A SURVEY FOR *ECHINOCOCCUS MULTILOCULARIS* IN COYOTES AND FOXES IN MICHIGAN

By

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ABSTRACT

A SURVEY FOR *ECHINOCOCCUS MULTILOCULARIS* IN COYOTES AND FOXES IN MICHIGAN

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Echinococcus multilocularis is a parasitic tapeworm with the potential to cause serious disease and even death in humans. The minute worm uses a rodent intermediate host and canid definitive host to complete its life cycle and humans can become accidentally infected through exposure to infective eggs. *Echinococcus multilocularis* had been identified in the north central portion of the United States, including Michigan and nearby states in the early 1990's; however little is known about current prevalence and distribution. In this study, 302 coyotes, gray foxes and red foxes collected from hunters, trappers, fur buyers and state and federal agencies in Michigan were examined for presence of the parasite. *Echinococcus multilocularis* was identified in 1/219 (0.46%) coyotes from the southwestern Lower Peninsula. The parasite was not identified in any of the red or gray foxes examined. Data generated in this study provides a greater understanding of the spatial distribution of the parasite, provides a baseline with which future research can be compared, and can be used to assess the level of risk to humans in the state. Although the prevalence in wild canids in Michigan appears to be low, the risk of encountering the parasite does exist and those handling wild canids are encouraged to take precautions to prevent exposure.

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Introduction

Echinococcus multilocularis, Leuckart, 1863, is a parasitic tapeworm that can occur in wild canids and rodents and is capable of causing severe disease and death in humans. *Echinococcus multilocularis* has been identified worldwide in the Northern Hemisphere, including the north central portion of the United States. The parasite was identified in red foxes (*Vulpes vulpes*) in Michigan in the early 1990's; however little is known about current prevalence and distribution in the state (Storandt and Kazacos 2012). The coyote (*Canis latrans*), whose populations have increased in distribution and abundance across North America over the past 2 centuries, have the potential to be an important definitive host in the state (Kritsky and Leiby 1978, Gehrt 2006, Mastro 2011). The coyote's ability to adapt to urban environments and live in close proximity to humans and domestic animals may lead to greater risk of human exposure to *E. multilocularis*, in addition to other parasites and infectious agents. Because current data on *E. multilocularis* in Michigan are lacking, surveillance is needed to determine prevalence and distribution, which will help assess risks to accidental hosts.

Description[1]

Four species of *Echinococcus* occur worldwide. Two occur in North America: *E. granulosus* and *E. multilocularis*. *Echinococcus* species have indirect life cycles, requiring an intermediate and definitive host to complete. Both a sylvatic and pastoral life cycle occur with *E. granulosus*; in North America the sylvatic cycle involves wild canids and ungulates and the

[1] Refer to Schmidt et al. 2009 for definitions of parasitological terms.

pastoral cycle involves domestic dogs and sheep, as the definitive and intermediate hosts, respectively (Olsen 1974, WHO/OIE 2001). Wild canids are also involved in the life cycle of *E. multilocularis*; however rodents serve as the intermediate hosts. Overlap in life cycles of *E. multilocularis* and *E. granulosus* can occur, with some canid species capable of harboring both species of parasite (Olsen 1974). Humans can become accidently involved in both life cycles as incidental hosts, and although infection in humans in North America is rare, infection with either species can be fatal (Olsen 1974, WHO/OIE 2001).

The tapeworm *E. multilocularis* is a member of the phylum Platyhelminthes, class Cestoda, order Cyclophyllidea and family Taeniidae. Eggs of members of the family Taeniidae are indistinguishable from one another and identification to species must be made by morphological characteristics of adult worms (Hildreth et al. 1991, Bowman 1999, Schmidt et al. 2009). Adult worms of *Echinococcus* spp. are distinguished from other members of the family Taeniidae by the small number of proglottids (Olsen 1974). Scoleces of *Echinococcus* spp. have 4 suckers and an armed rostellum. Adult *E. multilocularis* range in length from 1.2-3.7 mm with 3-5 proglottids, with the gravid proglottid being less than half of the entire body length (Figure 1). Other distinguishing characteristics of adult *E. multilocularis* include position of the genital pore (at or anterior to the midline), the shape of the uterus (saclike and lacking lateral branches), the number (17-26) and position (posterior to the genital pore) of the testes, the shape of the ovary (bilobed with fine tubes) and the number (20-36) of rostellar hooks (Hildreth et al. 1991). In contrast, adult *E. granulosus* are slightly larger in size (2-6 mm) with the gravid proglottid being greater than half of the entire body length. Additionally, in *E. granulosus* the genital pore is posterior to the midline, the uterus is saclike with lateral branches, and a larger number of testes (45-65 anterior or posterior to the genital pore) and rostellar hooks (28-48) are present

(Hildreth et al. 1991). As an alternative to identification by morphological characteristics, adult worms and worm fragments (e.g. proglottids, scoleces) can be identified by polymerase chain reaction (PCR) (Trachsel et al. 2007, Liccioli et al. 2012).

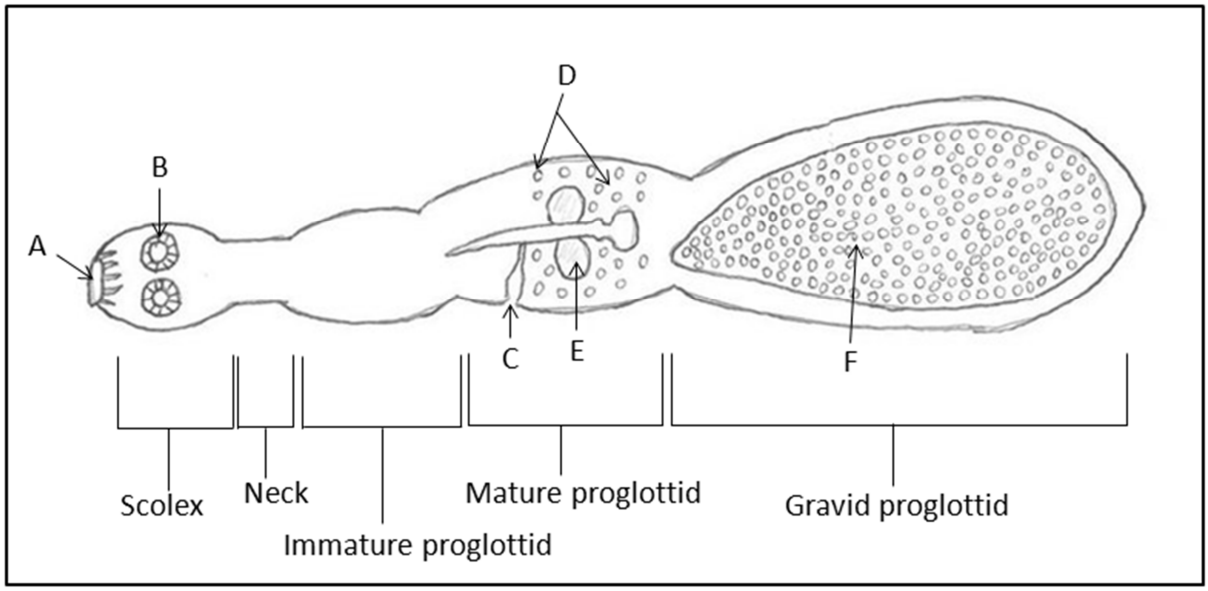


Figure 1. Morphological characteristics of adult *Echinococcus multilocularis*: 3-5 proglottids with the gravid proglottid being less than half the entire length; scolex with armed rostellum (A) and 4 suckers (B); genital pore anterior to midline (C); 17-26 testes posterior to genital pore (D); bilobed ovary (E); and saclike uterus (F).

Life cycle

The life cycle of *E. multilocularis* begins with a sexually mature, adult worm in the intestinal tract of the canid definitive host (Figure 2). Adult worms begin producing eggs 28-35 days post infection and survive in the definitive host 3-4 months before being eliminated (Rausch and Richards 1971, Hildreth et al. 1991). Consumption of additional infected intermediate hosts insures infections over longer periods of time (Hildreth et al. 1991). Eggs are excreted with fecal

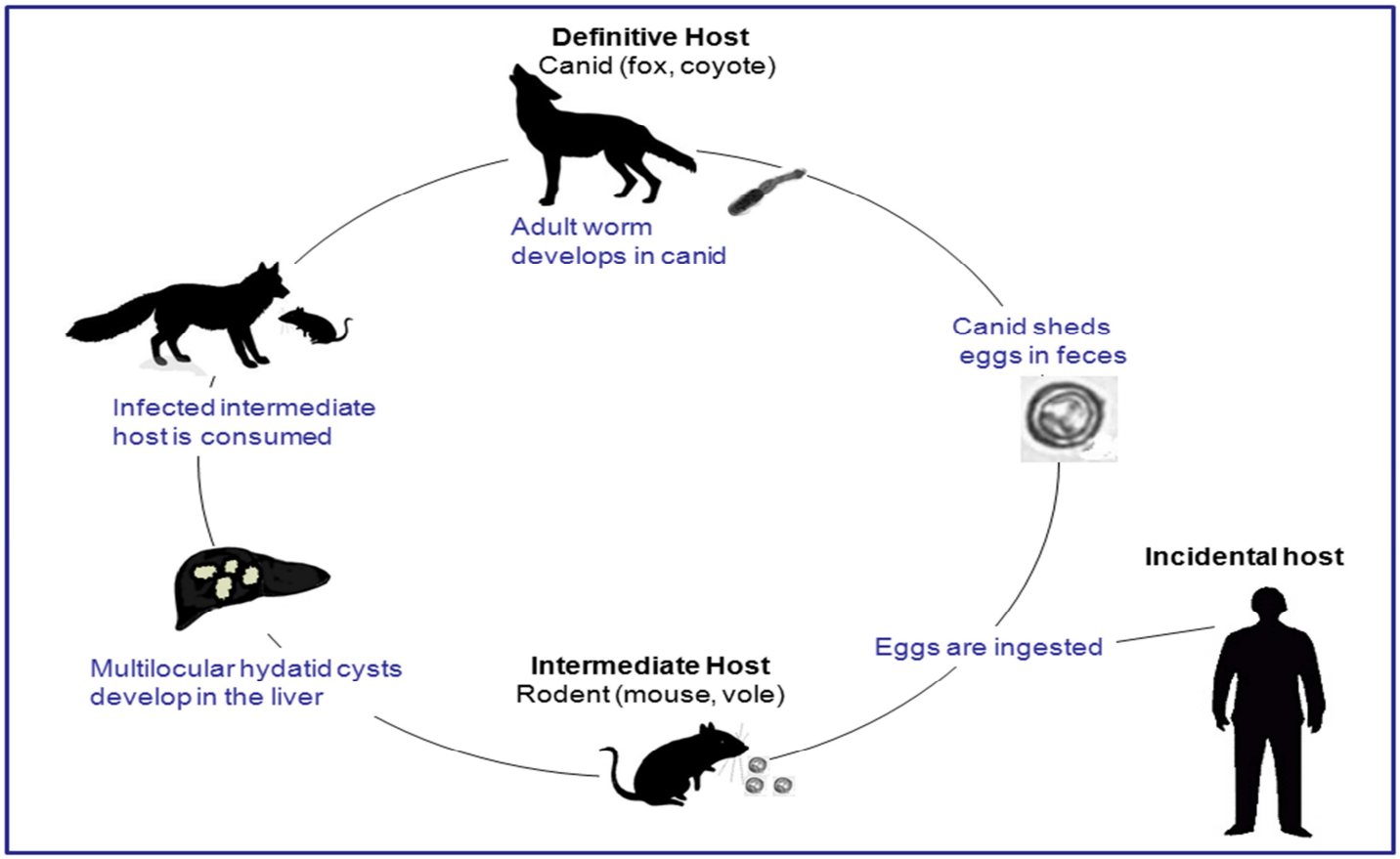


Figure 2. Life cycle of *Echinococcus multilocularis*. Adult worms develop in a canid definitive host and infective eggs are shed in fecal material. Rodent intermediate host ingests infective eggs while foraging on insects or vegetation. Oncospheres hatch from eggs in the rodent host, travel to the liver and develop into a multilocular hydatid cyst (metacestode stage) in which protoscoleces are formed. When an infected rodent is consumed by a canid definitive host, the protoscoleces develop into adult worms and the cycle repeats. Humans can accidently become involved in the cycle through ingestion of infective eggs. For interpretation of the reference to color in this and all other figures, the reader is referred to the electronic version of this thesis.

material and are accidently ingested by the rodent intermediate host while foraging on vegetation and/or insects. These eggs are quite tolerant of environmental conditions and can survive over one year in suitable, moist conditions at low temperatures (WHO/OIE 2001). Once eggs are ingested, oncospheres hatch in the small intestine and via hooks and enzymes, travel out of the intestinal tract through the hepatic portal system and to the liver or another visceral organ and develop into multilocular hydatid cysts, or the metacestode stage (Hildreth et al. 1991, Olsen 1974). Within the metacestode, protoscoleces develop rapidly, and the proliferative growth of the cyst causes extensive damage and death in rodents within several weeks to several months of infection (Hildreth et al. 1991). The cycle is completed when the infected rodent is consumed by a definitive host that ingests the protoscoleces, which pass into the small intestine and attach to the crypts of the intestinal mucosa and develop into sexually mature, adult worms.

Intermediate hosts

The primary intermediate hosts for *E. multilocularis* in north central North America are the eastern meadow vole (*Microtus pennsylvanicus*) and the deer mouse (*Peromyscus maniculatus*) (Leiby et al. 1970, Rausch and Richards 1971, Jones and Pybus 2001). Examination of nearly 8,000 mammals of 32 different species, including rodents, small carnivorous mammals and domestic dogs (*Canis familiaris*) and cats (*Felis catus*) by Leiby et al. (1970) during the late 1960's found the following prevalence in intermediate hosts from north central North America: 1.92% in eastern meadow voles (n= 1,033), 4.8% in deer mice (n= 4,209) and 1.1% in house mice (*Mus musculus*; n=91). Similar examination for presence of *E. multilocularis* of over 1,800 small rodents representing ten species of shrews, mice, voles and ground squirrels from North Dakota in the late 1960's found infection in only deer mice (3%)

and eastern meadow voles (6%) (Rausch and Richards 1971). Despite low prevalence in intermediate hosts, high prevalence in definitive hosts can be maintained (WHO/OIE 2001, Storandt et al. 2002).

Both eastern meadow voles and deer mice are common and abundant throughout most of North America and Michigan where suitable habitat is available (Table 1) (Baker 1983, Kurta 1995). Home range sizes of both species are 1 ha or less, with home ranges of eastern meadow voles being 2 to 3 times smaller than that of deer mice (Table 2). Densities of both rodent species fluctuate based on food availability, habitat, predation, weather and disease outbreaks and eastern meadow vole populations can fluctuate drastically in 4 year cycles (Baker 1983, Kurta 1995). Eastern meadow vole populations peak in January and February, hit lows in late spring and then gradually increase until mid-winter (Baker 1983). Deer mice populations peak in late spring and again in the fall and then gradually decrease through winter (Baker 1983). Life expectancy of rodent species is short and in the wild is usually less than one year (Baker 1983, Kurta 1995).

Experimental research has found that the eastern meadow vole becomes infected with larger numbers of protoscoleces than the deer mouse; however in a free-ranging condition, the eastern meadow vole is less likely, based on different feeding habits (Table 3), to become infected than the deer mouse (Leiby and Nickel 1968, Leiby et al. 1970, Rausch and Richards 1971, Kritsky and Leiby 1975). Deer mice are more likely than meadow voles to inhabit areas around carnivore dens and thereby more likely to consume contaminated vegetation or contaminated insects (Leiby and Nickel 1968, Leiby and Nickel 1970, Kritsky and Leiby 1975).

Table 1. Habitat preferences of host species (Baker 1983, Kurta 1995, University of Michigan 2012).

Species	Host Type (D=Definitive, I=Intermediate)	Habitat Preference
Eastern meadow vole	I	Agricultural fields, fragmented patches of grass, grassy fields and marshes, lowlands, meadows, river banks, woodlands
Deer mouse - southern subspecies	I	Agricultural fields, grass lands, meadows, open lands, pastures
Deer mouse - northern subspecies	I	forests, shrubby areas
Coyote	D	Agricultural fields, clear cuts, lowland brush, patchy landscapes, woodland edges; nearly any habitat type including urban areas
Red fox	D	Agricultural fields, bushy fence rows, field edges, meadows, shrubby areas, woodland edges
Gray fox	D	Cedar (*Thuja occidentalis*) swamps, coniferous forests, hardwoods, tamarack (*Larix laricina*) stands, woodlands

Table 2. Home range sizes of host species ((Baker 1983, Kurta 1995, Kamler and Gipson 2000, Grinder and Krausman 2001, Gehrt 2006, Gehrt et al. 2009).

Species	Home range sizes
Eastern meadow vole	0.1 to .3 ha
Deer mouse	0.2 to 1.0 ha
Coyote (resident)	1.7 to 50.7 km²
Coyote (transient)	2.0 to 180 km²
Red fox	0.6 to 6.0 km²
Gray fox	0.6 to 8.7 km²

Table 3. Dietary habits of host species (Baker 1983, Kurta 1995).

Species	Dietary habits
Eastern meadow vole	Leaves, grasses, plants, seeds, fruit, invertebrates, small vertebrates and occasionally other meadow voles
Deer mouse	Insects and invertebrates, vegetation, seeds, nuts and fruit
Coyote	Small mammals (e.g. rabbits, squirrels, mice, voles), carrion, birds, reptiles, amphibians, insects, fruit and vegetation
Red fox	Small mammals (e.g. rabbits, squirrels, mice, voles), ground nesting birds, carrion, reptiles, amphibians, insects, nuts and fruit
Gray fox	Small mammals (e.g. rabbits, small rodents), birds, amphibians, reptiles, insects and vegetation

Definitive hosts

Historically, in North America, the Arctic fox (*Alopex lagopus*) and red fox have been considered the primary definitive hosts, although the coyote is also a suitable host (Jones and Pybus 2001). Arctic foxes do not occur in Michigan; however, red foxes, coyotes and gray foxes (*Urocyon cinereoargenteus*) can be found throughout the state in suitable habitat (Table 1). Michigan represents the northern edge of the range for gray foxes and this species is not as abundant as other canids in the state (Baker 1983, Kurta 1995, Whitaker 2001). Maximum home range sizes of resident coyotes (adult, non-dispersing coyotes and their offspring) can be 5-8 times larger than that of gray and red foxes, while home range sizes of transient coyotes can be 3 times larger than that of residents (Table 2). All three species have omnivorous feeding habits, consuming small mammals, birds, insects, fruits and vegetation (Table 3). Life expectancy in the wild for canids can be as high as 8-10 years, however most live less than 3 years due to human persecution (Bekoff 1978, Baker 1983, Kurta 1995).

In the Great Lakes region, prevalence as high as 35.3% in coyotes and 27.3% in red foxes has been found (Storandt and Kazacos 1993). The highest prevalence in North America documented in both species has occurred in South Dakota, with prevalence of 44.4% in coyotes and 88.9% in red foxes (Hildreth et al. 2000). *Echinococcus multilocularis* was identified in 1 of 6 (16.7%) gray foxes from Minnesota by Vande Vusse et al. (1978) but not in any other published surveys where gray foxes were examined (Table 4). Although gray foxes can harbor the parasite, infection in this species is presumed to be rare (Hildreth et al. 1991, WHO/OIE 2001).

Neither age nor sex of the definitive host has been shown to influence prevalence; however, adult intermediate hosts are more likely to become infected than juveniles because over

time they have a greater potential to be exposed to infective eggs (Rausch and Richards 1971, Kritsky and Leiby 1978, Ballard 1984). The definitive host does not appear to be adversely affected by infection even with high worm burdens (Hildreth et al. 1991). Rodents infected with *E. multilocularis* often have thousands of protoscoleces; thereby canids can harbor hundreds to thousands of adult worms (Hildreth at al. 1991). Worm intensities in definitive hosts have ranged from 1,100 to as high as 180,000 in Arctic foxes, 1 to 1,860 in red foxes, and 1 to 52,000 in coyotes (Kritsky and Leiby 1978, Ballard 1984, Storandt and Kazacos 1993, WHO/OIE 2001). In South Dakota where high prevalence occurs in red foxes and coyotes, mean worm intensities of 125 in red foxes and 127 in coyotes have been observed (Hildreth et al. 2000).

Accidental hosts

Domestic dogs, cats and humans can all become accidently involved in the cycle. Dogs and cats can become definitive hosts through consumption of infected rodents. Humans are incidental hosts and become infected after accidental ingestion of eggs, via consumption of contaminated vegetation, exposure through pets or by handling wild canids and canid carcasses. Hunters, trappers, fur buyers, wildlife workers, veterinarians and animal control personnel who handle wild canids are all at risk of exposure.

Although human infection is rare in North America, *E. multilocularis* can cause alveolar hydatid disease, which destroys the liver in a manner similar to a malignant tumor, has high fatality rates and can go undetected for years, at which time treatment is often unsuccessful (Schantz 1991, WHO/OIE 2001). Infection with *E. multilocularis* is considered to be more harmful than infection with *E. granulosus* and is considered worldwide to be one of the most important helminthic zoonoses based on the destructive damage and severity of disease, high

fatality rates, high cost of treatment (over 240,000 USD per case) and the ability to be transmitted naturally between humans and animals (Hildreth et al. 1991, Thompson and Lymbery 1995, Kraus et al. 2003, Eckert et al. 2011). Unlike *E. granulosus*, which is contained in a unilocular cyst and remains relatively isolated from host tissue, the multilocular hydatid cyst of *E. multilocularis* is composed of multiple thin walled vesicles that infiltrate host tissue (Thompson 1986, Shakespeare 2002). Progression of the disease in humans is a slow, gradual process and an infected individual can remain asymptomatic for 5-15 years, during which time severe damage to the liver has occurred (McManus et al. 2003). Even with treatment, which often involves chemotherapy and/or surgery to remove the infected portion of the liver, humans with this disease have a poor prognosis with 50-70% of cases ending in death (Hildreth et al. 1991).

Data on infection worldwide varies and human infection in some cases may be poorly documented or not documented at all. According to the WHO/OIE (2001), annual incidence rates in Europe range from 0.1-1.4 per 100,000 individuals, 10 per 100,000 in Russia and as high as 200 per 100,000 in endemic areas in China. On St. Lawrence Island, Alaska, between 1947 and 1990 cases in Eskimos living in isolated communities were as high as 53 per 1,000 individuals (WHO/OIE 2001). It is presumed these high infections in Alaska were a result of a large number of infected voles being present and easily preyed upon by the village dogs, leading to heavy environmental contamination with eggs in a situation where humans and dogs were living in close proximity (WHO/OIE 2001). In some of these villages dogs had prevalence rates as high as 12% (WHO/OIE 2001). Likewise, similarly high prevalence in dogs in rural areas has been observed in some areas in China and endemic areas in Switzerland (WHO/OIE 2001). In

Europe, individuals working in agricultural areas in close proximity to infected fox appear to be at increased risk by handling contaminated food and soil (WHO/OIE 2001).

Only two cases have been documented in north central North America, one in a man from Manitoba in 1937 and another in a woman from Minnesota in 1977. The man from Manitoba was a 54 year old fisherman who emigrated from Iceland to Canada at the age of seven. It is presumed however, that he became infected with the parasite while living in Canada, as it is not known to occur in Iceland (James and Boyd 1937). Despite surgery, he died seven years after diagnosis due to severe liver damage caused by the parasite (James and Boyd 1937). The Minnesota case involved a 56 year old woman who lived for 22 years on a farm that housed at least one outdoor dog and five to ten outdoor cats at any given time. No one in the family was a hunter or trapper, nor was anyone else in the family infected and it was therefore assumed that she contracted the parasite through exposure to contaminated feces of one of her domestic dogs or cats, who had consumed an infected rodent (Gamble et al. 1979). She recovered after treatment. No additional documented cases of *E. multilocularis* in humans in North America could be found in the literature.

Several hypotheses have been proposed to explain the lack of infection in humans in n orth central North America in the presence of high prevalence in definitive hosts. These include differences in infectivity between the North American and European strains of *E. multilocularis*; humans in North America being less susceptible to the parasite; lowered risk because fewer dogs and cats are infected; differences in personal hygiene (e.g. hand washing, donning gloves) deter infection; and misdiagnosis of infection (Hildreth et al. 1991, Hildreth et al. 2000). In 1990-91, Hildreth et al. (2000) conducted serological surveys on 115 hunters and trappers in South Dakota, who had trapped foxes and coyotes an average of 15.9 and 11.4 years respectively.

Prevalence in red foxes in some areas of South Dakota at the time of the study was nearly 90%, however only one individual exhibited a weak titer, which could not be confirmed; all others were negative (Hildreth et al. 2000).

Distribution

Worldwide, *E. multilocularis* has been identified in Russia, China, Japan, Mongolia, the Middle East, Europe and North America. In 1952, Robert Rausch documented the first occurrence of *E. multilocularis* on the mainland of Alaska along the Arctic Coast in Arctic foxes and red foxes (Rausch 1956). Subsequently, Choquette et al. (1962) identified *E. multilocularis* in an Arctic fox at Eskimo Point, in the province of Nunavut, documenting the first occurrence on the Canadian mainland. In 1964, *E. multilocularis* was documented for the first time in the contiguous United States in North Dakota, when 6 of 9 red foxes examined were infected with the parasite (Leiby and Olsen 1964). The finding of this parasite beyond its range in Alaska and the Arctic Tundra sparked surveillance and research in several north central states, extending as far east as Ohio, through the early 1990's (Table 4). New geographical distributions were documented as well as new natural hosts, including the coyote (Leiby and Nickel 1970). Along with research on prevalence and distribution, the ecology of *E. multilocularis* and such factors as seasonality of sample collection, age and sex of definitive hosts, and geographic landscapes were examined. Despite the plethora of research and published data compiled through the early 1990's, little if any, subsequent research has been conducted and/or published to determine recent prevalence in any of these states where *E. multilocularis* was previously found. Distribution of *E. multilocularis* in Michigan and surrounding states based on prior surveillance is displayed in Figure 3. *Echinococcus multilocularis* was first identified in Illinois during

Table 4. Known prevalence of *E. multilocularis* in coyotes and foxes in North America.

State	# Positive/ # Tested	Prevalence	Year	Source
Coyote				
Illinois	6/17	35.3%	1990-91	Storandt and Kazacos 1993
Indiana	13/87	14.9%	1990-91	Storandt and Kazacos 1993
Iowa	0/1	0.0%	1965-69	Leiby et al. 1970
Kansas	0/89	0.0%	1991-92	Storandt et al. 2002
Michigan	0/54	0.0%	1993-94	Storandt and Kazacos 2012
Montana	0/30	0.0%	1965-69	Leiby et al. 1970
	9/219	4.1%	1977-78	Seesee et al. 1983
Nebraska	0/31	0.0%	1994-96	Storandt et al. 2002
North Dakota	7/111	6.3%	1965-69	Leiby et al. 1970
Ohio	2/7	28.6%	1993-94	Storandt and Kazacos 2012
South Dakota	0/29	0.0%	1965-69	Leiby et al. 1970
	4/9	44.4%	1990-91	Hildreth et al. 2000
Gray Fox				
Illinois	0/607	0.0%	1955-63	Dyer and Klimstra 1980
Indiana	0/33	0.0%	1990-91	Storandt and Kazacos 1993
Michigan	0/1	0.0%	1990-91	Storandt and Kazacos 1993
Michigan	0/11	0.0%	1993-94	Storandt and Kazacos 2012
Minnesota	1/6	16.7%	1977-78	Vande Vusse et al. 1978
Ohio	0/13	0.0%	1993-94	Storandt and Kazacos 2012
Wisconsin	0/31	0.0%	1982-83	Ballard 1984
Red Fox				
Illinois	4/40	10.0%	1981-82	Ballard and Vande Vusse 1983
Illinois (cont'd)	4/40	10.0%	1981-1982	Ballard and Vande Vusse 1983
Indiana	16/125	12.8%	1990-91	Storandt and Kazacos 1993
Iowa	1/200	0.5%	1965-69	Leiby et al. 1970
Kansas	0/22	0.0%	1991-92	Storandt et al. 2002

Table 4 (cont'd)

Red Fox				
State	**# Positive/ # Tested**	**Prevalence**	**Year**	**Source**
Michigan	0/1	0.0%	1990-91	Storandt and Kazacos 1993
	4/97	4.1%	1993-94	Storandt and Kazacos 2012
Minnesota	14/277	5.1%	1965-69	Leiby et al. 1970
	14/278	5.0%	1966-67	Carney and Leiby 1968
	134/261	51.3%	1977-78	Vande Vusse et al. 1978
Montana	0/11	0.0%	1965-69	Leiby et al. 1970
Nebraska	10/36	27.8%	1981-82	Ballard and Vande Vusse 1983
	27/72	37.5%	1994-96	Storandt et al 2002
North Dakota	6/9	66.70%	1964	Leiby and Olsen 1964
	26/44	59.1%	1964-65	Kritsky and Leiby 1978
	35/161	21.7%	1965-66	Kritsky and Leiby 1978
	115/830	13.9%	1965-69	Leiby et al. 1970
	6/60	10.0%	1966-67	Kritsky and Leiby 1978
	35/435	7.7%	1967-68	Kritsky and Leiby 1978
	12/97	12.4%	1968-69	Kritsky and Leiby 1978
	36/180	20.0%	1969-70	Kritsky and Leiby 1978
	34/158	21.5%	1970-71	Kritsky and Leiby 1978
Ohio	6/22	27.3%	1990-91	Storandt and Kazacos 1993
	9/55	16.4%	1993-94	Storandt and Kazacos 2012
South Dakota	1/222	0.5%	1965-69	Leiby et al. 1970
	16/25	64.0%	1987-88	Hildreth et al. 2000
	6/8	75.0%	1988-89	Hildreth et al. 2000
	40/59	67.8%	1989-90	Hildreth et al. 2000
	40/45	88.9%	1990-91	Hildreth et al. 2000
Wisconsin	6/72	8.3%	1982-83	Ballard 1984
Wyoming	0/31	0.0%	1994-96	Storandt et al. 2002

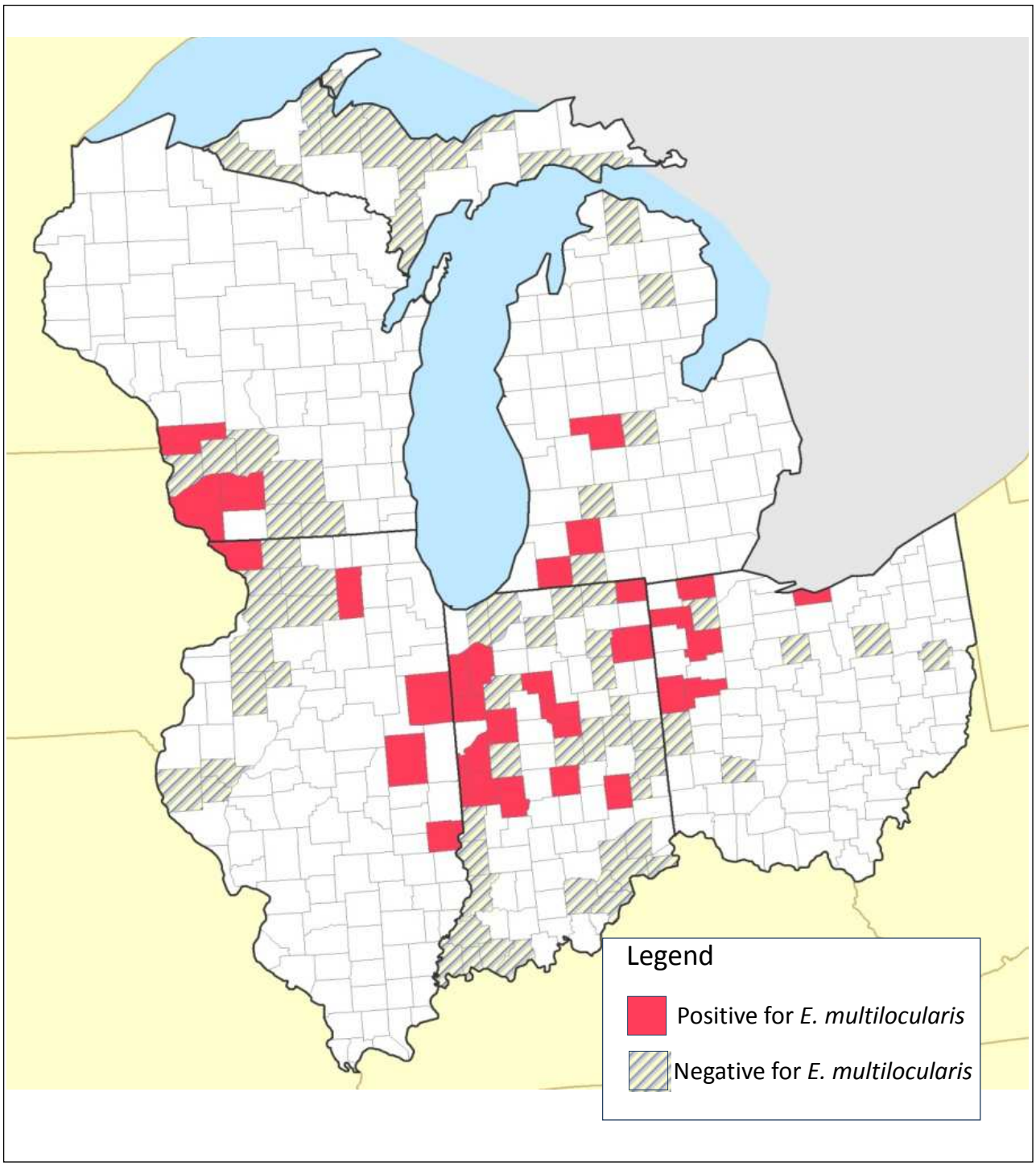


Figure 3. Previous surveillance results by county for *E. multilocularis* in Michigan and bordering states (Ballard 1984, Storandt and Kazacos 1993, Storandt and Kazacos 2012).

surveillance conducted on red foxes from Illinois and Nebraska during the 1981-82 trapping season. Forty red foxes from Illinois were examined and 4 were found infected with the tapeworm (Ballard and Vande Vusse 1983). Ten years later, *E. multilocularis* was identified in a coyote in Indiana, which prompted surveillance in Indiana, Illinois, Ohio, Kentucky and Michigan. Prevalence in the states bordering Michigan as a result of this study were: Illinois: 6/17 (35.3 %) coyotes; Indiana: 13/87 (14.9 %) coyotes and 16/125 (12.8 %) red foxes; and Ohio: 6/22 (27.3%) red foxes (Storandt and Kazacos 1993). *Echinococcus multilocularis* was not identified in either of two foxes collected from Michigan. Subsequent surveillance in 1993-1994 by Storandt and Kazacos (2012) included 162 animals from the Upper and Lower Peninsulas of Michigan and 75 animals from Ohio. *Echinococcus multilocularis* was identified in 4/97 (4.1 %) red foxes in Michigan and in 2/7 (28.6 %) coyotes and 9/55 (16.4 %) red foxes from Ohio (Storandt and Kazacos 2012). The parasite was not identified in any of the 54 coyotes examined from Michigan.

Detection of E. multilocularis

To detect *E. multilocularis* in an area, large numbers of intermediate hosts must be tested compared to relatively small numbers of definitive hosts; therefore definitive hosts are most often used in surveillance programs. *Echinococcus multilocularis* infection is confirmed by identification of the adult worm in the intestinal tract of the definitive host. Hunter and trapper harvested wild canids are the primary source of samples used by researchers for *E. multilocularis* surveillance, allowing for a large number of specimens to be collected over a wide geographic range by individuals with expertise in harvesting these animals. Time and monetary constraints may otherwise make such large scale collection impossible for the researcher.

Several methods have been utilized for detection of *E. multilocularis* in definitive hosts, which involve examination of the small intestine and morphological identification of adult worms. These methods include scraping or washing of the intestinal mucosa and examining retained material either directly under a stereomicroscope, by concentrating parasites through a series of sieves, or by sedimentation (Leiby et al.1970, Kritsky and Leiby 1978, Storandt and Kazacos 1993, Jacobs et al. 1994, Hildreth et al. 1991, Hildreth et al. 2000, WHO/OIE 2001). Polymerase chain reaction (PCR), rather than morphological characteristics, can be used to confirm *E. multilocularis* when suspected adult worms are found (Trachsel et al. 2007, Liccioli et al. 2012).

Very low temperatures kill the infectious eggs of *E. multilocularis,* and it is recommend that carcasses or intestinal tracts be frozen at -70° C for 96 hours or at – 80° C for 48 hours to eliminate infectivity of eggs (WHO/OIE 2001). In addition to freezing at low temperatures, proper personal protective equipment (PPE) is recommended when processing intestinal tracts including a disposable suit, rubber boots, rubber gloves, eye shield and respirator (WHO/OIE 2001). A concentration of at least 3.75% sodium hypochlorite (NaOCl) applied for 3 to 5 minutes can be used to decontaminate work stations, instruments and glassware (WHO/OIE 2001).

Ecology of definitive hosts and need for surveillance

Prevalence and distribution of *E. multilocularis* have not been determined for twenty-years or longer in many regions of the United States. In other areas, surveillance for *E. multilocularis* has never been conducted. Anthropogenic changes in land use and landscape are resulting in changing vertebrate communities that are causing many ecological changes, such as

changes in species abundance and distribution, including parasites and pathogens causing human diseases (e.g., Lyme disease) (Barbour and Fish 1993). A striking trend has been the rise of coyote populations corresponding with a decrease in red fox numbers (Gosselink 2003, Levi et al. 2012).

During the past two centuries coyotes have increased in abundance and distribution and have expanded their range from the Plains and southwest deserts across North America into all but the northern-most regions of the continent (Gompper 2002, Gompper et al. 2003, Gehrt 2006, Berger and Gese 2007, Mastro 2011). Extirpation of larger predator species, specifically the gray wolf, and post-European settlement land use patterns resulting in clear cutting, an increase in cultivated lands, fragmented landscapes and small scale farming with confined livestock have created ideal habitat for the coyote, a generalist species, allowing them to attain higher densities than other, more specialized predators (Bekoff 1978, Gompper 2002, Berger and Geese 2007).

The top down effects of coyotes on red foxes, gray foxes and other mesopredators (intermediate sized predators that often increase in abundance as larger, top predators decrease) have been well documented (Bekoff 1978, Sargeant et al. 1987, Sovada et al. 1995, Crooks and Soule 1999, Gompper 2002, Gosselink et al. 2003, Levi et al. 2012, Levi and Wilmers 2012). Coyotes and red foxes have similar food and habitat requirements (Tables 1 and 3) and coyotes will directly kill or displace red foxes through interference competition, forcing them into less desirable habitats and excluding red foxes in sympatric populations (Major 1983, Sovada et al. 1995, Gosselink et al. 2003, Gehrt 2006). Coyotes may also have effects on gray fox populations and have been observed as an important cause of mortality in gray foxes as well as bobcats in California (Fedriani et al. 2000). In addition to competition for food and resources, coyote home ranges are generally 5 to 7 times larger than that of red foxes (Table 2), contributing to a limited

number of red foxes being present in areas dominated by coyotes (Voigt and Earle 1983). Areas regularly used by coyotes for travel and rearing of young are generally avoided by red foxes; however coyotes will utilize and establish territories in areas used by red foxes (Voigt and Earle 1983, Caron 1986, Sargeant et al. 1987). Over time, displacement of established red foxes by coyotes, lack of immigration into red fox populations and coyote induced mortality will cause red fox populations to decline (Sargeant et al. 1987).

In Michigan, population estimates of coyotes and foxes do not exist; however annual hunter and trapper harvest surveys have been collected. Where population estimates are lacking, harvest trends have been used as an index to make inferences about population trends over time with the assumption that population size and harvest rates are linearly related and that increases in annual harvest correlates with an increase in the animal population being harvested (Ecker 2003, Konig et al. 2005). Harvest trends in Illinois, when pelt prices were controlled for, support trends that red foxes have been declining while coyotes have been increasing since the mid-1970's (Gosselink et al. 2003). Frawley (2002*a*, 2012*c*), based on analyses of Michigan furbearer harvest surveys, suggested red fox populations have been declining since the mid 1980's, while coyote populations have been on the rise. An increase in harvest effort (days afield) for red foxes can be observed with a simultaneous decrease in harvest, further supporting trends of declining red fox populations in Michigan (Figure 4). Harvest effort and the number of coyotes harvested have both been on the rise since the late 1980's/early 1990's; however whether the increase in coyote harvest is a true reflection of an increase in coyote populations or is a result of an increase in harvest effort is uncertain (Figure 4). None the less, trends of increasing coyote populations have been well documented across North America (Gompper 2002, Gompper et al. 2003, Gehrt 2006, Berger and Gese 2007, Mastro 2011).

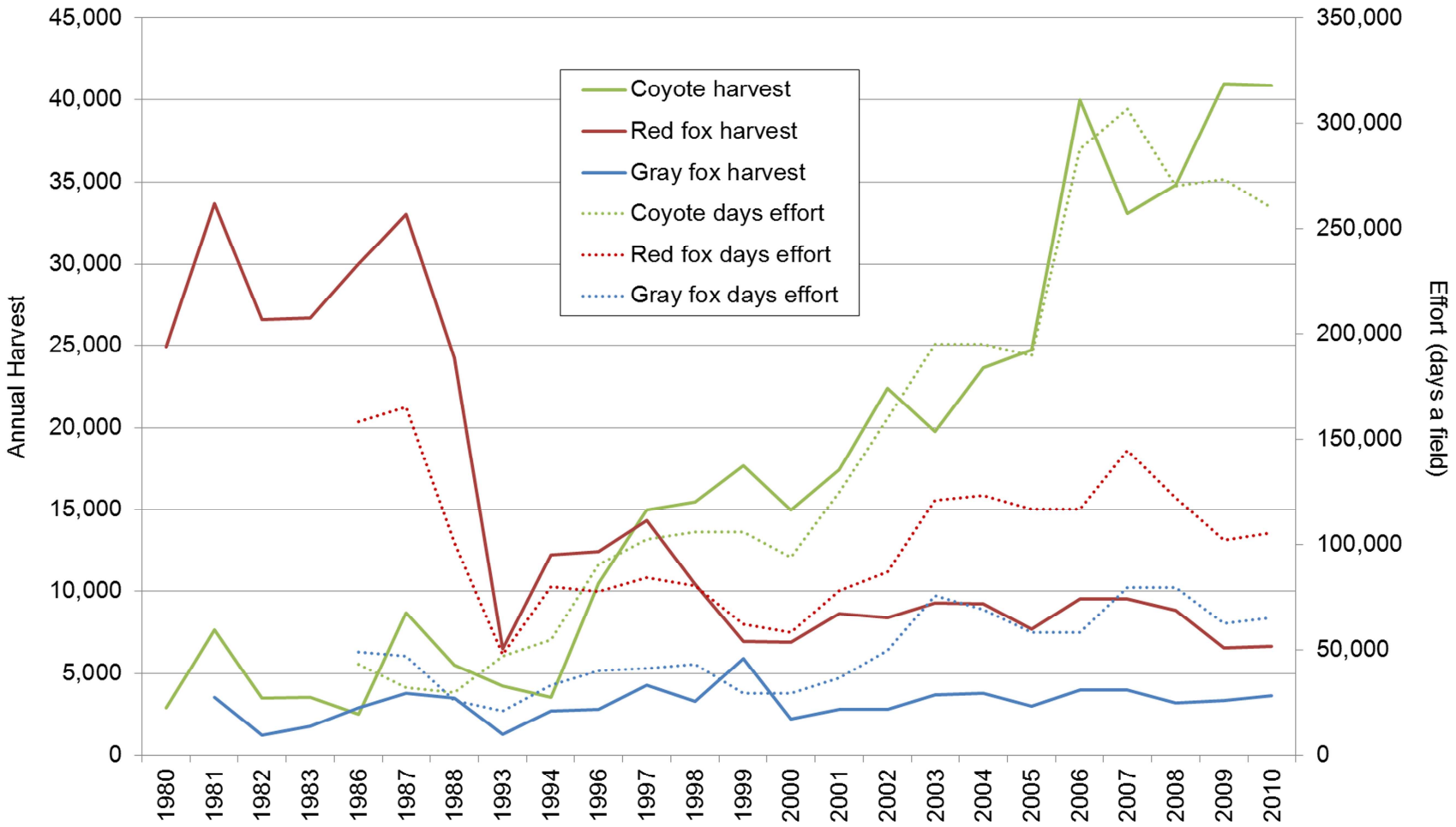


Figure 4. Annual harvest and harvest effort of hunters and trappers for coyotes, gray foxes and red foxes in Michigan (Hawn 1981, Hawn 1982, Hill 1983, Stuht and Hill 1983, Reis 1985, Reis and Hill 1985, Reis 1986, Reis 1989, Karasek and Moritz 1996, Karasek and Moritz 1997, Karasek 1998, Frawley 2001, Frawley 2002a, Frawley 2002b, Frawley 2003, Frawley 2004, Frawley 2006, Frawley 2007*a*, Frawley 2007*b*, Frawley 2007*c*, Frawley 2008*a*, Frawley 2008*b*, Frawley 2012*a*, Frawley 2012*b*, Frawley 2012*c*, Frawley 2012*d*, Frawley 2012*e*, Frawley 2012*f*).

Coyotes have adapted well to human dominated landscapes and will utilize habitat with high human densities, allowing them to live in close proximity to humans (Gompper 2002, Laliberte and Ripple 2004). The coyote's ability to tolerate human dominated landscapes, including metropolitan areas, is evident in popular media reports such as "Michigan DNR says coyote sightings on the rise in urban, suburban areas" (The Oakland Press 2012), "Coyote packs are in the rise in west Michigan" (MLive 2011) and "Coyotes make themselves at home in Michigan cities" (Michigan Radio 2011). Incidents of nuisance coyotes have been on the rise in Chicago, Illinois, from 20 per year in the early 1990's to 350 per year by the late 1990's (Gehrt 2006). Increasing presence of coyotes in urban and suburban areas has been attributed to increasing coyote populations in the state (Michigan Department of Natural Resources 2013).

Likewise, in Europe, red foxes have increased in abundance and distribution and have become urbanized following rabies vaccination programs (Deplazes et al. 2004). These increasing urban fox populations have been considered important in increasing exposure in domestic dogs and cats and have been considered to play a role in the epidemiology and emergence of *E. multilocularis* in some European cities (WHO/OIE 2001, Konig et al. 2005). Similar patterns are possible in the United States with the urbanization of the coyote.

Coyotes have high reproductive capabilities, the ability to disperse long distances, have larger home ranges than foxes and are opportunistic in dietary and habitat choices, allowing for colonization of areas where specialist species may not flourish (Major 1983). Kritsky and Leiby (1978) speculated the coyote could serve as a suitable definitive host capable of maintaining high prevalence in South Dakota in areas with low red fox densities. Urbanization, expanding coyote populations and the species' ability to adapt and live in close proximity to humans may increase the risks of exposure to *E. multilocularis* in both humans and domestic pets. Due to lack of

knowledge of recent prevalence of *E. multilocularis* in Michigan and the on-going increase in population size and urbanization of this definitive host, I propose to survey coyotes and foxes to answer the following questions:

- What is the prevalence of *E. multilocularis* in Michigan?
- Are there significant differences amongst geographical regions within the state, or amongst species, age or sex of the definitive host?

Such data would allow comparison with prior findings, provide a current understanding of the spatial distribution of prevalence of *E. multilocularis*, and provide a baseline with which to compare data in the future as vertebrate wildlife communities change. These data will also help inform public health officials of the level of risk for human infection.

Methods and Materials

In order to address the study objectives, wild coyotes and foxes from Michigan were collected between winter 2009 and spring 2012. Coyotes, due to increasing populations, ability to adapt to urban environments and ubiquitous nature in Michigan (Kurta 1995, Gompper et al. 2003, Gehrt 2006, Gehrt et al. 2009) were chosen as the species of primary focus in this study; however red foxes and gray foxes were also accepted. Intact or skinned carcasses were requested *i)* to avoid exposing collectors to *E. multilocularis* and other parasites, and *ii)* to insure that the entire small intestine was present and other data could be collected, such as age class and sex.

Sample seasons

The study began with collection of coyotes and foxes in December 2009 and continued through April 2012 with the majority of collection taking place during the hunting and trapping seasons. In Michigan, a fur harvester or small game license is required for trapping or hunting coyotes. Trapping season begins October 15 and ends March 1 and hunting season begins July 15 and ends April 1. Additionally, coyotes that are "doing or are about to do damage" can be taken on private property year-round by the landowner. A fur harvester license is required to take gray foxes or red foxes in the state. Trapping and hunting seasons for fox species run from October 15 to March 1. Two main collection periods were established corresponding with the hunting and trapping seasons: the 2010 season (fall 2010 through spring 2011) and the 2011 season (fall 2011 through spring 2012).

Sample area

The state of Michigan consists of an Upper Peninsula and Lower Peninsula, separated by the Straits of Mackinac and accessible to travel by the Mackinac Bridge. The state is divided into 83 counties of which 15 are in the Upper Peninsula and 68 are in the Lower Peninsula (Appendix A). The Upper Peninsula is 537 km wide by 346 km long and the Lower Peninsula is 354 km wide by 460 km long (City–data.com 2012). Coyotes, capable of traversing distances of 160 km to 640 km could easily travel across multiple counties in either peninsula and potentially across an entire peninsula (Bekoff 1978, Baker 1983, Whitaker 2001). Foxes also have the ability to traverse long distances, with a distance of 394 km documented for a male red fox (Baker 1983). Therefore, coyote and fox populations for this study were distinguished as either Upper Peninsula or Lower Peninsula populations. It was assumed for sampling purposes that the parasite was distributed homogeneously across the landscape and along with host distribution, presumed capable of existing in any county in either peninsula in the state, although more likely to occur in the southern Lower Peninsula based on prior research.

During the first year of the study, coyotes and foxes were collected from any county in the state for which they could be provided. During the second year of the study, collection was focused on the southern half of the Lower Peninsula. Modifications to focus sampling primarily on the southern Lower Peninsula were made based on previous findings of *E. multilocularis* in the bordering states of Indiana and Ohio and on recently published data that included prevalence and distribution of the parasite based on survey work from the early 1990's in Michigan (Storandt and Kazacos 1993, Storandt and Kazacos 2012).

Sample size

To estimate prevalence of *E. multilocularis*, Win Episcope 2.0 (De Blas et al. 2000) was used to calculate desired sample size based on an estimated population size, a presumed prevalence of *E. multilocularis* in the population, an acceptable Type 1 error rate (here a 95% or 99% confidence level) and a desired level of absolute precision or error (here 5 or 10%), all of which are set by the investigator. Population estimates for Michigan coyotes and foxes do not exist; therefore, calculations were performed using an estimated population size of 35,000 animals, which is a conservative estimate of the coyote population based on a proportion of the yearly harvest in the Lower Peninsula in the state. This estimated population size for sample size calculations was effectively an infinite population based on iterative calculations in Win Episcope 2.0. Because *E. multilocularis* has not yet been detected in coyotes in Michigan, prevalence previously identified by Storandt and Kazacos (1993) in coyotes in Illinois, Indiana and Ohio, the states nearest Michigan, was assumed to be most similar. The required sample sizes based on different scenarios are displayed in Table 5. For example, in order to be 95% confident that the parasite was detected at an assumed prevalence of 10% with 5% absolute precision, a sample size of 139 animals would be required and the absolute precision of +/- 5% of the 10% prevalence would yield an acceptable range of 5-15%. Required sample size was chosen assuming a prevalence of 15%, an acceptable Type I error rate of 5% and a desired absolute precision of 5%. Based on these choices, a sample size of 196 coyotes was required. A sample size of 82 red foxes was required based on an estimated prevalence of 5.6%, the value previously found by Storandt and Kazacos (2012) in red foxes in the Lower Peninsula. This estimated prevalence was also used to calculate the sample size of gray foxes. It was assumed that each animal collected was random and had an equal chance of being sampled.

Table 5. Calculation of desired sample size based on an estimated prevalence (Deblas et al. 2000).

	Confidence Level (1 – Type I error rate)			
	95%		99%	
	Desired Absolute Precision		Desired Absolute Precision	
Expected Prevalence	5%	10%	5%	10%
2%	31	8	53	14
4%	60	15	102	26
6%	87	22	150	38
8%	114	29	196	49
10%	139	35	239	60
15%	196	49	339	85
20%	246	62	425	107
25%	289	73	498	125
30%	323	81	558	140
35%	350	88	604	151

Specimen collection

Collection of coyotes and foxes began in December of 2009 with animals submitted for general necropsy to the Michigan Department of Natural Resources (DNR) Wildlife Disease Laboratory (WDL). The DNR WDL receives only a small number of coyotes and foxes per year and these were also collected when opportunities arose. Wild canids euthanized as part of research projects or on depredation permits by the DNR or by the United States Department of Agriculture (USDA), Animal Plant Health Inspection Services (APHIS), Wildlife Services (WS) were collected when available. Additionally, animals were requested from trappers, hunters and fur buyers in the state. An exemption from filing an Institutional Animal Care and Use Committee (IACUC) Animal Use Form with Michigan State University was granted by IACUC as specimens would be obtained from animals that were already dead.

Prior to the start of the 2010 season, contact was made with the National Trappers Association (NTA) and the Michigan Trappers and Predator Callers Association (MPTCA) to

ask for assistance from hunters, trappers and fur buyers in obtaining coyotes and foxes. The MPTCA posted the request on their website forum (http://www.mtpca.com/phpBB3/) and the NTA recommended posting to the Michigan Sportsman's Forum (http://www.michigan-sportsman.com/forum/). Fourteen hunters, trappers and fur buyers from various locations responded and were interested in providing animals. Those wishing to participate were sent a packet that included instructions and a specimen tag (Appendix B and C). Some hunters and trappers were willing to take animals to their nearby DNR field offices for transport to the WDL. In other cases, weekly trips were made to different areas of the state to pick up animals. Most of the animals collected were properly tagged by the individual collecting them. The majority of the carcasses were skinned and many were frozen when picked up and therefore allowed to thaw overnight prior to processing.

During the 2011 season, in addition to requests via the MPTCA and Michigan Sportsman forums, telephone contact was made with nine licensed fur buyers from eight counties in southern Michigan. While most of these handle a limited number of wild canids, successful contact was made with a large scale fur buyer from Calhoun County who was extremely cooperative in providing a large number of coyotes and foxes. Contact was made on a weekly basis and trips to pick up animals were made weekly or as needed.

Carcass processing

Coyotes and foxes were transported to the WDL and allowed to thaw overnight if frozen or processed immediately (Figure 5). Animals were processed at the WDL in the Bio-level 2 laboratory with proper PPE being worn which included scrubs, Tyvek suit, disposable inner gloves, rubber outer gloves, rubber boots, googles and a respirator. Each animal was given

Figure 5. Skinned coyote carcasses collected from a southwest Michigan fur buyer ready to be processed at the DNR, WDL.

an identification number and data was recorded including collector's name, date collected, date received, location of harvest (county, town, range and section or nearest city and crossroads), age, sex and comments. Male sex was determined by presence of a penis and female sex determined externally by absence of a penis and confirmed by the presence of a uterus observed when the initial incision was made. Animals were categorized as either juvenile or adult by time of year collected, body size and tooth wear patterns (Missouri Department of Conservation 2011). The first upper pre-molar tooth was extracted from each animal in the event that determining a definitive age would become a necessary component of the research project (Figure 6). The small intestine (bounded by the stomach anteriorly and the cecum posteriorly) was excised, bagged in a Whirl-pak (VWR sterile sample bag #89000-650, 1650 ml, 17.8 x 30.5 cm) labeled with the specimen identification number and frozen at -20° C (Figure 7).

Figure 6. Removal of the first upper premolar from a coyote for aging.

Figure 7. Removal of the intestinal tract from a coyote.

Additional biological samples were collected from select coyotes for other research projects, in return for providing coyotes and foxes for this project. Skulls and muscle tissue were saved from over 100 coyotes for a researcher in the Upper Peninsula and sections of spleen,

tongue and large intestine were saved from 29 coyotes for a parvovirus study being conducted by USDA, APHIS, WS. Assistance was also provided to a student conducting a study on canine heartworm (*Dirofilaria immitis*) in coyotes from southern Michigan in exchange for coyotes for this study.

Intestinal tract processing

The gold standard for the diagnosis of *E. multilocularis* is the sedimentation and counting technique (SCT), through which, during a series of sedimentation and decanting steps, adult worms can be recovered from the small intestine of the definitive host, providing 98 to 100% sensitivity and 100% specificity (Eckert 2003, Deplazes et al. 2004). Although this technique is time consuming and labor intensive, it is relatively inexpensive and allows for adult worms to be individually counted. This technique is performed as follows, per the Manual on *Echinococcus* in Humans and Animals (WHO/OIE 2001):

- After deep freezing at -80 C for 5 days the intestine is incised longitudinally and examined macroscopically for large helminthes and then cut into 20 cm segments.
- The segments of the intestine are transferred to a glass bottle containing 1 L physiological saline solution. After vigorous shaking for a few seconds, the mucosa is stripped between two pressed fingers, and the segments of the intestine are removed from the flask.
- The washing fluid with the intestinal material is sedimented several times for 15 minutes, and the supernatant decanted until the sediment is sufficiently cleared of colored particles.

- The sediment is examined in small portions (5 ml-10 ml) in rectangular plastic dishes with a counting grid (9cm x 9 cm Falcon, No. 1012) under a stereomicroscope at a magnification of x120.

Similar methods of scraping intestinal material into tap water and decanting to concentrate parasites had been used by Leiby et al. (1970) and Kritsky and Leiby (1978).

An alternative to the SCT recommended by WHO/OIE (2001) is the intestinal scraping technique (IST), which is performed by dividing the intestinal tract into segments, making scrapings of the intestinal mucosa at 5 intervals and examining the material under a stereomicroscope. This method may be preferred to the SCT because it is less time consuming, however sensitivity is 78% as compared to the SCT and it does not allow for the quantification of infection (Deplazes et al. 2004). Researchers have also filtered out the parasite by scraping and washing intestinal contents over a series of sieves ranging in size from 1 mm to 120 µm (Hildreth et al. 1991, Storandt and Kazacos 1993, Jacobs et al. 1994, Hildreth et al. 2000). The SCT was modified by Duscher et al. (2005) to the shaking in a vessel technique (SVT) which included the addition of a mesh screen (0.5 mm) in the cap of the jar to prevent the small parasite from accidently being decanted during the process.

Prior to examination, intestines were frozen at a minimum of -70° C for at least one week to inactivate eggs and were then returned to -20° C until processing. Whirl-paks containing intestines were allowed to thaw overnight in a cooler between 1-2 ° C. Once thawed, the intestine was elongated, measured, incised longitudinally and divided in half into the anterior and posterior sections, which were processed individually using the SCT method (Figure 8).

Figure 8. Coyote intestinal tracts elongated, separated into anterior and posterior sections and incised longitudinally.

Each section was cut into approximately 20 cm segments and placed into a glass jar containing 1 liter of tap water (Figure 9). The jar was capped and shaken vigorously for 5-10 seconds and segments stripped between the thumb and forefinger two times and then removed (Figure 10). The remaining solution was allowed to sediment for 15 minutes, after which the supernatant was decanted, additional tap water added, and the jar shaken again (Figures 11 and 12). This process was repeated until the solution was sufficiently cleared. The remaining sediment was then poured into one or multiple specimen cups (VWR specimen container, #25384-148, 133 ml) and either examined immediately or preserved in 70% ethanol and set aside for examination several days later. The sediment and remaining solution were examined in a square, gridded Petri dish (Falcon 9x9 square dish with grid, VWR #25378-047) under a stereomicroscope at 10 to 63 power and all parasites observed were counted, given unique accession numbers and retained in 70% ethanol for later identification. All parasites observed were categorized based on phylum or class (i.e. Nematoda, Cestoda, Trematoda), size and basic

Figure 9. Segments of a coyote intestinal tract in glass jars, separated by anterior and posterior sections, prior to shaking.

Figure 10. Stripping of coyote intestinal tract segments between thumb and forefinger prior to sedimentation.

Figure 11. Glass jars containing material from coyote intestinal tracts in different stages of sedimentation.

Figure 12. Pouring off supernatant during the sedimentation and counting technique.

morphological features. Five to seven intestinal tracts were processed at a time, taking 4-7 hours to complete, depending on the amount of material in the intestinal tract and quantity of parasites present.

Alternative methods were performed on a small number of intestinal tracts during the first year of the project, including direct examination of intestinal tracts and washing and sieving. Five intestinal tracts were examined directly under the stereomicroscope prior to washing. For this process, samples were thawed and separated into anterior and posterior sections and 5-7 cm segments were examined under a stereomicroscope. After direct examination and removal of any parasites observed, the segments were processed through the SCT to assure additional parasites were not missed. Samples were processed by direct examination to insure that *E. multilocularis* worms were not being broken apart and therefore rendered unidentifiable or lost during the SCT process. Additionally, three samples were processed by washing through a series of sieves per methods described by previous researchers (Hildreth et al. 1991, Storandt and Kozacos 1993, Jacobs et al. 1994, Hildreth et al. 2000, Liccioli et al. 2012). For the washing and sieving technique, intestines were again separated into anterior and posterior sections and intestinal contents were scraped and washed over a series of sieves with pore sizes 1.0 mm (#18, US Standard Series 8" brass sieve, VWR 57334-264), 300 um (#50, US Standard Series 8" brass sieve, VWR 57334-278 and 150 um (#100 US Standard Series 8" brass sieve, VWR 57334-286) and retained material was examined under the stereomicroscope.

Evaluation of intestinal tract processing methods was made with members of the graduate committee and additional contact was made with Dr. Kevin Kazacos, DVM, PhD, Dipl ACVM (Professor of Veterinary Parasitology, Department of Comparative Pathobiology, Purdue University College of Veterinary Medicine, West Lafayette, IN, USA), Dr. Emily Jenkins, PhD,

DVM, BSc (Department of Veterinary Microbiology and School of Public Health, University of Saskatchewan, Saskatoon, SK, Canada) and Dr. Alessandro Massolo, MSc, PhD (Assistant Professor, Wildlife Health Ecology Department of Ecosystem and Public Health, Faculty of Veterinary Medicine, University of Calgary, AB, Canada) based on their past and present research on *E. multilocularis* for input on methods. Recommendations included a more vigorous scraping of the intestinal tract at the start of the process to insure that tightly adhered parasites would be removed and the use of a screen on the glass jar lid when decanting to safeguard against pouring off the tiny parasite. Both modifications were performed on all samples processed after January 2012.

Duscher et al. (2005) modified the SCT into the SVT by adding a screened cap when processing red fox intestinal tracts for *E. multilocularis*. To create a screen cap, a circular area of plastic on the cap of the jar was removed using a Dremel tool. Screen of mesh sizes 1.0 mm and 0.5 mm were cut to size to cover the hole in the cap and placed inside (Figure 13). Both screen sizes were tested by decanting material into a dish and examining material for parasites that may have escaped the mesh screen. With pore size of 1.0 mm, material decanted quickly and it was immediately observed that adult digenetic trematodes, similar in size to *E. multilocularis* were passing through the screen, and therefore this screen size was discarded. The same process was repeated with a screen size of 0.5 mm and no parasites were observed passing through the screen. This modified screen was utilized during the final decantation to pour off most of the water while retaining parasites and other sedimented material. In addition, to validate methods, two gray wolves from the Upper Peninsula, where *E. granulosus* is known to occur, were processed using the SCT method modified with the screen cap.

Figure 13. Cap for glass jar modified with screen (pore size 0.5 mm).

Parasite identification

For identification of *E. multilocularis*, morphological features as previously described (Figure 1.1) can be used to identify the parasite after it has been stained and mounted. Alternatively, adult worms and worm fragments (separated proglottids, pieces of proglottids and scoleces) can be confirmed by PCR. Due to a lack of expertise and equipment, worm fragments (proglottids and scoleces) suspected of being *E. multilocularis* and *E. granulosus* were sent to Dr. Emily Jenkins at the University of Saskatchewan, Saskatoon, Canada for identification by PCR. PCR was performed as described in Traschel et al. (2007) and Catalano et al. (2012) and modified using 50 ul, of extraction buffer (rather than 25 ul) which was found to produce more consistent results (Karen Gesy, University of Saskatchewan, personal communication).

Analysis of Results

Win Episcope (De Blas 2000) was used to calculate the maximum possible prevalence at which the parasite could be present per Thursfield et al. (2001) using the estimated population size and the number of negative results. Free Calc (Aus Vet Animal Health Services 2002) was used to calculate the probability of freedom from disease (or in this case, absence of the parasite) per Cameron and Baldock (1998) using expected prevalence, estimated population size, and sensitivity and specificity of the diagnostic test.

Results

Three hundred two wild canids representing 38 counties were examined for the presence of *E. multilocularis. Echinococcus multilocularis* was found in one coyote from Calhoun County in southern Michigan in this study. The parasite was not found in any of the gray foxes or red foxes examined. Other species of helminths were recovered from 89.5% of animals examined. Additionally, *E. granulosus* was recovered from one of two gray wolves from the Upper Peninsula examined to validate the method used for processing intestinal tracts.

Specimen collection and processing

Four hundred ten wild canids (329 coyotes, 45 gray foxes and 36 red foxes) were collected between December 2009 and April 2012, with 83% provided by hunters, trappers and fur buyers and 17% obtained through non-harvest means (Table 6). Fur buyers were the greatest sources of animals, providing over half of the animals collected. Collection preference was given to coyotes originating from the central and southern Lower Peninsula, although gray and red foxes were also collected when they were available. Because the method of processing intestinal tracts is very time consuming, not all animals collected were examined for *E. multilocularis*. All gray foxes and all useable red foxes were examined. Eight animals collected were determined to be insufficient based on the deteriorated condition of the intestinal tract. Preference for processing intestinal tracts was given to coyotes from the south and central counties of the Lower Peninsula although a small number of coyotes from the Upper Peninsula and northern Lower Peninsula were examined.

Table 6. Coyotes and foxes collected for *E. multilocularis* survey by species and by season. Animals collected as "other" were obtained from WDL, DNR or USDA personnel.

Season	Coyote	Gray Fox	Red Fox	Total (%)
2010	214	9	18	58.8%
2011	83	5	11	24.1%
other	32	31	7	17.1%
Total (%)	80.2%	11.0%	8.8%	100%

Two hundred twenty three coyotes, 45 gray foxes and 34 red foxes were examined for the presence of *E. multilocularis* in Michigan. Ninety-eight percent of the animals examined came from 36 of the 68 counties (53%) in the Lower Peninsula and 2% of the animals came from 2 (13%) of the 15 counties in the Upper Peninsula, with the majority (84%) originating from the south and central Lower Peninsula. Location to county was provided on 99% of the animals examined and 80% had a specific enough location that town and range could be determined. Of the 3 animals for which no specific location data were provided, the region of the state (i.e. southwestern Lower Peninsula, northwestern Lower Peninsula, Upper Peninsula) from which the animal came was known. The largest number of animals examined were adult female coyotes (34.4%) followed by adult male coyotes (25.8%) (Table 7).

Intestinal tract processing

Processing of intestinal tracts began during late spring of 2011 and concluded in August of 2012. All intestinal tracts processed after January 2012 (73.5% of the total sample) were done by the sedimentation and counting technique (SCT) modified to include a screen cap in the lid. Intestinal tracts processed prior to this date were done using the SCT (23.9 %) or while developing study protocols (2.6%) as previously mentioned (i.e. 97.4% were examined via

Table 7. Coyotes and foxes examined for *E. multilocularis* by species, sex and age class.

Coyote	Male	Female	Unknown	Total (%)
Juvenile	12	25	0	16.6%
Adult	78	104	0	81.6%
Unknown	1	3	0	1.8%
Total (%)	40.8%	59.2%	0.0%	100%
Red Fox	Male	Female	Unknown	Total (%)
Juvenile	1	1	0	5.9%
Adult	11	19	0	88.2%
Unknown	1	1	0	5.9%
Total (%)	38.2%	61.8%	0.0%	100%
Gray Fox	Male	Female	Unknown	Total (%)
Juvenile	10	9	0	42.2%
Adult	10	14	0	53.3%
Unknown	1	0	1	4.5%
Total (%)	46.7%	51.1%	2.2%	100%

at least the gold standard test). The SCT modified with the screen cap was preferred as this method provided extra assurance that minute parasites would not be poured off while decanting. Using this modification, parasites less than 0.5 mm were recovered, including metacercariae of *Alaria* sp. and another species of digenetic trematode that will be identified in the future. This method was further validated by processing intestinal tracts from two gray wolves from the Upper Peninsula, where *E. granulosus* is known to occur. Tens of thousands of proglottids and hundreds of scoleces from adult *E. granulosus* were recovered from one of these wolves from Gogebic County.

Mean intestinal tract length ($\pm$ standard deviation) by species was 3.2 m ($\pm$ 0.3) for coyotes, 2.1 m ($\pm$ 0.3) for gray foxes and 1.5 m ($\pm$ 0.2) for red foxes. The number of times

required for a specimen to clear sufficiently through the SCT process of sedimentation, decanting and repeating (one cycle) ranged from 2 to 9 cycles and varied based on the length of the intestinal tract and the amount of ingesta present. Intestinal tracts that contained massive amounts of hair and organic material required more cycles to clear sufficiently.

Recovery of parasites

Echinococcus multilocularis was confirmed in 1 (0.33%) of the 302 animals examined. The parasite was identified from an adult female coyote from Calhoun County, harvested on 01/19/2011. Prevalence of *E. multilocularis* in coyotes as a result of this study was 1.54% (1/65) in Calhoun County and 0.46% (1/219) in the Lower Peninsula population. One scolex from an adult worm was recovered from this animal. Due to the small piece of material present, the scolex was assayed by PCR and confirmed to be *E. multilocularis*. Strain typing on this small fragment of *E. multilocularis* was unsuccessful. The identification of *E. granulosus* recovered from one gray wolf similarly was confirmed by PCR and strain typed as the North American cervid strain (G8).

Additionally over 5,800 individual helminths were recovered from the coyotes, red foxes and gray foxes processed, ranging from minute digenetic trematodes approximately 1 mm or less in size to lengthy cestodes of which hundreds of proglottids were present. The greatest proportions of helminths found were digenetic trematodes (48.3%), followed by cestodes (32.8%) and nematodes (18.9 %). Of the 302 animals examined, 89.5% had at least one helminth present in the small intestine. By species, 90.6% of coyotes, 86.7% of gray foxes and 82.4% of red foxes had at least one helminth present (Table 8).

Table 8. Number of coyotes and foxes examined by county and species for *E. multilocularis* including the number and percentage of animals in which at least one helminth was found in the small intestine.

	Coyotes		Gray Foxes		Red Foxes		Total	
County	Number Examined	Number with ≥1 Helminth Present (%)	Number Examined	Number with ≥1 Helminth Present (%)	Number Examined	Number with ≥1 Helminth Present (%)	Number Examined	Number with ≥1 Helminth Present (%)
Upper Peninsula (UP)								
Gogebic	4	2 (50%)					4	2 (50%)
Menominee					1	1 (100%)	1	1 (100%)
Total UP	4	2 (50%)	0	0	1	1 (100%)	5	3 (60%)
Lower Peninsula (LP)								
Allegan	25	22 (88%)	2	2 (50%)	6	5 (83%)	33	28 (85%)
Antrim	12	8 (67%)					12	8 (67%)
Benzie	1	1 (100%)					1	1 (100%)
Branch	25	24 (96%)					25	24 (96%)
Calhoun	65	60 (92%)	6	6 (100%)	6	6 (100%)	77	72 (94%)
Cass	1	1 (100%)					1	1 (100%)
Charlevoix			3	3 (100%)			3	3 (100%)
Cheboygan	1	1 (100%)	4	2 (50%)			5	3 (60%)
Clare	1	1 (100%)	2	2 (100%)			3	3 (100%)
Clinton	15	14 (93%)					15	14 (93%)
Crawford	1	1 (100%)					1	1 (100%)
Eaton	5	5 (100%)					5	5 (100%)
Gladwin	1	0 (0%)					1	0 (0%)
Grand Traverse			2	2 (100%)			2	2 (100%)
Ingham	7	7 (100%)	1	0 (0%)	2	1 (50%)	10	8 (80%)
Isabella			1	1 (100%)			1	1 (100%)
Jackson	4	4 (100%)					4	4 (100%)

Table 8. (cont'd)

County	Coyotes Number Examined	Coyotes Number with ≥1 Helminth Present (%)	Gray Foxes Number Examined	Gray Foxes Number with ≥1 Helminth Present (%)	Red Foxes Number Examined	Red Foxes Number with ≥1 Helminth Present (%)	Total Number Examined	Total Number with ≥1 Helminth Present (%)
Lower Peninsula (LP)								
Kalkaska			1	1 (100%)			1	1 (100%)
Kent	12	11 (92%)	4	4 (100%)	10	8 (80%)	26	23 (88%)
Lake			1	1 (100%)			1	1 (100%)
Leelanau			3	1 (33%)			3	1 (33%)
Lenawee	1	0 (0%)					1	0 (0%)
Livingston			1	1 (100%)			1	1 (100%)
Mecosta	19	16 (84%)					19	16 (84%)
Monroe			1	1 (100%)			1	1 (100%)
Montmorency	4	4 (100%)					4	4 (100%)
Newaygo	3	3 (100%)	3	3 (100%)	1	1 (100%)	7	7 (100%)
Oakland					1	1 (100%)	1	1 (100%)
Oscoda					1	1 (100%)	1	1 (100%)
Otsego			2	2 (100%)	1	1 (100%)	3	3 (100%)
Ottawa	8	8 (100%)	2	2 (100%)	3	3 (100%)	13	13 (100%)
Sanilac					1	0 (0%)	1	1 (100%)
Unknown - SW County	2	2 (100%)	1	1 (100%)			3	3 (100%)
Tuscola					1	1 (100%)	1	1 (100%)
Washtenaw			1	1 (100%)			1	1 (100%)
Wayne	6	6 (100%)					6	6 (100%)
Wexford			4	4 (100%)			4	4 (100%)
Total LP	219	199 (91%)	45	39 (87%)	33	28 (85%)	297	266 (90%)
Total	**223**	**201 (90%)**	**45**	**39 (87%)**	**34**	**28 (82%)**	**302**	**269 (89%)**

Discussion

The objectives of this study were to determine prevalence of *E. multilocularis* in wild coyotes and foxes in Michigan and use the data collected to determine if significant differences in prevalence occurred amongst geographic regions, or between sex or age classes of the definitive host. Because the parasite was found in only one animal, such comparisons were not possible. However, this data can be compared with past surveillance in the state, used as a baseline for future research, and can aid in assessing the level of risk for human infection. Prevalence found in this study was lower than what was expected based on previous research in Michigan and nearby states (Ballard and Vande Vusse 1983, Ballard 1984, Storandt and Kazacos 1993, Storandt and Kazacos 2012). Several explanations for the low prevalence are discussed below including how study design and ecological and environmental factors may have influenced the prevalence found.

Historical prevalence

The earliest focus of *E. multilocularis* in the contiguous United States was found in the Dakotas in the early to mid-1960's (Leiby and Olsen 1964). The means by which the parasite became established in the north central region of the United States from endemic areas in the Arctic tundra are unknown, but likely occurred by the natural movement of wild canids or through importation of domestic dogs (Rausch 1967, Rausch and Richards 1971). *Echinococcus multilocularis* is hypothesized by several researchers to have spread across north central North America in a southeast direction over time based on both negative and positive surveillance results from several states (Figure 14) (Hildreth et al. 1991, Storandt and Kazacos 1993, Storandt

et al. 2002, Storandt and Kazacos 2012). Surveillance in the 1960's identified the parasite east of the Dakotas in Minnesota and Iowa, but not to the west in Montana until the late 1970's (Leiby et al. 1970). Subsequently in the early 1980's the parasite was detected in Nebraska, Wisconsin and Illinois, and in the early 1990's in central Illinois and Indiana, north central Ohio and southern Michigan (Ballard and Vande Vusse 1983, Ballard 1984, Storandt and Kazacos 1993). The parasite may have become established in new areas, including Michigan, through the natural movement of hosts, specifically coyotes and foxes, or by the translocation of wild canids for hunting enclosures (Hildreth et al. 1991, Storandt and Kazacos 1993, Jones and Pybus 2001).

In several states when subsequent surveillance was conducted approximately 10 years after the initial finding of *E. multilocularis*, an increase in prevalence was observed. For example, prevalence increased from 0 to 4.1% in coyotes in Montana, and in red foxes from 5.1 to 51.3% in Minnesota, from 27.8 to 37.5% in Nebraska, and from 0.5 to 88.9% in South Dakota (Leiby et al. 1970, Vande Vusse et al. 1978, Ballard and Vande Vusse 1983, Seesee et al. 1983, Hildreth et al. 2000, Storandt and Kazacos 2012). Although this trend appears to be reversed in Illinois red foxes, during the latter survey the sample size (4 animals) was inadequate to conclude that prevalence had truly decreased in the state. It was expected that over time the parasite would increase in geographic distribution and prevalence across North America due to adequate populations of host species and by translocation of foxes and coyotes from infected areas to non-infected areas (Hildreth et al. 1991, WHO/OIE 2001, Storandt and Kazacos 2012).

Based on the predicted geographic expansion of the parasite and the amount of elapsed time since the last known surveillance in the Midwest, it was expected that the current prevalence in Michigan would be comparable to those previously found in nearby states, 14.9-35.3% in coyotes and 12.8-27.3% in red foxes (Storandt and Kazacos 1993). During the final

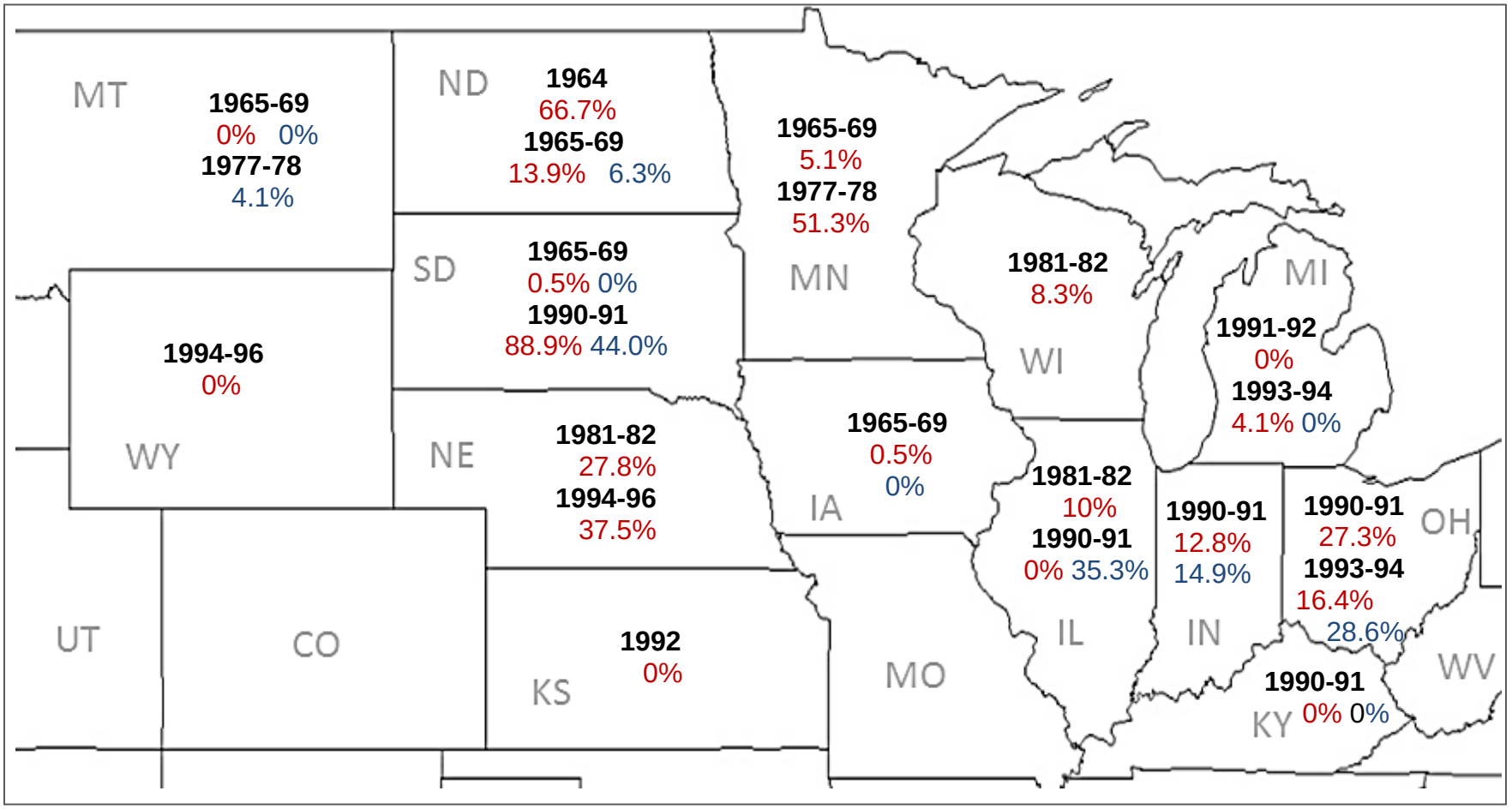


Figure 14. Prevalence in coyotes and red foxes as a result of previous surveillance for *E. multilocularis* in North America. Text in red indicates prevalence in red foxes and text in blue indicates prevalence in coyotes (Leiby and Olsen 1964, Leiby et al. 1970, Vande Vusse et al. 1978, Ballard and Vande Vusse 1983, Seesee et al. 1983, Ballard 1984, Storandt and Kazacos 1993, Hildreth et al. 2000, Storandt et al. 2002, Storandt and Kazacos 2012).

year of this project, prevalence values in Michigan collected in the early 1990's by Storandt and Kazacos (2012) became available, with prevalence of 0% in gray foxes (0/9) and coyotes (0/46) and 5.6% in red foxes (4/71) in the Lower Peninsula and 0% in gray foxes (0/2), coyotes (0/8) and red foxes (0/26) in the Upper Peninsula. *Echinococcus multilocularis* has not been found in the Upper Peninsula (although sample sizes in both this and the aforementioned study were small); therefore discussion will focus on the Lower Peninsula.

Prevalence in Michigan

Echinococcus multilocularis has only been documented in one gray fox in North America and based on negative surveillance results here and in other studies (Table 4) it appears that this parasite is not common in gray foxes in North America or in Michigan. *Echinococcus multilocularis* was not found in any of the forty-five gray foxes examined from 20 counties in the Lower Peninsula in this study (Figure 15). The maximum possible prevalence based on these results calculated per Thursfield et al.(2001) is relatively low (6.4%) in this species (Table 9). A larger sample size of gray foxes would have been required (n=82) to conclude that this parasite does not occur in this species in the state based upon an expected prevalence of 5.6% (that previously found by Storandt and Kazacos (2012) in red foxes in the state) (Table 9). Although they can serve as a definitive host, based on historical surveillance records, gray foxes are probably not important in maintaining the life cycle of *E. multilocularis* in Michigan or in North America.

Thirty-three red foxes were examined from 11 counties in the Lower Peninsula resulting in 0% prevalence (Figure 16). In previous surveys by Storandt and Kazacos (1993) and Storandt

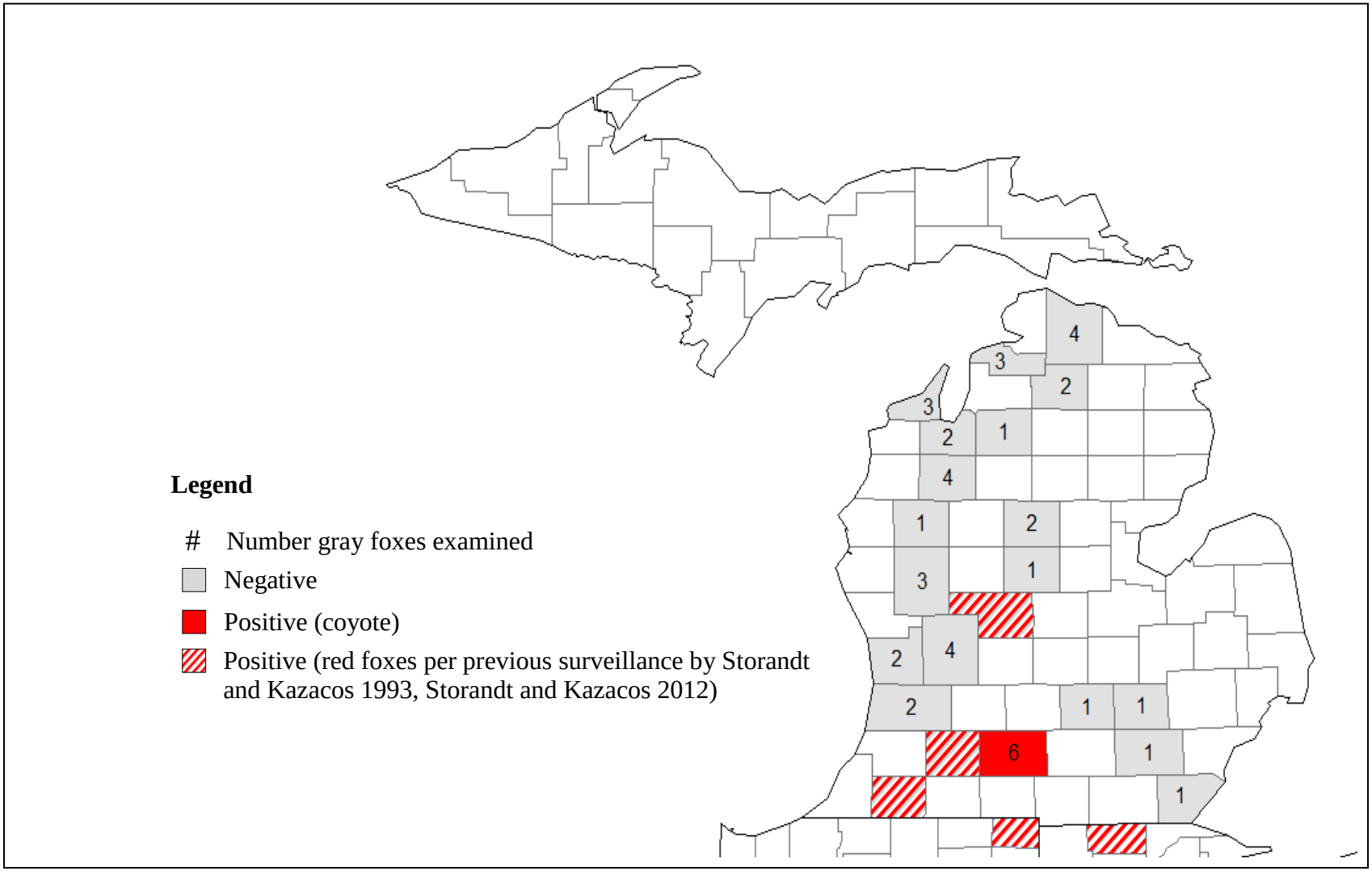


Figure 15. Total number gray foxes sampled by county for *E. multilocularis*, with counties where *E. multilocularis* has been identified in red foxes and coyotes in this and previous surveillance activities highlighted. The location to county for one gray fox is unknown.

Table 9. Comparison of results of this study with previous surveillance in the Lower Peninsula of Michigan, maximum possible prevalence and probability of freedom from disease (i.e. absence of the parasite) at the 95% confidence level (Cameron and Baldock 1998, Thursfield et al. 2001).

Species	Lower Peninsula Population Estimate*	Number Positive/ Number Sampled	Prevalence	Prevalence by Storandt and Kazacos (2012)	Maximum Possible Prevalence	Probability of freedom from disease (i.e. absence of the parasite)
Gray Fox	3000	0/45	0.0%	0.0%	6.4%	Inadequate sample size
Red Fox	6900	0/33	0.0%	5.6%	8.7%	Inadequate sample size
Coyote	35000	1/219	0.5%	0.0%		Prevalence is $\geq$ 0.46% but < 2.2%

* Since population sizes for foxes and coyotes are unquantified for Michigan, a portion of the yearly harvest representative of the animals harvested in the Lower Peninsula was used, assuming at a minimum, the population would be equal to the number of animals harvested (i.e., this was a conservative estimate).

and Kazacos (2012), *E. multilocularis* was identified in red foxes in the south and central Michigan counties of Cass (1/36), Kalamazoo (1/2) and Montcalm (2/20), and in red foxes in Fulton County, OH (5/16) and an unspecified canid in Steuben County, IN (1/3) which border Michigan to the south. Overall prevalence determined by Storandt and Kazacos (2012) in the Lower Peninsula in red foxes was 5.6%. The calculated maximum possible prevalence (8.7%) and prevalence found by Storandt and Kazacos (2012) are still lower than what was previously found in other, nearby states (12.8-27.3%) (Storandt and Kazacos 1993). The sample size in this study was too small to confirm that *E. multilocularis* still occurs in red foxes in the state. Based on previous prevalence and the maximum possible prevalence, *E. multilocularis* might still occur in red foxes in the state but has probably not increased substantially over time; however a larger sample of red foxes would need to be examined to confirm these conclusions.

Coyotes were the greatest number of animals examined, with 219 animals being examined from 22 counties in the Lower Peninsula (Figure 17). Prevalence in coyotes as a result of this study is 0.46 % in the Lower Peninsula and 1.54 % in Calhoun County. The positive coyote found in this study was from Athens Township, which is in the southeast corner of Calhoun County. Prevalence in coyotes in nearby states has ranged from 14.9 to 35.3%, and as high as 44.4% in South Dakota (Storandt and Kazacos 1991, Hildreth et al. 2000). The parasite was not found in any coyotes in Michigan during the previous survey by Storandt and Kazacos (2012), which may have been influenced by sample size and distribution. Of the 46 coyotes that they examined from the Lower Peninsula, the majority (91%) came from 2 counties in the northern portion of the peninsula. If the parasite did occur previously in coyotes in Michigan, it may have been missed due to the smaller sample size and the low prevalence or lack of specimens from the southern Lower Peninsula.

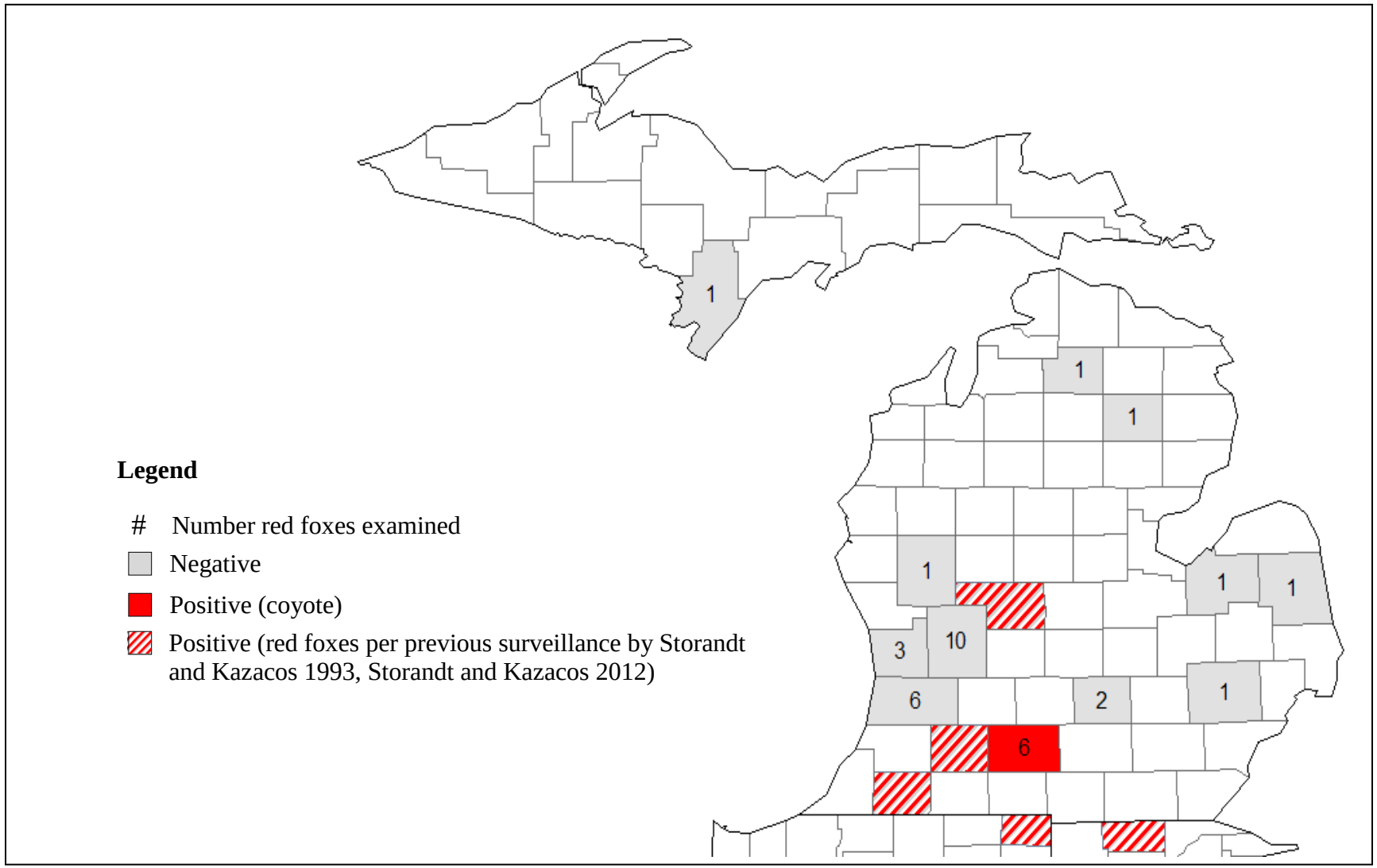


Figure 16. Total number red foxes sampled by county for *E. multilocularis*, with counties where *E. multilocularis* has been identified in red foxes and coyotes in this and previous surveillance activities highlighted.

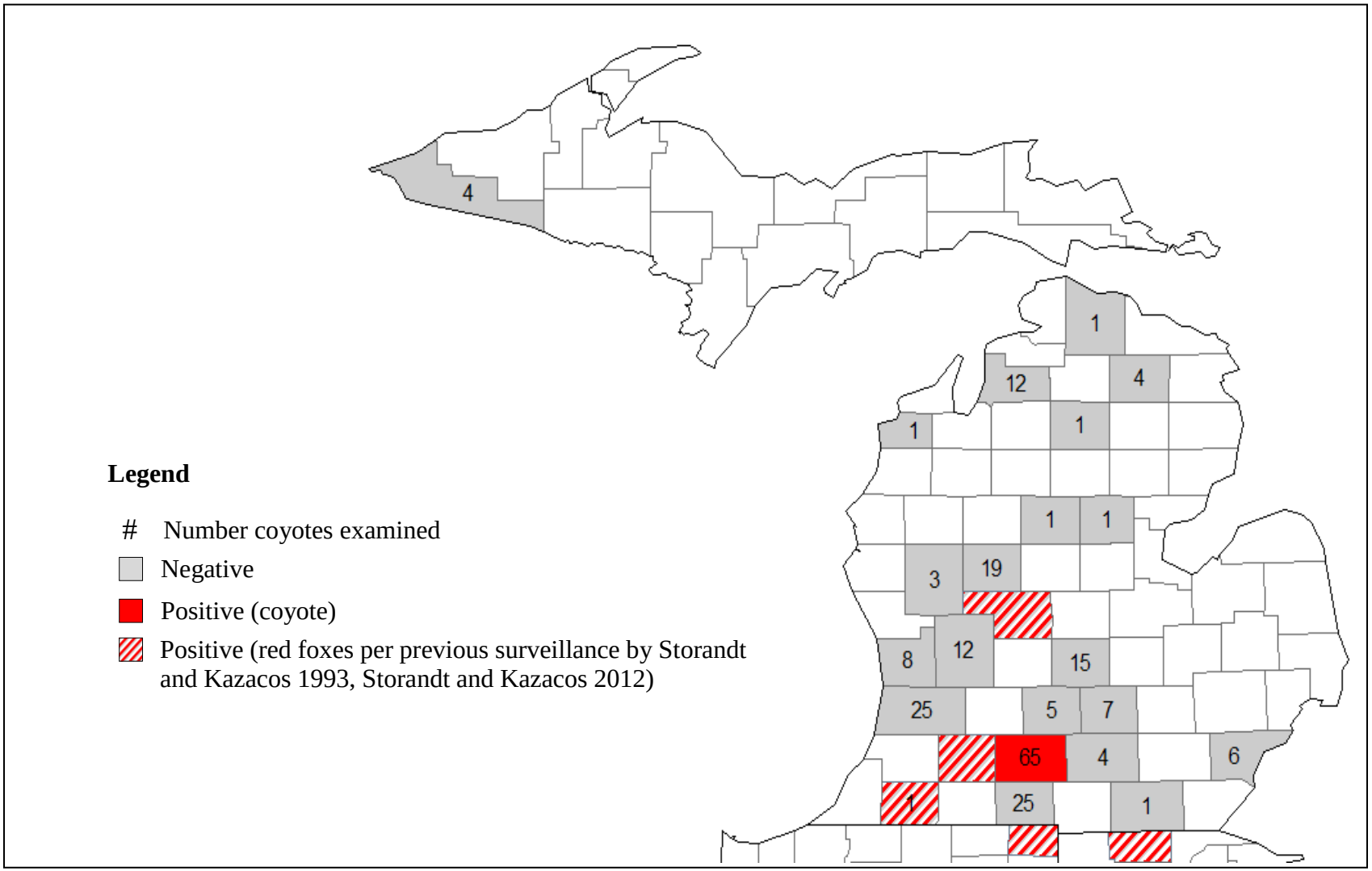


Figure 17. Total number coyotes sampled by county for *E. multilocularis*, with counties where *E. multilocularis* has been identified in red foxes and coyotes in this and previous surveillance activities highlighted. The location to county for 2 coyotes is unknown.

Coyotes were the focus of this study and therefore greatest effort was made in collecting specimens of this species. Red and gray foxes were more difficult to obtain than coyotes when collecting at fur buyer operations, perhaps a reflection of the decrease in harvest in red fox and the increased harvest of coyotes. A larger sample of gray and red foxes might have been collected with increased effort and would have been necessary to increase our level of confidence in results when drawing conclusions about prevalence in these species in the state.

It is not surprising that *E. multilocularis* would be found in a southern county based on the hypothesized spread of the parasite and where it had been previously identified in wild canids. However, the parasite was also predicted to spread north into Michigan and based on the expansion of the parasite's range and trends of increasing prevalence over time, it was expected that this would be reflected in the results of this study (Storandt and Kazacos 2012). It appears that based on this study and previous work, prevalence has historically been low and remains low in Michigan.

Influence of study design

The design of this study was modeled after published surveys for *E. multilocularis* and recommendations made by the WHO/OIE (2001) utilizing hunter harvested canid samples collected during the fall and winter trapping and hunting seasons and processing intestinal tracts according to guidelines considered to be the gold standard (Hofer et al. 2000, Eckert 2003, Sreter et al. 2003, Deplazes et al. 2004, Siko et al. 2011). Although sample size of coyotes in this study was adequate to provide a high level of statistical power, the method of sampling (convenience sampling) has the potential to create biases and requires assumptions be made in order to extrapolate or interpret results to the entire population.

One such assumption is that all animals were harvested at random and that all animals in the study population had an equal chance of entering the sample. Another assumption is that the study population, and in this case the parasite, are distributed homogenously across the landscape. Convenience sampling likely does not provide a true representation of the study population and canid species will be distributed where habitat and food sources are available. *Echinococcus multilocularis* which exhibits spatial heterogeneity and can be found in one county but not in an adjacent county and prevalence can vary among areas as small as a few hectares (WHO/OIE 2001).

Specimens collected for *E. multilocularis* surveys are collected through hunting, trapping, euthanasia due to abnormal behavior or from animals found dead, and biases associated with this method of collection can occur (Conner et al. 2000). One bias associated with convenience sampling is the susceptibility or insusceptibility of infected animals to being harvested. For example, a disease that may cause abnormal behavior in an animal may contribute to an animal being less alert and more easily harvested. On the other hand, a disease that causes an outwardly sick appearance may deter hunters or trappers from taking the animal and decrease chances of being harvested. In these instances, prevalence may be overestimated or underestimated. *Echinococcus multilocularis* is not known to impact its canid host by causing abnormal behavior or causing an animal to appear unhealthy and therefore would not be expected to create this bias.

Eleven percent of the animals collected in this study were found sick or dead by the public and submitted to the DNR, WDL for general necropsy, the majority (65%) of which were gray foxes. Animals afflicted with an illness (such as canine distemper) might be less likely to display normal behavior and/or consume a typical diet, which could potentially influence their likelihood of becoming infected. Adult *E. multilocularis* live for several months in the canid

host and an animal could become infected prior to becoming ill; however these animals could potentially lead to an underestimation of prevalence.

Convenience sampling of hunter harvested animals can create biases associated with seasonality of specimen collection including seasonal fluctuations in prevalence (Conner et al. 2000). Seasonal trends in prevalence of *E. multilocularis* occur, with highest prevalence in both intermediate and definitive hosts being observed in spring and summer and lower prevalence in fall and winter (Kritsky and Leiby 1978). Leiby & Kritsky (1974) examined over 5,600 deer mice from North Dakota between 1965-1972 and found the highest seasonal prevalence observed in spring (6.5%) and summer (5.5%), followed by fall (2.2%) and winter (2.0%). Subsequently, Kritsky and Leiby (1978) examined over 1,100 red foxes from North Dakota between 1965-1972 finding the highest average prevalence correlating with that found in deer mice, with highest prevalence in the spring (25.3%) and summer (32.4%), followed by fall (13.7%) and lowest prevalence in the winter (6.4%). Most animals provided by hunters and trappers were collected from November through early March, corresponding with a time of year when prevalence is known to be lowest, which might contribute to added difficulty in sampling infected animals.

Spatial distribution of samples may also influence prevalence and when convenience samples are used obtaining specimens from specific geographic areas can be difficult. In similar surveys when data per county were listed, only a small number of animals per county were sampled for most counties. Coyotes and foxes collected by Storandt and Kazacos (2012) were patchy in distribution with as few as one specimen to as high as 37 specimens per county being collected. In surveillance in Illinois and Nebraska by Ballard and Vande Vusse (1983) 76 red foxes were examined from 23 counties. In Illinois, Indiana, Ohio, Michigan and Wisconsin when the number examined per county was provided, less than 5 animals per county were

examined in over 60% of the counties and in only 6% of the counties were 20 or more animals examined (Ballard 1984, Storandt and Kazacos 1993, Storandt and Kazacos 2012). In this study, 5 or fewer animals were collected and processed from 68% of the counties sampled, and from 11% of the counties 20 or greater animals were processed. In 42% of the counties sampled, only one animal was collected. Attempts to collect a larger number of coyotes and foxes per county, particularly in southern Michigan were unsuccessful. Hunters and trappers that provided carcasses in this and other studies were likely limited to a specific geographic area where they hunt and trap. Depending on the size of the area, the animals trapped and hunted may represent family groups or animals that live in close proximity to one another, and thereby be more apt to share diseases and parasites, or be free of diseases and parasites, if distribution is spatially heterogeneous with isolated geographic foci.

The task of finding this parasite becomes even more difficult since prevalence can vary significantly within small geographic areas (WHO/OIE 2001). The patchy distribution of animals collected could lead the researcher to sample in areas where the parasite does not occur causing prevalence to be underestimated or to sample in areas where the parasite is highly clustered, causing prevalence to be overestimated. Designing a study with specific study locations and animals being collected by the researcher could still easily miss this parasite, unless it was known to occur in the area being sampled. Convenience sampling, although having the potential to create biases, is the most efficient means of obtaining a large number of animals across a wide geographic range, while exerting minimal effort and expense.

The method used for processing intestinal tracts (SCT) has high specificity and sensitivity; although when only a small number of worms are present, the ability to detect the parasite may decrease and parasites could be decanted with the supernatant or accidently

removed with the intestinal tract (Karamon et al. 2010). Karamon et al. (2010) evaluated the SCT by processing small intestines experimentally infected with a known number of *E. multilocularis* adult worms and found when 30 or more worms were present, the ability to detect the parasite was 100%, but dropped to 60% detection when 10 or fewer worms were present. The SCT remains the acknowledged gold standard test and even with alternative methods, when only a small number of *E. multilocularis* were present, they could be lost during steps in the processing.

The low prevalence found in this study is not believed to be attributed to the intestinal tract processing, although if worm burdens were small, it is possible that parasites could have been lost during the sedimentation and decantation process. However, the one *E. multilocularis* parasite recovered was only a scolex of an adult worm; thousands of *E. granulosus* proglottids and hundreds of scoleces were recovered from one of the wolves examined and numerous scoleces from other cestode species, as well as digenetic trematodes as small as 1 mm in diameter were recovered. Given that these are the same size or smaller than *E. multilocularis*, it is unlikely that *E. multilocularis* infections were missed due to the processing protocol. Additionally, careful examination was made when intestinal villi resembled what could have been deformed worms or proglottids.

Ecological and environmental influences

Although *E. multilocularis* occurs in Michigan, contrary to apparent trends in other states, prevalence has remained relatively low since being first documented in the 1990s. Ecological or environmental changes may have occurred leading to unfavorable conditions for the parasite to increase in prevalence and distribution. For *E. multilocularis* to persist definitive

and intermediate host species must be present and interactions between the two must occur (e.g. mice must be exposed to contaminated vegetation and/or insects, canids must consume infected mice). The complex life cycle of this parasite requires multiple hosts working in concert and environmental conditions favorable to egg survival. Both the intermediate rodent host as well as the adult worm in the definitive host is short lived, requiring interactions between the hosts to continue during a relatively short period of time (several months) under specific conditions in order for the cycle to continue.

The influence of landscape changes in prevalence of *E. multilocularis* is restricted to the extent at which it affects the abundance and distributions of hosts. When habitat is supportive of high densities of intermediate and definitive hosts, prevalence is expected to be higher (Leiby and Kritsky 1974). Anthropogenic landscape changes have favored increases in coyote populations and abundance, inadvertently leading to declines in red fox populations (Whitaker 2001, Gompper 2002, Gosselink et al. 2003, Levi et al. 2012). Changes in abundance of these two species have occurred over the last 20-30 years, incidentally around the same time that the last surveillance efforts of *E. multilocularis* in the United States were conducted. Subsequent studies have not been conducted to determine if a decline in red foxes could influence prevalence of *E. multilocularis*.

Prevalence was not consistently higher in one species over another when both red foxes and coyotes were examined in the same study. For example, Storandt and Kazacos (1993, 2012) found higher prevalence in coyotes (14.9%, 28.6%) than in red foxes (12.8%, 16.4%) from Indiana and Ohio respectively. Hildreth et al. (2000) found higher prevalence in red foxes (88.9%) than in coyotes (44.4%) in South Dakota. Higher prevalence was also observed in North Dakota red foxes (13.9%) as compared to coyotes (6.3%) (Leiby et al. 1970). These

studies, conducted at least twenty years ago, do not address population dynamics nor do they indicate if one species was more abundant than the other. In a recent survey of intestinal helminths of coyotes in Alberta, Canada, *E. multilocularis* was found in 25.3% of the animals examined (Catalano et al. 2012). However, species' dynamics differ from Michigan and although coyote densities are high, red foxes have also been increasing in abundance and distribution (Alberta Swift Fox Recovery Team 2007). In the absence or reduction of red foxes, certain life history traits of coyotes might limit their ability to maintain a high prevalence of *E. multilocularis*.

Dietary preferences of coyotes might limit prevalence and because the adult worm is short lived in the canid host, consumption of additional infected rodents needs to occur in order for infections to be maintained. Coyotes and red and gray foxes have similar feeding habitats, although coyotes may consume rodents less frequently than foxes. A review of the literature of coyote stomach content analyses from over 17 states by Landry and Van Kruiningen (1979) found rabbits were the primary food source, followed by carrion and then rodents. Other studies on stomach contents have found hare and small rodent remains constituting 20-33% and 18-23% of contents respectively, while carrion, including deer can make up a significant portion of the diet (3 to 48.6%), becoming more important during winter months (Bekoff 1978, Baker 1983). In Illinois coyotes, Gehrt (2006) found small rodents to be the most common food item (42%), with voles being the most common rodent found. Deer (22%) and rabbits (18%) were the next most common food items observed (Gehrt 2006). In winter months, carrion, deer, livestock and hare are important food sources, while in the summer mice, birds, rabbits and plant material comprise a higher portion of the diet (Bekoff 1978). Rabbits and rodents were found as the dominant food items in a review of the literature on stomach content analyses of red foxes from

15 states (Landry and Van Kruiningen 1979). Identification of other parasites recovered from nearly 90% of foxes and coyotes examined in this study may provide insight into the dietary habits of these canids based on the intermediate hosts of these other parasites. For example, 50.7% of the coyotes examined had tapeworms that appear to be either *Taenia pisiformis* or *T. hydatigena*, based on initial morphological identification, neither of which uses a rodent intermediate host but rather rabbits and white-tailed deer respectively. Only 5.8% of the red foxes examined were infected with these same parasites.

While coyote and red fox family units are comparable in size (8-10 animals) home range sizes are up to 8 times larger for resident coyotes and 30 times larger for non-resident coyotes than those of red foxes (Table 2) (Baker 1983, Gehrt 2006, Gehrt et al. 2009). Coyotes and foxes defecate along trails, roads and regular travel routes, around territory borders and near dens or bedding sites and feces may accumulate in these areas (Elbroch 2003, Gehrt 2006). Although coyotes could be responsible for transporting the parasite greater distances the increased mobility of this species may result in feces being distributed more widely across the landscape, leading to a lower concentration of feces containing eggs, thereby decreasing exposure and infection in rodents.

Infection with a small number of adult *E. multilocularis* in the canid host would also decrease the potential for environmental contamination. Only one scolex from an adult worm was recovered from the positive coyote in this study. Based on research by Karamon et al. (2010), if coyotes or foxes in this study harbored worm infections of 30 worms or greater, the parasite would have been detected. If foxes and coyotes in Michigan harbor low worm intensities, fewer eggs will be deposited into the environment, leading to a lower degree of contamination, resulting in fewer rodents becoming infected.

Similar to canid populations, composition of rodent populations has changed. Myers et al. (2009) hypothesized that climate change is leading to the replacement of the deer mouse by the white-footed mouse (*Peromyscus leucopus*) in the Great Lakes Region, including Michigan. Through the study of museum records and capture studies of woodland mammal species dating back to 1883, Myers et al. (2009) have found that capture rates of white-footed mice in the northern Lower Peninsula have nearly doubled since the early 1980's, while capture rates of deer mice have experienced a 5 fold decrease, correlating with shifts in relative abundance of both species. In previous surveys on *E. multilocularis*, eastern meadow voles and deer mice were abundant and present across a wide geographic range, and were considered the main intermediate hosts, with little mention of the white-footed mouse (Leiby and Olsen 1964, Leiby et al. 1970, Rausch and Richards 1971, Kritsky and Leiby 1975). If indeed the white-footed mouse has replaced the deer mouse in Michigan, it is unclear what, if any, affect this may have on transmission dynamics of *E. multilocularis*. The white-footed mouse has not been thoroughly examined as a host for *E. multilocularis* (only 13 mice were examined and found negative) and it is unknown what impact changes in rodent species composition may have on the distribution of the parasite (Leiby et al. 1970).

Rodent populations can experience fluctuations based on weather, abundance of resources and predation and some species, such as voles, fluctuate in regular cycles (Baker 1983, Witmer and Prouix 2010). Kritsky and Leiby (1978) found prevalence ranges from 7.7 to 59.1% in red foxes in North Dakota over an 8 year study period, with higher prevalence correlated with high population levels in both host species. The low prevalence found in this study may have been a factor of sampling during a low in rodent populations. Fewer rodents in the environment

becoming infected and available as a food source to coyotes and foxes could make the cycle more difficult to maintain.

Echinococcus multilocularis is a parasite of northern latitudes and cold and temperate regions (WHO/OIE 2001). Yearly and seasonal variations in temperature and relative humidity as well as local climate could influence prevalence and lead to variability of prevalence between years and geographic locations. Eggs of *E. multilocularis* can survive for up to a year in a cool, moist environment, but with higher temperatures and lower humidity, survival time is reduced. At 4°C, *E. multilocularis* can survive for 16 months in water (WHO/OIE 2001). When temperatures are increased to 25°C and at 27% relative humidity, survival time is decreased to 2 days, and is reduced to 2 hours at 45°C at 15% relative humidity (WHO/OIE 2001). Myers et al. (2009) have attributed climate change and warming temperature in the Great Lakes Region to an increase in historically southern small mammal species (common opossum, *Didephis virginiana*; eastern chipmunks, *Tamias striatus*; southern flying squirrels, *Glaucomys volans*; white-footed mice) with a simultaneous decrease of some of the more northern small mammal species (northern subspecies of deer mice; least chipmunks, *Tamias minimus*; northern flying squirrels, *Glaucomys sabrinus*; southern red-backed voles, *Myodes gapperi*, woodland jumping mice *Napaeozapus insignis*). Impacts of weather and climate change on prevalence of *E. multilocularis* should be explored.

Recommendations for future research

Understanding how host population dynamics have influenced prevalence of *E. multilocularis* in North America should be addressed. Perhaps the red fox is the maintenance host and the coyote is a spillover host, requiring red fox densities to occur above a certain

threshold in order for the parasite to persist. The worldwide distribution of *E. multilocularis* overlaps with the range of the red and Arctic foxes and presumably one or both of these fox species would be present where *E. multilocularis* is found (WHO/OIE 2001, University of Michigan 2012). Studies on prevalence when both coyotes and red foxes are present and abundant should be compared to studies where coyotes are abundant but red foxes are absent or reduced in numbers. Surveillance in states where prevalence has been historically high could be conducted to determine what prevalence is now, while also examining if changes in species composition have occurred. The white-footed mouse should also be examined as a potential host, either experimentally or through surveillance.

Harvested animals present the best opportunity for sampling and thousands of specimens are potentially available for use in research projects. To obtain a greater number of samples, a much larger scale effort would have to be undertaken requiring extensive manpower and funding. Individuals willing to collect and store specimens in various locations would be required. State natural resource agency field offices could serve as potential collection and storage sites for specimens; however with limited staff and other job priorities, unless this collection was given a priority by the agency, it could not be accomplished. If whole carcasses were being collected, they could be frozen, however most collection sites do not have the capacity to freeze numerous carcasses. Intestinal tracts could be collected if collectors were provided with the proper PPE and the ability to freeze samples until they could be collected. Although the possibilities exist, the biggest obstacles would be manpower and funding; thus, surveillance for this disease would need to be given high priority, which is unwarranted in Michigan at this time due to the low prevalence in definitive hosts in the state and the rare occurrence of zoonotic infections.

Public health implications

Alveolar hydatid disease, caused by infection with *E. multilocularis* in humans, can lead to serious disease with high fatality rates (50-70%) even after treatment, which often involves invasive surgery and long term chemotherapy (Hildreth et al. 1991, WHO/OIE 2001). With increases in red fox distribution and abundance in Europe and Asia, there is concern that infections in humans are increasing (Deplazes et al. 2004, Pleydell et al. 2004, Konig et al. 2005, Eckert et al. 2011). For example in Switzerland, incidence rates of alveolar hydatid disease have increased from 0.10 to 0.25 per 100,000 over the last 10-15 years, corresponding with increases in red fox populations (Eckert et al. 2011). Infections in humans in North America are rare and given the low prevalence in wild canids in Michigan, the risk to public health is minimal. Despite the low risk of exposure, because infections in humans can cause serious disease and precautions to prevent exposure are inexpensive and require minimal effort, it is recommended that they be taken by those handling wild canids.

This study represents the first finding of *E. multilocularis* in a Michigan coyote. Coyotes have increased in abundance and will reside in both urban and rural areas and will utilize space in close proximity to humans, potentially contaminating backyard gardens and vegetation. Coyotes will consume watermelon and other fruit crops, suggesting the possibility for fecal contamination of vegetation and gardens in rural areas where coyotes may feed upon backyard gardens (Chamberlain et al. 2000). Fresh fruits and vegetables should be washed prior to consumption to remove any eggs of this or other parasites. Infection in urban and suburban coyotes could result in infected rodents in closer proximity to human residences and therefore more easily preyed upon by domestic dogs and cats. In areas where domestic pets and wild animals share space, dogs and cats can be given anti-helminthic medication to eliminate

gastrointestinal parasites and reduce potential exposure to pet owners. Coyotes also represent the largest number of wild canids harvested in Michigan. Hunters, trappers and fur buyers when handling wild canids should avoid hand to mouth contact (e.g. eating, smoking) wear disposable gloves and wash their hands thoroughly when finished. In addition clothing and/or footwear could be designated for use specifically when handling these animals. Finally, knowledge that the parasite occurs in Michigan needs to be communicated to those most likely to encounter this parasite so they can choose to take the proper precautions. In May of 2013 an article was published in the Trapline, a newletter distributed by the MTPCA, indicating that the parasite had been found in Michigan and outlining precautions that could be taken to reduce exposure.

A note on E. granulosus in Michigan

Although surveillance for *E. granulosus* was not a part of this study, adult worms were recovered from one of two gray wolves examined from the Upper Peninsula of Michigan. The parasite is known to occur in Michigan but the prevalence at which it occurs in the gray wolf population is unknown. Additionally, a coyote pup examined for routine necropsy in August 2013 from Delta County in the Upper Peninsula was found infected with numerous adult *E. granulosus* worms. This animal was not part of this research project; however these findings are worth mentioning and this species of parasite may be present at a much higher prevalence in the state than *E. multilocularis*. Gray wolves and coyotes are routinely handled in the Upper Peninsula by wildlife biologists and other wildlife workers as part of research projects and euthanized on depredation permits. This year also marks the first hunting season for gray wolves in the state of Michigan. Information on *E. granulosus* is available on the DNR's website on this parasite, including precautionary measures for those handling wild canids. Further research

should be conducted on this parasite to determine prevalence of coyotes and gray wolves in the Upper Peninsula.

APPENDICES

APPENDIX A

Map of the state of Michigan showing location of counties by number listed in table 8.

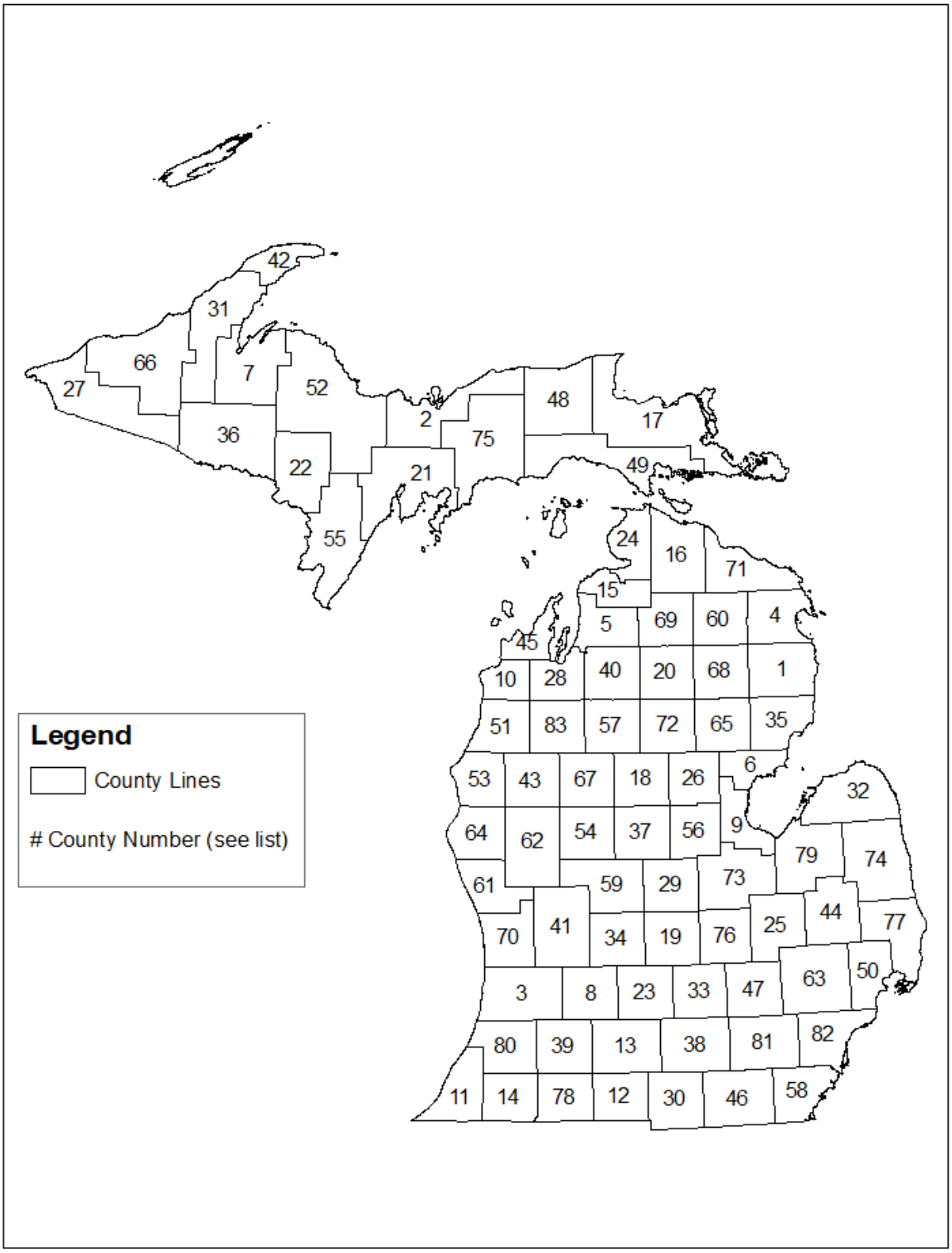


Figure 18. Map of the state of Michigan showing location of counties by number listed in Table 10.

Table 10. List of counties by number corresponding with Figure 18.

County	Number	County	Number
Alcona	1	Lake	43
Alger	2	Lapeer	44
Allegan	3	Leelanau	45
Alpena	4	Lenawee	46
Antrim	5	Livingston	47
Arenac	6	Luce	48
Baraga	7	Mackinac	49
Barry	8	Macomb	50
Bay	9	Manistee	51
Benzie	10	Marquette	52
Berrien	11	Mason	53
Branch	12	Mecosta	54
Calhoun	13	Menominee	55
Cass	14	Midland	56
Charlevoix	15	Missaukee	57
Cheboygan	16	Monroe	58
Chippewa	17	Montcalm	59
Clare	18	Montmorency	60
Clinton	19	Muskegon	61
Crawford	20	Newaygo	62
Delta	21	Oakland	63
Dickinson	22	Oceana	64
Eaton	23	Ogemaw	65
Emmet	24	Ontonagon	66
Genessee	25	Osceola	67
Gladwin	26	Oscoda	68
Gogebic	27	Otsego	69
Grand Traverse	28	Ottawa	70
Gratiot	29	Presque Isle	71
Hillsdale	30	Roscommon	72
Houghton	31	Saginaw	73
Huron	32	Sanilac	74
Ingham	33	Schoolcraft	75
Ionia	34	Shiawassee	76
Iosco	35	St. Clair	77
Iron	36	St. Joseph	78
Isabella	37	Tuscola	79
Jackson	38	Van Buren	80
Kalamazoo	39	Washtenaw	81
Kalkaska	40	Wayne	82
Kent	41	Wexford	83
Keweenaw	42		

APPENDIX B

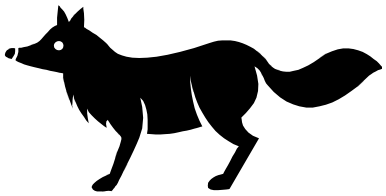
Instruction sheet provided to hunters, trappers and fur buyers willing to provide carcasses for the project.

WANTED: COYOTE, RED FOX and GRAY FOX CARCASSES

Contact: Julie Rose Melotti

MSU Graduate Student
Department of Fisheries and Wildlife

MI DNRE – Wildlife Disease Lab
517-336-5042
Melottij@michigan.gov



Your help is needed to collect carcasses statewide for a research project looking for the tapeworm *Echinococcus multilocularis* in wild canids. This tapeworm is found in the intestinal tract of the wild canids, but because this tapeworm can be passed to humans, whole carcasses are being requested.

Time Period: **Fall 2010 thru Spring 2012**

What is Needed: **Carcasses of coyotes, red fox and gray fox**

Data Needed:

- **Name**
- **Date of Harvest**
- **Location** (County and township, range and section or nearest cross roads)

Carcasses will not be returned so if you are keeping the hides, please have them skinned first. No need to worry – I am used to handling dead, slimy, stinky, rotting animals so a skinned carcass is no problem!

Figure 19. Copy of the instruction sheet for carcass collection.

Figure 19 (Cont'd)

Transportation: I can come out and pick up carcasses as needed. If you have freezer capacity and are willing to store them, or can store them outside in colder weather, call me when you have some ready to be picked up and I will come and get them at your convenience. Carcasses can also be delivered to DNR Wildlife Division Field Offices for storage and transportation down to the lab. If you are delivering to a DNR office, make sure to call first to make sure that someone is there to accept the carcasses.
This project will help us learn more about *Echinococcus multilocularis* in our state and to establish safety guidelines and educational material for hunters, trappers, fur buyers, wildlife workers, veterinarians and animal control personnel.

Thank you for your assistance with this project … it is greatly appreciated! I look forward to sharing the results of the research with you.

APPENDIX C

Tag provided to hunters, trappers and fur buyers to complete and attach to carcasses collected for the study.

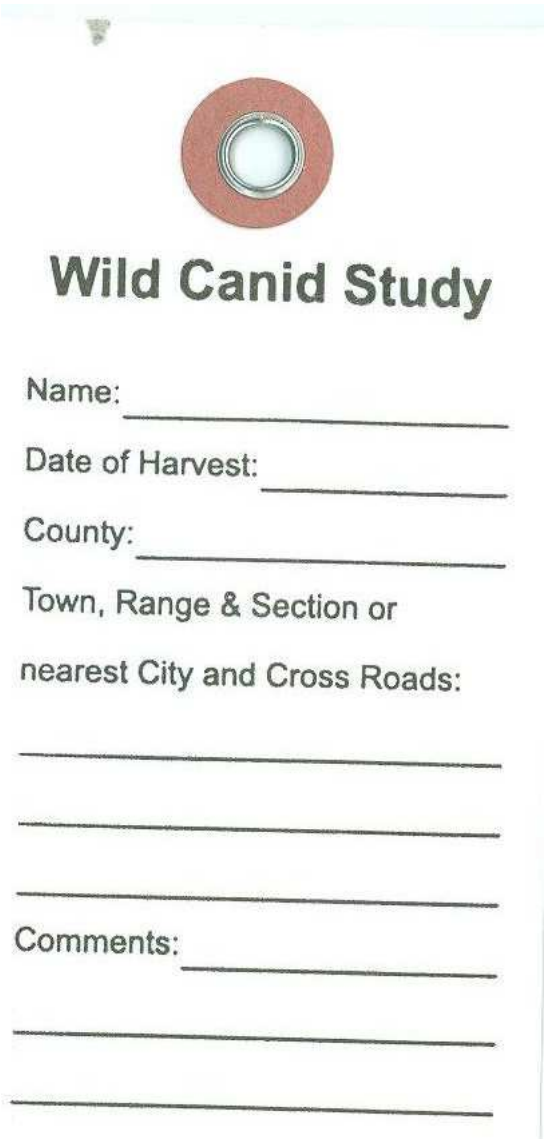


Figure 20. Tag provided for carcass collection.

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