalence range was determined for feces collected from animal species with no individual animal identification.

Feces from jackrabbits, striped skunks, Virginian opossums, and coyotes tested PCR positive for *L. intracellularis*, whereas all feces collected from feral cats, Brewer's Blackbirds, raccoons, and ground squirrels tested PCR negative for *L. intracellularis* (Table 2). The PCR testing on DNA extracted directly from feces was positive for *L. intracellularis* in six of 164 fecal samples. When DNA purification from feces was followed by a precipitation and preamplification step, five additional fecal samples tested PCR positive for *L. intracellularis* (11/164). The largest number of PCR positive *L. intracellularis* fecal samples was observed in striped skunks, followed by Virginian opossums, jackrabbits, and coyotes. With the exception of the PCR-positive coyote, all other PCR-positive animals originated from farm 1. The exact prevalence was 50% for coyote and 22% for Virginian opossums, whereas the prevalence for striped skunks and jackrabbits ranged from 4.5% to 22% and from 1% to 2%, respectively. Prevalence range for the last two animal species was determined based on the inability to identify individual animals.

Epidemiologic studies determining the exposure rate of free-living animals to *L. intracellularis* are hampered by the lack of established and validated serologic assays. As an alternative method, molecular assays have been established with the goal to document the presence of *L. intracellularis* nucleic acid in feces. Although PCR is one of the most sensitive nucleic acid detection assays, the use of fecal material for molecular diagnostics has been associated with false negative results due to the presence of inhibitory substances that can interfere with nucleic acid purification or amplification (Machiels et al., 2000; Ja-

TABLE 2. Polymerase chain reaction results for the detection of *Lawsonia intracellularis* from fecal samples collected from free-living animals from two equine premises with documented occurrence of equine proliferative enteropathy.

Animal species	PCR protocol (no. of samples/PCR-positive samples)		Prevalence (%)[a]
	Real-time PCR	Real-time PCR with preamplification	
Black-tailed jackrabbit (*Lepus californicus*)	1/100	2/100	1–2
Striped skunk (*Mephitis mephitis*)	3/22	6/22	4.5–27
Feral cat (*Felis catus*)	0/14	0/14	0
Brewer's Blackbird (*Euphagus cyanocephalus*)	0/10[b]	0/10[b]	0
Virginian opossum (*Didelphis virginiana*)	2/9	2/9	22
Raccoon (*Procyon lotor*)	0/4	0/4	0
California ground squirrel (*Spermophilus beecheyi*)	0/3	0/3	0
Coyote (*Canis latrans*)	0/2	1/2	50

[a] Results are expressed as minimal and maximal prevalence when individual animals were not identified.

[b] Each sample represents a pool of 10 bird droppings.

cobson et al., 2003). However, development and use of specific extraction protocols has improved the yield of nucleic acid from feces (Li et al., 2003). The inhibitory effect of feces on the molecular detection of *L. intracellularis* was documented in the present study by the 10-fold difference in analytical sensitivity between *L. intracellularis* extracted from a noninhibitory substrate (PBS) and *L. intracellularis* extracted from feces. Several PCR assays have been established and used to document *L. intracellularis* shedding in pigs and other animals, with a reported analytical sensitivity ranging from 1 to 1,000 target molecules per reaction tube (Jones et al., 1993; Lindecrona et al., 2002; Herbst et al., 2003; Jacobson et al., 2004). Although the detection limit of these assays may be sensitive enough to document fecal shedding in affected animals, the sensitivity may not be high enough to document low fecal shedding in reservoir hosts or animals with intermittent shedding. Therefore, to increase the analytical sensitivity of the fecal samples tested, the present study also used a two-step PCR process. This process has been successfully used for the study of gene expression from very small tissue samples (Baumgarth et al., 2004). This approach allowed us to increase the analytical sensitivity from 40 target molecules per reaction tube to one single target molecule per total volume of purified fecal material. This represents a 4,000-fold increase in sensitivity, thereby increasing the proportion of fecal samples positive for *L. intracellularis* from six to 11 out of 164 tested samples. One must, however, bear in mind that the molecular detection of *L. intracellularis* in the feces of animals does not allow any conclusion to be drawn regarding the biologic state of the organisms or its origin. The direct link between wild animals and EPE has still to be proven by either characterization of the detected bacterial isolates or by experimental challenges using isolates from free-living hosts. Traditionally, genomic and proteomic analyses of *L. intracellularis* of porcine origin have shown great homogeneity between strains, making a differentiation between field and vaccine isolates very difficult (McOrist et al., 1995). However, recent work using multiple-locus variable number tandem repeat analysis has shown promise in differentiating *L. intracellularis* isolates (Gebhart, pers. comm.). Such an approach could be used to characterize the isolates from free-living animals in addition to the detection of *L. intracellularis* from their gastrointestinal tract.

The largest number of PCR-positive *L. intracellularis* fecal samples was observed

in striped skunks (six), followed by Virginian opossums (two), jackrabbits (two), and coyote (one). Based on individual identification or the detection of only one positive animal for a specific species, exact prevalence of 50 and 20% was determined for coyote and Virginian opossum, respectively. The high prevalence determined for coyote was influenced by the small sample size, and additional samples are needed to assign a more accurate prevalence. In comparison with our results, a recent study performed in the Slovak carpathians reported 7 and 9% of fecal samples from red foxes and wolves, respectively, testing PCR positive for *L. intracellularis* (Tomanová et al., 2003). Due to the inability to identify feces from individual jackrabbits collected from the field and from striped skunks trapped in cages, the proportion of fecal samples positive for *L. intracellularis* was expressed as a range defined by all positive samples originating from either one single animal or from all different animals. Interestingly, none of the fecal samples from feral cats, raccoons, Brewer's Blackbirds, and ground squirrels had detectable *L. intracellularis*. The lack of molecular pathogen detection in these species may be related to the small sample size, a potential intermittent mode of pathogen shedding, or the inability of *L. intracellularis* to infect these species. One of the potential limitations of the study was the inability to analysis feces from a similar cohort of animal species from each farm. Differences in habitat and number of trapping events between the two farms were at the origin of this discrepancy. However, the results highlight the variety of animal species potentially involved in the shedding of *L. intracellularis* on farms with documented cases of EPE.

The mode of transmission of *L. intracellularis* to susceptible weanlings has remained speculative. However, it is likely that foals become exposed to *L. intracellularis* after the ingestion of feed or water contaminated by *L. intracellularis* containing feces from domestic or free-living animals. In addition, the role of clinically and subclinically infected foals in the fecooral transmission of *L. intracellularis* needs to be further investigated. From the different free-living animals tested in the present study, the potential reservoir host must fulfill several criteria to serve as the source of infection for *L. intracellularis*, knowing that up to 45% of the resident weanlings tested seropositive for *L. intracellularis* (data not shown). Potential reservoir hosts must be abundant on the premises, must have unlimited access to feeding and drinking areas of the susceptible weanlings, and must be able to maintain the agent indefinitely in their populations. From the study results, jackrabbits, Virginian opossums, and striped skunks would be considered prime reservoir host candidates. It is possible that additional domestic or free-living animals may play a role in the transmission of *L. intracellularis* on the two study premises.

This project was supported by the Center for Equine Health with funds provided by Marcia MacDonald Rivas Endowment, School of Veterinary Medicine, University of California, Davis, with additional contributions from public and private donors.

LITERATURE CITED

Baumgarth, N., R. Szubin, G. M. Dolganov, M. R. Watnik, D. Greenspan, M. Da Costa, J. M. Palefsky, R. Jordan, M. Roederer, and J. S. Greenspan. 2004. Highly tissue substructure-specific effects of human papilloma virus in mucosa of HIV-infected patients revealed by laser-dissection microscopy-assisted gene expression profiling. American Journal of Pathology 165: 707–718.

Bednar, V. 2006. Detection of *Lawsonia intracellularis* in mice captured in pig farms with the occurrence of porcine proliferative enteropathy. *In* Proceedings of the 19th International Pig Veterinary Society Congress, Copenhagen, Denmark, 16–19 July, 180 pp.

Dezorzova-Tomanova, K., J. Smola, I. Trcka, J. Lamka, and I. Pavlik. 2006. Detection of *Lawsonia intracellularis* in wild boar and fallow deer bred in one game enclosure in the Czech Republic. Journal of Veterinary Medicine Series B 53: 42–44.

DROLET, R., D. LAROCHELLE, AND C. J. GEBHART. 1996. Proliferative enteritis associated with *Lawsonia intracellularis* (ileal symbiont intracellularis) in white-tailed deer. Journal of Veterinary Diagnostic Investigation 8: 250–253.

FEARY, D. J., C. J. GEBHART, AND N. PUSTERLA. 2006. *Lawsonia intracellularis* proliferative enteropathy in a foal. Schweizer Archiv für Tierheilkunde 132: 129–133.

HERBST, W., B. HERTRAMPF, T. SCHMITT, R. WEISS, AND G. BALJER. 2003. Diagnostik von *Lawsonia intracellularis* mittels Polymerasekettenreaktion (PCR) bei Schweinen mit und ohne Diarrhoe and anderen Tieren. Deutsche Tierärztliche Wochenschrift 110: 361–364.

JACOBSON, M., S. ENGLUND, AND A. BALLAGI-PORDÁNY. 2003. The use of a mimic to detect polymerase chain reaction-inhibitory factors in feces examined for the presence of *Lawsonia intracellularis*. Journal of Veterinary Diagnostic Investigation 15: 268–273.

———, A. ASPAN, M. H. KÖNIGSSON, C. H. SEGERSTAD, P. WALLGREN, C. FELLSTRÖM, M. JENSEN-WAERN, AND A. GUNNARSON. 2004. Routine diagnostics of *Lawsonia intracellularis* performed by PCR, serological and post mortem examination, with special emphasis on sample preparation methods for PCR. Veterinary Microbiology 102: 189–201.

JONES, G. F., G. E. WARD, M. P. MURTAUGH, G. LIN, AND C. J. GEBHART. 1993. Enhanced detection of intracellular organism of swine proliferative enteritis, ileal symbiont intracellularis, in feces by polymerase chain reaction. Journal of Clinical Microbiology 31: 2611–2615.

LAVOIE, J.-P., AND R. DROLET. 2007. *Lawsonia intracellularis*. *In* Equine infectious diseases, D. C. Sellon and M. T. Long (eds.). Saunders Elsevier, St. Louis, Missouri. pp. 313–316.

LAWSON, G. H. K., AND C. J. GEBHART. 2000. Proliferative enteropathy. Journal of Comparative Pathology 122: 77–100.

LI, M., J. GONG, M. COTTRILL, H. YU, C. DE LANGE, J. BURTON, AND E. TOPP. 2003. Evaluation of QIAamp DNA Stool Mini Kit for ecological studies of gut microbiota. Journal of Microbiological Methods 54: 13–20.

LINDECRONA, R. H., T. K. JENSEN, P. H. ANDERSEN, AND K. MØLLER. 2002. Application of a 5′ nuclease assay for detection of *Lawsonia intracellularis* in fecal samples from pigs. Journal of Clinical Microbiology 40: 984–987.

MACHIELS, B. M., T. RUERS, M. LINDHOUT, K. HARDY, T. HLAVATY, D. BANG, V. A. SOMERS, C. BAETEN, M. VON MEYENFELDT, AND F. B. THUNNISSEN. 2000. New protocol for DNA extraction of stool. Biotechniques 28: 286–290.

MCORIST, S., C. J. GEBHART, R. BOID, AND S. M. BARNS. 1995. Characterization of *Lawsonia intracellularis* gen. nov., sp. nov., the obligately intracellular bacterium of porcine proliferative enteropathy. International Journal of Systematic Bacteriology 45: 820–825.

STEGE, H., T. K. JENSEN, K. MØLLER, K. VESTERGAARD, P. BAEKBO, AND S. E. JORSAL. 2004. Infection dynamics of *Lawsonia intracellularis* in pig herds. Veterinary Microbiology 104: 197–206.

TOMANOVÁ, K., I. LITERÁK, J. KLIMES, L. PAVLACÍK, V. MRLÍK, AND J. SMOLA. 2003. *Lawsonia intracellularis* in wild mammals in the Slovak Carpathians. Journal of Wildlife Diseases 39: 407–411.

VAN DER HEIJDEN, H. M., J. BAKKER, A. R. ELBERS, J. H. VOS, A. WEYNS, M. DE SMET, AND S. MCORIST. 2004. Prevalence of exposure and infection of *Lawsonia intracellularis* among slaughter-age pigs. Research in Veterinary Science 77: 197–202.

Received for publication 25 August 2007.