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Surveillance of *Salmonella* Enteritidis in Layer Houses: A Retrospective Comparison of the Food and Drug Administration's Egg Safety Rule (2010–2011) and the California Egg Quality Assurance Program (2007–2011)

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SUMMARY. Between July 2007 and December 2011, 2660 environmental drag swab samples were collected in total from California layer flocks on behalf of the California Egg Quality Assurance Program (CEQAP), the egg safety rule (21 CFR Parts 16 and 118) of the Food and Drug Administration (FDA), or both. The samples were processed by the California Animal Health and Food Safety Lab, and positive or negative results for *Salmonella enterica* serovar Enteritidis (SE) were recorded. This study retrospectively compares the differences between the FDA and CEQAP programs with respect to their SE environmental sampling surveillance results. To accomplish this comparison, two different CEQAP (new and old) data sets representing different SE environmental surveillance approaches in the life of the flock were compared against each other and against the FDA's SE environmental testing plan. Significant differences were noted between the CEQAP and FDA programs with respect to the prevalence of SE in the farm environment. Analyses of the prevalence of SE at different stages in the flock's life cycle (chick papers, preproduction, midproduction, postmolt, and premarket) found the highest prevalence of SE in premarket (11.9%), followed by postmolt (3.5%) and midproduction (3.4%), and there was a tie between chick papers and preproduction (2.1%). To assess the main effects of the presence of SE in the farm environment, backwards binary logistic regression was used. Of six independent variables examined (age of flock, year, season, owner, CEQAP membership, and analysis of pooled samples *vs*. individual swabs), only age of flock, owner, and year were determined to be significant factors in the final model. Although CEQAP membership and pooling *vs.* individuals swabs were not included in the final model, Pearson chi-square tests did show significantly higher odds of SE for non-CEQAP member farms and higher odds of SE in pooled samples *vs*. individual swabs.

RESUMEN. Vigilancia de *Salmonella* Enteritidis en casetas de aves de postura: Comparación retrospectiva de la reglamentación de la Administración de Alimentos y Medicamentos en la seguridad del huevo (2010–2011) y del Programa de Aseguramiento de la Calidad del Huevo en California (2007–2011).

Entre julio del 2007 y diciembre del 2011, se recolectaron 2660 muestras de hisopos de arrastre ambientales de parvadas completas de gallinas ponedoras de California bajo el Programa de Aseguramiento de la Calidad del Huevo en California (con las siglas en inglés CEQAP), bajo las reglamentaciones en seguridad del huevo (Código de Regulaciones Federales número 21, partes 16 y 118) de la Administración de Alimentos y Medicamentos (con las siglas en inglés FDA), o bajo ambos. Las muestras fueron procesadas en el Laboratorio de Salud Animal y Seguridad Alimentaria de California y se registraron los resultados positivos o negativos para *Salmonella enterica* serovar Enteritidis (SE). Este estudio retrospectivo comparó las diferencias entre los programas de la FDA y del CEQAP con respecto a sus resultados de muestreos ambientales de vigilancia. Para llevar a cabo esta comparación, dos conjuntos diferentes de datos del CEQAP (nuevos y antiguos) que representaban diferentes enfoques de muestreos de vigilancia ambiental para *Salmonella enterica* serovar Enteritidis durante toda la vida de la parvada se compararon entre sí y con el plan de muestreo ambiental para *S. enterica* serovar Enteritidis de la FDA. Se observaron diferencias significativas entre los programas de la CEQAP y de la FDA con respecto a la prevalencia de esta bacteria en el ambiente de la granja. Mediante los análisis de la prevalencia para *S. enterica* serovar Enteritidis en las diferentes etapas del ciclo de vida de la parvada (del papel que cubre las charolas de pollo, preproducción, a la mitad de la producción, postmuda, y previa a la comercialización) se encontró la mayor prevalencia de esta bacteria en pre-mercado (11.9%), seguido por el periodo postmuda (3.5%) y mitad de la producción (3.4%), y se observaron resultados similares entre las muestras de papeles de las charolas de pollitos y preproducción (2.1%). Para evaluar los principales efectos de la presencia de esta bacteria en el entorno de granja, se utilizó el método de regresión logística binaria inversa. De las seis variables independientes analizadas (edad de la parvada, año, estación, propietario, membresía al programa CEQAP y análisis de muestras combinadas vs hisopos individuales), sólo la edad de la parvada, el propietario, y el año se determinaron como factores significativos en el modelo final. Aunque la participación en el CEQAP y el análisis de las muestras combinadas en comparación con las muestras individuales no se incluyeron en el modelo final, la prueba de ji cuadrado de Pearson mostró una probabilidad significativamente más alta para *para S. enterica* serovar Enteritidis para las explotaciones que no son miembros CEQAP y mayores probabilidades en muestras combinadas en comparación con los hisopos individuales.

Key words: *Salmonella* Enteritidis, Food and Drug Administration's Egg Safety Rule, (CFR Parts 16 and 118), California Egg Quality Assurance Program, environmental sampling for *Salmonella* Enteritidis

Abbreviations: CAHFS = California Animal Health and Food Safety Laboratory; C&D = cleaning and disinfection; CDFA = California Department of Food and Agriculture; CEQAP = California Egg Quality Assurance Program; CFR = Code of Federal Regulations; FDA = Food and Drug Administration; NPIP = National Poultry Improvement Program; OR = odds ratio; ROC = receiver operating characteristic curve; SE = *Salmonella* Enteritidis; USDA = United States Department of Food and Agriculture

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More than 2500 discrete seroytpes of *Salmonella* have been identified (10). *Salmonella enterica* serovar Enteritidis (SE) is a zoonotic pathogen that is primarily attributed to eggs (18). SE is relatively common in laying hens due in part to the persistence of SE in the environment and in part to the fact that layers may harbor SE without showing any signs of infection (10). Once a layer is infected, SE can translocate to the hen's reproductive tract and subsequently vertically infect eggs destined for human consumption (11). In addition, SE can be transmitted to eggs horizontally from the environment (e.g., from contaminated litter, feed, rodents, fomites) once the egg has been laid (15). Consequently, preharvest food safety at the poultry farm and postproduction interventions (such as refrigeration) are essential toward minimizing opportunities for the introduction, persistence, and transmission of SE on poultry farms with the ultimate goal of preventing human infections.

Because *Salmonella* and SE can be introduced into flocks from so many different sources, a comprehensive quality-assurance approach, encompassing both risk reduction and testing components, has been associated with a significantly lower incidence of SE in egg-laying flocks and humans (16). To address this issue, some state governments and, more recently, the federal government have implemented programs designed to reduce the incidence and prevalence of SE at the farm level.

The Food and Drug Administration (FDA) issued 21 Code of Federal Regulations (CFR), parts 16 and 118 (commonly referred to as The Egg Rule) in July 2009 (8). The Egg Rule requires that shell-egg producers implement measures to prevent SE from contaminating eggs on the farm and from further growth during storage and transportation, and it requires these producers to maintain records concerning their compliance with the rule and to register with the FDA (6). One of the measures requires egg producers to swab the farm environment at preproduction (14–16-week-old pullets), midproduction (40–45-week-old hens), and postmolt (4–6 weeks after molt) (6).

The rule was phased in to cover large producers (laying operations with >50,000 hens) effective July 2010 and small producers (laying operations with >3000 hens) effective July 2012.

The California Egg Quality Assurance Plan (CEQAP) is a voluntary program started in 1995 by United States Department of Agriculture (USDA), FDA, California Department of Food and Agriculture (CDFA), University of California extension, California Animal Health and Food Safety (CAHFS) Laboratory, and the table-egg industry as a response to human cases of SE that were traced to table eggs (17). CEQAP uses production, management, and monitoring practices focused on risk reduction of SE. Components of the plan include environmental monitoring for SE, rodent control, best practices, and flock health programs such as SE vaccination. By all indications, CEQAP and Egg Quality Assurance Programs in general have been successful in SE mitigation (16).

Initially, CEQAP only required SE environmental sampling once in the lifetime of the flock (usually a premarket sample), although most egg companies sampled chick papers (papers or material on the bottom of the chick delivery box) as well. Over the years, CEQAP's SE environmental monitoring program has become more robust. Specifically, as of 2010, five different SE environmental swabs in total are required (17). In addition to the three samples listed above as part of the FDA egg safety rule, California exceeds FDA standards by also requiring the testing of chick papers at delivery and a premarket sample (CEQAP, 2010). These changes to the CEQAP program coupled with the implementation of the FDA egg safety rule have resulted in a dramatic increase in sampling for SE.

This paper retrospectively reviews samples submitted for SE monitoring by the California egg industry to the CAHFS lab system over the past 5 yr in compliance with either the CEQAP, the FDA program, or both. The overall objective was to compare the value of the FDA and CEQAP programs with respect to SE prevalence and to build a statistical model to assess the main factors that contribute to SE positive farm environments in California.

MATERIALS AND METHODS

Study flocks. A query of the CAHFS Laboratory Information Management System for SE results submitted on behalf of the FDA egg safety rule, the CEQAP program, or both from July 2007 to December 2011 provided 2660 unique records upon which the study was based. The data were downloaded as a Excel (Microsoft, Redmond, WA) spreadsheet that was then imported into an Access (Microsoft) relational database. Stages in the production cycle of the flock at the time of sampling were categorized into one of the following six categories: chick papers, preproduction, midproduction, postmolt, premarket, and unknown.

If any of multiple tests performed on the submission were positive, the whole submission was classified as being positive for SE. Although the FDA program was not in effect before July 2010, samples that were collected for routine surveillance at points in the production cycle consistent with the FDA's sampling including preproduction, mid-production, and postmolt were categorized as FDA equivalent for purposes of this study.

Sample collection and testing. *FDA*. Environmental samples were collected according to 21 CFR Parts 16 and 118. In brief, the FDA requires environmental testing for SE at the following stages of production of a laying flock: preproduction (14–16-week-old pullets), midproduction (40–45-week-old hens), and postmolt (4–6 weeks postmolt).

Environmental samples are collected using four-by-four sterile gauze pads that are moistened in evaporated milk and dragged across the relevant environment (e.g., floors, belts, scrappers, tiers) of the poultry house(s). The area that each swab is dragged across is based on parameters outlined by the FDA (7). Once the swabs are collected, each swab is individually placed into separate Whirl-pak® bags (Fisher Scientific, Fair Lawn, NJ) and sent to a reference laboratory for detection of the presence of SE using the SE isolation procedure of the National Poultry Improvement Plan (NPIP) (7).

CEQAP. The CEQAP program requires members to implement an SE environmental monitoring program that includes environmental testing for SE at the three stages required by the FDA plus the chick papers at delivery and the premarket environment.

For purposes of this study, old CEQAP is defined as premarket samples. This old testing program was in place until 2010. Also for purposes of this study, new CEQAP is defined as samples taken at all five time points listed above. This testing program has been in place since 2010. Premarket samples, which were part of the old CEQAP and the new CEQAP, were included in both data sets.

Environmental samples were collected using a similar approach as described above for the FDA program. However, the CEQAP program allows pooling of up to four swabs in a single Whirl-pak bag for laboratory analysis.

Statistical analysis. To determine statistically significant ($P < 0.05$) differences between the SE monitoring program used by the FDA and that of CEQAP, 2×2 and $2 \times n$ Pearson chi-square tests were constructed (SYSTAT 13; Systat Software, Chicago, IL). Odds ratios (ORs) and P values are reported. To determine the most useful predictors of SE presence in the farm environment, multiple types of logistic regression were evaluated (SYSTAT 13). The regression models were evaluated based upon receiver operator characteristic curve (ROC) values and Naglekerke R^2 values. For logistic regression, the reference for the owner category was selected based on the owner with the greatest amount of sample submissions ($n = 213$). The reference year for the

Table 1. Number of SE-negative and SE-positive results for samples taken according to the old CEQAP program before 2010 (only premarket samples), the new CEQAP program (chick, papers, preproduction, midproduction, postmolt, and premarket), or FDA's sampling procedure (preproduction, midproduction, and postmolt). FDA equivalent includes samples taken before the FDA rule's implementation in 2010 that were equivalent to the FDA's sampling protocol. The OR reflects the odds that the FDA sample is positive relative to CEQAP. The 2010–2011 data were separated out from the 2007–2011 data to directly assess the FDA rule.

	Samples taken 2007–2011				Samples taken 2010–2011			
	Premarket (old CEQAP)	FDA equivalent	New CEQAP	FDA equivalent	Premarket (old CEQAP)	FDA	New CEQAP	FDA
SE negative	257	1109	1994	1109	177	803	1299	803
SE positive	34	35	82	35	11	15	29	15
Total	291	1144	2076	1144	188	818	1328	818
% Positive	10.46	3.06	3.94	3.06	5.85	1.83	2.23	1.83
OR	4.19		1.30		3.33		1.20	
P	<0.05		0.20		<0.05		0.58	

logistic regression was 2011, and the reference production stage was the premarket stage.

RESULTS

Pearson chi-square tests showed significant differences between the old CEQAP sampling procedure (SE sampling only at the premarket stage) and the FDA sampling protocol (sampling at the preproduction, midproduction, and postmolt stages; Table 1). Specifically, looking at data from 2007 to 2011, samples collected under the old CEQAP methodology were 4.19 times as likely to be positive for SE compared with samples collected under FDA methodology ($P < 0.05$). For those same years, the new CEQAP methodology (sampling from five stages in the flock's life cycle) was 1.30 times as likely to be positive compared with samples taken according to the FDA program ($P = 0.20$; Table 1). When looking at the results between 2010 and 2011, which includes the date that the FDA egg safety rule was implemented, samples collected under the old CEQAP methodology were 3.33 times ($P < 0.05$) and 1.20 times ($P = 0.58$) as likely to have positive SE samples relative to the current FDA program's methodology (Table 1). Over the 5 yr encompassed by the study, non-CEQAP members were 2.03 times as likely to have a positive SE samples relative to CEQAP members ($P < 0.05$; Table 2). In addition, pooled swabs were 2.62 times as likely to be positive compared with individual swabs ($P < 0.05$; Table 2).

Percentages of SE positives at each of the five stages in the production cycle were determined and are summarized in Table 3. The premarket samples (predepopulation) had the highest prevalence of SE, with 11.9% SE positive, followed by the postmolt samples with 3.5% SE positive, and the midproduction samples with 3.4% SE positive. Both the chick paper and the preproduction samples had the lowest prevalence of SE (2.1%; Table 3). An additional category containing samples of unknown origin had 7.3% SE-positive samples (Table 3).

A 2×5 Pearson chi-square test showed significant differences in SE prevalence between the five stages. Stratifying the SE results by year showed the highest prevalence of SE in 2007 (12.6%) followed by a significant decline until the completion of the study in 2011, for which the SE prevalence was 3.5% ($P < 0.05$). Of the 86 post-cleaning and disinfection (C&D) samples, 36% were positive for SE. We were unable to determine how many of those 86 samples were repeat samples from environments that tested positive for SE more than once.

Logistic regression. After testing multiple types of logistic regression, a best-fit model was derived using backwards binary logistic regression. The final ROC value was 0.84, with a Naglekerke R^2 value of 0.27 (the predictive value). Of the six covariates used (date, year, season, age, CEQAP status, pooled *vs*. individual swabs, and owner), only owner, date, and age of the flock were determined to be significant in the final model (Table 4). By removing each of the remaining significant covariates, owner was determined to be the most important variable, followed by year and age of flock. Of the 58 different farms included in this study, only eight farms had an OR > 1, with a range between 1.0 and 10.8.

DISCUSSION

The overall prevalence, 4.8%, of SE found in the environment of California poultry farms between July 2007 and November 2011 was consistent with previous statewide studies that showed the prevalence of SE on farms between 2.6% and 10% (13). The prevalence of *Salmonella* species was not captured for all the records supplied for this study, so an assessment of *Salmonella* at the genus level was not done. When stratifying the prevalence based on stage of production, the results were consistent with previous literature in that older birds (premarket) were more likely to have SE in their environment compared with younger birds (Table 3). A relatively low prevalence of SE in the postmolt samples (3.48%) relative to the midproduction samples (3.39%) and the premarket samples (11.92%)

Table 2. Number of SE negative and positive samples taken between 2007 and 2011 for CEQAP and non-CEQAP members and for pooled and nonpooled environmental samples. The ORs showed that non-CEQAP members are 2.03 times as likely to have a positive SE sample compared with CEQAP members ($P < 0.05$) and that pooled samples are 2.62 times as likely to have a positive SE sample compared with nonpooled samples ($P < 0.05$).

	CEQAP members	Non-CEQAP members	Total	Pooled	Nonpooled	Total
SE negative	1652	799	2451	2163	279	2442
SE positive	76	74	150	143	7	150
Total	1728	873	2601	2306	286	2952
% Positive	4.40	8.48	5.77	6.20	2.45	5.79

Table 3. Number of SE-negative and SE-positive samples taken at six different stages of production in the layers' life cycle between 2007 and 2011. Pearson chi-square tests for a 2 × 5 (unknown was not included) showed significant differences ($P < 0.05$) between the different stages of production.

	Chick papers	Preproduction	Midproduction	Postmolt	Premarket	Unknown	Total
SE negative	621	382	370	194	251	560	2378
SE positive	13	8	13	7	34	44	119
Total	634	390	383	201	285	604	2497
% Positive	2.05	2.05	3.39	3.48	11.92	7.28	4.8

was observed. Historically, molting has been identified as an important risk factor for SE infection (9). Our data seem to offer further support for this observation.

A low prevalence of SE in the chick paper samples (2.05%) also was observed (Table 3). The current procedure for sampling chick papers only tests approximately 10% of the chick papers, but this number of papers may not be enough. Assuming a delivery of 100,000 chicks, divided into 1000 boxes, the current sampling protocol would test the chick papers from 100 boxes. Given a disease prevalence of 2% (Table 3), and an SE test sensitivity and specificity of 100%, we would have 88% confidence that the boxes are SE free. Using the same parameters, 138 boxes would need to be tested to have 95% confidence that the boxes are SE free (1). We would recommend further studies to determine the cost-benefit ratio of testing a larger proportion of chick papers.

When comparing the prevalence of SE by using the FDA's time points (preproduction, midproduction, and postmolt) with that of the old CEQAP testing procedure (premarket samples), the prevalence of SE is significantly higher for the old CEQAP methodology (Table 1). This difference is primarily due to the high prevalence (11.9%) of SE in the premarket samples (Table 3). It should therefore be noted that a higher OR for the old CEQAP methodology does not make that test ''superior'' to that of the FDA or to the new CEQAP methodology because the old CEQAP only tested the premarket sample and hence never tested stages in production with lower odds of SE positivity. Because many producers do not molt their birds, no testing occurs for up to 40 wk after the midproduction sample in birds that are not molted, assuming the birds are depopulated at approximately 80 wk. This gap would constitute the largest time span between samples for the FDA program. In contrast, the new CEQAP program has a premarket sample that samples the environment approximately 2 wk before depopulation. Consequently, in the CEQAP program, the time gaps between testing are narrower. Due to the higher risk of SE in older birds coupled with the importance of detection of SE in the environment of the house before the introduction of a new flock, there may be value in a sampling protocol that includes the premarket sample. For example, if SE was present at or introduced to a farm after the postmolt sample, the FDA's sampling procedure would not test that SE-positive layer house environment until the midproduction sample of the following flock. At the minimum, this would be >40 wk later. In contrast, the CEQAP's sampling procedure would test that same environment in the premarket sample of the affected flock before a new flock was introduced to the farm.

When comparing the prevalence of SE using the FDA's time points relative to the new CEQAP testing procedure (applied retroactively to the entire data set), there was no statistical difference between the two programs. Although the statistics call into question the utility of the extra two time points that the new CEQAP requires, SE sampling of chick papers may be integral toward controlling, mitigating, or both the effect of an SE outbreak. For example because no chick papers are tested in the FDA program, no SE testing occurs until the 14–16-wk sample in the FDA program. In contrast, if a producer is following the CEQAP program, the chick papers would have been sampled at week 1. Consequently, if there was an infection in a flock that was only using the FDA's sampling procedure, the potential for a larger SE outbreak exists. An incident demonstrating the value of chick paper testing by producers occurred in 2007 when SE-infected chicks were detected at several farms that bought their chicks from a common hatchery. If these farms had waited to test for SE until the 14–16-wk sample, a much larger and costly SE outbreak may have occurred. In addition, it is important to recognize that the sensitivity of SE testing assays is never 100%. Consequently, having more than one time point to test the environment for SE before production begins may be of some value from a risk perspective. However, it is also important to recognize that most (95%) of the pullets in pullet-raising facilities came as chicks from breeder flocks that are monitored by the NPIP to prevent and control egg-transmitted, hatchery-disseminated poultry diseases, including SE (19).

Although the comparisons between the SE environmental testing results for the new CEQAP, old CEQAP, and FDA cover identical time periods, a more accurate comparison should be made by comparing SE results at the farm or flock level instead of comparing individual samples. In our current analysis, for example, we are comparing one farm's chick papers against another farm's premarket samples. Because SE is persistent and linked to the environment, an ideal comparison would look at the SE environment within each flock or farm from chick papers to premarket. The current data provided for this analysis do not provide enough information for this type of comparison. However, because of the FDA egg safety rule, this type of data analysis may be possible in the future. A mixed statistical model with both fixed and random effects would allow for linking repeated SE measurements with a single farm and or flock.

Table 4. OR for final selected independent variables for binary backwards logistic regression. For each year, 2011 was used as the reference year. Therefore, as an example and holding all other variables constant, a farm in 2007 was 6.11 times as likely to test positive for SE compared with a farm in 2011. For the stage of production category, the premarket sample was used as a reference stage. Therefore, as an example and holding all other variables constant, the environment where preproduction birds live was 0.19 times as likely to have SE than the premarket environment. Of the 58 different farms included in this study, only eight farms had an OR > 1, with a range between 1.0 and 10.8.

Parameter	OR
2007	6.11
2008	2.19
2009	1.81
2010	1.52
Chick paper	0.15
Preproduction	0.19
Midproduction	0.52
Postmolt	0.72

Of the 86 post-C&D samples recorded, 36% of those samples were positive for SE. Although a post-C&D sample is currently not required, the relatively high prevalence of positive SE in that sample category may reflect a potential risk for future flocks associated with that house. Although for this study we were unable to determine how many of the 86 samples were repeats of the same houses, the relatively high prevalence and persistence of SE post-C&D is not surprising (4,10). In a previous study, C&D did not eliminate SE from 50% of contaminated laying houses in a Pennsylvania study and from none of 12 laying houses in the United Kingdom (10).

Of the six independent variables considered for the final model, only age of flock, year, and owner were selected as main effects for the final model (Table 4). Of those three variables, we determined that the owner had the most significant affect by removing each of the three variables separately and calculating the ROC and Naglekerke R^2 values. It seems reasonable that ownership would play an important role in predicting whether a farm is SE positive or SE negative because the persistence of SE in the environment has been well established. Farms with a history of SE are more likely to have an SE problem in the future than farms without any history of SE. In one case, *Salmonella* was determined to survive in litter and feed for >2 yr after the removal of a flock (3). Consequently, the historical presence of SE on a farm should be considered a valuable piece of information in determining the risk of future outbreaks.

The significance of the year variable is primarily due to an outbreak of SE in 2007 linked to a hatchery. Specifically, with all other independent variables held constant, the model predicts that brooder farms, layer farms, or both in 2007 were 6.11 times as likely to have SE in their environment compared with the reference year 2011 (Table 4). This finding again highlights the potential importance of testing the chick papers instead of waiting until the preproduction sample for the first SE test.

Naglekerke R^2 and ROC values are common ways to assess the fitness of logistic regression models (5). Briefly, Naglekerke R^2 values are considered pseudo-R^2 measures that range between 0 and 1, and they are used to evaluate the quality of the model (5). The ROC curve plots the true-positive fraction versus the false-positive fraction (5). The final Nagelkerke R^2 (0.27) and ROC (0.84) values show that the model was not a very effective predictor of SE positivity or negativity. This outcome may be because the independent variables selected did not include factors such as vaccination status, flock size, stocking density, manure management, ventilation, and watering systems, any of which may play an important role in preharvest food safety (2,10,12,20,21).

Besides the differences in testing protocols with respect to the age of the flock, two other differences between the current CEQAP program and FDA egg safety rule should be noted: The CEQAP program requires an SE vaccination program that includes a killed or inactivated vaccine, and the CEQAP program also requires employee training and mentoring (17). Although CEQAP membership was not included in the final model, it was interesting to note that non-CEQAP members were 2.03 times as likely to have SE in their farm environment than CEQAP members (Table 2). This reflects that although there was a significant difference ($P < 0.05$) between CEQAP members and nonmembers, the difference was not considered significant enough to contribute to the overall strength of the model.

This phenomenon of having a significant difference via a Pearson chi-square test that is not significant enough to contribute to the final logistic regression model also was noted with respect to pooling *vs*. nonpooling of environmental samples (Table 2). Interestingly, pooled samples were 2.62 times as likely to test positive for SE than nonpooled samples (Table 2). It is important to note that this was not a direct comparison (i.e., both techniques were not performed side by side on the same flock) of the two methodologies. A study by Kinde *et al*. (14) in 2004 showed there was no statistical difference between single and pooled swabs with respect to identifying *Salmonella* on a row or flock basis. A pending study by Kinde *et al*. is being designed to help determine the equivalency of pooling versus individual swabs using the FDA testing protocol.

Continued surveillance of retrospective data is essential for monitoring and optimizing both the FDA and CEQAP program. Both programs have limited resources; therefore, identifying the significant factors that affect the likelihood of SE positivity or negativity is essential for effectively allocating state and federal resources with respect to inspecting the farms with the most potential risk for having SE. In addition, with the inclusion of smaller farms (e.g., 3,000–50,000 hens) in the FDA egg safety rule, a new line of epidemiologic surveillance is needed to understand the effect of farm size on SE.

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