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SOURCES AND RESERVOIRS OF *TOXOPLASMA GONDII* INFECTION ON 47 SWINE FARMS IN ILLINOIS

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ABSTRACT: Field studies were conducted on 47 swine farms in Illinois during 1992 and 1993 to identify sources and reservoirs of *Toxoplasma gondii* infection. Blood samples were obtained from swine and from trapped wildlife. Serum antibodies to *T. gondii* were determined using the modified agglutination test, incorporating mercaptoethanol. Antibodies to *T. gondii* (titer ≥25) were found in 97 of 4,252 (2.3%) finishing pigs, 395 of 2,617 (15.1%) sows, 267 of 391 (68.3%) cats, 126 of 188 (67.0%) raccoons, 7 of 18 (38.9%) skunks, 29 of 128 opossums (22.7%), 6 of 95 (6.3%) rats, 3 of 61 (4.9%) white-footed mice (*Peromyscus* sp.), and 26 of 1,243 (2.1%) house mice (*Mus musculus*). Brains and hearts of rodents trapped on the farm were bioassayed in mice for the presence of *T. gondii*. *Toxoplasma gondii* was recovered from tissues of 7 of 1,502 (0.5%) house mice, 2 of 67 (3.0%) white-footed mice, and 1 of 107 (0.9%) rats. Feces of 274 cats trapped on the farms and samples of feed, water, and soil were bioassayed in mice for the presence of *T. gondii* oocysts. *Toxoplasma gondii* was isolated from 2 of 491 (0.4%) feed samples, 1 of 79 (1.3%) soil samples, and 5 of 274 (1.8%) samples of cat feces. All mammalian species examined were reservoirs of *T. gondii* infection. All farms had evidence of *T. gondii* infection either by detection of antibodies in swine or other mammalian species, or by detection of oocysts, or by recovery from rodents by bioassay. The possibility of transmission of *T. gondii* to swine via consumption of rodents, feed, and soil was confirmed.

Humans become infected with *Toxoplasma gondii* usually by ingesting oocysts in food and water contaminated by cat feces or by consuming tissue cysts in undercooked meat (Dubey and Beattie, 1988). Pork is considered to be the most important meat source of *T. gondii* infection in the U.S. (Dubey, 1986). There are potentially serious consequences of *T. gondii* infection in humans. Exposure of women to *T. gondii* for the first time during pregnancy can result in perinatal mortality and birth defects (Alford et al., 1974; Wilson et al., 1980; Dubey and Beattie, 1988; Frenkel, 1990). Infection of immunocompromised humans, e.g., cancer and AIDS patients can result in encephalitis, blindness, and death (Dubey and Beattie, 1988; Frenkel, 1990).

Infection of swine with *T. gondii* is widespread. In a national survey in 1983–1984, antibodies to *T. gondii* were found in 23% of 11,229 market-aged <7 mo and 41.4% of 613 adult swine (Dubey et al., 1991). Recently, viable *T. gondii* was isolated from 17% of 1,000 sows from Iowa (Dubey, Thulliez, and Powell, 1995).

There are several potential reservoirs of *T. gondii* on swine farms. Cats are the definitive host; the shedding of *T. gondii* oocysts in cat feces may contaminate feed, water, and soil that can be ingested by swine. Transmission to swine may also occur by the consumption of tissues of animals such as rodents and birds infected with *T. gondii* tissue cysts and by cannibalism (Penkert, 1973; Dubey and Beattie, 1988).

In order to develop control strategies to reduce or eliminate *T. gondii* in the nation's swine herds, it is imperative to obtain detailed information on the reservoirs of infection and the modes of transmission of *T. gondii* to swine. Epidemiologic investigations of the reservoirs of *T. gondii* on swine farms have been limited. Lubroth et al. (1983) in a study of 2 swine farms in Georgia found serological evidence of *T. gondii* infection in house mice, white-footed mice, and rats, as well as recovering *T. gondii* by bioassay of rodent tissue in mice; they concluded that rodents were a major source of *T. gondii* infection for swine. Smith et al. (1992) trapped wildlife on 19 swine farms in 3 counties in central Iowa, and found serologic evidence for *T. gondii* infection in cats, rodents, raccoons, skunks, and opossums; cats were implicated as the primary source of *T. gondii* infection for swine. Neither of these studies attempted detection of *T. gondii* oocysts on swine farms.

We have conducted extensive epidemiological investigations on *T. gondii* infection on swine farms in Illinois (Weigel, Dubey, Siegel, Hoefling et al., 1995). In the first paper of this series, we report the results of an investigation of sources and reservoirs of *T. gondii* infection on 47 swine farms in Illinois. The companion paper (Weigel, Dubey, Siegel, Kitron, et al. 1995) examines risk factors for transmission of *T. gondii* to swine.

MATERIALS AND METHODS

Field methods

Selection of farms: The epidemiologic investigations of *T. gondii* infection on swine farms was conducted during the spring and summer of 1992 and 1993. In each year, 24 farms were selected. In 1992, 14 of the farms were selected during recruitment of farms for the cross-sectional survey described in the companion paper (Weigel, Dubey, Siegel, Kitron et al., 1995). In addition, 10 farms used previously for research and clinical training were recruited. The sample of 24 farms was selected to represent the diversity of Illinois swine operations in herd size, type of confinement, and geographic location.

In 1993, a sample of 24 farms was selected from the sampling frame of farms with completed risk factor interviews that had not been selected in 1992. Selection was by stratified random sampling, with geographic region (3 levels: north, central, south), type of housing (4 levels: total confinement, partial confinement with sows outside, partial confinement with growing or finishing pigs outside, pasture), and herd size (2 levels: above median breeding herd size [=167.5 sows], below median breeding herd size) as strata. No distinction was made between swine housed outside on dirt or on concrete. However, on the farms selected, concrete lots were covered with mud. *Toxoplasma gondii* serologic prevalence was not a factor taken into account in farm selection.

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One pasture operation studied in 1992 was reselected for the 1993 study because the farm had become a farrow-to-finish production unit. Thus, there were 47 farms in the 2-yr sample. Each farm in the study was visited 3 times between late March and early October.

Survey of swine for T. gondii*:* On each visit, a blood sample was obtained from 30 market-weight finishing pigs (or the largest finishing pigs on the premises). In 1992, an additional sample of blood from 30 sows was taken on the first of the 3 visits. In 1993, blood was obtained from 30 sows on each of the 3 visits. A sample of 30 sows provides a 95% confidence of detecting a *T. gondii* seroprevalence of at least 10%; a sample of 90 sows or finishing pigs provides a 99% confidence of detecting a seroprevalence of at least 5% (Beal, 1983).

Trapping of animals: On each visit, overnight live trapping of small and medium size mammals was conducted. Traps were set for mice indoors (at a density of 1 per 150 m^2 of building floor space, with additional traps set in areas where sightings of mice or mouse feces confirmed their presence) and outdoors (at 30-m intervals along perimeters of swine buildings and lots). These traps were designed to trap small rodents; however, occasionally small birds were also trapped. A total of 32 traps were set for rats, both indoors and outdoors. These were mostly distributed evenly throughout the indoor facilities except when the farm personnel identified an area where rats were sighted, in which case more traps were placed in these areas. The bait for rodent traps was bread with peanut butter. A total of 20 medium-sized mammal traps baited with sardines were set outdoors. Their primary purpose was to trap cats, although raccoons, opossums, and other medium-sized mammals were also trapped. These traps were dispersed over the farm, with 10 placed near swine buildings and lots, and 10 placed in fields, woods, and along ponds and streams (within 0.8 km of the farm). In addition, whenever possible, additional cats were caught by hand.

Animal processing: Blood samples were obtained from each animal captured. All animals except pet cats and rats were anesthetized by injection with a ketamine HCl and acepromazine maleate (Aveco Co., Inc., Fort Dodge, Iowa) cocktail. Rats were anesthetized with ether. Rodents and birds were necropsied in the field, and the hearts and brains were collected. All other live-trapped animals were released. Feces from cats were obtained from anesthetized cats by inserting a latex-gloved finger into the anus, or by abdominal massage, or by a water enema (second year only).

Collection of feed, soil, and water samples: Samples of feed, soil, and water were collected each time the farm was visited. Approximately 0.5 kg of pig feed was collected from feed bins in all sections of the herd facility. Soil samples of approximately 250 g were collected only from farms where hogs were kept outdoors. Dry soil was collected in plastic bags; muddy soil was collected in centrifuge tubes. Water samples were collected in 50-ml centrifuge tubes. Water was obtained only from pens where it was delivered in open troughs or bowls where cats might defecate.

Samples of tissues, cat feces, sera, feed, water, and soil were transported by overnight air express mail to the Parasite Biology and Epidemiology Laboratory, Beltsville, Maryland, for *T. gondii* examination.

Laboratory methods

Serological examination for T. gondii*:* Sera from all animals were examined at Beltsville for anti-*T. gondii* IgG antibodies with the modified agglutination test (MAT) as described by Dubey and Desmonts (1987). For MAT, sera were diluted 1:25, 1:50, and 1:500. Results of the 1:25 dilution were selected as the threshold titer for determining seropositive status. Certain sera were retested at the Paris laboratory for MAT antibodies and for the Sabin–Feldman dye test (DT) antibodies as described by Desmonts and Remington (1980). Previous studies in our laboratory have estimated the MAT to have a sensitivity of 83% and a specificity of 90% (Dubey, Thulliez, Weigel et al., 1995).

Isolation of T. gondii *from feces of naturally infected cats:* Feces of each cat were emulsified in approximately 40 ml of water, filtered through 2 layers of gauze, poured into a 50-ml tube, and centrifuged. All centrifugations (either feed, water, or tissues) were performed in a floating head centrifuge at 1,180 g for 10 min, irrespective of source of sample. The supernatant was discarded and the sediment was mixed with approximately 40 ml of sucrose solution (specific gravity 1.28), and centrifuged. A drop of the film from the very top of the fecal float was examined microscopically for oocysts between a coverslip and a slide. Approximately 5 ml of the supernatant from each fecal float was pipetted into a 50-ml tube, mixed with 45 ml of water, and centrifuged. After discarding the supernatant, 5 ml of 2% aqueous H_2SO_4 was added to the sediment and the capped tube was aerated at room temperature (20–22 C) for at least 7 days. Thereafter, tubes were stored at 4 C until bioassayed in mice.

For bioassays in mice, contents of each tube were neutralized with 3.3% NaOH, and after adding 40 ml of saline, the tube was centrifuged. The supernatant was discarded and 1–2 ml of saline was added to the sediment and contents were inoculated into mice (Dubey and Beattie, 1988).

Examination of water samples for T. gondii*:* Water samples were centrifuged, the supernatant was discarded, and after adding 5 ml of 2% H_2SO_4, the sample was aerated at room temperature for 7 days. Thereafter, samples were treated the same way as fecal floats.

Examination of feed and soil samples for T. gondii*:* Each feed or soil sample was poured into a 500- or 1,000-ml jar. Water was added to at least 3 cm above the level of the feed. After soaking overnight at room temperature, the sample was filtered through 2 layers of gauze and the entire filtrate was centrifuged in 250-ml bottles. The sediment from all bottles from 1 sample was pooled and recentrifuged in a 250-ml bottle. After pouring off the supernatant, the sediment was floated in approximately 200 ml of sucrose solution. Twenty-five milliliters of the supernatant were removed from the top layer of the sucrose float, mixed with 225 ml of water, and centrifuged. After decanting the supernatant, the sediment was mixed with water and centrifuged in a 50-ml tube with a conical bottom; the water supernatant was removed and the sediment was suspended in 5 ml of 2% H_2SO_4 and aerated at room temperature for at least 7 days. Thereafter, the feed sample was treated the same way as cat feces.

Preparation of rodent tissues for T. gondii *bioassay:* The brain and heart of each rodent were ground together in a mortar with pestle in 1–2 ml of aqueous 0.85% NaCl solution (saline). When large particles had settled, the fine tissue suspension was drawn into a 3-ml syringe containing 1.5 ml of antibiotic saline solution containing 2,000 units of penicillin and 200 μg of streptomycin per ml of saline. After 30–120 min at room temperature, the tissue suspension was used for inoculation into mice.

Bioassay in laboratory mice to identify T. gondii *infection:* All mice used for bioassays were Swiss-Webster albino females weighing approximately 20–25 g. Each sample of rodent tissue, feed, water, and soil was inoculated subcutaneously (s.c.) into 2 mice. The processed samples of cat feces were inoculated orally or s.c. into 2–4 mice. The mice were examined for *T. gondii* infection. Impression smears of lung and/or brain of the mice that died after inoculation with test material were examined microscopically for *T. gondii* tachyzoites (lungs) or tissue cysts (brain). Survivors were bled from the orbital sinus 2 mo after inoculation with test material; a 1:50 dilution of each mouse serum was tested for anti-*T. gondii* antibodies using the MAT. All mice, irrespective of their serologic status, were killed 2 mo after inoculation, and a 2–4-mm portion of their cerebrum was crushed between a glass slide and coverslip and examined microscopically for *T. gondii* tissue cysts (Dubey and Beattie, 1988). Mice were considered *T. gondii* positive only when tachyzoites or tissue cysts were demonstrated in their tissues and antibodies in the serum.

Brains of mice containing tissue cysts of the isolates from feed, water, cat feces, or rodent tissues were fed directly to *T. gondii*-free cats or were first cultivated in *T. gondii*-free laboratory mice and then fed to *T. gondii*-free cats. These procedures were undertaken to exclude *Hammondia hammondi* infection. *Toxoplasma gondii* and *H. hammondi* are structurally similar coccidians, however, *H. hammondi* cannot be maintained by mouse to mouse passage (Frenkel and Dubey, 1975). Oocysts obtained from experimental cats were orally inoculated into mice to complete the transmission cycle. The cats were from the *T. gondii*-free cat colony at the Beltsville Agricultural Research Center, Beltsville, Maryland (Dubey, 1995). Antibodies to *T. gondii* were not found in 1:25 dilution of the preinoculation serum of any of these cats when examined in MAT.

RESULTS

Table I presents a summary of the trapping and serological testing results by species and year for all 47 farms in the study.

TABLE 1. *Toxoplasma gondii* seroprevalence by species.*

Species	Year	Sera tested	Titer <25	25	50	100	500	No. of positive sera	% Positive sera
Cats									
Adult	1992	161	40	12	20	0	89	121	75.2
	1993	134	32	8	8	4	82	102	76.1
	Both	295	72	20	28	4	171	223	75.6
Juvenile	1992	48	30	8	6	0	4	18	37.5
	1993	48	22	5	2	4	15	26	54.2
	Both	96	52	13	8	4	19	44	45.8
All	1992	209	70	20	26	0	93	139	66.5
	1993	182	54	13	10	8	97	128	70.3
	Both	391	124	33	36	8	190	267	68.3
Raccoons	1992	126	46	17	57	0	6	80	63.5
	1993	62	16	14	9	9	14	46	74.2
	Both	188	62	31	66	9	20	126	67.0
Skunks	1992	10	6	0	1	0	3	4	40.0
	1993	8	5	1	0	0	2	3	37.5
	Both	18	11	1	1	0	5	7	38.9
Opossums	1992	69	55	5	8	0	1	14	20.3
	1993	59	44	7	7	1	0	15	25.4
	Both	128	99	12	15	1	1	29	22.7
Rats	1992	22	22	0	0	0	0	0	0.0
	1993	73	67	3	2	1	0	6	8.2
	Both	95	89	3	2	1	0	6	6.3
White-footed mice	1992	31	28	2	1	0	0	3	9.7
	1993	30	30	0	0	0	0	0	0.0
	Both	61	58	2	1	0	0	3	4.9
House mice	1992	577	571	2	3	0	1	6	1.0
	1993	666	646	14	0	4	2	20	3.0
	Both	1,243	1,217	16	3	4	3	26	2.1
Swine									
Sows	1992	743	639	34	47	0	23	104	14.0
	1993	1,874	1,583	87	38	53	113	291	15.5
	Both	2,617	2,222	121	85	53	136	395	15.1
Finishing pigs	1992	2,147	2,109	15	12	0	11	38	1.8
	1993	2,105	2,046	21	6	12	20	59	2.8
	Both	4,252	4,155	36	18	12	31	97	2.3

* Other species (seropositive/samples): sparrow (0/21), vole (0/5), rabbit (0/4), ground squirrel (0/3), mole (0/2), shrew (0/2), groundhog (0/1).

Table II shows the variation among farms in serological testing results. Seroprevalence is defined as the percentage of samples that tested positive at the 1:25 dilution. Overall, the *T. gondii* seroprevalence was highest in cats (68.3%) and raccoons (67.0%). Adult cats had a higher seroprevalence (75.6%) than juvenile cats (45.8%). Cats were found on all farms except 1, with seropositive cats on 44 farms (93.6%). The median cat seroprevalence among farms was 66.7%. Except for house mice, relatively few rodents were trapped. The overall seroprevalence among house mice was low (2.1%). House mice were trapped on all 47 farms, with 14 farms (29.8%) having at least 1 seropositive mouse. The highest seroprevalence in *M. musculus* among farms was 14.3%. With respect to swine, 15.1% of sow samples were seropositive. There was a wide, positively skewed distribution of sow seroprevalence rates among farms (0% to 93.9%; median = 6.7%), with 37 farms (78.7%) having at least 1 seropositive sow. The seroprevalence among finishing pigs was 2.3%. Among farms, the median seroprevalence was 1.1% (maximum = 16.7%), with 26 farms (55.3%) having seropositive finishing pigs.

Toxoplasma gondii was isolated from the tissues of 7 of 1,502 house mice (0.5% of samples) on 5 farms (10.6% of farms), 2 of 67 (3.0%) white-footed mice on 2 farms (4.3%), and 1 of 107 (0.9%) rats (Table III); no *T. gondii* was isolated from tissue samples of 8 voles (*Microtus* spp.), 3 ground squirrels, 2 moles, 2 shrews, and 21 sparrows. It is noteworthy that of the 9 infected rodents identified, 6 of them had no detectable antibodies to *T. gondii* in their sera (Table III).

Toxoplasma gondii was isolated by bioassay in mice from 5 of 274 (1.8%) cat fecal samples on 4 farms (8.5%), 2 of 491 (0.4%) feed samples on 2 farms (4.3%), and 1 of 79 (1.3%) soil samples (Table IV). Experimental cats fed tissue cysts of all strains isolated from rodents, soil, and feed shed *T. gondii* oocysts in their feces. Two of the *T. gondii* strains isolated from house mice were lethal to mice on their original passage (Table III). Oocysts of most isolates from Illinois were lethal to mice.

When considering both the serological testing and *T. gondii* isolation from tissues, evidence for the presence of *T. gondii* infection in rodents (either *Mus, Peromyscus,* or rats) was present for 22 farms (46.8%). When considering the serological test-

TABLE II. Variation among farms in *Toxoplasma gondii* seroprevalence by species.

Species	Year	Farms tested		No. tested per farm				Farms positive		Seroprevalence by farm (%)			
		No.	%	Minimum	Maximum	Mean	Median	No.	%	Minimum	Maximum	Mean	Median
Cats	1992	23	100.0	2	27	9.1	8	22	95.7	0.0	100.0	66.0	66.7
	1993	23	95.8	0	27	7.6	6	22	91.7	0.0	100.0	69.4	66.7
	Both	46	97.9	0	27	8.3	8	44	93.6	0.0	100.0	66.7	66.7
Raccoons	1992	18	78.3	0	26	5.5	2	15	65.2	0.0	100.0	58.0	57.4
	1993	16	66.7	0	16	2.6	1	14	58.3	0.0	100.0	73.6	95.0
	Both	34	72.3	0	26	4.0	2	29	61.7	0.0	100.0	65.4	65.8
Opossums	1992	19	82.6	0	7	3.0	2	9	39.1	0.0	57.1	17.0	0.0
	1993	16	66.7	0	8	2.5	3	8	33.3	0.0	75.0	20.3	7.2
	Both	35	74.5	0	8	2.7	3	17	36.2	0.0	75.0	18.5	0.0
Swine	1992	22	95.7	0	56	33.8	30	17	73.9	0.0	55.6	14.7	6.7
Sows	1993	24	100.0	19	110	78.1	88.5	20	83.3	0.0	93.9	15.9	6.0
	Both	46	97.9	0	110	56.9	42	37	78.7	0.0	93.9	15.3	6.7
Finishing	1992	23	100.0	86	120	93.3	93	13	56.5	0.0	6.7	1.8	1.1
	1993	24	100.0	50	92	87.7	90	13	54.2	0.0	16.7	2.7	1.1
	Both	47	100.0	50	120	90.5	91	26	55.3	0.0	16.7	2.3	1.1
White-footed mice	1992	11	47.8	0	7	1.3	0	3	13.0	0.0	100.0	18.2	0.0
	1993	13	54.2	0	7	1.3	1	0	0.0	0.0	0.0	0.0	0.0
	Both	24	51.1	0	7	1.3	1	3	6.4	0.0	100.0	8.3	0.0
Rats	1992	10	43.5	0	5	1.0	0	0	0.0	0.0	0.0	0.0	0.0
	1993	12	50.0	0	36	3.0	0.5	4	16.7	0.0	100.0	14.1	0.0
	Both	22	46.8	0	36	2.0	0	4	8.5	0.0	100.0	7.7	0.0
House mice	1992	23	100.0	1	176	25.1	11	5	21.7	0.0	14.3	1.6	0.0
	1993	24	100.0	5	84	27.8	18.5	9	37.5	0.0	11.1	2.7	0.0
	Both	47	100.0	1	176	26.4	14	14	29.8	0.0	14.3	2.2	0.0

ing of all species, isolation from rodents, and the detection of oocysts on the farm, all 47 farms had evidence of reservoirs of *T. gondii* infection.

DISCUSSION

As expected, there was a high seroprevalence for cats (68.3%), indicating a high rate of prior exposure to *T. gondii* and probably also a high degree of past oocyst shedding. Seroprevalence rates from the 1992 (66.5%) and the 1993 (70.7%) samples were comparable, suggesting the absence of sampling bias. The 46% seroprevalence for juvenile cats indicates that approximately half of the farm cats were exposed to *T. gondii* before reaching maturity.

Compared with the high prevalence of serum antibodies to *T. gondii* in the current survey, it was possible to isolate viable

TABLE III. Isolation of *Toxoplasma gondii* from tissues of wild rodents from swine farms in Illinois.

Year	Animal species	Farm no.	*T. gondii* antibody titer of donor rodent		No. of mice infected with *T. gondii**
			MAT	DT	
1992	*Mus musculus*	92-4	6,400	ND†	2
1992	*M. musculus*	92-20	No sample		1
1992	*M. musculus*	92-10	<25	ND	1
1992	*M. musculus*	92-10	<25	ND	1
1992	*M. musculus*	92-22	<25	ND	1
1992	*Peromyscus* sp.	92-15	12,800	ND	2
1993	*Peromyscus* sp.	93-20	<25	ND	2
1993	*Rattus norvegicus*	93-19	≥500	≥640	1
1993	*M. musculus*	93-18	<25	<10	1 (Died)
1993	*M. musculus*	93-18	<25	<10	1 (Died)

* Of 2 mice inoculated.
† ND = not done.

TABLE IV. Isolation of *Toxoplasma gondii* from samples of cat feces, pig feed, and soil from swine farms in Illinois.

Year	Sample type	Farm no.	Days between collection and bioassay	No. of mice positive for *T. gondii*
1992	Cat feces*	92-13	84	1†
1992	Cat feces	92-3	59	1
1992	Cat feces	92-17	62	1
1992	Pig feed	92-12	101	1
1992	Pig feed	92-4	100	1
1993	Cat feces	93-24	158	1
1993	Cat feces	93-24	158	2
1993	Soil	93-24	236	2

* Serum antibody titers to *T. gondii* of these 5 cats were 6,400, 1,600, ≥25,600, 1,600, and 200, respectively.
† Of the 2 mice inoculated with the sample.

oocysts from only 1.8% (5 of 274) of cat fecal samples. These 5 cats represent 4 farms. All 5 cats that were shedding *T. gondii* oocysts in feces had a relatively high (≥200) titer for *T. gondii* antibodies, using the MAT. The most likely explanation for this low isolation rate is that most of the cats (75%) caught were adults. Cats shed oocysts in feces usually for 1 wk soon after infection, and most cats are infected as juveniles (Dubey and Beattie, 1988). The 46% seroprevalence rate for juvenile cats in this study confirms this observation. Thus, most of the cats caught had presumably shed oocysts in the past. Another factor contributing to the failure to isolate oocysts is the small sample size of feces (usually <1 g) obtainable by massage or digital insertion. Use of an enema in the second year of the study increased the size of fecal samples.

Finding of *T. gondii* oocysts in feces of only 1.8% of cats does not diminish the importance of oocysts in maintaining *T. gondii* in the environment. A cat may excrete millions of oocysts in a day and the oocysts can survive in the environment for >1 yr (Dubey and Beattie, 1988).

The stage of *T. gondii* found in feed and soil samples is also the oocyst because the samples were stored for several mo, soaked in water, and then suspended in 2% H_2SO_4. All of these procedures would kill tissue cysts of *T. gondii* (Jacobs et al., 1960; Dubey et al., 1970). Theoretically, tissue cysts from dead animals can contaminate the pig feed. However, tissue cysts are killed even by water (Jacobs et al., 1960).

Finding viable *T. gondii* oocysts in 2 of 491 feed samples and 1 of 79 soil samples is noteworthy because only a small proportion of feed was sampled, the samples were stored for several months before bioassay, and there is a tremendous loss of oocysts during the recovery process. Conservatively, >90% of *T. gondii* oocysts are estimated to be lost during the recovery and bioassay procedures.

Little information has been available previously concerning *T. gondii* infection in farm cats in the United States. Lubroth et al. (1983) found 2 seropositive cats on 1 farm. Using the same MAT for serum antibodies to *T. gondii* (but using a higher titer of 32 as the cutoff for seropositivity), Smith et al. (1992) found a 41.9% seroprevalence rate for cats on swine farms in Iowa, with 6 of 13 farms (46.2%) having seropositive cats. There may indeed be more widespread *T. gondii* infection in Illinois farm cats, or the fewer number of farms with seropositive cats in the Iowa study may have been due to the lower trapping rate (mean = 3.9 cats trapped per farm, compared to a mean of 8.3 cats per farm trapped in Illinois). In the present study, there was only 1 farm on which cats were not trapped, and there were 3 farms (including the former) on which no seropositive cats were trapped. However, all of these farms had seropositive swine. Sampling error can account for failure to trap cats.

Neither Lubroth et al. (1983) nor Smith et al. (1992) attempted detection of oocysts on the farm. Although contamination of feeds and soil in swine housing by feces of infected cats is logical and expected, the present study is the first to provide definitive evidence that pig feed and soil can be contaminated by *T. gondii* oocysts.

Although cats can acquire infection transplacentally and by ingesting sporulated oocysts, the concentration of *T. gondii* infection in the tissues of prey (in contrast to dispersion of oocysts throughout the habitat) and epidemiologic data suggest that most cats become infected by consuming the encysted tissue of intermediate hosts (Dubey, 1973). Birds and small mammals are considered to be the main sources of *T. gondii* infection for feral cats. In the present study, blood and tissues from 22 birds were tested and none was found to be infected. This small sample size may be insufficient to detect *T. gondii* infection in bird populations. However, *T. gondii* infection was indicated by serologic examination and tissue bioassay for house mice, white-footed mice, and rats. Evidence of *T. gondii* infection in rodents was found on 47% of the farms. Further studies are needed to determine the role of feral birds and rodents in the epidemiology of *T. gondii* infection on swine farms.

The prevalence of infection in rodents may have been underestimated. The small number of house mice (median n = 14) and the even smaller number of white-footed mice and rats captured on some farms is insufficient to detect low prevalence of infection where it exists. In addition, less than perfect diagnostic sensitivity contributes to underestimation. In the present study, 5 of the 7 mice that had viable *T. gondii* in their tissues were seronegative. There are several explanations for this phenomenon. The infection may have been derived congenitally (Jacobs, 1964). When infection had been recent, antibodies may not have developed yet. In cases of long-term infection, antibody concentration may have dropped below the level of detection. This indicates that the use of a serum dilution of 1:25 for the detection of *T. gondii* antibodies in the present study may have underestimated the prevalence of *T. gondii* infection in mice. On the other hand, bioassay will not always be successful in isolating *T. gondii* from infected animals. Although concentration of *T. gondii* tissue cysts in either the heart or the brain is expected, it is possible for infection to occur without invasion of these organs. With respect to the bioassay itself, inoculation with infected tissue may not always be successful in establishing infection in the host's brain or heart.

Sampling bias in trapping also may result in underestimation of the prevalence of infection. Mice are generally more susceptible to *T. gondii* infection than other hosts and the ingestion of a few oocysts can produce clinical toxoplasmosis in mice, which will make them more subject to predation by cats (Dubey and Beattie, 1988). It is also likely that some mice die of acute toxoplasmosis. Both of these factors contribute to underrepresentation of infected rodents in trapping surveys.

Thus, small sample sizes, imperfect diagnostic sensitivity, and sampling bias may have resulted in the underestimation of the prevalence of *T. gondii* infection in rodents on swine farms. It is very likely that >50% of the farms studied had rodent reservoirs of *T. gondii* infection.

The relative abundance of different species of rodents on the swine farms sampled was not estimated precisely. The larger number of mice trapped reflects in part the greater number of mouse traps set. Rats may also be more trap shy than mice, which contributes further to their underrepresentation. Thus, the relative sizes of *T. gondii* reservoirs in these species remains unclear. However, the existence of seropositive test results and the successful isolation of *T. gondii* by bioassay indicates that on swine farms in Illinois, at least 3 rodent species (house mice, white-footed mice, and rats) are a reservoir and potential source of *T. gondii* infection.

In a study of 2 swine farms in Georgia, Lubroth et al. (1983) found 60% (n = 20) of house mice, 100% (n = 3) of white-footed mice, 50% (n = 6) of cotton rats, and 100% (n = 2) of

Norway rats seropositive for *T. gondii,* using the indirect immunofluorescent antibody test. These higher seroprevalences suggest that *T. gondii* infection in rodents is higher in Georgia than in Illinois. In contrast, in the study by Smith et al. (1992) of 19 Iowa farms, the house mouse seroprevalence was 0.3% (2 positive out of 588 sampled), with 10% of farms (2 of 20) having seropositive mice; serologic testing of 21 white-footed mice and 9 Norway rats yielded negative results. It appears the Iowa farms have a lower house mouse seroprevalence because the mean number of samples per farm (29) was comparable to the current study, and the narrower range (21–36) would have allowed better detection of seropositive mice on each farm than existed for the current study.

How rodents become infected in nature is not known. Carnivorism, ingestion of oocysts from contaminated feed, and congenital transmission are all efficient routes of infection. *Toxoplasma gondii* can be repeatedly transmitted congenitally in mice and several infected litters may be produced by chronically infected, clinically normal mice (Beverley, 1959). However, epidemiologic data on remote islands suggests that *T. gondii* is not maintained in the environment without oocysts (Wallace, 1969; Munday, 1972).

The seroprevalence for raccoons (67.0%) in the current study was almost the same as that estimated for cats. Previously, Dubey et al. (1992) estimated the seroprevalence among raccoons sampled from 6 states to be 50%. As omnivores, raccoons feed on carrion and vegetation, and are considered as good monitors of environmental contamination with *T. gondii.* However, despite the high prevalence, raccoons are unlikely to serve as a primary source of *T. gondii* infection for swine because they do not excrete the *T. gondii* oocysts (Dubey et al., 1993), and swine are unlikely to consume raccoon flesh.

Comparative data on the prevalence of infection in mammalian and avian reservoirs of *T. gondii* infection are available from several studies in the United States. In a recent survey (Brillhart et al., 1994), 2 of 115 white-footed mice from Kansas had *T. gondii* antibodies. In the survey from Montana (Dubey, 1983), *T. gondii* was not isolated from tissues of 500 ground squirrels (*Spermophilus richardsoni*), 99 deer mice (*Peromyscus maniculatus*), 84 muskrats (*Ondatra zibethicus*), and 52 meadow voles (*Microtus pennsylvanicus*). The isolation of *T. gondii* from 7 of 1,502 house mice, 2 of 67 *Peromyscus* sp., and 1 of 107 rats in the present study, combined with the serologic evidence from swine farms in Georgia and Iowa, suggests that *T. gondii* is more concentrated on swine farms, most likely due to the higher concentration of cats.

The seroprevalence of *T. gondii* in swine was lower in the present study than in the 1983–1984 national survey that also used the MAT (Dubey et al., 1991), where 23% of 11,229 finishing pigs and 41.4% of 613 breeder hogs had *T. gondii* antibodies at a serum dilution of 1:25. In the national survey, *T. gondii* antibodies were found in 24.2% of 1,330 hogs from Illinois (Dubey et al., 1991). Although the proportion of finishing versus breeding swine from Illinois was not recorded, the majority (>95%) of the hogs surveyed in the entire sample were finishing pigs. The swine seroprevalence estimates in the current field study (15.1% for 2,617 sows, 2.3% for 4,252 finishing pigs) were slightly lower than the 1992 statewide Illinois serological survey of pseudorabies testing samples from a state diagnostic laboratory, where 20.8% of 5,080 breeding hogs and 3.1% of 1,885 finishing pigs were seropositive at 1:25 dilution (Weigel, Dubey, Siegel, Hoefling et al., 1995). These differences probably represent sampling variation. Overall, estimates of swine seroprevalence from the 2 recent Illinois studies have high precision because of the large sample sizes.

Sera for all 3 Illinois surveys were examined in 1 laboratory at Beltsville using the MAT. Overall, the *T. gondii* seroprevalence estimates for swine in the 2 recent surveys from Illinois are substantially lower than the national survey conducted 8–10 yr ago. Without comparative data on herd characteristics, the reasons for this decline in seroprevalence are not clear, but changes in management and housing are potentially important factors. Confinement housing can reduce exposure of pigs to cats and rodents and the use of rodenticides can affect the numbers of rodents on the swine premises.

In a study of Iowa swine, Smith et al. (1992), using the MAT with a titer of 32 as the cutoff for seropositivity, estimated the *T. gondii* seroprevalence for sows to be 14.3% (n = 273); this estimate was similar to the field study conducted here in Illinois. It is not possible to compare the results of the present study with other surveys using other serologic tests.

When the serologic testing for *T. gondii* antibodies in all species, the bioassay of rodent tissues, and the detection of oocysts are considered as evidence of *T. gondii* infection on swine farms, all 47 farms had evidence of *T. gondii* infection. Thus, the risk of transmission of *T. gondii* to swine is a potential problem for all swine farms.

The current investigation has identified all mammalian species captured in large numbers on swine farms in Illinois (cats, raccoons, opossums, skunks, house mice, white-footed mice, rats) as reservoirs of *T. gondii* infection. Rodents are probably the only direct animal source of *T. gondii* infection for swine. Rodents also assist in maintaining the infection in the cat population. The shedding of oocysts by cats on swine farms has been verified, and confirmation has been provided that pig feed and soil are 2 direct sources of oocysts that can infect swine.

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LITERATURE CITED

ALFORD, C. A., S. S. STAGNO, AND D. W. REYNOLDS. 1974. Congenital toxoplasmosis: Clinical, laboratory, and therapeutic considerations, with special reference to subclinical disease. Bulletin of the New York Academy of Medicine **50:** 160–181.

BEAL, V. S. 1983. Regulatory statistics, Vol. I. U.S. Department of Agriculture Animal and Plant Health Inspection Service, Washington, D.C., 6th edition. Part III, p. 7–21.

BEVERLEY, J. K. A. 1959. Congenital transmission of toxoplasmosis through successive generations of mice. Nature (London) **183:** 1348–1349.

BRILLHART, D. B., L. B. FOX, J. P. DUBEY, AND S. J. UPTON. 1994. Seroprevalence of *Toxoplasma gondii* in wild mammals in Kansas.

Journal of the Helminthological Society of Washington **61:** 117–121.

DESMONTS, G., AND J. S. REMINGTON. 1980. Direct agglutination test for diagnosis of *Toxoplasma* infection: Method for increasing sensitivity and specificity. Journal of Clinical Microbiology **11:** 562–568.

DUBEY, J. P. 1973. Feline toxoplasmosis and coccidiosis: A survey of domiciled and stray cats. Journal of the American Veterinary Medical Association **162:** 873–877.

———. 1983. *Toxoplasma gondii* infection in rodents and insectivores from Montana. Journal of Wildlife Diseases **19:** 149–150.

———. 1986. Toxoplasmosis. Journal of the American Veterinary Medical Association **189:** 166–170.

———. 1995. Duration of immunity to shedding of *Toxoplasma gondii* oocysts in cats. Journal of Parasitology **81:** 410–415.

———, AND C. P. BEATTIE. 1988. Toxoplasmosis of animals and man. CRC Press, Inc., Boca Raton, Florida, 220 p.

———, AND G. DESMONTS. 1987. Serological responses of equids fed *Toxoplasma gondii* oocysts. Equine Veterinary Journal **48:** 1239–1243.

———, J. K. FRENKEL, AND N. L. MILLER. 1970. Characterization of the new fecal form of *Toxoplasma gondii*. Journal of Parasitology **56:** 447–456.

———, A. N. HAMIR, C. A. HANLON, AND C. E. RUPPRECHT. 1992. Prevalence of *Toxoplasma gondii* infection in raccoons. Journal of the American Veterinary Medical Association **200:** 534–536.

———, ———, S. K. SHEN, P. THULLIEZ, AND C. E. RUPPRECHT. 1993. Experimental *Toxoplasma gondii* infection in raccoons (*Procyon lotor*). Journal of Parasitology **79:** 548–552.

———, J. C. LEIGHTY, V. C. BEAL, W. R. ANDERSON, C. D. ANDREWS, AND P. THULLIEZ. 1991. National seroprevalence of *Toxoplasma gondii* in pigs. Journal of Parasitology **77:** 517–521.

———, P. THULLIEZ, AND E. C. POWELL. 1995. *Toxoplasma gondii* in Iowa sows: Comparison of antibody titers to isolation of *T. gondii* by bioassays in mice and cats. Journal of Parasitology **81:** 48–53.

———, ———, R. M. WEIGEL, C. D. ANDREWS, P. LIND, AND E. C. POWELL. 1995. Sensitivity and specificity of various serologic tests for detection of *Toxoplasma gondii* infection in naturally infected sows. American Journal of Veterinary Research **56:** 1030–1036.

FRENKEL, J. K. 1990. Toxoplasmosis in human beings. Journal of the American Veterinary Medical Association **196:** 240–248.

———, AND J. P. DUBEY. 1975. *Hammondia hammondi* gen. nov., sp. nov., from domestic cats, a new coccidian related to *Toxoplasma* and *Sarcocystis*. Zeitschrift für Parasitenkunde **46:**3–12.

JACOBS, L. 1964. The occurrence of *Toxoplasma* infection in the absence of demonstrable antibodies. Proceedings of the First International Congress of Parasitology **1:** 176–177.

———, J. S. REMINGTON, AND M. L. MELTON. 1960. The resistance of the encysted form of *Toxoplasma gondii*. Journal of Parasitology **46:** 11–21.

LUBROTH, J.-S., D. W. DREESEN, AND R. A. RIDENHOUR. 1983. The role of rodents and other wildlife in the epidemiology of swine toxoplasmosis. Preventive Veterinary Medicine **1:** 169–178.

MUNDAY, B. L. 1972. Serological evidence of *Toxoplasma* infection in isolated groups of sheep. Research in Veterinary Science **13:** 100–102.

PENKERT, R. A. 1973. Possible spread of toxoplasmosis by feed contaminated by cats. Journal of the American Veterinary Medical Association **162:** 924.

SMITH, K. E., J. J. ZIMMERMAN, S. PATTON, G. W. BERAN, AND H. T. HILL. 1992. The epidemiology of toxoplasmosis of Iowa swine farms with an emphasis on the roles of free-living mammals. Veterinary Parasitology **42:** 199–211.

WALLACE, G. D. 1969. Serologic and epidemiologic observations on toxoplasmosis on three Pacific atolls. American Journal of Epidemiology **90:** 103–111.

WEIGEL, R. M., J. P. DUBEY, A. M. SIEGEL, D. HOEFLING, D. REYNOLDS, L. HERR, U. D. KITRON, S. K. SHEN, P. THULLIEZ, R. FAYER, AND K. S. TODD. 1995. The prevalence of antibodies to *Toxoplasma gondii* in swine in Illinois in 1992. Journal of the American Veterinary Medical Association **206:** 1747–1751.

———, ———, ———, U. D. KITRON, A. MANELLI, M. A. MITCHELL, N. E. MATEUS-PINILLA, P. THULLIEZ, S. K. SHEN, O. C. H. KWOK, AND K. S. TODD. 1995. Risk factors for transmission of *Toxoplasma gondii* on swine farms in Illinois. Journal of Parasitology **81:** 736–741.

WILSON, C. B., J. S. REMINGTON, S. STAGNO, AND D. W. REYNOLDS. 1980. Development of adverse sequelae in children born with subclinical *Toxoplasma* infection. Pediatrics **60:** 767–774.