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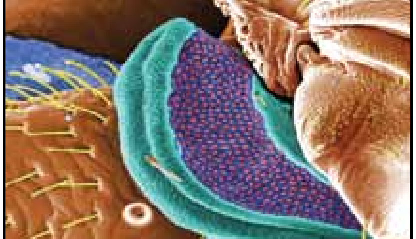

Zoonotic *Baylisascaris procyonis* Roundworms in Raccoons, China

To the Editor: *Baylisascaris procyonis*, an intestinal roundworm that infects raccoons (*Procyon lotor*), causes fatal or severe neural larva migrans in animals and humans (*1*,*2*). Globally, ≈130 species of wild and domesticated animals are susceptible (*2*). Infections in humans typically occur in children who have the disorders pica or geophagia and ingest *B. procyonis* eggs in items contaminated with raccoon feces (*3*). Clinical manifestations include ocular disease, eosinophilic encephalitis, and eosinophilic cardiac pseudotumors; severe infection can lead to death. Since 1984, ≈24 cases of *B. procyonis*–related human neural larva migrans have been reported, mainly in the United States (*1*,*3*–*5*; K.R. Kazacos, pers. comm.). Despite few cases among humans, lack of effective treatment and widespread distribution of infected raccoons in close association with humans make *B. procyonis* a potentially serious public health threat (*2*,*6*). The current distribution of *B. procyonis* is poorly recorded in Asia (*2*,*7*), except for Japan (*8*). We describe *B. procyonis* infections among raccoons in China as part of a series of ongoing surveys of helminthic zoonoses linked to captive exotic animals in zoologic gardens (ZGs) in China.

More than 90% of raccoons in China (n >320) are raised as exotic ornamental animals in 18 ZGs. During 2011–2013, we collected 2×308 fecal samples (i.e., 1 repeat within each sampling) from 277 raccoons in 12 randomly selected ZGs (online Technical Appendix Figure 1, wwwnc.cdc.gov/EID/article/20/12/14-0970-Techapp1.pdf). Samples were stored in individual plastic bags at –20°C until use. We examined raccoons (n = 31) at the Sichuan ZGs twice, in June 2012 and May 2013. We identified *B. procyonis* eggs in feces using morphologic and molecular analyses (*1*,*2*,*9*). The nuclear first internal transcribed spacer (428 bp) and mitochondrial cytochrome c oxidase subunit 1 (*cox-1*, 938 bp) genes in each sample were PCR-amplified and sequenced. *B. procyonis* infection was confirmed by sequencing and phylogenetic analyses of both genes (*7*,*9*). We reexamined ≈60% of fecal samples to validate results. Prevalence (95% CI) was calculated for the overall population and independently for female, male, juvenile, and adult raccoons. We determined differences between the tested ZG prevalence and prevalence by sex or age of raccoons using χ^2 or Fisher exact tests in SAS (SAS Institute, Cary, NC, USA); p values <0.05 were considered significant.

Building on egg-based morphologic characterization and internal transcribed spacer 1 and *cox-1* gene-based phylogenies using neighbor-joining trees (online Technical Appendix Figure 2), we found *B. procyonis* in raccoon feces from 5/12 ZGs (42%; 95% CI 14%–70%), including 2 in the most densely populated provinces, Henan and Sichuan. More infections were found in western than central and eastern ZGs (4/6 and 1/6, respectively; Table, online Technical Appendix Figure 1) (p = 0.079). Fecal samples of 35 raccoons (13%; 95% CI 9%–17%) tested positive for *B. procyonis*. The mean intensity of egg shedding was 5,000 eggs per gram (range 800–11,200 eggs per gram; data not shown). No significant difference was observed in the intensity of shedding by comparing sex and age of animals, and no significant differences were noted in the mean prevalence between female and male raccoons (12% versus 14%; p = 0.677) or between adult and juvenile animals (13% versus 10%; p = 0.536).

This investigation documents the presence and prevalence of *B. procyonis*

Table. Prevalence of *Baylisascaris procyonis* roundworm infections among captive raccoons, China, 2011–2013*

Location, zoological gardens	No. *B. procyonis*–positive samples/total no. samples (%)				
	Sex		Age group		
	M	F	Adult	Juvenile	Total
Western region					33/146 (23)
Chongqing	–	0/4	0/4	–	0/4
Bifengxia	0/8	0/14	0/13	0/9	0/22, 0/22
Chengdu	0/4	1/5 (20)	0/6	1/3 (33)	1/9 (11), 0/9
Xi'an Wildlife	0/9	1/27 (4)	0/28	1/8 (13)	1/36 (3)
Kunming	12/12 (100)	15/15 (100)	22/22 (100)	5/5 (100)	27/27 (100)
Kunming Wildlife	1/24 (4)	3/24 (13)	4/48 (8)	–	4/48 (8)
Central region					2/56 (4)
Harbin Northern Forest	0/12	0/18	0/21	0/9	0/30
Zhengzhou	1/5 (20)	1/11 (9)	2/12 (17)	0/4	2/16 (13)
Changsha	0/3	0/7	0/5	0/5	0/10
Eastern region					0/75
Beijing	0/16	0/24	0/22	0/18	0/40
Guangzhou	0/5	0/15	0/20	–	0/20
Shanghai Wildlife	0/4	0/11	0/9	0/6	0/15
Total	14/102 (14)	21/175 (12)	28/210 (13)	7/67 (10)	35/277 (13)
p value	0.677		0.536		–

*Raccoons, considered to be exotic ornamental animals, are mainly kept in 18 zoologic gardens (ZGs) in China; 12 ZGs were examined for *B. procyonis* prevalence during the study period. Sichuan ZGs, including Bifengxia ZG and Chengdu ZG, were tested twice during this surveillance period. –, no raccoons in the group or no data available.

among raccoons in China. The findings imply that raccoons harboring this parasite have the potential for spreading it to humans. One reason is that captive raccoons adapt readily to humans and easily take food offered by hand; another is that communal raccoon latrine sites in ZGs are usually close to areas where humans gather, so ZG visitors may be exposed to large numbers of eggs (online Technical Appendix Figure 3). These eggs can remain viable and infective for years (*2*), and latrines are recognized as primary sources of transmission of *B. procyonis* to humans (*4*). Current public health initiatives to prevent *B. procyonis* infections in humans rely on the education of veterinary and human health care professionals, who in turn inform the public (*1*,*6*,*10*). Thus, veterinarians, clinicians, and public health officials in China should be more informed about this pathogen, especially in regions with large raccoon populations.

Because of a lack of clinical awareness of this illness and subsequent lack of early diagnosis and effective treatment, prevention of *B. procyonis* infection by education is essential. In addition, a strategy for eradication is needed. Heat, in the form of boiling water, steam-cleaning, or fire, is the optimal tool for killing *B. procyonis* eggs (*2*) and therefore can be used to decontaminate areas surrounding latrines. Within heavily contaminated areas, removing and then sterilizing the top few inches of surface soil with heat would be effective and practical (*1*,*2*). Among captive raccoon populations, particularly in China, regular deworming is also likely to be helpful in reducing novel and existing sources of infection (*1*–*3*).

Finally, although no cases of human infection have been reported in China to our knowledge, physicians should consider including *B. procyonis* infections in their differential diagnoses of patients with indicative features: clinical (eosinophilic encephalitis, ocular disease), epidemiologic (raccoon exposure), radiologic (white matter disease), and laboratory results (blood and CNS eosinophilia) (*1*,*10*). This study lays the foundation for future steps to educate the population of China about *B. procyonis* infection and to create programs to prevent the spread of this disease to humans.

Acknowledgments

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Novel Divergent Rhabdovirus in Feces of Red Fox, Spain

To the Editor: Rhabdoviruses (family *Rhabdoviridae*) are enveloped single-stranded negative-sense RNA viruses belonging to the Mononegavirales order. The International Committee on Taxonomy of Viruses recognizes 11 genera (*Cytorhabdovirus*, *Ephemerovirus*, *Lyssavirus*, *Novirhabdovirus*, *Nucleorhabdovirus*, *Perhabdovirus*, *Sigmavirus*, *Sprivivirus*, *Tibrovirus*, *Tupavirus*, *Vesiculovirus*) (*1*). In addition, many recently described rhabdoviruses remain unassigned. Rhabdoviruses contain 5 major genes, encoding for nucleoprotein (N), phosphoprotein (P), matrix (M), glycoprotein (G), and RNA-dependent RNA polymerase (L). The *Rhabdoviridae* family includes pathogens of various animal species, humans, and plants. Viruses of the genus *Lyssavirus* are the most relevant to public health because they can cause rabies. Bats are the driving force within this genus; foxes and various other species of wild carnivores also can be infected with lyssaviruses and transmit them to humans and dogs (*2*).

During a viral metagenomic survey, conducted as described previously (*3*), of fecal samples collected from 4 red foxes (*Vulpes vulpes*) that were found dead in Álava, Basque Country, Spain, we identified the complete coding sequence and the partial leader and trailer sequence of a novel rhabdovirus, tentatively called red fox fecal rhabdovirus (RFFRV; 15,541 nt, GenBank accession no. KF823814; online Technical Appendix, http://wwwnc.cdc.gov/EID/article/20/12/14-0236-Techapp1.pdf) by mapping 8,287 of the 56,519 sequence reads in the sample of a red fox. A proportion of obtained reads contained sequences that were ≥99% identical to mitochondrial DNA of *V. vulpes*, which confirmed that the sample was collected from a red fox.

The obtained sequence of RFFRV was partially confirmed by specific primers and Sanger sequencing of PCR amplicons. Five major and 3 minor open reading frames (ORFs) were identified that had a genome organization similar to that of other rhabdoviruses (Figure, panel A). No significant hits were obtained by BLAST analysis (http://blast.ncbi.nlm.gov/Blast.cgi) of N, P, M, and G nucleotide and amino acid sequences, which was reported previously for novel divergent rhabdoviruses (*4*).

Predicted N, P, and M genes of RFFRV consist of 1,629, 2,490, and 813 nt, respectively, encoding for 543, 830, and 271 aa (online Technical Appendix Table 1). In addition to the absence of significant hits observed by BLAST analysis, no significant sequence homology was observed with known rhabdovirus proteins in pairwise alignments. Furthermore, no conserved motifs were detected in N, P, and M genes of RFFRV that are commonly observed in rhabdoviruses. However, intergenic regions between all major ORFs contained relatively conserved motifs that could be transcription termination/polyadenylation sequences (A/U) CU_7, similar to other rhabdoviruses (*5*). Adjacent to this termination signal was a stretch of conserved nucleotides that might function as a transcription initiation signal (online Technical Appendix Table 1).

The amino acid sequence of the G protein consisted of 669 aa and contained an N terminal signal peptide (1-MYHLIVLLVMLGQRAVA-17), a noncytoplasmic domain (aa 18–646), a transmembrane domain (647-ITAILMPLLSLAVVVGIIMCC-667), and a cytoplasmic tail of 2 aa, similar to other rhabdovirus G proteins as predicted by using Phobius and TMHMM (http://www.cbs.dtu.dk/services/TMHMM) (*6*,*7*). We predicted 3 potential glycosylation sites in the ectodomain at positions 38–40

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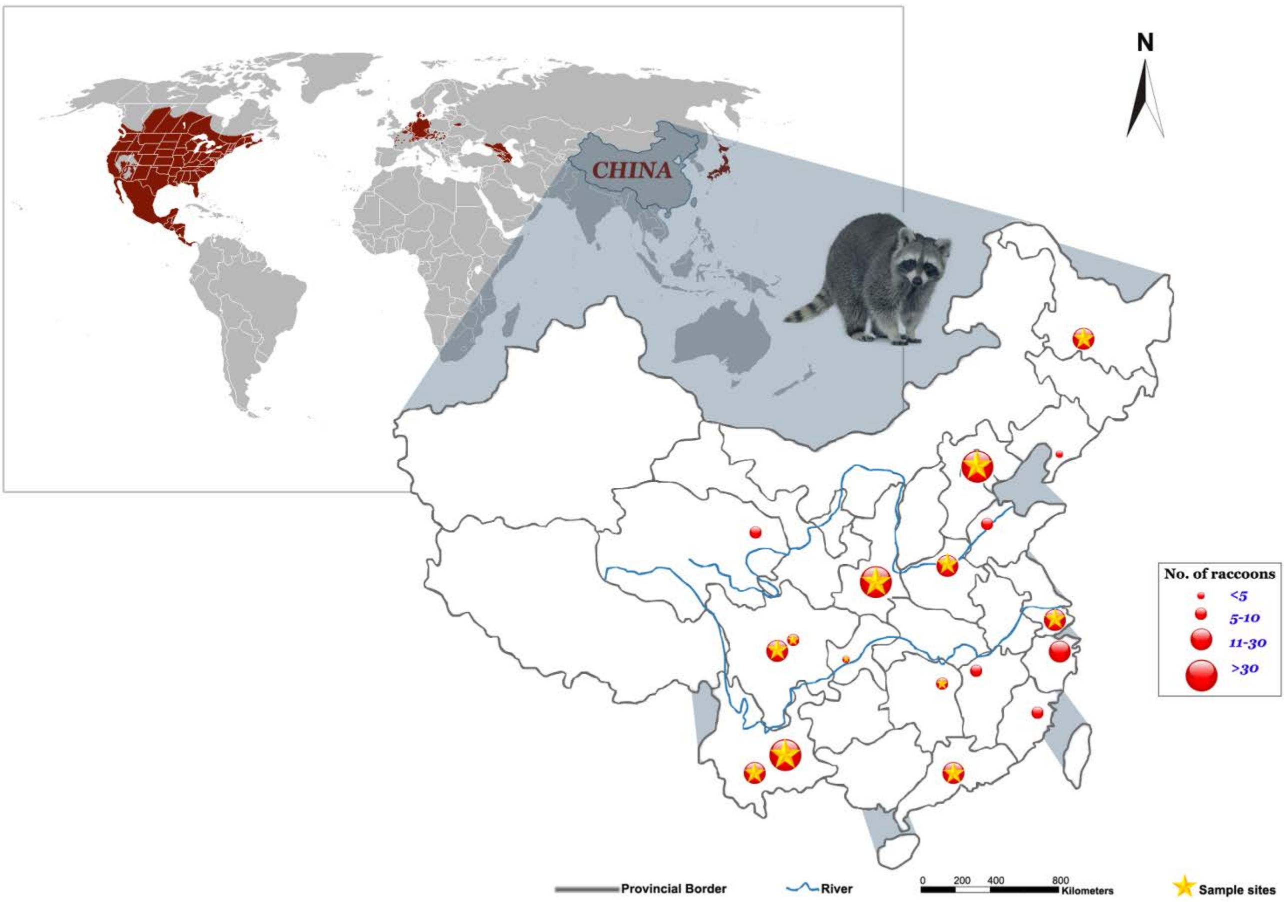
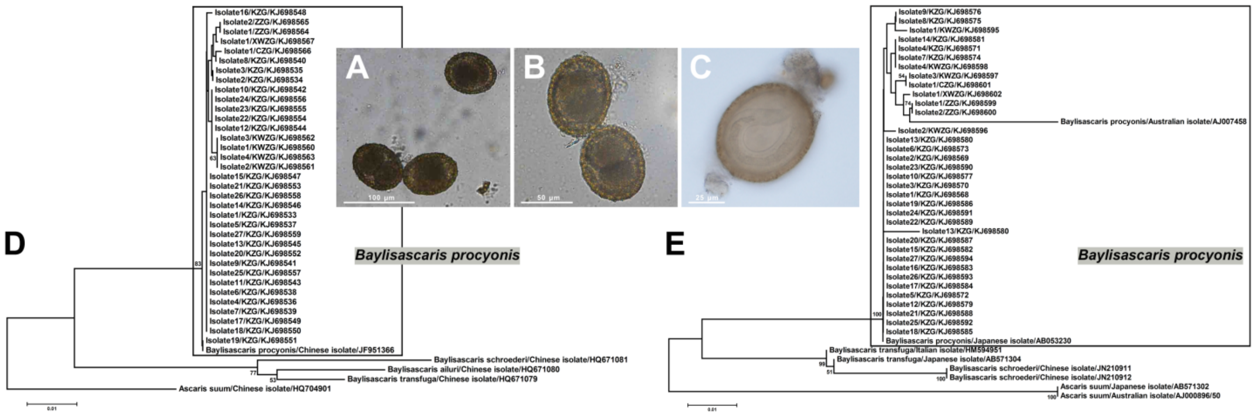
Zoonotic *Baylisascaris procyonis* Roundworms in Raccoons, China

Technical Appendix

Distribution of raccoons in China, morphological and molecular characterization of *Baylisascaris procyonis* parasitic roundworm eggs in captive raccoons in China, and the potential risk of human infection with *B. procyonis* in China.



Technical Appendix Figure 1. Distribution of raccoons in China, 2011–2013. As exotic ornamental animals, >320 raccoons are kept in 18 Zoological Gardens (ZGs) in China. The size of the red circles represents the density of raccoons in each ZG during the study period. Sampling sites are marked with yellow stars.



Technical Appendix Figure 2. Morphological and molecular characterization of *Baylisascaris procyonis* parasitic roundworm eggs in captive raccoons in China, 2011–2013. Eggs with 1–2 cells (A and B) or infective larvae (C) detected in the feces of raccoons by the flotation method with NaNO3 were identified as B. procyonis on the basis of their size and morphologic features (*1,2*). Phylogenetic relationships inferred by using GTR + I + G model-based neighbor-joining analysis on the basis of the partial sequences of mitochondrial cox-1 (D) and nuclear ITS-1 (E) from isolates of Baylisascaris species and related ascaridoids. Ascaris suum was used as an outgroup. Neighbor-joining trees were constructed using MEGA 5.0 (www.megasoftware.net). Bootstrapping with 1,000 replicates was performed to calculate the percentage reliability for each node in both genes; only values of ≥50% are shown. Horizontal branch lengths are proportional to genetic distances. Black boxes containing the 35 isolates from this study represent the B. procyonis clusters (sequences are in GenBank: KJ698533–KJ698567 for cox-1 and KJ698568–KJ698602 for ITS-1). Scale bars indicate the number of nucleotide substitutions per site. KZG, Kunming ZG; ZZG, Zhengzhou ZG; XWZG, Xi'an Wildlife ZG; CZG, Chengdu ZG; KWZG, Kunming Wildlife ZG.

A B C

Technical Appendix Figure 3. An image series showing the artificial "niche" created for raccoons in Chinese Zoological Gardens (ZGs) and depicting the potential risk of human infection with *Baylisascaris procyonis* parasitic roundworms. A) Different living environments of raccoons in the studied ZGs. B) Several typical raccoon latrines found in ZGs. C) Human-raccoon interactions, particularly between young children and raccoons. The red arrows within panel C) denote the locations of latrine sites and indicate that these sites are in close proximity to humans and therefore potentially put large numbers of infective *B. procyonis* eggs in the immediate environment of visitors.

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