

nutalli parasites are as virulent as *E. histolytica* parasites in animal models, it remains unclear whether they are as virulent in humans (4). We recommend that caretakers of NHPs be screened on a regular basis and be provided with appropriate medications if needed.

Acknowledgments

We thank all caretakers who participated in this study and the laboratory staff of the Institute of Tropical Medicine (Antwerp).

This study received financial support from the Centre of Research and Conservation of The Royal Zoological Society of Antwerp, which is supported by the Flemish government (Dehousse grant).

References

1. Levecke B, Dreesen L, Dorny P, Verweij JJ, Vercammen F, Casaert S, et al. Molecular identification of *Entamoeba* spp. in captive nonhuman primates. *J Clin Microbiol*. 2010;48:2988–90. <http://dx.doi.org/10.1128/JCM.00013-10>
2. Loomis MR, Britt JO Jr, Gendron AP, Holshuh HJ, Howard EB. Hepatic and gastric amebiasis in black and white colobus monkeys. *J Am Vet Med Assoc*. 1983;183:1188–91.
3. Haq A, Sharma A, Ahmad S, Khan HM, Khan N. Experimental infection of rhesus monkeys with *Entamoeba histolytica* mimics human infection. *Lab Anim Sci*. 1985;35:481–4.
4. Tachibana H, Yanagi T, Pandey K, Cheng XJ, Kobayashi S, Sherchand JB, et al. An *Entamoeba* sp. strain isolated from rhesus monkey is virulent but genetically different from *Entamoeba histolytica*. *Mol Biochem Parasitol*. 2007;153:107–14. <http://dx.doi.org/10.1016/j.molbiopara.2007.02.006>
5. Tachibana H, Yanagi T, Akatsuka A, Kobayashi S, Kanbara H, Tsutsumi V. Isolation and characterization of a potentially virulent species *Entamoeba nuttalli* from captive Japanese macaques. *Parasitology*. 2009;136:1169–77. <http://dx.doi.org/10.1017/S0031182009990576>
6. Levecke B. The importance of gastrointestinal protozoa in captive non-human primates [doctoral dissertation]. Ghent (Belgium): Ghent University; 2010.
7. Verweij JJ, Oostvogel F, Brienen EA, Nang-Beifubah A, Ziem J, Polderman AM. Short communication: Prevalence of *Entamoeba histolytica* and *Entamoeba dispar* in northern Ghana. *Trop Med Int Health*. 2003;8:1153–6. <http://dx.doi.org/10.1046/j.1360-2276.2003.01145.x>
8. Stensvold CR, Lebbad M, Verweij JJ. The impact of genetic diversity in protozoa on molecular diagnostics. *Trends Parasitol*. 2011;27:53–8. <http://dx.doi.org/10.1016/j.pt.2010.11.005>
9. Parkar U, Traub RJ, Kumar S, Mungthin M, Vitali S, Leelayoova S, et al. Direct characterization of *Blastocystis* from faeces by PCR and evidence of zoonotic potential. *Parasitology*. 2007;134:359–67. <http://dx.doi.org/10.1017/S0031182006001582>
10. Feng M, Komiyama T, Yanagi T, Cheng X, Sherchand JB, Tachibana H. Correlation between genotypes of tRNA-linked short tandem repeats in *Entamoeba nuttalli* isolates and the geographical distribution of host rhesus macaques. *Parasitol Res*. 2014;113:367–74. <http://dx.doi.org/10.1007/s00436-013-3664-0>

Address for correspondence: Bruno Levecke, Department of Virology, Parasitology and Immunology, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium; email: bruno.levecke@UGent.be

***Alaria alata* Mesocercariae among Feral Cats and Badgers, Denmark**

Nao Takeuchi-Storm, Mohammed N.S. Al-Sabi, Stig M. Thamsborg, Heidi L. Enemark

Author affiliations: Technical University of Denmark, Copenhagen, Denmark (N. Takeuchi-Storm, M.N. Solaiman Al-Sabi, H.L. Enemark); University of Copenhagen (S.M. Thamsborg)

DOI: <http://dx.doi.org/10.3201/eid2110.141817>

To the Editor: The digenean trematode *Alaria alata* is considered an emerging zoonotic parasite in Europe because of increased findings in wild boars during *Trichinella* inspection. No human illness caused by *A. alata* mesocercariae (infective larvae) has been reported, but concern remains because the closely related North American species *A. americana* has caused illnesses among humans, including 1 death (1).

In Denmark, high prevalence of *A. alata* trematodes in final hosts has been shown (2), but limited data on potential paratenic hosts are available. Therefore, samples from 406 domestic pigs, 130 wild boars, 9 badgers, and 99 cats were collected by convenience sampling during October 2013–September 2014. We used pig and wild boar samples from multiple geographic areas of Denmark were leftover tissue samples from ongoing *Trichinella* spp. surveillance. Badgers had died naturally or were hit by vehicles (8 from Jutland, 1 from Zealand) and collected as part of a wildlife monitoring program. Cats (all from Zealand) were either feral (n = 92) or domesticated (n = 7) and had been euthanized as part of a national control program. Carcasses were necropsied in our laboratory; we collected 30 g of tissue samples according to Riehn et al. (3). All samples were analyzed by the modified *A. alata* mesocercariae migration technique (3). In brief, the sample was cut into ≈0.5-cm edge pieces, wrapped in gauze, and suspended for 90 min by 2 wooden sticks in a conical glass with ≈300 mL of water (46°C–48°C). Approximately 15 mL of sediment was collected from the bottom of the glass by suction by using a glass pipette and examined by microscopy (magnification ×20).

A. alata mesocercariae were isolated from 3 cats and 6 badgers (online Technical Appendix Table 1, <http://wwwnc.cdc.gov/EID/article/20/10/14-1817-Techapp1.pdf>). All 3 cats were female (2 pregnant, 1 lactating); prevalence was significantly higher in pregnant or lactating females (3/12) than other intact females (0/24) (p = 0.031 by Fisher exact test). This finding might be related to increased exposure because an increase in predation by the cats during pregnancy and lactation to meet higher protein and energy demand. However, because *A. marciana* mesocercariae can be

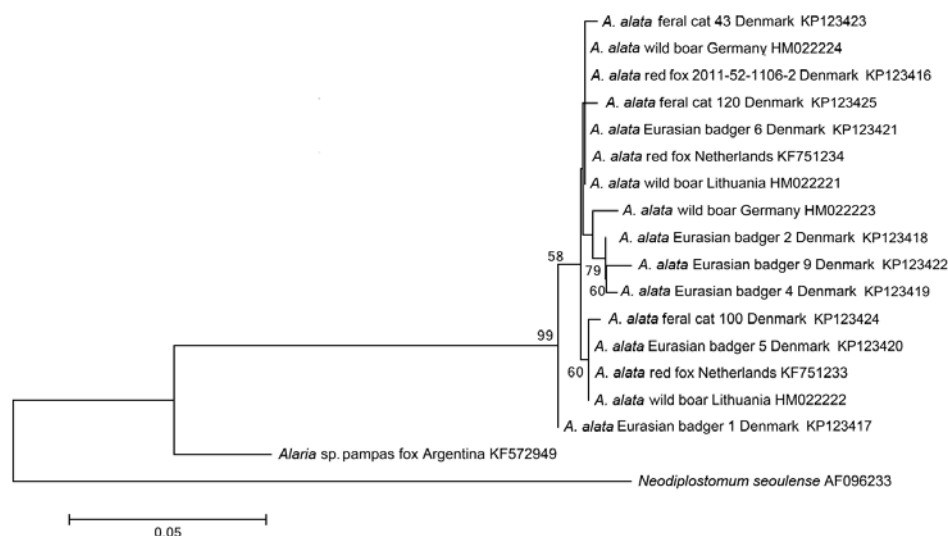


Figure. Neighbor-joining phylogenetic tree of *Alaria alata* isolates based on the analysis of partial mitochondrial cytochrome c oxidase subunit 1 gene sequences (332 bp). Bootstrap values are indicated to the left of the nodes and are based on 10,000 replicates. GenBank accession numbers are listed to the right. Scale bar indicates base substitutions per site.

transmitted through milk in cats (4), lactating females may also be predisposed to an increased chance for *A. alata* mesocercariae reaching their offspring. Examination of the intestines of all cats by sedimentation and counting technique (5) revealed no *A. alata* adults. Although *A. alata* adults have been found in cats in Uruguay (6), reports from Europe are lacking, and thus it is still uncertain whether cats can act as amphiparatenic or final hosts. Natural infection of cats with other *Alaria* spp. has been reported in the United States (7), indicating biologic differences among *Alaria* spp.

Zoonotic risk for *A. alata* infection through ingestion of cat meat is probably minimal in Europe but may be important in Asia and South America, where cats are occasionally consumed. Badgers are, however, sometimes consumed as game meat or road kill meat in Europe. In Russia, 10.6% of trichinellosis outbreaks during 1998–2002 were caused by consumption of badger meat (8). Thus, the zoonotic potential of infections in this animal, although a protected species, should not be ignored.

Negative findings in domestic pigs and wild boars in this study may reflect underestimation because those samples were below the recommended 30 g and often taken from sites that are not typically infected with mesocercariae (3). A follow-up study with better sampling strategy would be of value to determine the risk for *A. alata* transmission from domestic pigs and wild boars.

Identification of isolated mesocercariae was confirmed by PCR and sequencing of a fragment (332 bp) of the mitochondrial cytochrome c oxidase subunit 1 gene (*cox1*) (9). By neighbor-joining analysis (10), the consensus *cox1* sequences were compared with the trematode *Neodiplostomum seoulense* (outgroup), 1 *A. alata* isolate from a Danish red fox, and all 7 *cox1* sequences of *Alaria* spp. available in GenBank as of October 2014. (Sequences from this study

have been deposited into GenBank under accession nos. KP123417–KP123422 [badgers] KP123423–KP123425 [feral cats].) The inferred phylogenetic tree (Figure) showed marked genetic variation among *A. alata* isolates from Denmark and other parts of Europe but no apparent separation of most *A. alata* isolates from Europe based on host species or country, except for that from badger 1 (online Technical Appendix Table 2). This animal originated from Northern Jutland, where host and parasite populations are geographically isolated by a large fjord separating the region from the rest of the country. The marked genetic variation within *cox1* sequences suggests the usefulness of this marker, but additional genetic markers should be included in future studies to explore the genetic flow of *A. alata* within natural hosts.

In conclusion, *A. alata* mesocercariae seem to favorably infect pregnant or lactating cats, thereby increasing the chance of vertical transmission. Further, detection of *A. alata* infection in numerous badgers suggests potential high zoonotic risk associated with ingestion of such exotic meat. These results should, however, be interpreted with caution because of the small sample size and unknown efficacy of the modified *A. alata* mesocercariae migration technique.

Acknowledgments

We thank the staff of the Danish Veterinary and Food Administration and Mariann Chriél, Helena Mejer, Caroline S. Olsen, Mia Jensen, and Tina V. Hansen for providing animals for the project. We thank Boi-Tien Thi Pham for conducting molecular analysis.

References

- Möhl K, Große K, Hamedy A, Wüste T, Kabelitz P, Lücker E. Biology of *Alaria* spp. and human exposition risk to *Alaria* mesocercariae—a review. *Parasitol Res.* 2009;105:1–15. <http://dx.doi.org/10.1007/s00436-009-1444-7>

2. Al-Sabi MNS, Chriél M, Jensen TH, Enemark HL. Endoparasites of the raccoon dog (*Nyctereutes procyonoides*) and the red fox (*Vulpes vulpes*) in Denmark 2009–2012—a comparative study. *Int J Parasitol Parasites Wildl.* 2013;2:144–51. <http://dx.doi.org/10.1016/j.ijppaw.2013.04.001>
3. Riehn K, Hamedy A, Große K, Zeitler L, Lückner E. A novel detection method for *Alaria alata* mesocercariae in meat. *Parasitol Res.* 2010;107:213–20. <http://dx.doi.org/10.1007/s00436-010-1853-7>
4. Shoop WL, Corkum KC. Maternal transmission by *Alaria marcianae* and the concept of amphiparatenesis. *J Parasitol.* 1987; 73:110–5. <http://dx.doi.org/10.2307/3282354>
5. Eckert J, Deplazes P, Craig P, Gemmell M, Gottstein B, Heath D, et al. Echinococcosis in animals: clinical aspects, diagnosis and treatment. WHO/OIE manual on echinococcosis in humans and animals: a public health problem of global concern. 2001:73–99. <http://apps.who.int/iris/bitstream/10665/42427/1/929044522X.pdf>
6. Castro O, Venzal JM, Felix ML. Two new records of helminth parasites of domestic cat from Uruguay: *Alaria alata* (Goeze, 1782) (Digenea, Diplostomidae) and *Lagochilascaris major* Leiper, 1910 (Nematoda, Ascarididae). *Vet Parasitol.* 2009;160:344–7. <http://dx.doi.org/10.1016/j.vetpar.2008.11.019>
7. Lucio-Forster A, Bowman DD. Prevalence of fecal-borne parasites detected by centrifugal flotation in feline samples from two shelters in upstate New York. *J Feline Med Surg.* 2011;13:300–3. <http://dx.doi.org/10.1016/j.jfms.2010.12.013>
8. Ozeretskovskaya NN, Mikhailova LG, Sabgaida TP, Dovgalev AS. New trends and clinical patterns of human trichinellosis in Russia at the beginning of the XXI century. *Vet Parasitol.* 2005;132:167–71. <http://dx.doi.org/10.1016/j.vetpar.2005.05.056>
9. Bowles J, Blair D, McManus DP. Genetic variants within the genus *Echinococcus* identified by mitochondrial DNA sequencing. *Mol Biochem Parasitol.* 1992;54:165–73. [http://dx.doi.org/10.1016/0166-6851\(92\)90109-W](http://dx.doi.org/10.1016/0166-6851(92)90109-W)
10. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol.* 2013;30:2725–9. <http://dx.doi.org/10.1093/molbev/mst197>

Address for correspondence: Heidi L Enemark, Norwegian Veterinary Institute, PO Box 750 Sentrum, NO-0106 Oslo, Norway; email: heidi.enemark@vetinst.no

Human Infections with *Pseudoterranova cattani* Nematodes, Chile

Thomas Weitzel,¹ Hiromu Sugiyama, Hiroshi Yamasaki, Cristian Ramirez, Reinaldo Rosas, Rubén Mercado¹

Author affiliations: Clínica Alemana-Universidad del Desarrollo, Santiago, Chile (T. Weitzel, R. Rosas); National Institute of Infectious Diseases, Tokyo, Japan (H. Sugiyama, H. Yamasaki); Universidad Mayor, Santiago (C. Ramirez); Universidad de Chile, Santiago (R. Mercado)

DOI: <http://dx.doi.org/10.3201/eid2110.141848>

To the Editor: Anisakidosis is an emerging foodborne zoonosis caused by nematode larvae of the Anisakinae

subfamily, which includes the genera *Anisakis*, *Pseudoterranova*, and *Contracecum* (1). In natural cycles, anisakid larvae are transmitted to marine mammals or piscivorous birds when they eat raw saltwater fish or squid. In the human incidental host, larvae attach to the mucosa of the gastrointestinal tract, causing clinical features ranging from asymptomatic carriage to severe abdominal pain with complications, such as gastric perforation (2). Microscopical diagnosis is hampered by the lack of distinguishing morphologic characteristics in larval stages (1). Recently, molecular genetic techniques have shown that the main species, *Anisakis simplex* and *Pseudoterranova decipiens*, are in fact species groups with distinct geographic and biologic characteristics (3,4). The *P. decipiens* complex consists of at least 6 sibling species (online Technical Appendix Table, <http://wwwnc.cdc.gov/EID/article/21/10/14-1848-Techapp1.pdf>). We report 4 human infections with *P. cattani* diagnosed during 2012–2014.

The case-patients were adults 22–59 years of age; 2 were female, and all lived in Santiago, Chile. Additional anamnestic and clinical data were available for 3 patients: all spontaneously regurgitated the parasites without having other gastrointestinal complaints. All 3 reported eating ceviche, a dish made of raw marine fish marinated in lemon juice. One patient reported a tingling sensation and coughs before the expulsion of a highly motile larva (Video, <http://wwwnc.cdc.gov/article/21/10/14-1848-V1.htm>). This patient was awaiting oral surgery after a bicycle accident and had eaten the last raw fish dish 2 weeks previously. Initially, parasites were identified by morphologic criteria. Larvae were 20 mm long, were of whitish to reddish color, and had 3 anterior lips (online Technical Appendix Figure 1). Because of the presence of an anteriorly directed cecum (online Technical Appendix Figure 2), they were assigned to *Pseudoterranova* species.

For further molecular identification, DNA samples were extracted by using a DNeasy Blood and Tissue Kit (QIAGEN K.K., Tokyo, Japan). The rRNA gene containing 2 internal transcribed spacer (ITS) regions was amplified by PCR using primers NC5 and NC2, as previously described (5). PCR products were sequenced by using a BigDye Terminator Cycle Sequencing Kit (Applied Biosystems Inc., Foster City, CA, USA) on an automated sequencer (ABI3100, Applied Biosystems). Sequence similarities were determined by a BLAST search of DDBJ (<http://blast.ddbj.nig.ac.jp/top-j.html>). The GENETYX-WIN program version 7.0 (Software Development Co., Tokyo, Japan) facilitated sequence alignment and comparison. Within the 4 ITS sequences of amplicons obtained, all were 100% identical, and alignment with the other *P. cattani* sequence differed only in 1 nt. ITS sequences of 2 isolates are available in GenBank (accession nos. KF781284 and KF781285). All *P. cattani* sequences showed a previously described

¹These authors contributed equally to this article.

Alaria alata Mesocercariae among Feral Cats and Badgers, Denmark

Technical Appendix

Technical Appendix Table 1. Information on domestic animals and wildlife examined for *Alaria alata* by using modified *Alaria alata* migration technique, Denmark October 2013–September 2014*

Host species	Origin	Examination period	Sample type	No. examined	Prevalence (%) (95% CI)
Pigs					
Outdoor	Z	Oct 2013–Mar 2014	Diaphragm or foreleg	260	0
ND	Z, F, J, ND	Oct 2013–Mar 2014	Diaphragm or foreleg	146	0
Wild boars					
Farmed	Z	Oct 2013–Mar 2014	Diaphragm or foreleg	39	0
Farmed	J	Nov 2013–Jan 2014	Diaphragm, foreleg, or intercostal muscles	58	0
ND	ND	Oct 2013 – Mar 2014	Diaphragm or foreleg	33	0
Badgers	J	Feb–Sep 2014	Tissue sample mixture†	9	66.7 (29.9–92.5)
Cats	Z	Mar–May 2014	Tissue sample mixture†	99	3.0 (0.6–8.6)

*Z, Zealand; F, Funen; J, Jutland; ND, no data available.

†30 g tissue sample mixture of diaphragm, tongue, and masticatory muscles;

Technical Appendix Table 2. Characteristics of *Alaria alata* mesocercariae–positive animal tissue samples identified by using modified *Alaria alata* migration technique, Denmark

Identity code	Species	Sex	Origin	No. of mesocercariae per 30 g sample	GenBank accession number
Cat 43	Cat (feral)	Female (pregnant)	Zealand	23	KP123423
Cat 100	Cat (feral)	Female (pregnant)	Zealand	2	KP123424
Cat 120	Cat (feral)	Female (lactating)	Zealand	12	KP123425
Badger 1	Badger	Not recorded	Northern Jutland	1*	KP123417
Badger 2	Badger	Female	Central Jutland	2	KP123418
Badger 4	Badger	Female	Southern Jutland	1	KP123419
Badger 5	Badger	Male	Central Jutland	2	KP123420
Badger 6	Badger	Male	Central Jutland	8	KP123421
Badger 9	Badger	Female	Zealand	2	KP123422

*30 g tissue sample mixture of diaphragm, tongue, and masticatory muscles were used.