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SHORT COMMUNICATIONS

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Lawsonia intracellularis in Wild Mammals in the Slovak Carpathians

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ABSTRACT: Feces of wild mammals were collected in the Bukovské Vrchy Hills (north-eastern Slovakia) in January and February 2002. The feces were examined for *Lawsonia intracellularis* by means of nested polymerase chain reaction. A total of 194 samples of feces from red deer (*Cervus elaphus*), 46 samples from roe deer (*Capreolus capreolus*), 31 samples from red fox (*Vulpes vulpes*), 23 samples from gray wolf (*Canis lupus*), and 12 samples from brown hare (*Lepus europaeus*) were examined. *Lawsonia intracellularis* was found in two samples from wolves, in two samples from foxes, and one sample from red deer. This is the first description of *L. intracellularis* in these three species.

Key words: *Canis lupus*, *Cervus elaphus*, deer, fox, *Lawsonia intracellularis*, *Vulpes vulpes*, wild animals, wolf.

The obligate intracellular bacterium *Lawsonia intracellularis* causes enteritis known as proliferative enteropathy, intestinal adenomatosis, and ileitis. *Lawsonia intracellularis* infection has worldwide distribution in domestic pigs (McOrist et al., 1995). In domestic animals other than pigs, *L. intracellularis* has been found in horses (Cooper et al., 1997), rabbits (Hotchkiss et al., 1996), ferrets (Fox et al., 1994), dogs (Leblanc et al., 1993), and hamsters (Cooper at al. 1997). In captive wild animals, the bacterium has been detected in white-tailed deer (*Odocoileus virginianus*; Drolet et al., 1996), rhesus macaques (*Macaca mulatta*; Klein et al., 1999), ostrich (*Struthio camelus*; Cooper et al., 1997), and emu (*Dromaius novaehollandiae*; Lemarchand et al., 1997). *Lawsonia intracellularis* probably caused adenomatosis in blue foxes (*Alopex lagopus*) reared in a fur farm (Eriksen et al., 1990).

Considering the wide range of known host species of *L. intracellularis*, we supposed that *L. intracellularis* may also affect wild mammals in their natural habitat. Recently, during examination of feces of wild mammals in Slovakia, we documented *L. intracellularis* in several cases.

Feces of wild mammals from the Bukovské Vrchy Hills (north-eastern Slovakia; 49°07′N, 22°21′E) near former villages Zvala and Ruské and around extant villages Runina, Topoľa, Ruský Potok, Nová Sedlica, and Zboj were examined. Feces were collected between 25 January and 4 February 2002 during regular winter survey of big carnivores. Winter was chosen so that the carnivores could be tracked on permanent snow cover. Feces were collected along tracks and the species were determined according to characteristic footprints.

A total of 303 samples of feces was collected, including 194 samples from red deer (*Cervus elaphus*), 46 samples from roe deer (*Capreolus capreolus*), 31 sample from red fox (*Vulpes vulpes*), 23 samples from gray wolf (*Canis lupus*), and 12 samples from brown hares (*Lepus europaeus*). In most cases the feces were formed, firm, and typical of the particular species. Feces were soft (diarrheic) in two cases from wolves and in two cases from foxes. Fecal samples collected in the field were refrigerated at 4 C for 1 wk and, after transport to laboratory, they were frozen at −20 C.

Fecal samples were incubated in lysis buffer for 1 hr and then centrifuged (1,400×G for 2 min). This was followed by incubation with diatomaceous earth sus-

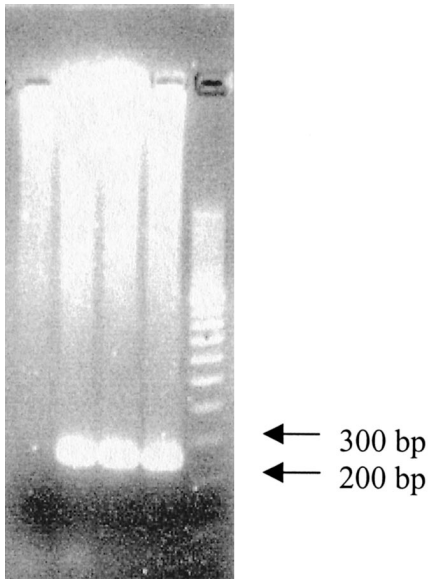

FIGURE 1. Nested PCR for detecting *L. intracellularis* DNA. Polymerase chain reaction products of templates amplified with primers C and D. Nested PCR products were visualized in 1% agarose gel, electrophoresed in TAE buffer and stained with ethidium bromide. Lane 1 = negative control; lane 2 = sample LI 103 (fox); lane 3 = sample LI 98 (wolf), and lane 4 = sample LI 168 (deer); lane M molecular size standards (Sigma Chemical Co., St. Louis, Missouri, USA).

pension for 10 min and subsequent centrifugation (1,400×G for 2 min) (Boom et al., 1990). The sediment was treated with wash buffer (twice), cold 70% ethyl alcohol (twice), acetone (once), and dried at 56 C for 15 min. Extracted DNA was resuspended in sterile water and kept at −20 C.

Nested polymerase chain reaction (PCR) was performed in a 50 μl total volume containing PCR buffer (10 mM Tris-HCl [pH 8.3], 50 mM KCl, 1.5 mM $MgCl_2$), 0.2 mM dNTP (Top Bio, Praha, Czech Republic), and 2 U of *Taq* DNA-polymerase (Top Bio, Praha, Czech Republic). Extracted DNA from the sample (1 μg) was added to the reaction. After denaturation of the template for 3 min at 94 C reaction was run for 35 cycles consisting of 40 sec each at 94 C, 55 C, and 72 C, and concluded by final extension 7 min at 72 C. The second amplification was performed with 2.5 μl of the first PCR product as DNA source and under conditions as describe above.

Two pairs of primers were added to reaction at a concentration of 1 pmol/μl (Jones et al., 1993). The outer pair of primers LIA: 5′-TAT GGC TGT CAA ACA CTC CG-3′and LIB: 5′-TGA AGG TAT TGG TAT TCT CC-3′ flanked a 319-bp fragment. The inner pair of primers LIC: 5′-TTA CAG GTG AAG TTA TTG GG-3′and LID: 5′-CTT TCT CAT GTC CCA TAA GC-3′ flanked a 270-bp fragment.

Negative control samples without DNA and positive control samples with known DNA were subjected to extraction and PCR amplification in all experiments.

Positive PCR products from the first amplification cycle were reamplified in the amount of 10 μl in the total reaction mixture volume of 100 μl. Nested PCR products (Fig. 1) were purified using a commercial kit (Qiagen, Hilden, Germany), subsequently sequenced by the ABI PRISM 310 Genetic Analyser (Applied Biosystem, Foster City, California, USA), and finally evaluated using the Omega v. 2.0 program (Oxford Molecular, Cambridge, UK).

Specific DNA sequence of *L. intracellularis* (Fig. 2) was found in two (9%) of 23 wolf feces, designated LI 98 and LI 122; in two (7%) of 31 fox feces, designated LI 103 and LI 142; and in one (0.5%) of 194 red deer feces, designated LI 168. By sequencing the 270 bp PCR product, the amplicons from the fox (LI 103), wolf (LI 98), and red deer (LI 168) were found to vary in three positions when compared to the type strain NCTC 12656^T. One positive sample of wolf feces and one of fox feces had diarrheic consistency. Both these diarrheic samples and the second positive fox sample were from Runina locality. Another positive wolf sample was from Ruské locality and the positive deer sample was from Topoľa locality. All three localities where *L. intracellularis* was detected in fe-

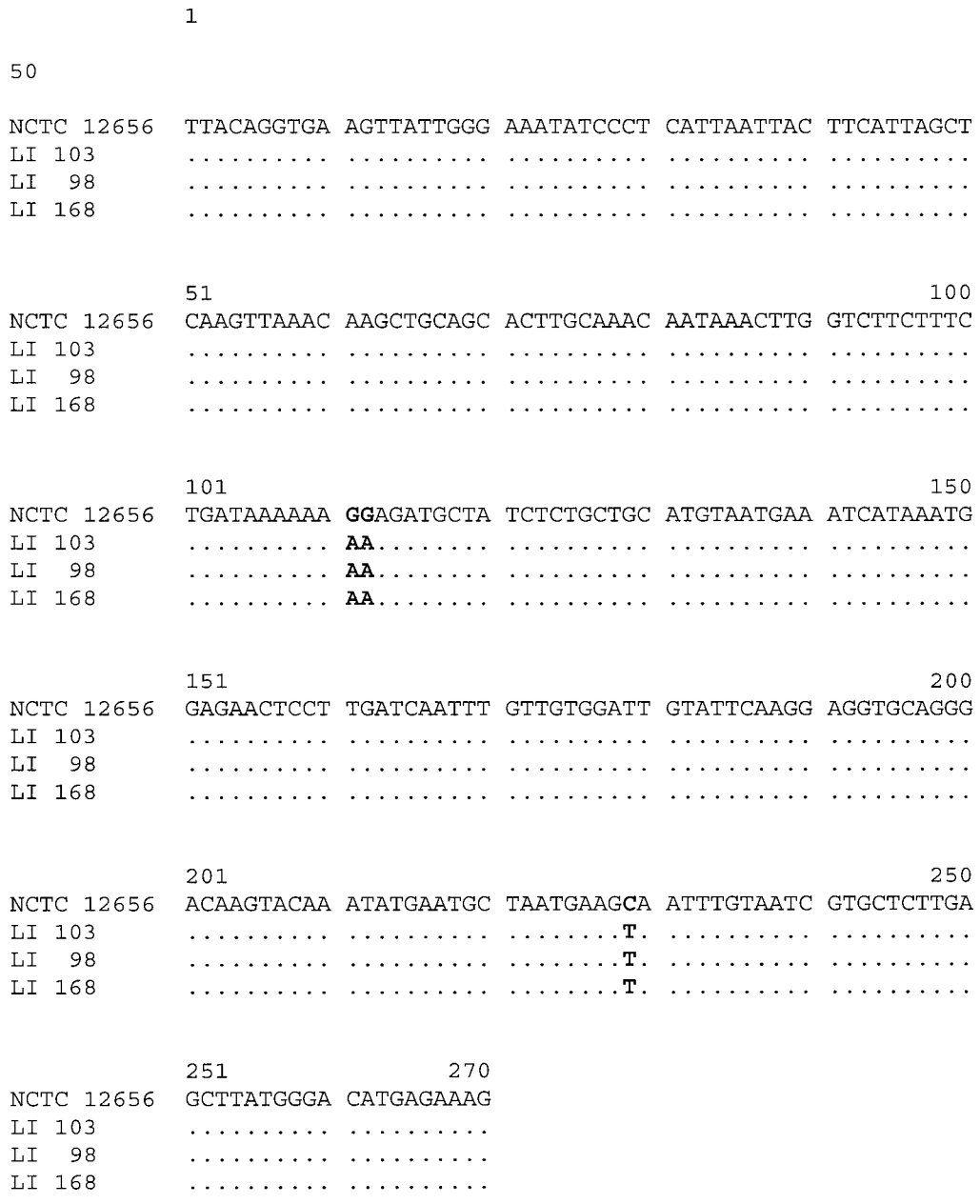

FIGURE 2. DNA sequences of a portion of the 16S rDNA of the intracellular agents of PE derived from the fox (LI 103), wolf (LI 98), and deer (LI 168), compared to the consensus sequences of a pig isolate of *L. intracellularis* (NCTC 12656^T). Dots indicate identical bases.

ces of wild mammals are situated close to one another.

Reports of infections likely caused by *L. intracellularis* in carnivores are scarce compared to numerous studies in swine (Lawson and Gebhart, 2000). Disease caused or likely caused by *L. intracellularis* have been described in ferrets, dogs, blue foxes, and white-tailed deer based on pathologic, histologic, electron microscopic, or immunohistochemical findings resembling porcine proliferative enteritis.

In ferrets, *L. intracellularis* caused proliferative colitis (Fox et al., 1994). In dogs, Collins et al. (1983) described proliferative enteritis in two pups where the gross necropsy, histologic, and ultrastructural findings were similar to those described for proliferative enteritis in the pig. The second reported case in a dog had hyperplastic gastritis morphologically similar to proliferative enteritis that was associated with intraepithelial *Campylobacter*-like organism (CLO) previously well-known in pigs (Leblanc et al., 1993). Numerous fluorescent organisms were seen in a section from the stomach after indirect immunofluorescence staining with specific rabbit antiserum. This was the first convincing evidence of *L. intracellularis* infection in dogs (Lawson and Gebhart, 2000).

According to clinical, histologic, immunoperoxidase, and electron microscopic examinations, it also is very likely that fur farm-reared blue foxes were infected with *L. intracellularis* (Eriksen et al., 1990). The infection in these foxes manifested with diarrhea and rectal prolapse.

In the present study we showed that two other species from the family Canidae, namely gray wolf and red fox, can also be affected with *L. intracellularis.* The infection was indicated by detection of *L. intracellularis* in feces with PCR, which is the most widely used technique for the detection of *L. intracellularis.* The length (270 bp) of the nested PCR product from fecal samples of these animals corresponded to the DNA fragment that was obtained in examinations of infected domestic pigs (Jones et al., 1993). Comparison of sequences of the *L. intracellularis* type strain (NCTC 12656^T) from a domestic pig (McOrist et al., 1995) and from the fox (LI 103) and wolf (LI 98) showed a high homogeneity (99%), which strongly indicates that they belong to the same bacterial species.

It is difficult to speculate about the clinical course of *L. intracellularis* infection in wolves and foxes. However, two of four fecal samples (one from a wolf and one from a fox) in which we demonstrated the presence of *L. intracellularis* had soft consistency. Diarrhea is one of the characteristic signs of porcine proliferative enteritis (Lawson and Gebhart, 2000) and was also observed in affected dogs and blue foxes (Collins et al., 1983; Eriksen et al., 1990).

The proportion of fecal samples positive for *L. intracellularis* in wolves (9%) and in foxes (7%) was relatively high. In the entire Bukovské Vrchy Hills, three to four packs of wolves hunted during the 2001–02 winter, each consisting of some five individuals (Š. Pčola, pers. com.). Approximately 15–20 wolves thus occurred in the study area. Even if only one wolf was infected, the prevalence of infection would represent a remarkable ≥5%. Such estimate is not possible in foxes that are much more numerous. However, two positive samples of 31 feces examined may also indicate relatively high prevalence of infection.

In our study, *L. intracellularis* was detected only in feces from one red deer found near the localities with infected wolves and foxes. *Lawsonia intracellularis* was found in white-tailed deer kept on a farm in Canada (Drolet et al., 1996), but it has, to our knowledge, never been detected in free-living ruminants.

The grey wolf, red fox, and red deer may play a role in circulation of *L. intracellularis* in natural habitats of certain areas of central Europe. Study of possible occurrence of *L. intracellularis* in wild mammals merits further attention.

LITERATURE CITED

BOOM, R., C. J. A. SOL, M. M. M. SALIMANS, C. L. JANSEN, P. M. E. WERTHEIM-VAN DILLEN, AND J. VAN DER NOORDAA. 1990. Rapid and simple method for purification of nucleic acids. Journal of Clinical Microbiology 28: 495–503.

COLLINS, J. E., M. C. LIBAL, AND D. BROST. 1983. Proliferative enteritis in two pups. Journal of the American Veterinary Medical Association 183: 886–889.

COOPER, D. M., D. L. SWANSON, AND C. J. GEBHART. 1997. Diagnosis of proliferative enteritis in frozen and formalin-fixed, paraffin-embedded tissues from a hamster, horse, deer and ostrich us-

ing a *Lawsonia intracellularis*-specific multiplex PCR assay. Veterinary Microbiology 54: 47–62.

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.

ERIKSEN, K, T. LANDSVERK, AND B. BRATBERG. 1990. Morphology and immunoperoxidase studies of intestinal adenomatosis in the blue fox, *Alopex lagopus.* Journal of Comparative Pathology 102: 265–278.

FOX, J. G., F. E. DEWHIRST, G. J. FRASER, B. J. PASTER, B. SHAMES, AND J. C. MURPHY. 1994. Intracellular *Campylobacter*-like organism from ferrets and hamsters with proliferative bowel disease is a *Desulfovibrio* sp. Journal of Clinical Microbiology 32: 1229–1237.

HOTCHKISS, CH. E., B. SHAMES, S. E. PERKINS, AND J. G. FOX. 1996. Proliferative enteropathy of rabbits: The intracellular *Campylobacter*-like organism is closely related to *Lawsonia intracellularis.* Laboratory Animals Science 46: 623–627.

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.

KLEIN, E. C., C. J. GEBHART, AND G. E. DUHAMEL. 1999. Fatal outbreaks of proliferative enteritis caused by *Lawsonia intracellularis* in young colony-raised rhesus macaques. Journal of Medical Primatology 28: 11–18.

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

LEBLANC, B., J. G. FOX, J. L. LE NET, M. T. MASSON, AND A. PICARD. 1993. Hyperplastic gastritis with intraepithelial *Campylobacter*-like organisms in a beagle dog. Veterinary Pathology 30: 391–394.

LEMARCHAND, T. X., T. N. TULLY, S. M. SHANE, AND D. E. DUNCAN. 1997. Intracellular *Campylobacter*-like organism associated with rectal prolapse and proliferative enteroproctitis in emus (*Dromaius novaehollandiae*). Veterinary Pathology 34: 152–156.

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.

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