Examination of Wild Birds and Feral Hogs from Oklahoma, USA, for Infection with *Trichinella*

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Abstract: *Trichinella* species infect many groups of vertebrates throughout the world. Data for *Trichinella* in wildlife in Oklahoma, USA, is limited, and key reservoir hosts have yet to be identified. We performed artificial digestions on feral hogs (*Sus scrofa*; n=42) and wild birds (19 species; n=36) from Oklahoma to detect the presence of *Trichinella* larvae. Artificial digestions involved heating and mixing host tissue in pepsin and hydrochloric acid and mimic the excystment of larvae in the host bowels once tissue is consumed. Additionally, a subset of feral hogs (n=31) was examined for antibodies to *Trichinella* spp. Among all wildlife species examined, no *Trichinella* spp. larvae were detected in the tissues via digestions. However, ELISA testing detected antibodies to *Trichinella* spp. in 2 of 31 (6.5%) feral hogs. These relatively low seroprevalence levels in feral hogs are consistent with other reports of *Trichinella* in Oklahoma wildlife and suggest feral hogs may not be key reservoir hosts in the sylvatic cycle. Differences in results from the two detection methods we used indicate the importance of utilizing an integrative approach when examining animals for *Trichinella* infections. Further surveillance in bird populations is necessary to adequately determine whether birds are serving as reservoirs for *Trichinella* species.

Introduction

The genus *Trichinella* includes species of nematodes infecting various groups of vertebrates throughout the world. To date, 13 species and/or genotypes of *Trichinella* have been described from carnivores, omnivores, and scavengers

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(Pozio and Zarlenga 2013; Sharma et al. 2020). Reports of *Trichinella* predominantly occur in wild mammals, comprising over 100 species of mammals from 11 orders. Additionally, all attempts to experimentally infect mammals with *Trichinella* have been successful, hinting at this parasite group's ability to infect and survive in new hosts, including domestic animals (Pozio and Murrell 2006). Therefore, the lack of reports in certain mammal species is likely a result of geographical limitations to host-parasite contact. Novel host infections and range expansions appear to be a result of human activity, specifically where poor feeding and farming practices (exposure to infected meat) exist (Pozio and Murrell 2006; Crisóstomo-Jorquera and Landaeta-Aqueveque 2022).

Species of *Trichinella* are known to have a complex, two host life cycle. Briefly, when tissue from an infected host that contains *Trichinella* larvae is ingested by another host, larvae are released and develop into adults in the small intestine. From here, male and female worms mate, release larvae that migrate to muscle, and become encapsulated or non-encapsulated inside muscle cells, until they are ingested by the next host (Pozio and Murrell 2006; Rawla and Sharma 2022). Unlike the majority of nematode species, transmission is strictly via trophic transfer, including predation, cannibalism, or scavenging. Two types of *Trichinella* life cycles exist: one in wildlife (sylvatic cycle), predominantly carnivores, and one in domestic animals (domestic cycle), predominantly scavengers. The difference between these types of life cycles is merely dependent on the context of the host species (i.e., wildlife vs domestic animals) and whether they become infected and transmit the parasite to subsequent hosts. Sylvatic cycles utilize many more host species than in domestic cycles and are the primary origin of human infections in the United States (Pozio and Murrell 2006; Casillas and Jones 2017). Humans, although dead-end hosts, can become infected with *Trichinella* if raw or undercooked meat containing larvae are ingested. In general, *Trichinella* appears to be a very opportunistic genus of nematodes, with a low degree of host specificity.

Strict government regulation as well as improved education has greatly reduced the transmission of *Trichinella* to humans, yet it remains an important zoonotic parasite due to its constant widespread existence (Pozio and Murrell 2006; Pozio and Zarlenga 2013; Crisóstomo-Jorquera and Landaeta-Aqueveque 2022). Despite the low number of human cases of trichinellosis (i.e., the disease resulting from *Trichinella* infection) in the United States, cases have remained relatively constant in recent years (Centers for Disease Control and Prevention 2019). Wild animals such as black bears and feral hogs have been implicated as the most common sources of infection (Casillas and Jones 2017). Birds have also rarely been reported to be infected with *Trichinella pseudospiralis* larvae, and these infections tend to have a patchy distribution in the United States (Pozio and Murrell 2006; Pozio and Zarlenga 2013). The role of birds as hosts in sylvatic cycles remains unclear but may be important due to their high mobility, allowing birds to facilitate the spread of *Trichinella* into new hosts and geographical areas. The rarity of *Trichinella* infection reports in birds may be due at least in part to limited sampling effort, emphasizing the need for increased surveillance efforts. Although studies have tested wildlife for *Trichinella* in Oklahoma (e.g., coyotes, *Canis latrans*, Reichard et al. 2011; bobcats, *Lynx rufus*, Reichard et al. 2021; feral hogs, *Sus scrofa*, Hill et al. 2014), this U.S. state remains under-sampled in general. Additionally, no studies have examined birds from Oklahoma for *Trichinella*. Therefore, the objective of this study was to survey feral hogs and various bird species from Oklahoma for *Trichinella*.

Methods

Bird carcasses used in this study were collected in two different ways. A subset of bird carcasses were collected in Stillwater, Oklahoma (Payne County), USA, as part of permitted research on bird-window collisions (Riding et al. 2020) and under appropriate federal and state permits (see acknowledgements for permit numbers). These birds included residents and migrants and were collected between April and

October of 2015-2017. They capture a variety of species, most of which were passerines (e.g., American robin, northern cardinal, and scissor-tailed flycatcher), but also include non-passerines (e.g., mourning dove and yellow-billed cuckoo). Additional birds were obtained as donations from the Oklahoma State University (OSU) Zoological Medicine service from Kay, Lincoln, Noble, and Payne Counties, Oklahoma throughout the year between 2015–2018 (Table 1); this sample included a similar mix of passerine and non-passerine species, but was notable in also including raptors (e.g., Mississippi kite, turkey vulture, and barred owl). The animals donated by the OSU Zoological Medicine service were injured animals presented for wildlife rehabilitation that were euthanized due to their injuries, unrelated to this study. Pectoralis muscle from birds was removed with forceps and a scalpel by dissection. Tongues and jowl tissues were obtained from feral hogs (*Sus scrofa*) through the United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) Wildlife Services during routine hunting and disease surveillance from Jefferson, Love, Osage, Pawnee, Pittsburg, and Tillman Counties, Oklahoma between March and April, 2015 (Johnson et al. 2017). All bird and hog tissues were transported on ice to the Oklahoma Animal Disease Diagnostic Laboratory and frozen at -18°C until evaluation.

For detecting *Trichinella* spp. infections, tissues from birds and hogs were thawed and immediately used for artificial digestions (Reichard et al. 2017). Briefly, tissues were weighed (to the nearest 0.1 g) and homogenized using a Polytron (Kinematica GmbH, Kriens-Luzern, Switzerland). Samples were then mixed with 10 ml of artificial digestive fluid (1% pepsin, 1:10,000 IU, and 1% hydrochloric acid) per gram of tissue and mixed on stir plates at 37°C for 30 min. After 30 min, samples were cooled on ice and allowed to settle for 20 min, in which the top transparent supernatant layers were then carefully removed and resuspended in tap water. This washing process was repeated 3–5 times depending on the amount of cellular debris, and larvae were searched for under a stereomicroscope at 20–40x magnification. All averages include ± 1 standard deviation and range, and prevalence include 95% confidence intervals.

Sera from a subset of hogs were tested for antibodies to *Trichinella* spp. using a

Table 1. Potential hosts from Oklahoma examined for *Trichinella* spp. via artificial digestion and ELISA methods. County names in bold indicate counties where antibodies to *Trichinella* spp. were detected in feral hogs.

Host class	Host order	Host species	Oklahoma county collected (sample size)	Hosts infected /hosts examined: via digestions	Hosts infected /hosts examined: via ELISA
Mammalia	Artiodactyla	Feral hog (*Sus scrofa*)	Jefferson (6), **Love** (4), **Osage** (13), Pawnee (9), Pittsburg (4), Tillman (6)	0/42	2/31
Aves	Accipitriformes	Mississippi kite (*Ictinia mississippiensis*)	Noble (1)	0/1	–
Aves	Accipitriformes	Red-tailed hawk (*Buteo jamaicensis*)	Kay (1)	0/1	–
Aves	Cathartiformes	Turkey vulture (*Cathartes aura*)	Lincoln (1), Payne (2)	0/3	–
Aves	Columbiformes	Mourning dove (*Zenaida macroura*)	Payne (1), Unknown (1)	0/2	–
Aves	Cuculiformes	Yellow-billed cuckoo (*Coccyzus americanus*)	Payne (2)	0/2	–
Aves	Falconiformes	American kestrel (*Falco sparverius*)	Lincoln (1), Payne (1)	0/2	–
Aves	Passeriformes	Eastern meadowlark (*Sturnella magna*)	Payne (1)	0/1	–
Aves	Passeriformes	American robin (*Turdus migratorius*)	Payne (8)	0/8	–
Aves	Passeriformes	Common grackle (*Quiscalus quiscula*)	Payne (1)	0/1	–
Aves	Passeriformes	Northern cardinal (*Cardinalis cardinalis*)	Payne (4)	0/4	–
Aves	Passeriformes	Scissor-tailed flycatcher (*Tyrannus forficatus*)	Payne (2)	0/2	–
Aves	Passeriformes	Eastern kingbird (*Tyrannus tyrannus*)	Payne (1)	0/1	–
Aves	Passeriformes	European starling (*Sturnus vulgaris*)	Payne (1)	0/1	–
Aves	Passeriformes	House sparrow (*Passer domesticus*)	Payne (1)	0/1	–
Aves	Passeriformes	Northern mockingbird (*Mimus polyglottos*)	Payne (2)	0/2	–
Aves	Passeriformes	Orchard oriole (*Icterus spurius*)	Payne (1)	0/1	–
Aves	Passeriformes	House finch (*Haemorhous mexicanus*)	Payne (1)	0/1	–
Aves	Piciformes	Northern flicker (*Colaptes auratus*)	Payne (1)	0/1	–
Aves	Strigiformes	Barred owl (*Strix varia*)	Payne (1)	0/1	–
Total				**0/78**	**2/31**

commercial ELISA kit (SafePath Laboratories, Carlsbad, CA, USA). Each sample was tested using the manufacturer's recommended testing procedure. Positive and negative control sera from hogs were included with the kit and used for each ELISA plate. Samples that had absorbance values ≥0.3 after subtraction of the negative control absorbance value were considered positive (SafePath Laboratories; Hill et al. 2014). Samples reading less than 0.3 after subtraction of the negative control were considered negative.

Results

A total of 78 wild animals were examined for *Trichinella* spp. infection. These included 42 tissue samples from feral hogs (17 females, 8 males, 17 sex unknown) and 36 bird carcasses (2 females, 4 males, 30 sex unknown) from 8 orders and 19 species (Table 1). An average of 5.5 g ± 4.6 (0.2–18.5) of bird tissue and 4.6 g ± 0.8 (2.5–5.5) of hog tissue were included in artificial digestions. No *Trichinella* larvae were detected in any bird or hog samples examined via artificial digestion. A subset of 31 feral hogs had sera that was available and was tested for antibodies to *Trichinella* spp. Of these 31 samples, 2 feral hogs (6.5%; 0.76–21.8%), one each from Love and Osage Counties, Oklahoma, had detectable antibodies to *Trichinella*.

Discussion

Previous reports of *Trichinella* in Oklahoma wildlife indicate an overall low prevalence. For example, prevalence of *Trichinella murrelli* in coyotes was 6.8% (Reichard et al. 2011); overall prevalence of *T. murrelli* and *T. pseudospiralis* in bobcats was 5.9% (Reichard et al. 2021); and seroprevalence of *Trichinella* spp. in feral hogs was 1.4% (Hill et al. 2014). The mapped distribution of all known Oklahoma counties with *Trichinella* in wildlife shows a patchy distribution, suggesting that other counties may contain infected hosts (Fig. 1). Our finding of a seroprevalence of 6.5% in feral hogs is similar to the findings of Hill et al. (2014), in which only 6 (1.4%) of the 425 feral hogs tested were positive for antibodies to *Trichinella*. Taken together, this suggests feral hogs may not be serving as important hosts in the sylvatic cycle in Oklahoma. However, the threat of feral hogs introducing pathogens, such as *Trichinella*, into domestic pig populations and vice versa may increase with the range expansion of invasive feral hogs (Hill et al. 2014; VerCauteren et al. 2019; USDA 2023). Feral hogs likely become infected via cannibalism, such as tail biting, a typical behavior in feral hogs (Hill et al. 2014). Common hunting practices such as leaving field-dressed pig carcasses behind may also facilitate the transmission of *Trichinella* to scavenging feral hogs (Hill et al. 2014). Thus, managing feral hog populations has the potential to help reduce *Trichinella* infections in animals and humans.

In Oklahoma, Reichard et al. (2011; 2021) conducted the most extensive surveys for *Trichinella* in carnivores, including bobcats and coyotes, and suggested rodents may be a potential source of *Trichinella* infection in bobcats. Coyotes have a variable diet but consume predominantly small mammals in Oklahoma (Best et al. 1981). Therefore, small mammals may be important in the sylvatic transmission cycle. However, some small mammals such as rodents may not live long enough to transmit the parasites to larger carnivores, and they may not be able to ingest enough infected tissue to permit transmission (Pozio and Murrell 2006). Regardless, many species of small mammals have been shown to harbor *Trichinella* larvae (Pozio 2005) and may be important reservoirs, especially in scavengers. Studies in Oklahoma have yet to examine small mammals for *Trichinella*, making them a useful candidate host group for future studies.

Trichinella reports in birds are overall few, and this has been suggested to be a result of the fewer examinations of birds compared to mammals (Pozio and Murrell 2006). To date, *T. pseudospiralis* is the only species reported in birds, which may be due to the non-encapsulated nature of the species, and can survive at higher temperatures of 40.5–42.5 °C in birds compared to all other *Trichinella* species (Pozio 2005). Reports of *Trichinella* spp. exist from at least 13

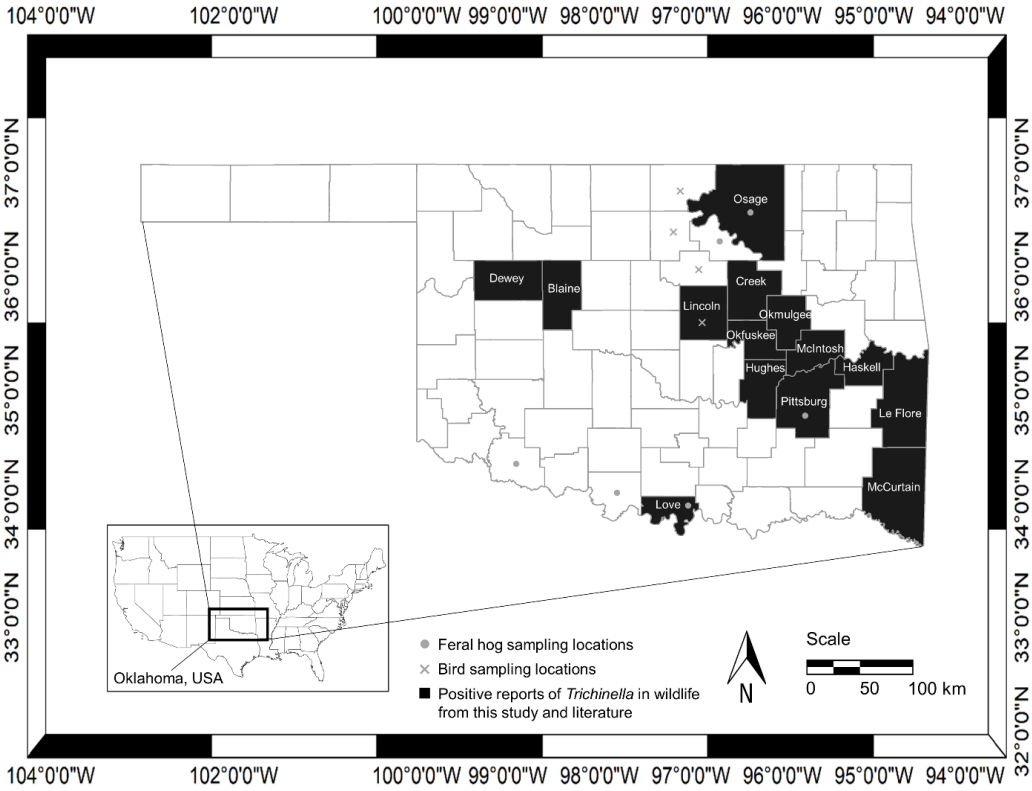

Figure 1. Map of Oklahoma showing all known* *Trichinella* reports in wildlife. Counties in which *Trichinella* larvae or antibodies to *Trichinella* spp. have been detected are shaded black. Counties sampled for feral hogs from this study are indicated as a grey circle. Counties sampled for birds from this study are indicated as a grey x (Reichard et al. 2011; Reichard et al. 2021).*County-level data on antibodies to *Trichinella* spp. in Oklahoma feral hogs reported by Hill et al. 2014 were not available for inclusion in this map.

species and five orders of birds: six species of Accipitriformes (osprey, kites, hawks, eagles), four species of Strigiformes (owls), one species of Charadriiformes (shorebirds), one species of Falconiformes (falcons, caracaras), and one species of Passeriformes (perching birds); however, *T. pseudospiralis* has only been confirmed in seven bird species (Pozio 2005). Reports in birds represent a cosmopolitan distribution, including occurrences in Australia, Asia, Europe, and North America, and prevalence of *T. pseudospiralis* in birds appears to be relatively low. For example, Rausch et al. (1956) reported a prevalence of 10% (1/10) in pomarine jaegers (*Stercorarius pomarinus*) from Alaska; Zimmermann and Hubard (1969) reported a prevalence of 0.4% (1/237) in great horned owls (*Bubo virginianus*) from Iowa; and Shaikenov (1980) reported a prevalence of 0.7% (2/296) in rooks (*Corvus frugilegus*) from Kazakhstan.

The lack of detections of *Trichinella* larvae in the birds in this study may similarly be due to low sample size, but may also be due to the majority of birds examined occupying a lower trophic position in the food web. A smaller proportion of birds (22%) were raptors, including the Mississippi kite, red-tailed hawk, turkey vulture, American kestrel, and barred owl (Table 1). These groups have a higher probability of ingesting *Trichinella* in carcasses, which may explain why most reports of natural infection are for meat-eating birds. Although not true

predators, additional birds sampled in this study may occasionally ingest meat via opportunistic scavenging or predation, such as house sparrows (MacGregor-Fors et al. 2020), adding to the importance of testing non-carnivorous birds for *Trichinella*. Interestingly, *T. pseudospiralis* has been shown experimentally to infect a wide range of bird species from at least five additional orders of birds, suggesting it has a very wide host spectrum in birds (Pozio 2005). Finally, it should be noted that birds were collected from various months throughout the year, yet the seasonality of *Trichinella* in birds remains poorly studied.

The detection of *Trichinella* antibodies in feral hog sera, despite not finding larvae in artificial digestions, suggests there are limitations of tissue digestion. A similar trend was also observed in Hill et al. (2014), in which antibodies for *Trichinella* spp. were detected in 2.9% (29/984) of feral hogs, but *Trichinella spiralis* larvae were only found in 1.81% (6/330) of tongues. The artificial digestion technique has also failed to detect *Trichinella* larvae in sheep while real-time PCR showed five positive samples (Wang et al. 2023), implying digestions have an overall low sensitivity. Several reasons may explain these differences. The current study examined a small portion of hog tissue (average 4.6 g), yet the recommended tissue sample to detect 1 larvae per gram (LPG) of *Trichinella* is 5 g (Gajadhar et al. 2019). If the actual intensity in feral hogs is less than 1 LPG, larvae may have been missed. This may also be true for bird tissues, although the average tissue amount examined was 5.5 g. Additionally, only tongues and jowl tissue were examined from feral hogs, which may have biased our detection results. Other striated muscles, such as diaphragm and foreleg tissues, have been recently suggested as predilection muscles for detecting *Trichinella* in feral hogs, and evaluation of additional tissue types may have revealed larvae that were not present in the tissues sampled in this study (Gajadhar et al. 2019). Another factor affecting the accuracy of digestions may be the freezing and thawing of tissues. Because the majority of *Trichinella* species have a low freeze resistance and some have a very thin collagen capsule (i.e., non-encapsulated clade), the artificial digestion process can potentially degrade individuals of these species and reduce sensitivity of their detection. Overall, the digestion technique has been considered the gold standard for *Trichinella* detection (Crisóstomo-Jorquera and Landaeta-Aqueveque 2022). However, there are limitations to this method, such as being insensitive and failing to detect low numbers of larvae in muscle samples (Wang et al. 2023). Utilizing additional techniques (e.g., antibody detection) may be important to consider in future studies.

This is the second study to detect *Trichinella* spp. in feral hogs in Oklahoma and the first to examine various bird species in Oklahoma for *Trichinella* spp. Identifying additional reservoir hosts for *Trichinella* species remains an important goal. This study examined potential host species in Oklahoma, a state known for few *Trichinella* reports. Further surveillance of Oklahoma wildlife for *Trichinella* is required to properly assess the unknown routes of transmission and potential reservoir hosts. Additionally, it is not known whether humans are increasing their risk of *Trichinella* infection with anthropogenic changes (e.g., increased interactions between humans and areas with infected wildlife). We recommend that studies incorporate multiple collecting methods such as blood and tissue sampling to help answer these questions.

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and disease surveillance.

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