Involvement of synanthropic mammals in the epidemiology of infectious agents of swine in Iowa

by

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TABLE OF CONTENTS

EXPLANATION OF THESIS FORMAT

This thesis was written in the alternate format. It begins with a general introduction. Four separate manuscripts (labeled as sections in the thesis) follow. The first manuscript is a review of the literature dealing with a main theme of the thesis. The following three manuscripts deal with specific aspects within the general theme of the thesis. The thesis concludes with a general summary. The master's candidate, Kirk Smith, is the principal investigator and first author of each manuscript and is responsible for their contents.

GENERAL INTRODUCTION

Synanthropic mammals, in an agricultural setting, may be defined as wild mammal species that use human-created buildings and animal production facilities as favorable environments for their existence, reproduction, and development. Foremost among these are the synanthropic rodents, namely house mice (*Mus musculus*) and Norway rats (*Rattus norvegicus*). Other species in Iowa that fit this description include feral "domestic" cats (*Felis domestica*), opossums (*Didelphis virginiana*), raccoons (*Procyon lotor*), and striped skunks (*Mephitis mephitis*).

Synanthropic mammals, particularly rodents, have been implicated in the epidemiology of several infectious agents of swine. The first objective of this study was to assimilate published knowledge of the role of synanthropic rodents in the epidemiology of infectious agents of swine. The second objective of this study was to investigate the role of synanthropic mammals in the epidemiology of three infectious agents of swine in Iowa: *Toxoplasma gondii*, *Leptospira interrogans* serovar *bratislava*, and encephalomyocarditis virus. The major importance of *T. gondii* for swine lies in the incrimination of pork as a major source of toxoplasmosis for human beings, whereas *L. interrogans* serovar *bratislava* and encephalomyocarditis virus may be significant causes of reproductive failure in swine.

SECTION I. A REVIEW OF THE ROLE OF SYNANTHROPIC RODENTS IN THE EPIDEMIOLOGY OF INFECTIOUS AGENTS OF SWINE

INTRODUCTION

Because of the attraction of the swine production environment for synanthropic rodents, swine-rodent interaction is a regular occurrence. Rodents have been implicated in the epidemiology of several infectious agents of swine, including those whose primary importance lies in their zoonotic potential. This review of the role of rodents in the epidemiology of infectious agents of swine will focus on the house mouse (*Mus musculus*) and the Norway rat (*Rattus norvegicus*), the two most common synanthropic rodent species on swine farms in the midwestern United States swine belt.

Treponema hyodysenteriae - Experimentally inoculated mice are susceptible to enteric infection with *T. hyodysenteriae,* (Chia and Taylor, 1978; Joens, 1980; Joens and Glock, 1979; Joens and Kinyon, 1982; Joens et al., 1981; Kinyon et al., 1977), can develop clinical signs and lesions characteristic of swine dysentery (Joens, 1980; Joens and Glock, 1979; Joens et al., 1981), and can shed *T. hyodysenteriae* in their feces for at least 14 days (Chia and Taylor, 1978) and as long as 180 days (Joens, 1980). Joens (1980) also demonstrated that experimentally inoculated mice could infect contact mice up to 180 days after inoculation, and that swine could become infected from direct exposure to feces of experimentally inoculated mice.

T. hyodysenteriae was isolated from the ceca of 3 wild house mice and 1 mouse of the genus *Peromyscus* from 3 swine farms with a history of swine dysentery (Joens

and Kinyon, 1982). All 3 house mouse isolates were pathogenic to swine upon intragastric inoculation.

Based on experimental and field data, investigators hypothesize that mice are a reservoir host of *T. hyodysenteriae* and that they are involved in the spread of swine dysentery (Harris, 1984; Joens, 1980; Joens and Kinyon, 1982). Since mice do not usually migrate from farm to farm, they are probably not involved in farm to farm transmission of the disease. The greatest significance of mice is probably as a constant reservoir of infection on farms attempting eradication of swine dysentery. Thus, it may be necessary to eradicate mice from a premises to completely disinfect it (Joens, 1980; Joens and Kinyon, 1982).

Braha (1983) reported the isolation of *T. hyodysenteriae* from laboratory rats and Norway rats captured in cattle barns in Germany. He hypothesized that *T. hyodysenteriae* is a normal inhabitant of the colon of healthy adult rats, and that rats can therefore be considered as a natural source of swine dysentery. In the United States, Joens and Kinyon (1982) did not isolate *T. hyodysenteriae* from 21 Norway rats captured on farms with a history of swine dysentery, or from 20 rats from farms without swine dysentery. Chia and Taylor (1978) could recover the organism from rat feces only 2 days after intragastric inoculation. Thus, rats are not considered to be reservoirs of *T. hyodysenteriae* in the United States (Harris, 1984), but it is apparent that this issue may be worthy of further investigation.

Encephalomyocarditis virus - The epidemiology of encephalomyocarditis virus (EMCV) is not well understood. EMCV has been isolated from a large number of species of mammals and wild birds, but a reservoir host has not been unequivocably identified (Acha and Szyfres, 1987). Rodents, especially the genus *Rattus*, have been considered by some as the natural reservoir of EMCV and the main source of infection for swine, through the contamination of pig feed with rodent carcasses or feces (Acland, 1989; Acland and Littlejohns, 1986; Boulton, 1984; Seaman et al., 1986). Support for this hypothesis includes the frequent detection of infection in wild rodents through serosurveys and/or virus isolation (Adamacova and Bardos, 1959; Bardos, 1957; Causey et al., 1962; Dick, 1953; Gainer and Bigler, 1967; Ghosh and Rajagopalan, 1973; Heredia et al., 1982; Jonkers, 1961; Paul et al., 1968; Pope, 1959; Pope and Scott, 1960; Tesh and Wallace, 1978; Vizoso and Hay, 1964; Warren et al., 1949; Wells and Gutter, 1986).

EMCV outbreaks in swine are often associated with "plagues" (population explosions) or heavy infestations of mice and rats, especially in Australia and New Zealand (Acland and Littlejohns, 1975; Acland et al., 1970; Boulton, 1985; Hill et al., 1985; Mercy et al., 1988; Sanford et al., 1989; Seaman et al., 1986). In several EMCV outbreaks in swine, the virus has been isolated from at least one rodent on the premises (Acland and Littlejohns, 1975; Gainer et al., 1968; Hill et al., 1985; Mercy et al., 1988; Ramos et al., 1983), or antibodies against EMCV have been found in rats on the premises (Kovatch et al., 1969; Murnane et al., 1960). Sick and dead mice (*Mus musculus*) have been noted during EMCV outbreaks in swine

(Acland and Littlejohns, 1975; Seaman et al., 1986). It has been suggested that EMCV infection is endemic in members of the genus *Rattus*, and that it occurs mainly as epidemic disease in mice, which would then serve as amplifiers of the disease rather than reservoir hosts (Boulton, 1984; Seaman et al., 1986).

Despite the frequency with which infected rodents are associated with clinical outbreaks in swine, no relationship between rodent infection and swine disease has been established. It has been suggested that the frequency of detection of EMCV infection in rodents compared to other species is due to an extreme sampling bias (Tesh and Wallace, 1978). Also, a chronic intestinal carrier state has not been demonstrated in rodents, and other investigators therefore contend that infected rodents are only indicators of EMCV activity rather than reservoir hosts (Adamcova and Bardos, 1959; Kilham et al., 1956; Tesh and Wallace, 1978).

Pseudorabies virus - Historically, there has been much interest in the role of rats in the epidemiology of pseudorabies (PR). Sick or dead rats have been observed in association with several outbreaks of PR in domestic animals, and pseudorabies virus (PRV) has been isolated from dead or apparently healthy trapped rats from PR-infected farms (Balas, 1908; Becker and Herrmann, 1963; Hutyra, 1910; Lamont, 1947; Maes et al., 1979; Shope, 1935a). Shope (1935b) and others (see Galloway, 1938; Lamont, 1947) demonstrated that wild rats could be infected with PRV by feeding on tissues of animals infected with PRV. Shope (1935b) also showed that swine could be infected by feeding them a single carcass of an experimentally

infected wild rat, and hypothesized that rats were the source of PRV for swine and that migration of rats may be one means of spread of PR from one swine herd to the next.

Nikitin (1959, 1960a, 1960b) reported that up to 25 percent of wild Norway rats inoculated intramuscularly with low doses of PRV could survive clinical PR, and that PRV could be isolated from these rats for up to 131 days after infection. Nikitin also reported the isolation of PRV from apparently healthy rats captured on farms with recent PR outbreaks in swine. These rats were held approximately two months before isolation attempts, leading the author to hypothesize that naturally infected rats could remain carriers for up to 100 days. Becker and Herrmann (1963) also reported the isolation of PRV from apparently healthy rats captured on a farm during an outbreak of PR in swine.

Despite the reports suggesting that rats may be a reservoir of PR, recent studies have repeatedly demonstrated that while rats are quite resistant to experimental oral inoculation, *infection* with PRV is uniformly fatal to rats under natural and experimental conditions (Aldasy and Mate, 1969; Maes et al., 1979; McFerran and Dow, 1970; Ulbrich, 1969). Evidence supporting this includes the failure to demonstrate the presence of PRV or virus-neutralizing antibodies in wild rats surviving experimental inoculation, the absence of virus-neutralizing antibodies in populations of wild rats on PR-infected farms (Lamont, 1947), and the failure to elicit resistance with increasing doses of virus. Also, lateral contact transmission in rats could not be demonstrated (Maes et al., 1979; McFerran and Dow, 1970).

The weight of the evidence suggests that the Norway rat has minimal importance in the epidemiology of PR relative to swine themselves, probably limited to the possibility of swine consumption of carcasses of rats that have died of PR. However, there is still the chance that a rat infected on one farm could migrate to a PR-free farm during the incubation period.

Mice are susceptible to experimental inoculation with PRV, but even less so than rats (Fraser and Ramachandran, 1969). Natural infection in mice has also been reported (Lukashev and Rotov, 1939). Fraser and Ramachandran (1969) reported that adult mice occasionally recover from clinical disease, that resistance to normally lethal doses of PRV could be elicited in mice by inoculating them with successively higher doses of virus, and that lateral transmission could occur in mice, probably by cannibalism. A search of the literature failed to uncover any studies in which virus or antibodies were looked for in mice surviving experimental inoculation with PRV. Mice may deserve more consideration as hosts of PRV, because maintenance of PRV by mice would have importance in farm eradication efforts.

Leptospira interrogans - Rodents are among the most important hosts of *L. interrogans* worldwide (Van der Hoeden, 1958; Torten, 1979). At least 35 serovars have been isolated from the Norway rat and the house mouse alone (Torten, 1979). Of the five serovars that have been isolated from swine in the United States - *pomona*, *grippotyphosa*, *icterohaemorrhagiae*, *canicola*, and *bratislava* (Hanson and Tripathy, 1986; Ellis and Thiermann, 1986), only *icterohaemorrhagiae* has been

isolated from Norway rats or house mice there (Roth, 1964; Schnurrenberger et al., 1970). The Norway rat is the primary reservoir of serovar *icterohaemorrhagiae* (Roth, 1964; Schnurrenberger et al., 1970), and apparent natural transmission from rats to swine has been described (Schnurrenberger et al., 1970). Serovar *ballum* has been commonly isolated from house mice and occasionally from rats in the U. S. (Brown and Gorman, 1960; Clark, 1961; Ferris et al., 1961; Schnurrenberger et al., 1970; Yager et al., 1953), but only serologic evidence of infection with this serovar has been demonstrated in swine (Hanson and Tripathy, 1986). In the U. S., *pomona* has been isolated from the deer mouse (*Peromyscus maniculatus*), and *grippotyphosa* from the meadow vole (*Microtus pennsylvanicus*).

All five serovars isolated from U. S. swine have been isolated from Norway rats, house mice (except for *canicola*), and numerous other rodents outside the U. S. (Galton, 1966; Hathaway et al., 1983; Sulzer, 1975). Serovars *pomona*, *grippotyphosa*, and *muenchen*, a close relative of *bratislava*, are all maintained by various field mice and voles in Europe (Hathaway et al., 1983; Sebek et al., 1976; Sebek et al., 1987), and several investigators consider free-living rodents as important sources of these serovars for swine in Europe (Hathaway et al., 1983; Sebek et. al., 1983; Waldmann, 1990).

Available information indicates that rodents should not be overlooked as sources of leptospires for swine, and that rodent control in swine housing may be important in reducing the incidence of porcine leptospirosis.

Toxoplasma gondii - Rodents are easily infected with the oocyst stage of *T. gondii* (Dubey et al., 1970; Frenkel et al., 1970; Miller et al., 1972), and survivors usually develop chronic infection with the organism (Wallace, 1973). It has been suggested that rodents may be appropriately considered reservoir hosts of *T. gondii* capable of maintaining the organism in their population for significant periods of time without reinfection from cats, the only definitive host (Chinchilla, 1978; Dreesen, 1990; Lubroth et al., 1983; Wallace, 1973). Repeated congenital infection can occur in mice, rats, and other small mammals (Beverly, 1959; Dubey, 1983; Remington et al., 1961; de Roever-Bonnett, 1969). Several litters may be born infected from an infected mouse without reinfection from an outside source. Congenitally infected mice can themselves produce congenitally infected mice for five or more generations (Beverly, 1959). Rats can be infected by eating infected mouse and swine tissue (Weinman and Chandler, 1954), so cannibalism and scavenging may also contribute to the maintenance of *T. gondii* in rodent populations.

Naturally infected populations of Norway rats and house mice have been discovered on numerous occasions (Chinchilla, 1978; Eyles et al., 1959; Franti et al., 1976; Lubroth et al., 1983; Perrin et al., 1943; Ruiz and Frenkel, 1980; Wallace, 1973; Zimmermann, 1975). In these surveys, prevalence estimates usually ranged up to about 5 percent (house mice) or 10 percent (Norway rats).

Because of the potential for prevalent *T. gondii* infections in rodents, it has been suggested that the ingestion of infected rodents, presumably with encysted bradyzoites, is an important route of transmission to swine (Dreesen and Lubroth

1981, 1983; Dubey et al., 1986; Lubroth et al., 1983; Weinman and Chandler, 1954). Experimentally, swine can be easily infected with *T. gondii* by feeding them tissues of infected rats and mice (Durfee and Chien, 1971; Weinman and Chandler, 1954).

Two studies that looked at the prevalence of *T. gondii* infection in rodents on swine farms yielded seemingly contradictory results. Lubroth et al. (1983) found antibodies against *T. gondii* in 12 of 20 house mice, 2 of 2 Norway rats, 3 of 6 cotton rats (*Sigmodon hispidus*), and 3 of 3 white-footed mice (*Peromyscus leucopus*) on 2 Georgia swine farms. A similarly high prevalence in rodents on both farms, and a much lower prevalence in swine relatively free from contact with rodents suggested that rodents may be important in the transmission of toxoplasmosis to swine. Smith et al. (submitted for publication), however, found *T. gondii* antibodies in only 2 of 588 house mice, 0 of 21 white-footed mice, and 0 of 9 Norway rats trapped on 20 Iowa swine farms, and hypothesized that house mice are not a major source of toxoplasmosis for swine in Iowa.

Until more is known about the epidemiology of *T. gondii* infections in swine, recommendations for the prevention of swine toxoplasmosis should include preventing rodent contact with swine.

Erysipelothrix rhusiopathiae - *E. rhusiopathiae* has been isolated from a large number of wild mammals, including over 20 species of rodents (Wood and Shuman, 1981). It was first recognized in wild mammmals when it caused an epizootic in meadow mice (*Microtus arvalis*) and house mice (*Mus musculus*) in California in 1926-1927

(Wayson, 1927). *E. rhusiopathiae* has also been isolated from Norway rats in the United States (Drake and Hall, 1947; Stiles, 1944) and from a black rat in Australia (Eamans, et al., 1988), and is commonly isolated from house mice, Norway rats, and numerous other rodent species in the U. S. S. R. (Moteyunas et al., 1974; Surkov et al., 1972; Ovasapyan et al., 1964; Timofeeva et al., 1975; Wood and Shuman, 1981).

Wellman (1954) reported that experimentally inoculated individuals of many wild mouse and rat species could become bacteremic without exhibiting clinical signs; he therefore considered wild mice as potential carriers and spreaders of erysipelas. Ovasapyan et. al. (1964) also reported that experimentally inoculated rats could become asymptomatic carriers of erysipelas. Wild mice were strongly suspected as the source of infection in an outbreak of erysipelas in captive monkeys (Wallach, 1977). Rodents have also been implicated in the contamination of straw and stream water with *E. rhusiopathiae* during the course of rodent epizootics (Olsuf'ev et al., 1959). Some investigators have suggested that rodents may play a role in the epidemiology of swine erysipelas (Stiles, 1944; Wayson, 1927; Wellman, 1954), but a search of the literature failed to uncover reports directly addressing this issue. Rodents do provide an extensive reservoir of *E. rhusiopathiae*, and their potential significance in the transmission of this organism to swine may warrant investigation (Wood, 1986).

Trichinella spiralis - The significance of rats as a source of trichinosis for swine has been controversial. Although trichinosis in rats has been found to be rare or absent

on some farms with infected swine (Martin et al., 1968; Peres, 1941; Zimmermann and Hubbard, 1969), it has been shown to be prevalent in rats on several swine farms (Hanbury et al., 1986; Hill et al., 1985; Moynihan and Musfeldt, 1949; Poole, 1952; Schad et al., 1987). Many authorities (Beck, 1970; Hall, 1938; Madsen, 1974; Merkushev, 1970) diminished the role of rats as a source of trichinosis for swine, postulating that infected rats from swine farms are only recipients of and indicators of infection in swine. Cameron (1970) supported this view under the assumption that pigs rarely catch live rats and are reluctant to eat dead ones.

Recent work has shown that swine readily eat dead rats, and that swine could be consistently infected with *T. spiralis* by ingesting infected rats (Murrell et al., 1984). It had previously been shown that the feces of recently infected rats and mice could serve as a short-term source of *T. spiralis* for swine (Robinson and Olson, 1960; Zimmermann et al., 1959).

Schad et al. (1986) demonstrated natural transmission of *T. spiralis* to swine through predation of Norway rats or consumption of dead rat carcasses. They also showed that rats could maintain trichinosis in a swine herd in the absence of swine to swine transmission and garbage feeding, and that an abundant rat population with a high prevalence of trichinosis could be as effective as cannibalism in maintaining a high prevalence of trichinosis in swine. They also demonstrated that minimizing rat-swine contact on such farms could markedly reduce the incidence of trichinosis in swine. They could not conclude that rats serve as a true reservoir in the long-term absence of swine; rat populations may require periodic access to

infected swine carcasses to maintain the infection long-term. However, the potential importance of rats as a source of trichinosis for swine was evident.

Rats may also be important as a link between the domestic and sylvatic cycles of trichinosis (Campbell, 1983). Wild or feral carnivores may become infected through predation of or scavenging infected rats, and represent a potentially important reservoir for the transmission of *T. spiralis* from one farm to another, or for the re-introduction into herds from which trichinosis has been eradicated (Murrell et al., 1987, Leiby et al., 1988).

Much less work has been done with the house mouse and *T. spiralis*, but up to 4.3 percent of house mice have been shown to be infected on a swine farm where trichinosis was prevalent in both swine and rats (Hanbury et al., 1986).

Miscellaneous infectious agents - Laboratory mice harbor a rotavirus designated epizootic diarrhea of infant mice (EDIM) virus (Kraft, 1957; Much, 1972), and laboratory rats harbor an atypical rotavirus designated infectious diarrhea of infant rats (IDIR) virus (Vonderfecht et al., 1984). EDIM virus is closely related to porcine rotavirus, but probably represents a distinct serotype of rotavirus (Gaul et al., 1982; Woode et al., 1976). Like rotaviruses from other species, EDIM virus can infect and cause disease in experimentally inoculated gnotobiotic swine (Woode et al., 1976). Wild rodents have not been investigated as a source of rotavirus to swine, but the ability of certain rotaviruses to cross the species barrier may have epidemiologic significance (Woode, 1986).

Deyoe (1986) reported that there have been instances of *Brucella suis* infection in rodents trapped near areas where swine brucellosis has occurred. *Brucella abortus* has been isolated from wild Norway rats that lived in close association with infected cattle (Bosworth, 1937; Fitch and Bishop, 1938; Karkadinovsky, 1936). Lateral transmission of *B. abortus* among rats and isolation of *B. abortus* from composite feces and urine samples of rats have been reported (Bosworth, 1937). *B. abortus* has also been isolated from wild house mice (Corey et al., 1964). Although not considered epidemiologically important in swine brucellosis (Deyoe, 1986), rodents may warrant consideration as maintenance hosts during eradication efforts.

Bordetella bronchiseptica infection can occur in local populations of Norway rats (Switzer et al., 1966). A carrier state has been demonstrated in laboratory rats (Burek et al., 1972), and it has been suggested that wild rat populations may serve as a source of infection for swine (Switzer et al., 1966). Non-swine isolates of *B. bronchiseptica* are capable of producing turbinate atrophy in swine (Ross et al., 1967), but the significance of rodent populations with regard to swine disease caused by *B. bronchiseptica* is unknown.

Yersinia enterocolitica has been isolated from Norway rats, black rats (*Rattus rattus*), house mice, and other rodents trapped in swine production environments (Aldova et al., 1980; Mraz et al., 1989). Rodents are thus potential reservoirs of *Yersinia* species for swine.

Salmonella species infections are common in rodents, particularly rats and mice (Clarenburg, 1964), and the list of serotypes isolated from rodents is extensive

(Taylor, 1969). *Salmonella typhimurium*, one of the two serotypes responsible for the vast majority of clinical disease due to salmonellosis in swine (Wilcock, 1986), is frequently isolated from rodents (Clarenburg, 1964; McKiel et al., 1970; Mesina, 1973; Robinson and Daniel, 1968; Sandstedt et al., 1980; Taylor, 1969). Mice are also susceptible to infection with *Salmonella cholerasuis*, as reflected by their widespread use as experimental models for studying *S. cholerasuis* (Griffith et al., 1984).

Schnurrenberger et al. (1968) reported the isolation of *Salmonella* species (*S. give* and *S. derby*) from 2 of 181 Norway rats captured on Illinois farms. Some authors consider rodents more as victims of pig-origin infection than as sources of infection for pigs (Newell and Williams, 1971), but studies on this subject are lacking; a search of the literature failed to uncover any attempts (except Schnurrenberger, 1968) to investigate *Salmonella* infections in mice and rats on swine farms.

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SECTION II. THE EPIDEMIOLOGY OF TOXOPLASMOSIS ON IOWA SWINE FARMS WITH AN EMPHASIS ON THE ROLE OF SYNANTHROPIC MAMMALS

SUMMARY

During the summer of 1990, a study was conducted to improve current knowledge on the epidemiology of toxoplasmosis on swine farms. A sample of multiparous sows from each of 20 central Iowa swine farms was tested for antibodies against *Toxoplasma gondii* by the modified direct agglutination test. Antibody titers $\geq$ 1:32 were considered positive. Cats, rodents, and wildlife species (opossums, raccoons, striped skunks) were live-trapped on each farm and similarly tested for antibody titers. Overall prevalence of *T. gondii* antibodies in the species tested was: 39/273 (14.3%) swine, 31/74 (41.9%) cats, 2/588 (0.3%) house mice, 0/21 mice of the genus *Peromyscus*, 0/9 Norway rats, 1/34 (2.9%) opossums, 4/14 (28.6%) raccoons, and 2/7 (28.6%) striped skunks. Overall prevalence was significantly greater in: adult cats vs. juvenile cats, adult male cats vs. adult female cats, and adult raccoons vs. juvenile raccoons.

The prevalence of *T. gondii* antibodies in sows was compared to the prevalence in each non-swine species on a farm basis in order to identify existing associations. The prevalence in sows (and each of the non-swine species) was also analyzed on a farm basis for association with farm characteristics or swine management practices including degree of confinement of swine, population size and average parity of breeding female swine, estimated cat population size, and estimated mouse and rat abundance. Average titers of positive animals were compared on a species basis.

The prevalence in sows which were totally and continuously confined was

lower than that of sows which were not totally and continuously confined. The prevalence in sows from farms with an average parity of less than 2.0 was significantly lower than that in sows from farms with an average parity of 2.0 or greater. These results suggested that the prevalence of *T. gondii* infection in swine increased with age and that prevalence in swine could be reduced through total confinement.

No associations could be established between prevalence in sows and prevalence in non-swine species or other farm characteristics/swine management practices. However, the high prevalence of *T. gondii* antibodies in cats suggested that fecal contamination of the environment by cats may be the most significant source of toxoplasmosis for swine. The extremely low prevalence of *T. gondii* antibodies in house mice suggested that this species was not an important source of *T. gondii* for swine in Iowa.

INTRODUCTION

There have been over 1500 papers dealing with *Toxoplasma gondii*; its life cycle, broad host range, and prevalence in and importance to human beings has been widely documented (Burridge, 1980; Dubey, 1986b; Dubey and Beattie, 1988; Fayer, 1981; Feldman, 1982; Frenkel, 1990a, 1990b). Food animals, especially swine, have been circumstantially implicated as the major source of exposure of people in the United States through the ingestion of undercooked meat and/or the contamination of hands, food preparation surfaces, and food handling utensils from contact with raw meat (Dubey, 1986b, 1988; Dubey et al., 1984; Frenkel, 1990a; Weinman and Chandler, 1956).

Serologic surveys have indicated that infection with *T. gondii* is quite prevalent in swine in the United States (Dubey, 1986a). In spite of the massive literature base on *T. gondii*, relatively little is known about the route(s) of transmission to swine and factors affecting the epidemiology of toxoplasmosis on farms where swine are raised. It is generally agreed that cats probably play a major role in transmitting *T. gondii* to swine through fecal contamination of soil, food, or water (Dubey, 1986a; Frenkel, 1990b). However, discussions of sources of *T. gondii* for swine have commonly also included reference to rodents, presumably with encysted bradyzoites, as another source for swine (Dreesen and Lubroth, 1981, 1983; Dubey et al., 1986a; Lubroth et al., 1983; Weinman and Chandler, 1954). Recommendations for the prevention of toxoplasmosis in swine have usually included preventing cat and

rodent contact with swine (Dubey, 1990; Hoefling and Todd, 1986). At least one study has suggested that doing so would markedly decrease the seroprevalence of toxoplasmosis in swine (Lubroth et al., 1983).

The purpose of this study was to add to current knowledge on the epidemiology of toxoplasmosis on Iowa swine farms, especially with regard to the relative roles of cats, rodents, and other wildlife species. It was felt that this study would provide further clues as to the specific source of *T. gondii* for swine, as well as other aspects of the epidemiology of this organism on swine farms in Iowa.

MATERIALS AND METHODS

Design of study

The approach used was to live-capture and collect a blood sample from rodents, cats, and wildlife species that live in, or in close proximity to, facilities used to house swine or store their feed. As many cats and wild animals as possible were captured during the period of rodent collection on each farm. A minimum of 25 mice were collected, if practical, from each farm. Blood samples were also collected from 15 home-raised multiparous sows on these farms, if practical. If not home-raised, sows that had inhabited the farm the longest were sampled. Based on serum antibody titers, the prevalence of toxoplasmosis was estimated for each species on each farm.

Farmers were asked to complete questionaires to provide information about the size and average parity of breeding herds, principal operation type, confinement status of swine by life stages (5 stages--breeding, gestation, farrowing, nursery, growing/finishing), estimated outdoor cat population, and frequency of observation (never, occasionally, frequently) of rodents and wildlife on the premises.

Study farms

Twenty swine farms in the central Iowa counties of Boone, Hamilton, and Story were surveyed. One farm was strictly a feeder pig-to-finish operation and thus no sows were available to sample. In the other 19 herds, the mean number of

breeding females was 169, with a range of 12 to 550. There were 7 herds of 1-100 breeding females, 7 herds of 101-200, and 5 herds of more than 200. The average parity of breeding females on the 16 farms where this information was available ranged from 1.35 to 7.00 (mean = 3.00). The degree of swine confinement varied from total and continuous confinement of all life stages to never having any of the life stages totally and continuously confined.

The mean outdoor cat population on the farms as estimated by farmers was 7.9, with a range of 2 to 26. Mice were observed by farmers frequently on 14 farms and occasionally on 6 farms. Rats were observed frequently on 5 farms, occasionally on 13 farms, and never on 2 farms.

Animal sampling

Rodents were live-trapped using Sherman[1] and Ketch-all[2] live traps set primarily within and around the periphery of swine housing and feed storage facilities. Sherman traps were baited with a mixture of peanut butter and oatmeal flakes, checked daily, and rebaited as needed. Ketch-all traps were not baited. Mice were transferred from the traps into plastic cages with wire covers for transport to the Iowa State University College of Veterinary Medicine. Rats were transported in the individual traps and anesthetized by placing a chloroform-saturated cotton ball in the trap before blood collection was carried out. Blood was

[1]H. B. Sherman Traps, Inc., Rt. 22, Box 365, Tallahassee, FL 32304.

[2]Kness Mfg. Co., Inc., Albia, IA 52531.

collected from rodents via orbital sinus puncture (mice) or by jugular vein puncture (rats). Rodents were euthanitized by cervical dislocation after blood collection.

Free-ranging cats (*Felis domestica*), opossums (*Didelphis virginiana*), raccoons (*Procyon lotor*), and striped skunks (*Mephitis mephitis*) were captured using Tomahawk[3] live traps baited with canned cat food. These animals were immobilized with an intramuscular injection of a mixture of approximately 20 mg/kg ketamine hydrochloride[4] and 0.2 mg/kg acepromazine maleate[5] administered with a pole syringe.[6] Animals were classified according to gender and age class (adult or juvenile). Juvenile cats, as defined in this study, were at least of weaning age but not older than 6-7 months. Approximately 5 ml of blood was collected via cardiac puncture. Animals were then released and monitored for recovery.

Serology

Sera were separated from blood samples and stored at -70° C until tested by the modified direct agglutination test (MAT) (Desmonts and Remington, 1980). Recent studies have shown that the MAT is a sensitive test for the serodiagnosis of toxoplasmosis (Dubey and Beattie, 1988; Dubey and Thulliez, 1989; Patton et al.,

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[3]Tomahawk, WI 54487.

[4]Ketaset, Bristol Laboratories, Syracuse, NY 13210.

[5]PromAce, Ayerst Laboratories, Inc., New York, NY 10017.

[6]Safe-T-Flex Pole Syringe, Kane Enterprises Ag-Tek Division, Sioux Falls, SD 57101.

1991). The MAT does not require the living organisms, expensive equipment, or species specific conjugates. The formalin-fixed tachyzoites used as antigen in the MAT were supplied by bioMerieux Laboratory Reagents, Lyon, France. The test was performed as previously described with mercaptoethanol added to the serum samples to inactivate IgM (Dubey and Desmonts, 1987; Patton et al., 1990). Trypan blue dye was added to the diluent solutions before they were mixed with the antigen so agglutination could be easily seen.

All sera were diluted in phosphate buffered saline (PBS), pH 7.2, and screened for antibodies at dilutions of 1:16 and 1:512. Sera which tested positive for anti-*Toxoplasma gondii* antibodies at the 1:512 dilution were then diluted two-fold and titered to endpoints. Sera which tested positive for anti-*T. gondii* antibodies at the 1:16 dilution, but negative at the 1:512 dilution were also titered to endpoints. Control sera[7] (positive and negative) were included with each batch of sera tested to assure accuracy and reproducibility of results. As a further control, anti-*Sarcocystis* serum prepared in rabbits (Granstrom et al., 1990) was also diluted two-fold in PBS, from 1:4 to 1:8192, and checked for antibodies that would cross react with the formalin-fixed *T. gondii* tachyzoites used as antigen in the MAT. Two samples were checked. Anti-*Sarcocystis* serum from one of the rabbits was positive at a titer of 1:8; the other rabbit serum was negative. Because of this and results

[7]The control sera used in this study were collected from laboratory personnel. They have reproducible titers on the serological tests used in this study.

from previous studies, titers of less than 1:32 were considered nonspecific reactions (Dubey and Beattie, 1988; Dubey, 1988; Patton et al., 1991).

Analysis of data

Prevalence in sows was compared to prevalence in each non-swine species on a farm basis in order to identify associations. Prevalence in sows was also analyzed for association with various farm characteristics and swine management practices. Prevalence within each species was analyzed according to differences in age, gender, and farm characteristics/swine management practices. Characteristics that were analyzed on a farm basis included population size and average parity of breeding female swine, estimated cat population size, number of cats captured, whether or not seropositive cats were captured, average titer of seropositive animals within a species, abundance estimates of mice and rats, and whether or not rats were captured.

Comparisons were accomplished using Fisher exact test, Yates corrected chi-square test, correlation analysis, or logistic regression analysis, depending on the sample size of species involved, the nature of the variables examined, and the number of variables examined.

Mean titers for positive animals were compared among species using analysis of variance.

RESULTS

Serologic evidence of toxoplasmosis was found on every farm. Two farms had a single raccoon as the lone seropositive animal and one farm had two skunks as the only seropositive animals of all the animals sampled. On the other 17 farms, toxoplasmosis was detected serologically in cats and/or swine. The overall serologic prevalence in sampled animals, by species, is given in Table 1.

Table 2 summarizes findings on the prevalence of *T. gondii* antibodies in sows. The overall prevalence in sows in this study was 14.3%. The 39 sows which tested positive came from 10 of 19 (53%) farms on which sows were tested; the prevalence in sows on these 10 farms was 28.3%. The prevalence in sows on farms on which sows were totally and continuously confined (2.2%) was significantly lower than on farms on which sows were not totally and continuously confined (16.7%) ($P = 0.02$). The essential difference between these two groups of farms was whether or not sows were totally confined during breeding and gestation in addition to the farrowing/lactation period. On three farms where sows were never totally confined, the prevalence in sows did not differ significantly from that on farms on which sows were kept in confinement during the farrowing/lactation period only.

The prevalence in sows on farms with an average parity of less than 2.0 was significantly lower than the prevalence on farms with an average parity of 2.0 or greater ($P < 0.005$). There was no significant difference in prevalence within the group of sows from farms with an average parity of greater than or equal to 2.0.

Table 1. *Toxoplasma gondii* serology: Number and percentage of animals seropositive (antibody titer $\geq$ 1:32) by the modified direct agglutination test, by species

Species	Number Tested	Number Positive	Percent Positive
Swine *-Sus scrofa*	273	39	14.3
Domestic Cat *-Felis domestica*	74	31	41.9
House Mouse *-Mus musculus*	588	2	0.3
Peromyscus species mice	21	0	0
Norway Rat *-Rattus norvegicus*	9	0	0
Opossum *-Didelphis virginiana*	34	1	2.9
Raccoon *-Procyon lotor*	14	4	28.6
Striped Skunk *-Mephitis mephitis*	7	2	28.6

Table 2. Prevalence of toxoplasmosis in sows from Iowa

	Prevalence	
Sow Category	Frequency[a]	% (95% Confidence Interval[b])
Cumulative	39/273	14.3 (10.1 to 18.5)
Herds with seropositive sows	39/138	28.3 (20.6 to 36.0)
Confined	1/45	2.2[c] (0.0[d] to 6.6)
Nonconfined	38/228	16.7 (11.8 to 21.6)
Herds with parity < 2.0	3/75	4.0[c] (0.0[d] to 8.5)
Herds with parity ≥ 2.0	32/153	20.9 (14.3 to 27.5)

[a]Number seropositive over total number tested.
[b]Galen and Gambino, 1975.
[c]Significantly different than corresponding category ($P < 0.05$).
[d]Truncated at 0% (Thrushfield, 1986).

For these analyses the oldest sows on a given farm were typically the ones sampled to maximize probabilities of exposure, but the exact parity of sows sampled was not known.

On 19 farms on which cat trapping was attempted, the mean number of cats sampled was 3.9, with a range of 0 (1 farm) to 9. Seropositive cats were detected on 13 of these farms. Seropositive swine were detected on 6 of the 13 farms with seropositive cats. The overall prevalence in cats was 41.9% with a 95% confidence interval (CI--Galen and Gambino, 1975) of 30.5% to 53.4%. There was a significantly higher prevalence in adults (49.2%) than juveniles (7.7%) and a significantly higher prevalence in adult males (68.8%) than adult females (27.6%) ($P < 0.01$). Data are summarized in Table 3.

A total of 618 rodents, mostly house mice (*Mus musculus*), were caught on the 20 farms, primarily within and around the periphery of swine housing and feed storage facilities. The mean number of house mice caught on each farm was 29, with a range of 21 to 36. The overall prevalence of *T. gondii* antibodies in house mice was 0.3% (2/588). Each of the 2 seropositive mice came from a different farm; each of these farms also had seropositive cats and swine. There were no seropositive individuals among 21 mice of the genus *Peromyscus* and 9 Norway rats (*Rattus norvegicus*).

Other wildlife species were captured on 18 farms. The number of animals of each species captured ranged from 1 to 5 opossums on 17 farms, 1 to 6 raccoons on 7 farms, and 1 to 3 skunks on 4 farms. The overall prevalence of *T. gondii*

Table 3. *Toxoplasma gondii* serology of cats: Prevalence by age class and gender

	Juvenile	Adult	Total
Male	1/5	22/32[a]	23/37[a]
Female	0/8	8/29	8/37
Total	1/13	30/61[a]	

[a]Significantly different than corresponding total or gender class ($P < 0.01$).

antibodies was significantly higher in cats vs. opossums, and in raccoons and skunks vs. opossums ($P < 0.01$), with the prevalence in cats not significantly different from that in raccoons and skunks. In raccoons, the seroprevalence was significantly higher in adults (4/6) than in juveniles (0/8) ($P < 0.05$). Positive raccoons consisted of 3/3 adult males and 1/3 adult females. In opossums, 16 adults and 18 juveniles were sampled; the lone positive animal was an adult male. The age class of the 2 positive skunks (one male and one female) was not determined.

The distribution of titers of seropositive animals within each species is given in Table 4. The average antibody titer was significantly higher in cats than in swine, mice, skunks, and the group of opossums, raccoons, and skunks ($P < 0.05$).

No association on a farm basis was found between the prevalence in sows and prevalence in any of the non-swine species. Furthermore, no association was found between prevalence in sows and farm characteristics or swine management practices other than degree of confinement and average parity of the breeding herd.

Table 4. *Toxoplasma gondii* serology: Distribution of titers of seropositive animals, by species

Species	Reciprocal of Modified Direct Agglutination Titer												
	32	64	128	256	512	1024	2048	4096	8192	16384	32768	65536	131070[a]
Cat				2	3				7	4	8	3	3
Swine	13	5	4	1	7	6	1	1	1				
House Mouse	1						1						
Raccoon			2		1					1			
Striped Skunk	1		1										
Opossum					1								

[a]One animal in this group had a titer of 4.1×10^6.

[b]Mean titers were significantly greater in cats than in swine, mice, skunks, and the group of wildlife species (opossums, raccoons, and skunks) ($P < 0.05$).

DISCUSSION

The results of this study are analyzed in the context of what is already known and what is hypothesized about the epidemiology of toxoplasmosis in cats, rodents, wildlife, and swine relevant to Iowa swine farms. Findings of this study are assessed for their support or lack of support of current hypotheses, and for identification of questions and research needs on the subject.

Cats

The overall prevalence of *T. gondii* antibodies in cats in this study and the significantly higher prevalence in adults vs. juveniles are compatible with existing reports. Serosurveys for *T. gondii* in cats in the continental United States have a reported prevalence ranging from 5% to 62% (Dubey and Beattie, 1988). In these surveys, however, several different serologic techniques were used and the age of cats was not always reported (Dubey and Beattie, 1988). Age of cats surveyed is important to note, because seropositivity has been shown to increase with age (Dubey, 1973; Ruiz and Frenkel, 1980), and most cats are infected post weaning (6 to 10 weeks of age) (Dubey and Beattie, 1988).

There are few reports on the prevalence of *T. gondii* antibodies in free-ranging rural cats in the midwestern United States. This is the population of cats with potential impact on the epidemiology of toxoplasmosis on Iowa swine farms. Dubey (1973) reported 58% of 157 older than 6 month-old "stray" cats from farms in Iowa

and northern Missouri as seropositive (antibody titer of $\geq$ 1:2) by the dye test. Zimmermann (1975) reported 61.7% of 60 feral cats in central Iowa as positive ($\geq$ 1:16) by the indirect hemagglutination test. Compatibility of these two studies with the present study can be demonstrated by 95% confidence intervals (CI) of the prevalence estimates. These three studies suggest that approximately 50-60% of adult rural Iowa cats have antibodies to *T. gondii*.

The higher prevalence in adult males vs. adult females in this study was unexpected, but might be explained by larger home ranges for adult male rural midwestern cats. In a study on demographics and movements of free-ranging domestic cats in rural Illinois (Warner, 1985), adult male cats ranged over significantly larger areas than adult females. This might result in adult males encountering more farms, each of which could be considered as a concentration of definitive (cat) and intermediate (rodent) hosts of *T. gondii*. It might be hypothesized that visitations to more farms would thus increase the chance for exposure to *T. gondii*.

It is not certain how most cats become infected with *T. gondii*. This is an important epidemiologic issue because cats are the only animals known to excrete oocysts (Miller et al., 1972). Transplacental transmission in cats is rare (Dubey, 1977; Dubey and Hoover, 1977; Dubey and Johnstone, 1982). Cats can be infected by ingesting oocysts (Dubey et al., 1970a; Frenkel et al., 1970), and direct cat to cat transmission of oocysts in nature seems logical. However, experimental and epidemiological data support an indirect cycle dependent on tissue cyst (bradyzoite)-

bearing intermediate hosts as the main cycle occuring in nature. A cat infected by ingesting an infected intermediate host sheds millions of oocysts (Dubey and Frenkel, 1972, 1976) capable of infecting a large number of additional intermediate hosts, especially those herbivorous animals that feed on the ground (Frenkel, 1990b).

Evidence supporting the importance of tissue cyst vs. oocyst transmission to cats includes the variable prepatent periods (time to the shedding of oocysts after infection) occuring with the ingestion of the different stages of *T. gondii*: from 3 to 10 days following ingestion of bradyzoites, but 19 days or longer following ingestion of oocysts (Dubey and Frenkel, 1976; Frenkel et al., 1970; Wallace, 1973b). Furthermore, oocysts are much less potent sources of infection for cats than are tissue cysts (Dubey and Frenkel, 1976), and relatively large doses of oocysts - more than might be expected to be encountered in natural exposure - are usually required to develop patency and elicit production of antibodies (Wallace, 1973b). Less than 20% of cats shed oocysts after ingesting oocysts, whereas nearly all cats shed oocysts after ingesting tissue cysts (Dubey and Frenkel, 1976; Wallace, 1973b). When patent infections do occur after ingestion of oocysts, patent periods are usually relatively short and quantity of oocysts shed is relatively small (Wallace, 1973b).

In further support of the importance of the indirect cycle, cats usually become infected after weaning, when they are old enough to either hunt or eat prey brought to them by their dam (Dubey, 1973; Wallace, 1971b, 1973b). If direct transmission

of oocysts was the principal route of transmission of *T. gondii* among cats, one would expect a higher prevalence of infection in younger animals (Wallace, 1973b).

Rodents

Considering the indirect cycle as described above to be the principal cycle occuring in nature, rats, mice, other small mammals, and birds would logically seem to be the key intermediate hosts of *T. gondii* since they are the natural prey of cats. Rats and mice in particular would seem to be important intermediate hosts because they are often abundant, readily available, and the preferred prey of cats. Rodents are easily infected with the oocyst stage of *T. gondii* (Dubey et al., 1970b; Frenkel et al., 1970; Miller et al., 1972), and survivors of infection usually develop chronic infection with the organism (Wallace, 1973a).

It has been suggested that rodents may be more appropriately considered reservoir hosts than intermediate hosts (Chinchilla, 1978; Dreesen, 1990; Frenkel, 1973; Lubroth et al., 1983; Wallace, 1973a). Theoretically, *T. gondii* could be maintained in nature by any mammals or birds whose habits include carnivorism, cannibalism, and/or scavenging. Rats can be infected by eating infected mouse and swine tissue (Weinman and Chandler, 1954). Cannibalism among rodents is not unusual, and was observed among house mice many times during the present study.

Repeated congenital infection can occur in mice, rats, guinea pigs, and hamsters, and perhaps other small mammals (Beverly, 1959; de Roever-Bonnett, 1969; Dubey, 1983; Remington et al., 1961). Several litters may be born infected

from an infected female mouse without reinfection from an outside source. Congenitally infected mice can themselves produce congenitally infected mice for five or more generations (Beverly, 1959).

Thus, it seems possible that rodents may maintain the organism in their population for significant periods of time, although probably not indefinitely, without reinfection from cats, the only definitive hosts of *T. gondii* (Ruiz and Frenkel, 1980a; Wallace, 1973a).

Table 5 lists some studies that have attempted to detect *T. gondii* in wild house mice and Norway rats. These studies have indicated that both rats and mice can be naturally infected with *T. gondii*, but that the prevalence of detectable infections in nature has usually been quite low. Wallace (1973a) warned that serologic surveys with negative results in highly susceptible species should be interpreted with caution since it is possible that most infections may be fatal. This would bias trapping efforts toward uninfected animals, making it difficult to obtain an accurate index of infection in that species. If most individuals of a species suffer from acute toxoplasmosis after infection, the species would tend to be an efficient intermediate host of toxoplasmosis since sick individuals would be easy prey for cats, yet might not be considered so on a *T. gondii* infection survey basis.

Mice are considered generally susceptible to *T. gondii* (Dubey and Beattie, 1988). However, pathogenicity to mice differs with the strain of *T. gondii* and the strain of mouse (Dubey and Beattie, 1988), and virulence of a particular isolate is dose dependent (Dubey et al., 1981; Wallace, 1973a). Wallace reported that all six

Table 5. Some previously published reports on the detection of *Toxoplasma gondii* or *T. gondii* antibodies in house mice and Norway rats

Species	Serology			Isolation[a]	Location	Ref.[c]
	Frequency[a]	Test	Titer[b]			
Mus musculus	-	-	-	0/61	Tennessee, Mississippi	a
"	2/51	Dye	≥16	9/220	Memphis, Tn	a
"	3/122	"	"	-	Hawaii	b
"	5/114	IHA	≥64	-	California	c
"	18/202	Dye	≥2	7/202	Costa Rica	d
"	5/100	"	"	2/100	Costa Rica	e
"	0/32	IHA	≥16	-	Iowa	f
"	12/20	IIF	≥32	-	Georgia	g
Rattus norvegicus	0/29	Dye	≥16	0/36	Tennessee, Mississippi	a
"	8/100	"	"	6/190	Memphis, Tn	a
"	-	-	-	14/157	Georgia	h
"	22/107	Dye	≥2	15/120	Costa Rica	d
"	0/64	CF	≥8	-	USA, east	i
"	1/73	Dye	≥16	-	Hawaii	b
"	1/28	IHA	≥64	-	California	c
"	1/10	IHA	≥16	-	Iowa	f
"	2/2	IIF	≥32	-	Georgia	g

[a]Number positive over number tested.

[b]Reciprocal of titer considered positive.

[c]References: a = Eyles et al., 1959; b = Wallace, 1973a; c = Franti et al., 1976; d = Ruiz and Frenkel, 1980; e = Chinchilla, 1978; f = Zimmermann, 1975; g = Lubroth et al., 1983; h = Perrin et al., 1943; i = Morris et al., 1956.

strains used in one study (1973a) were capable of producing fatal disease in mice, depending on dose. Dubey et al. (1981) reported that lethal $dose_{50}$ in mice ranged from 1 to $10^{3.7}$ after oral inoculation with oocysts of 10 different isolates. Ruiz and Frenkel (1980a) reported that 90% of 31 *T. gondii* isolates from the feces of Costa Rican cats resulted in asymptomatic infection on original passage in laboratory mice. Even the 10% of mouse-pathogenic isolates did not kill mice until after bradyzoites were formed, at least 10 days after infection with oocysts. Work on the pathogenicity of Iowa swine farm isolates of *T. gondii* oocysts to mice after oral challenge needs to be done to determine the probability of infected mice being excluded from *T. gondii* prevalence surveys due to acute disease.

Interpretation of serologic findings on apparently healthy mice was another factor to consider when attempting to estimate prevalence of *T. gondii* infection in wild house mice. Of particular concern was the possibility that a proportion of seronegative mice (or mice with low titers considered nonspecific) may actually be infected, and that serologic surveys would thus underestimate prevalence in mice. Dubey (1981) isolated *T. gondii* from four house mice that were seronegative ($<$ 1:2) by the dye test, as did Ruiz and Frenkel (1980a) from a single seronegative house mouse. Questions also arose about the specificity of the dye test in house mice. The combined results of two Costa Rican surveys (Ruiz and Frenkel, 1980a; Chinchilla, 1978) showed that of 23 seropositive house mice, *T. gondii* was isolated from all 8 with dye test titers $\geq$ 1:64, but from 0 of 15 mice with titers of 1:2 to 1:64.

This issue becomes even more complex when one considers that some isolation methods may not be highly sensitive in detecting *T. gondii* in mice with legitimate antibody titers. Dubey (1983) isolated *T. gondii* from only 3 of 6 experimentally inoculated mice, all of which had developed dye test titers > 1:256.

These examples are cause for concern, although studies with larger numbers of mice have indicated that serologic findings correlate well with infection status. Remington et al. (1961) found no discrepancies between dye test results (at a test dilution of 1:16) and microscopic findings. Dubey et al. (1970b) reported that all of over 100 mice that survived feeding with 10^1-10^5 mouse infectious $dose_{50}$ of oocysts at least 6 days developed dye test titers of 1:64-1:8000. Lubroth et al. (1983) found that infectivity of wild mice whose tissues were inoculated into laboratory mice correlated well with the results of the indirect immunofluorescence test on the serum of the wild mice. In two surveys in Costa Rica (Chinchilla, 1978; Ruiz and Frenkel, 1980a), *T. gondii* was isolated from only 1 of 279 mice seronegative (< 1:2) by the dye test.

The modified agglutination test for *T. gondii* antibodies has not been previously used in rodent surveys, and how it performs in mice is unknown. Overall, it is the most sensitive test for the serodiagnosis of toxoplasmosis known (Dubey and Beattie, 1988; Dubey and Thulliez, 1989; Patton et al., 1991). Evaluation of agglutinating antibody response to oral infection with oocysts and corresponding success of isolation attempts needs to be made to be able to more accurately evaluate rodent serosurveys. This should include study of the immune response of

congenitally infected mice, which, if the mice were immunotolerant, would not be detected in serosurveys for *T. gondii*.

The 0.3% prevalence of *T. gondii* antibodies found in house mice in the present survey indicated a very low prevalence of infection in this species on farms in the study area. Even if a proportion of the house mice on these farms were highly susceptible to the local strains of *T. gondii*, or if serologic tests failed to detect some infected individuals, the large number of mice sampled in this survey would likely have included more than two seropositive animals if, in fact, infection with *T. gondii* was significantly more prevalent.

The fact that mice of the genus *Peromyscus* were infrequently trapped in this study probably reflected their relative rarity in the trapping environment compared to the house mouse. This, and the fact that all of the *Peromyscus* species mice sampled were seronegative, suggested that this genus was not an important source of toxoplasmosis for swine.

Norway rats were known to be common in the environment of swine on certain of the study farms, but were infrequently captured, probably because the Sherman live-traps were not quite large enough to efficiently capture them. Worldwide, infection in Norway rats is considered to be more common than in mice (Dubey and Beattie, 1988). Rats are highly resistant to toxoplasmosis (Dubey and Beattie, 1988), commonly becoming chronic carriers rather than showing illness (Wallace, 1973a; Weinman and Chandler, 1954). Thus, rats are potentially important hosts of

T. gondii for swine. The results of this study, based on only nine rats, failed to provide evidence that this is the case.

Wildlife

Opossums, raccoons, and striped skunks commonly inhabit swine farms in Iowa, often living in or under buildings (barns, corn cribs, machinery or storage sheds, etc.) in close association with swine. Surveys for *T. gondii* antibodies in wildlife indicate that these three species are commonly seropositive (Burridge et al., 1979; Ferguson and Heidt, 1981; Franti et al., 1975, 1976; Tizard et al., 1976; Zimmermann, 1975). Surveys of large numbers of these species have a reported prevalence of 16%-18% for raccoons, 20%-31% for striped skunks, and 8%-11% for opossums (Burridge et al., 1979; Ferguson and Heidt, 1981; Tizard et al., 1976; Zimmermann, 1975). Burridge et al. (1979) reported a significantly higher prevalence in adults than juveniles for both raccoons (23% vs. 5%) and opossums (15% vs. 1%). Findings of the present study, though based on a smaller sample size, were compatible with those surveys.

Concerning the involvement of these species in the epidemiology of swine toxoplasmosis, the only demonstrable way an infected individual of one of these species could infect swine is for the individual to die within access to swine, which would then have to eat the animal (Miller et al., 1972). This probably does not occur often enough for these species to be an important source of toxoplasmosis for swine.

Swine

The 14.3% prevalence of *T. gondii* antibodies in sows in this study is compatible with existing reports. Past serologic surveys for *T. gondii* in swine in the United States have reported seropositivity rates from < 1% to 69% (Dubey, 1990; Zimmerman et al., 1990). It was difficult to interpret or compare many of these surveys because of differences in the sensitivity and specificity of different serologic tests used, lack of standardization of each test, and lack of information on the types of swine surveyed and management conditions under which swine were raised (Dubey, 1990). Some of the more recent surveys, although employing different serologic tests, are more informative because of one or more of the following factors: large number of swine sampled, information on the type of swine sampled and swine management conditions, and better standardization of the tests.

In Georgia, 30.9% of mature swine were seropositive (Dreesen and Prestwood, 1980). In Louisiana, 24.6% of sows and gilts were seropositive (Hugh-Jones et al., 1986). In a recent survey of Iowa swine, 10.0% (95% CI of 7.6 to 12.5%) of 587 sows and gilts were seropositive (Zimmerman et al., 1990). These along with other surveys (Hellesnes et al., 1978; Ikegami, 1970) have indicated that toxoplasmosis was more prevalent in breeding swine than in finishing swine and that the prevalence of infection with *T. gondii* in swine generally increased with age. The present study supported these findings in that prevalence in sows from farms with an average parity of 2.0 or greater was higher than that in sows from farms with an average parity of less than 2.0.

Considering that the present study typically sampled the oldest sows on the farms and that prevalence of infection has been shown to increase with age, the 14.3% prevalence in sows (95% CI of 10.1-18.5%) corresponded very well with the prevalence reported in the Iowa survey by Zimmerman et al. (1990). The latter survey also reported that 68% of 25 breeding herds were infected and that within infected herds, 16.4% of sows and gilts were seropositive. The corresponding values of the present study were that 53% of 19 breeding herds were infected and that 28.3% of sows were seropositive within infected herds. These agreed quite well with the former values considering the sample size in the present study may not have been adequate to detect all infected herds, and that only older sows were sampled.

The relatively low prevalence of *T. gondii* antibodies in Iowa swine vs. the prevalence in swine in the southern United States may be partially due to climatic differences in the two areas. Georgia has a much warmer climate with far less severe winters than Iowa (Dreesen et al., 1989), and infection with *T. gondii* is more prevalent in warm, humid climates than cold, dry climates (Dubey and Beattie, 1988). It has been hypothesized that environmental conditions may influence natural transmission rates of *T. gondii* through effects on sporulation and survival of oocysts in the environment (Dubey and Beattie, 1988).

This reasoning could also be used to explain the very low prevalence found in rodents in the present study. Frenkel (1990b) suggested that the lack of detection of toxoplasmosis in a large number of rodents from Montana (Dubey, 1983) may

indicate that transmission rates are lower in the rural north central United States than in suburban San Jose, Costa Rica, where infection in rodents was relatively common. However, when comparing prevalence in intermediate hosts in different areas, the density of cats in the areas should be considered before attributing differences in prevalence solely to environmentally influenced transmission rates.

The lower prevalence in sows totally and continuously confined vs. the prevalence in sows not totally confined is not unusual. Lubroth et al. (1983) reported a marked difference in the seropositivity rate in all ages of swine in a herd raised under strict confinement (0.8%) compared to that in a herd maintained in a semi-confined to open management system (27.6%). Zimmerman et al. (1990) did not find a difference in prevalence in confined vs. nonconfined sows and gilts, but suggested that facility type exerted some degree of influence on seroprevalence within a herd in that finishing swine in confinement appeared to have a lower inherd prevalence than nonconfined finishers.

In the present study, there was no relationship between breeding herd size and herd infection rate or infection rate within a herd. In contrast, Zimmerman et al. (1990) found that breeding herds of less than 100 animals were more likely to be infected than were larger herds, but that prevalence within infected breeding herds was not influenced by size.

The present study supported previous observations that prevalence of *T. gondii* infection in swine increased with age and that prevalence of infection could be reduced through confinement. The question, particularly with respect to the latter

observation, was why? Is it due to the fact that confinement units exclude cats, or rodents and other intermediate hosts, or both?

How most pigs naturally acquire *T. gondii* infection is not precisely known. Transplacental infection has been demonstrated in pigs but probably is not a common route of transmission; it is presumed that most pigs acquire infection postnatally (Dubey, 1986a, 1990). The only demonstrable means of postnatal transmission in nature are ingestion of feed and water contaminated with oocysts from feline feces, and ingestion of tissue cysts in the flesh of animals (Dubey, 1990).

The ingestion of oocysts from the environment would have logically appeared to be the main source of infection (Dubey, 1986a). One cat may shed millions of oocysts (Dubey and Frenkel, 1972, 1976) capable of surviving in soil for a year or more (Frenkel et al., 1975). Cats often defecate in stored feed, barns, and lots or pastures inhabited by livestock (Penkert, 1973; Dubey, 1986b). Oocysts of *T. gondii* are small and are easily dispersed by wind, rain, and by movement of animals, people, machinery, etc. (Fayer, 1981). Also, organisms such as flies, cockroaches, and earthworms have been shown experimentally to be able to carry oocysts and may act as transport hosts to disseminate oocysts and facilitate infection of intermediate hosts (Chinchilla and Ruiz, 1976; Dubey et al., 1970; Frenkel et al., 1975; Wallace, 1971a, 1972, 1973a). *T. gondii* has been isolated under natural conditions from earthworms, which probably pick up oocysts while burrowing in the soil (Ruiz and Frenkel, 1980a).

The above information has underscored the tremendous transmission potential

of oocyst contamination of the environment for swine. The ingestion of tissue cysts has also received much attention as a potentially important source of toxoplasmosis for swine. It is possible for toxoplasmosis to be transmitted to swine in this way through ingestion of uncooked or undercooked garbage, cannibalism, or ingestion of infected rodents or other hosts (Dubey and Beattie, 1988).

Garbage feeding can be a direct source of toxoplasmosis through meat scraps, and can also attract rats and cats, intensifying the cat-rodent cycle of toxoplasmosis in the environment of swine (Dubey et al., 1986). It has also been suggested that cannibalism may be an important mode of transmission of toxoplasmosis to swine (Dubey et al., 1986). Cannibalism (defined here as scavenging of dead pigs by other pigs) is not unusual, and one carcass could theoretically infect many pigs. Weinman and Chandler (1954) indicated that pigs could easily be infected by feeding them tissues of pigs infected with *T. gondii*. Tail-biting among swine has also been suggested as a transmission route (Dubey et al., 1986).

It has been suggested that the ingestion of infected rodents is an important route of transmission to swine (Lubroth et al., 1983; Weinman and Chandler, 1954). It is not uncommon for swine to eat dead rodents and, on occasion, to catch and eat rodents in their environment (Dreesen and Lubroth, 1981). Infected dead rodents may contaminate feed at a central storage place (Dubey et al., 1986; Dreesen and Lubroth, 1983). Weinman and Chandler (1954) indicated that pigs could be easily infected with *T. gondii* by feeding them tissues of infected rats and mice.

Lubroth et al. (1983) investigated rodent involvement in the epidemiology of

swine toxoplasmosis in Georgia. The prevalence of *T. gondii* antibodies in a swine herd totally and continuously confined was 0.8%, compared to 27.6% in a herd raised under semi-confinement and open range conditions. Swine confinement facilities on the first farm were relatively rodent free and prevented easy access by other animals. Antibodies to *T. gondii* were commonly found, at about the same prevalence, in rodents on both farms. Thirteen of 21 (62%) rodents, including 7 of 12 house mice, 3 of 6 cotton rats (*Sigmodon hispidus*), both of 2 Norway rats, and a white-footed mouse trapped on the farm with the totally confined swine were seropositive. Seven of 10 (70%) rodents, including 5 of 8 house mice and both of 2 white-footed mice trapped on the other farm were seropositive. The exclusion of cats from swine confinement facilities was considered to be an important factor in the lower prevalence of toxoplasmosis in the totally confined herd. However, the similarly high prevalence in rodents on the two farms, and the much lower prevalence in swine relatively free from contact with rodents suggested that rodents may also be important in the transmission of toxoplasmosis to swine, possibly as reservoir hosts.

Rodents are intimately associated with swine on most farms. Abundance varies, but rodents are invariably present. On the 20 farms in the present study, rodents were common in the environment of swine, as indicated by the farmers' and author's observations. Many total confinement facilities, even those considered good ones by most standards, were inhabited by numerous mice. In fact, these buildings often held the greatest concentrations of mice encountered, probably

because such buildings provide an ideal food source, shelter, and protection from natural predators. These buildings often are not totally sealed from the outside environment, and access by these small animals is not difficult. Confinement buildings with minimal or no mouse activity were encountered, but they were the exception. Confinement buildings, however, usually excluded rats.

Given the information about rodents and *T. gondii* presented by a number of investigators in this discussion, and the abundance of mice in the immediate environment of swine on the study farms, it was projected that rodents might be further implicated as an important source of *T. gondii* for swine. It was hypothesized that prevalence in rodents on a farm basis might correlate with prevalence in swine, or at least to prevalence in cats, which likely depend on intermediate hosts for infection. The extremely low prevalence found in house mice was unexpected, and suggested that house mice did not serve as an important source of toxoplasmosis for swine in Iowa.

Although the lack of seropositive rats from the small sample hinted that toxoplasmosis was not highly prevalent in rats on these farms, a much larger number of rats would need to be sampled before a definitive statement could be made regarding their involvement in swine toxoplasmosis in Iowa. Further study of rats and other intermediate hosts that live in close association with swine, such as some species of birds, needs to be done to elucidate their importance in the epidemiology of swine toxoplasmosis.

Even though prevalence of *T. gondii* antibodies in sows could not be associated

with cat factors, the high prevalence of *T. gondii* antibodies in cats suggested that oocysts from cat feces contaminating the environment deserve considerable attention as the most significant source of toxoplasmosis for swine in Iowa.

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SECTION III. A SURVEY OF MICE ON IOWA SWINE FARMS FOR INFECTION WITH *LEPTOSPIRA INTERROGANS* SEROVAR *BRATISLAVA*

SUMMARY

Five hundred and thirty-five house mice (*Mus musculus*) and 21 mice of the genus *Peromyscus* from 20 Iowa swine farms were tested for antibodies against *Leptospira interrogans* with the microscopic agglutination test. Serovars *bratislava*, *icterohaemorrhagiae*, *autumnalis*, *tarassovi*, *pomona*, *hardjo*, *grippotyphosa*, and *canicola* were included in the initial survey. Mice with antibody titers $\geq$ 1:25 against any of these serovars were then tested for antibodies against serovar *ballum*. Forty-three house mice (8.0%) had antibody titers $\geq$ 1:25 against serovar *bratislava*. Twenty of these 43 mice also had antibody titers $\geq$ 1:25 against other serovars, including 10 against serovar *icterohaemorrhagiae* and 4 against serovar *ballum*.

Subsequently, 24 additional house mice were captured from 2 of the farms which had previously yielded several house mice with MAT antibody titers $\geq$ 1:25 against serovar *bratislava* alone. These mice were examined for infection with serovar *bratislava* by the MAT, fluorescent antibody tests (FAT) of kidneys and urine with fluorescein-labeled rabbit anti-*bratislava* serum, and bacteriologic culture. Five of the 24 mice, including 4 of 10 mice on one farm, had antibody titers $\geq$ 1:25 against serovar *bratislava*. Positive FAT were obtained on the kidneys of 2 mice seronegative for the serovars included in the initial survey, including *bratislava*. Suspect FAT were obtained on the kidneys of 3 mice, including 2 with antibody titers $\geq$ 1:25 against *bratislava*. Isolation attempts on all 24 mice from the additional sample were unsuccessful.

Leptospira interrogans serovar *bratislava* has previously been isolated from house mice in Europe (Galton, 1966). The MAT and FAT results of the present study suggest that serovar *bratislava* infections occur in house mice on Iowa swine farms. Further study is needed to confirm the presence of serovar *bratislava* in house mice in the North America and to assess their role as possible hosts of *bratislava* and a source of infection for swine.

INTRODUCTION

Infection of swine with serovars of *Leptospira interrogans* belonging to the Australis serogroup, particularly *bratislava* and *muenchen*, is an important cause of porcine reproductive failure in Europe (Hathaway and Little, 1981; Hathaway et al., 1981; Ellis et al., 1985, 1986a, 1986b, 1986c). In the United States, there is serologic evidence that infection of swine with serovar *bratislava* is common (Frantz, 1987; Hanson, 1987; Miller et al., 1990), and *bratislava* has been isolated in Iowa from aborted, stillborn, and weak neonatal pigs, placentae, and the urogenital tract of sows experiencing reproductive failure (Bolin and Cassells, 1990; Bolin et al., 1991).

In Europe, it is hypothesized that free-living reservoir host species may provide a source of Australis serogroup infection for swine (Hathaway et al., 1983a). Serovar *bratislava* appears to be maintained by the hedgehog (*Erinaceus europaeus*) (Broom and Coghlan, 1960; Hathaway et al., 1983a; Ellis, 1988), and has also been isolated from house mice (*Mus musculus*) (Galton, 1966), and a brown rat (*Rattus norvegicus*) (Hathaway et al., 1983a). The closely related serovar *muenchen* appears to be maintained by a number of small rodents, including wood mice (*Apodemus sylvaticus*), short-tailed voles (*Microtus agrestis*), and bank voles (*Clethrionomys glareolus*) (Ellis, 1988; Hathaway et al., 1983a). Other serovars of the Australis serogroup (i.e. *lora* and *jalna*) also appear to be maintained by small rodents in Europe (Pritchard et al., 1985).

Despite the reports from Europe, free-living non-porcine species in North America have not been examined for infection with serovar *bratislava*. The house

mouse is a particularly important species to consider in this context since several serovars of *L. interrogans* infectious for swine, including *bratislava*, have been isolated from house mice outside of North America (Galton, 1966; Sulzer, 1975) and because house mice are frequently abundant where swine are raised due to ready access to food and shelter. The objective of this study was to examine house mice from swine farms in Iowa for evidence of infection with *Leptospira interrogans* serovar *bratislava* with the specific aims of estimating the frequency of infection in house mice and evaluating their possible role in the transmission of serovar *bratislava* to domestic swine.

MATERIALS AND METHODS

Design of study

Mice were collected from 20 swine farms in central Iowa and screened for antibodies against *Leptospira interrogans* serovars *bratislava*, *icterohaemorrhagiae*, *tarassovi*, *autumnalis*, *hardjo*, *pomona* type *kennewicki*, *grippotyphosa*, and *canicola* by the microscopic agglutination test (MAT). All mice with antibody titers $\geq$ 1:25 against any of the serovars in this initial survey were then tested for antibodies against serovar *ballum*. On 2 farms which had yielded several mice with antibody titers $\geq$ 1:25 against *bratislava* alone, additional mice were trapped and examined for serovar *bratislava* infection by the MAT, fluorescent antibody tests (FAT) on kidneys and urine, and bacteriologic culture. All testing and culturing was carried out by the Leptospirosis and Mycobacteriosis Research Unit, National Animal Disease Center, USDA, Agriculture Research Service, PO Box 70, Ames, Iowa 50010.

Mouse sampling

Mice were live-trapped using Sherman[1] and Ketch-all[2] live traps set primarily within and around the periphery of swine housing and feed storage facilities. Sherman traps were baited with a mixture of peanut butter and oatmeal flakes,

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[1]H. B. Sherman Traps, Inc., Rt. 22, Box 365, Tallahassee, FL 32304.

[2]Kness Mfg. Co., Inc., Albia, IA 52531.

checked daily, and rebaited as needed. Ketch-all traps were not baited. Mice were transferred from the traps into plastic cages with wire covers for transport to the Iowa State University College of Veterinary Medicine. Blood was collected via orbital sinus puncture. Mice were then euthanitized by cervical dislocation. Serum was separated from blood samples and stored at -70° C until testing.

Serology

Serum samples were initially examined for antibodies against 8 serovars of leptospires at a serum dilution of 1:25 by the MAT (Cole et al., 1973). The following serovars were used as antigens: *bratislava* (As-05), *icterohaemorrhagiae* (Ic-02), *tarassovi* (Ta-01), *autumnalis* (At-01), *hardjo* (Hb-15A), *pomona* type *kennewicki* (Po-06), *grippotyphosa* (Rm-52), and *canicola* (Ca-01). Mice that were seropositive to any of these serovars at the 1:25 dilution were then tested for antibodies against serovar *ballum* at a serum dilution of 1:25.

Fluorescent antibody testing

Kidney suspensions and urine collected aseptically from the urinary bladder of euthanitized mice were examined for leptospires by staining with fluorescein-labeled rabbit anti-*bratislava* serum (Bolin et al., 1989). Leptospires were identified by typical shape and specific fluorescence when examined by incident-light fluorescence microscopy. A "suspect" FAT was used to designate instances where specific

fluorescence occured, but where the fluorescing antigen did not appear to conform to characteristic leptospiral morphology.

Bacteriologic culture

Culture was attempted from kidneys and urine collected aseptically from the urinary bladder of euthanitized mice. Samples were cultured for *Leptospira interrogans* serovar *bratislava* as described previously (Bolin, 1990).

RESULTS

Results of the initial serosurvey are summarized in Table 1. Five hundred and fifty-six mice, including 535 house mice and 21 mice of the genus *Peromyscus*, were tested in the initial survey. None of the mice had antibody titers $\geq$ 1:25 against serovars *grippotyphosa* or *canicola*. None of the white-footed mice had antibody titers against any of the serovars included in the tests.

Seventy-five (14.0%) house mice had antibody titers $\geq$ 1:25 against 1 or more of the serovars in the initial survey. Forty-three (8.0%) house mice had antibody titers $\geq$ 1:25 against serovar *bratislava*. Of these 43 mice, 20 also had antibody titers $\geq$ 1:25 against other serovars, including 10 with antibody titers against serovar *icterohaemorrhagiae*, 4 against serovar *ballum*, 3 against serovar *autumnalis*, and 3 against serovar *pomona*. Three mice had antibody titers $\geq$1:25 against 3 serovars: one mouse against *bratislava*, *icterohaemorrhagiae*, and *tarassovi*; one against *bratislava*, *hardjo*, and *tarassovi*; and one against *bratislava*, *autumnalis*, and *pomona*. Of the 75 house mice seropositive to 1 or more serovars in the initial survey, 13 also had antibody titers $\geq$ 1:25 against serovar *ballum* (Table 2).

Farms 6 and 8 were chosen for further study because of the number of mice from those farms with antibody titers $\geq$ 1:25 against serovar *bratislava* alone. Fourteen and 10 additional mice, respectively, were captured and examined for serovar *bratislava* infection by the MAT, FAT on kidneys and urine (Table 3), and

Table 1. *Leptosira interrogans* serology of mice from Iowa swine farms

Farm	# of mice tested	Number of mice with a MAT antibody titer of ≥ 1:25 against serovar[a]:						Number of mice with a titer of ≥ 1:25 against two serovars[b]:			
		As-05	Ic-02	At-01	Ta-01	Po-06	Hb-15a	As-05 + Ic-02	As-05 + At-01	As-05 + Po-06	As-05 + Ta-01
1	21										
2	20	1									
3	28	3	1					1			
4	23	1		1	1						
5	19	1		1							
6	31	7	4	3	1			1	2		
7	27	3	1		2			1			
8	27	9	4	1		1	1	3		1	
9	33	4	7	1	2		1	2			1
10	32	1	6	1				1			
11	32	2									
12	26	1	1					1			
13	29		3								
14	32	5				1				1	
15	31										
16	28	2		4		1			1		
17	31	2									
18	29										
19	25	1									
20	32			1							
Total	556	43	27	13	6	3	2	10	3	2	1

[a]As-05 = *bratislava*, Ic-05 = *icterohaemorrhagiae*, At-01 = *autumnalis*, Ta-01 = *tarassovi*, Po-06 = *pomona*, Hb-15a = *hardjo*. No mice had titers to serovars *canicola* or *grippotyphosa*.

[b]Mice in these categories were also counted in their respective single-serovar categories.

Table 2. *Leptospira interrogans* serology on farms with mice seropositive (antibody titer $\geq$ 1:25) for serovar *ballum* in addition to other serovars[a]

Farm	Number of mice seropositive for serovar(s):[b]			Number of mice seropositive for serovar *ballum* also seropositive for serovar(s):		
	As-05	Ic-02	As-05 + Ic-02[c]	As-05	Ic-02	As-05 + Ic-02[d]
3	3	1	1	1		
6	7	4	1		1	
8	9	4	3	1		
9	4	7	2			1
10	1	6	1		3	1
12	1	1	1			1
13		3			1	
14	5			1		
17	2			1		
Total	32	26	9	4	5	3

[a]All of the mice seropositive for any serovar on the initial survey were then tested for antibodies against serovar *ballum*. All of the mice which were seropositive for *ballum* were seropositive for As-05 and/or Ic-02, except for 1 mouse on farm 12, which was seropositive for Hb-15a.

[b]From Table 1. See Table 1 for abbreviations.

[c]Mice in this category were also counted in the corresponding single-serovar categories.

[d]Mice in this category were not counted in the corresponding single-serovar categories.

Table 3. Fluorescent antibody test results on additional mice from farms 6 and 8

Farm	Number of mice	Number of mice with a positive FAT[a]		Number of mice with a suspect FAT	
		bratislava sero-positive	*bratislava* sero-negative	*bratislava* sero-positive	*bratislava* sero-negative
6	14[b]		2		1
8	10[c]			2	

[a]Fluorescent antibody test, on kidney.

[b]One of these 14 mice was seropositive (antibody titer $\geq$ 1:25) for serovar *bratislava*.

[c]Four of these 10 mice were seropositive (antibody titer $\geq$ 1:25) for serovar *bratislava*.

bacteriologic culture. For these mice, only the serovars included in the initial survey were used as antigens in the MAT.

From farm 6, one of the 14 mice had an antibody titer $\geq$ 1:25 against serovar *bratislava* and 1 against serovar *tarassovi*. Positive FAT were obtained on the kidneys of 2 other mice from farm 6, both of which were seronegative for all serovars included in the initial survey, including *bratislava*. A suspect FAT was obtained on the kidney of an additional mouse, also seronegative for all serovars included in the initial survey.

From farm 8, four of the 10 mice had antibody titers $\geq$ 1:25 against serovar *bratislava*, but not against the other serovars included in the initial survey. Thus, of a total of 37 mice captured on farm 8, 13 (35.1%) had antibody titers $\geq$ 1:25 against serovar *bratislava*. Nine of the 13 *bratislava* positives were seronegative for all other serovars included in the initial survey, and 8 of the 9 *bratislava* positives which were tested for *ballum* were seronegative for that serovar. Suspect fluorescent antibody tests were obtained on the kidneys of 2 mice that were seropositive for *bratislava* but seronegative for all other serovars included in the initial survey. Culture attempts on all 24 mice of the additional sample were negative for *L. interrogans*.

DISCUSSION

Some investigators (Hathaway et al., 1983a) have hypothesized that free-living host populations constitute the main source of Australis serogroup infection for swine. Ellis (1988) also suggested that voles and wood mice might act as reservoirs of serovar *muenchen* infection for pigs in England. Consequently, it is reasonable to search for small rodent hosts of serovar *bratislava* in North America. The house mouse, common in both confinement and open swine units on most farms, was considered to be a good candidate. Although *ballum* and *icterohaemorrhagiae* are the only serovars which have been isolated from house mice in North America (Galton, 1966; Sulzer, 1975), *bratislava* and several other serovars infectious for swine have been isolated from this species in Europe (Galton, 1966).

The fact that 10 mice had antibody titers $\geq$ 1:25 against both serovar *bratislava* and *icterohaemorrhagiae* was not unexpected. Concurrent antibody titers against these 2 serovars have been reported in swine and probably represented serologic cross-reactivity between the 2 serovars rather than concurrent infections (Bolin and Cassells, 1990; Ellis and Thiermann, 1986). Thus, infections of mice with serovar *icterohaemorrhagiae* might have accounted for some of the antibody titers against serovar *bratislava*. Farms 9 and 10 are good examples of where this might have occured. Norway rats (*Rattus norvegicus*), the main reservoir host of serovar *icterohaemorrhagiae* (Roth, 1964), were abundant on these 2 farms.

Leptospira interrogans serovar *ballum* has commonly been isolated from house

mice throughout the United States (Brown and Gorman, 1960; Clark, 1961; Ferris et al., 1961; Schnurrenberger et al., 1970; Yager et al., 1953). Thus, *ballum* represented a potentially significant source of false positive reactions in tests for other serovars, including *bratislava*. In this study, however, only 4 of 43 mice seropositive for *bratislava* in the initial survey were also seropositive for serovar *ballum*. Thus, serovar *ballum* did not appear to be the source of a significant number of *bratislava* positives.

MAT results provided evidence that serovar *bratislava* infection has occured in mice on at least some farms, particularly farms 6, 8, and 14. Although leptospiral antibody titers found in serologic surveys must always be regarded as only "potentially serotype indicative" (Hathaway et al., 1983b), the presence of *bratislava* antibody titers without serologic reactions to other serovars is highly suggestive of infection.

Positive FAT results were also supportive of infection with serovar *bratislava*. As is the case with the MAT, FAT results are suggestive, not definitive, evidence of infection with serovar *bratislava*. That is, a diagnosis of leptospirosis can be made with confidence on the basis of a positive FAT, but identification at the serovar level is difficult with the currently available conjugates (Bolin and Cassells, 1990). The positive FAT on kidneys of the seronegative mice is not necessarily contradictory; leptospires have frequently been isolated from rodents seronegative for the homologous serovar (Schnurrenberger et al., 1970; Torten, 1979). Negative culture results on kidneys with positive FAT could be explained by the fastidious

nature of *bratislava*, the presence of low numbers and/or nonviable leptospires, or inhibition by antimicrobials in swine feed eaten by mice.

The results of the present study may be interpreted as suggesting that house mice living on Iowa swine farms might be infected with *L. interrogans* serovar *bratislava*. Confirmation of this will require the isolation of the organism from house mice. The results of this study and the known association of specific Australis serogroup members with small rodents in Europe indicate that further study of house mice and other small rodents to assess their role as possible hosts of serovar *bratislava* infections in North America is warranted.

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SECTION IV. A SURVEY OF SWINE AND FREE-LIVING SPECIES ON IOWA FARMS FOR ANTIBODIES AGAINST ENCEPHALOMYOCARDITIS VIRUS

SUMMARY

Swine and free-living non-porcine species from 20 Iowa swine farms were surveyed for antibodies against encephalomyocarditis virus (EMCV). The microtitration serum neutralization test was used, and antibody titers $\geq$ 1:8 were considered positive. The prevalence of antibodies in sows was analyzed for association with various swine management practices and farm characteristics.

The overall prevalence in 267 sows was 37.8 percent, and the prevalence on individual farms ranged from 20 to 86 percent. The prevalence in sows maintained in total confinement was significantly lower than in sows not maintained in total confinement ($P = 0.01$). Prevalence in sows was not associated with size of breeding herd, average parity of breeding females, estimated abundance of rats, estimated abundance of mice, residence on farms where rats were captured, or any combination of these factors.

Free-living non-porcine animals tested included 74 domestic cats (*Felis domestica*), 203 house mice (*Mus musculus*), 15 mice of the genus *Peromyscus*, 9 Norway rats (*Rattus norvegicus*), 34 opossums (*Didelphis virginiana*), 14 raccoons (*Procyon lotor*), and 7 striped skunks (*Mephitis mephitis*). Of these, the only seropositive animals were 2 domestic cats.

The results of this study failed to implicate the free-living species surveyed as important reservoirs of EMCV for swine, and suggested that swine and/or species not included in this survey are the main reservoir of EMCV for swine in Iowa.

INTRODUCTION

Encephalomyocarditis virus (EMCV) infections in swine have been found throughout the world, and numerous outbreaks of EMCV-induced clinical disease in swine herds have been described (Acland et al., 1970; Acland and Littlejohns, 1975; Gainer and Murchison, 1961; Hill et al., 1985; Kovatch et al., 1969; Mercy et al., 1988; Murnane et al., 1960; Ramos et al., 1982; Roehe et al., 1985; Sanford et al., 1989; Seaman et al., 1986; Sutherland, 1977). In the last decade, EMCV has also been implicated as a cause of reproductive failure in swine (Gomez et al., 1982; Joo et al., 1988; Kim et al., 1989; Links et al., 1986; Littlejohns, 1984; Love and Grewal, 1986; Mercy et al., 1988). In the United States, EMCV has been isolated from swine in Florida, Georgia, Hawaii, Maryland, and Minnesota, and serosurvey results have indicated that EMCV infection in swine is widespread in Iowa (Zimmerman, 1991).

The epidemiology of EMCV is not well understood. EMCV has been isolated from a large number of species of mammals and wild birds, but a reservoir host has not been unequivocably identified (Acha and Szyfres, 1987). Rodents, especially the genus *Rattus*, have been considered by some as the natural reservoir of EMCV and the direct source of infection for swine (Acland, 1989; Acland and Littlejohns, 1986; Boulton, 1984). EMCV has also been isolated from a raccoon (*Procyon lotor*) (Gainer and Bigler, 1967) and an opossum (*Didelphis virginiana*) (Wells and Gutter,

1986) in areas of clinical outbreaks of EMCV, and antibodies against EMCV have been found in domestic cats (*Felis domestica*) (Tesh and Wallace, 1978).

The objective of the present study was to improve current knowledge of the epidemiology of EMCV infections in swine on Iowa swine farms. Specific aims were to: **1.** determine the prevalence of EMCV infections in swine, **2.** identify any association between prevalence in swine and management practices or farm characteristics, including rodent abundance, and **3.** determine the prevalence of EMCV infections in free-living non-porcine species living in close association with swine with the purpose of identifying potential reservoirs of EMCV for swine.

MATERIALS AND METHODS

Design of study

Rodents, cats, and wildlife species that live in, or in close proximity to, facilities used to house swine or store their feed were live-captured for collection of a blood sample. Blood samples were also collected from 15 home-raised, multiparous sows per farm, if practical. If not home-raised, sows that had inhabited that farm the longest were sampled. From the blood samples, a determination of the prevalence of antibodies against EMCV was made for each species on each farm.

Farmers were asked to complete a questionaire to obtain information about the size and average parity of the breeding herd, principal operation type, confinement status of swine by life stage, and frequency of observation (never, occasionally, or frequently) of rats, mice, and wildlife on the premises.

Study farms

Twenty swine farms in the central Iowa counties of Boone, Hamilton, and Story were surveyed. One farm was strictly a feed-to-finish operation and thus no sows were available for sampling. For the other 19 herds, the mean number of breeding females was 169, with a range of 12 to 550. There were 7 herds of 1-100 breeding females, 7 herds of 101-200, and 5 herds of more than 200. The average parity of breeding females on the 16 farms where this information was available

ranged from 1.35 to 7.00 (mean = 3.00). The degree of swine confinement varied from total and continuous confinement of all life stages to never having any of the life stages totally and continuously confined. None of the herds under study had been vaccinated for EMCV.

Animal sampling

Rodents were live-trapped using Sherman[1] and Ketch-all[2] live traps set primarily within and around the periphery of swine housing and feed storage facilities. Sherman traps were baited with a mixture of peanut butter and oatmeal flakes, checked daily, and rebaited as needed. Ketch-all traps were not baited. Mice were transferred from the traps into plastic cages with wire covers for transport to the Iowa State University College of Veterinary Medicine. Rats were transported in the individual traps and anesthetized by placing a chloroform-saturated cotton ball in the trap before blood collection was carried out. Blood was collected from rodents via orbital sinus puncture (mice) or jugular vein puncture (rats). Rodents were euthanitized by cervical dislocation after blood collection.

Cats and wildlife species, including opossums, raccoons, and striped skunks (*Mephitis mephitis*), were captured using Tomahawk[3] live traps baited with canned cat food. These animals were immobilized with an intramuscular injection of a

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[1]H. B. Sherman Traps Inc., Rt. 22, Box 365, Tallahassee, FL 32304.

[2]Kness Mfg. Co. Inc., Albia, IA 52531.

[3]Tomahawk, WI 54487.

mixture of approximately 20 mg/kg ketamine hydrochloride[4] and 0.2 mg/kg acepromazine maleate[5] administered with a pole syringe[6]. Approximately 5 ml of blood was collected via cardiac puncture. Animals were then released and monitered for recovery.

Serology

Serum samples were assayed for the presence of antibodies against EMCV by the microtitration serum neutralization (SN) test, as previously described (Zimmerman, 1990). The EMC-NVSL strain[7] was used as the challenge virus in the SN test. The challenge virus concentration for the SN test was determined to be 421 tissue culture infectious $dose_{50}$ ($TCID_{50}$). Antibody titers $\geq$ 1:8 were considered positive for this study, with an estimated specificity of 95.7 percent and sensitivity of 97.8 percent at this dilution (Zimmerman et al., 1990). Analyses were also performed using an antibody titer of 1:16 as the cutoff. Zimmerman et al. (1990) reported an estimated specificity of 100 percent and a sensitivity of 93.9 percent at this dilution.

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[4]Ketaset, Bristol Laboratories, Syracuse, NY 13210.

[5]PromAce, Ayerst Laboratories, Inc., New York City, NY 10017.

[6]Safe-T-Flex Pole Syringe, Kane Enterprises Ag-Tek Division, Sioux Falls, SD 57101.

[7]US Department of Agriculture, National Veterinary Services Laboratories, Ames, IA 50010.

Analysis of data

Prevalence in sows was compared to prevalence in each non-swine species on a farm basis in order to identify associations. Prevalence in sows was also analyzed for association with confinement status of swine, breeding herd size, average parity of breeding female swine, estimated abundance of rats, estimated abundance of mice, residence on farms where rats were captured, or a combination of the above factors.

Comparisons were accomplished using Fisher exact test, Yates corrected chi-square test, correlation analysis, or logistic regression analysis, depending on the sample size of the species involved, the nature of the variables examined, and the number of variables examined.

RESULTS

Results of the serosurvey by species, based on an antibody titer cutoff of $\geq$ 1:8, are summarized in Table 1. The overall prevalence of EMCV infection in sows was 37.8 percent. Prevalence in sows on individual farms ranged from 20 percent to 86 percent, and 15 of the 19 farms had a prevalence of 20 percent to 40 percent. The prevalence in sows maintained in total confinement (20.0%) was significantly lower ($P = 0.01$) than the prevalence in sows not maintained in total confinement (41.4%) (Table 2). There was no identifiable association of prevalence in sows with breeding herd size, average parity of breeding females, estimated abundance of rats, estimated abundance of mice, residence on farms where rats were captured, or any combination of the above.

Using an antibody titer of 1:16 as the cutoff, the overall prevalence in sows was 25.1 percent, with a range of 7 percent to 50 percent on individual farms. Other results, and the conclusions drawn from them, remained the same as for results using a cutoff of 1:8. Even at a cutoff of 1:32, all herds would have been designated as infected with EMCV, with an overall prevalence in sows of 15.7 percent. At a cutoff of 1:64, only 2 herds would not have been designated as infected, with an overall prevalence of 10.5 percent. Tables 3 and 4 give the distribution of antibody titers on each farm and prevalence using different antibody titer cutoffs, respectively.

The true prevalence, a more accurate estimate of the level of infection in a

Table 1. Encephalomyocarditis serology: number and percentage of animals seropositive (antibody titer $\geq$ 1:8) by the microtitration serum neutralization test, by species

Species	Number Tested	Number Positive	Percent Positive
Swine *-Sus scrofa*	267	101	37.8
Domestic Cat *-Felis domestica*	74	2	2.7
House Mouse *-Mus musculus*	203	0	0
Peromyscus species mice	15	0	0
Norway Rat *-Rattus norvegicus*	9	0	0
Opossum *-Didelphis virginiana*	34	0	0
Raccoon *-Procyon lotor*	14	0	0
Striped Skunk *-Mephitis mephitis*	7	0	0

Table 2. Prevalence of EMCV in sows from Iowa

	Prevalence	
Sow Category	Frequency[a]	% (95% Confidence Interval[c])
Cumulative	101/267	37.8 (31.9 to 43.7)
Confined	9/45[b]	20.0 (8.1 to 31.9)
Nonconfined	92/222	41.4 (34.8 to 48.0)

[a]Number seropositive (antibody titer $\geq$ 1:8) over total number tested.
[b]Significantly different from nonconfined sows (P = 0.01).
[c]Galen and Gambino, 1975.

Table 3. Distribution of antibody titers against EMCV in sows

Farm	# of sows sampled	# of sows with a titer of the reciprocal of the following:								
		<2	2	4	8	16	32	64	128	≥256
1	15	4	3	3	1	-	2	1	1	-
2	7	-	-	1	3	2	-	1	-	-
3	15	6	2	1	2	2	-	1	1	-
4	15	4	3	3	3	-	-	-	-	2
5	15	9	-	1	1	3	1	-	-	-
6	15	7	1	2	3	-	1	1	-	-
7	14	5	1	3	3	1	-	-	1	-
8	11	4	1	2	-	2	-	1	1	-
10	15	4	2	4	1	2	1	1	-	-
11	15	11	1	-	2	-	-	-	-	1
12	15	5	1	-	3	3	2	-	1	-
13	15	10	-	2	-	1	-	-	-	2
14	15	7	-	2	1	2	2	1	-	-
15	15	7	1	4	1	-	1	1	-	-
16	15	7	-	3	-	2	-	1	1	1
17	15	9	-	-	2	2	1	1	-	-
18	15	4	-	-	5	2	1	1	1	1
19	10	4	-	1	-	1	1	2	-	1
20	15	7	1	3	3	-	1	-	-	-
Total	267	114	17	35	34	25	14	13	7	8

Table 4. Prevalence of EMCV at different antibody titer cutoffs

	Herds		Animals	
Cutoff	Frequency[a]	Percent	Frequency[a]	Percent
≥ 1:8	19/19	100.0	101/267	37.8
≥ 1:16	19/19	100.0	67/267	25.1
≥ 1:32	19/19	100.0	42/267	15.7
≥ 1:64	17/19	89.5	28/267	10.5
≥ 1:128	10/19	52.6	15/267	5.6

[a]Number positive over total number tested.

population, can be calculated using the sensitivity and specificity of a test (Thrushfield, 1986). At the $\geq$ 1:8 cutoff and using the sensitivity of 97.8 percent and specificity of 95.7 percent reported for the microtitration serum neutralization test (Zimmerman et al., 1990), the true prevalence of infection in sows was 35.8 percent.

Mice were observed by farmers frequently on 14 farms and occasionally on 6 farms. Rats were observed frequently on 5 farms, occasionally on 13 farms, and never on 2 farms. Mice were captured on all 20 farms, but rats were captured on only 7 farms. Free-living non-porcine animals tested included 74 domestic cats, 203 house mice (*Mus musculus*), 15 mice of the genus *Peromyscus*, 9 Norway rats (*Rattus norvegicus*), 34 opossums, 14 raccoons, and 7 striped skunks. Of these, only 2 domestic cats were seropositive, one with an antibody titer of 1:16 and one with an antibody titer of 1:32.

DISCUSSION

Zimmerman et al. (1991) reported a prevalence of 17.2 percent in Iowa sows and gilts, and concluded that in excess of 90 percent of Iowa swine herds are infected with EMCV. The results of the present study confirm that EMCV infection is widespread in swine in Iowa. Using an antibody titer cutoff of 1:8, every herd sampled (n = 19) was infected and had a minimum prevalence of 20 percent. Prevalence estimates at higher antibody titer cutoffs were included to address questions about the specificity of the test for natural infections. These analyses showed that EMCV infection in sows was prevalent even when these higher antibody titer cutoffs were used.

The higher overall prevalence found in the present study (37.8%) vs. the Zimmerman et al. study may be due to the age distribution of animals sampled. Zimmerman et al. (1991) found that prevalence of EMCV infection increases with age. They sampled gilts as well as sows, whereas the present study sampled the oldest sows on each farm.

The significantly lower prevalence in confined sows vs. nonconfined sows in the study is in conflict with Zimmerman et al. (1991), who found the opposite to be true. An explanation for this was not evident, but may be due to the difficulty in defining "confinement" and obtaining accurate sow life histories.

This study represented a valuable opportunity to identify non-porcine species of potential importance in the epidemiology of EMCV infections of swine. EMCV

infection was present in all of the swine herds studied, and at least 7 species of free-living mammals representing 3 orders (*Rodentia*, *Carnivora*, and *Marsupiala*) lived in close association with swine on these farms (Table 1).

Rodents were of particular concern in this study because of their implication by some investigators as the main source of infection for swine (Acland, 1989; Acland and Littlejohns, 1986; Boulton, 1984). Support for this hypothesis includes the frequent detection of infection in wild rodents through serosurveys and virus isolation (Adamacova and Bardos, 1959; Bardos, 1957; Causey et al., 1962; Dick, 1953; Gainer and Bigler, 1967; Ghosh and Rajagopalan, 1973; Heredia et al., 1982; Jonkers, 1961; Paul et al., 1968; Pope, 1959; Pope and Scott, 1960; Tesh and Wallace, 1978; Vizoso and Hay, 1964; Warren et al., 1949) and the presence of large numbers of rodents and/or EMCV-infected rodents in association with clinical outbreaks of EMCV in swine (Acland et al., 1970; Boulton, 1984; Gainer et al., 1968; Hill et al, 1985; Kovatch et al., 1969; Love and Grewal, 1986; Mercy et al., 1988; Murnane et al., 1960; Ramos et al., 1983; Sanford et al., 1989; Seaman et al., 1986). However, a chronic intestinal carrier state has not been demonstrated in rodents, and other investigators thus contend that infected rodents are only indicators of EMCV activity rather than reservoir hosts (Adamcova and Bardos, 1959; Kilham et al., 1956; Tesh and Wallace, 1978).

The results of the present study failed to show evidence of EMCV infection in any of the rodents or other free-living species captured in the proximity of swine facilities, except for 2 seropositive domestic cats. The sample size of each free-

living species was thought to be indicative of the relative abundance of each species on the swine farms, excluding rats. Rats were present on most of the study farms but could not be captured efficiently with the live-traps used. However, the nine rats that were caught were seronegative for EMCV. Also, prevalence of EMCV virus in sows was not associated with abundance estimates of rats and/or mice. Thus, results of this study provided evidence that the free-living species surveyed in this study are not important reservoirs of EMCV for Iowa swine. By implication, swine themselves, or some species not yet identified, are the reservoir hosts of EMCV in Iowa.

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GENERAL SUMMARY

This thesis hopefully has contributed in a significant way to the knowledge on the role of synanthropic mammals, particularly rodents, in the epidemiology of infectious agents of swine. A review of the literature established that rodents are of known or potential significance in the epidemiology of a variety of these infectious agents, ranging from those that are primarily disease agents of swine to those agents for which swine are implicated as reservoirs for human beings. For most of these agents, however, relatively little has been done to specifically investigate the significance of rodents and other synanthropic mammals as disease carriers on swine farms, particularly in recent years. It was this fact that prompted the studies reported in this thesis.

Evidence reported in this thesis suggested that, except for cats, synanthropic mammals are not important sources of *Toxoplasma gondii* for swine in Iowa. Synanthropic mammals also could not be implicated as important sources of encephalomyocarditis virus for swine in Iowa. This study did, however, raise the possibility that the house mouse may be a reservoir host for *Leptospira interrogans* serovar *bratislava*, which has not previously been reported.

Although the field studies reported in this thesis did not totally bear it out, the potential significance of synanthropic mammals in the epidemiology of infectious agents of swine remains. Even if swine themselves are clearly the primary reservoir of particular agents for other swine, rodents may have significance in the eradication

of these agents. It is the hope of this author that the manuscripts in this thesis will prompt investigators to at least consider synanthropic mammals when investigating the epidemiology of infectious agents of swine.

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