Pulmonary *Paragonimus* infection and other pathologic findings in feral swine (*Sus scrofa*) from Macon County, Alabama

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Summary

Tuskegee University College of Veterinary Medicine (TUCVM) was integrated into a feral swine surveillance program to aid in monitoring feral swine in Macon County, Alabama. The program was initiated by the Wildlife Services division of the Animal Plant and Health Inspection Services of the United States Department of Agriculture. Feral swine were captured, humanely euthanized, and

blood was collected for various serological analyses. The carcasses were then submitted to the TUCVM diagnostic laboratory for postmortem examination and tissues were collected for additional disease surveillance. This report highlights pathologic findings identified in 15 feral hogs captured from Macon County, Alabama between March 14, 2012 and April 16, 2013, and serves as a record of some of the diseases the feral swine

in this area harbor. Some of the pertinent pathologic findings identified include pulmonary metastrongyliasis, pulmonary paragonimiasis and severe ectoparasitism.

Keywords: swine, Alabama, feral swine, *Metastrongylus, Paragonimus*

Received: October 25, 2018 Accepted: January 23, 2019

Resumen – Infección pulmonar por *Para-gonimus* y otros hallazgos pulmonares en cerdos salvajes (*Sus scrofa*) del Condado de Macon, Alabama

El Colegio de Medicina Veterinaria de la Universidad Tuskegee (TUCVM por sus siglas en inglés) se integró a un programa de vigilancia de cerdos salvajes para ayudar en el monitoreo de cerdos salvajes del condado de Macon, Alabama. El programa fue iniciado por la división de Servicios de Fauna Silvestre de los Servicios de Inspección de Animales, Plantas y Salud del Departamento de Agricultura de los Estados Unidos. Los cerdos salvajes fueron capturados, sacrificados

humanamente, y se recolectó sangre para varios análisis serológicos. Las canales fueron entregadas al laboratorio de diagnóstico de TUCVM para la examinación post mortem y se recolectaron tejidos para vigilancia adicional de enfermedades. Este reporte resalta los hallazgos patológicos identificados en 15 cerdos capturados en el condado de Macon, Alabama entre marzo 14, 2012 y abril 16, 2013, y sirve como un registro de algunas de las enfermedades que los cerdos salvajes albergan en esta área. Algunos de los hallazgos patológicos pertinentes identificados incluyen metastrongiliasis pulmonar, paragonimiasis pulmonar y ectoparasitismo severo.

gonimus et autres trouvailles pathologiques chez des porcs sauvages (Sus scrofa) dans le comté de Macon, Alabama

Résumé - Infection pulmonaire à Para-

La Tuskegee University College of Veterinary Medicine (TUCVM) a été intégrée dans un programme de surveillance des porcs sauvages afin d'aider à surveiller les porcs sauvages dans le comté de Macon, Alabama. Le programme fut initié par la division des Services de la faune du Animal Plant and Health Inspection Services du département de l'agriculture des États-Unis. Des porcs sauvages ont été capturés, euthanasiés de façon humanitaire, et du sang prélevé pour différentes analyses sérologiques. Les carcasses furent ensuite soumises au laboratoire de diagnostic de TUCVM pour un examen post-mortem et des tissus ont été prélevés pour la surveillance de maladies additionnelles. Le présent rapport souligne les trouvailles pathologiques identifiées chez 15 porcs sauvages capturés dans le comté de Macon, Alabama entre le 14 mars 2012 et le 16 avril 2013, et sert de registre de quelquesunes des maladies rencontrées chez les porcs sauvages dans cette région. Quelques-unes des trouvailles pathologiques pertinentes identifiées incluent la metastrongylose pulmonaire, la pargonimiase pulmonaire et un ectoparasitisme sévère.

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This article is available online at http://www.aasv.org/shap.html.

Gilbreath ET, Gorham SL, Anderson DL, et al. Pulmonary *Paragonimus* infection and other pathologic findings in feral swine (*Sus scrofa*) from Macon County, Alabama. *J Swine Health Prod.* 2019;27(3):125-132.

eral swine (Sus scrofa) are highly prolific and lack natural predators. Therefore, once established, they can readily overpopulate an area. Their numbers have progressively increased to high numbers in the United States, currently estimated at over 6 million, with highest populations in Texas, California, Florida, and Hawaii.² They are also becoming more common in other regions of the nation, including Alabama. Their presence and increasing prevalence warrants concern of their potential impact on humans and other animals that share their habitat. Feral swine readily interact with and breed with domestic swine (Sus scrofa domesticus) and the ability for disease transmission between wild and domestic swine is high. 4,5 Transmissible diseases known to be harbored and display seroprevalence by feral swine in the United States include pseudorabies virus^{6,7} and zoonotic diseases such as Brucella, ⁵ Toxoplasma gondii, 8 Trichinella spiralis, 8 and influenza A virus. 9,10 Furthermore, consumption of improperly cooked products (ie, skeletal muscle and intestine) of paratenic hosts of Paragonimus, such as wild boar, could result in human and animal infection as has been previously reported. 11,12 Feral swine also have the potential to propagate foreign animal diseases, such as foot-and-mouth disease, 13 hog cholera, 14 and African swine fever. 15 In addition to the potential for disease transmission to humans and various domestic species, their natural foraging behavior can result in massive crop destruction. They are also known to prey on small mammals, including goat kids and neonatal

lambs, ¹⁶ resulting in their classification as agricultural nuisances.

In light of the growing prevalence of feral swine in Alabama and the United States^{1,17} and their ability to cause adverse effects on humans, domestic animals, and other wildlife species, monitoring these animals for pathologic conditions is paramount for public health, public education, and epidemiologic surveillance. This report highlights significant pathology identified in 15 feral swine captured in Macon County, Alabama between March 14, 2012 and April 16, 2013.

Case description

The Manually Initiated Nuisance Elimination trapping system (Jager Pro Hog Control, Fortson, GA) was used to capture feral swine. The capture devices were installed on the Russell Plantation which comprises 1687 acres of forestry land owned by Tuskegee University in Macon County, Alabama. Once pigs were captured in the trap, they were humanely killed by gunshot. All pigs appeared to be in good health based on observation of adequate activity, sufficient body condition score, and absence of external lesions or adverse clinical signs. Sterile swabs were used to obtain nasal samples and blood was collected via cardiac puncture using a 60 mL syringe with a 16-gauge, 10.16-mm needle. While in right lateral recumbency, the blood collection site was between the fourth and fifth rib behind the left elbow. Enough blood was collected from each animal to fill three 8.5 mL BD vacutainer blood tubes (Becton, Dickinson and Co, Franklin Lakes, NJ).

Serum samples were obtained and frozen until analysis by the US Department of Agriculture. Serum was analyzed for antibodies to pseudorabies virus (n = 15) via glycoprotein B enzyme-linked immunosorbent assay (ELISA), classical swine fever virus (n = 15), porcine hemagglutinating encephalomyelitis virus (n = 8), influenza A virus (n = 4), and Trichinella (n = 4) via ELISA, Toxoplasmavia ELISA (n = 4) and microagglutination assay (n = 7), and Brucella suis (n = 15) via fluorescence polarization assay. Nasal swabs were analyzed for influenza A virus (n = 7)via real-time reverse transcription polymerase chain reaction. Tests were performed at various laboratories (Table 1). Carcasses were submitted to the Tuskegee University College of Veterinary Medicine for postmortem evaluation.

Case findings

Three of 7 serum samples submitted for *Toxoplasma* microagglutination assay were positive. The 4 samples submitted for *Toxoplasma* ELISA were negative. One of 15 samples submitted for pseudorabies virus was a suspect positive. Results of all other tests previously listed were negative.

Postmortem examinations were performed on feral swine that ranged from juvenile to adult animals weighing 9 to 57 kg. There were 10 females and 5 males. Of these, 1 female was pregnant with 8 fetuses with crown-torump lengths of 25 cm, which is most consistent with a gestational age of approximately 99 days based on an established prediction equation for gestational age. ¹⁸ Feral swine

Table 1: Laboratory locations where various tests were performed on samples from 15 feral pigs captured in Macon County, Alabama

Test performed	Laboratory
Pseudorabies virus – glycoprotein B ELISA	University of Georgia Tifton Veterinary Diagnostic Laboratory, Tifton, GA and Wisconsin Veterinary Diagnostic Laboratory, Madison, WI
Classical swine fever virus – ELISA	Foreign Animal Disease Diagnostic Laboratory, Greenport, NY
Hemagglutinating encephalomyelitis virus - ELISA	National Institutes of Health, Bethesda, MD
Influenza A virus – ELISA	USDA APHIS National Wildlife Disease Program, Fort Collins, CO
Trichinella - ELISA	USDA APHIS National Wildlife Disease Program, Fort Collins, CO
Toxoplasma - ELISA	USDA APHIS National Wildlife Disease Program, Fort Collins, CO
Toxoplasma - microagglutination	USDA Agricultural Research Service, Beltsville, MD
Brucella - fluorescence polarization assay	Kansas State Federal Brucellosis Laboratory, Topeka, KS
Influenza A virus - rRT-PCR (nasal swab)	Thompson Bishop Sparks State Diagnostic Laboratory, Auburn, AL

ELISA = enzyme-linked immunosorbent assay; USDA = US Department of Agriculture; APHIS = Animal and Plant Health Inspection Service; rRT-PCR = real-time reverse transcription polymerase chain reaction.

have a gestational period of approximately 115 days and usually have 1 to 2 litters per year, with an average of 4 to 8 piglets per litter.⁴

All 15 pigs were infected with numerous ectoparasites; 46 ectoparasites were examined. Of the 36 ticks, 33 (91.7%) were identified as *Amblyomma americanum* and 3 (8.3%) were identified as *Dermacentor variablis*. The ticks were characterized by a cephalothorax and 4 pairs of legs. ¹⁹ Ten *Haematopinus suis* lice, (21.7% of the total ectoparasites collected) were identified. Lice had a distinct head, thorax, abdomen, and 3 pairs of legs (Figure 1).²⁰ Speciation of ectoparasites was done by a veterinary parasitologist via observation through a dissecting microscope.

Intrabronchial and intrabronchiolar nematodes were macroscopically observed in 7 of 15 pigs (46.7%). In pigs with pulmonary nematodes, lung color was diffusely mottled, variably firm on palpation, and were associated with tracheobronchial lymph node hyperplasia. The pulmonary nematodes were white, thin, cylindrical, and 4 to 6 cm in length (Metastrongylus species; Figure 2). Histologically, bronchi and bronchioles contained cross-sections of intraluminal nematodes that measured 500 to 700 µm. They contained a body cavity, thin cuticle, coelomyarian musculature, intestinal tract lined by few multinucleated cells, and ovaries and uterus filled with oocytes and developing larva (Figure 3). Bronchi and bronchioles contained moderate amounts of intraluminal edema, fibrin, and mucus admixed with predominately eosinophils and fewer macrophages, lymphocytes, plasma cells, and neutrophils. Occasional free nematode eggs were present in bronchi. Bronchial and bronchiolar epithelium was hyperplastic with goblet cell metaplasia. There was marked peribronchial and peribronchiolar smooth muscle hypertrophy and bronchial associated lymphoid tissue hyperplasia. There were multifocal to coalescing areas of alveolar capillary congestion with associated intra-alveolar edema. To confirm species identity of the pulmonary nematodes, genomic DNA was extracted from 0.1 g of formalin-fixed paraffin embedded (FFPE) lung tissue.

Briefly, pulverized FFPE samples were suspended in 600 μ L of TSK buffer (567 μ L of TE buffer [10 mM Tris and 1 mM EDTA, pH = 7.5], 30 μ L of 10% sodium dodecyl sulfate, and 3 μ L of 20 mg/mL proteinase K)

Figure 1: Representation of arthropod ectoparasites on the feral pig carcasses. The larger ectoparasite on the left is a female *Amblyomma americanum* tick, the middle ectoparasite is a male *A americanum* tick, and the ectoparasite on the right is a *Haematopinus suis* louse.



Figure 2: Moderate numbers of white cylindrical *Metastrongylus* parasites (indicated by arrow) lie on the mucosal surface of 3 bronchi in the lung of a feral pig.



and incubated at 50°C overnight. Then, 114 μL of 5 M NaCl and 91 μL of 1 M NaCl and 10% (vol/vol) hexadecyltrimethylammonium bromide mixture were added. After a 15-minute incubation at 65°C, DNA was extracted twice with an equal volume of phenol-chloroform-isoamyl alcohol (25:24:1, vol/vol) and then once with an equal volume of chloroform-isoamyl alcohol (24:1, vol/vol). After precipitation with 900 µL of isopropanol, the DNA was washed with 70% (vol/vol) ethanol and resuspended in TE buffer. The lung samples came from 9 different pigs. Conventional polymerase chain reaction (PCR) was performed on 20 ng of DNA using primer sets specific for detection of Metastrongylus salmi, Metastrongylus pudendotectus, Metastrongylus elongatus (apri), Paragonimus westermani, and Paragonimus kellicotti (Table 2). The cycling conditions included an initial denaturation for 1 minute at 95°C, 35 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, elongation at 72°C for 30 seconds, and a final extension step at 72°C for 5 minutes. The amplified gene fragments were sequenced and found to match the gene sequences reported in the GenBank corresponding to the genes' accession numbers. The PCR amplification targeted the 28s ribosomal RNA gene for M salmi and M pudendotectus, and the 18s ribosomal RNA gene for M elongatus (GenBank accession numbers AJ305404, AF210046 and AJ920363, respectively). As loading control, all samples were positive for S scrofa 12s ribosomal RNA gene (Figure 4A; GenBank accession number EF027294). Among the 9 samples tested by PCR, 8 were positive for M salmi (Figure 4B), while all 9 samples were

negative for both *M pudendotectus* and *M elongatus*.

An intrapulmonary trematode was observed in a pig that did not have grossly observable pulmonary nematodes. The trematode was 1.5 cm long, brown, flat, and tapered at both ends, consistent with *Paragonimus* species. Microscopically, the trematode cross section was 8×3 mm², contained multiple cuticular ridges and spines, had a body filled with parenchyma, and contained peripherally

located vitelleria (Figure 5A). Ceca, testes with mature sperm (Figure 5B), and a uterus with yellow-shelled eggs (Figure 5C) were present. Histologically, the lung of the pig infected with pulmonary trematodes contained multinodular aggregates of innumerable oocytes surrounded by thick bands of fibrosis (Figure 6). The oocytes were ovoid, 50 to 70 \times 80 to 100 μm^2 with a distinct 3 μm thick, yellow-pigmented refractile cell wall, typical of trematode eggs. A single operculum with opercular ridges was occasionally evident

Figure 3: Cross sections of nematode parasites in a pulmonary bronchiole of a feral pig. There is a body cavity (asterisk), digestive tract (arrow), cuticle (arrowhead), and an ovary filled with eggs (hashtag). H&E stain; Magnification \times 100.

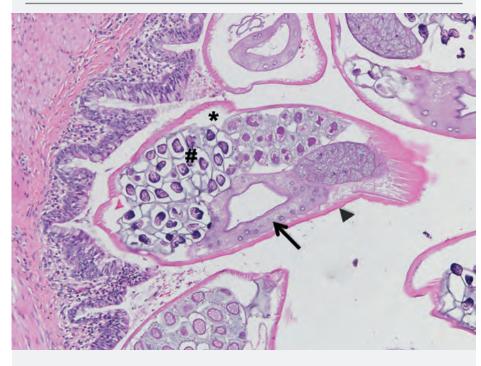


Table 2: Primer pairs used for detection of pulmonary parasites by polymerase chain reaction

Genus and species tested	Primer pairs
Metastrongylus salmi	Forward 5'-TTCAGGGTTGTTAACGAT-3' and Reverse 5'-TTGCTTGAACGGGTAA-3'
Metastrongylus pudendotectus	Forward 5'-CAGTGACCGGGTCGGTT-3' and Reverse 5'-TCCGTACCAGTTCCA-3'
Metastrongylus elongatus	Forward 5'-TGCATGTCGAGTTCAACTTC-3' and Reverse 5'-ATGCTGCGTTATTCAGAGTC-3'
Paragonimus westermani	Forward 5'-AGGCAATGTGGTGTTCAGGT-3' and Reverse 5'-ATCGGACTCGTGCAAGTA-3'
Paragonimus kellicotti	Forward 5'-ATATTGCGGCCACGGGTTA-3' and Reverse 5'-ACGTGGCACATACATAGATCA-3'
Sus scrofa	Forward 5'-AAACTGGGATTAGATACCCCA-3' and Reverse 5'-AGAACAGGCTCCTCTAGGT-3'

(Figure 7). The observed opercular ridges are a characteristic feature of Paragonimus species oocytes.²¹ Oocytes were surrounded by moderate to numerous lymphocytes, plasma cells, and macrophages, which often contained abundant brown granular pigment (hemosiderin) and few to moderate scattered eosinophils. In the surrounding lung tissue, within bronchi, bronchioles, and alveoli, there were small to moderate numbers of eosinophils, lymphocytes, plasma cells, and histiocytes, which were sometimes infiltrating peribronchiolar regions. The interstitium was mildly expanded by macrophages. Bronchioles and alveoli occasionally contained small amounts of hemorrhage, fibrin, and edema. There was a focally extensive abscess with a central area of necrosis, hemorrhage, and numerous oocytes admixed with numerous degenerate and few viable eosinophils, lymphocytes, plasma cells, macrophages, and neutrophils. The central necrosis was surrounded by lymphocytes, plasma cells, and

macrophages and rimmed by fibrosis which was further lined by hemorrhage, fibrin, and edema. The PCR was performed on DNA extracted from the 9 samples which included the FFPE trematode-infected lung tissue using primer pair for P westermani 28s ribosomal RNA gene (GenBank accession number HM172630) and P kellicotti 28s ribosomal RNA gene (GenBank accession number HQ900670). Among the 9 samples tested, 2 were positive for P westermani (Figure 4C) and P kellicotti was not detected. These results suggest that the trematode P westermani was present. Because this species of Paragonimus is not endemic in North America, additional ancillary diagnostics should be performed in future studies to definitively confirm the presence of P westermani in our samples. The PCR results do, nonetheless, confirm the macroscopic and microscopic diagnosis of Paragonimus.

One pig (6.7%) had a tortuous nematode within the mucosa of the dorsal tongue. The

nematode in the tongue was histologically observed in cross-section within the lingual epithelium. It was 60 to 70 µm in diameter and had a 5 to 7 µm cuticle, platymyarian musculature, a body cavity, and contained a small intestine and a uterus with unembryonated, thin-shelled eggs (Figure 8). The most common nematode in the tongue of the wild pig is *Eucoleus (Capillaria) garfiai*,²² and is the likely species in this case. This was considered an incidental finding of minimal pathologic significance.

In 1 pig (6.7%), there was a firm, red, $2 \times 2 \times 2$ cm³, nodular area of consolidation that extended into the underlying pulmonary parenchyma. Microscopically this was an area of lymphofollicular hyperplasia. Immunohistochemistry confirmed the presence of a mixed population of B and T lymphocytes, ruling out pulmonary lymphoma.

Additional incidental findings included 3 pigs with enlarged inguinal lymph nodes, 1 pig

Figure 4: Conventional polymerase chain reaction of formalin-fixed paraffin-embedded lung tissue from feral pigs for parasite identification. (A) All samples were positive for *Sus scrofa* 12s ribosomal RNA. (B) Rows 1-3 and 5-9 were positive for *Metastrongylus salmi*. Row 4 was negative for *M salmi*. All samples were negative for *Metastrongylus pudendotectus* and *Metastrongylus elongatus*. (C) Among the 9 samples tested from the lung tissue of the wild pig with the pulmonary trematode, 2 samples (rows 4 and 6) were positive for *Paragonimus westermani*.

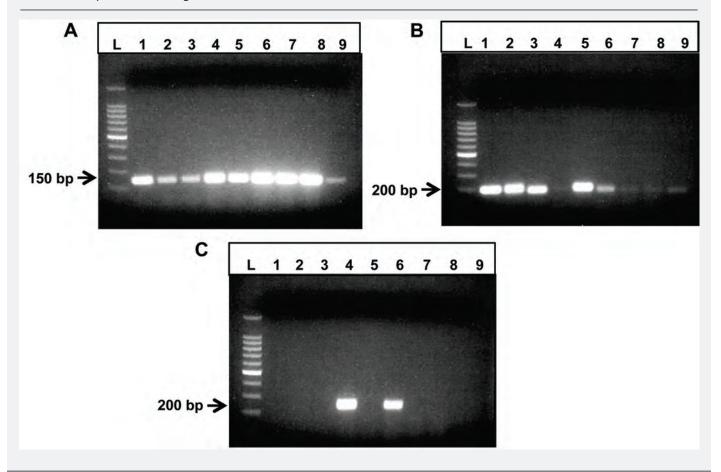
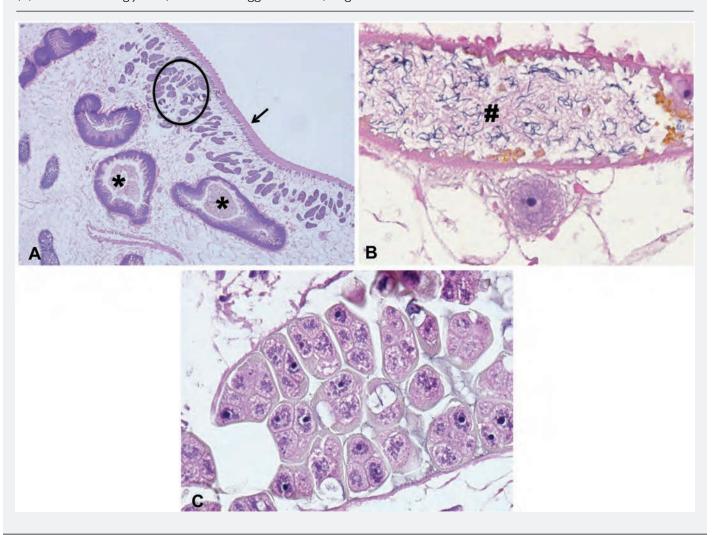


Figure 5: Sections of a trematode obtained from the lung of a feral pig. (A) The trematode has a thin cuticle with cuticular ridges and spines (arrow), prominent subcuticular vitellaria (circle), multiple cross-sections of a digestive tract (asterisk), and absence of a coelomic space. H&E stain; magnification \times 40. (B) Testis filled with mature sperm (hashtag). H&E stain; magnification \times 600. (C) Uterus containing yellow, thick-shelled eggs. H&E stain; magnification \times 400.



with englarged mandibular lymph nodes, and 1 pig with enlarged mesenteric lymph nodes. Other significant macroscopic and microscopic findings were not observed.

Discussion

Feral swine in Macon County, Alabama were infested with several external parasites: the *H suis* louse, *D variablis* tick (American dog tick), and *A americanum* tick (Lone star tick). *Haematopinus suis* can transmit swine pox virus²³ and *Mycoplasma suis*,²⁴ *D variablis* can transmit Rocky Mountain Spotted Fever (*Rickettsia rickettsia*)²⁵ and tularemia (*Francisella tularensis*),²⁶ and *A americanum* can transmit tularemia, *Ehrlichia chaffensis*, and *Ehrlichia eweingii* (the cause of human ehrlichiosis).²⁷ Feral swine in Macon County, Alabama were infected with *Metastrongylus* organisms, which is the pig lung worm that

can be transmitted between feral and domestic pigs. A novel finding in this case was the presence of the lung fluke, a Paragonimus organism. This trematode was macroscopically and microscopically observed in 1 pig; however, PCR detected DNA for P westermani in this pig as well as in 1 additional pig. This suggests that even though the trematode was not observed macroscopically or microscopically, the infection was still present. Paragonimus organisms are zoonotic agents that can be transmitted to humans and other animal species. Transmission commonly occurs by way of consuming infected crayfish, which are intermediate hosts, but can also occur through consumption of undercooked meat from paratenic hosts, such as feral swine. 11,12,21 Antibodies to Toxoplasma organisms were detected in 3 pigs. This indicates that the animals were

exposed to this organism and generated an immune response. *Toxoplasma* is a zoonotic protozoan parasite that can be transmitted to humans and other homeothermic species via ingestion of oocysts from feline (definitive host) feces, ingestion of bradyzoites in tissues of intermediate hosts (ie, wild pigs), and transplacentally.²⁸ Transplacental transmission can cause fetal infection and abortion²⁹; therefore, pregnant women should be especially cautious when handling feral swine tissues.

It is important to be aware of diseases prevalent in feral swine that may come in contact with domestic animals and humans. Proper precautionary measures should be taken when coming in contact with feral swine, thereby minimizing the risk of disease transmission.

Implications

The sample of feral swine in Macon County, Alabama were:

- infested with ectoparasites that can transmit a variety of diseases to domestic swine, other animal species, and humans.
- infected with endoparasites that are transmissible to domestic swine, other animal species, and humans.
- serologically positive for *Toxoplasma* which is transmissible to domestic swine, other animal species, and humans.

Acknowledgements

Appreciation is extended to the Auburn College of Veterinary Medicine Histopathology Laboratory for performing immunohistochemistry for this study. This study was supported by US Department of Agriculture's Animal and Plant Health Inspection Service – Wildlife Services Cooperative Agreement (16-7100-0357-CA) and the National Institutes of Health-Tuskegee University Research Centers in Minority Institutions Core Facility (Grant G12MD007585).

Conflict of interest

None reported.

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Figure 6: In the lung of a feral pig, aggregates of trematode oocytes have effaced normal lung architecture and are separated by bands of fibrous tissue and associated with inflammatory cells, hemorrhage, and hemosiderin-laden macrophages. Oocytes are round to oval with thick refractile walls. H&E stain; magnification \times 40.

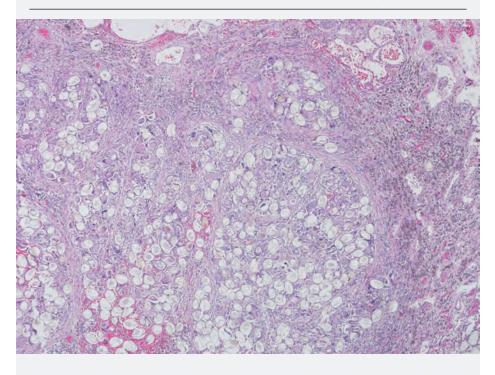
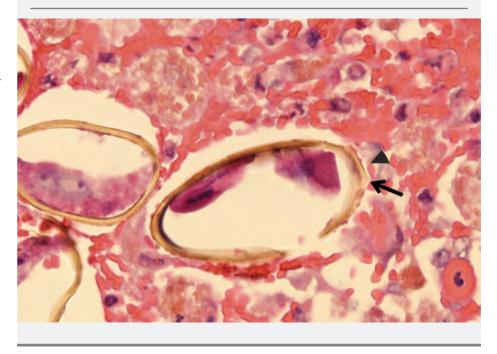
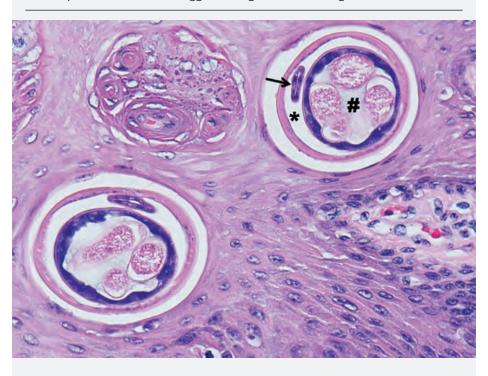


Figure 7: A trematode oocyte with a single operculum (arrowhead) and opercular ridges (arrow) in the lung of a wild pig. H&E stain; magnification \times 600.



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Figure 8: Cross sections of a nematode in the lingual mucosa of a feral pig. The nematode contains a body cavity (asterisk), digestive tract (arrow), and uterus with unembryonated, thin-shelled eggs (hashtag). H&E stain; magnification \times 400.



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