Salmonella enterica
serotype Enteritidis
in Table Egg Layers in the U.S.

National Animal Health Monitoring System   **October 2000**

Acknowledgments

This report has been prepared from material received and analyzed by the U.S. Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Veterinary Services (VS) during a nationwide study of management and flock health on layer operations.

The Layers '99 study was a cooperative effort between State and Federal agricultural statisticians, animal health officials, university researchers, extension personnel, and table egg layer operators. We want to thank the industry members who helped determine the direction and objectives of this study by participating in focus groups.

Thanks to the National Agricultural Statistics Service (NASS) enumerators and State and Federal Veterinary Medical Officers (VMO's) and Animal Health Technician's (AHT's) for their hard work visiting the operations and collecting the data and for their dedication to the National Animal Health Monitoring System (NAHMS). The roles of the producer, Area Veterinarian in Charge (AVIC), NAHMS Coordinator, VMO, AHT, and NASS enumerator were critical in providing quality data for Layers '99 reports. Special recognition goes to the following individuals for their guidance and advice:

Thomas E. Walton,
Director
Centers for Epidemiology and Animal Health

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Introduction

Salmonella

What is *Salmonella*?

Salmonella is a group of bacteria that can cause diarrheal illness in humans and animals and is a safety issue for foods from all animal sources. There are many different kinds of *Salmonella* bacteria. *Salmonella typhimurium* and *Salmonella enterica* serotype Enteritidis are the most common serotypes in the United States.[1]

What are the signs of salmonellosis in humans?

Signs of illness due to *Salmonella* infection, including diarrhea, fever, and abdominal cramps, appear 12 to 72 hours after infection. The illness usually lasts 4 to 7 days, and most people recover without treatment. In some patients, the *Salmonella* infection may spread from the intestines to the blood stream and to other body sites. Severe dehydration may require hospitalization and fluid treatment. The elderly, infants, and those with impaired immune systems are more likely to develop severe illness. Acute salmonellosis may cause death without prompt antibiotic treatment, although deaths are rare.

A small number of people infected with *Salmonella* will develop pains in their joints, irritation of the eyes, and painful urination. This is called Reiter's syndrome. It can lead to chronic arthritis which is difficult to treat.

Salmonella enterica serotype Enteritidis

Salmonella enterica serotype Enteritidis is referred to as SE throughout this report. SE is the primary serotype of concern with regard to food safety from poultry sources and is of particular concern in the U.S. to the layer industry as SE can infect the reproductive tracts of laying hens. Eggs can be contaminated if the layers are infected and the SE is deposited in or on the egg. The birds show no sign of infection, and the eggs they produce appear normal. The rate of egg contamination with SE is sporadic and is estimated between 1 and 11 positive eggs per 100,000 eggs.[2]

What can producers and consumers do to reduce the risk of SE infection?

Producers potentially can reduce risk of spreading SE infection and other disease problems in their flocks by cleaning and disinfecting layer houses thoroughly between flocks and using good rodent control practices. Immediate refrigeration of eggs will also prevent multiplication of bacteria in the eggs. Quality assurance programs have been developed to help producers implement best management practices to reduce risk of SE in eggs.

Consumers can prevent illness by discarding cracked eggs, thoroughly cooking eggs, keeping eggs and egg-containing foods refrigerated, and washing hands and utensils in hot, soapy water after handling raw eggs.

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1   Salmonellosis. U.S. Department of Health and Human Services; Centers for Disease Control and Prevention. 2000. (www.cdc.gov/ncidod/dbmd/diseaseinfo/salmonellosis_g.htm)

2   Salmonella Enteritidis Risk Assessment: Shell Eggs and Egg Products. USDA:APHIS, Food Safety and Inspection Service. 1998. (www.fsis.usda.gov/OPHS/risk/index.htm)

Layers '99 Study

The National Animal Health Monitoring System's (NAHMS) Layers '99 study was designed to provide both participants and the industry with information on the nation's table egg layer population for education and research. NAHMS is sponsored by the USDA:APHIS:Veterinary Services (VS).

Layers '99 is the first NAHMS national study of the layer industry. NAHMS developed study objectives by exploring existing literature and contacting industry members and researchers about their informational needs and priorities. The objectives are listed inside the back cover of this report.

The USDA's National Agricultural Statistics Service (NASS) collaborated with VS to select a statistically-valid sample from 15 states for Layers '99 (see map below). The 15-state target population accounted for over three-quarters of the table egg layers in the U.S. on December 1, 1998.

NASS enumerators collected data for *Part I: Reference of 1999 Table Egg Layer Management in the U.S.* from 208 single- and multiple-farm companies via a questionnaire administered February 1-26, 1999. These respondents provided information on 526 farm sites which formed the basis of that report.

States Participating in the Layers '99 Study

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The second phase of data collection was done by federal and state Veterinary Medical Officers (VMO's) and Animal Health Technicians (AHT's) in the 15 states. Data were collected on 252 farm sites for *Part II: Reference of 1999 Table Egg Layer Management in the U.S.* via a questionnaire administered from March 22 through April 30, 1999.
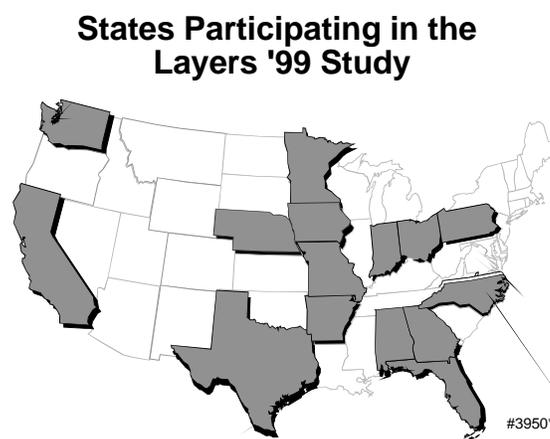
Information in both Parts I and II is operator-reported reflecting the operator's knowledge or opinion, which may or may not be based on laboratory results or veterinary advice (see Section II for methodologic information).

Environmental sampling was conducted in 200 layer houses from May 3 through October 22, 1999. Rodents were trapped in a a subset of these houses (137) to measure rodent index and house mice were cultured. Eggs were collected from 97 houses for yolk antibody testing for SE.

Results of the Layers '99 and other NAHMS studies are accessible on the World Wide Web at http://www.aphis.usda.gov/vs/ceah/cahm. For questions about this report or additional Layers '99 and NAHMS results, please contact:

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www.aphis.usda.gov/vs/ceah/cahm

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* Identification numbers are assigned to each graph of this report for public reference.

Terms Used in This Report

Bacterin: A killed bacterial product administered to immunize the host against a specific bacterial disease.

Competitive exclusion: Administration of a product containing bacteria that compete with SE bacteria in the digestive tract, thereby limiting growth of SE bacteria.

Egg yolk antibody test: A measurement of exposure to SE via an ELISA test to detect antibodies to SE in the yolk.

Environmental sample: Swabs were taken from surfaces in the layer house which included manure piles, egg belts, elevator/equipment, and walkways.

Farm site: A contiguous land unit that makes up a single premises. A farm site may have one or more layer houses on it.

Flock: A group of birds of similar age (ages may have varied several weeks from the median age of the flock) considered as a production unit. A flock usually fills only one layer house, but it may take up more or less than one house.

Last completed flock: The most recent flock that completed its production cycle and then was removed from the farm.

Layer: A chicken that produces eggs for table use or egg products.

Molt: That period of time when birds are taken out of production (usually around 65-70 weeks of age) until they return approximately to their 18-week weight. After a rest period, they are returned to production for another laying cycle.

N/A: Not applicable.

Non-business visitor: Anyone who did not have a business reason for visiting the operation, such as friends, family members, and tours.

Odds ratio: Estimate of relative risk, or increased risk, compared to reference level (where odds ratio=1).

P-value: Probability of obtaining a difference at least as large as the observed difference by chance alone given that the null hypothesis is true.

Population estimates: Averages and proportions *weighted* to represent the population. For this report, the reference population was all company-owned and contract farms associated with (companies) operations that had 30,000 or more layers on December 1, 1998, in the 15 participating states. Most of the estimates in this report are provided with a measure of precision called the *standard error*. If the only error is sampling error, chances are 95 out of 100 that the interval created by the estimate plus or minus two standard errors will contain the true population value. In the example illustrated on the next page, an estimate of 7.5 with a standard error of 1.0 results in a range of 5.5 to 9.5 (two times the standard error above and below the estimate). The second estimate of 3.4

shows a standard error of 0.3 and results in a range of 2.8 to 4.0. Similarly, the 90 percent confidence interval would be created by multiplying the standard error by 1.65 instead of two. ***Where differences between groups are noted in this report, the 90% confidence intervals do not overlap.*** Most estimates in this report are rounded to the nearest tenth. If rounded to 0, the standard error was reported. If there were no reports of the event, no standard error was reported.

Probability distribution: The likelihood of getting the data that is in the sample, given that the true prevalence in the population was at various levels.

Pullet: A female chicken less than 20 weeks of age. A pullet placed in the laying house is called a layer.

Regions:
    Great Lakes: Indiana, Ohio, and Pennsylvania.
    Southeast: Alabama, Florida, Georgia, and North Carolina.
    Central: Arkansas, Iowa, Minnesota, Missouri, and Nebraska.
    West: California, Texas, and Washington.

Sample profile: Information that describes characteristics of the operations from which Layers '99 data were collected.

SE: *Salmonella enterica* serotype Enteritidis.

Size of farm site: Size groupings based on number of layers 20 weeks of age or older present on December 1, 1998. For this report, sizes of farm sites were less than 100,000 and 100,000 or more.

Standardized rodent index: A measurement of rodent population in a house standardized to be equivalent to the number of rodents trapped in one house using 12 traps for 7 days (see Section II for methodology information).

**Examples of a
95% Confidence Interval**

95%
Confidence
Intervals

(1.0)   (0.3)
Standard Errors

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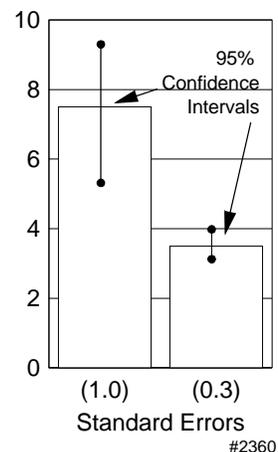
Section I: Population Estimates

A. SE Monitoring/Prevention Practices

1. SE programs during the pullet growing period

Overall, layers on 69.6 percent of farm sites came from pullet facilities that monitored for SE. The West region had the largest percentage of farm sites (83.0 percent) that obtained their layers from SE monitored pullet facilities.

Note: Estimates for farm sites that monitored for SE may be low because about 4 percent of producers overall (20 percent of producers in the Central region) did not know whether or not these procedures were done. These farm sites were included among those farm sites where monitoring was not done.

a. Percent of layer farm sites that used the following methods to monitor SE in pullets at the growing operation by region:

| | Percent Farm Sites by Region* | | | | | | | | | |
| | Great Lakes | | Southeast | | Central* | | West | | All Farm Sites | |
SE Monitoring Methods	Percent	Standard Error	Percent	Standard Error	Percent	Standard Error	Percent	Standard Error	Percent	Standard Error
Dead chick/chick paper testing	41.5	(7.4)	41.3	(9.5)	13.9	(2.8)	70.6	(4.2)	43.5	(3.9)
Environmental/manure culture	55.6	(8.1)	54.7	(9.4)	54.0	(6.7)	43.8	(5.6)	52.4	(4.1)
Bird culture	4.2	(1.2)	1.7	(0.8)	8.2	(2.3)	23.9	(4.1)	8.9	(1.2)
Serology	4.6	(1.6)	17.0	(4.9)	13.4	(3.1)	49.3	(6.5)	19.2	(2.7)
Any of the above	63.6	(8.4)	69.1	(7.7)	65.1	(6.0)	83.0	(2.6)	69.6	(3.9)

* Producers on 20 percent of farm sites in the Central region did not know if these procedures were done. The remaining regions had less than 2 percent of producers who did not know.

Percent Layer Farm Sites that Used Any Method to Monitor SE in Pullets at the Growing Operation by Region

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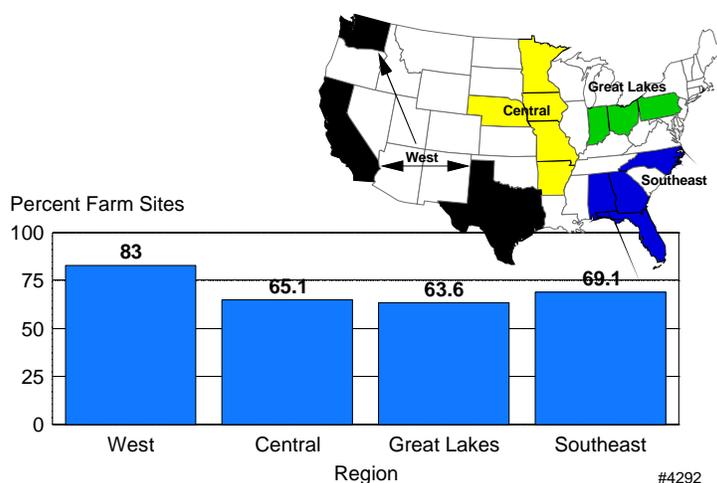
> Ten percent of farm sites obtained replacement pullets from facilities that used a competitive exclusion product in pullets. An additional 20.5 percent of farm sites did not know whether or not a competitive exclusion product was used.

b. Percent of layer farm sites on which a competitive exclusion product had been used to reduce SE in pullets at the pullet growing operation:

Use of Competitive Exclusion Product	Percent Farm Sites	Standard Error
Yes	10.3	(2.9)
Didn't know	20.5	(3.3)
No	69.2	(3.9)
Total	100.0	

Percent of Farm Sites on Which a Competitive Exclusion Product Had Been Used to Reduce SE in Pullets at the Growing Operation

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i. Percent of layer farm sites on which a competitive exclusion product had been used to reduce SE in pullets at the pullet growing operation by region:

Percent Farm Sites by Region

Great Lakes		Southeast		Central		West		All Farm Sites	
Percent	Standard Error	Percent	Standard Error	Percent	Standard Error	Percent	Standard Error	Percent	Standard Error
17.7	(6.8)	14.3	(4.1)	3.0	(1.2)	0.0	(--)	10.3	(2.9)

A total of 14.6 percent of layers (on 5.4 percent of farm sites) had been vaccinated as pullets against SE, with an additional 5.4 percent of layers for which vaccination status was unknown. Layers '99 data did not determine if immunization products used against SE were bacterin or live vaccine, however at the time of the Layers '99 study, most of the commerically available, licensed products were SE bacterins.

c. Percent of layer farm sites (and percent of layers on those farm sites) where the most recently placed flock had been vaccinated as pullets against SE:

| | Percent Farm Sites and Layers | | | | | | |
| | Yes | | Didn't Know | | No | | |
Measure	Percent	Standard Error	Percent	Standard Error	Percent	Standard Error	Total
Farm sites	5.4	(0.9)	10.4	(1.8)	84.2	(2.1)	100.0
Layers	14.6	(3.0)	5.4	(0.9)	80.0	(3.1)	100.0

The Great Lakes region had the highest percentage of farm sites where layers had been vaccinated as pullets against SE (10.2 percent of layer farm sites).

   i. Percent of layer farm sites (and percent of layers on those farm sites) where the most recently placed flock had been vaccinated as pullets against SE by region:

| | Percent by Region | | | | | | | | | |
| | Great Lakes | | Southeast | | Central | | West | | All Farm Sites | |
Measure	Percent	Standard Error	Percent	Standard Error	Percent	Standard Error	Percent	Standard Error	Percent	Standard Error
Farm sites	10.2	(2.5)	0.0	(--)	5.3	(1.3)	2.7	(0.8)	5.4	(0.9)
Layers	27.0	(7.7)	0.0	(--)	13.0	(3.6)	9.9	(3.5)	14.6	(3.0)

Percent Farm Sites Where the Most Recently Placed Flock had been Vaccinated Against SE as Pullets by Region
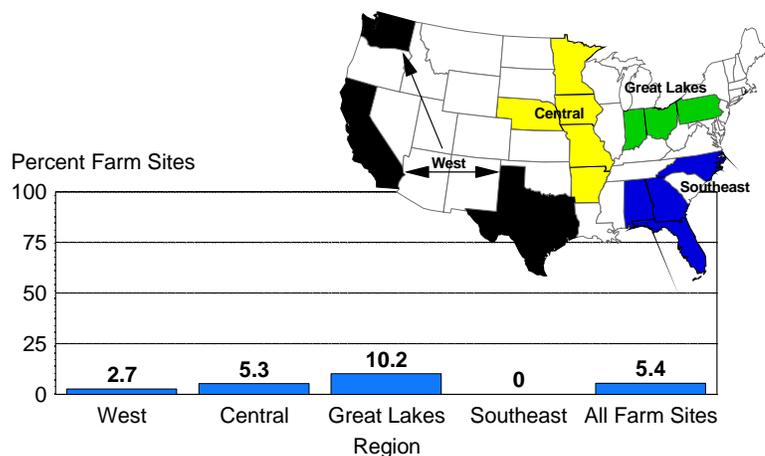
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2. Testing feed

The percentages of farm sites where either finished feed or feed ingredients were tested for SE ranged from 28.8 percent of farm sites in the Central region to 80.7 percent of farm sites in the West. Testing of feed ingredients was most common for farm sites in the West (76.0 percent) and Southeast (74.5 percent) regions.

a. Percent of farm sites that routinely tested finished feed or any feed ingredients for SE by region:

<u>Percent Farm Sites by Region</u>

	Great Lakes		Southeast		Central		West		All Farm Sites	
Item Tested for SE	Percent	Standard Error	Percent	Standard Error	Percent	Standard Error	Percent	Standard Error	Percent	Standard Error
Finished feed	41.0	(7.8)	51.5	(9.9)	25.9	(4.9)	67.6	(5.1)	46.8	(4.1)
Any feed ingredients	38.9	(7.7)	74.5	(6.7)	18.5	(4.5)	76.0	(3.8)	51.6	(4.0)
Either feed or feed ingredients	43.8	(7.8)	78.5	(6.6)	28.8	(5.1)	80.7	(3.4)	57.2	(4.0)
Both feed and feed ingredients	36.1	(7.7)	47.5	(9.8)	15.6	(4.3)	62.8	(5.5)	41.2	(4.1)

Percent of Farm Sites that Routinely Tested Finished Feed or Any Feed Ingredients for SE

Item Tested

Item Tested	Percent
Finished feed	46.8
Any feed ingredients	51.6
Feed or feed ingredients	57.2
Feed & feed ingredients	41.2

Percent

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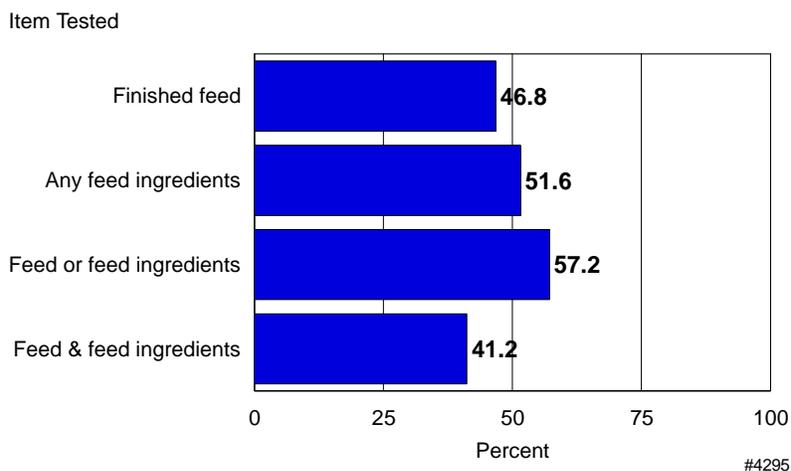
3. Testing in the layer house

> Only 15.7 percent of farm sites routinely tested for SE in 1994, whereas nearly three out of five (58.0 percent) farm sites routinely tested for SE in 1999. In 1999, the percentage of farm sites with a SE testing program ranged from 25.6 percent of farm sites in the Central region to 83.8 percent of farm sites in the Southeast region.
>
> Results of tests conducted by the producers were not collected for the Layers '99 study.

a. Percent of farm sites that were routinely testing for SE in the layer houses at the time of the Layers '99 interview and in 1994 by region:

Percent Farm Sites by Region

Time Frame	Great Lakes Percent	Standard Error	Southeast Percent	Standard Error	Central Percent	Standard Error	West Percent	Standard Error	All Farm Sites Percent	Standard Error
1999	57.3	(12.3)	83.8	(6.2)	25.6	(6.2)	58.8	(9.2)	58.0	(5.7)
1994*	23.4	(13.3)	1.6	(1.5)	13.7	(5.0)	18.5	(6.6)	15.7	(5.1)

* Excluded farm sites that were less than 5 years old at the time of the Layers '99 interview.

Percent of Farm Sites that Routinely Tested for SE in the Layer Houses by Time Frame and by Region
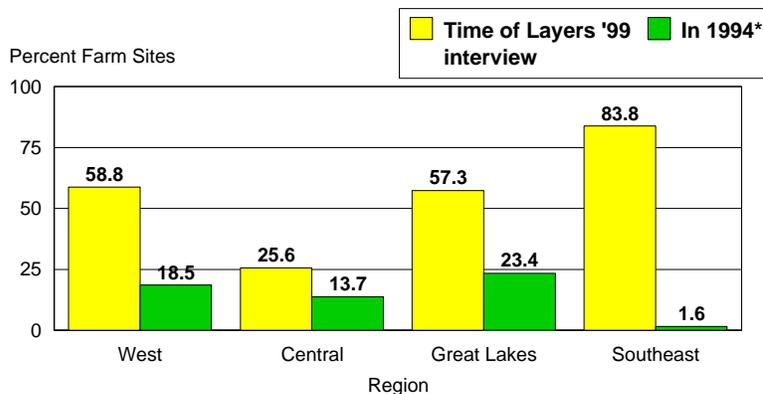
* Excluded farm sites that were less than 5 years old at the time of the Layers '99 interview..   #4180

NOTE: The following tables describe those farm sites that tested for SE at the time of the Layers '99 interview and those farm sites that tested for SE in 1994. Fewer than one in five farm sites tested in 1994, whereas nearly three in five farm sites tested during Layers '99 (Table I.A.3.a).

The most common method of testing for SE was by manure culture (89.7 percent of farm sites that tested). Approximately one-half of the farm sites that tested for SE cultured swabs from egg belts and elevator equipment. More than one test method may have been used on a farm site.

i. For farm sites that tested for SE in the layer houses for each time period, percent of farm sites that used the following methods to test for SE in the layer houses at the time of the Layers '99 interview and in 1994:

| | Percent Farm Sites by Time Frame | | | |
| | 1999 | | 1994 | |
Method of Testing (Source)	Percent	Standard Error	Percent	Standard Error
Culture				
Manure (swab)*	89.7	(3.6)	84.2	(11.1)
Egg belts (swab)*	52.6	(9.2)	41.3	(18.2)
Elevator/equipment (swab)*	42.0	(8.7)	34.7	(16.7)
Egg	10.4	(3.5)	26.8	(12.1)
Serology	12.7	(3.9)	27.7	(13.0)
Other	0.6	(0.4)	0.0	(--)

* For those farm sites that had such equipment.

Company or farm personnel collected samples for SE testing in 1999 on nearly three out of four farm sites (70.1 percent). A private veterinarian was the most frequent sample collector included in the Other category.

ii. For farm sites that tested for SE in the layer houses, percent of farm sites by primary sample collector for SE testing at the time of the Layers '99 interview and in 1994:

| | Percent Farm Sites by Time Frame | | | |
| | 1999 | | 1994 | |
Primary Sample Collector	Percent	Standard Error	Percent	Standard Error
Company or farm personnel	70.1	(6.3)	59.1	(15.3)
State or Federal personnel	8.5	(2.4)	17.2	(10.9)
Other	21.4	(5.4)	23.7	(13.0)
Total	100.0		100.0	

In 1999, approximately equal percentages of farm sites tested (by any sampling method) for SE before and during the last 4 weeks of production. Testing during the last 4 weeks of production was more common in 1999 than in 1994 for farm sites that tested for SE. About one in three farm sites in each time frame tested before layers were placed. Farm sites may have tested more than once during a production cycle.

iii. For farm sites that tested for SE in the layer houses, percent of farm sites by when testing for SE was usually performed at the time of the Layers '99 interview and in 1994:

	Percent Farm Sites by Time Frame			
	1999		1994	
Time Testing Was Performed	Percent	Standard Error	Percent	Standard Error
Before layers were placed	29.4	(6.7)	33.7	(12.8)
After layers were placed but before the last 4 weeks of production	59.8	(8.1)	62.1	(15.1)
During the last 4 weeks of production	59.2	(9.0)	24.5	(9.9)

Producers in the Great Lakes region who tested for SE primarily tested *before* the last 4 weeks of production (91.7 percent of farm sites). In the other regions, the majority of producers who tested conducted the tests *during* the last 4 weeks of production.

iv. For farm sites that tested for SE in the layer houses, percent of farm sites by when testing for SE was usually performed at the time of the Layers '99 interview and by region:

	Percent Farm Sites by Region							
	Great Lakes		Southeast		Central		West	
Time Testing Was Performed	Percent	Standard Error	Percent	Standard Error	Percent	Standard Error	Percent	Standard Error
Before layers were placed	3.2	(2.9)	44.4	(14.7)	47.8	(11.0)	40.0	(10.3)
After layers were placed but before the last 4 weeks of production	91.7	(6.6)	36.4	(16.0)	40.1	(9.9)	52.2	(10.5)
During the last 4 weeks of production	8.3	(6.6)	98.1	(1.9)	81.6	(6.3)	72.3	(9.2)
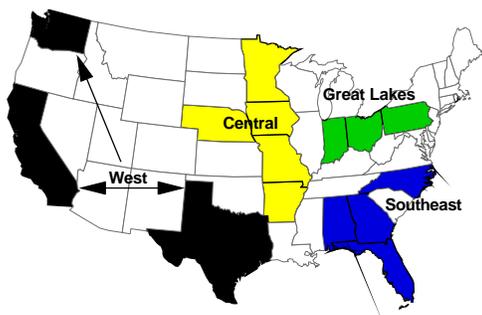
Percent of Farm Sites* by When Testing for SE Was Usually Performed by Region

■ Before placed
■ During production
□ Last 4 weeks of production
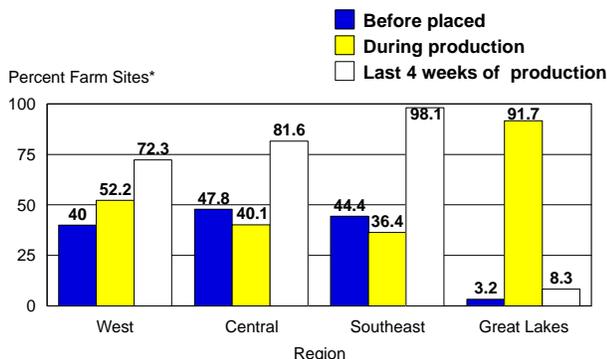
* For farm sites that tested for Salmonella enteritidis (S.e.) in the layer houses.   #4296

4. SE quality assurance programs

Over one-half (56.1 percent) of farm sites participated in a SE quality assurance program, with the most common being a company sponsored program (40.3 percent of farm sites). The percentage of farm sites participating in any program ranged from 22.9 percent in the Central region to 83.8 percent in the Southeast. In some states, a state or company program may have been the same as the commodity program and may have been included in one or both categories.

Estimates of participation in programs were based on producer reports with no further confirmation. Note that the percentages of farm sites participating in any quality assurance program are similar to the percentages of farm sites testing for SE (see Table I.A.3.a).

a. Percent of farm sites that participated in the following SE quality assurance programs by region:

Percent Farm Sites by Region

SE Quality Assurance Program	Great Lakes		Southeast		Central		West		All Farm Sites	
	Percent	Standard Error	Percent	Standard Error	Percent	Standard Error	Percent	Standard Error	Percent	Standard Error
State program	25.1	(13.4)	9.4	(3.6)	0.0	(--)	48.2	(8.4)	22.7	(5.3)
Company sponsored program	29.5	(9.7)	72.4	(8.5)	21.8	(5.5)	39.5	(8.8)	40.3	(5.3)
Commodity group program (e.g., United Egg Producers)	18.1	(12.1)	59.6	(12.1)	10.2	(3.0)	27.2	(6.8)	28.4	(6.2)
Other	0.0	(--)	0.0	(--)	0.0	(--)	0.0	(--)	0.0	(--)
Any	52.0	(12.4)	83.8	(6.2)	22.9	(5.5)	60.2	(9.2)	56.1	(5.7)

Percent of Farm Sites that Participated in the Following SE Quality Assurance Programs

SE Quality Assurance Program

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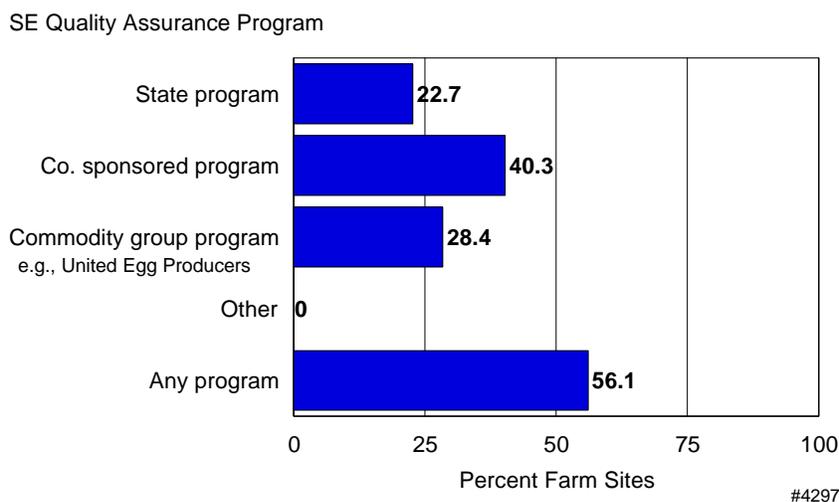
> Over one-half (55.0 percent) of farm sites that participated in a SE quality assurance program had an inspection by someone not associated with the farm (i.e., independent third-party verification). The percentage ranged from 6.1 percent of farm sites in the Central region to 88.0 percent of farm sites in the West region. State involvement in verification of SE quality assurance plans is described for Layers '99 states in Appendix II.

i. For farm sites that participated in a SE quality assurance program, percent of farm sites that had an inspection by someone not associated with the farm site or company to verify compliance with the SE quality assurance program by region:

<u>Percent Farm Sites by Region</u>

Great Lakes		Southeast		Central		West		All Farm Sites	
Percent	Standard Error	Percent	Standard Error	Percent	Standard Error	Percent	Standard Error	Percent	Standard Error
48.2	(18.8)	46.3	(14.7)	6.1	(4.8)	88.0	(3.8)	55.0	(8.2)

B. Environmental Culture Results

1. Descriptive results

Overall, SE was found in 7.1 percent of layer houses. Regional prevalence estimates ranged from 0 percent in the Southeast to 17.2 percent in the Great Lakes region. Note the large standard error in the Great Lakes region due to a small sample size as a result of low participation in this region. However, this small sample was similar to the large sample from this region that participated in Phase I of the study in terms of many management practices (see the discussion at the end of Section II). These results are specific for SE; presence of other serotypes were not recorded.

a. Percent of layer houses with at least one environmental culture swab positive for SE by region:

Percent Layer Houses by Region

Great Lakes		Southeast		Central		West		All Farm Sites	
Percent	Standard Error	Percent	Standard Error	Percent	Standard Error	Percent	Standard Error	Percent	Standard Error
17.2	(13.7)	0.0	(--)	9.0	(7.2)	4.4	(2.5)	7.1	(3.6)

Percent Layer Houses with at Least One Environmental Culture Swab Positive for SE by Region
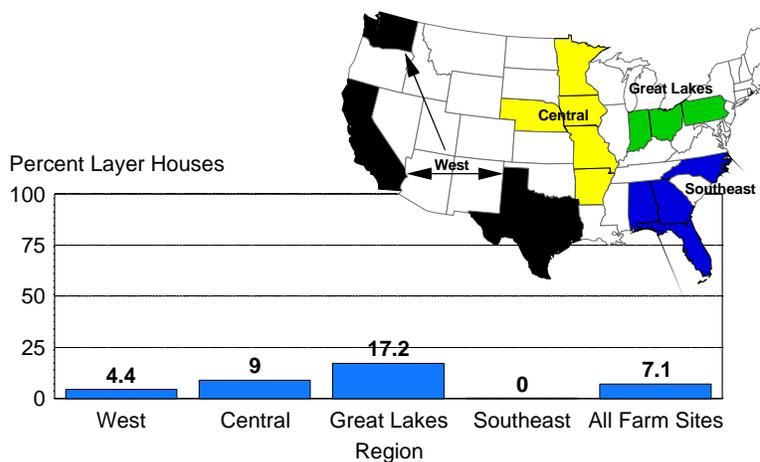
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Approximately 4 percent of houses with fewer than 100,000 layers were environmentally positive for SE, while 16.5 percent of houses with 100,000 or more layers were environmentally positive for SE.

b. Percent of layer houses with at least one environmental culture swab positive for SE by flock size (number layers placed in the house):

Percent of Layer Houses by Flock Size (Number Layers Placed)			
Less than 100,000		100,000 or More	
Percent	Standard Error	Percent	Standard Error
3.9	(2.0)	16.5	(10.4)

Environmentally positive houses had an average of more than 109,000 layers (median = 120,000) versus an average of fewer than 65,000 layers (median = 54,000) in environmentally negative houses.

i. Average flock size (number of layers placed) for layer houses environmentally positive and negative for SE:

Layer House Status	Average Flock Size (Number Layers Placed)	Standard Error
Positive	109,777	(19,968)
Negative	64,356	(5,124)

Nearly one-half of the positive flocks were identified via the egg belt (47.8 percent) or elevator (45.2 percent) samples. Although a goal of this study was to be able to compare the SE recovery rates by different collection sites, too few positive samples were found to make this comparison.

Note: Sixty percent of positive houses had only one positive sample, and no houses had more than two positive samples.

c. For layer houses environmentally positive for SE, percent of houses that were positive by source:

Source	Percent Layer Houses	Standard Error
Manure	16.9	(13.6)
Egg belt	47.8	(18.8)
Elevator	45.2	(14.2)
Walkway	18.1	(8.6)

The odds of a flock having at least one environmental sample testing positive for SE was evaluated for rodent index (Table I.B.2.a), several flock characteristics (Table I.B.2.b), farm management practices (Table I.B.2.c), and cleaning and disinfecting practices (Table I.B.2.d). These variables were modeled with region and flock size as covariates to adjust for possible confounding influences. Other potential confounders may exist, but due to the low number of positive flocks, additional covariates could not be modeled. Tables in this section show potential factors related to presence of SE, the percent of flocks with the factor that were positive for SE (and standard error), the odds ratio (adjusted for region and flock size), and the p-value.

2. Risk factors for having a positive flock

After adjusting for region and flock size, houses with a standardized rodent index of 20 or more were nearly nine times more likely to have SE found within the house than were houses with a rodent index of less than 20. Calculation of the rodent index is described in the Methodology section (Section II.D.1) of this report.

a. Percent of layer houses with at least one environmental culture swab positive for SE by standardized rodent index (rodents trapped in 12 traps per week):

Rodent Index	Percent Layer Houses Positive	Odds Ratio	p-value
0 - 19	2.0	1	.04
20 or more	10.1	8.9	

The average rodent index in SE positive houses was more than twice that of negative houses.

i. Average rodent index for layer houses environmentally positive and negative for SE:

Layer House Status	Average Rodent Index	Standard Error
Positive	38.9	(7.1)
Negative	16.7	(2.4)

Flocks of the Hy-line breed had a lower prevalence of SE (5.2 percent) than other white egg breeds combined (12.3 percent). There were too few flocks of any other specific white egg breed to evaluate separately. None of the brown egg flocks tested positive, but there were too few to evaluate statistically. After adjusting for region and flock size, flocks that were 0-16 weeks post-molting were 9.3 times more likely to test positive compared to flocks that were 60 or more weeks of age and unmolted, but flocks more than 16 weeks post-molt had very little increased risk. Younger flocks (less than 60 weeks of age) were 4.7 times more likely to test positive than older, unmolted flocks. Flocks that were reported to be in excellent health and that had no concurrent diseases were less likely (although marginally insignificant) to test positive than other flocks. None of the flocks that had been vaccinated against SE or that had been given a competitive exclusion product tested positive, however very few flocks received these practices and therefore, these factors could not be evaluated statistically.

b. Percent of flocks positive for SE (based on environmental cultures) by the following flock characteristics:

Flock Characteristics	Percent Positive	Standard Error	Odds Ratio	p-value
Breed/strain				.03
Hy-line	5.2	(3.7)	0.21	
Other white	12.3	(5.7)	1	
Brown	0.0		Too few	
Age/molt				.02
Less than 60 weeks of age, not molted	8.0	(4.5)	4.7	
60 weeks or more of age, less than 16 weeks post-molt	11.3	(6.2)	9.3	
60 weeks or more of age, 16 weeks or more post-molt	3.9	(3.6)	1.4	
60 weeks or more of age, not molted	4.9	(4.3)	1	
Any concurrent disease				.12
Yes	11.1	(6.3)	3.4	
No	5.1	(2.5)	1	
Flock health				.16
Excellent	4.5	(2.6)	0.3	
Good/fair	10.1	(5.6)	1	
SE vaccination (this flock)				
Yes	0.0		Too few	
Don't know	2.8	(2.5)		
No	8.7	(4.6)		
Competitive exclusion product administered (this flock)				
Yes	0.0		Too few	
Don't know	2.1	(1.9)		
No	8.5	(4.5)		

Flocks that had been primarily floor reared as pullets were 5.9 times more likely to test positive for SE than were flocks that had been cage reared. The SE prevalence was slightly higher for flocks on farms that fed poultry by-products, however this difference was not statistically significant. None of the flocks tested positive on farms that fed feeds without animal products. Also, none of the flocks tested positive that drank chlorinated water, although this practice was relatively uncommon and therefore could not be evaluated statistically. Flocks where pests such as flies, wild birds, and rodents had access to the feed prior to it being fed (hoppers, lines, etc.) were 6.2 times more likely to test positive. Flocks where visitors were not allowed in the layer houses had decreased odds of testing positive. None of the houses that used a flush system to handle manure tested positive compared to 13.4 percent of houses with high rise or deep pits for manure. The association with manure handling method may be related to the regional distribution of these practices (Layers '99 Part II, Table I.E.1.a). For houses with pits, the SE prevalence was lower (although not statistically significant) for those that had cleaned out the pit within the previous 6 months (3.4 percent) compared to those that had gone a longer time since cleaning the pit (15.7 percent). Additional factors evaluated and not found to be significant included testing the feed for SE, the age of the house, and the square inches of space per bird.

c. Percent of flocks positive for SE (based on environmental cultures) by the following farm management factors:

Farm Management Factors	Percent Positive	Standard Error	Odds Ratio	p-value
Floor reared during rearing				.04
Yes	10.5	(8.3)	5.9	
No	5.4	(2.7)	1	
Feed contains poultry by-products				.67
Yes	8.6	(7.2)	1.5	
No	6.0	(3.1)	1	
Feed contains animal products				*
Yes	8.9	(4.4)	1	
No	0.0		<1	
Water chlorinated				*
Yes	0.0		<1	
No	8.3	(4.6)	1	
Pests have access to feed (prior to feed trough)				.03
Yes	9.6	(4.6)	6.2	
No	5.8	(4.9)	1	
Visitors allowed (non-business)				.04
Yes	17.0	(10.3)	5.0	
No	3.6	(2.2)	1	
Manure handling method				*
High rise/deep pit	13.4	(7.6)	2.3	
Flush system	0.0		<1	
Other (shallow pit, manure belt, and scraper)	4.1	(2.3)	1	
Pit cleaned out in previous 6 months				.20
Yes	3.4	(3.3)	0.26	
No	15.7	(9.3)	1	

* p-value was not generated where no positive flocks were identified for one level of the variable.

None of the houses tested positive for SE on farms where the feeders or hoppers were cleaned and disinfected between each flock. Also, no houses tested positive where cages, walls, and ceilings were washed between each flock, whether or not they were fumigated. Houses that only fumigated between each flock had a lower prevalence of SE than houses that neither fumigated nor washed. A reduced risk was not identified in this study for dry cleaning cages and walls or for cleaning egg belts and elevators.

d. Percent of flocks positive for SE (based on environmental cultures) by the following cleaning and disinfecting practices:

Cleaning and Disinfecting (Between Each Flock) (V73a-t)	Percent Positive	Standard Error	Odds Ratio	p-value
Feeders				*
Yes	0.0		<1	
No	11.2	(5.3)	1	
Hoppers				*
Yes	0.0		<1	
No	10.1	(4.9)	1	
Cages, walls, ceiling				*
Wash and fumigate	0.0		<1	
Wash only	0.0		<1	
Fumigate only	5.3	(3.2)	1	
Neither	12.2	(6.5)	3.2	

* p-value was not generated where no positive flocks were identified for one level of the variable.

C. Mouse Culture Results

Because farms participating in rodent trapping were part of a convenience sample subset of the larger, Phase I sample, analysis was not weighted. To optimize regional representation, the number of houses targeted to participate in each region was roughly proportional to the size of the industry in that region.

1. Descriptive results

Overall, 3.7 percent of house mice cultured were positive for SE. None of the mice collected from the Southeast region were positive. The Great Lakes region had the highest SE prevalence in mice (7.3 percent). This regional distribution in mice was roughly consistent with the environmental results (see Table I.B.1.a).

a. Percent of house mice culture positive for SE by region:

Percent Mice by Region

| Great Lakes	Southeast	Central	West	All Regions
Percent	Percent	Percent	Percent	Percent
7.3	0.0	2.3	3.7	3.7

Percent Layer Houses with at Least One Environmental Culture Positive and Percent Mice Culture Positive for SE by Region
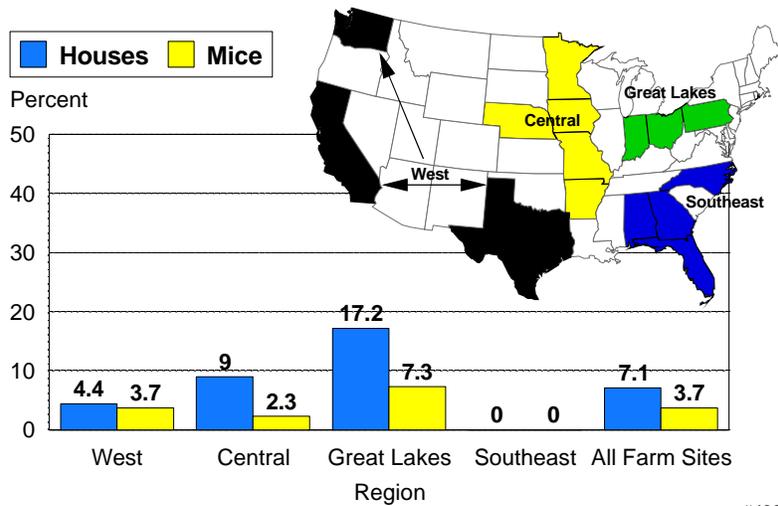
#4325

Because the rodent data analysis was unweighted, a standard error was not calculated. In order to put some bounds on the estimates, likelihood analysis was performed. This analysis gives a probability distribution for the estimates, i.e. the graph below shows the probability of obtaining the results in our data if the true prevalence were at various levels. It shows the possible true prevalence in mice in the West region was a fairly narrow range around the estimate (3.7 percent), whereas the estimate for the Great Lakes region (7.3 percent) had a much wider possible range. The probability of obtaining our results (no positive mice) in the Southeast region peaked at a true prevalence of 0 percent, and the probability of obtaining this result decreased with a true prevalence greater than 0 percent.

b. Probability distribution of SE prevalence in house mice by region:
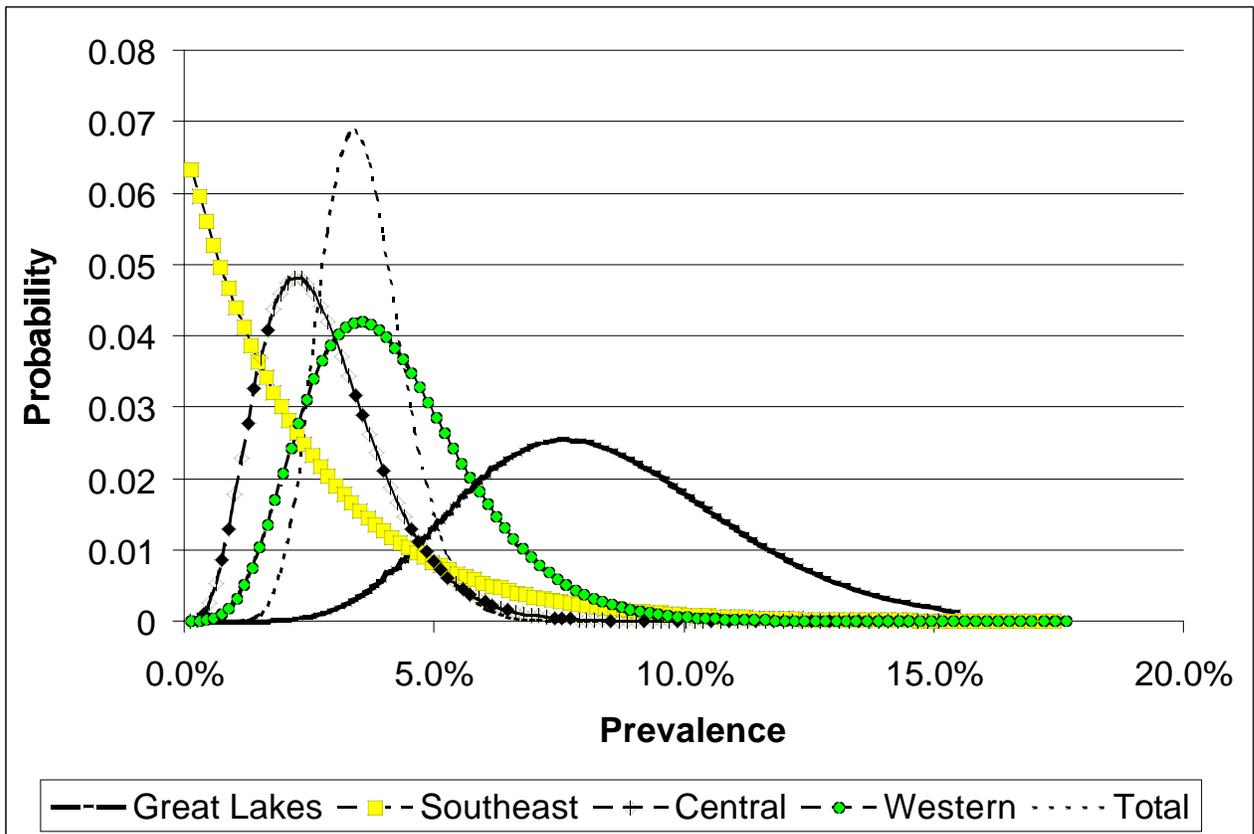
The percent of mice culture positive for SE was similar for large and small flocks.

c. Percent of house mice culture positive for SE by flock size (number layers placed in house):

Percent of Mice by Flock Size (Number Layers Placed)	
Less than 100,000	100,000 or More
Percent	Percent
3.5	3.9

Not only did environmentally positive houses have a higher rodent index (Table I.B.2.a), the prevalence of SE in house mice from environmentally positive houses was nearly four times that of mice from environmentally negative houses.

d. Percent of house mice culture positive for SE by house environmental status for SE:

Layer House Status	Percent Mice
Positive	11.2
Negative	2.9

D. Egg Yolk Antibody Test

One objective of the Layers '99 study was to evaluate the testing of egg yolks for the presence of SE antibodies as a possible tool for monitoring for SE in layer flocks. For the most part, monitoring SE in layer flocks has relied, to date, on bacterial culturing, which is expensive and requires at least 3 days to yield results. An inexpensive, more timely alternative was sought. The plan was to determine if egg yolk antibody testing might present a solution as it is economical, fast, and non-invasive to chickens (it relies on collection of eggs).

Previously developed ELISA tests that used flagella antigens were prone to cross-react with antibodies against other *Salmonellae* found in poultry. Because of that, we chose to go with an experimental fimbrial antigen ELISA test for the yolk survey with the hope that it would be more specific for SE antibodies.

For unexplained reasons, the results of the yolk tests using the fimbrial antigen do not appear to be compatible with the SE culture results using proven methods. We believe that until the immunology of SE is better understood and we have a test available that is proven to be specific for SE antibodies, this egg yolk test with fimbrial antigen will not be an effective monitoring tool for the presence of SE in layer flocks.

Section II: Methodology

A. Needs assessment

NAHMS was approached by United Egg Producers with a request for a national table egg layer study addressing the issue of SE. U.S. Poultry and Egg supported such a project. To further identify information needs, four focus groups were assembled to represent a broad spectrum of information users. These focus groups represented researchers/academia, industry, state and federal government, and West coast interests. Conference calls were held to brainstorm potential study topics. Focus group members then voted on topics to set the study objectives. Key participants from each focus group continued to provide advice on the study objectives and to provide guidance throughout the study design, implementation, and analysis. These individuals met twice in person and communicated regularly via telephone and e-mail discussions.

B. Sampling and estimation

1. State selection

The goal for NAHMS national studies is to include states that account for at least 70 percent of the animal and farm population in the U.S. The National Agricultural Statistics Service (NASS) Layers and Egg Production, 1997 Summary (released January 1998) was used to determine state ranking for table egg layers. All states with 4.0 percent or more of the U.S. table egg layers were included in the study. In addition, five states were added to provide better geographic coverage (Missouri, Washington, North Carolina, Arkansas, Alabama), resulting in a total of 15 states participating, representing 82 percent of 1997 U.S. table egg layers. NASS does not publish the total number of layer farms (some data were received from the 1992 Census of Agriculture), and therefore, number of layer farms per state did not contribute to state selection for this study.

2. Operation selection

NASS maintains a list of all egg-laying operations with 30,000 or more laying hens which is the basis for estimating monthly egg production. An operation may have one farm or multiple farms. Farms from multiple-farm operations may be company owned or contract farms. The individual farms may have fewer than 30,000 layers, but the total layers for all farms associated with a company must equal or exceed 30,000. All operations (companies) that had 30,000 or more laying hens (20 weeks of age or older) in the 15 selected states were eligible to participate.

3. Farm selection

NASS enumerators made the first personal contact to the operations. Enumerators visited company headquarters except for single-farm operations, where the farm was visited. If a company had farms in more than one state, each state was treated as a separate operation (assigned a unique operation identification code), and the NASS enumerator contacted the person who reported for the company in that state. The NASS enumerator selected a random sample of farms to participate. All farms were selected for operations with 10 or fewer farms. If the operation had 11 to 29 farms, 10 farms were selected. If there were 30 or more farms, 15 farms were selected.

4. Population inferences

All operations (companies) that had 30,000 or more laying hens (20 weeks of age or older) in the 15 selected states were eligible to participate in the NAHMS Layers '99 study. Therefore, the probability of selection (selection weight) was one for all operations. This selection weight was adjusted for non-response within state and size group strata. For each participating farm, a farm-level weight was created, equal to the operation weight multiplied by an expansion factor (number of farms in the operation divided by number of the operation's farms participating). This weight was adjusted again for non-response at the VS phase.

For the environmental sampling results, the farm level weight was expanded to account for the number of houses the farm had versus the number of houses sampled. Because farms participating in rodent trapping were part of a convenience sample subset of the larger NASS sample and to optimize regional representation, analysis was not weighted. The number of houses allowed to participate in each region was roughly proportional to the size of the industry in that region.

C. Data collection

1. Marketing

NASS mailed a pre-survey letter, letters of support from the U.S. Poultry & Egg Association and United Egg Producers, and information on the NAHMS Layers '99 study to each eligible operation (company). Additional information about NAHMS and the Layers '99 study were delivered at the time of the first personal contact. Some focus group participants made additional contacts to encourage participation.

2. Phase I: Layers Management Report, February 1 - 26, 1999

The NASS enumerator administered a Layers Management Report. This questionnaire was limited to items that could more readily be answered by company headquarters than by personnel on farm (e.g., pullet sources, feed sources). Practices that were expected to be the same on every farm were asked once of the operation, whereas a separate questionnaire for each farm was completed for those practices that may differ among farms. If an operation was willing to continue to the next stage of the study, a consent form was signed. The Layers '99 Part I report is from this phase of the Layers '99 study.

3. Phase II: Initial VS Visit, March 22 - April 30, 1999

Farms for which the operation had signed a consent form were contacted by Veterinary Services (VS) for the second phase (on-farm) of the study. Veterinary Medical Officers (VMO's) contacted each farm for participating operations, explained the program, and administered a questionnaire that could most readily be answered by farm personnel (e.g., housing, biosecurity). Although these questionnaires were scheduled to be completed by April 30, some states were given an extension in order to increase the number of participants. The last questionnaire was completed July 14, 1999. Layers '99 Part II reports results of this phase of the Layers '99 study.

4. Environmental sampling, May 3 - September 30, 1999

Environmental culturing was offered to all farms. One house per farm was randomly selected for culturing. On a few large farms, more than one house was selected. Samples were collected from surfaces throughout the house including manure (five samples per house), egg belts (five samples per

house), elevators (five samples per house), and walkways (two samples per house). If the house did not have egg belts or elevators, then 10 samples were collected from cage floors. Each sample consisted of two swabs. Samples were placed in whirl-pak bags containing skim milk, and shipped overnight on ice to the Agriculture Research Service in Athens, GA, for culture and serogrouping. Group D isolates were then sent to National Veterinary Services Laboratories (NVSL) in Ames, IA, for serotyping. Information about the flocks and houses being sampled was recorded on a Clinical Evaluation Record.

5. Rodent collection

Rodent collection was offered to 150 farms that also participated in environmental sampling. Twelve traps were placed per house. VMO's returned 4 to 7 days later to count the number of rodents caught. Rodents were euthanized using dry ice. House mice (*mus musculus*) were placed in large whirl-pak bags and shipped overnight on ice to NVSL for culture of internal organs. Other species (e.g., deer mice) were not tested because of human safety concerns due to the association with hantavirus. Up to five mice were cultured together as a pool. The number of rodents trapped, number submitted, trap location, and whether the trap had functioned properly were recorded on a rodent submission form.

6. Egg yolk antibody

Egg yolk antibody testing was offered to 100 farms that also participated in environmental sampling and rodent collection. There were 150 eggs collected per farm. The egg yolks were aspirated from the eggs and shipped overnight on ice to the University of Minnesota for testing for presence of antibody to SE. An ELISA test using the SF14 fimbrial antigen was performed. (See Section I.D for additional information.)

D. Data analysis

1. Editing and estimation

Initial data entry and editing for the Layers '99 Part I report were performed in each individual NASS state office. Data were entered into a SAS data set. NAHMS personnel performed additional data edits on the entire data set after data from all states were combined.

Data entry and editing for Part II were done by the NAHMS national staff in Fort Collins, CO. VS field staff followed up with producers where necessary. Summarization and estimation for questionnaire data and environmental sampling results were performed by NAHMS national staff using SUDAAN software. Odds ratios and p-values were obtained by modeling each variable using logistic regression. Region and flock size were included as covariates to adjust for the confounding influence of these variables.

Standardized rodent index was calculated as
        # rodents trapped * 7/# days * 12/# functional traps
so that all houses were standardized to the equivalent of having 12 traps function for 7 days. SE prevalence in mice was estimated using previously described methods for prevalence estimation from pooled samples.[1] Rodent culture results were summarized using SAS software. Likelihood analysis modeling of rodent culture results by region was performed using @Risk software.

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1   Sacks JM, Bolin SR, Crowder SV. Prevalence estimation from pooled samples. Am J Vet Res 1989; 50:205-206.

2. Response rates

The sample for Part I included 341 operations, of which 328 were considered eligible to participate. Thirteen operations in the sample were ineligible (e.g., broiler operations, or pullet growers). Of the 328 eligible operations, 208 operations agreed to participate (63 percent). These 208 operations provided information on 526 individual farms (see Farm selection on page 24). Consent was given to contact 393 of these farms for the second phase of the study (75 percent). Of the 393 farms contacted by VS, 11 were ineligible (no longer in business). Of the 382 eligible farms, 252 participated in the VS phase of the study (66 percent).

Rodents were collected from 129 houses, and egg yolks were collected from 97 houses. A total of 200 houses provided environmental samples for culture. Participation in environmental subsampling by state ranged from 1 house to 43 houses. In order to get some measure of the response bias caused by the poor participation of the Great Lakes region in this phase of the study, the small sample from this region for the VS phase of the study was compared to the relatively large sample this region provided for the NASS phase.

The table below shows the 27 VS-phase participants were very similar to the larger NASS sample from the Great Lakes region in terms of size, testing feed for SE, and vaccination practices.

Measure	Percent Farms Phase I (n=142)	Percent Farms Phase II (n=27)
Test feed for SE	43.8	51.0
Farm size (number layers)		
Less than 50,000	35.2	38.3
50,000-99,999	29.8	26.7
100,000-199,999	35.0	35.0
Total	100.0	100.0
Vaccinate pullets against:		
Laryngotracheitis (LT)	76.3	74.6
Mycoplasma gallisepticum (MG)	16.1	23.7
Fowl pox	91.5	87.1
Salmonella enteritidis (SE)	10.2	7.7
Avian infectious coryza	10.4	0.0

Appendix I: Sample Profile

The following tables present the numbers of flocks in the sample that contributed data. Each flock's data were expanded in order to make inferences to the population (all farm sites in the 15 participating states). The expansion factor for each flock was related to how many flocks it represented within its region. (See Section II.D for more specific information on population inferences.)

A. Number of Houses Environmentally Sampled

1. Flock size (number of layers placed)

Flock Size (Number Layers)	Number Houses
Less than 50,000	94
50,000-99,999	68
100,000 or more	38
Total	200

2. Region

Region	Number Houses
Great Lakes	23
Southeast	50
Central	44
West	83
Total	200

Appendix II

> State departments of agriculture are involved in third-party monitoring of quality assurance plans in Alabama, California, Missouri, Ohio, and Pennsylvania.
>
> Several states have adapted the United Egg Producers 5-Star Plan as their state program, while others, such as California, have their own plan. Some states have laws that address refrigeration and carton identification. For those states that have laws regarding refrigeration, maximum temperatures vary from 45-55° F.

Layers '99 states by state department of agriculture involvement in verification (e.g., third party) of a SE quality assurance plan:

State	Yes/No	Clarifications
Alabama	Yes	
Arkansas	No	
California	Yes	
Florida	No	
Georgia	No	A plan was being developed as of September 2000.
Indiana	No	
Iowa	No	
Minnesota	No	
Missouri	Yes	The State Veterinarian was prepared, however no producers were enrolled in third-party verification, as of September 2000.
Nebraska	No	
North Carolina	No	
Ohio	Yes	
Pennsylvania	Yes	
Texas	No	
Washington	No	

Appendix III: U.S. Table Egg Layers

During the Month of December in Flocks with 30,000 and Above*, 1998 and 1999

Region	State	Table Egg Layers (Thousand)	
		December 1998	December 1999
Central	Arkansas	4,565	5,151
	Iowa	24,261	26,399
	Minnesota	11,403	12,138
	Missouri	5,179	4,724
	Nebraska	10,522	11,700
	Total	55,930	60,112
Great Lakes	Indiana	21,265	22,107
	Ohio	29,639	30,778
	Pennsylvania	21,389	21,306
	Total	72,293	74,191
Southeast	Alabama	3,793	3,462
	Florida	10,244	10,036
	Georgia	11,892	11,800
	North Carolina	3,847	3,587
	Total	29,776	28,885
West	California	25,657	24,282
	Texas	13,719	13,830
	Washington	4,893	4,616
	Total	44,269	42,728
Total (15 states)		202,268 (78.6% of US)	205,916 (77.9% of US)
Total U.S. (50 states)		257,455	264,441

* There were 263,524,000 table egg layers during December 1998 and 270,367,000 table egg layers during December 1999 in flocks of all sizes.

Source: National Agricultural Statistics Service (NASS), *Chickens and Eggs*, February 22, 2000.

Outputs and Related Study Objectives

1. Describe baseline health and management practices used by the U.S. layer industry, such as disposal methods for manure/waste/dead birds/spent hens, pest control (rodents, birds, flies), molting practices, vaccination/preventive practices, and housing/ventilation.

➡ Part I: Reference of 1999 Table Egg Layer Management in the U.S., October 1999

➡ Part II: Reference of 1999 Table Egg Layer Management in the U.S., January 2000

2. Estimate the national prevalence of *Salmonella enteritidis* in layer flocks by testing the environment and other sources of contamination on layer operations.

➡ ***Salmonella enterica* serotype Enteritidis in Table Egg Layers in the U.S.**, October 2000

3. Identify potential risk factors associated with the presence of *S. enteritidis* to support and enhance quality assurance programs.

➡ ***Salmonella enterica* serotype Enteritidis in Table Egg Layers in the U.S.**, October 2000

4. Describe biosecurity practices used in the layer industry and how they benefit flock health.

➡ Part II: Reference of 1999 Table Egg Layer Management in the U.S., January 2000

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