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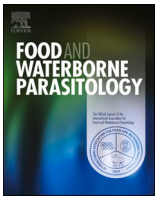

Over a century of progress on *Trichinella* research in pigs at the United States Department of Agriculture: Challenges and solutions☆

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ABSTRACT

Trichinellosis, caused by 13 species/subspecies/genotypes in the nematode genus *Trichinella*, is a worldwide zoonosis. In the United States, trichinellosis was of historical and economic significance because of European restrictions on the import of U.S. pork. Before the advent of effective protective measures, most cases of trichinellosis were derived from consumption of undercooked or inadequately processed, infected pork. Research conducted at the United States Department of Agriculture (USDA) since 1891, and policies established by USDA regulatory agencies, have helped to reduce *Trichinella* infections in commercially raised domestic pigs to negligible levels. Here, we review the history of this scientific progress, placing special emphasis on research conducted at the USDA's Beltsville Agricultural Research Center.

1. Introduction

Trichinellosis is a parasitic disease of humans, that occurs worldwide. Trichinellosis has been known for more than two centuries (Table 1). Moreover, paleopathological findings provide evidence that trichinellosis existed at least 3500 years ago (Gaeta and Bruschi, 2021). Although species endemic to North American wildlife hosts likely have a long history here, *Trichinella spiralis* was introduced to the new world only since European colonial expansion (Rosenthal et al., 2008). Until 1970, *T. spiralis* was the only species recognized in the genus *Trichinella*. Currently, 13 species/subspecies/genotypes have been identified and USDA scientists played a major role in this effort as summarized in Table 2.

Once a common and serious human infection, trichinellosis was historically linked to the consumption of raw or undercooked pork. Through many years of research and changes in the pork industry, most cases of trichinellosis in the United States now result from consuming game meats including wild boar, bear among others (Murrell and Pozio, 2011).

For more than a century, the United States Department of Agriculture (USDA) has conducted research on *Trichinella* infection in pigs and other animal species, developing control and preventive measures that have reduced prevalence in pigs to negligible levels (Gamble et al., 2024) (Table 1). Here, we summarize the USDA's contributions to *Trichinella* research in animals, particularly in the last

☆ This paper is a tribute to Kenneth Darwin Murrell, retired from USDA, for his monumental contributions to research and control of *Trichinella.*

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Table 1
Historical landmarks concerning *Trichinella* and trichinellosis with particular reference to studies at USDA laboratories (**in bold**).

Year	Contribution	Reference
1835	*Trichina spiralis* discovered and described based on cysts found by a British first- year medical student, James Page, while dissecting a human cadaver that had died of tuberculosis.	Owen (1835); history detailed by Campbell (1979)
1842	*T. spiralis* detected in a human cadaver in the USA.	Bowditch (1842)
1846	*Trichina* found in pork that the 23-year-old human physician, Joseph Leidy was having for dinner; cooked pork contained dead larvae.	Leidy (1846); details provided by Ward (1923)
1850	Experimental transmission of *Trichina* in animals. Trichina were found in muscles of a pet badger that had been fed scraps of muscles from dogs and cats naturally infected with trichina. Three pups fed muscles of badger died of trichinosis.	Herbst (1853): full account in Reinhard (1958)
1857	Trichina from human flesh found infective to mice, dogs, and pigs. Morphology of adult *T. spiralis* described.	Leuckart (1860); details in Campbell (1983)
1859	Trichina from human flesh was infective to a dog and pig. Development of *T. spiralis* first described, and trichinoscopic testing of pigs at slaughter proposed to monitor infections in pigs.	Virchow (1859); details in Campbell (1983)
1860	A previously healthy 20-year-old female servant who ate and served pork at the Christmas dinner to a farmer family in Germany died of acute trichinosis; an autopsy performed by Zenker identified thousands of trichina in her muscles. Zenker found adult *T. spiralis* in intestines of this woman that had been in cold storage for 1 month. The farmer and his wife also had died. Two months later, Zenker visited the butcher who prepared ham and sausages sold to the farmer. The butcher also developed severe muscle pains but survived. Zenker found trichinae in ham and in pork sausages that had been stored at the butcher shop for about 2 months. First demonstration of human as an intermediate and definitive host for *T. spiralis*.	Zenker (1860); full account in Reinhard (1958)
1863–1879	Mandatory inspection of pork introduced in Germany. In trichinoscope method around 28 or more samples in 2 rows of wheat grain sized diaphragmatic muscle are arranged on a glass slide and compressed under another slide, clamped with screws, and examined in a projection microscope; illustrated by Gould (1970) and Zimmermann (1983). The procedure cannot detect light infections (1 larva/g).	Zimmermann (1983); Gould (1970); Brantz (2008)
1879–1888	Several European countries banned importation of pork from USA.	Gignilliat (1961)
1891	**Trichinoscopic testing of pork for export introduced in USA. In 8 years of testing (1898–1906) of > 8 million pigs for export to Germany tested, trichina was found in 1.41%.**	Hall (1937)
1895	Trichinosis outbreaks observed in Germany, some involving 100 cases at a time.	Kozar (1970)
1895	Amended name *Trichina* to *Trichinella* because the genus *Trichina* was preoccupied with flies. Henceforth, the parasite was recognized as *Trichinella spiralis* (Owen, 1835) Railliet, 1895.	Railliet (1895, 1896)
1897	**Artificial digestion of pork in pepsin and hydrochloric acid proposed to liberate encysted larvae from muscle. *Trichinella* first found in horse meat.**	Thornbury (1897)
1898	**The USDA's Bureau of Animal Industries posts C.W. Stiles, an eminent American parasitologist, to the Embassy in Berlin to test German claims that American pork was the source of outbreaks of trichinellosis in Germany.**	Stiles (1901); Cassedy (1971)
1911	First serological diagnosis test (complement fixation test) described.	Ströbel (1911)
1914, 1990,2009	**Demonstration that freezing kills *Trichinella* in pork, including different *Trichinella* species (genotypes) circulating in USA. Freezing standards proposed for meat industry (**Kotula et al., 1990**)**	Ransom (1914); Ransom (1915); Ransom (1916); Kotula et al. (1990)
1919,1939, 1983	**Heating to 58 ^{o} C kills trichina in pork. Only dead larvae were found in sausages heated to 58 °C (137 °F). Time and temperatures parameters established for FSIS by** Kotula et al. (1983)**.**	**Ransom and Schwartz (1919)**; Schwartz (1939); Schwartz (1929); Kotula et al. (1983)
1920, 1985	**Demonstration that irradiation kills *Trichinella* in pork.** Brake et al. (1985) **tested consumer acceptable levels of gamma irradiation.**	**Schwartz (1921)**; Brake et al. (1985)
1920, 2017	**Curing of pork can kill *Trichinella*. Combining NaCl concentrations above 1.3% with fermentation to pH 5.2 or below inactivates > 96% of *Trichinella* muscle larvae in stuffed sausages within 24–28 h.**	Ransom et al. (1920); Schwartz (1939, 1940); Hill et al. (2017)
1930	**Muscle larva antigen enables *T. spiralis* diagnosis in pigs.**	Schwartz et al. (1930)
1935, 1936	**Elevated (> 4 times) prevalence in in garbage-fed (vs. grain-fed) pigs.**	Hall (1937); Schwartz (1940)
1949–1970	*Trichinella* prevalence in Arctic and Alaska, USA documented.	Rausch (1970)
1952	Feeding uncooked garbage to pigs outlawed to control the viral disease vesicular exanthema, reducing prevalence of *Trichinella* in pigs.	Jefferies et al. (1966)
1958–1972	International Commission on trichinellosis established.	Dupouy-Camet et al. (2020); **Supplementary file 2**
1961	Benzimidazole treatment introduced as a drug against trichinellosis.	Campbell and Denham (1983)
1967	Pooled muscle digestion procedure proposed for detection of *Trichinella* for surveillance.	Zimmermann (1967); Gamble (1996, 1998, 1999)
1961–1966	National survey of *Trichinella* in pigs by peptic digestion of diaphragms of 43,868 revealed low prevalence in farm-raised pigs. *Trichinella* infections in pigs and humans in the USA reviewed.	Zimmermann and Brandly (1965); Zimmermann (1970); Zimmermann and Zinter (1971)
1969	**First commercial slaughterhouse testing of *Trichinella* in pork at a plant in Iowa. Based on 5–8 g samples of diaphragm from each pig tested 42 (0.008%)**	Andrews et al. (1969)

(*continued on next page*)

Table 1 (*continued*)

Year	Contribution	Reference
	of 482,392 pigs during a 32-weeks period were positive for *Trichinella* larvae. The cost of testing was estimated to be 0.1$ per pig.	
1972	Multiple species within the genus *Trichinella* proposed. *Trichinella nelsoni* and *T. nativa* recognized (species characteristics and biology of each species currently recognized are summarized in Table 2.	Britov and Boev (1972); see Table 2 for other authors contributions
1974	First ELISA test developed for serological diagnosis in pigs.	Ruitenberg et al. (1974); van Knapen et al. (1976)
1980	Outbreaks of clinical trichinellosis derived from consumption of horse meat recognized in Europe. Experimental demonstration that *Trichinella* from human is infective to horses.	Mantovani et al. (1980); Ancelle (1998); Soule et al. (1989)
1983, 1988	**A sensitive and specific enzyme-linked immunoassay using excretory-secretory products from *T. spiralis* larvae developed for the detection of *Trichinella* antibodies.**	Gamble et al. (1983, 1988), Gamble (1996)
1986	**Cannibalism, not rodents, demonstrated as a major source of infection in an endemic herd of 1000 pigs.**	Hanbury et al. (1986)
1987–2006	**Morphology, isoenzymes, geography, and genetics discriminate species of *Trichinella.***	**(**see Table 2**)**
1988	Establishment of the International *Trichinella* Reference Centre.	Dupouy-Camet et al. (2020); Marucci et al. (2022); **Supplementary file 2**
1996,2007	**Viable *T. spiralis* can persist in muscles of experimentally infected horses for >12 months in the absence of detectable level of antibodies. 5 g samples of horsemeat found necessary to detect viable *Trichinella* infections.**	Gamble et al. (1996); Hill et al. (2007a, 2007b)
1999	Freeze resistance of *Trichinella nativa* established.	Kapel et al. (1999); additional details in Pozio (2016, 2020, 2022)
1999-2001	**Development of Multiplex PCR to diagnose all genotypes of *Trichinella* that became the international standard for genotyping.**	Zarlenga et al. (1999, 2001)
2005	**A USDA, pork industry initiative of *Trichinella* certification program for pork issued in USA. All 11,713 pigs tested from certified farms tested negative for *Trichinella.***	Pyburn et al. (2005)
2006	**Evolutionary and biogeographic hypothesis for Trichinellae.**	Zarlenga et al. (2006)
2007	**Joint publication of FAO/OIE/WHO Guidelines for the for the surveillance, management, prevention, and control of trichinellosis.**	Dupouy-Camet and Murrell (2007)
2008	**Documentation of especially inbred *T. spiralis* in Europe and the Americas, impairing outbreak tracing.**	Rosenthal et al. (2008)
2011	**First draft sequence of any *Trichinella* genome, revealing marked differences from the *C. elegans* "model nematode."**	Mitreva et al. (2011)
2015	**Natural introgression among *T. spiralis* and *T. britovi.***	Franssen et al. (2015)
2016	**Draft genomes of all known species of *Trichinella.***	Korhonen et al. (2016)
2017	**First chromosomal assembly of a *Trichinella* genome.**	Thompson et al. (2017)
2018	Microsatellite markers readily trace transmission of *T. britovi* **but less readily trace transmission of *T. spiralis* in Europe.**	La Rosa et al. (2012, 2018); **Bilska-Zając et al., 2022**
2022	**Demonstration that genome variation can trace *T. spiralis* outbreaks.**	Rosenthal et al. (2021); **Bilska-Zając et al. (2022)**
2024	**Testing over 3 million PQA+ pigs via artificial digestion revealed none infected with *Trichinella,* establishing this production compartment as one of "negligible risk."**	Gamble et al. (2024); see text

50 years. Recognizing essential partnerships with valued international research teams, our present focus is to summarize the achievements of USDA agencies (including the Animal Plant and Health Inspection Service [APHIS], Food Safety and Inspection Service [FSIS], and the Agricultural Research Service [ARS]) in collaboration with the National Pork Board. The National Agricultural Library provided literature not otherwise easily accessible.

Landmarks concerning *Trichinella* biology are summarized in Table 1 with special emphasis on contributions by the USDA scientists. Dupouy-Camet (2024) recently narrated events and lives of European scientists seminal to the discovery of trichinellosis.

2. Brief early history of trichinellosis research at USDA

Research on *Trichinella* at USDA began as early as 1890.The Bureau of Animal Industry (BAI) was created within the USDA by the United States Congress in 1884. The mission of the BAI was to promote livestock disease research, enforce animal import regulations, and regulate the interstate movement of animals. In the 1880's, the U.S. became the world's leading exporter of pork. During this time, some European countries banned import of U.S. pork owing to the lack of mandated testing for *Trichinella* in pork (Table 1). Stiles was appointed in 1891 as a zoologist in the Zoological Division of BAI in Washington, DC and in 1898–1899 he was posted to the U.S. Embassy in Berlin to report on German claims that American pork was the source of outbreaks of trichinellosis (Stiles, 1901; Campbell, 1983). This American-born scientist was chosen for this mission because he was fluent in French and German, having studied at the Institute of Pasture in Paris and having obtained a Ph.D. from the University of Leipzig, Germany. Stiles (1901) in a 110- page report listed all cases of trichinellosis in Germany from 1881 to 1898 including reports from Prussia, Saxony, Empire, and other states; none of these were due to pork imported from the U.S. He found that outbreaks occurred despite samples being found negative by

Table 2
Biology of *Trichinella* species/subtypes/genotypes.

Genotype	Lineage designation/name	General location	Muscle phase encapsulated	Main hosts	Additional references
T1	*Trichinella spiralis* (Owen, 1835) Railliet, 1895	Cosmopolitan	Yes	Suids, rodents, humans	**Dame et al. (1987); Zarlenga and Gamble (1990); La Rosa et al. (1992); Pozio et al. (1992b); Lichtenfels et al. (1983); Zarlenga et al. (2002); Murrell et al., (2000); Pozio and Murrell (2006)**; **Zarlenga and La Rosa (2000); Zarlenga et al. (2020); Pozio and Zarlenga (2021)**
T2	*Trichinella nativa* Britov and Boev, 1972	Circumpolar Arctic	Yes	Suids, carnivores	**Lichtenfels et al., 1983); Pozio et al. (1992a); La Rosa et al. (1992); Murrell et al. (2000); Pozio and Murrell (2006) Pozio and Zarlenga (2021)**
T3	*Trichinella britovi* Pozio, La Rosa, **Murrell**, **Lichtenfels**, 1992b	Temperate Europe and Northern Africa	Yes	Suids, carnivores	**Pozio et al. (1992a); La Rosa et al. (1992); Murrell et al. (2000); Pozio and Murrell (2006); Pozio and Zarlenga (2021)**
T4	*Trichinella pseudospiralis,* Garkavi, 1972	Cosmopolitan	No	Mammals, birds	**Lichtenfels et al. (1983); Pozio et al. (1992a); La Rosa et al. (1992); Zarlenga et al. (1996); Murrell et al. (2000); Pozio and Murrell, 2006) Pozio and Zarlenga (2021)**
T5	*Trichinella murrelli* Pozio and La Rosa, 2000	Temperate North America	Yes	Carnivores	**Zarlenga et al. (1991); Pozio and Zarlenga (2021)**
T6	*Trichinella* genotype T6 (Pozio, La Rosa**, Murrell**, **Lichtenfels**, 1992a)	Northern temperate North America	Yes	Carnivores	**Pozio et al. (1992b); Murrell et al. (2000); La Rosa et al. (1992); Pozio and Murrell (2006); Pozio et al. (2009); Pozio and Zarlenga (2021)**
T7	*Trichinella nelsoni,* Britov and Boev, 1972	Southeastern Africa	Yes	Carnivores	**La Rosa et al. (1992); Pozio et al. (1992b); Murrell et al. (2000); Pozio and Murrell (2006); Pozio and Zarlenga (2021)**
T8	*Trichinella* genotype T8 (Pozio, La Rosa, **Murrell**, **Lichtenfels**, 1992b)	Southern Africa	Yes	Carnivores	**La Rosa et al. (1992); Pozio et al. (1992); Murrell et al. (2000); Pozio et al. (2009); Pozio and Murrell, (2006)**
T9	*Trichinella* genotype T9 (Pozio, La Rosa, **Murrell**, **Lichtenfels**, 1992b)	Japan	Yes	Carnivores	Nagano et al. (1999)*; **Murrell et al. (2000); Pozio and Murrell (2006); Pozio et al. (2009); Pozio and Zarlenga (2021)**
T10	*Trichinella papuae,* Pozio, Owen, La Rosa, Sacchi, Rossi, Corona, 1999	Southeast Asia	No	Suids, crocodiles	**Murrell et al. (2000); Pozio and Murrell (2006)**
T11	*Trichinella zimbabwensis* Pozio, Foggin, Marucci, LaRosa, Sacchi, Corona, Rossi, Mukaratirwa, 2002	Southern Africa	No	Crocodiles, reptiles	**Murrell et al. (2000); Pozio and Zarlenga (2005, 2021)**
T12	*Trichinella patagoniensis* Krivokapich, Pozio, Gatti, Prous, Ribicich, Marucci, La Rosa, and Confalonieri, 2012 .	Southern Temperate South America	Yes	Carnivores	**Murrell et al. (2000); Pozio and Zarlenga (2021)**
T13	*Trichinella chanchalensis,* Sharma, **Thompson**, Hoberg, Scandrett, Konecsni, Harms, Kukka, Jung, Elkin, Mulders, Larter, Branigan, Pongracz, Wagner, Kafle, Lobanov, **Rosenthal**, and Jenkins, 2020	Northwest North America	Yes	Carnivores	

In **bold (USDA-affiliated)**.
* excepting Nagano et al 1999, all entries in this column were authored or co-authored with USDA scientists.

trichinoscope examination because this method failed to detect light infections (Stiles, 1901; Dupouy-Camet, 2024). Greater confidence in the veracity of negative tests would require broad application of the more sensitive artificial digestion test.

Thereafter, Schwartz, Ransom, and Hall continued research on trichinellosis for the BAI in Washington, DC (Table 1). In addition to parasitologists at the DC laboratory, scientists were employed by BAI and posted at various swine slaughterhouses, especially those supporting pork exports to Germany.

Thornbury, a MD, was among such supervising microscopists at an abattoir in Buffalo, New York. His observations on *Trichinella* in pigs and humans are noteworthy (Thornbury, 1897). He examined muscles from 197,948 pigs in 11 months and found *Trichinella* in

Table 3
Prevalence of *Trichinella* in domestic pigs tested at **USDA laboratories**.

Year tested	Region*	No. tested	Method	No. positive (%)	Notes	Reference
1898-1906	North central states	8,257,928	Trichinoscope (discontinued in 1906)	212,228 (2.57)	1.41% contained live and 1.126% dead or degenerated larvae	Ransom (1915); Schwartz (1929)
1936		2,341	Digestion	130 (5.5)	Garbage fed	Schwartz (1936)
		4,740	Digestion	53 (1.11)	Grain fed	
1933-1937	11 states	6,622	Digestion	60 (0.91)	Grain fed	Schwartz (1938,1939)
		6,484	Digestion	286 (4.41) 11 (0.55)	Garbage fed	
		1987	Digestion		Cooked garbage fed	
1935		1973	Digestion	95 (4.8)	Garbage fed	Hall (1935, 1937)
		2146		33 (1.5)	Grain fed	
		3254		0	Processed pork products	
1930's		13,000	Digestion	126 (0.95)	Farm-raised, 1-5 larvae/100g	Schwartz (1940, 1952)
		10,500		599 (5.7)	Garbage fed	
1948-1952		3,500	Digestion	20 (0.57)	1-5 larvae/100g pork	Schwartz (1960)
1969	1 commercial plant in Fort Dodge, Iowa	482,392	Digestion	42 (0.008%)	The cost of testing was estimated to be 0.1$ per pig (see text).	Andrews et al. (1969)
1971-1975	Illinois	50,235	Digestion	67 (0.13)	30,644 herds tested. See text	Hill et al. (1985)
1982-1983	New England (CT, ME, MA, NH, RI, VT)	5,315	Digestion	39 (0.73)	Infected pigs were from small farms. Prevalence was higher in pigs slaughtered in small custom slaughterhouses versus commercial slaughterhouses	Schad et al. (1985b)
1981-1983	Mid -Atlantic (PA, NJ, IN, IL, VA, OH, NY, DE)	33,482	Digestion	196 (0.58)	Infected pigs from small backyard pigs in PA, NJ.	Duffy et al. (1985) ; Schad et al. (1985a)
1983	New Jersey	63	Digestion	56 (88.9)	Poorly managed farm-see text for on farm epidemiology. *T spiralis* genotyped. Fecundity compared with wildlife *T. spiralis* isolates	Schad et al. (1987); Murrell et al. (1987); Leiby et al. (1985)
1984-1988	Illinois (East St. Louis), poorly managed farm)	66,854	Digestion	0	See text for epidemiological studies	Doby and Murrell (1989)
1989-1990	Hawaii	509	ELISA	2 (0.3)	Infected pigs were garbage fed	Dubey et al. (1992)
1990	NAHMS	3048 (lactating sows)	ELISA	5 (0.16)	Sows from 24 states. 5 infected sows were from different herds in NC, OH, PA	USDA-APHIS information sheet (2011); Gamble and Busch (1999)
1995	NAHMS	7987 (finishers)	ELISA	1 (0.013)	16 states	Gamble and Busch (1999)
1994-1995	North Carolina	2183	ELISA	1 (0.046)	Infected pig housed outdoors on dirt lot	Davies et al. (1998)
2000	NAHMS	14,328	ELISA	0	17 states	USDA-APHIS information sheet (2018)
2006	NAHMS	6238	ELISA	0	17 states	USDA-APHIS info sheet (2011)
Not stated	New England	2132	ELISA, digestion	10 (0.47), larvae in 4 of 10	Risk assessment study (see text), 90 farms	Gamble et al. (1999)
	New Jersey	1946	ELISA, digestion		90 farms	

(*continued on next page*)

Table 3 (*continued*)

Year tested	Region*	No. tested	Method	No. positive (%)	Notes	Reference
				5 9 (0.26)		
	Trichina Certification Project	11,713	ELISA, digestion	0	461 farms	Pyburn et al. (2005)
2007	Maryland, poorly managed farm	50	Digestion	17 (34.0)	*T. spiralis* genotyped in all pigs	Hill et al. (2010)
2012	NAHMS	5705	ELISA	1	13 states	USDA-APHIS information sheet (2018)
2024	Commercial pigs slaughter	>3,000,000	Digestion	0	Risk assessment	Gamble et al. (2024)

* CT = Connecticut, DE = Delaware, IN=Indiana, IL = Illinois, ME = Maine, MD = Maryland, MA = Massachusetts, NH=New Hampshire, NJ = New Jersey, NY=New York, OH=Ohio, PA = Pennsylvania, RI = Rhode Island, VA = Virginia, VT = Vermont.

1043 (0.05%) of the carcasses. In a comparative study, prevalence of *Trichinella* was higher in pork loin muscles than in muscles of the neck or the diaphragm; but the intensity of infection was greatest in the diaphragm. As many as 1023 larvae were found in a single histological slide (Thornbury, 1897). As many as 50,000 larvae were estimated in one ounce (~ 28 g) of pork. Thornbury was first to describe the sensitive pepsin digestion method to liberate *Trichinella* from muscle tissues (Table 1). Also noteworthy is his documentation of severe trichinellosis in residents, of German descent, in Milwaukee, Wisconsin. Seven of the nine people who feasted on one sausage "roost Wurst" (probably undercooked/uncooked) died of acute trichinellosis. Large numbers of *Trichinella* larvae were found in muscles of two humans examined microscopically, and in the sausages they consumed. The Secretory of Agriculture, the Honorable Jerry Rusk was briefed on the episode (Thornbury, 1897).

In 1953, the functions of the BAI were transferred to the newly established Agricultural Research Service (ARS). Staff of the Zoological Division were transferred from Washington, DC to the Beltsville Parasitology Laboratory (BPL). In 1960–1961, BPL moved to its current location and the name was changed to the Animal Parasitology Institute (API) in 1972 (Andrews, 1987).

3. *Trichinella* research at the animal parasitology institute (now animal parasitic diseases laboratory, APDL), ARS, Beltsville, USDA

After the retirement of Swartz in 1959, work on *Trichinella* was put on hold until the appointment of Dr. K. D. Murrell in 1978 as a scientist in the Animal Parasitology Institute, Beltsville Agricultural Research Center (BARC). (Supplementary file 1).

While modernization of pork production systems, including a ban on feeding raw garbage in the mid 20th century, had a major impact in reducing exposure of pigs to *Trichinella*, documenting the safety of pork to domestic consumers and for purposes of trade remained a high priority. Gaps in knowledge existed regarding the risks associated with various management practices, as well as the epidemiology of *Trichinella* in the sylvatic cycle. Questions remained regarding processing requirements to render pork safe in ready to eat products and for home preparation. Much was to be learned concerning the parasite itself (genetics and phylogeny) as well as the biology of the parasite in its broad range of hosts. Here, we summarize contributions of Murrell and the APDL staff who worked with, and followed, him in the study of trichinellosis. Contributions include aspects of (1) prevalence of *Trichinella* in pigs in the U.S., (2) epidemiology and transmission, (3) wildlife reservoirs as sources of *Trichinella* infections for humans and pigs, (4) horses as a source of trichinellosis in humans, (5) post-harvest treatment of pork (heating, freezing, curing, irradiating) to kill *Trichinella,* (6) pre-harvest control strategies, and (7) phylogenetics, molecular epidemiology, and evolution. Murrell also supported establishment, and supplied materials for, the International Trichinella Reference Center in Rome, Italy which became an indispensable resource for understanding the biology and transmission of *Trichinella* spp. (Marucci et al., 2022).

4. Prevalence of *Trichinella* in pigs in the U.S

Trichinella testing of U.S. pigs/pork for purposes of export, commenced in 1898 but was terminated in 1906, when methods then employed were deemed unreliable (Table 3). Later surveys, employing the more reliable pepsin digestion method, yielded prevalence estimates of around 1% in farm-raised pigs. The prevalence was reduced drastically when feeding uncooked garbage to pigs was outlawed in the 1950s (Tables 1, 3). A pilot project concerning the feasibility of using a digestion method for *Trichinella* testing at a commercial slaughterhouse reported the cost of testing to be around 10 cents per pig (83 cents in 2024, adjusting for inflation) (Andrews et al., 1969). The method was deemed costly and logistically impractical at that time, given the large number of pigs produced.

Since the 1980s, surveys revealed a declining prevalence and reduced risk associated with *Trichinella* infection in the U.S. (Table 3). These studies, predominantly in commercial pigs, affirmed that modern pork production systems prevent exposure of pigs to sources of *Trichinella*. A recent survey used the gold standard artificial digestion method to test over 3 million pigs raised in the United States under confined housing and related biosecurity measures defined in the Pork Quality Assurance Plus (PQA+) pigs, (https://porkcheckoff.org/certification-tools/training-certification/pqa-plus/); it found no positive animals, providing a statistical

Table 4
Prevalence of *Trichinella* in wildlife tested at or in collaboration **USDA, APDL, Beltsville, Maryland.**

Host	Region	Year	No. tested	Method	#Pos. (%)	Notes (**in bold, species characterized**)	Reference
Wild pig (*Sus scrofa*)	Texas-North central	1997–1998	226	Digestion	0		Gamble et al. (2005)
	Newcastle		1	Digestion, bioassay, genotyping	1	***T. pseudospiralis***	
	Nationwide (APHIS)	2012–2013	3247 sera	ELISA	98 (3.0)		Hill et al. (2014)
	Nationwide (APHIS)	2012–2013	330-tongues	Digestion, genotyping	6	All 6 isolates were ***T. spiralis***	Hill et al. (2014)
Black bear (*Ursus americanus*)	New Hampshire	1986–1992	1515	Digestion	160 (10.5)	Private farm	Worley et al. (1993)
	Pennsylvania	1981–1983	2056	Digestion	37 (1.8)	Hunter killed. Biological characteristics of 9 *Trichinella* isolates described (see text-section 6.1.3). Two isolates (ISS345 and ISS 346) were used by Pozio and La Rosa (2000) for original description of ***T. murrelli.***	Leiby et al. (1985); Murrell et al. (1985); Schad et al. (1986)
		1992	63 muscle 319 sera	Digestion	2 (3.2)	*Trichinella* seen in histological sections of 3 of 162 bears	Dubey et al. (1994)
				ELISA	6 (1.8)		
	New Hampshire	2003	1 bear meat frozen at minus 20 °C for 6 weeks	Digestion, bioassay	1	***T. nativa***	Hill et al. (2005)
	Maryland	2005–2011	389-tongues	Digestion	2 (0.5)	Hunter killed, ***T. murrelli***	Dubey et al. (2013)
	Pennsylvania	2015–2016	181 adults	ELISA	6 (3)	Live, hibernating	Dubey et al. (2016)
			8 yearlings		1 (3.6)		
			44 nursing cubs		0		
	North Carolina	1996	79	ELISA	0		Nutter et al. (1998)
Grizzly bear (*Ursus arctos*)	Alaska	1973–1987	878	ELISA	427 (48.6)	355 (82.5%) of 430 from North, 62 (24.6%) of 252 from Interior, and 10 (5.1%) of 196 from South	Zarnke et al. (1997)
Raccoon (*Procyon lotor*)	Pennsylvania	1982–1983	1170	Digestion	31 (2.6)	Biological characteristics of *Trichinella* isolate described (see text-section 6.1.3)	Leiby et al. (1985); Murrell et al. (1985)
	Illinois	1986–1988	143	Digestion	12 (8.3)		Doby and Murrell (1989)
	New Jersey	1983–1985 (?)	1	Digestion	1 (100)	Biological characteristics of *Trichinella* isolate described (see text-section 6.1.3)	Murrell et al. (1985); Leiby et al. (1988)
	Illinois	1987–1989	323	Digestion	5 (1.3)	***T. murrelli***	Snyder et al. (1993)
	Wisconsin	2005–2006	59	Digestion, histology, serology, bioassay	11 (18.6)	***T. murrelli*** was isolated by bioassay from tongue	Hill et al. (2008)
	Maryland	2007 (?)	38	Digestion	6	***T. spiralis***	Hill et al. (2010)
Coyote (*Canis latrans*)	Illinois	1986–1988	5	Digestion	0		Doby and Murrell (1989)
	Illinois	1987–1989	1	Digestion	0		Snyder et al. (1993)
	Wisconsin	2005–2006	42	Digestion	11 (26.1)	Bioassay of tongue positive	Hill et al. (2008)
Skunk (*Mephitis mephitis*)	Pennsylvania	1982–1983	51	Digestion	2 (3.9)		Leiby et al. (1985)

(*continued on next page*)

Table 4 (*continued*)

Host	Region	Year	No. tested	Method	#Pos. (%)	Notes (**in bold, species characterized**)	Reference
	New Jersey	1983–1985 (?)	15	Digestion	7 (47)	***T. spiralis.*** Biological characteristics of *Trichinella* isolate described (see text-section 6.1.3)	Murrell et al. (1985); Leiby et al. (1988)
	Wisconsin	2005–2006	7	Digestion	0		Hill et al. (2008)
Foxes	Illinois	1986-1988	28	Digestion	1 (3.5)		Doby and Murrell (1989)
Red fox (*Vulpes fulva*)	Pennsylvania	1982–1983	73	Digestion	11 (15.1)		Leiby et al. (1985)
		2024	21	Compression, PCR	7 (33.3)	***T. murrelli***	Dubey et al. (2024b)
	Illinois	1987–1989	9	Digestion	2 (17.1)	***T. murrelli***	Snyder et al. (1993)
Gray fox (*Urocyon cinereoargenteus*)	Pennsylvania	1982–1983	90	Digestion	6 (6.7)	Fecundity of *Trichinella* isolates compared in hamsters, jirds, deer mice, rats, and multimmate rats	Leiby et al. (1985); Murrell et al. (1985)
		2004	1	Compression, PCR		***T. murrelli***	Thompson et al. (2024)
Black vulture (*Coragyps atratus*)	Alabama	Not stated	1	Digestion, bioassay, genotyping	1	**T. *pseudospiralis,*** infective to pigs, mice, and chickens	Lindsay et al. (1995)
Opossum (*Didelphis virginianus*)	Pennsylvania	1982–1983	384	Digestion	11 (2.9)		Leiby et al. (1985)
	Illinois	1986–1988	48	Digestion	1 (2.0)		Doby and Murrell (1989)
	New Jersey	1983–1985 (?)	3	Digestion	1 (33.3)	***T. spiralis.*** Biological characteristics of *Trichinella* isolate described (see text-section 6.1.3)	Murrell et al. (1985); Leiby et al. (1988)
	Maryland	2007(?)	4	Digestion	2	***T. spiralis***	Hill et al. (2010)
Mice (unspecified)	Illinois	1986–1988	8	Digestion	0		Doby and Murrell (1989)
Deer mice (*Peromyscus* spp.)	New Jersey	1983–1985 (?)	18	Digestion	0		Leiby et al. (1988)
Shorttail shrew (*Blarina brevicaudata*)	New Jersey	1983–1985 (?)	5	Digestion	0		Leiby et al. (1988)
Rat (*Rattus norvegicus*)	Illinois	1986–1988	117	Digestion	1 (0.8)		Doby and Murrell (1989)
	New Jersey	1983–1984	443	Digestion	188 (42.4)	On an endemic, poorly managed on farm	Leiby et al. (1990)
Muskrat (*Ondatra zibethicus*)	Pennsylvania	1982–1983	201	Digestion	0		Leiby et al. (1985)
Mink (*Mustela vison*)	Illinois	1986–1988	35	Digestion	0		Doby and Murrell (1989)
	Pennsylvania	1982–1983	17	Digestion	1 (5.9)		Leiby et al. (1985)
Feral domestic cat (*Felis catus*)	New Jersey	1983–1985 (?)	2	Digestion	2 (100)	***T. spiralis***	Leiby et al. (1988)
Bobcat (*Lynx rufus*)	Mississippi	2017	25	Histology	1		Dubey et al. (2024a)
Dog (*Canis familiaris*)	Virginia	2004	1 (muscle & tongue)	Histology, bioassay	1	***T. murrelli***	Dubey et al. (2006)
Gray wolf (*Canis lupus*)	Montana	1987	1	Digestion, bioassay	1	**Not freeze resistant**	Worley et al. (1990)

prevalence of <1 infection in 1 million pigs (Gamble et al., 2024). As in most countries, backyard pigs raised and slaughtered outside of veterinary services, may still pose a risk to public health (Gamble, 2022).

5. Epidemiology and transmission

In the early 1980's, a major challenge was to identify *Trichinella*-infected pig farms and assess the risk of reservoir hosts. Within a very short time, >100,000 pig diaphragms, from major slaughterhouses, were tested for *Trichinella* infection (Table 3). The results culminated in the launch of an extensive program of research summarized below.

Table 5
Game as source of human trichinellosis in the USA.

Year	State	No of persons affected	Suspected source	*Trichinella* in game meat	Notes	Reference
2022	AZ, MN SD	6	Bear meat grilled	Viable larvae in bear meat frozen 45 days	Bear from northern Saskatchewan, Canada	Cash-Goldwasser et al. (2024)
2016–2017	CA	12	Raw pork dish	Larvae in left over pork	Farm raised wild boar	Heaton et al. (2018)
2016	AK	9- First outbreak −4 family members	Raw or pan-fried walrus meat	Walrus meat not available for testing	Hunted walruses were from same area	Springer et al. (2017)
2017	AK	Second outbreak −5 neighbors	Shared walrus meat	Walrus meat not available for testing		Springer et al. (2017)
2011	MN	2- carcass dressed gloveless, meat consumed		Larvae in frozen boar meat	Wild boar hunted from private farm in Iowa	Holzbauer et al. (2014)
2008–2012	25 states and DC	90 cases	Pork products in 22 cases, non-pork products in 45 cases	No data	No data	Wilson et al. (2015)
2008	CA	23 confirmed, 6 probable	Bear meat consumption	Larvae in bear paw muscle	*Trichinella murrelli* -associated	Hall et al. (2012)
2005	NH	1 patient	Bear meat consumption suspected	Viable larvae from bear meat frozen -20 °C for 4 months	*Trichinella nativa* -associated	Hill et al. (2005)
2003	NY	1 patient	Ate nearly 1 kg of raw bear meat	Viable larvae recovered from frozen bear meat	*Trichinella nativa* -associated	Smith et al. (2004)
2003	TN	2 patients, husband, wife	Ate medium rare bear meat	Larvae in histological sections of bear meat	Bear was shot in Canada and transported to TN.	Smith et al. (2004)
1997–2003		33 outbreaks	Implicated meat			Roy et al. (2003)
	AK 4 patients, CA 8 patients IL 4 patients MN 5 patients, OH8 patients		Bear jerkey, Bear meat, Pork sausages, pork jerkey Pork sausages, Bear jerkey, Bear meat			
1995	ID	7 of 15 who ate cougar jerkey		*Trichinella* larvae recovered from frozen cougar meat	*Trichinella nativa* suspected	Dworkin et al. (1996)

AK = Alaska, AZ = Arizona, CA = California, DC=District of Columbia, ID=Idaho, IL = Illinois, MN = Montana, NH=New Hampshire, NY=New York, OH=Ohio, SD=South Dakota.TN = Tennessee.

5.1. On farm epidemiology and modes of transmission

5.1.1. The role of cannibalism

The relative contributions of cannibalism, wildlife, and rats as sources of infection for pigs remained controversial until 1985. An opportunity arose to investigate this topic on a 1000 head, pig farm in Eastern Illinois with ongoing transmission of *Trichinella* (Hanbury et al.,1986). Initially, *Trichinella* larvae were detected in digested tissues of 124 (52.9%) of 234 pigs surveyed from 1973 to 1984. Pigs were raised with minimal biosecurity (non-controlled housing) but there was no feeding of garbage on the farm (Hanbury et al., 1986). Using *Trichinella*-free tracer pigs, and controlling for rat infestation, it was demonstrated that cannibalism was a major mode of *Trichinella* transmission.

5.1.2. The role of rats with access to infected pig carcasses

Until 1980, rats were considered important in the natural transmission of *Trichinella*, given that sows can kill and swallow a whole rat (Murrell et al., 1984). The role of rats was investigated on a poorly managed 123 head pig farm in New Jersey (Schad et al., 1987). Before starting the experiment, the farm was depopulated of pigs; tissues from 42 of 44 pigs, and sera of 20 of 41 pigs, tested positive for *Trichinella.* After depopulation, the farm was restocked with 102 *Trichinella*- free pigs supplied by USDA researchers. At the

termination of the 12-month experiment, *Trichinella* larvae were detected in tissues of 43 of 46 pigs of the group with maximal exposure to rats, in 13 of 42 pigs with intermediate contact with rats, but not in any of the 14 pigs with minimal rat contact. Results of the experiment indicated that rat exposure could contribute to transmission as vector hosts where rats feed on dead pigs, given that pigs can also feed on rats.

Further epidemiological investigations were conducted on this farm (Murrell et al., 1987; Leiby et al., 1988). During a 21-month period, wildlife were trapped around this farm. *Trichinella spiralis* was found in seven of 15 (46.6%) skunks (*Mephitis mephitis*), one of three opossums (*Didelphis virginianus*), two of two feral domestic cats (*Felis catus*), and one of one raccoon (*Procyon lotor*), but not in any of 18 deer mice (*Peromyscus* spp.) or any of five shorttail shrews (*Blarina brevicauda*) (Leiby et al., 1988). Genetic typing indicated that all wildlife isolates of *Trichinella* resembled *T. spiralis* from domestic pigs on the farm. It was concluded that wildlife became infected with *Trichinella* from scavenging tissues from infected pigs.

5.1.3. Biological distinctions between the parasites predominating in domestic and sylvatic transmission cycles

Uncertainty prevailed concerning the identity of *Trichinella* isolates occurring in domestic pigs and wildlife prior to the advent of differential diagnostic tools exploiting genetic differences. The meat of black bears, feral pigs, and several furbearing mammals were all sources of potential human exposure to *Trichinella*, prompting USDA efforts to compare the characteristics of parasites derived from these sylvatic sources to those of parasites derived from domestic pigs (Leiby et al., 1985; Murrell et al., 1985). Notably, most parasites derived from wild carnivores demonstrated poor infectivity to pigs and mice. Only two of nine isolates from black bears, two of three isolates from raccoons, one isolate from a skunk, and one from opossum from the US were able to infect pigs as well as isolates derived from pigs (Murrell et al., 1985) (see Table 4 for sources of wildlife isolates). Some isolates from wildlife demonstrating strong infectivity for pigs came from the immediate vicinity of pig farms known to be circulating *Trichinella* infections. Infectivity to other laboratory animals (hamsters, jirds, deer mice, rats, and multimammate rats) also varied. Infectivity of a polar bear isolate of *Trichinella* (imported from Canada) was 15 times higher in foxes as compared with the Beltsville *T. spiralis* isolate from a pig (Murrell et al., 1985). It was concluded that meat from furbearers and other scavenging wildlife likely posed a threat to human health, and that such wildlife were susceptible to biologically distinct forms of *Trichinella,* only one of which reproduced efficiently in mice and pigs; they further, correctly concluded that, "…new methods, perhaps biochemical, are needed" to characterize wildlife samples and determine the genetic evidence for distinctions among species (then all diagnosed as *T. spiralis*). Ultimately (see below) such tools bore out distinctions among species of *Trichinella* (Murrell et al., 1987). One genotype, native to sylvatic hosts in North American carnivores, would ultimately be recognized as a new species named in his honor, *T. murrelli* (Pozio and La Rosa, 2000). Survey data indicates this is the most prevalent species circulating among wildlife in the temperate regions of North America (Table 4).

5.1.4. Risks posed to wildlife from poorly managed pig farms

An investigation was conducted over 18 months on a pig farm in Maryland with very poor management (Hill et al., 2010). This farm was quarantined because of animal welfare concerns. Cannibalism was discovered to be taking place in pigs and pigs were also feeding on wildlife carcasses. Necropsied tissues were tested for *Trichinella* infections by muscle digestion and serology. *Trichinella spiralis* was isolated from 17 of 50 pigs, after which the property was depopulated of all pigs.

USDA researchers trapped wildlife on and near the farm over an 18-month period, starting six months after pig depopulation. Initially, five of 14 raccoons and two of three opossums were found positive for *T. spiralis*. Twelve months later, only one of ten raccoons (old enough to have been alive during the swine farm's operation) was found infected with *T. spiralis.* In the last trapping, none of 14 raccoons were infected; one, older opossum was infected. At follow-up, the infected raccoons were adult males with an average weight of six kg; younger and light weight raccoons were not infected. These data led the team to conclude that wildlife acquired infection from a focus of *Trichinella* in pigs maintained by cannibalism on the farm, and that wildlife infection risk waned after cessation of transmission in pigs. Although scavenging wildlife acquired infection from the pigs, transmission among wildlife in the absence of pigs did not appear to be sustainable (Hill et al., 2010).

6. Wildlife reservoirs as sources of *Trichinella* for humans and pigs

The prevalence of human infections in the United States declined drastically between 1936 and 1971, based on the detection of *Trichinella* in cadavers, coinciding with a declining prevalence of *Trichinella* in domestic pigs (Zimmermann et al., 1973). Surveillance reports by the Centers for Disease Control and Prevention (CDC) between 1997 and 2012, reported outbreaks of trichinellosis epidemiologically associated with ingestion of pork products and game meats, with a predominance of cases originating from the latter (Roy et al., 2003; Wilson et al., 2015).

Feral pigs and black bears have been a significant source of *Trichinella* infections for humans in the mainland U.S. (Zimmermann et al., 1973; Murrell and Pozio, 2011). The number of feral pigs (*Sus scrofa*) in the U.S. is estimated to exceed 5 million, and their geographic range continues to expand. Feral pigs pose a threat to those raised in non-controlled housing by serving as reservoirs for a variety of pathogens including *Toxoplasma* and *Trichinella* (Dubey et al., 2020b). The USDA's Wildlife Services has been charged with controlling feral pigs to mitigate environmental damage. They routinely collect sera from a subset of feral pigs for pathogen surveillance. In two such surveys, conducted 2006–2010 from 32 U.S. states, *Trichinella* antibodies were detected in 3.0% of samples tested; viable *T. spiralis* larvae were recovered from 6 of 330 (1.8%) tongues sampled (Table 4). In a follow up survey from 2014 to 2020, antibodies were detected in 12.4% of 7467 feral pigs tested by ELISA (Cleveland et al., 2024). These data indicate that a sylvatic cycle of *T. spiralis* continues, and surveillance will be needed to monitor outdoor herds exposed to feral pigs. The carcass of a single improperly cooked infected pig can be a source of trichinellosis for many people. In addition to *T. spiralis, T. pseudospiralis* has been

documented in feral pigs (Table 4).

Bears are another important wildlife reservoir of *Trichinella* infection in the U.S. Thousands of bears are hunted in the U.S. each year. Approximately 3500 black bears (*Ursus americanus*) are legally harvested each year in Pennsylvania, alone, during the November (Thanksgiving week) hunting season (Dubey et al., 2016).Outbreaks of trichinellosis continue to occur in the U.S., mostly associated with ingestion of raw or undercooked bear meat (Table 5). Proper cooking is the only way to prevent trichinellosis, because freezing will not kill all *Trichinella* genotypes (e.g. *T. nativa*) (Table 5).

7. Horses as a source of human trichinellosis

Typical hosts for *Trichinella* are predatory and scavenging carnivores and omnivores, given that ingestion of infected tissue constitutes the sole means of contracting infection. Surprisingly, the herbivorous nature of horses did not prevent them, when intentionally fed meat, from contracting infection and serving as a public health risk (Murrell et al., 2004a, 2004b). Horses will eat rats or food augmented with meat scraps (Murrell et al., 2004a, 2004b). Outbreaks of clinical trichinellosis have occurred in Europe in people who ate raw or undercooked horse meat, including meat imported from other countries (see Table 1). The biology of *Trichinella* in horses has been shown to differ from that in pigs. In horses, tongue is the most parasitized tissue (Gamble et al., 1996., Hill et al., 2007a, 2007b). Horses can be successfully infected by feeding *Trichinella* infected tissues (Table 1). In experimentally infected horses, IgG antibodies peaked six-ten weeks post-inoculation (p.i.) but waned by 26 weeks p.i.; however, horses continued to harbor viable *Trichinella* larvae even after turning serologically negative (Hill et al., 2007b). Additionally, *T. spiralis* exhibited resistance to freezing in tissues from experimentally infected horses (Hill et al., 2007a). Horse meat is rarely eaten in the U.S., where it is banned as a human food product (Whiting, 2007).

8. Thermal, irradiation, and chemical (curing) treatments of pork to kill *Trichinella*

The USDA's Food and Safety Inspection Service (FSIS) provides guidelines and regulatory oversight for the safety of meat and meat products. The FSIS depends on the USDA's research agency, ARS, to develop a scientific basis for such guidelines. The efficacy of various interventions to kill *Trichinella* in pork had been established by various studies (see Table 1). A USDA effort standardized this assessment, recruiting the talents of meat scientists, statisticians, radiation biologists, and food science specialists (Kotula et al., 1983). For example, for cooking/freezing parameters, temperatures of water or chemical baths were recorded digitally by thermocouples embedded in homogenized samples of infected meat pressed to uniform thickness. Similar procedures were adapted not only for *Trichinella* but also for *Toxoplasma gondii* (Dubey, 2010), so that data could be used to standardize safe processing requirements for these two organisms.

8.1. Cooking

Thermal death curves were generated for killing of *T. spiralis* in pork at different temperatures (Kotula et al., 1983). Using conventional cooking methods (not microwave), *Trichinella* was killed in 47 min at 52 °C, in 6 min at 55 °C, and in 1 min at 60 °C (Kotula et al.,1983). USDA (2018) used these data to require that pork be cooked for 2 h at 52.2 °C, for 15 min at 55.6 °C, or for 1 min at 60 °C. Currently, USDA recommends consumers cook fresh pork until the internal temperature reaches 63 °C (145 °F) (Gamble, 2021), based in large part on research conducted in collaboration with scientists at APDL.

8.2. Freezing

Low temperature death curves for *T. spiralis* were developed using samples frozen at 19 temperatures ranging from -1 °C to -193 °C (Kotula et al., 1990). *Trichinella spiralis* in pork was killed instantaneously at -23 °C. USDA (2018) guidelines specify temperatures for freezing pork intended for use in processed products (Gamble, 2021). Further studies indicated that in addition to *T. spiralis*, other North American genotypes of *Trichinella* (*T. murrelli, T. pseudospiralis, T. nativa*) are also killed by freezing (Hill et al., 2009). However, these data do not apply to horse meat infected with *T. spiralis*. USDA researchers established that unlike in pork, *T. spiralis* in horse meat can survive for at least eight weeks in meat stored at -18 °C (Hill et al., 2007a). It should be noted that some freeze-resistant parasites circulating among wildlife hosts (*T. nativa* and T6) can survive for years at subzero temperatures in native host tissues.

8.3. Curing

Preservation of pork in salt and spices and drying (curing) has been used for a long time to produce ready-to-eat hams, sausages, pepperoni, and other pork products (Lin et al., 1990a, 1990b). Therefore, USDA scientists examined how curing efficacy responds to changes in pH and to the concentration of one such salt, NaCl on *Trichinella* and *Toxoplasma* (Hill et al., 2017; Dubey et al., 2020a). Until recently, producers lacked a model to judge the efficacy of the curing process. Previous studies judged the efficacy of curing by assessing larval motility. However, physical appearance is a poor judge of the viability of the parasite; some motile larvae are not infectious, and some apparently inert larvae remain infective to mice. The viability of larvae was tested by bioassay in mice. Salt and pH proved important in the efficacy of curing. Salt concentrations above 1.3%, in combination with a pH of 4.6, had deleterious effects on larvae. *Trichinella* larvae were killed after eight days incubation in a salt concentration of 2.8%. Other salts, such as nitrous salts, may have different effects.

8.4. Irradiation

The United States has a huge stockpile of cessium-137, and food irradiated at low doses does not affect the taste, color, or texture of meats. Cessium-137 has excellent penetration qualities. In the 1980's, pork producers envisaged irradiating whole pig carcasses to kill parasites in pork. In collaboration with the U.S. Department of Energy, USDA parasitologists and radiobiologists at the Sandia National Laboratories determined that pork experimentally infected with the Beltsville strain of *T. spiralis* could be rendered noninfectious by exposure to a low dose (30 krads) of cessium-137 (Brake et al., 1985). This was the basis for the first FDA and FSIS approval of irradiation for meat (irradiation of strawberries was first for any food). Lack of public acceptance of irradiated foods, however, dissuaded implementation of this measure.

8.5. Hydrodynamic pressure

In an initial study USDA research determined that the hydrodynamic pressure (MPa 55–60) typically used for meat tenderization, had no demonstrable effect on the viability of *T. spiralis* (Gamble et al., 1998). In subsequent experiments, *T. spiralis* was inactivated in pig masseter by all treatments of HPP as confirmed by both microscopy and mouse bioassays; infected pig masseter muscles were pressurized at 483 and 600 MPa for 0.5 to 5 min (Porto-Fett et al., 2010). Additionally, this HPP level treatment drastically reduced-other microbial pathogens (*Listeria monocytogenes*, *Escherichia coli* O157:H7, *Salmonella* spp.).

9. Preventive strategies

9.1. Prospects for vaccination of pigs against Trichinella and development of resistant breeds of swine

In the 1980's, strategies to control transmission of *T. spiralis* on high-risk farms included efforts to vaccinate pigs and to breed swine resistant to *Trichinella* (Murrell, 1983, 1985a-c). Immunity to *T. spiralis* in pigs and mice was explored at Beltsville for the development of immune-based diagnostic methods and to foster immune protection (Alizadeh and Murrell, 1985; Gamble, 1985a, 1985b; Gamble and Murrell, 1986; Lunney and Murrell, 1988). Although there is no in utero transmission of *T. spiralis* in pigs, protective antibodies were found to be transferred via colostrum (Marti and Murrell, 1989). Pigs inoculated with a low dose of live *T. spiralis* larvae, but not with crude antigens, were found to induce acquired resistance to challenge with heavy doses of larvae (Gamble, 1985a; Murrell, 1985a; Marti and Murrell, 1986b; Marti et al., 1987; Lunney and Murrell, 1988). Pigs immunized with excretory secretory larval antigens (Gamble et al., 1986) or stichosome antigens alone, were not effective (Murrell, 1985c; Murrell and Despommier, 1984). Immunity to *Trichinella* infection in mice was found to be mediated by both humoral cellular immune components (Urban Jr. et al., 2000). Differences in immune responses were noted in mice versus pigs. Immunity in pigs was directed against the muscle dwelling larvae but not against adults in the intestine, whereas worms were expelled from the intestine of immune mice; this discovery posed a challenge for developing an effective vaccine for pigs (Gamble and Murrell, 1987). Marti et al. (1987) showed that immunized pigs responded most strongly to newborn larvae during their humeral migration. Notably, immunizing pigs with only inactivated newborn larvae proved effective. Importantly, this distinguished rodent and porcine responses to infection and immunization, rendering rodents of limited value as an experimental model in testing candidate vaccines. Using an inbred miniature swine herd at Beltsville, major histocompatibility genes were found to regulate swine immune responses to *Trichinella*; only pigs of the $SLA^{a'a}$ phenotype demonstrated high resistance to *Trichinella* (Lunney and Murrell, 1988; Madden et al., 1990, 1993; Dillender and Lunney, 1993). These research efforts demonstrated that the parasite is quite capable of subverting innate host resistance as well as acquired immunity, rendering impractical immunization, or breeding as widely applicable control strategies.

9.2. Educating hunters

Clinical trichinellosis in humans in the U.S. is now exceedingly rare; most cases stem from consuming meat of feral pigs or bears (Hall et al., 2012; Holzbauer et al., 2014). Prevalence of *Trichinella* in bears (Table 4) is not likely to decrease soon; viscera of hunted animals, when left in open or shallow coverings, are scavenged by other carnivores that in turn could serve as food for bears, perpetuating the cycle of *Trichinella* in wildlife. Educating hunters concerning *Trichinella* transmission can minimize the prevalence of this parasite, and there is some scientific evidence of success as illustrated by the first such effort initiated by ARS researchers.

An epidemiologic investigation was conducted on feral pigs in a private game park in New Hampshire in the U.S. (Worley et al.,1993). In 1987, a control program was introduced in the game park to reduce transmission of *Trichinella* in feral pigs. Hunters were issued specific permits, and they were required to incinerate viscera rather than field dressing each hunted carcass. Samples of tongues, diaphragms, and muscle scraps were collected from each hunted pig and shipped cold to ARS laboratory in Beltsville for testing (see Table 4). During the 7-year control program, *Trichinella* was detected in a total of 160 (10.5%) of 1515 hunted pigs. Before the intervention, prevalence was 15% in 1986 and 20% in 1987. Thereafter, prevalence decreased from 20% in 1988 (15/77) to 12% in 1989 (34/284), 11.2% in 1990, (17/152) 6.9% in 1991; (19/273); and 3.6% in 1992 (13/373), when this experiment was terminated (Worley et al., 1993).

9.3. Preharvest control and Trichinella certification programs

The USDA Animal and Plant Health Inspection Service (APHIS) is charged with regulating efforts to prevent livestock infections.

APDL scientists at Beltsville played a major role in helping APHIS achieve its goals to reduce the risk of *Trichinella* transmission from eating pork (Gamble, 2022; Gamble et al., 2000, 2001; Pyburn et al., 2005). These investigations involved developing and optimizing testing methods (ELISA) and direct testing of pork for *Trichinella* larvae (Gamble, 2021).

Following the recommendations of the International Commission on Trichinellosis (ICT) in 2000 (Gamble et al., 2000), regarding pre-harvest control, USDA scientists worked with APHIS and the U.S. pork industry to develop a voluntary certification program based on good management practices to exclude risk for exposure to *Trichinella*. This program included producer education, and several levels of auditing. While this voluntary program did not achieve widespread participation due to a lack of incentives, many of the principles developed were ultimately incorporated into the U.S. Pork Quality Assurance Plus program (https://lms.pork.org/Tools/View/pqa-plus), which includes participation by >90% of U.S. pork producers.

9.3.1. Development of specific and sensitive ELISA

A highly specific and sensitive ELISA was developed using excretory and secretory (ES) products from in vitro cultured *T. spiralis* larvae (Gamble et al., 1983, Murrell et al., 1986; Gamble, 1998; Oliver et al., 1989; Ivanoska et al., 1989) to overcome sub-optimal specificity when using somatic antigens in the ELISA originally developed by Dutch researchers (Ruitenberg et al., 1974; Van Knappen et al.,1976). For preparation of ES antigens, muscle larvae from experimentally infected rats were incubated in a cell culture medium, filtered to remove larvae and the filtrate dialyzed (Gamble et al., 1983). Further advances in ELISA technology for detecting *Trichinella* included identifying, purifying, cloning, and expressing diagnostic antigens (Gamble and Graham, 1984; Zarlenga and Gamble, 1990). The ELISA has been extensively validated, using the digestion method for comparison (Murrell et al., 1986; Pyburn et al., 2005) in pigs infected with *T. spiralis* and other *Trichinella* species (Kapel and Gamble, 2000) and is widely used for surveillance purposes.

9.3.2. Large scale testing of pork for evidence for muscle larvae

A variety of studies performed since the 1980's assessed the prevalence of *Trichinella* infection in U.S. pigs. Some of these studies were conducted in collaboration with other USDA agencies, including the 1990, 1995, 2000 and 2006 National Animal Health Monitoring Surveys (NAHMS). Other studies were regional in nature, focusing on farms and regions with elevated likelihood of infection. Additional studies were performed to inform the industry about progress of eradication of infection in commercial pigs; some results were only reported internally. These studies are summarized in Table 3.

Beginning in 1988, the APDL initiated a program at the request of the Agricultural Marketing Service (AMS) to train and monitor the testing of horses slaughtered for export (AMS Trichinae Export Program). This program responded to outbreaks of trichinellosis in France and Italy linked to consumption of horsemeat, purportedly from the U.S. The success of this program attracted participation by the U.S. pork industry and opened new export markets. All testing performed in the AMS program employed artificial digestion according to standard practices. From 1996 to 2010, six pork slaughter facilities tested a total of 38,755,374 samples, all of which tested negative. No positive horses were ever documented in the U.S., despite a testing program required for all horse slaughter plants commencing after the outbreaks in 1987. Naturally infected horses have been reported from Serbia, Romania and Poland (Murrell et al., 2004a, 2004b; Liciardi et al., 2009; Iacob et al., 2022). A horse testing positive for *T. murrelli* was reported to have been imported from the U.S. (Scandrett et al., 2018).

9.3.3. Continued support for FSIS personnel performing surveillance testing

A recent USDA review of methods (serology, DNA detection, muscle digestion) for the detection of *Trichinella* in pork, reaffirmed that pepsin muscle digestion provides the most efficient and cost-effective method for surveillance, but pointed to future possibilities to realize gains in other diagnostic methodologies (Barlow et al., 2021). The ARS's APDL propagates the Beltsville *T. spiralis* isolate in mice and rats. It employed this method in its recent comprehensive survey of Pork Quality Assurance Plus pigs (Gamble et al., 2024) and provides "check samples" to the Agricultural Marketing Service for use in testing the proficiency of personnel performing tests required for export to certain markets. Results of experimental *T. spiralis* infections in pigs and rats, conducted four decades ago at Beltsville, indicated that tongue is one of the most heavily infected tissues and most convenient for epidemiological studies (Kotula et al., 1984; Marti and Murrell, 1986a).

9.3.4. Chemotherapeutic inactivation of parasites

Although most commercial pigs are raised under conditions of biosecurity that protect them from infection risk, USDA researchers verified that it is possible to render muscle larvae of *T. spiralis* incapable of causing further infection by administration of mebendazole (Fredericks et al., 2024). Treating with 100 mg/kg (but not 5 or 50 mg/kg) for three- five days renders encysted *Trichinella* muscle larvae non-infective. This provides producers of pigs at higher risk (e.g., those raised on pasture) with means to mitigate such risk.

10. Genetics, molecular epidemiology, evolution

Reviews regarding the systematics, molecular epidemiology, and evolution of *Trichinella* demonstrate the breadth of research from an international community dedicated to understanding the biology of these worms (Zarlenga et al., 2020; Rosenthal et al., 2021; Bilska-Zając et al., 2022). Contributions of USDA researchers are summarized here.

10.1. Taxonomy

As stated previously, USDA researchers played an important role in taxonomy of *Trichinella* species as summarized in in Table 2.

10.2. Diagnostics-PCR

Early efforts to identify DNA differences among *Trichinella* lineages were focused on restriction fragment length polymorphisms (RFLPs) and the development of DNA hybridization probes. (Dame et al., 1987). Subsequently, Zarlenga et al. (1991) developed a DNA probe to differentiate *T. murrelli* from *T. spiralis*. These findings led to the conclusion that, contrary to popular belief, *T. murrelli* and not *T. spiralis* is the predominant species in the U.S. wildlife.

Progress was made concerning molecular diagnostics for *Trichinella* by identifying a size polymorphism in expansion segment 5 of the 28S ribosomal subunit that differed among species (Zarlenga and Dame, 1992). Subsequently, microsatellite repeat markers were developed that differentiated among different populations (Zarlenga et al., 1996). Ultimately, development of a multiplex PCR that amplified several different loci in the ribosomal DNA in a single reaction differentiated unique banding patterns for all lineages of *Trichinella* then known (Zarlenga et al., 1999). The multiplex assay was refined over the years to include newly identified species (Zarlenga et al., 2001) and remains the gold standard for diagnostics laboratories worldwide.

10.3. Epidemiology, outbreak tracing

Researchers at the USDA continue to advance efforts to understand the epidemiology of *Trichinella* and develop new molecular tools to differentiate *Trichinella* isolates and track outbreaks in near real-time (La Rosa et al., 2012). Results indicated that *T. spiralis* in Europe and the Americas harbor far less variation than do *T. spiralis* in East Asia, and far less variation than European populations of *T. britovi,* despite occupying an especially large geographic expanse. Microsatellites were later used to trace *Trichinella* outbreaks in Poland (Bilska-Zajac et al., 2021; Bilska-Zając et al., 2022). Findings from this collaborative team between USDA and European researchers helped differentiate local outbreak samples, connect them to wildlife genotypes, and separate them from circulating strains in wild boars (Bilska-Zając et al., 2022).

10.4. Evolution

USDA researchers have also been central to uncovering the ancient and more recent evolutionary history of *Trichinella*. Using ribosomal and mitochondrial DNA sequences to reconstruct the relationships among the extant species of *Trichinella* were uncovered and findings provided as to how these species evolved (in mammals) and moved across the globe (Zarlenga et al., 2006). This biogeographic hypothesis was affirmed by whole genome sequencing data (Korhonen et al., 2016).

USDA researchers contributed additional insights concerning more recent evolutionary events, including ongoing processes such as hybridization between lineages (Franssen et al., 2015).

The advent of affordable genomic sequencing enabled further studies into the evolutionary history of *T. spiralis* populations. Researchers showed that European *T. spiralis* populations appear to have grown and ebbed with the fate of European pigs, in contrast to the history of Asian pig populations (Hecht et al., 2018) and that European *T. spiralis* diverged from Asian *T. spiralis* prior to the domestication of swine (Thompson et al., 2021).

To understand the circumstances that enabled *Trichinella*'s ancestors to transition from free-living to intracellular parasites genes present in parasites but absent from free-living nematodes were identified (Mitreva et al., 2011). Additionally, a detoxifying enzyme (cyanase) that enables *Trichinella* to thrive inside mammalian cells was identified (Zarlenga et al., 2022). *Trichinella* evidently acquired the gene encoding this enzyme from a plant or fungus, via horizontal gene transfer (Zarlenga et al., 2019). This work helps to explain the ability of *Trichinella* to survive inside a muscle cell for decades.

10.5. Genomics

USDA researchers led efforts to understand *Trichinella* genomics, providing essential research resources for the wider community the first draft genome for *T. spiralis* was published (Mitreva et al., 2011). Using the emerging shotgun sequencing approach the mitochondrial genome of *T. spiralis* was sequenced and compared with *T. murrelli*. They uncovered cryptic variation across the mitochondrial genome by sequencing to great depth, demonstrating that pooled isolates are not uniform (Webb and Rosenthal, 2010, 2011; Thompson et al., 2017).

11. Conclusions

USDA scientists have contributed to aspects of control of *Trichinella* infection in pigs and concomitant prevention of public health risk to humans for >125 years. In the latter part of the 19th century and the first half of the 20th century, these efforts were primarily reactionary in support of domestic and export markets for fresh pork. Renewed interest in documenting pork safety began in the 1960s, and with a focus on this parasite by Murrell and colleagues, the Beltsville Agricultural Research Center became a hub of research of all aspects of *Trichinella* and trichinellosis. The work of various contributors included studies to support USDA regulatory agencies FSIS and APHIS in domestic and foreign markets including strategies for pre- and post-harvest mitigations, as well as studies on aspects of

basic biology, biochemistry, host immunology, detection and surveillance, epidemiology, and phylogeny and evolutionary relationships. USDA scientists also served in leadership and advisory roles in a variety of national and international organizations (e.g. ICT, WOAH, FAO). Today, modern production systems drive the incidence of *Trichinella* to negligible levels. The U.S., like many countries, does not have cases of trichinellosis acquired from commercial pork. Consistent with HACCP principles, responsibility has shifted to producers and processers to assure a safe and wholesome product. Production standards like PQA+ in the U.S. pork industry facilitate success in assuring absence of infection in commercial pork products. Nevertheless, *Trichinella* remains a fascinating model for research studies in areas such as the host parasite relationship, genetic diversity and molecular evolution.

CRediT authorship contribution statement

Jitender P. Dubey: Writing – original draft, Methodology, Investigation, Conceptualization. **Peter C. Thompson:** Writing – original draft. **Valsin Fournet:** Writing – review & editing. **Dolores E. Hill:** Writing – review & editing, Writing – original draft. **Dante Zarlenga:** Writing – review & editing, Writing – original draft. **H. Ray Gamble:** Writing – review & editing, Writing – original draft. **Benjamin M. Rosenthal:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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