Descriptive survey and *Salmonella* surveillance of pastured poultry layer farms in California

Naomi Dailey,[*] Deb Niemeier,[†] Carine Elkhoraibi,[‡] C. Gabriel Sentíes-Cué,[§] and Maurice Pitesky[#,1]

[*]*UC Davis College of Agriculture and Environmental Sciences, Geography Graduate Group, One Shields Ave., Davis, CA 95616, USA;* [†]*UC Davis College of Engineering, Department of Civil & Environmental Engieering, One Shields Ave, Davis, CA 95616, USA;* [‡]*UC School of Veterinary Medicine, Poultry Health and Food Safety Epidemiology, One Shields Ave, Davis, CA 95616, USA;* [§]*California Animal Health and Food Safety Laboratory System-Turlock Branch, 1550 N. Soderquist Rd. P.O. Box 1522, Turlock, CA 93274, USA; and* [#]*UC Davis School of Veterinary Medicine, Department of Population Health and Reproduction, One Shields Ave, Davis, CA 95616, USA*

ABSTRACT While pasture-raised poultry comprises a small portion of the commercial poultry industry in North America, these alternative rearing systems have become increasingly popular. As such, it is critical to improve our understanding of husbandry practices and prevalence of zoonotic and epizoonotic diseases in these systems. This research reviews the results of a survey sent to 82 commercial pastured poultry farms in California. While the survey response was low (13.4%), it was enhanced by detailed in-person interviews and farm visits. In addition, we conducted drag swabs for *Salmonella* Enteritidis. On average, farms utilized 12.3% of their total farmland for pastured poultry operations, which often coexisted with other livestock (45%), touch crops (27%), and non-touch crops (45%). While the mean (44.6 sq. ft./hen) and median (22.2 sq. ft./hen) pasture stocking densities were within auditing guidelines, the mean (1.2 sq. ft./hen) and median (0.5 sq. ft./hen) coop stocking densities were below the pending USDA (2016) guidelines recommended in 7 CFR Part 205. Drag swab results showed the presence of *Salmonella* Enteritidis (SE) in the environment of one of the 11 farms (9.1%). In addition, *Salmonella* Pullorum (SP) whole blood agglutination tests were used to understand the prevalence of *Salmonella spp.* in laying hens within the studied farms. Results showed the presence of antibodies in flocks at six of the seven non-SE vaccinated farms, with a mean on-farm prevalence of 25.6% in laying hens. Logistic regression was used to determine risk factors for Group D *Salmonella* exposure in non-vaccinated flocks, using the SP blood agglutination data as the dependent variable and the survey questions as the independent variables. Statistically significant ($P < 0.05$) risk factors included exposed wire floors and flock size. These results improve our understanding of *Salmonella* prevalence and husbandry practices on commercial pastured poultry farms in California.

Key words: pastured poultry, *Salmonella*, husbandry

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INTRODUCTION

Free-range poultry production has rapidly increased in popularity in the United States (Colles et al., 2008; Kijlstra et al., 2009). Consumers and farmers alike have expressed concern over food and health safety (Yeung and Morris, 2001) as well as animal welfare conditions in conventional production systems (Harper and Makatouni, 2002). Heightened consumer awareness has opened up a niche market for farmers to sell premium priced eggs from free-range systems (Jones et al., 2003; Sossidou et al., 2015; van Bommel and Spicer, 2011). Pasture-raised poultry is an extension of free-range systems, and refers to the husbandry practice in which flocks of birds are housed in a mobile structure or "eggmobile" at night, with continuous access to outdoor vegetation during the day (Sossidou et al., 2015). The USDA (2015) does not have a regulatory definition for pastured poultry systems, but does stipulate that free-range hens must have access to the outdoors (USDA, 2015). This is changing, however, as a recently proposed rule (7 CFR Part 205) by the USDAs Agricultural Marketing Service (AMS, 2016) outlined space and husbandry requirements for poultry with access to pasture or the outdoors. Many non-government stakeholders and academics have attempted to refine consumer concepts of free-range or pasture-raised poultry with external certification programs such as Certified Humane and Animal Welfare Approved (**AWA**) (HFAC, 2014;

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[1]Corresponding author: mepitesky@ucdavis.edu

AWA, 2015). While these external programs provide a foundation for understanding pastured poultry, geographic, and environmental variation among farms limit its utility (e.g., the 2012 to 2015 California drought restricted access to fresh-growing vegetation).

Often pastured poultry producers have relatively small flocks that fall below regulatory thresholds, resulting in a dearth of information on food safety parameters and basic production strategies. For example, while there is extensive research on identifying paratyphoidal and non-paratyphoidal *Salmonella* in conventional poultry operations (Henzler et al., 1994; Gast and Holt, 1999; Garber et al., 2003), there is a lack of similar research on pasture-raised layer flocks. In addition, pastured poultry production is regionally unique with respect to predator exposure, pasture rotation cycles, weather, and avian diseases (Sossidou et al., 2015; Xu et al., 2014). Specifically, because pastured poultry interact spatially with their individual micro-environments, which may be uniquely influenced by localized vegetation, predator systems, and microbiomes, it may not appropriate to extrapolate and generalize data from pastured poultry studies in different geographical areas.

The changes in poultry rearing systems and production practices can influence the safety and quality of eggs produced, particularly when hens have access to the outdoors (Holt et al., 2011). Indoor flocks have reduced exposure to predators and pathogens, and indoor rearing can control and mitigate potentially harmful variables in the environment (Sims, 2008; Holt et al., 2011). When raised outdoors, additional stress from thermal extremes, rehousing, and predation can exacerbate the prevalence of *Salmonella*, particularly with breeds that are not well-suited to outdoor lifestyles (Holt et al., 2011; Wallner-Pendleton et al., 2014). *Salmonella* control programs, such as the California Egg Quality Assurance Plan (CEQAP), are often costly to implement on small pastured poultry farms with mobile coops (Kinde et al., 2005). Yet these programs require management practices such as environmental monitoring for *Salmonella* Enteritidis (**SE**), flock vaccination, biosecurity, and rodent control that can help to reduce the incidence of *Salmonella* (Kinde et al., 2005; Wallner-Pendleton et al., 2014). While producers rearing egg layers indoors often monitor their flocks for bird health and food safety purposes (Schaar et al., 1997; Kinde et al., 2005), many pastured poultry producers do not participate in industry standard quality assurance programs like CEQAP. There is a significant amount of research on *Salmonella* prevalence in free-range broilers (Bailey and Cosby, 2005; Siemon et al., 2007; Melendez et al., 2010; Trimble et al., 2013), but since *Salmonella* prevalence varies widely between free-range farms, it is difficult to compare free-range or pasture-raised poultry with what we know about *Salmonella* incidence in conventional indoor-raised poultry (Bailey and Cosby, 2005; Siemon et al., 2007). Consequently, research on *Salmonella* prevalence in pasture-raised flocks remains very limited.

Federal and state regulations and monitoring systems apply to pasture-raised poultry farms, despite the limited knowledge of disease risks on pasture. The Food and Drug Administration (**FDA**) issued a guidance document that includes disease surveillance recommendations for *all* layer farms with more than 3,000 hens: Title 21 CFR, parts 16 and 118, also known as the "Egg Safety Rule" (2013). In addition, the California Department of Food and Agriculture (**CDFA**) recently codified the Shell Egg Food Safety Rule (SEFS) (2013) that also requires further surveillance and vaccination against *Salmonella*. For both the FDA and CDFA rules, producers with less than 3,000 hens are exempt. Since pastured poultry farms typically have less than 3,000 hens, and pastured poultry farming is a relatively new husbandry practice for commercial egg production in the United States, little is known about basic husbandry and management approaches.

The main objectives of this study are to provide an overview of the management practices of 11 commercial pastured poultry layer operations in California, including the identification of SE environmental prevalence and *Salmonella* Pullorum (**SP**) exposure in flocks as detected by positive blood agglutination. While similar studies of pastured broilers have been done in other regions (Siemon et al., 2007; Melendez et al., 2010; Trimble et al., 2013), to our knowledge this is the first such study of pastured layers in California.

MATERIALS AND METHODS

Recruitment

During the spring and summer of 2015, a total of 82 pastured poultry farms were identified via contacts from the 2015 Ecological Farming Conference in Asilomar, California, and from the National Center for Appropriate Technology's program for pasture-raised layer farms. Pasture-raised broiler farms were excluded from the list. The farms were invited to participate in the study by phone or email; 11 farms (13.4%) participated in the final study. An online survey, on-site field surveys, and informal interviews were conducted at each farm. In addition, SE environmental testing and SP whole blood agglutination surveillance was conducted via environmental drag swabs and a whole blood agglutination test, respectively.

Online Survey Questionnaire

A 69 question online survey questionnaire was administered to the 11 participating farms. The survey focused on collecting data associated with flock history, flock health and disease prevention, biosecurity practices, mortality management, predation, vaccination programs, egg processing and pricing, land cover, irrigation and fertilization, certification, and data collection. Questions were determined based on preliminary surveys with local backyard poultry owners, and based

on advice from animal behavior specialists from University of California Cooperative Extension (**UCCE**). The survey consisted of primarily multiple choice or rating questions, with the opportunity to expand answers further in comment boxes. It was pretested with backyard poultry owners in Davis, CA and with UCCE animal behavior and poultry welfare specialists. A total of 11 surveys were sent via email to participating farms, with phone or email reminders sent two to four weeks later. A paper copy of the survey was brought to each non-responding farm to complete in person if necessary.

On-site Data Collection

Field data collected included: a description of mobile coop structures, dimensions, building materials, type of equipment, ventilation, and stocking density per rotation plot (Table 1). Next, descriptions of the watering system (well, spring, municipal), drinkers, feeders, nest boxes, and perches were recorded (Table 1). Land cover type (e.g., cropland, pasture, bare soil) was recorded, and included both touch (i.e., crops where the edible portion touches the ground) and no-touch crops (i.e., crops where the edible portion does not touch the ground), as well as California native grasses. Specific information about flock age, flock behavior, and livestock was also identified.

Salmonella Pullorum Whole Blood Agglutination Test

Blood samples for the SP whole blood agglutination test were collected at each individual farm. The number of hens needed for a representative sample was determined using the EpiTools Services AusVet (Sergeant, ESG, 2016. Epitools epidemiological calculators. Ausvet http://epitools.ausvet.com.au). SP is considered very rare in conventional commercial layer farms in North America (Gast, 1997), but has an unknown prevalence in non-conventional commercial production, including pastured poultry production. Therefore, we made relatively conservative assumptions that the true SP prevalence was 0.5%, based on a search of the literature (Waltman and Horne, 1993). The assumed test sensitivity and specificity, confidence level, and precision for the SP whole blood plate agglutination test (Charles River Laboratories, Material No. 10100762, Charles River Laboratories, Wilmington, MA) was 0.99, 0.70, 0.90, and 0.2 respectively. Sample sizes ranged from four to 28 hens at each farm. Of these farms, only one had more than 3,000 birds, while ten had less than 3,000 birds and were therefore not subject to state or federally mandated SE monitoring and vaccination requirements.

We established a positive control sample at each farm to ensure the antigen's viability for each blood sample. Approximately 0.05 mL of the positive control was placed on a glass slide and mixed with 0.05 mL of the Pullorum antigen. The slide was agitated for two minutes, and if precipitate formed, the antigen was considered viable for blood sampling. A 0.1 mL blood sample was taken from the left wing of each hen using a sterile. 21-gauge needle and syringe. The blood sample was mixed with 0.05 mL of Pullorum antigen on a glass slide and agitated for two minutes for visual measurement of blue precipitate (positive).

Salmonella Enteritidis Test

Specimens tested for environmental SE were collected according to the protocol outlined in Title 21 CFR 118 of the FDA Egg Safety Rule (FDA, 2013). Testing areas were consistent between each farm included mobile coop floors, nest boxes, and the outside ground underneath the coops. The specimens were labeled and sealed in Whirl Pak bags, and immediately placed over ice until delivery to the laboratory for *Salmonella* enrichment and PCR by the California Animal Health and Food Safety Laboratory (**CAHFS**) lab in Turlock, CA for *Salmonella* enrichment and PCR (Charlton et al., 2005). Each mobile coop unit was sampled and recorded

Table 1. Field survey data results (selection).

Statistic	N	Mean	St. Dev.	Min	Median	Max
Coop area (m^2)	11	32.3	63.6	3.0	14.9	223.0
Number of coops	11	3	2	1	3	6
Birds per coop (no.)	11	525	1,057	12	200	3,666
Coop stocking density (m^2/bird)	11	0.07	0.1	0.01	0.04	0.4
Pasture stocking density (m^2/bird)	11	4.2	4.4	1.1	3.3	16.2
Waterers (no.)	11	7	9	1	3	30
Feeders (no.)	11	8	8	1	6	30
Nest boxes (no.)	11	416	1,061	4	103	3,600
Nest box area (m^3)	11	0.05	0.04	0.02	0.03	0.2
Nest box height from floor (m)	11	0.3	0.2	0	0.4	0.6
Birds per nest box (no.)	11	8.0	6.4	1.9	6.1	25.0
Roosts (no.)	11	37.9	48.0	1	24	168
Roost length (m)	11	5.5	4.9	1.8	3.7	18.3
Roost space per bird (m)	11	0.6	0.5	0.2	0.5	1.7
Roost height from floor (m)	11	0.4	0.1	0.1	0.4	0.7
Average temperature (F)	11	76.5	11.0	60	81	89
Average humidity (%)	11	54.0	24.5	22	47	80

as a separate entity. The environmental drag swabs were delivered to the CAHFS lab within eight hours of collection.

Statistical Analysis

Survey and sample data from all farms ($n = 11$) were included in the analysis. In the regression models and odds ratio (**OR**) calculations, only farms without *Salmonella spp.* vaccinations were included ($n = 7$), due to confounding results with vaccinated birds. Because not all survey questions were answered by each participant, the denominator used in the calculation was the total number of responses collected for each question. Data was refined using Microsoft Excel (2015, Microsoft Corp, Redmond, WA), and was further coded and analyzed using R 3.2.2 (2013, R Foundation for Statistical Computing, Vienna, Austria). Statistical tables were produced using R 3.2.2 and LaTeXiT 2.7.5 (Chatelier et al., 2014), and maps were produced using QGIS 2.10.1 (QGIS Project, 2016). Because we had a small sample, associations between categorical variables were determined using nonparametric statistical tests in R and Minitab (Minitab 16, State College PA). Odds ratios, *P*-values, and 95% confidence intervals were constructed for each variable. For all tests, significance was defined as $P < 0.05$. Note that the American Statistical Association recently called for a cessation of the use of *P*-values; we have provided *P*-values here simply for convention (Wasserstein, 2016). For continuous variables, the mid-point between the mean and median was used as a break-point for construction of a two by two square.

RESULTS AND DISCUSSION

All of the farms rotated laying hens on pasture, crops, or orchards. Each farm had mixed livestock species in addition to poultry, as well as touch and no-touch crops. Common livestock included geese, turkeys, cattle, sheep, and goats. Each farm used cage-free mobile chicken coops that were rotated among variably sized rotation plots and provided shelter for egg laying, roosting, and feed and water (Table 1). The mobile coops were predominantly built on trailer frames with metal or wooden roofs, a combination of wood and wire walls, and solid or slatted (permeable) flooring. Coops were rotated at different time scales on each farm, ranging from once per week to once every three months, depending on the farm. Time of rotation varied depending on the levels of denuded vegetation or farmer preference. Each coop was sized to hold ten to 500 hens, depending the type of structure used (i.e., homemade wooden trailer vs. factory-made fifth wheel trailer). Farms allotted between 0.25 to 300 total acres for poultry farming, and divided this acreage into individual rotation plots of 0.13 to 18 acres, separated via temporary fencing (Table 2). Eight of the 11 farms (73.7%) rotated their hens with other livestock or crops, presenting an opportunity for further food safety studies. Seven of the 11 farms (63.6%) also fertilized their rotation plots, using either cattle manure, poultry manure, horse manure, decomposed fish, or compost. The remaining farms did not report any type of compost use on pasture or cropland.

The most common source of mortality across all farms was predation (90.9%), followed by farmer culling because of old flock age (18.2%). Predators observed most frequently (reported daily or weekly) included hawks, coyotes, raccoons, owls, and rodents. Rodents and their feces have been commonly cited as vectors for *Salmonella* (Gast, 2007; Wallner-Pendleton et al., 2014), but only three farms reported seeing rodents on a daily or weekly basis. Only half of the farms used some form of rodent control, most commonly cats (27.2%) or snap traps (18.2%). All farms reported some flock loss, with six of the 11 farms (54.5%) estimating between 10 to 40% mortality of their flocks per year. These measures were all self-reported, taken from the online survey.

A total of 80 environmental drag swabs were collected across the participating farms (Table 3). Our small number of sampled farms, combined with the narrow environmental sampling dates of July and August, restricts our ability to generalize findings more broadly to pastured poultry farms across California (Figure 1). Despite the small sample size, the study's findings provide important and valuable insights of potential risk factors on pastured poultry layer farms. General vaccinations for Marek's and Newcastle disease virus had been applied on five of the 11 farms (45.5%). There was no apparent relationship between vaccination and the size of the flock. For example, one of the farms that vaccinated had over 3,000 hens, one had 1,600 hens, and one had 75 hens. Only four of the 11 farms (36.4%)

Table 2. Online survey data results (selection).

Statistic	N	Mean	St. Dev.	Min	Median	Max
Total acres	11	370	788	0.3	35	2,596
Total acres used for poultry	11	46	88	0.3	15	300
Rotation plot (acre)	11	2.4	5.4	0.004	0.5	18
Annual irrigation water (acre-feet)	8	1.1	1	0	0.9	3
Number of hens	11	2,670	6,469	12	600	22,000
Number of pullets	11	1,084	2,978	0	30	10,000
Cost per dozen eggs (USD)	11	6.7	2.5	0	7	9
Feed used per month (tons)	10	11	26	0.02	2	84.0

Table 3. *Salmonella* Enteritidis (SE) and *Salmonella* Pullorum (SP) prevalence.

Farm	Hens (total no.)	SE vaccination status	SE status	SP prevalence
A	300	No	Negative	19.2%
B	2,900	Yes	Positive*	100%
C	900	No	Negative	36.4%
D	75	Yes	Negative	100%
E	800	No	Negative	32.1%
F	1,600	Yes	Negative	20.0%
G	12	No	Negative	22.2%
H	30	No	Negative	0%
I	22,000	Yes	Negative	100%
J	150	No	Negative	29.2%
K	600	No	Negative	15.0%

*Environmental drag swab tested positive for SE.

Note: SE vaccination status indicates whether or not flocks received 2 live ST and 1 killed SE vaccine.

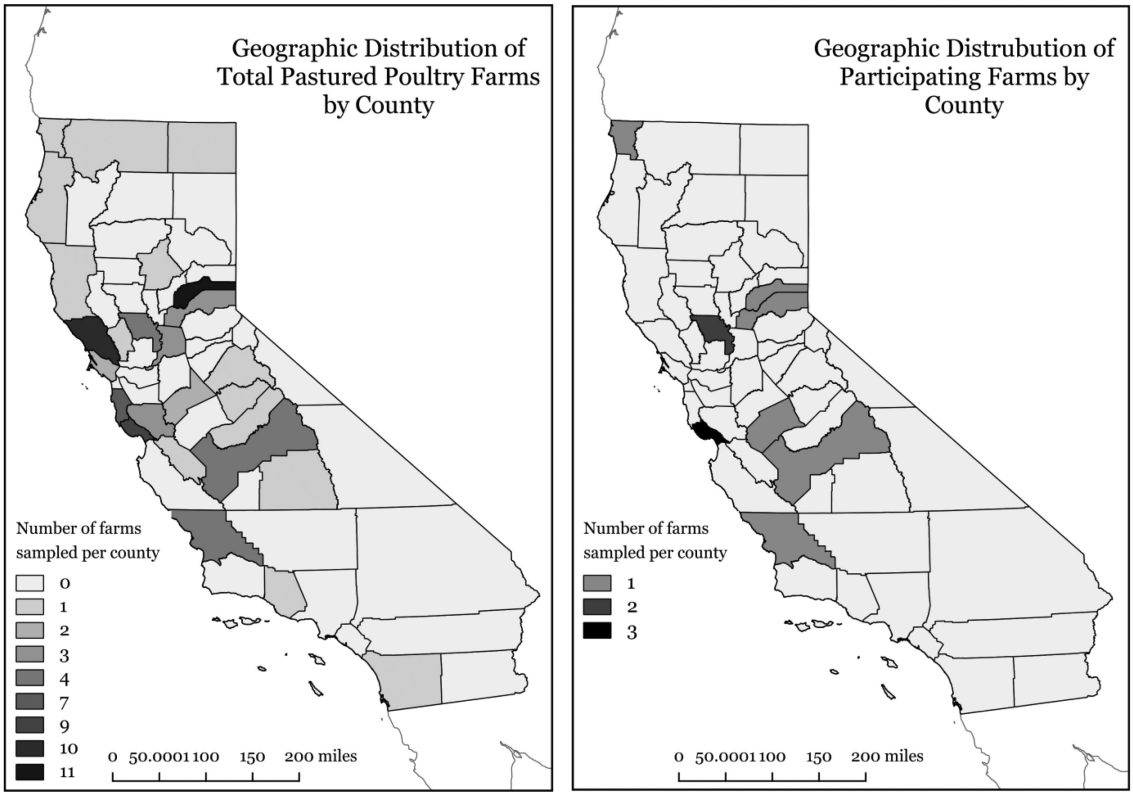

Figure 1. Geographic distribution of commercial pastured poultry farms in California by county (1A.) and farms participating in this study by county (1B.).

vaccinated against SE with a combination of live and killed vaccines consistent with the California Shell Egg Food Safety Rule (Table 3) (Zhang-Barber et al., 1999). Of the four farms that vaccinated against SE, one had above 3,000 hens and three had below 3,000 hens. Additionally, three of these vaccinated farms had conducted SE surveillance using drag swabs prior to this study.

SE was only detected on one of the 11 farms (Table 3). Interestingly, this flock was reported to have been vaccinated against SE. The SE vaccine used at the SE positive farm was two live *Salmonella* Typhimurium (ST) and one killed SE, and the test conducted on the drag swab sample was processed with enrichment followed by PCR, according to the FDAs Bacteriological Analytical Manual (Center for Food Safety and Applied Nutrition, 1998). The main disease prevention measures highlighted by farmers were vaccinations (54.5%), limiting wildlife contact (36.3%), and culling hens that appeared sick (27.2%). Only one of the 11 farms reported that their flock had been "sick" in the last 12 months.

SP positive agglutination tests were detected on ten of the 11 farms (Table 3) with an overall flock prevalence of 25.6%. After removing vaccinated flocks from this calculation, SP positive agglutination tests were detected on six of the seven unvaccinated farms, with a flock prevalence of 23.1%. The whole blood agglutination test is highly sensitive and has a high likelihood of false positives, particularly when tested on SE vaccinated flocks (Gast, 1997). Due to the high sensitivity and low specificity of the whole blood agglutination

Table 4. Statistically significant and other *Salmonella* Pullorum indicators identified by logistic regression. Higher odds ratio values indicate higher *Salmonella* Pullorum prevalence.

Indicator	Odds Ratio	*P*-value	CI: 2.5%	CI: 97.5%
Flock size	2.65	0.04	1.06	6.59
Flock density	1.84	0.07	0.77	3.64
Standing water	2.26	0.07	0.03	133.62
Wire floors	4.64	0.02	1.33	16.19
Temperature	1.00	0.82	0.99	1.01
Humidity	0.99	0.64	0.99	1.00
Soil type: Entisols	1.17	0.34	0.88	1.57
Soil type: Mollisols	0.98	0.88	0.80	1.21

Note: Significant indicators are boldface. *P*-values above 0.05 were considered statistically insignificant.

test, it is widely considered to only be a screening test (Gast, 1997). Confirmation that flocks are infected is done by bacteriological culturing or PCR of tissue samples (Gast, 1997), which was not granted for this study. In a previous study, over 13 different serotypes (including SP) were isolated from chickens that tested positive by tube agglutination, reflecting the poor specificity of this test (Waltman and Horne, 1993). Therefore, the prevalence of reactors or positive blood agglutination test noted in this study most likely reflects a relatively high exposure to *Salmonella spp.* Group D in the positive flocks.

Using Wallner-Pendleton et al. (2014), we identified numerous potential indicators for *Salmonella* on each farm, as well as positive SP as detected by blood agglutination and management decisions unique to pastured poultry farming (Table 4). The indicators include husbandry and environmental characteristics that had either been cited in the literature (Gast, 2007; Wallner-Pendleton et al., 2014) or had a significant effect ($P < 0.05$) on *Salmonella* prevalence on pastured poultry farms. It should be noted that the sample size for the risk indicator calculations was very low ($n = 7$), because four farms with SE-vaccinated flocks were removed from the analysis. Despite the small sample size, the associated OR values provide interesting and potentially important trends that should be further explored in future studies. Primary indicators for positive SP blood agglutination prevalence were analyzed via simple logistic regression. Significant indicators are outlined below.

Flock Density and Size

Stocking density has been identified as a critical indicator of *Salmonella* prevalence (Wallner-Pendleton et al., 2014), though the relevance of stocking density to *Salmonella* detection is widely debated (Estevez, 2007; Buijs et al., 2009). Recently, the USDA AMS proposed a rule (7 CFR Part 205) that would require 1.0 square foot per bird in pastured poultry mobile coops, and 1.8 square feet per bird outdoors (AMS, 2016). By comparison, caged layer operations outside of California require 0.47 and 0.53 square feet per White Leghorn Hen and Brown Egg Layer, respectively (United Egg Producers, 2016). For the purpose of this study, two types of stocking density were calculated on each farm: *pasture* stocking density is based on the average size of outdoor rotation plots per farm, and *coop* stocking density is based on the average size of indoor mobile coops. We have modified the definition of stocking density to reflect the fact that pasture-raised hens have continuous access to the outdoors, spending time both inside and outside of the mobile coop. Our mean pasture stocking density across all farms was 44.64 square feet per hen, with a median pasture stocking density of 22.22 square feet per hen. The mean coop stocking density across all farms was 1.22 square feet per hen, and the median coop stocking density was 0.53 square feet per hen.

Many independent auditing groups have stringent requirements on stocking density for pastured poultry operations. Animal Welfare Approved requires a minimum of 1.8 square feet per bird indoors, and 4.0 square feet per bird outdoors (AWA, 2015). Certified Humane has less stringent requirements, instead requiring 1.5 square feet per bird indoors, and 2 square feet per bird outdoors (HFAC, 2014). Interestingly, the pasture and coop stocking density were found to have an insignificant impact on predicting positive SP agglutination tests ($P < 0.05$), although as the coop density increased there was a trend toward increased positive SP agglutination tests ($P = 0.065$). When flock size was used rather than stocking density, it was found to be a significant indicator in predicting positive SP agglutination tests ($P = 0.04$). The associated OR value (OR = 2.65) indicates that as flock size increased there was a positive trend toward positive SP blood agglutination prevalence (Table 4). Since our sample size was relatively limited, it would be useful to conduct additional research.

Standing Water

Standing water has also been cited as a potential risk factor for *Salmonella* incubation (Bryan and Doyle, 1995; Bailey et al., 2001). At the time of this study, drought conditions in California were still considered extreme and forced many farms to reduce overall crop

watering. Yet, many of the participating farms continued to use flood irrigation to water their pasture. Hens had access to pools of standing water on three of the 11 farms (27.2%), all of which were flood irrigated. Standing water was found only on farms in the Central Valley and Sierra Nevada foothills of California, where July daytime temperatures range from 90°F to 110°F. These conditions have the potential to create an optimal reservoir for bacterial growth (Doyle and Erickson, 2006). Using a simple logistic regression model ($P < 0.05$), we found that standing water was a significant indicator of SP prevalence as detected by positive blood agglutination. The OR value for standing water as an indicator of SP prevalence as detected by positive blood agglutination was 2.26 ($P < 0.05$) (Table 4). Standing water was also present at the single farm that tested positive for environmental SE; the positive sample was swabbed from one of these pools outside of the coop.

Wire Floors

While the above mentioned indicators have been shown to be risk factors for *Salmonella* in large and medium-scale commercial layer operations, structural characteristics unique to mobile coops in operation on pastured poultry farms were also significant predictors of SP prevalence as detected by positive blood agglutination. Mobile coops at many of the farms were constructed using heavy gauge chicken wire or wood slats for flooring (63.6%). The open access allows feces to fall through the floor onto the pasture, fertilizing the soil and reducing the need for constant cleaning or bedding replacement. Yet, it also leads to higher concentration of feces directly under the wire flooring. Many mobile coops were also constructed on lifted trailer frames, with a vertical height ranging from 0.6 to 2.5 feet between the ground and base of the trailer—ample room for hens to forage and hide from predators underneath. Free access underneath the open bottomed trailer exposes hens to feces that may contaminate grass and other foraging material, as well as exposing hens to potentially infected feces droppings.

Horizontal transmission of *Salmonella* is common when hens are in close quarters (Gast and Holt, 1999), or if they are consuming feed or water contaminated with infected feces (Nakamura et al., 1994; Patterson et al., 2014). Likelihood of horizontal transmission may increase in pastured poultry systems, since hens prefer to cluster and forage underneath coops for added protection from predators (Clark and Gage, 1996). This behavior increases the risk of disease transmission via feces from the permeable wire flooring above. Using a simple logistic regression model ($P < 0.05$), the presence of a wire floor was found to be a significant risk factor for positive SP agglutination tests (OR = 4.76, $P = 0.02$) (Table 4). This variable was the most significant in our study with respect to predicting positive SP agglutination tests. This is a clear example of how a husbandry design decision (e.g., slotted floors in an eggmobile) and behavior response to predators (e.g., hiding under the eggmobile) lead to a food safety and animal disease challenge (e.g., horizontal *Salmonella* transmission). Creating additional habitat near the eggmobile for the pastured birds or changing coop flooring materials should be prioritized in design decisions, as it would help to mitigate this type of disease transmission.

Because pastured poultry spend a significant amount of time outside, environmental parameters including temperature, humidity and soil type were also analyzed. While none of these variables were significant (possibly due to the small sample size and short duration of the study), a summary of their importance to pastured poultry production is provided.

Temperature and Humidity

Ambient temperature and humidity specific to the geographic location of each farm were analyzed in relation to SP blood agglutination prevalence and SE prevalence. Climate and environmental data are important in understanding pasture-raised poultry, since the birds spend much of their lives outdoors exposed to varied weather and vegetation conditions. Average temperature and humidity measurements were recorded for the field sampling dates using the Weather Underground website (www.wunderground.com). While temperature and humidity were not influential on *Salmonella* detection, they do provide interesting geographic measurements to be analyzed on future farms in variegated climates over a longer period of time. Litter surface water activity (i.e., equilibrium relative humidity) levels above 0.85 appear to promote environmental survival and multiplication of *Salmonella* (Dufour-Zavala, 2008). Moist environments allow enteric pathogens to persist and proliferate (Bryan and Doyle, 1995), and high humidity was recorded at farms along the California coastline. Interestingly, the only farm where SP was not detected was located in Fresno County, a semi-arid region with low humidity and high temperatures in summer.

Soil Type

Soil characteristics specific to the geographic location of each farm were also analyzed in relation to SP blood agglutination prevalence (Figure 1). Soil order was recorded for each farm using the University of California's interactive soil map, SoilWeb (UC Davis California Soil Resource Lab, 2016). In addition to temperature and humidity, soil type was also found to be an insignificant indicator of SP prevalence on pastured poultry farms using a simple logistic regression model ($P < 0.05$). The most common soil types observed were mollisols and alfisols, with only one farm located on entisol soils. Entisols are soils that have little to no horizon development, and are found in sloped regions or in flood

plains, such as the Sacramento-San Joaquin River Delta (e.g., Yolo County) (USDA NRCS, 1999). Mollisols are common to soft grasslands along the coastline (e.g., Santa Cruz County), and alfisols form in semiarid to humid areas under hardwood forest cover (e.g., Placer, Fresno, and Nevada counties) (USDA NRCS, 1999). Further research on soil characteristics and environmental parameters could lend insight to geographic differences among pastured poultry farms and *Salmonella* prevalence.

CONCLUSION

Despite the small sample size of participating farms, this study confirmed many risk indicators for *Salmonella* commonly seen in large-scale commercial poultry operations, suggesting that pastured poultry rotation systems should be treated with similar caution and disease prevention measures. Positive SP blood agglutination tests were found on ten of the 11 farms, while SE was noticeably absent, with one farm as an exception. Due to the high level of false positives, the high prevalence of positive SP blood agglutination tests was interpreted as a high load of *Salmonella* exposure to multiple serotypes of *Salmonella* in the flocks, as opposed to a high level of only Pullorum disease. This result was similar to the prevalence of SE in conventional commercial layer farms in California (Pitesky et al., 2013). We also identified husbandry design practices (i.e., wire floors) that are unique to pastured poultry systems that appear to be risk factors for *Salmonella spp.* exposure. In addition, our results suggest that risk factors generally seen in conventional production systems are also risk factors for pastured poultry systems, including flock size (Pitesky et al., 2013).

We modified the definition of stocking density to reflect pasture conditions. For our study, we found a mean and median pasture stocking density in this study was 44.64 and 22.22 square feet per hen respectively; the mean and median coop stocking density was 1.22 and 0.53 square feet per hen respectively. While these values are within United Egg Producer guidelines, they do not meet requirements of certification programs such as Certified Humane and Animal Welfare Approved, which have unique requirements for pastured raised poultry. Further studies on the scientific basis of the density requirements listed by the certification programs is necessary to better understand the relationship between welfare and density on pastured poultry.

During informal discussions at field visits, many participating farms reported they were unaware of the husbandry and environmental indicators typically associated with *Salmonella* and other enteric pathogens, and even fewer were familiar with *Salmonella* surveillance methods and available diagnostic laboratories that could help diagnose avian diseases.

While this study is not an exhaustive review of pastured poultry farms in the United States, it does provide important -introductory information regarding husbandry practices. Further study could provide useful insights with respect to feed efficiency, cost and crop food safety related to integrative farming techniques (i.e., growing crops and raising hens on the same land). As pastured poultry rotation schemes increase in popularity on farms across California, there is an opportunity and need for extension professionals and veterinarians to develop resources to improve the food safety and health of pastured poultry flocks. The lack of requisite monitoring on pastured poultry farms grants producers the flexibility to implement preventative measures that can reduce their flocks' risk of *Salmonella*, improving the reputation of their product and safeguarding public health.

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