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The Impact of Livestock Density on Hospital-Acquired MRSA Infections in California: A Spatial Analysis

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Bachelor of Science in Integrative Biology
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ABSTRACT

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By Sydney Koelper

Increasing global food demands exert pressures for agricultural practices such as densely populated livestock facilities, but these productivity enhancements come at the cost of public health risks through increased antibiotic use and disease proliferating conditions. While the relationship between livestock and community-acquired MRSA has been studied, the association with hospital-acquired MRSA (HA-MRSA) is less known. This study analyzed 2022 California county-level livestock densities and their impact on HA-MRSA using negative binomial regressions and spatial epidemiological methods. Findings show a persistent presence of HA-MRSA since 2013, with significant clusters of infections present in Los Angeles, Yuba, Stanislaus, and Sacramento counties. Negative binomial regression shown a significant positive correlation between higher livestock densities and HA-MRSA incidences, yielding a risk ratio as high as 2.53 times compared to lower livestock density counties (95% CI = (1.17, 5.49)). Poverty was investigated as an effect modifier but was not found to significantly modify this relationship ($p = 0.99$). Findings highlight the importance of livestock management on public health, emphasizing the need for integrative approaches to infection control that consider both hospital and community-based factors on antibiotic-resistant infections cultivated in the hospital or beyond.

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INTRODUCTION

Balancing global food demand and the hazards of high-density livestock facilities is an involved task that will likely become more strenuous in the future. The livestock-derived food demand is estimated to increase by 14% between 2020 and 2050, should the current trajectory of income and population continue[1]. Yet, agriculture is already the largest land use in the world, with 52% of the U.S. total land areas being used as agriculture in 2023[2]. As land cannot be spared, efficiency of livestock facilities must be prioritized. One of the ways to improve productivity is to increase the density of animals at any one livestock facility. This method comes with complications, such as the high likelihood of disease spread between densely packed animals.

Antibiotic Use in Livestock

Infection in animals is treated similarly to infection in humans: with antibiotics. However, in contrast with humans, livestock regularly receive antibiotics to prevent and control future infectious events. The livestock industry uses approximately 50% of all antibiotics produced globally[3]. Resistance often occurs once usage begins, regardless of dose, but becomes persistent when antibiotics are used long-term compared to short-term. Antibiotic use in the livestock industry has steadily increased since the 1950s, and although determining timing of resistance is difficult, some studies have shown resistance developing as early as 11 days after beginning the treatment [4,5]. Once resistance becomes extensive, researchers are left to create a new antibiotic entirely, or suffer the consequences of resistant bacteria[6]. Livestock handlers have difficulty relinquishing antibiotics as they provide many benefits to their operations. Treating animals prior to being sick is said to be ethical given their living conditions, and it also relieves handlers of any financial stress that may come from treating an outbreak[7]. Further, antibiotic usage promotes efficiency in livestock facilities by the curation of the bacterial colonies that are present in the rumen (one of four stomachs in cattle). As antibiotics kill off unwanted bacteria, handlers can selectively increase cattle growth-encouraging compounds[7]. These compounds result in more meat per head, increasing profits and helping to mitigate global food demand.

Epidemiology of Antibiotic Resistance in Livestock

The prevalence of antibiotic resistance in livestock has been particularly challenging to quantify, partially because of its presence varying significantly among different animal groups and geographic regions, and partially because handlers will not often sample isolates from their livestock to determine resistance without a motivating factor. Of the studies that have obtained isolates from intensive animal production, resistance prevalence is alarming. One study in Malaysia finds that routine clinical samples obtained from 2015-2017 from both ruminants like cattle, goats, and sheep, and non-ruminants such as pigs and chickens, have a wide distribution of several species of bacteria that have high levels of resistance. Of the ruminants, specifically cattle were frequently affected by antibiotic-resistant infections. The resistance affects multiple drug types, including critical antibiotics like fluroquinolones, penicillin, tetracycline, and aminoglycosides, and among the many bacterial species that the study isolated, *Escherichia coli* and *Staphylococcus aureus* were identified most often, indicating potential target species. 701 routine, diagnostic clinical samples were investigated, and more than 72% of bacteria were found to be multi-drug resistant in ruminants, (i.e. resistant to at least three or more antibiotic classes) and more than 82.1% in non-ruminants[8].

The trends in drug-resistant bacteria in livestock over time is not well-known. While it somewhat uncertain if it is a growing health issue, studies from as recent as 2021 have documented substantial prevalence rates, underscoring its status as a significant public health concern that demands immediate action.

Human Antibiotic Resistance

Resistance does not just affect the livestock industry; the World Health Organization has recognized antibiotic resistance as a serious threat to humans[9]. Most commonly, antibiotic resistance originating in animal populations is transmitted to humans via food consumption. This food-borne transmission route is not only prevalent but also well-recognized and studied by the scientific community. In a systematic review of 332 studies on food-borne pathogen isolates published as recently as 2022, antimicrobial resistant food-borne pathogens found in food samples was greater than 10%. In clinical specimens isolated from humans, the mean prevalence of these food-borne pathogens found in human clinical specimens was more than 19%. Resistance profiles observed in the isolates from human samples closely mirror those in the food samples, supporting the hypothesis that the bacteria present in humans are of foodborne origin rather than introduced through alternative pathways. This similarity held true for all isolates except those affected by Listeria monocytogenes, which still exhibited resistance, but to lesser extent[10]. The most common antibiotic-resistant food-borne pathogens are *Campylobacter* spp., *Salmonella* spp., *Escherichia coli* and *Listeria monocytogenes*, and most are resistant to beta-lactam antibiotics, such as penicillins and cephalosporins[11].

The other major route between livestock-associated resistant pathogens to humans is through direct contact, commonly taken by *Staphylococcus aureus.* Direct contact transmission is more complicated than just contact with the animals affected; transmission routes may include contact with animal-derived products and contact with their environment, including air, as dust and particulate matter can carry the bacteria[12]. Manure, specifically, is a livestock-derived byproduct that can host live resistant pathogens, potentially contaminating vegetables, soil, and other fauna. Thus, reservoirs of resistant pathogens that are livestock-associated can transcend beyond animals and livestock-derived products, blending origins and making genealogy difficult to trace[13].

Methicillin-Resistant Staphylococcus Aureus and HAIs

Antibiotic resistant infections are also common within healthcare facilities, deemed a part of a larger umbrella term, healthcare-associated infections (HAIs). There is some evidence to show that an individual's income may influence their risk of contracting an HAI. In fact, according to the World Health Organization, 7% of patients staying in acute-care hospitals in high-income countries, and 15% in low-income countries, will acquire at least one healthcare-associated infection (HAI) during their stay. Further, on average, 10% of those who acquire an HAI will die from it[14]. Within the United States, most studies indicate that minoritized groups face a higher incidence of HAIs compared to their white counterparts[15, 16, 17]. The trend is hypothesized to be attributable to many factors, including unconscious bias by healthcare professionals in care, income affecting access to quality care, and overall insufficient research evaluating HAIs[18].

MRSA is a particularly dangerous and common hospital-acquired infection. Methicillin-resistant staphylococcus aureus is an antibiotic-resistant bacterium with the ability to cause serious bloodstream infections in humans. One study finds that nearly 32% of MRSA bloodstream infections result in death and the proportion of MRSA bloodstream infections has risen by 21% from 2016 to 2020, indicating intense need for intervention by public health specialists[19,20]. MRSA can live harmlessly on the skin and is resistant to many antibiotics, including methicillin, oxacillin, penicillin, and amoxicillin.

MRSA infections are categorized based on their settings of contraction: community-acquired (CA-MRSA) and healthcare-acquired (HA-MRSA). CA-MRSA will occur outside of healthcare facilities and typically involves skin infections. HA-MRSA is associated with severe outcomes, often involving invasive disease, soft tissue infections, and bloodstream infections. Studies have shown that the distinction between CA-MRSA and HA-MRSA is blurred, as CA-MRSA has been increasingly found in healthcare related settings and vice versa[21]. One study in San Francisco found that the most predominant clone of MRSA was the same both in CA-MRSA and HA-MRSA cases, disproving the notion that the strain was only capable of community acquisition. It is hypothesized that these discrepancies occur when someone that has become colonized with CA-MRSA enters the hospital, either as an employee or a patient[22]. However, when diagnosed with MRSA in a healthcare facility, treatment and prevention interventions are focused internally: on timely treatment with an antibiotic regimen, behaviors of healthcare employees, and treatment course changes in the hospital[23]. Investigations of prevention methods beyond the hospital and into communities is sorely overlooked.

MRSA is present in livestock communities worldwide but is commonly untreated as livestock are often asymptomatic carriers of the bacteria. This bacterium can colonize human skin and noses without causing immediate harm, potentially leading to higher MRSA carriage rates in individuals living near densely populated livestock facilities. Such residential proximity to these facilities is more commonly experienced by low-income communities. In the U.S., for instance, nearly half of the slaughterhouses are located in areas with a poverty rate exceeding 30%[24]. With potential unequal risk in populations, studying the risk factors of MRSA becomes even more imperative. There is a wide range of evidence to show that humans are at risk of developing infections from their community-associated encounters, such as exposure to livestock and livestock byproducts, but the link between HA-MRSA and local livestock density is significantly less understood.

Study Prospects

This study aims to use spatial analytical tools to identify whether locational data related to livestock is associated with hospital-acquired MRSA infections. Hospitals in the United States are required to submit reports on hospital-acquired infections to the Centers for Disease Control and Prevention, and this data is largely unavailable to the public. The California Department of Public Health is unique in that it published data on each hospital's count of MRSA bloodstream infections from 2013-2022, open sourced. The spatial analysis will thus take place across the state of California.

Spatial analyses are a robust tool in identifying hotspots and factors that may explain disease patterns in a way that is easily comprehended by researchers. Further, a spatial analysis is uniquely useful because it lends a perspective that can determine remaining confounders or effect modifiers that are present only by examining spatiotemporal dimensions. The specific spatial methods used in this study will be disease mapping by county in California from 2013-2022, mapping livestock density by county as per data from the USDA Census of Agriculture, testing for spatial statistical significance with Local Indicators of Spatial Association cluster determination, determining significant clustering with Kulldorff's spatial scan statistics, and using Poisson regression to determine the relationship between county livestock-density and local HA-MRSA cases, while considering poverty as an effect modifier. This study is conducted with intention to understand one external factor that have been overlooked yet may contribute to HA-MRSA, a persistent challenge in healthcare. In doing so, it reaffirms a foundational public health principle of prioritizing prevention over response.

METHODS

Study Design

This is an observational, cross-sectional study using online available, deidentified hospital acquired MRSA cases and deidentified livestock operations across the state of California from 2013-2023.

Data Acquisition

Counts of cases of hospital acquired MRSA bloodstream infections by hospital from 2013-2022 were obtained from the California Department of Public Health HA-MRSA bloodstream infections data archive. California is one of the few states that offers this data publicly. There is a lack of publicly accessible data regarding CA-MRSA case incidence both within California and elsewhere. Each California hospital reported the count of hospital onset MRSA bloodstream infections, diagnosed with a positive blood specimen sample that was collected more than three days after admission. All hospital categories, including acute care, long term acute care, critical access, and rehabilitation hospitals or units were used in the analysis. Hospital names and counties listed in the dataset were used to collect coordinates via Google Maps. The study examined data provided from 454 hospitals and rehabilitation units of various types. All 58 counties were divided into Superior, Central, Bay Area, Southern, and Los Angeles regions, with full details provided in Supplementary Figure 1. The Californian livestock estimates from 2019-2023 were collected from USDA National Agricultural Statistics Service, in collaboration with the California Department of Food and Agriculture. California's livestock statistics as provided by the USDA NASS only provide data on cattle, specifically, beef cows, milk cows, and the collective count of all cattle and calves (ACC) measures. Cattle inventory by county was provided for the first of January each year. Counties with livestock data that was 'suppressed' due to individual operation counts per county were considered missing in the analysis. Milk cow statistics had over 50% missingness, so the measure was excluded in the analysis, focusing solely beef cows and the overall count of ACC. Percent of total population in poverty in California in 2022 was acquired from the U.S. Census Bureau, Small Area Income and Poverty Estimates. The California counties shapefile was gathered from the ArcGIS Hub, which is cited by the US Census Bureau.

HA-MRSA Incidence Analysis

Raw infections counts were initially used to report regional-specific qualities about the hospitals and their infection rates. In the later spatial and regression analyses, to mitigate the influence of population size, these counts underwent normalization. This process involved aggregating the total number of reported infections by county and then dividing by the corresponding county population. This normalized value was mapped over the period from 2013 to 2022 to assess the temporal persistence of HA-MRSA across counties. Additionally, in the regression analysis, the raw infection count was employed, with an offset factor representing county population size integrated into the model to ensure further normalization.

Livestock Analysis

Livestock statistics as provided by the USDA NASS details livestock counts by county, annually, for the period between 2019 and 2022. For the regression, data from 2022, the most recent publishing, was used. Specifically, the variables for beef cow inventory and the overall inventory of all cattle and calves were utilized in the regression, while data on milk cow density was omitted. Both metrics were examined, as there is no evidence to assume that one metric is superior in its relationship to HA-MRSA than the other. The relationship is not central to the type of cow product produced, but rather on insights into the living conditions or environmental factors that may facilitate disease spread. The non-suppressed data on beef cow density was substantial enough, with 20 suppressed or missing counties. In contrast, milk cows had over 50% missing data, rendering them unusable for analysis. ACC data had the least number of missing values (2 counties). Therefore, both metrics were included in the analysis to ensure comprehensive coverage of the livestock population and to account for any potential variability between them. The data exhibited a significant range that indroduced skewness, so the variables were stratified into quartiles to transform them from continuous to categorical.

Poverty Analysis

The percentage of those in poverty by county was used as the poverty metric. Poverty was coded as a dichotomous variable, subtracting the maximum percentage of poverty by county in 2022 (22.0%) from the minimum percentage of poverty by county (6.2%). The difference of 15.8% was divided into 2 and added to the minimum, setting the threshold value of poverty to 14.1%. All counties above this percentage were considered “high-poverty” and those below were considered “low-poverty” to dichotomize the groups as effect modification by poverty was to be considered.

Spatial Analysis

Geospatial analyses were conducted in R, utilizing the sf, tmap, spdep, and SpatialEpi packages. Spatial analysis was used to visualize the incidence of HA-MRSA, which was defined as the total infection count in each county per the county population. It was also used to visualize the locational data of livestock density across the state. The spdep package was used to conduct both the global Moran’s I test and the local indicators of spatial autocorrelation (LISA) analysis. These tests determine whether spatial autocorrelation of HA-MRSA incidence exists and in what magnitude. The SpatialEpi package was used to detect statistically significant clusters via

Kulldorff spatial scan statistics testing. The tmap package was used to create choropleth maps of the normalized incidence density of HA-MRSA and density of livestock at the county level and to create maps that visualize the results of all diagnostic spatial testing, including the LISA analysis and scan statistics testing.

Regression Analysis

Due to the skewness present in the variables of interest, negative binomial regression was the best suited regression method. Four base models were run to examine the relationship between exposure and outcome by county. In all models, the varying population sizes of Californian counties were standardized by using an offset factor, which normalized the infection counts by incorporating population per county. All four models were run both with and without poverty alone to assess confounding. Poverty was defined as a confounder of the relationship when any beta estimates changed by more than 10% due to including poverty in the model. If confounding was found to be present, the base models 1 and 2 would be adjusted to contain poverty to control for it. If poverty was not considered to be a confounding variable, it was dropped from Models 1 and 2 completely.

Model 1 considers only the effect that beef cows per county has on HA-MRSA. Similarly, Model 2 measures the sole effect of ACC on HA-MRSA. Model 3 considers the effect that count of beef cows per county categorized into quartiles has with effect modification by poverty group (0 or 1; low or high) on hospital acquired MRSA infection counts. Model 4 examines the effect of the count of ACC on HA-MRSA, also considering poverty as an effect modifier.

Negative binomial regression was run on the four base models with the glm.nb() function in R. Model diagnostics such as a residual analysis and a collinearity assessment were performed to ensure no gross violations of model assumptions. Choosing the best model relied on investigating the variables for significance and analyzing fit via the Akaike information criterion score (AIC).

Models

Model 1 – Beef Cows Alone:

$$\log(\lambda_j) = \alpha + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \varepsilon$$

where:

$$\begin{cases} \lambda = infection\ count \\ j = counties = 58 \\ X_1 = indicator\ variable\ for\ the\ 2nd\ quartile\ of\ beef\ cow\ countt \\ X_2 = indicator\ variable\ for\ the\ 3rd\ quartile\ of\ beef\ cow\ count \\ X_3 = indicator\ variable\ for\ the\ 4th\ quartile\ of\ beef\ cow\ count \\ reference\ category = 1st\ quartile\ of\ beef\ cow\ count \\ \varepsilon = error\ term \end{cases}$$

Model 2 – All Cattle and Calves Alone:

$$\log(\lambda_j) = \alpha + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \varepsilon$$

where:

$$\begin{cases} \lambda = infection\ count \\ j = counties = 58 \\ X_1 = indicator\ variable\ for\ the\ 2nd\ quartile\ of\ all\ cattle\ and\ calves\ count \\ X_2 = indicator\ variable\ for\ the\ 3rd\ quartile\ of\ all\ cattle\ and\ calves\ count \\ X_3 = indicator\ variable\ for\ the\ 4th\ quartile\ of\ all\ cattle\ and\ calves\ count \\ reference\ category = 1st\ quartile\ of\ all\ cattle\ and\ calves\ count \\ \varepsilon = error\ term \end{cases}$$

Model 3 – Beef Cows + Poverty:

$$\log(\lambda_{ij}) = \alpha + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \gamma_1 X_4 + \delta_1(X_1 \times X_4) + \delta_2(X_2 \times X_4) + \delta_3(X_3 \times X_4) + \varepsilon$$

$$\left\{\begin{array}{c} \lambda = infection\ count \\ i = poverty\ groups = 2 \\ j = counties = 58 \\ X_1 = \ indicator\ variable\ for\ the\ 2nd\ quartile\ of\ beef\ cow\ count \\ X_2 = \ indicator\ variable\ for\ the\ 3rd\ quartile\ of\ beef\ cow\ count \\ X_3 = \ indicator\ variable\ for\ the\ 4th\ quartile\ of\ beef\ cow\ count \\ reference\ category = 1st\ quartile\ of\ beef\ cow\ coun\square\square \\ X_4 = 1\ if\ county\ percent\ poverty > 14.1\%, else\ 0 \\ \varepsilon = error\ term \end{array}\right.$$

$\boldsymbol{Model\ 4 - All\ Cattle\ and\ Calves + Poverty}$:

$$\log(\lambda_{ij}) = \alpha + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \gamma_1 X_4 + \delta_1 (X_1 \times X_4) + \delta_2 (X_2 \times X_4) + \delta_3 (X_3 \times X_4) + \varepsilon$$

$$\left\{\begin{array}{c} \lambda = infection\ count \\ i = poverty\ groups = 2 \\ j = counties = 58 \\ X_1 = \ indicator\ variable\ for\ the\ 2nd\ quartile\ of\ all\ cattle\ and\ calves\ count \\ X_2 = \ indicator\ variable\ for\ the\ 3rd\ quartile\ of\ all\ cattle\ and\ calves\ count \\ X_3 = \ indicator\ variable\ for\ the\ 4th\ quartile\ of\ all\ cattle\ and\ calves\ count \\ reference\ category = 1st\ quartile\ of\ all\ cattle\ and\ calves\ count \\ X_4 = 1\ if\ county\ percent\ poverty > 14.1\%, else\ 0 \\ \varepsilon = error\ term \end{array}\right.$$

RESULTS

HA-MRSA incidence data and hospital type was collected from 456 hospitals across California for the year 2022. Though county level data was used for the entirety of the analysis, regional data was compiled for the purpose of relaying summary statistics, as shown in Table 1. Of the total hospitals included (456), 94 hospitals were in the Bay Area region (20.6%), 66 in the Central California region (14.5%), 114 in Los Angeles region (25.0%), 147 in the Southern region (32.2%), and 35 in the Superior region (7.7%). The overall distribution of hospital categories shows that 71.7% of statewide hospitals were Acute Care, 7.9% were Critical Access, 4.8% were Long-Term Acute Care, and Rehabilitation Hospitals or Units accounted for 15.6%.

Acute Care Hospitals were the most prevalent hospital category among all regions except Superior, whose most prevalent was Critical Access Hospitals.

HA-MRSA bloodstream infection reporting varied across and within regions, with wide ranges of infection counts. The region with the highest average infection count per county was Los Angeles with an average of 2.79 infections (standard deviation 3.73, range 0 to 20 infections). This average was followed closely by the Central California region, though it varied more widely, with an average of 2.78 infections (standard deviation 5.19, range 0 to 31) per county. Next was the Southern, Bay Area, and Superior regions of California which presented means at 1.92 (standard deviation 2.82, range 0 to 16 infections), 1.71 (standard deviation 2.73, range 0 to 17 infections), and 0.47 (standard deviation 1.05, range 0 to 4 infections) infections per county, respectively. There were minimal missing data of infection counts reported by hospitals amongst the regions, with the highest missing data reported among hospitals in the Superior region (2.9%) and the lowest in Southern California (0.7%). The overall percentage of missing infection count data was less than 1% (0.9%) (Table 1).

Normalized incidence density of HA-MRSA from 2013-2022 shows fluctuating incidence within counties, but a persistent overall occurrence of HA-MRSA in California. There is no consistent reduction of cases over the 10 years that it has been reported. Counties steadily reporting high HA-MRSA incidence rates include Yuba and Shasta. In the year 2022 specifically, Yuba, Stanislaus, and Amador county exhibited higher incidence rates (Figure 1). The mapping of 2022 livestock head quantities overlayed with proportional symbols to represent HA-MRSA incidence showed areas where high livestock density coincides with increased cases (Figure 2). However, there are nuances to the association, as shown with increased MRSA incidence in highly dense populations with more hospitals, such as Los Angeles county.

The global Moran's I test showed an absence of any significant autocorrelation patterns (Moran's I statistic = -0.12, p = 0.90), which supports the findings of the Local Indicators of Spatial Autocorrelation. The LISA map showed no significant autocorrelation patterns of HA-MRSA incidence in every county of California.

Kullfdorff's scan statistic revealed four statistically significant clusters of HA-MRSA incidence, specifically in Los Angeles, Yuba, Sacramento, and Stanislaus counties, with an observed log-likelihood ratio of 15.75 (p = 0.002, Figure 3).

The confounding assessment performed on the poverty variable revealed that no estimates were changed by more than 10% in any of the models when poverty was included. Poverty alone was therefore excluded from the base models 1 and 2 (Table 2). Further, poverty alone did not significantly affect the relationship in any models .

Model 1 showed that when poverty is excluded from the model, the density of beef cows has a significant positive effect of HA-MRSA incidence when the county falls in the third quartile (RR = 2.53, 95% CI = (1.17, 5.49)). Model 1 had an AIC value of 211.56. The model that excluded poverty and measured the effect of ACC of HA-MRSA, Model 2, showed a significant positive association between counties in the second and fourth quartile range of ACC and the occurrence of HA-MRSA. The second quartile supported a rate ratio of 2.27 (95% CI = (1.04, 4.95)), and the fourth quartile a rate ratio of 2.28 (95% CI = (1.11, 4.69)). The AIC value of Model 2 was 275.43. It is to be noted that five out of six estimates between Models 1 and 2 were statistically significant at the *90% confidence level*, with rate ratios ranging from 1.92 to 2.53 (Table 2). The regression on Model 3 and Model 4 presented highly insignificant results (p-value ~1) at the 95% confidence level for all variables included and had AIC scores of 211.53 and 279.36, respectively (Table 3).

DISCUSSION

This study explores the nexus between livestock density and the prevalence of hospital-acquired MRSA infections of California in 2022. Amidst the growing global demand for food, which necessitates densely populated livestock facilities, HA-MRSA poses a significant and often fatal health risk. The study finds a significant positive correlation between density of beef cows, as well as the combined count of ACC, and the occurrence of HA-MRSA in hospitals across California. These findings are consistent with the hypothesis that highly dense facilities can contribute to an increased concentration of antibiotic resistant pathogens, which in turn may affect human health through hospital acquired infections.

Intriguingly, the study does not uncover a direct linear progression in the risk of HA-MRSA infections with escalating livestock numbers. Instead, the data reveal a fluctuating effect; Among the estimates significant at the 95% confidence interval, results show a modest linear increase by quartile in Model 2, with risk ratios increasing from 2.27 in the second quartile to 2.28 in the fourth quartile. However, after lowering the statistical significance threshold to 90% confidence, we find a curvilinear pattern of the significant values among Model 1, with outputted ratios at 1.95, 2.53, and finally, 1.92. If this pattern is not due to data collection problems, there may be many possibilities that result in this, including the existence of an optimal proximity where humans coexist with livestock, beyond which the risk of HA-MRSA increases. Alternatively, smaller livestock operations could be situated in more residentially attractive areas, influencing human-livestock interactions. Another consideration is that the handling of livestock byproducts, such as the use of manure in nearby crop fields, could be a more significant risk factor for HA-MRSA than previously recognized. While the regression certainly underscores a concerning, heightened risk of HA-MRSA associated with specific livestock densities, it also opens the door to a myriad of additional questions regarding the multifaceted nature of this relationship. It emphasizes the urgency of broadening our investigative lens to encompass external contributors to hospital infections, thereby shifting the paradigm from an exclusively in-hospital focus to a more holistic view of infection control.

The spatial analysis comparing MRSA incidence with livestock density suggested a complex interplay between the variables. The LISA and Moran's I test did not detect any spatial autocorrelation in HA-MRSA across the state. The Kulldorff's scan statistics revealed several possible localized clusters of HA-MRSA that varied from the expected risk in the area. These clusters seem associated with highly dense livestock counties visually, which is supported by the regression output. These results may suggest that localized factors may interact with HA-MRSA incidence. If found to be true, public health professionals may isolate areas that may benefit most from intervention programs. Additional research may reveal the specific localized factors that may affect the HA-MRSA rates in these counties, as they are not evenly distributed. Finally, scan statistics led to significant and informative results, that directly contradict the findings of the spatial homogeneity assessments, like LISA mapping and Moran's I analysis. Moran's I tests whether any certain county is not spatially independent of another nearby county. The Kulldorff's scan statistic test has a similar purpose, with different assumptions. The scan statistic does not test spatial independence; it seeks out counties that violate the "constant-risk" null-hypothesis. Both tests aim to identify clusters, albeit through different methodologies. Therefore, the findings are generally expected to agree, but the study uncovered a discrepancy between them. This incongruity may suggest that the findings should be warily concluded on. Alternatively, it may indicate that one spatial analysis tool may be insufficient for thorough spatial research. Similar to how regression diagnostics are employed, spatial analysis tools should be used in conjunction, with each result being critically evaluated against others to formulate a well-supported conclusion.

Poverty was not found to be a significant contributing factor or effect modifier in the relationship between livestock density and HA-MRSA incidence. While it surely does not imply health equity overall, the data in this analysis show that poverty can be safely ruled out as a disproportionate variable in the context of livestock density and HA-MRSA. This allows public health officials to focus on the primary factors at play, which can simplify future research by avoiding tailoring of strategies and messaging to different socioeconomic groups. Not finding socioeconomic status as a contributing factor to this relationship will also allow for streamlined policy implementation in livestock farming practices to control such outbreaks. Further research is warranted to assess the relationship between other community or environmental factors that may play a role in HA-MRSA transmission.

The WHO has recognized antibiotic resistance as a major hazard to human health, making the findings of this study especially pertinent and relevant. The findings add to the literature by emphasizing the possible threat that livestock proximity and its relationship to HA-MRSA poses to human health. Further, the findings call attention to the strict distinction of HA-MRSA and CA-MRSA. The delineation between the two are becoming increasingly obfuscated, suggesting a possible need to revise or move beyond this classification system. For instance, it was found that counties with an ACC counts of greater than 64,000 had a risk of developing HA-MRSA that was 2.28 times the risk of developing HA-MRSA in counties with an ACC density of lower than 11,350. As demonstrated, livestock density, a “community” attribute, emerges as a factor influencing MRSA acquisition *within* healthcare settings, supporting the hypothesis that the delineation between CA-MRSA and HA-MRSA is ambiguous. Prevention methods focusing only on hospital settings may be insufficient, given the potential for people to carry resistant bacteria from community settings into healthcare facilities. Moreover, this study probes the previously overlooked, and still unexplored, external factors that could contribute to all HAIs, thus advocating for a more holistic approach to infection control and prevention. In doing this, public health officials will be putting resource into prevention over response, which will address the underlying causes of antibiotic resistance spread that could reduce the incidence of HAIs.

The findings of this study should be taken in stride with its limitations. The cross-sectional and ecological design of the study by nature does not allow for causal inferences. The designs also limit the data to groups, instead of individuals, which can overlook finely grained factors that may be relevant to the relationship at the individual level. Further, there are surely confounders that are present the in the relationship between livestock density and MRSA rates that were not accounted for in the analysis. The use of reported data from hospitals could be subjected to reporting biases, inconsistencies, and mistakes. Suppressed data from the livestock data file was an especially large blow to the study, as it results in loss of data for entire counties, of which were some of the highest HA-MRSA contributors. Table 4 shows the percent difference in average estimates between suppressed versus non-suppressed data. In some cases, these differences were in substantial, emphasizing the need for extra caution in interpreting our findings based on the available data. It should be noted that these suppressed counties, while only having a small number of active livestock operations, are not necessarily the least livestock dense areas. Some counties may have a single, very large operation that surely would have contributed valuable data to the analysis. This study relied on open-source and deidentified data for analysis. Access to classified and more granular data, such as individual proximity to livestock-dense facilities or exposure levels to individuals in close proximity to such facilities, could enhance the robustness of future studies and provide deeper insights into the relationship between livestock density and public health outcomes.

This study sheds light on the role that livestock density, and more broadly, external factors, may have on hospital acquired infections. The significant interactions found between livestock density and HA-MRSA, and the identified localized clusters of infection provide new insights for public health policy, intervention strategies, and future research genesis. As antibiotic resistant infections and demand for livestock-derived food grow in tandem, the findings of this study highlight the importance of sustainable livestock management practices that aim to not only improve productivity, but to also protect health by mitigating the risk of antibiotic resistance spread.

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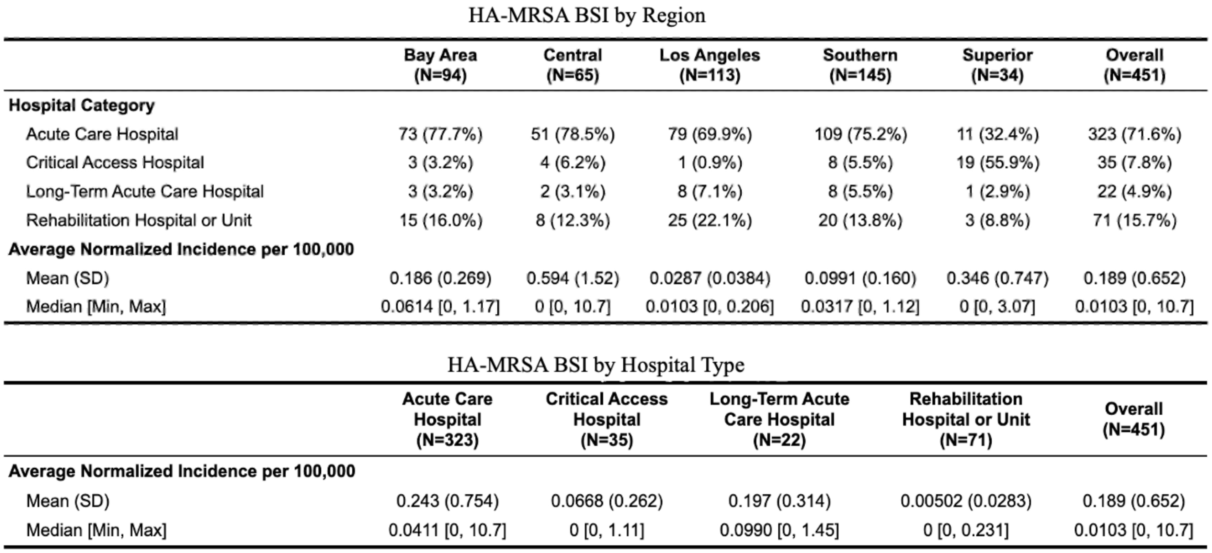
FIGURES AND TABLES

Table 1. HA-MRSA Hospital Statistics (California, 2022)



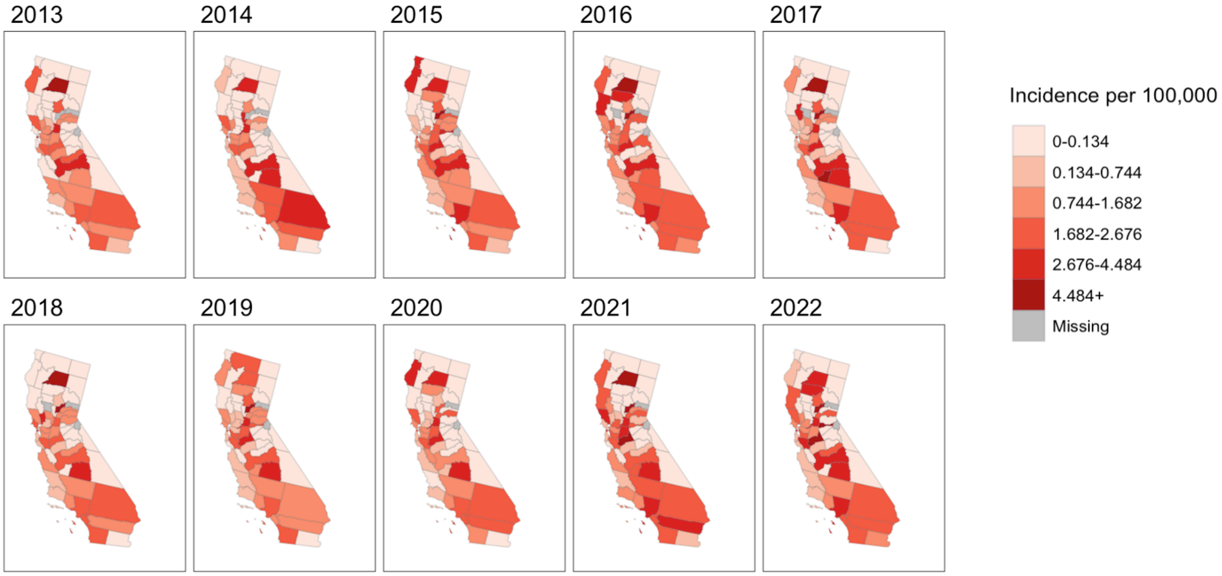
HA-MRSA BSI by Region

	Bay Area (N=94)	**Central (N=65)**	**Los Angeles (N=113)**	**Southern (N=145)**	**Superior (N=34)**	**Overall (N=451)**
Hospital Category						
Acute Care Hospital	73 (77.7%)	51 (78.5%)	79 (69.9%)	109 (75.2%)	11 (32.4%)	323 (71.6%)
Critical Access Hospital	3 (3.2%)	4 (6.2%)	1 (0.9%)	8 (5.5%)	19 (55.9%)	35 (7.8%)
Long-Term Acute Care Hospital	3 (3.2%)	2 (3.1%)	8 (7.1%)	8 (5.5%)	1 (2.9%)	22 (4.9%)
Rehabilitation Hospital or Unit	15 (16.0%)	8 (12.3%)	25 (22.1%)	20 (13.8%)	3 (8.8%)	71 (15.7%)
Average Normalized Incidence per 100,000						
Mean (SD)	0.186 (0.269)	0.594 (1.52)	0.0287 (0.0384)	0.0991 (0.160)	0.346 (0.747)	0.189 (0.652)
Median [Min, Max]	0.0614 [0, 1.17]	0 [0, 10.7]	0.0103 [0, 0.206]	0.0317 [0, 1.12]	0 [0, 3.07]	0.0103 [0, 10.7]

HA-MRSA BSI by Hospital Type

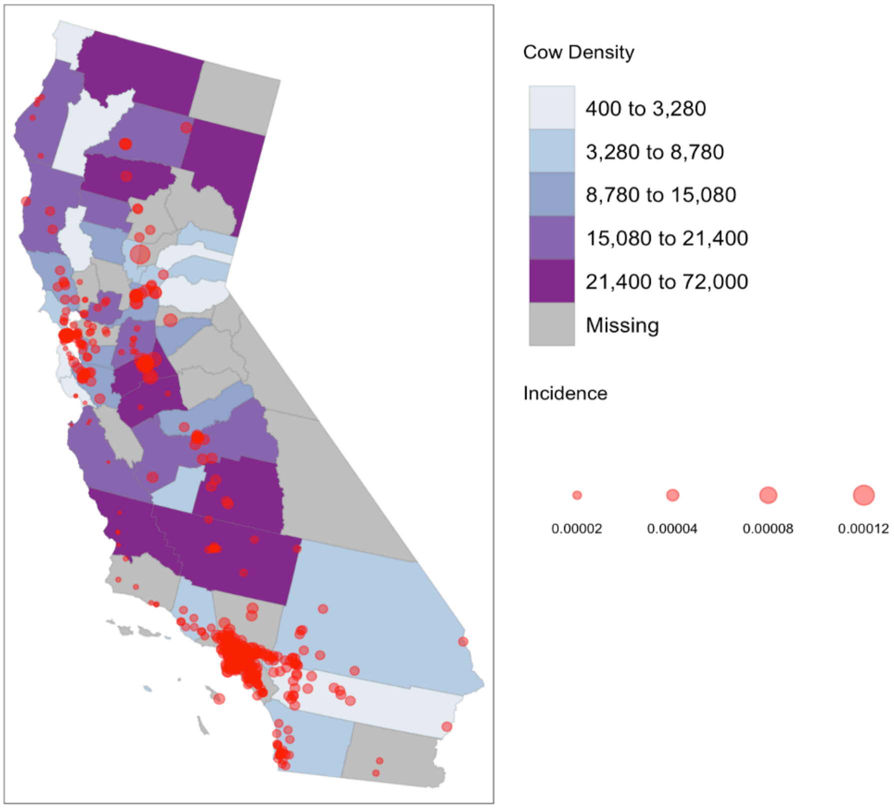
	Acute Care Hospital (N=323)	**Critical Access Hospital (N=35)**	**Long-Term Acute Care Hospital (N=22)**	**Rehabilitation Hospital or Unit (N=71)**	**Overall (N=451)**
Average Normalized Incidence per 100,000					
Mean (SD)	0.243 (0.754)	0.0668 (0.262)	0.197 (0.314)	0.00502 (0.0283)	0.189 (0.652)
Median [Min, Max]	0.0411 [0, 10.7]	0 [0, 1.11]	0.0990 [0, 1.45]	0 [0, 0.231]	0.0103 [0, 10.7]

Figure 1. HA-MRSA Incidence by County 2013-2022



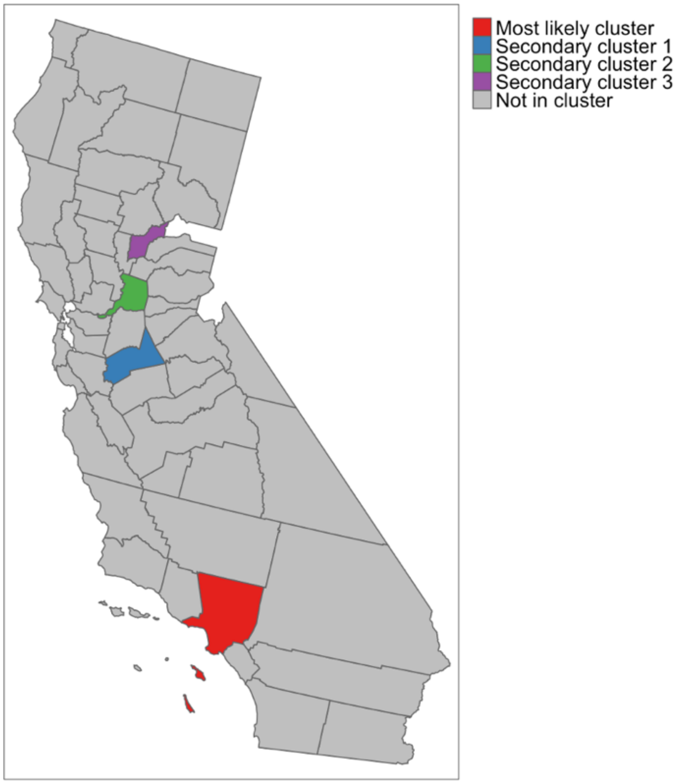
Source: California Department of Public Health

Figure 2. HA-MRSA Incidence by Beef Cow Count, 2022



Source: California Department of Public Health

Figure 3. Kulldorff's Scan Statistics of HA-MRSA, 2022



Source: California Department of Public Health

Table 2. Poverty Confounding Assessment

Variable	Estimates including Poverty	Estimates excluding Poverty	Greater than 10% difference?	Confounding
Model 1 – Beef Cows				
2nd Quartile	1.94	1.95	No	No
3rd Quartile	2.48	2.53	No	No
4th Quartile	1.83	1.92	No	No
Poverty	1.08	Poverty 95% Confidence Interval (0.591, 1.96)		
Model 2 – All Cattle and Calves				
2nd Quartile	2.24	2.27	No	No
3rd Quartile	1.53	1.54	No	No
4th Quartile	2.21	2.28	No	No
Poverty	1.05	Poverty 95% Confidence Interval (0.585, 1.90)		

The figure shows the estimate outputs of the negative binomial regressions performed on Models 1 and 2, and replicas of the models that contain the poverty variable. If estimates are more than 10% different when including the poverty variable, the variable is considered a confounder of the relationship between exposure and outcome. Figure also shows the insignificance of the poverty variable alone in these models via confidence intervals.

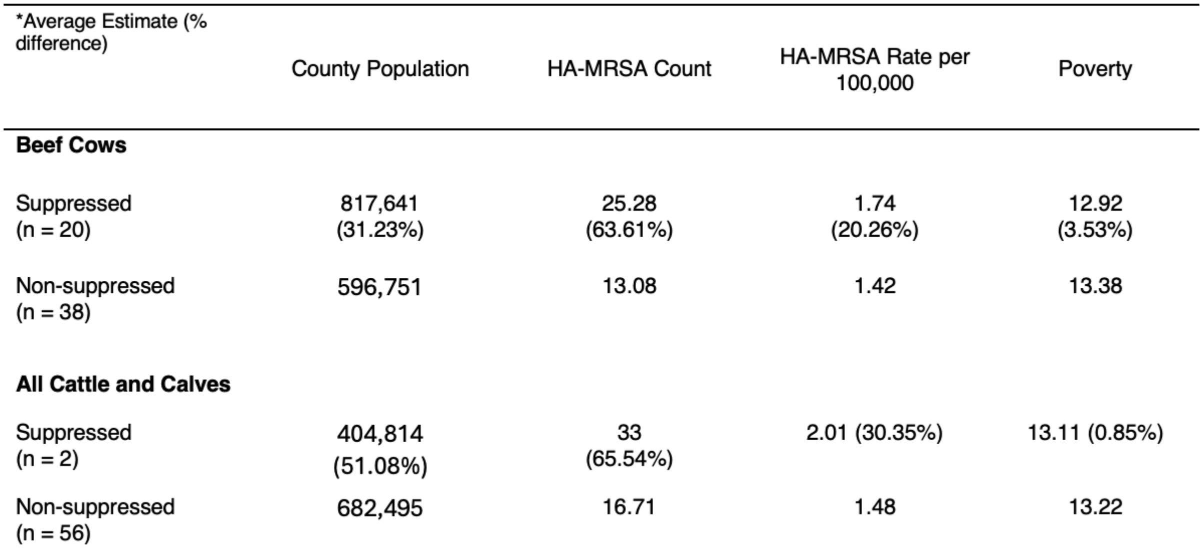
Table 3. Negative Binomial Regression Model Outputs

Variable	Number of Heads	Rate Ratio	95% Confidence Interval	90% Confidence Interval
Model 1 – Beef Cows Alone				
2nd Quartile	$3,550 \le x < 11,200$	1.95	(0.90, 4.20)	(1.00, 3.78)†
3rd Quartile	$11,200 \le x < 17,300$	2.53	(1.17, 5.49)†	(1.30, 4.93)†
4th Quartile	$x \ge 17,300$	1.92	(0.92, 3.98)	(1.02, 3.60)†
Model 2 – All Cattle and Calves Alone				
2nd Quartile	$11,350 \le x < 26,000$	2.2	(1.04, 4.95)†	(1.16, 4.42)†
3rd Quartile	$26,000 \le x < 64,000$	1.54	(0.69, 3.42)	(0.77, 3.05)
4th Quartile	$x \ge 64,000$	2.28†	(1.11, 4.69)†	(1.23, 4.22)†
Model 3 – Beef Cows + Poverty EMM				
2nd Quartile	$3,550 \le x < 11,200$	1.93	(0.93, 4.01)	(1.03, 3.64)†
3rd Quartile	$11,200 \le x < 17,300$	2.22	(1.0, 4.94)†	(1.11, 4.44)†
4th Quartile	$x \ge 17,300$	1.27	(0.55, 2.94)	(0.61, 2.63)
Poverty	-	2.85e-16	(0, Inf)	(0, Inf)
2nd Quartile * Poverty	$3,550 \le x < 11,200$	0.087	(0, Inf)	(0, Inf)
3rd Quartile * Poverty	$11,200 \le x < 17,300$	3.88e15	(0, Inf)	(0, Inf)
4th Quartile * Poverty	$x \ge 17,300$	5.86e15	(0, Inf)	(0, Inf)
Model 4 – All Cattle and Calves + Poverty EMM				
2nd Quartile	$11,350 \le x < 26,000$	1.70	(0.74, 3.93)	(0.83, 3.49)
3rd Quartile	$26,000 \le x < 64,000$	1.51	(0.66, 3.43)	(0.75, 3.05)
4th Quartile	$x \ge 64,000$	2.11	(0.88, 5.03)	(1.00, 4.44)†
Poverty	-	5.48e-16	(0, Inf)	(0, Inf)
2nd Quartile * Poverty	$11,350 \le x < 26,000$	3.75e15	(0, Inf)	(0, Inf)
3rd Quartile * Poverty	$26,000 \le x < 64,000$	1.10e15	(0, Inf)	(0, Inf)
4th Quartile * Poverty	$x \ge 64,000$	1.82e15	(0, Inf)	(0, Inf)

The figure shows the output of the negative binomial regressions performed on Models 1-4 with descriptions of the model aspects. Note that significant values' confidence intervals and estimates are signified with †. Model 1 AIC = 211.56, Model 2 AIC = 275.43, Model 3 AIC = 211.53, Model 4 AIC = 279.36.

SUPPLEMENTARY MATERIAL

Table 4. Supplementary Suppressed County Information



*Average Estimate (% difference)	County Population	HA-MRSA Count	HA-MRSA Rate per 100,000	Poverty
Beef Cows				
Suppressed (n = 20)	817,641 (31.23%)	25.28 (63.61%)	1.74 (20.26%)	12.92 (3.53%)
Non-suppressed (n = 38)	596,751	13.08	1.42	13.38
All Cattle and Calves				
Suppressed (n = 2)	404,814 (51.08%)	33 (65.54%)	2.01 (30.35%)	13.11 (0.85%)
Non-suppressed (n = 56)	682,495	16.71	1.48	13.22

The figure shows the comparison of county estimates used in the analysis (non-suppressed) with county estimates not used in the analysis (suppressed) due to suppression in county livestock estimate data.

Supplementary Figure 1. Regional County Distribution

Region	**County**
Superior	Butte
	Colusa
	Del Norte
	Glenn
	Humboldt
	Lake
	Lassen
	Mendocino
	Modoc
	Nevada
	Plumas
	Shasta
	Sierra
	Siskiyou
	Tehama
	Trinity
Central	Alpine

	Amador
	Calaveras
	El Dorado
	Fresno
	Inyo
	Kings
	Madera
	Mariposa
	Merced
	Mono
	Placer
	Sacramento
	San Joaquin
	Stanislaus
	Sutter
	Yuba
	Tulare
	Tuolumne
	Yolo
Bay Area	Alameda
	Contra Costa
	Marin
	Monterey
	Napa
	San Benito
	San Francisco
	San Mateo
	Santa Clara
	Santa Cruz
	Solano
	Sonoma
Southern	Imperial
	Kern
	Orange
	Riverside
	San Bernardino
	San Diego
	San Luis Obispo
	Santa Barbara
	Ventura
Los Angeles	Los Angeles