THESIS

EPIDEMIOLOGY OF REPORTED SCRAPIE IN THE UNITED STATES: 1947-1991

Submitted by
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WE HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER OUR SUPERVISION BY NORA E. WINELAND ENTITLED EPIDEMIOLOGY OF REPORTED SCRAPIE IN THE UNITED STATES: 1947 - 1991 BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE.

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ABSTRACT OF THESIS

EPIDEMIOLOGY OF REPORTED SCRAPIE IN THE UNITED STATES: 1947 - 1991

Data collected in support of the United States Department of Agriculture (USDA) scrapie eradication program between 1947 and September 30, 1991 were evaluated to determine the presence of trends or patterns which might help further the understanding of natural sheep scrapie. The USDA records from 957 confirmed positive cases of natural scrapie in 581 flocks from 39 states were reviewed and compiled into a database. Possible host and management risk factors for scrapie such as age at death, within-flock mortality, breed, sex, sire and dam disease status, flock size, and location were examined.

There were several significant findings from the study. The proportion of reported positive flocks in those states reporting positive cases showed a steady increase between 1965 and 1991. In addition, the average flock mortality showed a slight increase between 1947 and 1991. These increases did not seem to be directly related to any changes in the USDA eradication program.

The average age at death for confirmed cases was 43.6 months. Rams died of scrapie an average of five months younger than did the ewes. This difference was statistically significant, but likely due to the small numbers of rams included in the study. There were insufficient numbers of twins (26 pairs) to allow any significant conclusions to be drawn. There were no statistically significant differences between age at death for the eight geographical regions or the various sheep breeds affected. The Suffolk breed comprised 88% of the reported cases, and Hampshire sheep accounted for 6% of the cases.

Attempts were made to further define the role of vertical transmission in natural scrapie. The scrapie disease status of the sire had no appreciable effect on the age of death of positive offspring. The scrapie disease status of the dam had a detectable effect with positive offspring from positive dams diagnosed at a significantly younger age than positive offspring from other dams. Unfortunately it was not possible to determine when a positive dam might begin shedding the scrapie agent and consequently present a threat to her offspring. All of the positive dams in the study gave birth to their positive offspring in flocks where there were other active cases of scrapie which might have been the source of infection for the offspring.

The source of infection could not be determined for over half of the reported cases. Several possible explanations for this situation were presented. Failure to detect the sources of infection may in part be responsible for the apparent increase in the magnitude of the scrapie problem in the United States.

Data quality and consistency was a major issue for this study. The records available from the technical program staff of USDA contained varying amounts of information about each of the positive animals and flocks. In addition to variation in the records, the eradication program itself went through several phases during the study period. These different phases may have had multiple effects on the levels of disease reported to USDA. Unfortunately these effects could not be measured or corrected for in the analysis.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Scrapie is not a new disease. It has been recognized for literally hundreds of years. Despite the fact that scrapie has been around since at least 1750 AD when it was recognized and recorded in four breeds of sheep in Britain[50], there is remarkably little known about its etiology, transmission, and control. It belongs to a group of diseases known as the transmissible spongiform encephalopathies. Recently there is increased cause for concern about this disease with the discovery of Bovine Spongiform Encephalopathy (BSE) in the United Kingdom. BSE is thought to be the first instance where scrapie has crossed the species barrier in a "natural" setting[44], although attempts to document this occurrence have not been completed[20]. Transmissible Mink Encephalopathy (TME) is a transmissible spongiform encephalopathy seen in ranch mink and has been postulated to develop as a result of feeding either scrapied sheep carcasses or downer cattle carcasses to the mink. Experimental studies have been unable to completely confirm either type of transmission[2,30,31]. Chronic wasting disease of captive mule deer and elk also shares the common classification of transmissible spongiform encephalopathy[1,63,64]. There are three similar diseases seen in humans: Kuru, Creutzfeldt-Jakob disease (CJD), and Gerstmann-Straussler Syndrome (GSS). Of the three, CJD is the most similar to scrapie with both a genetic component and an infectious component[6,17] similar in nature to the patterns observed with sheep scrapie. To date, attempts to link the human spongiform encephalopathies with scrapie have not been successful[18].

CLINICAL PRESENTATION

Scrapie presents as a slowly progressive neurologic disorder in sheep 30 - 60 months (range 18 - 142 months) of age[32]. The clinical signs in individual animals are somewhat variable. The signs are non-specific and yet consistent throughout the reports in the literature[50]. The disease has an insidious onset with animals remaining afebrile throughout the course of the disease. Behavioral changes may be as subtle as an animal which lags behind the rest of the flock or an animal that is startled sooner by the approach of the shepherd. These initial signs worsen over a course of several weeks to several months. Later in the course of the disease, many affected animals will show wool loss as a result of rubbing and poor body condition despite normal appetites. They may also exhibit a stiff and somewhat uncoordinated gait. A "nibbling reflex" may be elicited in some animals by scratching the dorsal lumbar region. The affected sheep will extend its neck and make nibbling movements of the lips in response to the scratching[35,60].

DIAGNOSIS AND TREATMENT

Since the only reliable diagnostic tests for scrapie are conducted postmortem, diagnosis is rarely attempted until all other differential diagnoses have been ruled out. Differentials include external parasites, listeriosis, pregnancy toxemia, and any other disease capable of producing pruritus, chronic wasting, or central nervous system (CNS) signs. After these differentials have been ruled out, diagnosis is made by histologic examination of the brain with a finding of cytoplasmic vacuolation of neurons, extensive gliosis, status spongiosis, and neuronal degeneration[11]. Failure to detect all of these conditions results in a histologic diagnosis of suggestive, according to standards used by the United States Department of Agriculture (USDA), and may only mean that the disease is not sufficiently advanced to show the characteristic neuronal lesions[61]. In suggestive cases, the only USDA

accepted way to definitively diagnose scrapie is to conduct a mouse inoculation study which may take up to two years to complete. There are several other diagnostic test protocols[27] used in research which have not been approved as official tests in the United States, but are currently undergoing approval.

Attempts to find effective therapeutic agents have not been successful. Amphotericin B lengthened the incubation period in experimentally inoculated hamsters but had no effect on clinically affected animals[55]. There are a few reports in the literature suggesting recovery may be possible[54]. With no diagnostic test suitable for live animals, it is difficult to determine if these "recovered" animals were true cases of scrapie. Despite these reported recoveries, scrapie is generally regarded as a fatal disease.

HISTORY OF THE DISEASE AND THE UNITED STATES ERADICATION PROGRAM

With such a lengthy history for scrapie, it is worthwhile to examine the historical progression of knowledge about the disease in so much as it is documented. As one would expect with a disease of this nature, clinical descriptions of affected animals and descriptions of the initial attempts to understand and diagnose the disease were the first published information about scrapie. In a review of the literature, it becomes apparent that there are many missing pieces to the epidemiological puzzle. H. B. Parry spent considerable time compiling a complete history of sheep domestication in north-western Europe as well as a complete history of the occurrence of scrapie. Parry undertook detailing this history in the late 1950's because people believed scrapie prevalence was linked with various breeds and genotypes.

Prior to the Roman Empire, the sheep kept were small, horned, pigmented hairy breeds. Through each phase of occupation, the movements of people were paralleled with sheep movements. Around 1000 AD, there was evidence of sheep movements from France and Spain into Britain. By

the 17th century there were two main groups of sheep in Britain, the white-faced and the black-faced breeds. Through all of the intermingling of new breeds, some of the indigenous hill breeds such as the Herdwick remained genetically untouched[50].

According to Parry, the 18th century was spent developing and refining many of the sheep breeds which are still being raised today. There was considerable effort to improve wool quality and it is from these efforts that our present sheep populations are derived. In reviewing the sketchy information available prior to the 18th century, Parry concluded that the present British sheep population was derived from four main sources: small brown-fleeced Soay sheep, Roman origin white-faced hornless stocks, Danish origin black-faced hairy horned breeds, and Spanish fine wooled merinos. Both the Soay and the Roman origin white-faced sheep have evolved into breeds not initially thought to be susceptible to scrapie. They were the ancestors of the South West horned types, and the Ryeland, Romney, Cotswold, Leicester, Lincoln, and Teeswater breeds. The Danish origin sheep breeds, also known as Heath sheep, gave rise to the Norfolk Horn, Scottish Blackface, Swaledale, and other speckled-face breeds. The Spanish merinos were not introduced until after 1760. Despite the fact that scrapie was reported prior to 1760 and the merino is not considered a predominant contributor to the genetic pool, it is often blamed for bringing scrapie to the United Kingdom[50].

Prior to 1800, scrapie-like symptoms were noted in the Norfolk Horn, Wiltshire Horn, Dorset Horn, and Hampshire Down breeds in Britain[50]. At this point, the disease was considered incurable and unpredictable and was thought to be either infectious or hereditary. In the 1800's the Cheviot, Border Leicester, and crosses of these two breeds were noted to have scrapie. In the early 1900's (prior to 1950), the Scottish Blackface, Oxford Down and Suffolk breeds began to contract scrapie for the first time. These last two breeds were the result of crosses between

existing breeds which had previously exhibited scrapie. It has been mentioned that the introduction of the Spanish merino was followed by an increase in the incidence of scrapie, making the merino a likely culprit[50]. This does not explain the seemingly high prevalence of scrapie in the Suffolk breed which was created by crossing a Southdown male and a Norfolk Horn female.

Scrapie was first seen in North America in 1938 where it occurred in a Suffolk animal in Ontario, Canada that had originated from the United Kingdom. Most of the early occurrences of the disease in the United States could be linked to Suffolk sheep imported from England as could many other instances of the disease outside Europe. Sheep imported into New Zealand[4,5] and Australia[9] that were diagnosed with scrapie during the early part of the twentieth century also came from England.

After its first recognition in Canada in 1938, scrapie appeared in Michigan in 1947 and was attributed to Suffolk sheep purchased from Canada and from England[29,50]. At this point, there was no scrapie eradication or control program in effect in the United States, and flock owners were left to handle the problem on their own. In 1952, scrapie was diagnosed in a California flock and a second Michigan flock and traced to a flock in Canada. The United States Animal Health Association requested that the Secretary of Agriculture (USDA) declare a state of emergency to eradicate the disease[3]. Initial eradication steps focused on quarantine and slaughter of the affected flocks and the animals sold from these flocks. Indemnity was paid to the owners of animals destroyed because of scrapie[29].

In 1957, the intensity of the eradication program was increased as source flocks and exposed animals were also made eligible for indemnity[29]. Source flocks were defined as flocks from which scrapied animals had been removed within 18 months prior to showing clinical signs. Exposed animals were those that scrapied animals or their progeny had contacted. In 1965, the focus of the program shifted to include an option for

bloodline depopulation as a result of the theory that certain relatives of scrapied animals were more likely to contract the disease than others. These bloodline animals included the progeny, sire, dam, and all the siblings of the affected animal. This theory was based largely upon the work that Parry and his colleagues had conducted in England and presented at an international conference in Washington, D.C. in 1964[29].

By the mid-1960's, the Suffolk breed was no longer the only breed contracting the disease in the United States. Initially the non-Suffolk animals affected in the United States were found to have been associated with positive Suffolk flocks. In addition, people began to notice the disease spreading to non-bloodline animals in severely affected flocks. In 1975, the focus of the program shifted again in response to new evidence supporting the possibility of lateral transmission and the bloodline option was eliminated[29]. Some of the research supporting these shifts in focus was occurring in the United States at the Scrapie Field Trial facility in Mission Texas[32].

In 1983, the scrapie eradication program was scaled back to providing indemnity payments for bloodline animals only. The eradication program also included an optional 42 month surveillance program. Complete flock depopulation was used if there was evidence of non-bloodline involvement, and if complete depopulation would be more cost beneficial to USDA than continued surveillance of the flock[29]. This was due in part to lack of funding and in part due to industry pressure and concern that valuable bloodlines were being destroyed needlessly. Producers felt that there had been no apparent progress in the eradication of scrapie despite the fact that they had been under an eradication program for over thirty years.

The United States Animal Health Association requested that USDA's Animal Plant Health Inspection Service (USDA-APHIS) review the effectiveness and scientific merit of the scrapie eradication program in 1985. After several meetings over the course of three years, the final

recommendation was to abandon the program. Notice of intent to discontinue the program was published in the Federal Register in 1988. The notice was met with overwhelming comments in favor of adopting a new scrapie program rather than abandoning the existing program.

The scrapie eradication program remained in effect, unchanged and poorly financed. A negotiated rulemaking committee was formed in 1990 and charged with devising a more effective and workable scrapie program. This committee was composed of representatives from industry, university researchers, and government regulatory officials. The committee spent considerable time discussing options for effectively decreasing the risk of scrapie in United States sheep flocks. They looked at information generated by various researchers and discussed how best to approach the hoped for goal of eradication. The product of this committee was published as a proposed voluntary flock certification program, similar in concept to those already in effect for brucellosis and pseudorabies[29,62]. Following the appropriate comment periods, regulations for the new scrapie program were published as a final rule with an effective date of October 1, 1992.

EPIDEMIOLOGIC UNKNOWNS

Progress in the scrapie eradication efforts in the United States has been slow at best. The scientific reviews conducted in the 1980's were quick to point out the lack of scientific merit in the scrapie program. These reviews cited lack of specific epidemiologic information about scrapie and its behavior under field conditions in the United States. There are two largely unknown aspects of the epidemiology of scrapie. The first involves the nature of the etiologic agent. Despite being such an old disease, repeated attempts to isolate and identify the cause of this condition have not been successful[40]. The second unknown is the exact mode of transmission of the disease. As a result, there are

several theories regarding the nature of the agent and the mechanism for transmission of the disease.

NATURE OF THE SCRAPIE AGENT

There are several schools of thought on the cause of scrapie that range from a completely hereditary disorder to a completely infectious condition. The truth most likely lies somewhere in the middle and includes genetic as well as infectious attributes. Suffice it to say that the exact cause of scrapie has eluded scientists for several hundred years. There are currently four or five controversial theories concerning the exact nature of the scrapie agent. It is postulated to be either a prion, a nonconventional virus, a viroid, a virino, or a genetically controlled disease[38,41,56,57]. Supporters of each of these theories are quick to point out specific evidence about the disease which can be used to explain observed patterns under their theory[33,36]. To date, there has not been sufficient evidence disclosed to completely refute any of these theories.

NATURAL SCRAPIE

The mechanism for transmission of scrapie under natural conditions remains a puzzle. Various excretions and secretions from infected animals have been used in an attempt to experimentally transmit the disease[54]. After several repeated attempts, Pattison and colleagues were able to produce scrapie in sheep by feeding them fetal membranes from affected sheep[52,53]. Transmission appeared to occur much more effectively if the oral cavity of the susceptible animal was scarified[10].

In the United States, disease transmission studies have been conducted at the Scrapie Field Trial facilities in Mission, Texas. Although these trials did involve natural scrapie, they were conducted under what likely were not natural conditions. Some of the sheep from flocks condemned for scrapie were transferred to Mission for further

observation and study. In many cases, only specific bloodline animals were transferred to the facilities at Mission, which may have led to the creation of an elevated level of infection in this flock as compared with naturally infected flocks. Researchers in Mission, Texas found that a higher proportion of the offspring from scrapie positive females became scrapie positive than did the offspring from scrapie positive males[22,32]. They also found that the proportion of affected offspring could be reduced by removing the offspring from affected animals at birth[32]. Taken together, these two separate observations suggest the possibility that the disease is spread at lambing time by affected females to their offspring. A further interpretation of these findings led to the hypothesis that animals diagnosed positive for scrapie at less than 54 months of age contracted the disease at or near birth, while animals diagnosed positive for scrapie at greater than 54 months of age may have contracted the disease later in life[21]. These hypotheses were based on studies conducted at Mission and were used very heavily in structuring the new voluntary flock certification program.

The role of the ram is less clear. The above studies indicate that the ram is relatively unimportant in terms of disease transmission. Kimberlin[37] suggests that the role of the ram may be genetic in nature. Evidence for the ram being responsible for introducing susceptible genotypes into flocks containing the scrapie agent and resistant sheep is anecdotal, but plausible. Dickinson and colleagues[22], also hypothesized that there was an inherited component to natural scrapie incidence as a result of their work with Scottish Blackface flocks.

Several studies and experiences have shown that premises contamination may play a role in natural scrapie transmission. Most of these studies have been experimental in nature with one exception. In efforts to eradicate scrapie from Iceland, pastures grazed by affected sheep were left vacant for several years and when new sheep were introduced, they developed scrapie. The newly introduced sheep were

brought in from a region of the country where scrapie had never been diagnosed. Officials in Iceland attributed this failure to premises contamination[48]. It may also have been due to restocking with sheep previously exposed to the scrapie agent. Following this failure in eradication, Iceland developed more stringent guidelines for cleaning the environment following removal of infected animals. They also lengthened the time period following removal of infected animals during which no sheep could be kept on the affected premises and neighboring premises. They reported greater success with this second attempt and only noted "re-infection" on those premises not strictly adhering to the established cleaning and disinfection guidelines[59].

Several reports indicate that the annual mortality due to scrapie for scrapie-affected flocks is highly variable, ranging from 3% to 50%[51,59,65]. The annual mortality due to scrapie in scrapie positive flocks in the United States has not been reported since 1964 when flocks were observed to experience mortality of 10% to 20%[65].

Reports in the literature also indicate that the average age at death within a flock starts out at four to five years of age and slowly declines to around two years of age as scrapie becomes established in the flock[15,16,29,59]. Unpublished observations of individual scrapie positive flocks in the United States also report this phenomenon[21]. A proposed explanation is offered by Foster and Dickinson[25] who postulate that increased exposure to the agent is responsible for the observed decline in age at death in infected flocks. They do not state if they believe such increased exposure to be related to premises contamination or increasing numbers of positive animals which may spread the agent directly to other animals.

The magnitude of the scrapie problem in most countries where scrapie is recognized remains unquantified. A study in Great Britain, where scrapie has not been a reportable disease until recently, attempted to determine the national prevalence of scrapie through a producer

survey[45]. Estimates resulting from this study published in 1990 showed a prevalence ranging from 17% to 34.5%. The survey was criticized for very low response rates and for utilizing producer assessment of clinical signs rather than definitive diagnostics[39]. However, the survey remains the most recent attempt to quantify the scrapie problem in any country. Scrapie is a reportable disease in most states in the United States, and is the subject of a federal regulatory control program. Based on the differences in regulatory efforts between great Britain and the United States, it could be postulated that scrapie is less prevalent in the United States than in Great Britain. There have been no published attempts to determine the prevalence of scrapie in the United States[21].

A study conducted in France and published in 1983[14] involved an assessment of diagnosed scrapie in purebred sheep during the 12-year period of 1968 to 1979. The study consisted of a series of inquiries of practicing veterinarians and government veterinarians and the figures gathered were presented in terms of infected flocks. Unfortunately, only 10% of the reported diagnoses were what could be considered confirmed cases. The denominator data used were numbers of sheep rather than flocks and the numerators were comprised of the total numbers of animals in each flock with a positive case.

EXPERIMENTAL SCRAPIE

Early transmission studies involved intracerebral inoculations of brain homogenates from clinically affected animals into apparently healthy susceptible laboratory animals[13]. The experimentally inoculated animals developed clinical disease and the characteristic lesions. Further work with these experimental animal models of scrapie led to the discovery of genes controlling the length of the incubation period in both sheep and mice[8,12]. Fraser and colleagues[26] were able to distinguish various scrapie isolates based upon differing incubation periods and the distribution of lesions within the neural tissues of mice. These

differences were postulated to be due to strain differences in the isolates. Gilmour and colleagues[28] noted differences in the presence and location of cerebral amyloidosis in scrapie infected sheep and postulated this to be a result of strain differences. In further studies, Bruce and colleagues[8] noted the possibility that various strains could mutate which further complicates matters. Those who believed scrapie to be an infectious process interpreted these findings to mean that there was an infectious particle or agent present in the brains of affected animals.

Early transmission studies were able to produce clinical scrapie in experimentally inoculated mice[13] and hamsters[34]. Those who believe the disease is completely genetic in nature dismissed these findings as experimental passage of genetic material[8]. Alternative explanations included the suggestion that these experimental animals were also genetically diseased. If scrapie is truly an infectious rather than solely a genetic disease, it would stand to reason that there might be horizontal transmission of infection between unrelated animals. Horizontal transmission of scrapie between unrelated sheep as well as between sheep and goats was demonstrated in studies conducted at the USDA Scrapie Field Trial facility in Mission, Texas[32] as well as in the United Kingdom[22] and has also been documented in a number of naturally infected flocks. Nevertheless, these findings are somewhat controversial. The genetic disease proponents argue that the "unrelated" animals had infected relatives somewhere in their pedigrees.

The possibility of premises contamination through long term survival of the scrapie agent under environmental conditions has been demonstrated by a study conducted in the United States by Brown and Gajdusek[7]. Scrapie-infected hamster brain homogenates were mixed with soil and placed in two petri dishes, one perforated and one not perforated, that were buried in a garden in the Washington, DC area for three years. This mixture was assayed at the end of three years and found infective for hamsters when inoculated intracerebrally. In

addition, soil collected from around the perforated petri dish was also found infective for hamsters.

Despite all the indications that the disease is able to spread laterally, there continues to be evidence that the disease can be genetically controlled. Millot and colleagues[42,43] found that the major histocompatibility complex in Ile-de-France sheep is linked to at least one of the genes responsible for the incubation period in these sheep. Several researchers have suggested that breeding for resistance is the best available control option[19,50]. Davis and Kimberlin worked with Swaledale sheep and found that natural selection of resistant sheep in affected flocks cause outbreaks to be self-limiting[19]. Others have noted somewhat conflicting results[25,35,46], some of which may be due only to differences in terminology. Sheep classified as resistant may in fact be infected with scrapie, but not show signs of disease until an older age due to possession of the long incubation gene.

The seemingly epidemic nature of the disease is explained as an autosomal recessive gene[23,24]. According to this theory, a single autosomal recessive gene that manifests itself after the reproductive age is responsible for scrapie expression. The epidemics seen are a result of selection for the scrapie expression gene[24]. Nussbaum and colleagues[46] suggested that the gene controlling incubation may actually be autosomal dominant.

CHAPTER 2

PURPOSE AND OBJECTIVES

PURPOSE OF THE STUDY

With the exact cause and transmission mechanisms of scrapie unknown, it has been extremely difficult to design effective prevention and control programs. This difficulty is further compounded because the early subtle signs of the disease are not detectable until rather late in the productive life of most sheep[18,35]. It is also unknown at what point and by what route the scrapie agent is shed from the body of the diseased sheep and enters susceptible sheep. Progress in determining the natural mode of transmission is further hampered by incomplete information on positive cases owing to the reluctance of many producers to acknowledge what many believe to be a genetic defect in their animals. As a result, the true prevalence of scrapie is unknown[14,21]. All of these outstanding issues point to the need for further epidemiologic investigations, particularly those aimed at determining the natural mode of transmission of the scrapie agent. With the occurrence of BSE, it is becoming increasingly important to understand the natural transmission mechanism for scrapie in order to improve the effectiveness of the scrapie control program. Much can be done in this area by analyzing existing information on reported scrapie cases rather than waiting until the agent has been identified and a live animal diagnostic test has been developed. The purpose of this study is to describe cases of scrapie in sheep reported to USDA through the scrapie control and eradication program between 1947 and 1991 in terms of host and environmental factors and to offer some

additional epidemiologic hypotheses concerning risk factors for scrapie which may warrant future investigation.

OBJECTIVES OF THE STUDY

In order to describe reported cases of scrapie, relevant information concerning these cases must first be gathered and stored in a uniform format conducive to epidemiologic analysis. The first objective of this study is to design a database for uniformly storing the pertinent facts about each reported scrapie infected flock and the individual affected animals. This information must be stored in a consistent and readily accessible manner to allow for meaningful analysis.

The second objective involves utilization of the information in the database to describe the trends and patterns of reported natural scrapie in the United States throughout the study period. A general description of reported scrapie cases and a description of any observed trends and patterns may allow the formulation of further hypotheses describing possible modes of natural scrapie transmission. The general description of reported cases and positive flocks will include the following specific items:

1. A curve depicting the number of newly reported infected flocks over the time period of 1947 through 1991.

2. Examination of the quarter of the year for diagnosis for each of eight geographic regions in the United States.

3. Determination of the likely source of infection for each of the positive cases and for each positive flock.

4. The average age at death. The average age at death will be reported by sex, breed, quarter of the year for diagnosis, and geographic region.

A third and final objective of this study is to explore possible host and management risk factors associated with scrapie in an effort to further increase the knowledge about the epidemiology of natural scrapie. The following specific hypotheses will be tested or evaluated:

1. The prevalence of scrapie in the United States is postulated to be far less than the prevalence of scrapie in the United Kingdom. The study will determine the proportion of reported positive scrapie flocks in the United States between 1947 and 1991.

2. Studies at Mission, Texas suggested that scrapie positive dams are more likely to pass the disease to their offspring than are scrapie positive sires. The data for reported scrapie cases will be examined to determine further information concerning the roles of the sire and dam in natural scrapie transmission.

3. Reports in the literature suggest that the average age at death due to scrapie declines over time in an infected flock. Observations of this phenomenon involved experimental rather than natural scrapie. The available data will be examined to determine if this pattern exists in natural scrapie.

4. If transmission occurs at birth and genotype plays a role in susceptibility, patterns of disease occurrence in twins may shed further light on the mechanisms of natural scrapie transmission. The available data will be examined to determine if twins are equally affected.

CHAPTER 3

MATERIALS AND METHODS

STUDY DESIGN

The study is designed as a retrospective, descriptive evaluation of scrapie positive sheep flocks and scrapie positive and suspicious animals reported to USDA between 1947 and September 30, 1991 through the scrapie eradication and control program. The information used in this study was abstracted from various records held by USDA in support of the scrapie eradication program. These records have been kept by the technical program staff of USDA since first official recognition of the disease in 1952.

SOURCES OF DATA AND DEFINITION OF TERMS

The number of flocks included in the study for each year is partially dependent upon the level of support for the USDA eradication program in terms of available personnel and money, in addition to being dependent on the level of agency interest in eradication. For those years when total flock depopulation was not an option due to budget or program changes (see Table 1), flock owners had less incentive to admit to having the disease and to ask for assistance. Conversely, owners may not have been willing to risk losing their entire flocks over one positive sheep during those times when total flock depopulation was the only solution available. These situations are accounted for in the analysis where possible and where known.

Between 1947 and September 30, 1991, there were a total of 581 infected flocks and 957 positive animals[58] that were reported and

Table 1. Key changes in the history of the U. S. scrapie eradication program: 1947 - 1991.

Date	Program Change/Event	Indemnity Rate
1947	First case diagnosed in Michigan flock.	N/A
November 1952	Secretary of Agriculture declares state of emergency. Laboratory diagnosis required. Infected flocks quarantined and depopulated, animals sold from infected flock slaughtered.	Grade - $25.00 Registered - $75.00
April 1957	Source flocks (defined as flocks from which confirmed positive animals removed within 18 months or less of showing signs) also quarantined and depopulated.	Grade - $25.00 Registered - $75.00
March 1965	Added option for bloodline slaughter if disease limited to one genetic line. This option included two year quarantine for non-bloodline animals. Infected flock and source flocks could still be completely depopulated if multiple bloodlines affected.	Grade - $25.00 Registered - $75.00
October 1975	Bloodline option eliminated.	Grade - $40.00 Registered - $90.00
October 1978	Change to indemnity.	2/3 Appraised value $300.00 Maximum
April 1983	Bloodline option reinstated along with bloodline surveillance program.	2/3 Appraised value $300.00 Maximum

confirmed by National Veterinary Services Laboratory (NVSL). Since data quality and the overall magnitude of the potential risk factors is unknown, examination of all the available data rather than only a sample is most apt to lead to reliable and reproducible conclusions.

It is not possible to calculate rates of disease occurrence, in terms of incidence or prevalence, within the U.S. sheep population solely from the information on infected flocks. The National Agricultural Statistic Service (NASS) conducts an annual survey to determine the numbers of sheep and operations with sheep in the United States. NASS

annual survey data is used as the denominator for calculating the proportion of positive flocks within the United States.

Infected flocks are those from which a positive histologic or mouse inoculation diagnosis has been made by NVSL. Although the definition of source and trace flocks has varied over the period of this study, for purposes of analysis, the new definitions of source and trace flocks that are specified in the voluntary scrapie flock certification program[62] will be used. Under the new program, a trace flock is one in which one animal was born and later diagnosed with scrapie at less than 54 months of age. Trace flocks are reclassified as source flocks when a second animal is born into them and is diagnosed scrapie positive at less than 54 months of age. The second animal must be diagnosed within five years of the first animal in order to classify the flock as a source flock.

Animals were classified as either clinically negative or clinically affected. Clinically affected animals include all of those noted to exhibit signs suggestive of scrapie. Animals were determined to be clinically affected based on any mention in the records of chronic debilitating disease or neurologic disorders of unexplained origin, in addition to any references to signs suggestive of scrapie without specific mention of the signs involved. Clinically negative animals include all of those for which no signs suggestive of scrapie were noted in the record. Based upon laboratory findings, clinically affected animals were classified as either negative, suggestive, or positive according to USDA accepted criteria.

CRITERIA FOR INCLUSION

In order to be included in the study, animals must either originate from or have been a member of an infected flock, be diagnosed as scrapie positive or suspicious, or be the sire or dam of a scrapie positive or suspicious animal. The histologic or mouse inoculation-based diagnosis

of scrapie must have been made or confirmed by NVSL between 1947 and September 30, 1991 for inclusion in the study.

STUDY FACTORS

Available USDA records were examined to determine factors to include in the analysis of scrapie positive flocks and cases. Host and management factors selected at both the flock level and the individual animal level were available for most of the flocks throughout the study period. A sample of records from the different time periods of the eradication program was selected and examined for availability of information. Due to changes in personnel at the technical program staff of USDA and due to changes in the eradication program, the format and amount of information available varied with time. A discussion of each of the selected factors as well as an explanation of the reason for including each of them in the study follows.

Flock Factors

Flock factors are those elements pertaining to the flock as a whole and include the following:

1. Number of sheep. The number of sheep in the flock reflects the number of animals in the flock at the time of diagnosis of the first positive case and is used to calculate the reported within-flock mortality. In the case of depopulated flocks, within-flock mortality rates are calculated based only on the number of confirmed positive cases discovered prior to the first flock depopulation. Replacement flocks which became infected following the complete depopulation of an infected flock presented some difficulties and were not included in the within-flock mortality rate calculations for two reasons. First, accurate inventories of the repopulated animals were unknown or not routinely recorded.

Second, flock mortality rates for repopulated flocks would be greatly affected by the source of repopulated animals and the possibility of premises contamination. If premises contamination plays a heavy role as suggested by experiences in Iceland[59], these flocks could bias the observed mortality rates and potentially overshadow any trends that might be occurring over time.

2. Location of the flock. Flock locations are examined for the possibility of geographical differences that may help explain possible modes of transmission. For purposes of geographic differences, the United States is divided into eight regions where climatic conditions and flock management schemes are relatively homogenous[49]. These eight geographic regions are depicted in Figure 1.

3. Status of the flock. The status of the flock refers to whether the flock is a source, trace, or infected flock. The following criteria were used to classify flocks into one of these three categories: Infected flocks are those in which an animal has been diagnosed positive for scrapie by NVSL. Source flocks are those in which two or more animals were born and later diagnosed scrapie positive at less than 54 months of age within 60 months of each other. Trace flocks are those in which a single animal was born that was later diagnosed scrapie positive at less than 54 months of age. Each flock included in the study was classified as either infected, source, trace, or unknown. These flock classifications were used to determine which positive animals could be identified or linked to a source of infection.

4. Flock status date. The flock status date is the date on which the flock became a trace, source, or infected flock. This date is

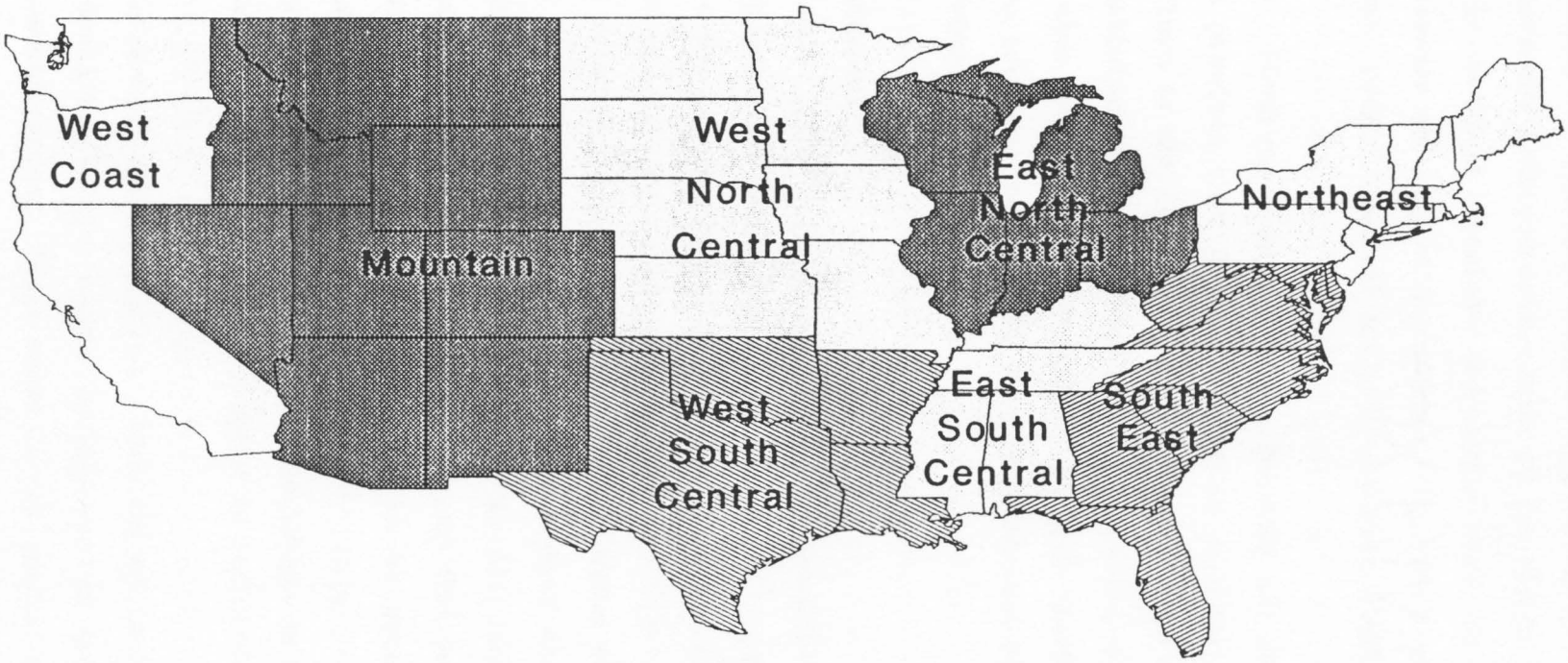

Figure 1. The eight geographic regions of sheep production in the United States.

either the date the infected animal was born in the flock, or the date the infected animal entered the flock. This date represents the earliest possible documented date for the introduction of disease and is used to determine the likely source of infection for each positive animal and each positive flock.

5. Flock depopulation date. The date all sheep were removed from a premises that was classified as a trace, source, or infected flock is the flock depopulation date. This date is important for calculating within-flock mortality rates. Within-flock mortality rates are only calculated for initial flock infections, not for replacement flocks which are found infected following a total flock depopulation.

Animal Factors

Animal factors are those attributes specific for each animal that were included in the study as host factors. Animal information is included for all animals submitted for diagnosis or noted as clinically affected and all their siblings, sires, and dams. This information is further examined to determine the disease status of the sire and dam as well as the age of the sire and dam at birth of the positive offspring. In addition, information is collected on all additions of animals to infected flocks within six years prior to the date on which clinical signs were first noted in the flock in an attempt to document all possible flock to flock transmissions. Six years was selected to comfortably allow for the 60 month incubation period described in the literature. The following information is included for each animal:

1. Animal identification. Each animal included in the study is identified with a unique combination of one to three pieces of identification. These three pieces consist of an eartag, a flock

tag, and a registration number. The animal identification information is used to link multiple records pertaining to the same animal together and is not used directly in any of the analyses.

2. Breed. Breed information is included to determine if any discernable patterns exist with respect to breeds affected.

3. Sex. Sex for each animal is included in order to examine trends and/or patterns with respect to sex. Sex is also included to make it easier to determine if an individual animal could be a sire or dam of another and to speed searches for sires and dams.

4. Whether the animal is a twin or triplet. Twins are of interest due to the proposed mechanisms of transmission. If the disease is transmitted by infected ewes at lambing time and young lambs are the most highly susceptible, then twins should be affected nearly equally. If genotype plays a strong role in determining infection, both twins might be affected more often than only one of the pair being affected if the twins are of identical genotypes.

5. Disease status. The individual disease status for each animal is two-fold, with both a clinical and a laboratory component. Animals are either clinically affected or clinically normal. Clinically normal animals include those animals examined and found normal as well as those animals not examined since these two situations cannot be distinguished when examining records kept to document only positive and suggestive cases. In addition to their clinical presentation, animals are laboratory negative, suggestive, positive, or not examined. In most cases when animals in entire flocks were subject to euthanasia under the scrapie eradication

program, further diagnostic evaluations were not conducted, regardless of the clinical presentations of the animals.

6. Date of birth. Birth dates are included for each animal where known in order to calculate age at death and the age of the animal for all movements. Date of birth is extremely important since most theories regarding possible modes of transmission involve the period between birth and nine months of age.

7. Entry date. The date an animal enters a flock is recorded where known and the age at entry calculated to ascertain possible source and trace flocks through comparisons with flock status dates. The entry date and birth date will be identical for animals born in the flock.

8. Disposition date. The disposition or exit date is recorded as the date the animal leaves the flock due to sale or loan to another flock, slaughter, euthanasia for diagnostic purposes, or death. In the case of the latter two, this date is used to calculate the age at diagnosis.

9. Sire and dam identification. Sire and dam information includes up to three pieces of identification for each and is used to look for commonalities between the sires and dams of infected sheep. Since all of the above information (animal factors 1 - 8) is also collected for each sire and dam where possible, the three pieces of identification are used to link back to the records containing birth and movement dates for parents of positive progeny.

DATABASE DESIGN

Once the data collection information needs for this study were defined through examining available records, a database was designed in ORACLE (version 5.1a)[47] to serve as a repository for the data. A relational database was desired in order to facilitate the association of information at both the flock and animal levels. Since animal movement information is critical to this study, the records for each animal were structured such that all movements could be recorded. The records were also structured to allow for incomplete movement and date information. A detailed description of the database structure is provided in Appendix I.

DATA COLLECTION AND VALIDATION

Flock information as well as information on individual animals was abstracted, coded, and entered into the ORACLE relational database. A sample of the coding forms is provided in Appendix II. Flock and animal level information was obtained from various records kept by the USDA technical program staff and NVSL. Although summarized information was available for the first 15 years of the scrapie eradication program, all information was abstracted or verified from the original records where possible to minimize any transposition errors present in the summarized data. The breed and sex information was obtained either from registration papers or from indemnity claim forms which have been submitted for animals sacrificed for diagnostic purposes and for animals which were depopulated at government expense. Registration information submitted with the indemnity claims and flock records was used to locate twins.

Sire and dam information was collected from the owner records as well as from the registration certificates. A computer program was written to match animal information with information for their sires and dams in order to determine the disease status of the sire and dam.

Positive animals born to positive dams and sires were identified utilizing this program and tagged as having possibly acquired the disease from their positive dam or sire. Positive animals not born to positive dams or sires were considered control animals for purposes of comparison with positive animals born to positive dams or sires in an effort to examine at what age a positive dam or sire becomes a disease threat to their offspring.

Date of birth was obtained from registration certificates where available or calculated based upon the stated age of the animal upon submission for diagnosis or indemnity. Date of entry into the flock was obtained from sale records, registry records, and owner records in that order of preference. In some cases these dates were incomplete or partial (ie. the year was known, but the exact month and day were not known). These partial dates were handled as follows: 1) if the year was not known, the date was considered missing for all calculations; 2) if the month and day were not known, it was assumed to be January 1; 3) if the day was not known, it was assumed to be the first day of the month; 4) if the season was mentioned, the following dates were assumed: March 1 for spring, June 1 for summer, September 1 for fall and December 1 for winter. In several cases, exact birth dates were not given, but animals were noted to be a certain age at death and these animals were assumed to have been born on January 1 of the year that would make them the age stated in the records.

Each time an animal was listed as having been present in a particular flock, the following information was written on one of the coding forms for later entry into the computer: premises ID, origin premises ID, disposition premises ID, twin, sex, breed, eartag, flock tag, registration number, disease status, sire eartag, sire flock tag, sire registration number, dam eartag, dam flock tag, dam registration number, birth date, entry date, disposition date, disposition, and remarks. The various premises IDs were assigned to all flocks involved

in the movement of animals included in this study. A master list of premises was maintained to prevent the assignment of duplicates and this list was checked each time an owner name showed up in the records. Identification of animals was coded where necessary and a master code list developed. Each time a flock tag was encountered, it was coded and recorded on the master flocktag code list. This type of coding was necessary since flock identification was sometimes a rather lengthy name and animals were often listed more than one way on different registration certificates. For example, an animal might be listed as "W. L. Smith2" one time and "WLS2" another time.

Three pieces of identification (eartag, flock tag, and registration number) were used to identify each animal in order to facilitate the identification of scattered bits and pieces of information to the correct animal. This was necessary since many of the records examined did not include all pieces of identification for each animal mentioned. Sire and dam information includes up to three pieces of identification for each in order to maximize the ability to match animals with their sires and dams. Once all of the data were entered, registered animal information was linked through the use of registration numbers and non-registered animal information was linked through the flock tag. Recorded movements of animals which did not carry any man-made identification devices were tracked through the assignment of sequentially incremented generic tag numbers.

Data validation involved checking to be sure that all identification, movement date, sire, and dam information was consistent across all of the records for each animal. Duplicates were eliminated when found and where necessary the original records were consulted to determine which dates and pieces of identification were correct. In addition, each flock listed on the national summary listings of infected flocks was checked to be sure the correct numbers and breeds of positive animals were present in the database. All the above data validation

procedures were performed prior to extracting this information from the ORACLE database for analysis in SAS[58].

DATA ANALYSIS

Data analysis initially consisted of evaluation of temporal and spatial relationships among the affected, trace and source flocks as well as frequency distributions of all the study variables within these flocks. For purposes of this study, the level of statistical significance was set at p-values of .10 or less.

The following statistical tests were performed using SAS[58]. For continuous outcomes with one independent variable having two groups, a Two Sample T Test was performed. If the variances of the two groups were not homogenous according to the folded form of the F statistic ($p < .50$), then a Wilcoxon Rank Sum Test was performed on the data. For continuous outcomes with one independent variable having three or more groups, a One-Way Analysis of Variance Test was performed. If the variances of the groups were not homogenous or the number of observations in each group was small or unequal, then a Kruskall-Wallis Test was performed on the data.

CHAPTER 4

RESULTS

A total of 957 clinical cases of sheep scrapie were confirmed positive by NVSL between 1947 and September 30, 1991. There were nine clinically affected animals which NVSL classified as suggestive, and 20 which were classified as histologically negative. A total of 82 animals that were classified as clinically affected were not examined histologically at NVSL. Due to the low numbers of clinically affected animals that did not have a positive laboratory diagnosis, the findings for these animals were not reported out separately.

Records on two animals that were diagnosed positive at NVSL without having exhibited any clinical signs were not included in the study analysis. These two animals were recorded as the clinically normal offspring of two clinically affected Suffolk ewes. Both of the animals were ewe lambs, one a 13 month old twin diagnosed by histopathology and the other a six month old single diagnosed by mouse inoculation. Data from these animals was not included in the study since they did not meet the initial criteria of being clinically affected.

The 957 confirmed cases involved a total of 581 flocks in 39 states and included multiple cases from several flocks. Two-thirds (642) of the confirmed cases were diagnosed between 1980 and 1991. Sufficient information was available for 870 (91%) of these cases to allow determination of the age of the animal at death to the nearest year. Only 443 (46%) of the confirmed cases contained month or season of birth and death allowing calculation of age to the nearest three months. In the cases that were diagnosed between 1980 and 1991, 583 (91%) contained

at least enough information to determine age of the animal to the nearest year and only 190 (30%) contained birth and death season or month.

For many of the analyses, sire and dam identification information was used to link records together for the purposes of evaluating patterns in the ages and disease statuses of the sires and dams of infected animals. The sire registration number or flock tag was known for 400 (42%) of the confirmed cases, and 125 (19%) of these cases were diagnosed between 1980 and 1991. The dam registration number or flock tag for positive animals was known in 418 (44%) of the cases, and 156 (24%) of these cases were diagnosed between 1980 and 1991.

In addition to infected flocks, information was available on 21 source flocks, 75 trace flocks, and 1,073 flocks which had contact with infected animals or their offspring, but did not meet the definitions of infected, source, or trace flocks. Tracing efforts also led back to 47 flocks in Canada and four flocks in England. Information from animals originating in these flocks was only included in the analysis if the animals were diagnosed in the United States by NVSL.

FLOCK FACTORS

Flock location was examined to look for trends and patterns. Figure 2 depicts the county locations for all of the infected flocks included in the study. The highest numbers of infected flocks discovered between 1965 and 1991 occurred in the east north central (192 flocks or 44% of the positive flocks), west south central (67 flocks or 15% of the positive flocks), and northeast (54 flocks or 12% of the positive flocks) regions. Using NASS figures, the average number of flocks in each region between 1965 and 1991 was calculated and these results along with the number of positive flocks are shown in Table 2. Figure 3 illustrates the percentage of positive flocks found in each region as compared to the percentage of average total flocks in each region. The east north central region accounted for an average of 24% of the sheep flocks, and

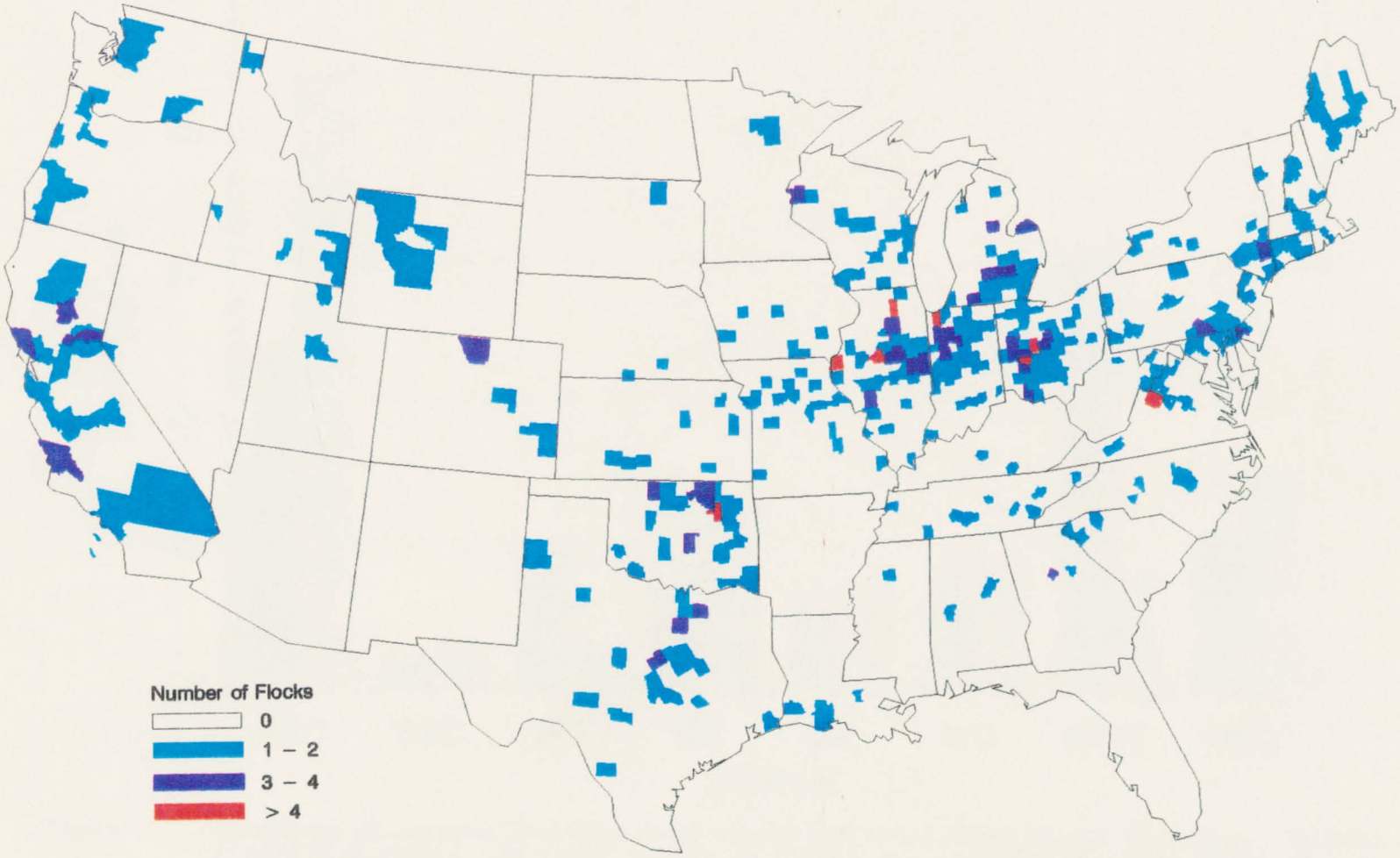

Figure 2. County location of scrapie positive flocks in the United States. Includes all flocks from which a positive diagnosis was made between 1947 and September 30, 1991.

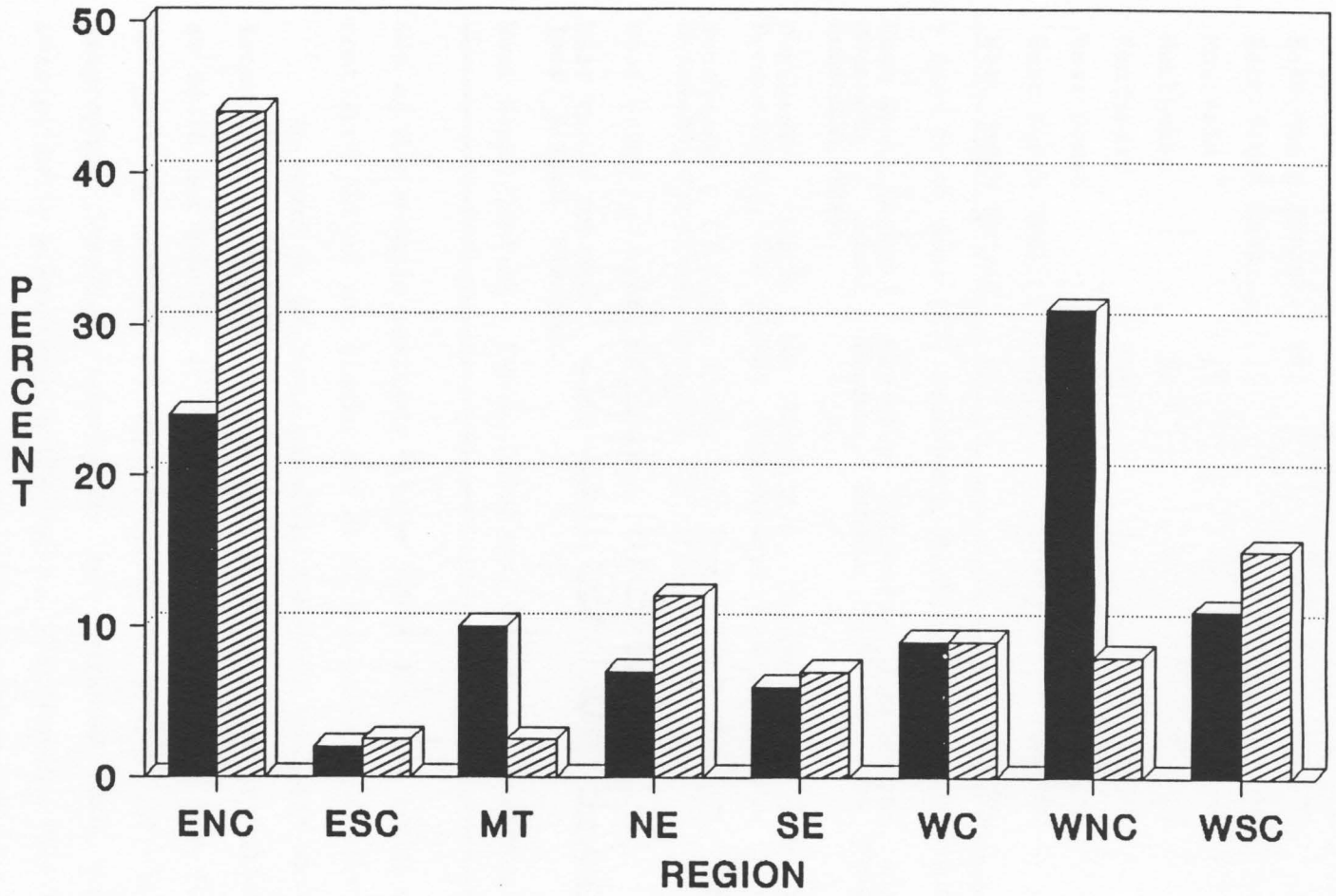

Figure 3. Percentage of scrapie positive sheep flocks and total sheep flocks by region. Numbers of positive flocks and total number of flocks are included for each state in each region where NASS figures were available between 1965 and 1991. Regions are abbreviated as follows: east north central (ENC), east south central (ESC), mountain (MT), northeast (NE), southeast (SE), west coast (WC), west north central (WNC), west south central (WSC).

Table 2. Regional distribution of U. S. sheep flocks: 1965 - 1991.

Region*	Number of Infected Flocks	Average Number of Flocks
East North Central	192	34,770
East South Central	11	2,781
Mountain	11	14,443
Northeast	54	10,429
Southeast	30	9,075
West Coast	39	12,537
West North Central	36	45,100
West South Central	67	14,994

* East North Central - Wisconsin, Michigan, Illinois, Indiana, Ohio.

East South Central - Kentucky, Tennessee, Mississippi, Alabama.
Mountain - Idaho, Montana, Nevada, Arizona, New Mexico, Wyoming, Colorado, Utah.

Northeast - Maine, New Hampshire, Vermont, New York, Rhode Island, Pennsylvania, New Jersey, Massachusetts, Connecticut.

Southeast - Florida, North Carolina, South Carolina, West Virginia, Delaware, Maryland, Georgia, Virginia.

West Coast - Oregon, Washington, California.

West North Central - North Dakota, South Dakota, Nebraska, Minnesota, Iowa, Kansas, Missouri.

West South Central - Texas, Oklahoma, Louisiana, Arkansas.

44% of the scrapie positive flocks, while the west north central region contained 31% of the flocks and 8% of the positive flocks.

In order to evaluate possible trends or patterns in the geographic location of confirmed cases, positive animals were evaluated both for age at death and quarter of the year when the disease was discovered. An analysis of variance on age at death by quarter of the year and by geographic location separately and together did not yield any statistically significant differences. Plotting the locations of flocks based upon the quarter of the year when positive animals were found did not produce any visible trends. Figures 4 - 7 show the location by

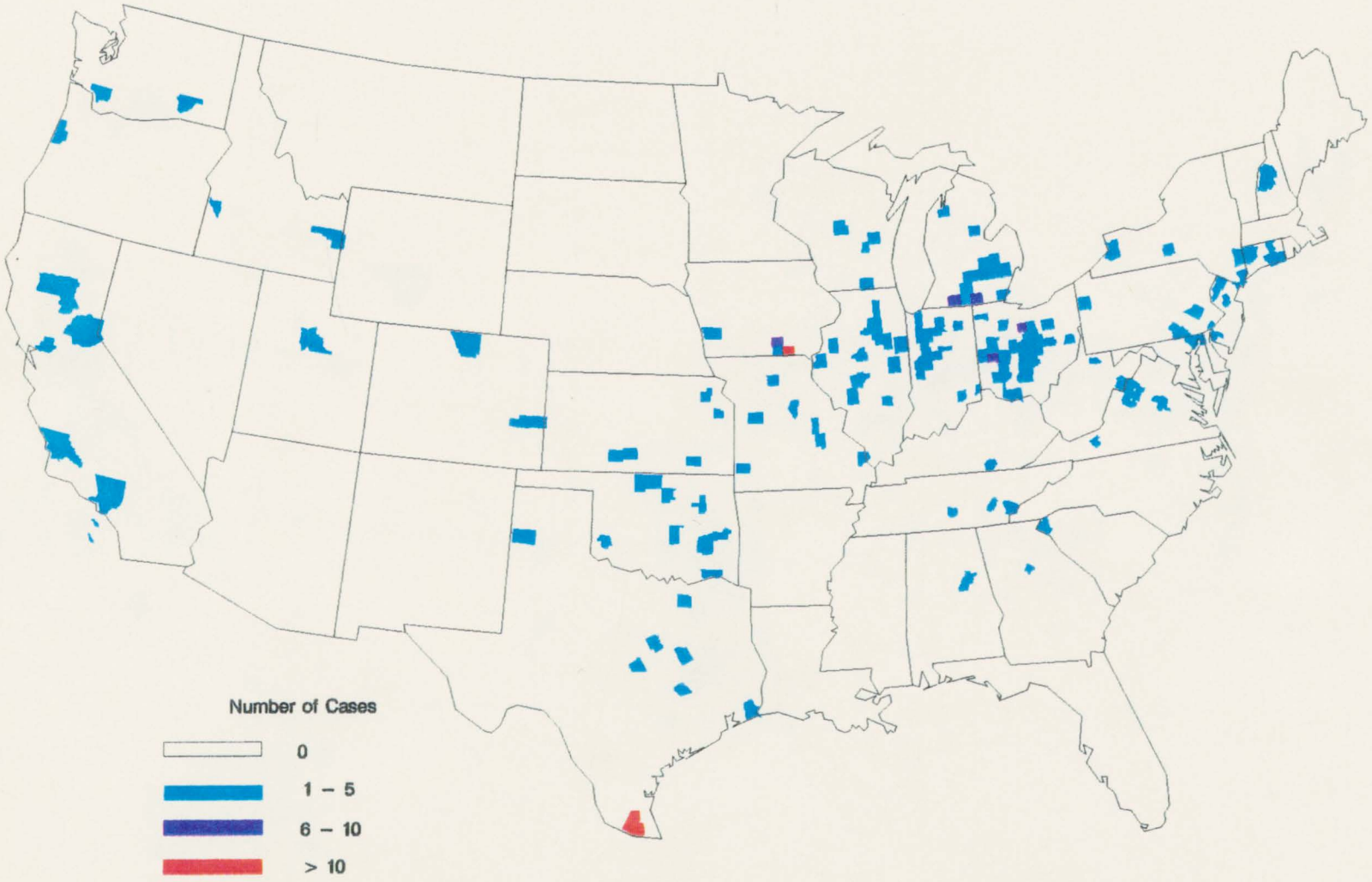

Figure 4. County location for all scrapie positive cases diagnosed between January and March. Includes all positive cases diagnosed between 1947 and September 30, 1991.

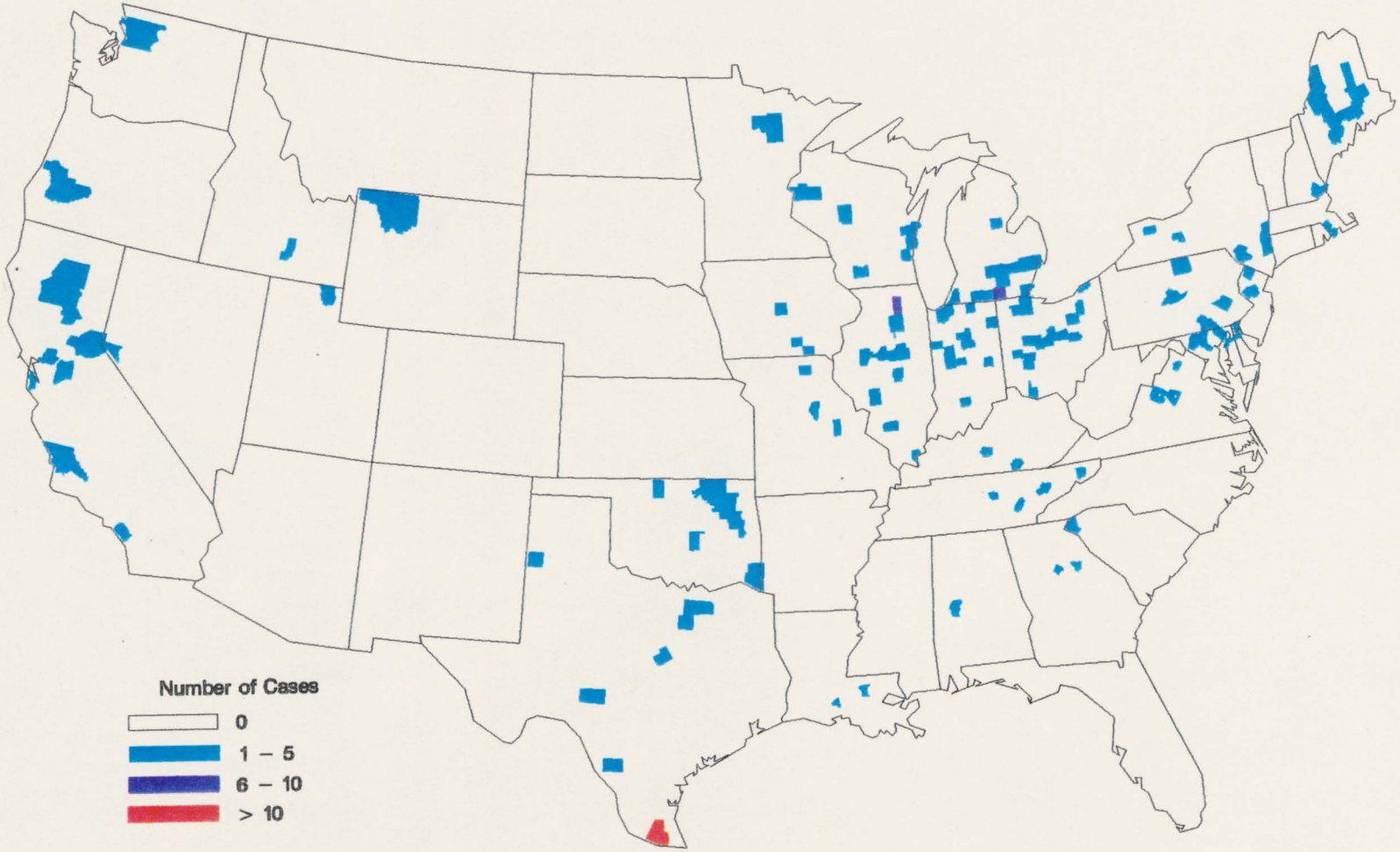

Figure 5. County location for all scrapie positive cases diagnosed between April and June. Includes all positive cases diagnosed between 1947 and September 30, 1991.

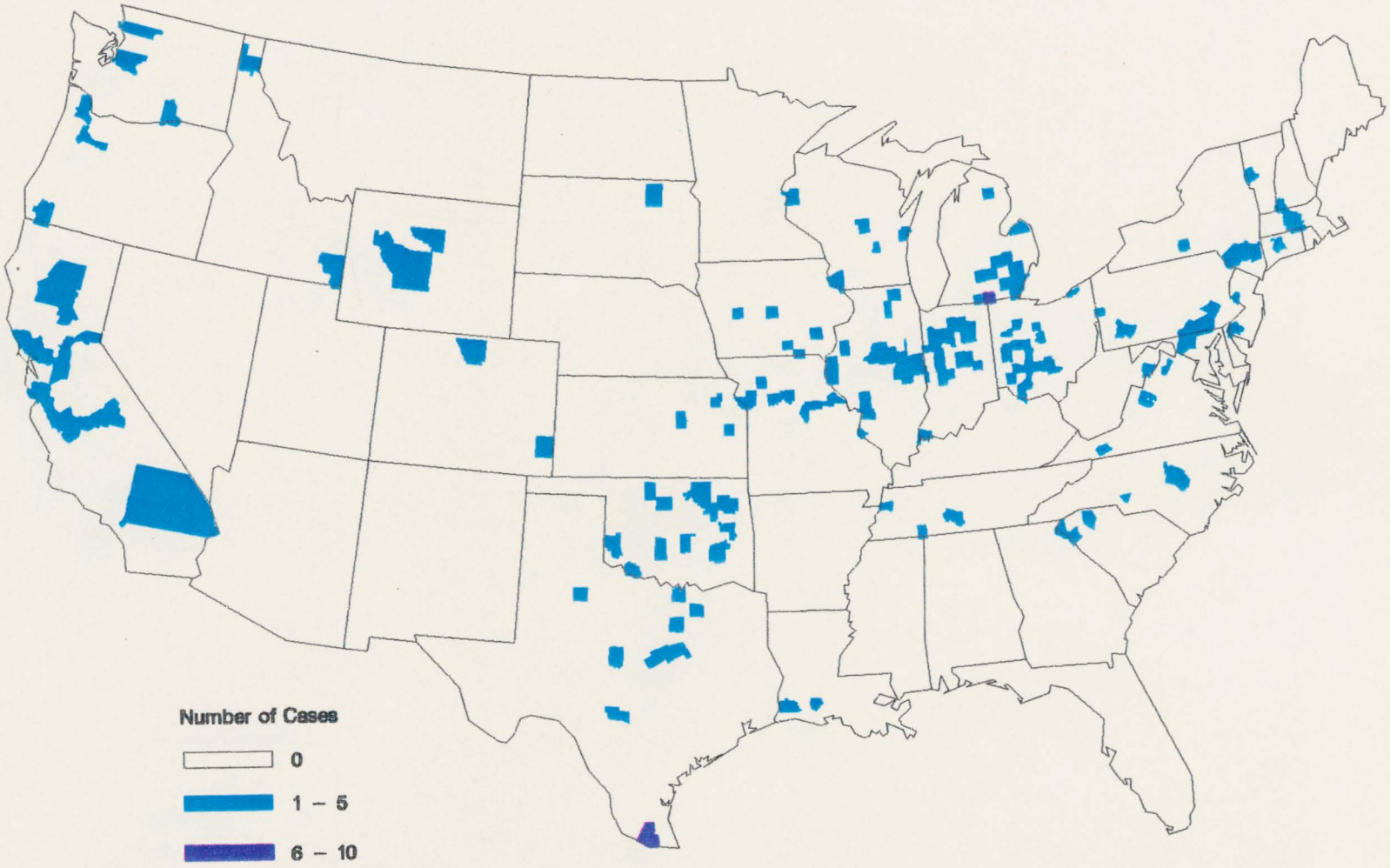

Figure 6. County location for all scrapie positive cases diagnosed between July and September. Includes all positive cases diagnosed between 1947 and September 30, 1991.

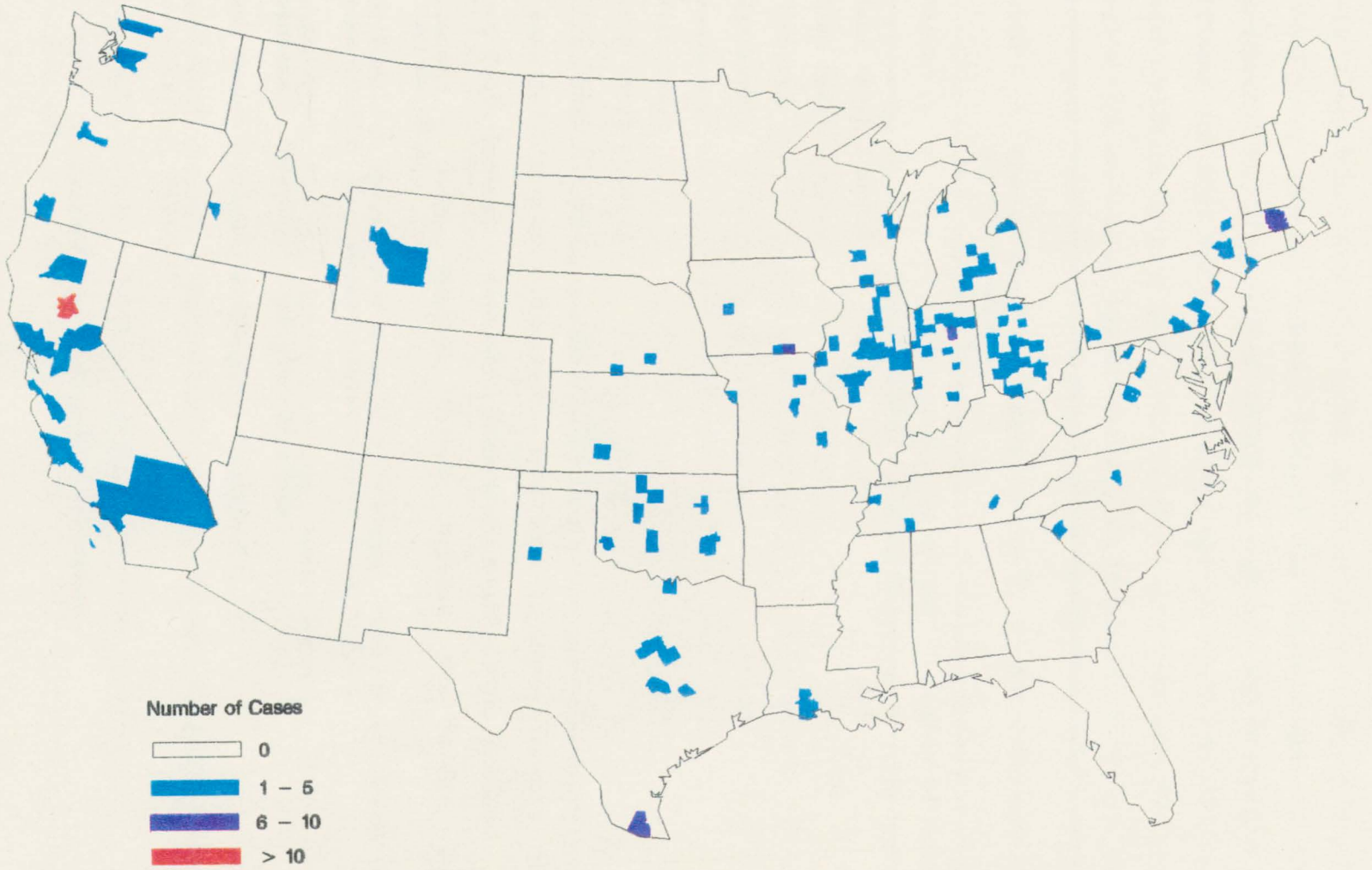

Figure 7. County location for all scrapie positive cases diagnosed between October and December. Includes all positive cases diagnosed between 1947 and September 30, 1991.

county for all of the positive cases based upon the quarter of the year in which they were detected. Each of the eight geographical regions contain cases for each of the four quarters and no specific patterns or trends were evident. Table 3 shows the average age at death or destruction for all positive animals by region. The two regions which differed the most in terms of average age at death were the mountain region with an average age at death of 48.44 months and the southeast region with an average age at death of 40.28 months, however these

Table 3. Age at death by region of the U. S. for confirmed scrapie positive cases.

Region*	Average Age in Months (Standard Deviation)**	Number of Animals
East North Central	43.43 (14.92)	219
East South Central	42.19 (13.85)	7
Mountain	48.44 (16.65)	14
Northeast	44.74 (14.89)	39
Southeast	40.28 (7.60)	27
West Coast	44.65 (13.46)	44
West North Central	43.11 (12.40)	18
West South Central	43.14 (10.07)	75

* East North Central - Wisconsin, Michigan, Illinois, Indiana, Ohio.

East South Central - Kentucky, Tennessee, Mississippi, Alabama.

Mountain - Idaho, Montana, Nevada, Arizona, New Mexico, Wyoming, Colorado, Utah.

Northeast - Maine, New Hampshire, Vermont, New York, Rhode Island, Pennsylvania, New Jersey, Massachusetts, Connecticut.

Southeast - Florida, North Carolina, South Carolina, West Virginia, Delaware, Maryland, Georgia, Virginia.

West Coast - Oregon, Washington, California.

West North Central - North Dakota, South Dakota, Nebraska, Minnesota, Iowa, Kansas, Missouri.

West South Central - Texas, Oklahoma, Louisiana, Arkansas.

** Number in parentheses is standard deviation.

differences were not statistically different when evaluated using the Wilcoxon Rank Sum Test.

The proportion of positive flocks in states reporting any positive animals for each year between 1965 and 1991 ranged from a high of .0062 in Tennessee in 1991 to .000050 in Ohio in 1966. Figure 8 shows the average yearly proportion of positive flocks in states reporting positive animals each year between 1965 and 1991. States contributing to the proportion positive include only those where positive flocks were found and where the number of operations with sheep as reported by NASS is known. The proportion of positive flocks is calculated for 1965 through 1991 only since NASS did not collect information concerning the number of operations with sheep prior to 1965. The number of operations for the entire United States is not used as the denominator since there were several years during which NASS estimates were not available for certain states. Regressing the average yearly proportion positive on year gave a positive and significant slope value, which means the slight upward trend noted in Figure 8 is statistically significant.

Flock sizes for infected flocks ranged from one to 7,080, with an average of 123 head and a median infected flock size of 49. Flock size information was used to calculate average within-flock mortality rates for infected flocks for each year between 1947 and September 1991. Results of these calculations are shown in Figure 9. The slight upward trend depicted in this figure with a trend line is statistically significant in that a regression line fitted to these data points has a positive and significant non-zero slope. Further examination of the effect of flock size showed no significant correlation ($r = .037$) between age at death and flock size.

The average flock mortality rate computed based upon the last recorded death of a confirmed positive animal in each flock prior to depopulation was 5.69% in 474 flocks for the entire study period. This is graphically depicted in Figure 9 as a plot of the average flock

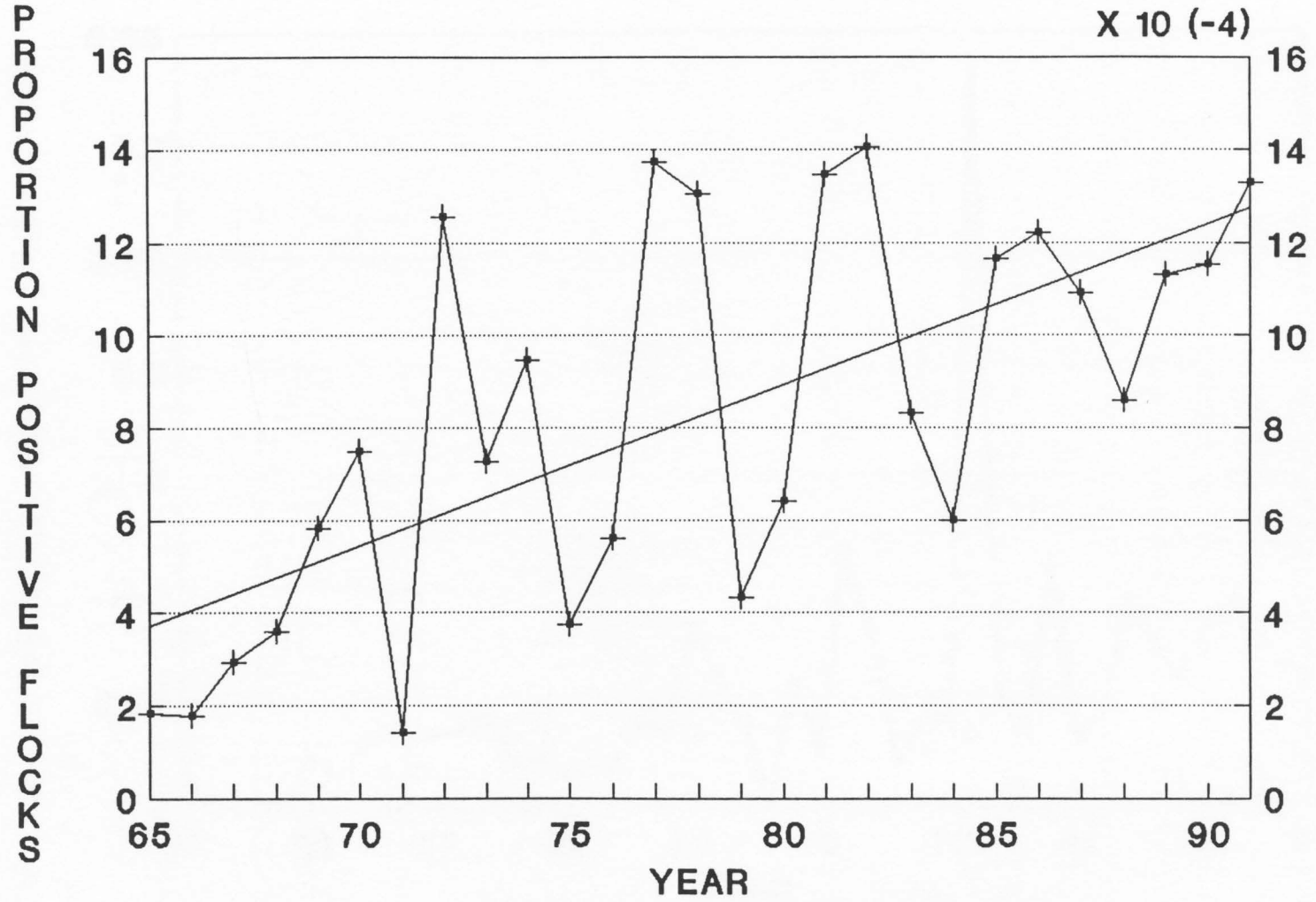

Figure 8. Proportion of flocks reported positive between 1965 and 1991 in states reporting scrapie.

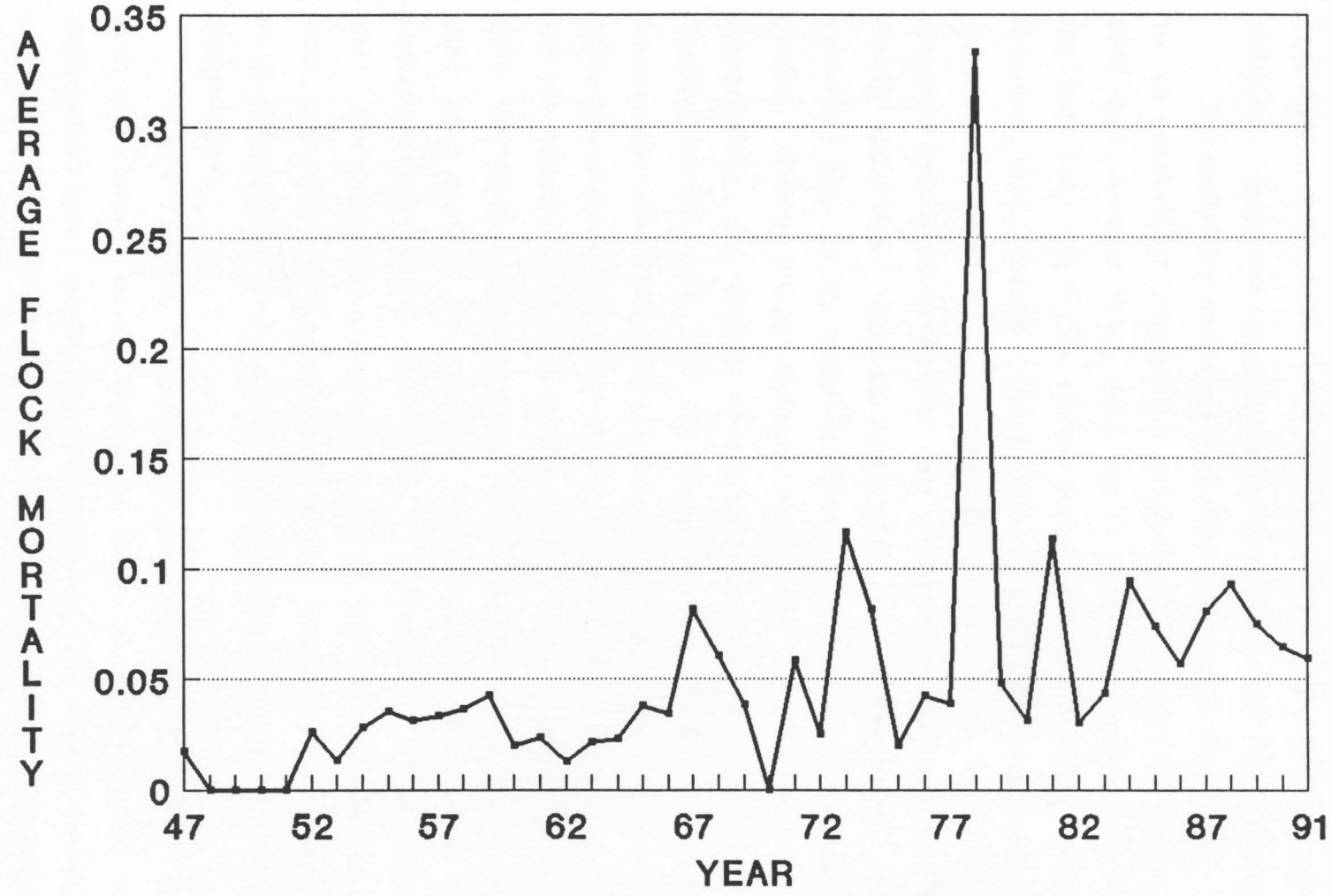

Figure 9. Average within-flock mortality rate by year of last death.

mortality rate based upon the year of death for the last confirmed positive case prior to flock depopulation. The average flock mortality rate varied quite a bit over time but exhibited a general upward trend which was statistically significant when evaluated using regression.

The mortality rate data were also evaluated with respect to changes in the eradication program and graphically depicted in Figure 10. From 1947 until October 1952, there was no eradication program in effect and the mortality was 1.92% within three infected flocks. Beginning in November 1952, infected flocks were quarantined and depopulated and animals sold from these infected flocks were also slaughtered. From November 1952 until March 1957, the average mortality rate in 53 infected flocks was 3.33%. Between April 1957 and February 1965, the average mortality rate in 87 infected flocks fell to 2.4%. During this time period, source flocks defined under the program were also being depopulated. The average mortality rate rose to 5.15% in 60 infected flocks between March 1965 and September 1975 which coincided with the introduction of the bloodline option. Between October 1975 and September 1978, the average mortality rate rose to 7.67% in eight infected flocks and the bloodline option was eliminated. From October 1978 until March 1983, the average mortality rate in 47 infected flocks was 5.48%. During this time period, the maximum indemnity payment allowed per sheep increased considerably (See Table 1). From April 1983 until September 1991, the average mortality was 7.42% in 223 infected flocks. In April 1983, the eradication program was further modified to include euthanasia of bloodline animals and flock surveillance, with much less emphasis on depopulation.

Figure 11 shows the number of newly affected flocks per year along with the changes to the indemnity rate. A total of 581 flocks are represented here. During the time period of 1947 through September 1991, several replacement flocks were known to have become infected following

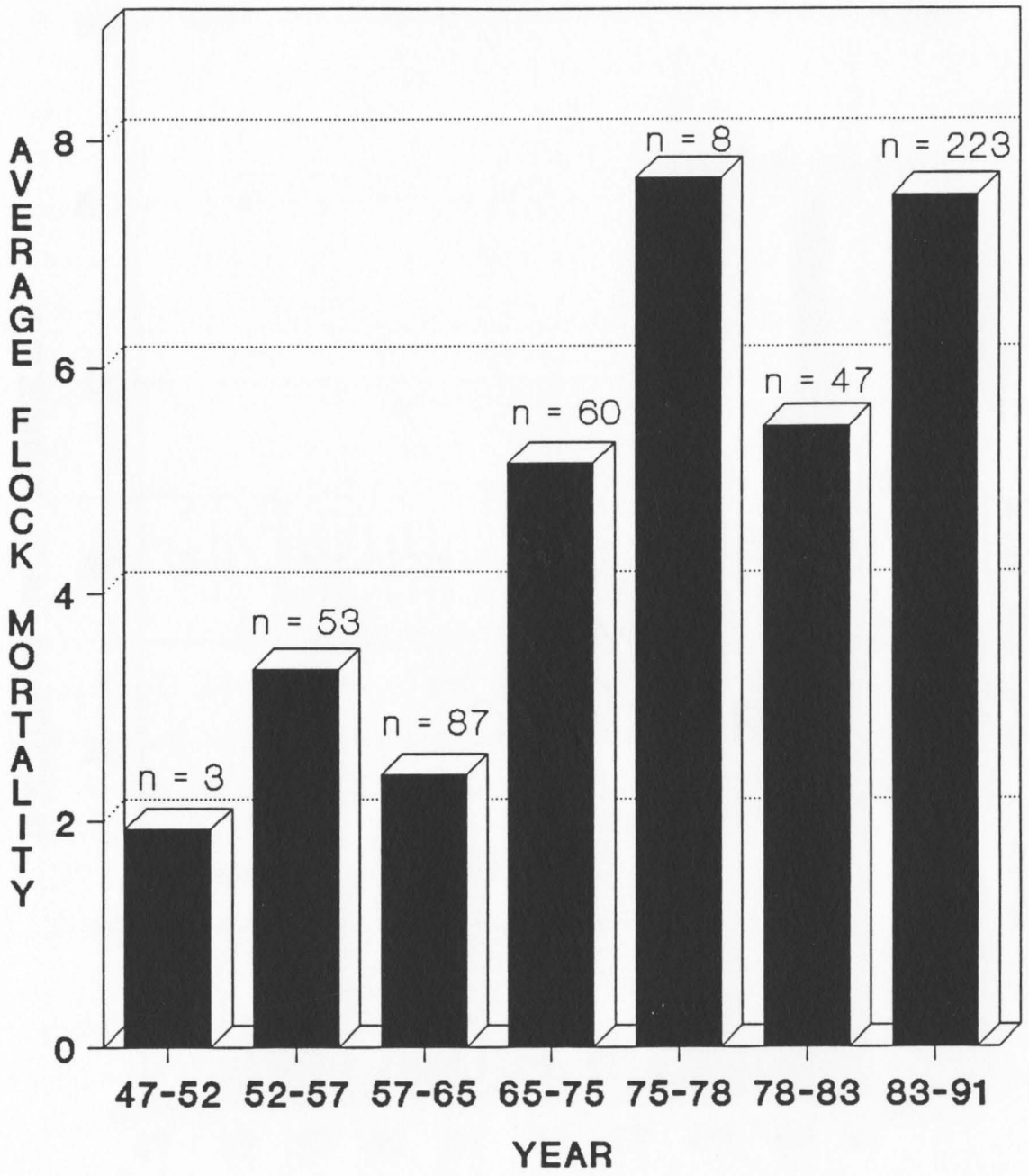

Figure 10. Average within-flock mortality rates by scrapie eradication program phases. For a more complete description of the specific changes for each phase, refer to Table 1.

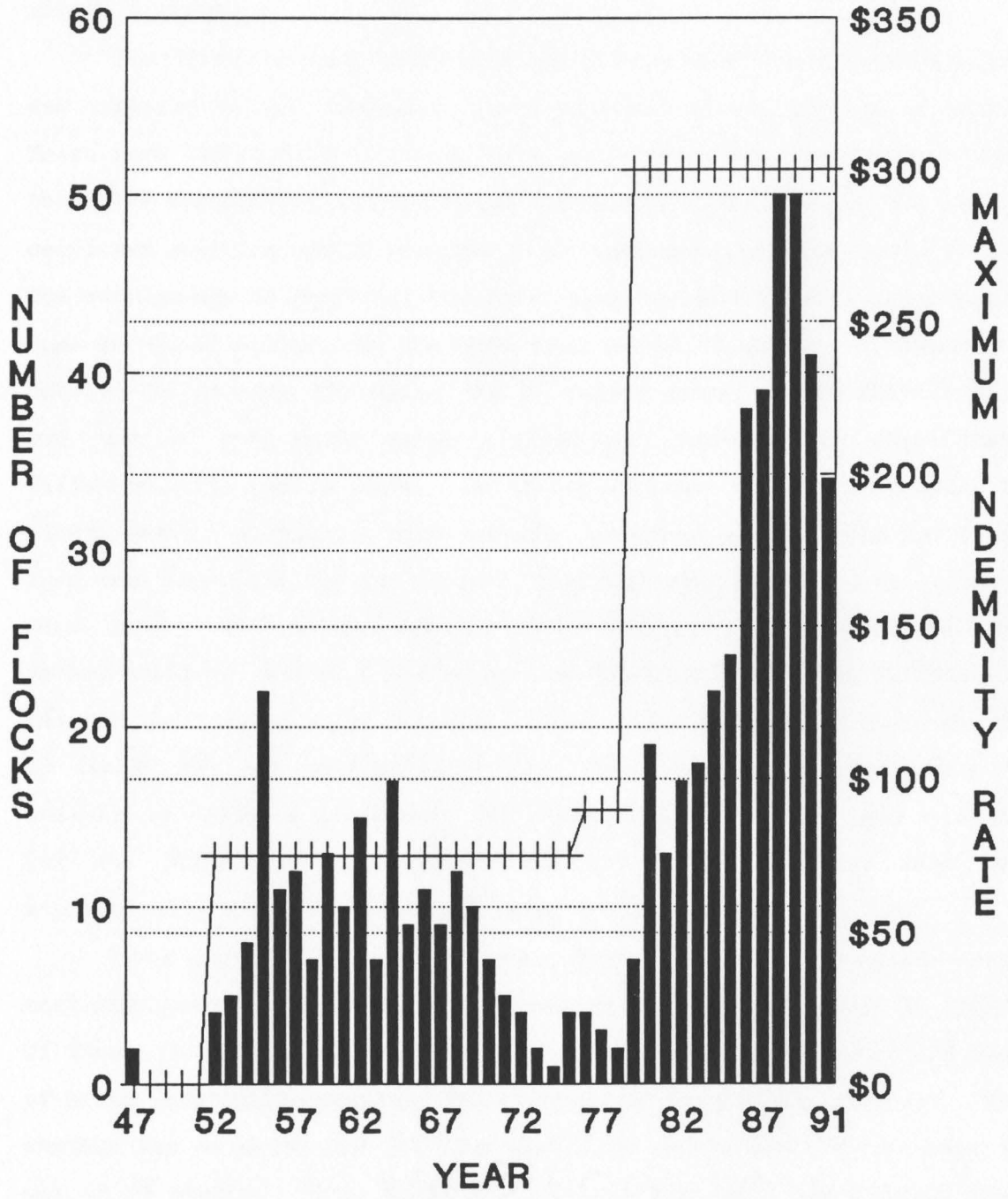

Figure 11. Number of newly reported scrapie flocks compared with the maximum allowable indemnity rate.

depopulation. The details for these situations are unclear and depopulation information was often not included in the records examined.

ANIMAL FACTORS

The first, second, and third cases from each flock were examined and compared to all subsequent cases in terms of average age at death. There were 440 cases containing birth month and year information and of these 309 represented initial cases within the flock, 67 were the second confirmed positive and 25 were the third confirmed positive in the flock. The average age at death for the first case was 43.25 months, the second case was 45.57 months, and the third case was 42.53 months. A comparison between the initial 309 cases, the 67 second cases, the 25 third cases, and the 39 additional cases yielded no statistically significant differences for age at death. In taking a closer look at only those 18 flocks which had three or more animals confirmed positive and for which ages were known for all three cases, the following average ages at death were noted: 49.4 months for the first positive, 45.2 months for the second positive, and 42.5 months for the third positive. The differences between the average ages for these first three positive animals in the 18 flocks was not statistically significant when evaluated using an analysis of variance procedure. The difference between the ages at death for the first case as compared to the third case was also not statistically different when utilizing a Wilcoxon Rank Sum Test.

There were 136 rams and 821 ewes, for a total of 957 clinical cases confirmed scrapie positive by NVSL between 1947 and September 30, 1991. Of these, 96 of the rams and 347 of the ewes had at least month and year of birth information enabling calculations of average age at death. The average age at death for the rams was 39.81 months and for the ewes it was 44.59 months. This difference was statistically significant when evaluated using a Wilcoxon Rank Sum Test ($p=.03$). Specific findings for sex are reported in Table 4 and Table 5. Table 5 represents findings for

Table 4. Age at death by sex for confirmed scrapie positive cases.

Sex	Average Age in Months	Number of Animals
Male	39.80 (8.07)*	96
Female	44.59 (14.62)	347

* Number in parentheses is standard deviation.

Table 5. Age at death by sex for all clinical scrapie cases.

Sex	Average Age in Months	Number of Animals
Male	40.27 (9.49)*	112
Female	45.03 (15.83)	442

* Number in parentheses is standard deviation.

all clinical cases and Table 4 includes only those which were confirmed positive by NVSL.

Breed information was examined for each of the 957 confirmed cases. Suffolk breed animals comprised 839 cases (88%), 55 of the cases (6%) were Hampshire's, 33 (3%) were white faced breeds, and 30 (3%) were cases where the breed was not specified. When these breed groupings were examined to determine the average age at death or destruction, no statistically significant differences were noted utilizing the Kruskal-Wallis Test. Specific results for breed are reported in Tables 6 and 7.

Table 6. Age at death by breed for confirmed scrapie positive cases.

Breed	Average Age in Months	Number of Animals
Suffolk	43.24 (13.46)*	400
Hampshire	45.62 (10.43)	21
White Faced Breeds	48.36 (17.96)	20
Other Breeds	37.18 (22.93)	2

* Number in parentheses is standard deviation

Table 7. Age at death by breed for all clinical scrapie cases.

Breed	Average Age in Months	Number of Animals
Suffolk	43.67 (14.51)*	496
Hampshire	46.40 (11.33)	24
White Faced Breeds	49.27 (18.39)	26
Other Breeds	45.08 (29.31)	8

* Number in parentheses is standard deviation

It is interesting to note that in reviewing the records, the first case of scrapie in a registered Hampshire in the United States occurred in a flock closely associated with an affected Suffolk flock.

Twins and triplets accounted for 152 of the 957 confirmed cases. Birth month and year information was available for 138 of the twins and triplets and 305 of the animals reported as singles. There were no statistically significant differences in the age at death for twins and triplets when compared to singles. Specific findings for singles, twins, and triplets are reported in Table 8 and Table 9. There were 26 twin

Table 8. Age at death by number of siblings for confirmed scrapie positive cases.

Number of Siblings	Average Age in Months	Number of Animals
1 or 2 (Twins & Triplets)	44.08 (13.19)*	139
0 (Singles)	43.31 (13.80)	304

* Number in parentheses is standard deviation.

Table 9. Age at death by number of siblings for all clinical scrapie cases.

Number of Siblings	Average Age in Months	Number of Animals
1 or 2 (Twins & Triplets)	44.68 (13.67)*	169
0 (Singles)	43.80 (15.40)	385

* Number in parentheses is standard deviation.

pairs included in the study where information for both members of the twin pair was available. One of these pairs consisted of two confirmed positive animals and one pair consisted of a confirmed positive and a clinically affected laboratory negative animal. The remaining 24 twin pairs consisted of 6 pairs where one twin was confirmed positive and the other was clinically affected but not confirmed and 18 pairs where one twin was confirmed positive and the other had an unknown disease status.

The sire and dam disease status was examined for each confirmed positive scrapie case. The sires and dams were each classified as confirmed positive, clinically affected, or unknown and the age at death for the offspring in each category was calculated and examined using the Kruskal-Wallis Test. The results of this analysis are presented in Table 10 and Table 11. The disease status of the sire had no appreciable effect on the age at death for the offspring, however the disease status of the dam had a statistically significant effect (p=.01) on the age at

Table 10. Age at death by disease status of sire for confirmed scrapie positive cases.

Disease Status of Sire	Average Age in Months	Number of Animals
Confirmed Positive	43.65 (13.05)*	5
Clinical - Not Confirmed	42.82 (10.33)	16
Unknown	43.58 (13.74)	422

* Number in parentheses is standard deviation.

Table 11. Age at death by disease status of dam for confirmed scrapie positive cases.

Disease Status of Dam	Average Age in Months	Number of Animals
Confirmed Positive	34.06 (8.34)*	5
Clinical - Not Confirmed	37.48 (8.04)	15
Unknown	43.88 (13.73)	423

* Number in parentheses is standard deviation.

death for her offspring when evaluated using the Kruskal-Wallis Test. The average age at death for offspring of dams of unknown disease status was 43.88 months, clinically affected dams had offspring which died at 37.48 months, and confirmed positive dams bore offspring which died on average at 34.06 months.

The average age and disease status of sires (Table 12) and dams (Table 13) at the birth of offspring later confirmed positive was examined in an effort to determine if patterns exist which might support a particular mode or timing for transmission. There were 98 confirmed positive cases which had a known birth year and for which the sire's birth month and year were also known. Four of the sires were confirmed positive, 10 were clinically affected and 84 of the sires were of unknown disease status. The confirmed positive sires were between 11 and 26

Table 12. Sire age at birth of scrapie positive offspring.

Category of Sire	Average Sire Age (in months)	Min. Sire Age	Max. Sire Age
All Sires	39.15 (n = 98)	10.98	105.26
Confirmed Positive	18.16 (n = 4)	10.98	25.60
Clinical - Not Confirmed	33.60 (n = 10)	13.28	51.33
Unknown Disease Status	40.81 (n = 84)	11.24	105.26

Table 13. Dam age at birth of scrapie positive offspring.

Category of Dam	Average Dam Age (in months)	Min. Dam Age	Max. Dam Age
All Dams	41.35 (n = 112)	14.13	96.02
Confirmed Positive	30.75 (n = 4)	23.92	36.01
Clinical - Not Confirmed	33.52 (n = 11)	21.66	70.03
Unknown Disease Status	42.68 (n = 97)	14.13	96.02

months of age at the birth of offspring later found positive. The clinically affected sires ranged in age between 13 and 52 months and the sires of unknown status were between 11 and 105 months of age at the birth of offspring later found positive.

There were 112 confirmed cases which had a known birth year and for which the dam's birth month and year were also known and of these, four cases were from confirmed positive dams, 11 were from clinically affected dams, and 97 were from dams with unknown clinical disease status. The confirmed positive dams were between 24 and 36 months of age at the birth of offspring later found positive. The clinically affected dams were between 22 and 70 months of age and the dams of unknown status were between 14 and 96 months of age at the birth of offspring later found positive. The four confirmed cases which were the offspring of positive dams had the following histories: one was born in a source flock, but was not the first positive born in that flock; and three were born into infected flocks which likely had been infected two years earlier.

There were 823 animals for which age at death could be calculated based upon at least birth year and death year. Most of these animals (633) died at less than 54 months of age and were therefore likely exposed at birth. A total of 184 (27.34%) of these were born in known infected flocks and died at an average age of 38.15 months which is statistically different than all others which died on average at 41.01 months ($p = .001$). Using the definition for source flock under the new voluntary certification program, 56 (8.32%) were born in source flocks and died at an average age of 41.90 months which was not statistically different than all others which died on average at 40.07 months. Trace flocks, as defined under the new program, were the birth flocks for 75 (11.14%) of the animals likely exposed at birth and animals born in trace flocks died at an average age of 40.95 months. For 53.19% of the animals that were likely exposed at birth, there was no birth flock information available in any of the records examined.

For the 190 animals exposed after birth by virtue of the fact that they died at greater than 54 months of age, the first two flocks to which they belonged were evaluated. Eighteen of these animals (9.33%) were exposed and diagnosed in their birth flocks. Of the remaining animals, 27 were at some point members of an infected flock, three passed through source flocks, and two passed through trace flocks. That leaves 143 animals (74.09%) which were born in and members of flocks of unknown status.

CHAPTER 5

DISCUSSION

DATA QUALITY

The overall condition, accuracy, and completeness of the records was highly variable over the study period. From 1952 until the late 1960s, the records were extremely complete and had been previously analyzed to some degree[32]. During the 1970s, it appeared as though the detailed information about each positive flock and each positive animal may not have been gathered, and by 1980 most of the detailed information such as registration certificates and movement dates were unavailable in records held by the technical program staff of USDA. Detailed records were obtained from the USDA-APHIS-VS office in Ohio for animals found positive after 1980 in Ohio. For animals found after 1980 in other states, only limited information such as breed, sex, estimated age, state, and county were available. These limits in available information may have significantly biased many of the study factors examined for several reasons. First of all by examining the area under the curve in Figure 9, it becomes apparent that over half of the positive flocks detected were found between 1980 and 1991. If there was a significant difference in factors affecting disease occurrence during this time period, the available data might not allow that determination. Secondly, when examining records, the limited information available was less accurate for cases confirmed between 1980 and 1991 since registration certificates, actual birth dates, and movement information were not available.

It was not possible to calculate the prevalence or cumulative incidence of scrapie in the United States using the available USDA records. It should also be kept in mind that calculations accomplished with the data from this study are reflective only of levels of reported disease occurrence and not true disease occurrence. The reported prevalence of scrapie could not be determined due to lack of consistent information concerning when confirmed cases became sick. The reported cumulative incidence could not be ascertained due to missing denominator information at the state level for several states which both contained sheep and confirmed scrapie cases. These problems were present for incidence and prevalence calculations at both the flock and individual animal levels. Mortality rates for infected flocks were calculated based on the number of confirmed cases divided by the reported flock population at the time of first diagnosis. The mortality rates calculated in this manner could arguably be called cumulative incidence since not all confirmed cases actually died due to scrapie. Some of these animals were sacrificed for the purposes of diagnosis and this may have led to a bias in the value arrived at as the average age at death for confirmed cases. Unfortunately, most of the records did not include information concerning the actual method of destruction, nor did they include the date of onset of clinical signs. Since scrapie is considered a uniformly fatal disease, all scrapie cases would eventually die due to scrapie if other causes of death did not intervene. For this reason, the number of confirmed cases divided by the flock population at the time of first diagnosis was referred to as a mortality rate. Errors and inconsistencies in the collection of flock population information values may have biased these calculations. There was not a uniform protocol followed to be sure that population values included only sheep in age groups at risk of contracting scrapie.

In evaluating individual animal information, it becomes apparent that much of the detail needed to assess trends and differences among

breeds, sex, number of siblings, and statuses of the sire and dam were unavailable. With the exception of breed and sex, nearly half of the animal level factors studied were unavailable in accurate format after 1980 since registration certificates were not included with the records. Age at death could be calculated for 443 of the 957 confirmed positive cases. Sire and dam information was available for an even smaller handful of the confirmed cases. It is possible that these missing pieces of data would significantly change the trends and patterns seen in the data. It is also possible that the confirmed cases reported to USDA are not reflective or representative of all scrapie affected animals in the United States.

With respect to the disease status of the individual animals, there is possible misclassification bias in this study due to the fact that not all clinically affected animals were examined histologically to establish their true disease status. Based on a subjective evaluation of the records available, it is highly likely that many clinically affected animals that were laboratory negative were not included in the records examined. It is possible that these animals might differ in some way from those animals which were subjected to laboratory diagnosis. Lack of complete disease status information for these clinically affected animals which were most often flock mates or bloodline animals may have significantly altered the findings of this study.

FLOCK FACTORS

Geographic and Seasonal Trends

Flock location appears to be relatively unimportant in determining the age at which death or destruction due to scrapie will occur. This indicates that the survival of the agent and/or transmission of the agent is not significantly affected by the variation in climate and husbandry practices found in the United States. This comes as no surprise in light

of the resistant character of the scrapie agent as documented by experimental studies.

Scrapie positive flocks were found in each one of the eight geographical regions, although two of the regions (mountain and west north central) had a substantially lower proportion of positive flocks. This may be explainable by differences in husbandry practices that make disease detection more difficult. Many flocks in the western regions of the country are raised under conditions conducive to coyote predation[49]. It is quite possible that many sheep clinically affected with scrapie are consumed by coyotes long before clinical signs are noted by the owner. It is also possible that there are differences in the predominant sheep breeds for each of the regions. Such a difference would tend to confound the effects due to geographic region alone and render any true regional differences undetectable. This would not be a problem if breed distributions were known on a state by state basis. Unfortunately, accurate information on state level breed distribution is not available.

There were no differences in seasonal occurrence of scrapie when the four quarters of the year were used as seasons and date of occurrence was the date of diagnosis. This lack of difference is not surprising when considering the multiple factors affecting the rapidity of disease recognition in positive flocks. Diagnosis of the first case in each flock often spurred the rapid diagnosis of other cases in the flock which could be at varying stages. In addition, the clinical course of each positive case is thought to vary in length, which further complicates any patterns of seasonal occurrence.

Magnitude of the Scrapie Problem in the United States

The proportion of reported scrapie affected flocks, a surrogate for the reported incidence, in the United States has been slowly increasing since 1965. The slowly increasing numbers of positive flocks being detected coupled with the steady decline in the number of sheep

operations in the United States have led to the increased proportion of reported scrapie positive flocks. The proportion of reported positive flocks shown in Figure 8 most certainly represent an under-reporting of disease. However, the available data do not allow detection of trends in the true level of scrapie infection in the United States.

The average within-flock mortality rates increased slowly during the study period. Many of the spikes noted in Figure 9 are the result of scrapie positive cases in flocks containing few animals. The changes in mortality over time depicted in Figure 9 cannot be explained by the various phases of the scrapie eradication program. If flock mortality rates are heavily influenced by indemnity rates, one might expect that the flock mortality rates would decrease in years following an increase in the indemnity rates since higher rates would influence flock depopulation before large numbers of animals succumb to disease. Indemnity rates increased in 1952, 1975, and 1978. In all cases, during the year or two following the increase, the average mortality rate within flocks dropped off slightly, but then resumed its steady climb suggesting that the influence of indemnity rates was relatively minor.

A possible explanation for the apparent lack of effect of the indemnity rates both on mortality and proportion of positive flocks is that the producers perception of the consequences for reporting clinically suspicious animals to regulatory officials had far more influence on their willingness to report a possible problem than did any other single item. The available USDA data did not include factors which could allow possible assessment of producer attitudes.

Evaluation of the number of newly affected flocks detected as compared with the indemnity rate suggests that the indemnity rate may have significantly influenced the willingness of the producers to report scrapie in their flock (Figure 11). The data available did not allow evaluation of this trend to determine if increased producer awareness, increased interest on the part of veterinarians, or a variety of other

possibilities might have had more to do with the increase in flocks found. The overall increase in the proportion of scrapie infected flocks in the United States (see Figure 8) may also have greatly influenced this apparent increase in detection of new positive flocks.

ANIMAL FACTORS

The available data showed very few differences between the age at diagnosis for the various animal level factors included in the study. In general, there is not sufficient denominator data for animal level characteristics of sheep in the United States. This makes any possible inferences based on available data nearly impossible.

Age at Death

The decreasing trend in age at death due to scrapie for the first through third cases within flocks was not significant. Unfortunately there were only 18 flocks with sufficient data to allow these calculations. It is quite likely that the observed trend would be significant if more flocks could be included in this type of analysis. It might then be possible to determine if age at diagnosis could be a useful tool in determining how long a flock has been infected with scrapie.

The significant difference between age at death for positive ewes as compared with positive rams is likely an artifact due to the large differences in numbers of ewes and rams. There were three and one-half times as many ewes as rams and this alone was likely the cause of the apparent differences. The difference in average age is only five months and when 90% confidence intervals are constructed for each, they overlap. It is also possible that this observed difference is a true difference. A difference of this nature could be related to husbandry practices. If ram lambs kept as breeding replacements are watched more closely and more prized than ewe lambs, one might postulate that their abnormal behavior

at the onset of initial clinical signs might cause more notice and concern on the part of the producer. Since the age at death as measured in this study is influenced by how early clinical signs are noted, this might provide a possible explanation for the five month difference in age at death for rams as compared to ewes.

The data are also unable to measure any differences in breed susceptibilities since exposures for the various breeds are unknown and likely vary. Within each of the breed categories, there is a rather large variance in the actual data which may be an artifact due to confounding by some other factor. The fact that there were no differences in average age at death between the various breeds of sheep affected may be a function of the sheer numbers rather than reflective of a true lack of difference. The Suffolk breed comprised nearly 88% of the confirmed positive cases, but there is no information available to precisely determine the percentage of Suffolk sheep in the entire United States sheep population. Without this type of information, it is difficult to determine if the observed pattern is due to biologic differences between the breeds of sheep in the United States. It is quite possible that specific industry and husbandry related factors not measurable in a study of this nature have a more profound affect on exposure potential than any possible breed predilections.

The low numbers of twin pairs (26) included in the study did not allow any significant conclusions to be drawn. With two-thirds of the confirmed cases having been diagnosed in the last eleven years of the study when registration certificates were not available, considerable numbers of twins and triplets may have been missed. For this reason, the lack of findings concerning twins is of limited significance.

Role of Vertical Transmission

The results on the disease status and average age of the sire and dam at birth of positive offspring are of interest when considering

transmission. There were insufficient data to determine if there is some threshold age which the sire or dam must cross before disease transmission to progeny can occur. In the case of the four positive dams, all of them gave birth to their positive offspring in flocks where transmission may also have occurred from other positive flock mates. For the positive sires, it is somewhat more difficult to know what conditions to consider likely indications of male scrapie transmission. With so little known or hypothesized about male scrapie transmission, the lack of a discernable trend in sire age is easily interpreted as a negative finding.

It is somewhat difficult to interpret the observed decline in age at death in the offspring of dams of unknown disease status as compared to the offspring of dams of clinically affected dams as compared to the offspring of confirmed positive dams. The decline in age at death may be another manifestation of the trend reported in the literature for a decline in the age at which signs are first seen in subsequent cases in a flock. For similar reasons, it is difficult to assess the apparent lack of differences between the average ages at death for offspring of positive sires. Earlier studies showed an increase in proportion of positive offspring born to positive dams. The differences seen in this data may be a reflection of increased scrutiny of the offspring of positive ewes combined with an increased likelihood for transmission from dams when compared to sires. It is difficult to tell if the difference between the age at death for offspring of positive dams is clinically significant since there was no real group of known negative dams with which to compare them.

Source of Infection

Most of the animals in this study were born in flocks which could not be classified as infected, source, or trace flocks under the new voluntary flock certification program. For many of these animals, there

was insufficient information available to determine the birth flock. This may be a result of some combination of one or both of the following:

1) Incomplete records were available to the technical program staff of USDA. There were 343 of the animals, which were probably exposed at birth based on age at diagnosis, which did not have any record of a birth flock and there were 131 of the animals exposed after birth that did not have a record of a birth flock.

2) The tracing activities needed to determine birth flock and likely source of infection did not take place or were inadequate for several possible reasons. USDA personnel involved with the tracing activities may have failed to completely follow out all of the leads on possible origins of the disease for each flock. The producers involved may have had insufficient records to determine flocks of origin, especially in the case of grade or unregistered animals. The producers involved may have refused to cooperate fully with USDA officials for a variety of possible reasons.

CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE STUDIES

There were many areas of inconsistent amounts and quality levels of the data included in this study. Although one might expect data gathered at a national level in support of an eradication or control program to be uniform in content and format, such was not the case with the scrapie data. This points out the need for more consistent data in order to accurately and adequately track the progress of the eradication or control program.

In general, it would be advantageous to seek to fill the gaps in the present set of information and re-run all of the analyses to see if any new or different trends appear. Areas that could benefit from filling in the gaps of missing information are as follows: breed

differences, time trends for all study variables, sire and dam information, depopulation dates, and information on laboratory negative animals. Roughly half of the confirmed Suffolk case records included birth date information and less than half of the confirmed positive Hampshire sheep had birth date information.

Despite these difficulties with data quality, there were several significant findings in this study that are noteworthy and may warrant further investigation. The study suggests the possibility of an increase in the magnitude of the scrapie problem in the United States in terms of overall reported incidence and mortality within infected flocks. Further investigations to determine if these trends are real would be beneficial in order to assess the potential risk of sheep scrapie to the sheep industry and other animal industries in the United States.

The Suffolk breed was the predominant breed affected in the cases reported to USDA. The available data did not allow evaluation of breed related differences in susceptibility, differences in husbandry practices, or possible natural scrapie strain differences. Further studies to evaluate these possibilities would be beneficial.

The role and timing of vertical transmission in the spread of natural scrapie could not be evaluated with the available information. It is possible that additional information could be gathered which would allow this evaluation without the need for time consuming prospective transmission studies. Additional pedigree information for positive animals as well as negative animals would also prove potentially valuable in assessing the validity of the theory concerning the role of the ram in the introduction of susceptible genotypes. Efforts to fill in the missing details in the study data set should be undertaken prior to conducting further experimental or observational transmission studies.

The source of infection could not be determined in well over half of the reported cases. Aside from lack of information on the birth flocks, there is lack of complete information on animal movements. In

addition, the possibility of premises contamination is not really addressed by this study, and may play a significant role in the epidemiology of natural scrapie. More information on the roles of premises contamination and the effects of genotype on age at death may prove valuable in determining the likely source of infection for reported cases of natural scrapie.

Unless or until all sources of scrapie infection are determined and systematically removed, the magnitude of the scrapie problem in the United States cannot be expected to diminish. The difficulties of scrapie diagnosis combined with the unknowns of natural transmission are a severe hindrance to the goal of eradication. Lack of complete and accurate information on the origins and movements of individual affected and exposed animals further complicate and slow efforts to control or eliminate this troublesome disease.

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APPENDIX 1

DATABASE STRUCTURE

The data used for this study was entered into a relational database prior to analysis. Data validation was completed while the data was in the database. Information was kept in several tables which could be linked by the Premises ID field and by the various animal identification fields. Each premises (or flock) was uniquely identified with the Premises ID and each animal was uniquely identified with a set of three pieces of identification.

The following table structure was used to store the data:

1. Flock Information was stored in the premises and premises species tables. The exact data elements contained in these tables are as follows:

PREMISES SPECIES TABLE

Name	Null?	Type
PREM_ID	NOT NULL	CHAR(10)
SPECIES	NOT NULL	CHAR(3)
TYPE_OP	NOT NULL	CHAR(3)
POPULATION		NUMBER(5)
SP_USER_CODE_1		CHAR(6)
SP_USER_CODE_2		CHAR(6)
SP_USER_CODE_3		CHAR(6)
SP_REMARK		CHAR(80)
SP_RECORD_STATUS		CHAR(1)

PREMISES TABLE

Name	Null?	Type
PREM_ID	NOT NULL	CHAR(10)
PREM_NAME	NOT NULL	CHAR(30)
PREM_STATE	NOT NULL	CHAR(2)
COUNTY	NOT NULL	CHAR(3)
PREM_ADDRESS		CHAR(30)
PREM_CITY		CHAR(20)
RANGE		CHAR(3)
TOWNSHIP		CHAR(3)
PREM_SECTION		NUMBER(2)
LATITUDE		CHAR(6)
LONGITUDE		CHAR(7)
LNAME		CHAR(15)
FNAME		CHAR(15)
MI		CHAR(1)
SOUNDEX_NAME		CHAR(4)
ADDRESS		CHAR(30)
CITY		CHAR(20)
STATE		CHAR(2)
ZIP_CODE		CHAR(10)
PHONE		CHAR(12)
PR_USER_CODE_1		CHAR(6)
PR_USER_CODE_2		CHAR(6)
PR_USER_CODE_3		CHAR(6)
PR_REMARK		CHAR(80)
PR_RECORD_STATUS		CHAR(1)

2. Depopulation information was stored in the premises status table. The exact data elements stored in the premises status table are as follows:

PREMISES STATUS TABLE

Name	Null?	Type
PREM_ID	NOT NULL	CHAR(10)
STATUS	NOT NULL	CHAR(6)
DISEASE	NOT NULL	CHAR(3)
SPECIES	NOT NULL	CHAR(3)
ISSUE_DATE	NOT NULL	DATE
ISS_ENTRY_DATE	NOT NULL	DATE
ISSUE_RSN		CHAR(6)
CASE_NR		CHAR(10)
NR_ANIMALS		NUMBER(5)
RELEASE_DATE		DATE
REL_ENTRY_DATE		DATE
RELEASE_RSN		CHAR(6)
FILING_NR		CHAR(10)
PERSON_ID		CHAR(6)
PS_USER_CODE_1		CHAR(6)
PS_USER_CODE_2		CHAR(6)
PS_USER_CODE_3		CHAR(6)
PS_REMARK		CHAR(80)
PS_RECORD_STATUS		CHAR(1)

3. Individual animal information was stored in the animal table. The exact data elements stored in the animal table are as follows:

ANIMAL TABLE

Name	Null?	Type
PREM_ID	NOT NULL	CHAR(10)
EARTAG		CHAR(9)
REG_NR		CHAR(9)
FLOCK_TAG		CHAR(15)
SEX	NOT NULL	CHAR(1)
BREED	NOT NULL	CHAR(2)
BIRTH_DATE_DD		CHAR(2)
BIRTH_DATE_MM		CHAR(2)
BIRTH_DATE_YY		CHAR(2)
ARRIVAL_DATE_DD		CHAR(2)
ARRIVAL_DATE_MM		CHAR(2)
ARRIVAL_DATE_YY		CHAR(2)
ORIGIN_PREM_ID		CHAR(10)
SIRE_EARTAG		CHAR(9)
SIRE_REG_NR		CHAR(9)
SIRE_FLOCK_TAG		CHAR(15)
DAM_EARTAG		CHAR(9)
DAM_REG_NR		CHAR(9)
DAM_FLOCK_TAG		CHAR(15)
DISEASE_STATUS		CHAR(5)
DISP		CHAR(1)
DISP_PREM_ID		CHAR(10)
DISP_DATE_DD		CHAR(2)
DISP_DATE_MM		CHAR(2)
DISP_DATE_YY		CHAR(2)
NECROPSY_RESULT		CHAR(1)
REMARKS		CHAR(80)
TWIN		CHAR(2)

APPENDIX 2

DATA COLLECTION FORMS

There were three forms used for data collection as part of this study. The first form was used to keep track of the unique Premises IDs being assigned to each flock. The IDs used included the postal abbreviation for the state in which the flock was located to simplify the assignment process. The second form was used to collect information about the individual animals and their movements. The third form was used to record flock level events such as depopulation and the dates that trace and source labels were applied to the flocks. Copies of each of these forms have been included on the pages that follow.

_____ STATE PAGE ___

ID#	NAME	COUNTY	CITY	OTHER

INFECTED SOURCE TRACE

FLOCK ID: _____ FLOCK NAME: _____ STATE: _____

FLOCK ADDRESS: _____ FLOCK CITY: _____

COUNTY: _____ ZIPCODE: _____

NR SHEEP: _____ BREED: _____ FLOCK MGMT: _____

OTHER SPECIES INVOLVEMENT: _____

DATES- DIAGNOSIS: __-__-__ DEPOPULATION: ___-___-___ REPOPULATION:__-___-__

SOURCE FLOCK: _____

FLOCK TAG: _____ REG NR: _____ EARTAG: _____

sex
TWIN M F W BREED: _____ DOB: __-__-__ ENTRY DATE: ___-___-___

SOURCE FLOCK: _____

SIRE FLOCK TAG: _____ REG NR: _____ EARTAG: _____

DAM FLOCK TAG: _____ REG NR: _____ EARTAG: _____

Disposition
S L D K U DISPOSITION DATE: ___-___-__ DISEASE STATUS: _____

DISPOSITIONFLOCK: _____

REMARKS/NECROPSY RESULT: _____

FLOCK TAG: _____ REG NR: _____ EARTAG: _____

sex
TWIN M F W BREED: _____ DOB: __-__-__ ENTRY DATE: ___-___-___

SOURCE FLOCK: _____

SIRE FLOCK TAG: _____ REG NR: _____ EARTAG: _____

DAM FLOCK TAG: _____ REG NR: _____ EARTAG: _____

Disposition
S L D K U DISPOSITION DATE: ___-___-__ DISEASE STATUS: _____

DISPOSITIONFLOCK: _____

REMARKS/NECROPSY RESULT: _____

SCRAPIE FLOCK STATUS FORM (Masters Project)

FLOCK ID _____ FLOCK NAME _____

FOLDER _____

FLOCK STATUS (Circle One) INFECTED TRACE SOURCE

For INFECTED flock, SOURCE is _____

For TRACE flock, origin flock is _____

STATUS ISSUE DATE _____ ISSUE REASON _____

STATUS RELEASE DATE _____ RELEASE REASON _____

FLOCK ID _____ FLOCK NAME _____

FOLDER _____

FLOCK STATUS (Circle One) INFECTED TRACE SOURCE

For INFECTED flock, SOURCE is _____

For TRACE flock, origin flock is _____

STATUS ISSUE DATE _____ ISSUE REASON _____

STATUS RELEASE DATE _____ RELEASE REASON _____

FLOCK ID _____ FLOCK NAME _____

FOLDER _____

FLOCK STATUS (Circle One) INFECTED TRACE SOURCE

For INFECTED flock, SOURCE is _____

For TRACE flock, origin flock is _____

STATUS ISSUE DATE _____ ISSUE REASON _____

STATUS RELEASE DATE _____ RELEASE REASON _____