Prevalence of *Alaria* infection in companion animals in north central Oklahoma from 2006 through 2015 and detection in wildlife

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**OBJECTIVE**
To determine the prevalence of *Alaria* infection in cats and dogs in north central Oklahoma over various periods and investigate whether wild animal species in this region were also infected.

**DESIGN**
Combined cross-sectional study and case series.

**SAMPLE**
Results of parasitological testing of fecal samples from 5,417 client-owned dogs and 1,246 client-owned cats (2006 through 2014); fecal samples from 837 shelter or rescue dogs and 331 shelter or rescue cats (2013 and 2014) and 268 feral cats (2015); tongue or jowl samples from cadavers of 43 wild pigs, 3 opossums, and 1 raccoon; and intestinal tract segments from cadavers of 48 cats and 5 coyotes.

**PROCEDURES**
Various parasite recovery techniques were performed to detect various *Alaria* stages in samples. Recovered adult trematodes and mesocercariae were used for PCR assay and sequencing of the 28S rRNA gene.

**RESULTS**
Prevalence of *Alaria* infection was significantly higher in feral cats (9.0%) than in shelter or rescue cats (0.6%) and client-owned cats (1.4%) and in shelter or rescue dogs (1.8%) than in client-owned dogs (0.2%). Mesocercariae were recovered from tissue samples from 11 (26%) wild pigs and 1 opossum. Amplicon sequences from adult trematodes and mesocercariae were 100% identical to each other and 99% homologous to GenBank sequences of *Alaria alata* and *Alaria mustelae*.

**CONCLUSIONS AND CLINICAL RELEVANCE**
Prevalence of *Alaria* infection in the study area has increased in dogs and cats since 1990, when infections were rare. Prevalence in wild pigs was similar to that in Eurasia, where *A alata* is considered an emerging zoonotic parasite. (J Am Vet Med Assoc 2017;250:881–886)

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Diplostomatid trematodes in the genus *Alaria* have a worldwide distribution. Domestic and wild carnivores are the definitive hosts. Adult trematodes in the small intestine pass eggs in the feces, and the eggs embryonate and hatch in fresh water. Newly hatched miracidiae penetrate susceptible species of fresh water snails (eg, *Helistoma, Planorbis, Lymnea*, and *Anisus* spp) that support asexual development of miracidiae into sporocysts and furcocercous cercariae. Cercariae penetrate the skin of tadpoles or frogs and become mesocercariae. Various species of birds, reptiles, and mammals (including humans) that ingest the infected frogs can serve as collector (paratenic) hosts.

Carnivores become infected when they ingest infected frogs or paratenic hosts or through transmammary transmission. The mesocercariae migrate to the lungs and encyst as metacercariae that are coughed up and swallowed. Recognized *Alaria* spp include *Alaria alata, Alaria mustelae, Alaria canis, Alaria arisaemoides, Alaria intermedia, Alaria taxidae, and Alaria marcianae*.

In reported cases of human alariosis in North America, *Alaria* infection was acquired through consumption of improperly cooked game meat. The only *Alaria* sp recorded to date in Europe is *A alata*. Although not definitively associated with human disease, *A alata* has been experimentally transmitted to a rhesus monkey. Over the past decade during mandatory testing of wild pigs (*Sus scrofa scrofa*) for *Trichinella* spp in the European Union, *A alata* mesocercariae have been discovered in wild pig tissues, primarily in muscle fascia and adipose tissue.

In Europe, alariosis had been classified as a low-risk zoonosis. However, with expansion of wild pig populations, an increase in the prevalence of mesocercariae in wild pigs, an increase in human consumption of meat from wild pigs, changes in cooking methods, and recognition of severe and fatal disease in hu-
mans, *A alata* is now considered an emerging threat to human health.1,6,14–20

Surveillance of *Trichinella* spp in the United States is not mandatory and does not include wild pig populations.21 Control has been achieved in domesticated pigs (*Sus scrofa domesticus*) through enforcement of laws that prevent feeding of raw garbage and through improvements in biosecurity and hygiene in confinement-type swine production units. Serologic testing (ELISA) is used in swine production herds to determine *Trichinella* status. No information is available on the role of wild pigs as a source of human infection with *Alaria* mesocercariae in the United States.

Efforts to control expanding wild pig populations in the United States through regulated hunting and trapping have been unsuccessful.22–23 This population expansion is believed to be due to periodic release of wild pigs from Eurasia into different areas of the United States for hunting and the escape of domesticated pigs that subsequently interbreed with these released wild pigs.22,23 Adaptability of wild pigs is related to their omnivorous feeding habits, high reproductive potential, intelligence, and reclusive nature. They are considered large game and are used for hunting purposes and human consumption in many parts of the world.20,24

Studies1,6,13,18,25 in Europe have revealed a high prevalence of *A alata* infection in red fox and wolf populations, which are also believed to be expanding. In North America, most reports4,5,26–32 of *Alaria* infections in domesticated and wild carnivores pertain to Canada, the northern United States, and Florida. In a study53 of endoparasitism in client-owned dogs conducted in north central Oklahoma from 1981 through 1990, no *Alaria* ova were detected in 12,515 fecal samples. The purpose of the study reported here was to obtain an updated estimate of the prevalence of *Alaria* infection in various dog and cat populations and to determine whether infection existed in wildlife reservoir hosts, primarily wild pigs, in north central Oklahoma.

**Materials and Methods**

**Animals and samples**

This study involved a retrospective and a prospective portion. For the retrospective portion, all results of parasitological examination of fecal samples from client-owned dogs and cats submitted to the Boren Veterinary Medical Hospital of Oklahoma State University or the Oklahoma Animal Disease Diagnostic Laboratory from 2006 through 2014 were included.

In the prospective portion approved by the Institutional Animal Care and Use Committee of Oklahoma State University, fecal samples were collected from shelter or rescue dogs and cats for 2 years (2013 and 2014) through a newly implemented spay-neuter program of the Boren Veterinary Medical Hospital. During this 2-year period, additional fecal samples from rescue cats were provided by 2 private feline practitioners located within 50 miles of the study area of Stillwater, Okla. Fecal samples collected from trapped feral cats as part of the teaching hospital’s neuter-and-release program, Operation Catnip, in 2015 were also included in the prospective portion of the study.

Forty-six cadavers of shelter cats, 1 stray cat, and 1 client-owned cat submitted to the Oklahoma Animal Disease Diagnostic Laboratory for disposal between March and July 2015 and 5 cadavers of coyotes that had recently been hit by cars and submitted to the same laboratory between September 10, 2014, and March 5, 2015, were included in the study. Also included were tongues and jowl tissues collected from 43 wild pigs through the cooperation of the USDA APHIS Wildlife Service during routine hunting and disease surveillance in 6 Oklahoma counties (Jefferson, Love, Osage, Pawnee, Pittsburg, and Tillman) between February 23 and May 12, 2015, as well as tongue and jowl tissues from 1 raccoon and 3 opossums that had been recently hit by cars. Tissues were shipped on ice overnight to the Oklahoma Animal Disease Diagnostic Laboratory for evaluation.

**Parasitological evaluation of fecal samples**

Historical laboratory results for fecal samples from owned cats and dogs were reviewed retrospectively for findings of *Alaria* ova. All other fecal samples were prospectively evaluated specifically for the study. For all fecal samples, including those represented in the retrospective portion of the study, a centrifugal fecal flotation technique with Sheather sucrose or 33% zinc sulfate solution was used to evaluate samples for evidence of parasites.34 Slides prepared in this manner were examined at 100X and 400X magnification with a binocular microscope.

**Parasitological evaluation of cadavers**

Cadavers were processed and examined for gastrointestinal parasites by use of the following technique. Intestinal tracts were removed. Segments (stomach, small intestine, and colon) were separated and placed into individual buckets. Each segment was opened into a bucket and washed with running tap water. Washed intestinal contents were allowed to sit undisturbed for 15 to 30 minutes, and the supernatant was carefully decanted. This procedure was repeated until the supernatant fluid appeared clear. Portions of sediment from each segment were transferred to clear glass pie plates, and grossly detectable parasites were removed and stored in jars containing 70% ethanol at 4°C. The sediment was then examined in 95 X 15-mm disposable polystyrene Petri dishes with a dissecting microscope (0.8X to 5.6X objective lenses) until all of the sediment from each segment had been examined and all parasites recovered. Trematode parasites from the intestinal tracts were identified by use of a taxonomic key.35

**Parasitological evaluation of tissue samples**

Individual tissue samples were evaluated with the mesocercariae migration technique.36 Samples
were weighed and cut into 2.5-cm³ cubes, then placed in an electric meat grinder. The ground tissue (47.5 to 525.0 g) was wrapped in 2 layers of cheesecloth, placed in disposable champagne glasses, and submerged in warm tap water for 90 minutes. Fluid from the stem ends of the glasses was transferred into a Petri dish by use of a disposable 5-mL pipet and examined with a dissecting microscope (0.8X to 5.6X objective lenses) for motile mesocercariae. Samples in which motile mesocercariae were not identified were allowed to sit overnight and reexamined. Equipment was washed with soap and water between each sample to prevent contamination.

**Molecular evaluation of Alaria spp**

Total DNA extraction and amplification of the partial 28S rRNA gene were conducted on 2 of 11 isolates of Alaria mesocercariae recovered from tissue samples from wild pigs, Alaria mesocercariae recovered from tissue samples from 1 opossum, and adult Alaria trematodes recovered from cadavers of 1 coyote, 1 stray cat, and 1 client-owned cat as described elsewhere. Three to 5 parasite stages/sample were used. The forward primer 5′-CTTAGCTGCGGTTCCTGCT-3′ and reverse primer 5′-CGGCACATAAGCAAATACCTCG-3′ were used for PCR amplification. Amplicons were sent for sequencing and alignment.

**Statistical analysis**

Prevalence of Alaria spp infection among various animal groups was compared by means of the χ² test. Values of P ≤ 0.05 were considered significant.

**Results**

Over the 9-year period, 9 of 5,417 (0.2%) fecal samples from client-owned dogs and 17 of 1,246 (1.4%) fecal samples from client-owned cats contained Alaria ova that measured 98 to 134 µm X 62 to 68 µm (Figure 1). Prevalence varied from year to year and ranged from 0% for both dogs (0/285) and cats (0/59) in 2006 to 0.3% (2/705) for dogs in 2013 and 2.3% (3/131) in cats in 2010. In 2013, 12 of 503 (2.4%) fecal samples from shelter or rescue dogs and 1 of 84 (1.2%) fecal samples from shelter or rescue cats contained Alaria ova. For 2014, these numbers were 3 of 334 (0.9%) and 1 of 247 (0.4%), respectively. Overall prevalence of Alaria infection in shelter or rescue dogs and cats for both years was 1.8% (15/837) and 0.6% (2/331), respectively. Twenty-four of 268 (9.0%) fecal samples collected in 2015 from trapped feral cats were positive for Alaria ova.

The prevalence of Alaria infection in feral cats was significantly (P < 0.001) greater than the prevalence in both shelter or rescue cats and client-owned cats. The prevalence of Alaria infection was also significantly (P < 0.001) greater in shelter or rescue dogs than in client-owned dogs.

Presence of Alaria spp in north central Oklahoma was further confirmed when the cadavers of a client-owned cat (May 2014) and a stray cat (July 2014) were processed for adult parasite recovery. Several 1.5- to 1.7-mm-long trematodes with distinct, spoon-shaped forebodies that contained oral and ventral suckers, a bulbous tribocytic organ, and 2 anterior spike-like pseudosuckers were recovered from the small intestinal contents (Figure 2). The flukes had cylindrical hind bodies that contained the reproductive organs. Typical Alaria ova were also identified via zinc sulfate centrifugal flotation of fecal samples collected from these cats. Five of 5 cadavers of coyotes that had been hit by cars from September 2014 through May 2015 were also found to harbor numerous adult Alaria trematodes in the small intestines. No adult trematodes were found in the intestinal tracts of the cadavers of 46 urban shelter cats submitted for disposal.

Mesocercariae were detected in tissues from 11 of 43 (26%) wild pigs and 1 of 3 opossums (Figure 3). These 11 wild pigs originated from 4 of 6 Oklahoma counties (Jefferson, Osage, Pawnee, and Tillman). The opossums and raccoon originated from Payne County. Distinct bands (approx 250 bp in length) were detected via agarose gel electrophoresis of PCR products from 3 samples of mesocercariae collected from the cadavers of 2 wild pigs and 1 opossum and adult flukes collected from the cadavers of 2 cats and 1 coyote. Amplicon sequencing and subsequent alignment revealed 100% sequence identity among the 6 animals.

![Figure 1—Photomicrograph of unstained Alaria ova recovered via zinc sulfate centrifugal flotation from the fecal sample of a dog. Bar = 20 µm.](image-url)
with 99% homology to GenBank sequences for *A alata* and *A mustelae*.

**Discussion**

In the present study, an increase in the prevalence of *Alaria* infection was detected in dogs and cats in north central Oklahoma since last measured in 1990. This increase was particularly high in free-roaming cats. The presence of *Alaria* spp (adult trematodes) in the study area was further confirmed in the cadavers of 1 client-owned cat, 1 stray cat, and 5 coyotes and in tissue samples (mesocercariae) from 1 opossum and 11 wild pigs. Recovery of *Alaria* ova in client-owned dogs and cats of the present study was unexpected because the parasite was not previously detected in the earlier study and was not considered endemic to the area. However, the prevalence of *Alaria* infection in these client-owned animals was generally low. A higher prevalence in shelter or rescue dogs and cats, feral cats, and coyotes in an endemic area might be expected owing to various factors. Transmammary infections in dogs and cats have been reported, free-roaming animals rely on predation and scavenging for food, and feral animals travel and hunt near accessible water sources, which is an important component in the epidemiology of *Alaria* infection because of the required snail and frog intermediate hosts.

Alariosis in definitive hosts such as dogs, cats, and wild carnivores is considered an infection of low pathogenicity, with clinical signs that are mild and nonspecific. However, these definitive hosts act as reservoirs of the adult parasite and contaminate the environment with ova.

Human infections with *Alaria* mesocercariae, acquired through ingestion of infected intermediate or paratenic hosts, can have serious sequelae and fatal outcomes. Vague and varied clinical presentations in humans and lack of noninvasive diagnostic tests make diagnosis of human alariosis difficult. Published reports of alariosis in humans often include a history of consumption of improperly cooked game meat.

The discovery of *A alata* mesocercariae in wild pigs from Europe over the past decade during mandatory testing for *Trichinella* larvae suggests that wild pig products, particularly those consumed raw or undercooked, could be a potential source of alariosis for humans. Identification of optimal methods for detection of infected carcasses and establishing risk factors for human exposure have led to several studies on the effects of tissue digestion solutions, sodium chloride and ethanol concentrations, simulated gastric solutions, curing processes, and temperatures on the viability and tenacity of *A alata* mesocercariae. In a 2% sodium chloride solution, mesocercariae were viable for up to 21 days but died within 24 hours in a 3% sodium chloride solution. Ethanol solutions from 8% to 70% were detrimental to mesocercariae viability in < 1 minute. Mesocercariae survived in simulated gastric solutions for 120 minutes. In raw, infected, homemade wild pig products prepared by curing, fermentation, cold smoking, and drying, the greatest danger of human exposure came from consumption of raw cured products (particularly certain types of sausage) within 24 hours after starting the curing process. Mesocercariae survived in meat products for 21 days at 2°C to -7°C and for 5 days at -18°C. In Ringer solution, temperatures > 60°C inactivated mesocercariae, as did 90 seconds in a microwave oven.
In the United States, the prevalence of infection with *Alaria* mesocercariae in wild pigs is unknown. In the present study conducted in north central Oklahoma, 26% of wild pigs tested were infected with *Alaria* mesocercariae, a prevalence similar to that in parts of Europe. Improperly cooked meat from infected wild pigs can be a potential source of human alariosis in the United States.

Factors that could account for emergence of *Alaria* spp in north central Oklahoma over the past 25 years likely include expansion of wild pig and coyote populations in the area; several years of drought with concentration of temporary water sources and subsequent concentration of snails, frogs, wild paratenic hosts, and wild definitive hosts around limited water sources; continuing expansion of suburban developments into wildlife habitats; or a combinations of all these factors. These factors would increase exposure risk for companion animals, particularly free-roaming dogs and cats in rural settings. Wild pigs, as paratenic hosts of *Alaria* spp and large game used for human consumption, increase the risk for human exposure.

**Acknowledgments**

The authors report that there were no conflicts of interest. Presented as a poster at the 60th Annual Meeting of the American Association of Veterinary Parasitology, Boston, July 2015, and at the 25th International Conference of the World Association for the Advancement of Veterinary Parasitology, Liverpool, England, August 2015.

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**Footnotes**

a. DNeasy Blood and Tissue Kit, Qiagen, Germantown, Md.
b. Eurofins Genomics, Eurofins MWG Operon LLC, Huntsville, Ala.
c. Clustal W2 EMBl-EBI, Wellcome Trust Genome Campus, Hinxton, Cambridgeshire, England.
d. SigmaPlot, Systat Software Inc, San Jose, Calif.

d. SigmaPlot, Systat Software Inc, San Jose, Calif.

**References**


From this month's AJVR

Assessment of contrast-enhanced ultrasonography of the hepatic vein for detection of hemodynamic changes associated with experimentally induced portal hypertension in dogs

Keitaro Morishita et al

OBJECTIVE
To assess the use of contrast-enhanced ultrasonography (CEUS) of the hepatic vein for the detection of hemodynamic changes associated with experimentally induced portal hypertension in dogs.

ANIMALS
6 healthy Beagles.

PROCEDURES
A prospective study was conducted. A catheter was surgically placed in the portal vein of each dog. Hypertension was induced by intraportal injection of microspheres (10 to 15 mg/kg) at 5-day intervals via the catheter. Microsphere injections were continued until multiple acquired portosystemic shunts were created. Portal vein pressure (PVP) was measured through the catheter. Contrast-enhanced ultrasonography was performed before and after establishment of hypertension. Time-intensity curves were generated from the region of interest in the hepatic vein. Perfusion variables measured for statistical analysis were hepatic vein arrival time, time to peak, time to peak phase (TTPP), and washout ratio. The correlation between CEUS variables and PVP was assessed by use of simple regression analysis.

RESULTS
Time to peak and TTPP were significantly less after induction of portal hypertension. Simple regression analysis revealed a significant negative correlation between TTPP and PVP.

CONCLUSIONS AND CLINICAL RELEVANCE
CEUS was useful for detecting hemodynamic changes associated with experimentally induced portal hypertension in dogs, which was characterized by a rapid increase in the intensity of the hepatic vein. Furthermore, TTPP, a time-dependent variable, provided useful complementary information for predicting portal hypertension.

IMPACT FOR HUMAN MEDICINE
Because the method described here induced presinusoidal portal hypertension, these results can be applied to idiopathic portal hypertension in humans. (Am J Vet Res 2017;78:465–471)