Distribution Agreement

In presenting this thesis or dissertation as a partial fulfillment of the requirements for an advanced degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis or dissertation in whole or in part in all forms of media, now or hereafter known, including display on the world-wide web. I understand that I may select some access restrictions as part of the online submission of this thesis or dissertation. I retain all ownership rights to the copyright of the thesis or dissertation. I also retain the right to use in future works (such as articles or books) all or part of this thesis or dissertation.

Signature:

_____ _____

Dayton Kassner Date

The impact of Culture-Independent Diagnostic Testing on *Salmonella* incidence and surveillance in the State of Georgia from 2008 – 2018.

By

Dayton Kassner

Master of Public Health

Epidemiology

_____

Anne Spaulding

Committee Chair

_____

Bethany LaClair

Committee Member

_____

Hope Dishman

Committee Member

The impact of Culture-Independent Diagnostic Testing on *Salmonella* incidence and surveillance in the State of Georgia from 2008 – 2018.

By

Dayton Kassner

B.A.

University of Wisconsin – Madison

2017

Faculty Thesis Advisor: Anne Spaulding, MD, MPH

An abstract of

A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University

in partial fulfillment of the requirements for the degree of
Master of Public Health
in Epidemiology

2019

Abstract

The impact of Culture-Independent Diagnostic Testing on *Salmonella* incidence and surveillance in the State of Georgia from 2008 – 2018.

By Dayton Kassner

Background: *Salmonella* has the potential to be a significant public health concern; the estimated burden of *Salmonella* in the U.S. is 1.2 million cases, 23,000 hospitalizations and 450 deaths every year. CIDT use for *Salmonella* detection began in 2011. CIDTs create challenges for surveillance epidemiologists who use historical data to establish trends for *Salmonella* incidence. The objectives of this study are to identify the impact of CIDT use on *Salmonella* surveillance and incidence in Georgia and determine whether any change in incidence differed amongst demographic groups.
Methods: The data was obtained through Georgia's State Electronic Notifiable Disease Surveillance System. *Salmonella* cases were arranged into three test type categories: culture positive only, culture positive and CIDT positive, and CIDT positive only. Differences in *Salmonella* incidence rates were analyzed with percent change calculations using Poisson regression. Analysis on the changes in the rates of positive cultures and positive CIDTs was done using Poisson regression. Analysis on *Salmonella* incidence amongst different demographic groups was conducted with Poisson regression.
Results: Among the 27,789 *Salmonella* cases, 24,704 (88.9%) were culture positive only, 1,564 (5.6%) were culture positive and CIDT positive, and 1,521 (5.5%) were CIDT positive only. 2010 and 2017 had the highest and lowest total *Salmonella* incidence rates of 28.77 and 22.48 *Salmonella* cases per 100,000 persons, respectively. Culture positive only *Salmonella* incidence rates decreased significantly after 2012. Both culture and CIDT positive or CIDT positive only *Salmonella* incidence rates increased significantly after 2012.
Discussion: The increased use of CIDTs in Georgia does not appear to have had any impact on *Salmonella* incidence rates. The impact of CIDTs was still noticeable for *Salmonella* surveillance. The total number of *Salmonella* cases with a positive culture decreased significantly. A loss of cultures will result in a loss of bacterial isolates. Bacterial isolates are used in variety of ways in *Salmonella* surveillance, such as monitoring trends in *Salmonella* subtypes, detecting outbreaks throughout Georgia, identifying vehicles in outbreaks and testing antimicrobial susceptibility. Future studies will need to be conducted to monitor CIDT use in *Salmonella* surveillance and other foodborne disease surveillance as CIDTs are still a relatively novel testing type.

The impact of Culture-Independent Diagnostic Testing on *Salmonella* incidence and surveillance in the State of Georgia from 2008 – 2018.

By

Dayton Kassner

B.A.
University of Wisconsin – Madison
2017

Faculty Thesis Advisor: Anne Spaulding, MD, MPH

A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
in partial fulfillment of the requirements for the degree of
Master of Public Health
in Epidemiology
2019

Table of Contents

BACKGROUND/LITERATURE REVIEW

Literature Review

In order to study the effect of culture-independent diagnostic tests (CIDTs) on *Salmonella* incidence in the state of Georgia, a literature review was needed to identify gaps in current knowledge on the topic. The objective of the literature review is to identify what CIDTs are currently in use for *Salmonella* detection in clinical samples, what the sensitivity and specificity are for the CIDTs in use and identify historical *Salmonella* incidence rates and how CIDTs may impact *Salmonella* incidence rates and public health surveillance.

There have been many studies conducted on the current CIDTs used for enteric disease diagnostic testing. Huang et al. conducted a study on the sensitivities and specificities of polymerase chain reaction (PCR) CIDTs for enteric disease detection including the BioFire FilmArray panel and Luminex panel (1). Huang found the sensitivity for the BioFire GI panel was 95.8 (95%CI: 78.9 – 100) and the specificity was 100 (95%CI: 97.2 – 100) for *Salmonella*. For the Luminex panel, the sensitivity was 79.2 (95%CI: 57.2 – 92.9) and the specificity was 100 (95%CI: 97.2 – 100) for *Salmonella*. The kappa between the BioFire and Luminex panels was 0.89. Both the BioFire GI and Luminex panel identified a positive *Salmonella* sample when the stool culture was unable to. Buss et al. compared how the BioFire panel compares to a stool culture, which had previously been named the gold standard for enteric testing (2). Buss found that the BioFire panel had a sensitivity was 100 (95%CI: 88.8 – 100) and the specificity was 99.6 (95%CI: 99.1 – 99.9) for *Salmonella*. Khare et al. conducted a study on the BioFire GI and Luminex panel compared to the gold standard of a stool culture (3). Khare found the that the sensitivity and specificity of the BioFire and Luminex panel for *Salmonella* was 100% and

99.6% respectively. These three studies show that while the BioFire and Luminex panel have been shown to perform well, both tests may result in both false positives and false negatives.

Several studies have been published on historical *Salmonella* incidence trends and how CIDT use may impact these trends using Foodborne Diseases Active Surveillance Network (FoodNet) data. FoodNet data is compiled using data from 10 different FoodNet sites located in select counties in California, Colorado, Connecticut, Georgia, Maryland, Minnesota, New Mexico, New York, Oregon, and Tennessee. Overall, the FoodNet surveillance area represents 15% of the United States population (4). A 2011 study by Gillis et al. on *Salmonella* incidence and trends using from 1996 to 2010 found that the total number of *Salmonella* cases in 2010 was 8,256 with an incidence of 17.6 per 100,000 persons (5). Gilliss reported that the 2010 incidence rate of *Salmonella* was significantly higher than the incidence rate from 2006 to 2008. The percent change from 2006 to 2008 was 10% higher with a confidence interval of 4% to 17%. Gilliss mention CIDTs only in reference to Shiga-toxin producing *E. coli* (STEC) and *Campylobacteria* but mentions CIDTs potential effect on reported incidence. The Gilliss study is important as it establishes a baseline for our study to come as all the results made on *Salmonella* incidence are before the introduction of CIDT use for *Salmonella*. A study by Iwamoto et al. on bacterial enteric infections detected by CIDTs for the years of 2012 to 2013 found that the total amount of *Salmonella* cases reported as culture positive was 15,034 (98%) (6). A total of a 115 (0.7 %) cases were reported as CIDT-positive and culture-positive, 8 (0.1%) cases were reported as CIDT-positive and culture-negative, and 185 (1.2%) were reported as CIDT-positive and no culture. The incidence for *Salmonella* was 16.0 per 100,000 persons for culture confirmed infections and 0.2 per 100,000 persons for positive CIDT reports with either no culture or negative culture. Another study by Huang et al. on CIDT use and its effect on foodborne illness

surveillance using data from 2012 to 2015 reported the total number of *Salmonella* cases reported as culture positive only was 7,354 (91%) for 2015 (7). Out of the rest of the *Salmonella* cases, 374 (5.0 %) were reported as CIDT-positive and culture-positive, 141 (2.0%) were reported as CIDT-positive and culture-negative, and 220 (3.0%) were reported as CIDT-positive and no culture. The incidence for culture-positive only *Salmonella* cases was 15.89 per 100,000 persons while the incidence for confirmed infections and CIDT positive reports was 16.63 per 100,000 persons. The difference between the two figures was not significant. When the 2012 to 2014 *Salmonella* incidence rate for confirmed infections was compared to the 2015 incidence rate, the result was not significant. Another study conducted by Marder et al. on the use of CIDT and its effects on foodborne illness surveillance using data from 2013 to 2016 found the total number of confirmed *Salmonella* cases was 7,554 (92.4%) and the total number CIDT-positive only cases was 618 (7.6%) (8). In 2016, the incidence for confirmed *Salmonella* cases was 15.4 per 100,000 persons while the incidence for confirmed or CIDT-positive only *Salmonella* cases was 16.66 per 100,000 persons. There was a 2% (95% CI -4-8%) increase in culture-confirmed *Salmonella* incidence in 2016 compared with the 2013-2015 incidence rate while there was a 6% (95% CI -1-12%) increase in CIDT-positive *Salmonella* incidence (with or without culture confirmation) during the same time period. Marder also reported that the number of laboratories performing CIDTs for *Salmonella* increased from 2 per 460 laboratories (<1%) in 2013 to 59 per 421 laboratories (14%) in 2016.

Background

Salmonella is a genus of rod-shaped Gram-negative bacteria that are part of the Enterobacteriaceae family. *Salmonella* is divided into 6 different subspecies with over 2,600 serotypes. Infection begins with the person ingesting *Salmonella* bacteria. Once in the intestinal tract, the bacteria will invade the intestinal lining and begin to proliferate, potentially spreading to the bloodstream. *Salmonella* symptom onset begins an average of 12 – 72 hours after ingestion but has been known to be longer. People infected with *Salmonella* present with clinical symptoms such as diarrhea (may contain blood), nausea, abdominal pain, fever or vomiting (9). There is a high burden of *Salmonella* both globally and domestically. The World Health Organization (WHO) reports around 1.9 billion people every year become ill with diarrheal illnesses resulting in 715,000 deaths worldwide. Of these 1.9 billion people that reported diarrheal illness, a 180 million were infected with *Salmonella* (10). According to the Centers for Disease Control and Prevention (CDC), the estimated burden of *Salmonella* in the U.S. is 1.2 million cases, 23,000 hospitalizations and 450 deaths every year (11).

The Foodborne Diseases Active Surveillance Network (FoodNet) is a collaboration between the CDC, 10 state health departments, the U.S. Department of Agriculture's Food Safety and Inspection Services (USDA – FSIS), and the Food and Drug Administration (FDA). The objective of the FoodNet program is to estimate the burden of foodborne illness in the United States, monitor trends over time, and disseminate information that can lead to improvements in public health practice and the development of interventions to reduce the burden of foodborne illnesses (4). FoodNet is an active, laboratory-based surveillance system that through which information on laboratory-positive cases of *Salmonella*, *Shigella*, *Campylobacter*, Shiga-toxin producing *E. coli*, *Vibrio*, *Yersinia*, *Listeria* and *Cylcospora* are

collected. *Salmonella* surveillance began in 1996 and data is collected on hospitalization status, patient outcome, travel history, and certain foodborne or environmental exposures for case patients. The Georgia Department of Public Health (GDPH) is one of the ten FoodNet sites. GDPH identifies *Salmonella* cases when an ill person tests positive for *Salmonella* via laboratory testing performed at a hospital or reference laboratory. In Georgia, all laboratories are required by law to report *Salmonella* positive tests to the GDPH. Once *Salmonella* is detected from a clinical sample and reported to the GPDH, the case-patient associated with that clinical sample will be defined as confirmed or probable based on the 2017 Council of State and Territorial Epidemiologists (CSTE) case definition (12). According to the 2017 case definition, a *Salmonella* case is considered to be confirmed if there is confirmatory lab evidence of isolation of *Salmonella* from a clinical sample i.e. *Salmonella* culture. A *Salmonella* case is considered to be probable if there is only supportive laboratory evidence of detection of *Salmonella* from a clinical sample using a CIDT such as PCR. A polymerase chain reaction (PCR) test is the only CIDT method used to identify *Salmonella*.

Previously, the gold standard for detecting *Salmonella* in clinical samples has been stool culture (13). The process involves using selective media specific to *Salmonella* to grow the bacteria for identification. The gold standard procedure involves incubating the media at 35 degrees Celsius and waiting 48 hours before considering the sample negative. If the sample is positive an additional 24 hours is needed to confirm the positive result (14, 15). Stool culturing is a time-consuming and costly procedure and, in an effort to improve clinical services and reduce costs, new methods of identifying gastrointestinal pathogens were developed (16). In 2011, Georgia first began receiving case reports with CIDTs being the primary method used to detect *Salmonella*. Since then there have been multiple PCR-based gastrointestinal syndromic panels

utilized to detect *Salmonella* in clinical samples. PCR-based GI syndromic panels are now used widely throughout the country as one of the methods used for identifying enteric diseases such as *Salmonella*. Two of the most common gastrointestinal panels used by laboratories are the BioFire FilmArray GI and Luminex panels. The BioFire panel includes 22 different gastrointestinal pathogens and results for a specimen can be available in one hour (16, 17). Some laboratories use a lab-developed PCR test to detect *Salmonella* in clinical samples instead of a commercial PCR panel test. As CIDT use increases across the country several challenges may occur for public health surveillance epidemiologists. One of the main challenges of CIDTs is varying sensitivity and specificity (1, 3, 18, 19, 20). CIDTs have been shown to have different results from stool cultures when the tests are run on the same sample. The Georgia Department of Public Health requires laboratories to submit clinical samples for all *Salmonella* positive reports to the Georgia Public Health Laboratory for culture testing. This allows GDPH to perform standardized testing on all *Salmonella* cases and to avoid the loss of important information gained by subtyping samples; however, not all *Salmonella* cases are identified using stool culture (7, 18, 19, 21).

Currently, there are several different brands of PCR tests being used to detect *Salmonella* in clinical samples. The Huang, Buss and Khare studies have shown that different CIDT PCR assays differ in sensitivities and specificities, which may cause challenges when compared to the sensitivity and specificity of culture testing. The Iwamoto, Huang and Marder studies have found that from 2010 to 2016 the incidence rate of *Salmonella* per 100,000 persons decreased if only confirmed cases were used for surveillance. However, it has also been shown that CIDT use was increasing throughout those years. CIDTs may improve medical care for patients by identifying *Salmonella* in clinical samples significantly faster, reduce total patient time spent in the hospital

and reduce total medical cost for both the patients and clinicians (15). The varying sensitivities and specificities among CIDTs, the differences in incidence rates between confirmed *Salmonella* cases throughout the past decade and the increased use of CIDTs creates challenges for surveillance epidemiologists who use historical data to establish trends for *Salmonella* incidence (23).

The purpose for this study is to understand the effect of CIDTs on *Salmonella* surveillance in Georgia. Previous studies have shown the effects of CIDTs on foodborne disease incidence vary amongst pathogens, but none have solely focused on *Salmonella* in Georgia (6, 7, 8). The number years analyzed by previous studies has been confined to a three to four-year time span. This time span is not an ample amount of time to draw a valid conclusion on whether or not a trend in *Salmonella* incidence rates can be established. There are a variety of factors, such as outbreaks, that contribute to foodborne disease incidence, especially for *Salmonella*, and there is always a degree of variance when it comes to the total amount of cases that occur in any given year. This study will include a total of 11 years of Georgia *Salmonella* data which may allow us to minimize the effect these factors have on year to year case counts due to the increased number of data available to analyze.

METHODS

Study Design

This study on the effect of CIDT use on *Salmonella* incidence in the state of Georgia is a retrospective cohort study. All analysis conducted on the data will be considered secondary analysis of data as primary data collection for *Salmonella* case data was not conducted for this study. The objectives of this study are to identify the impact of CIDT use on *Salmonella* surveillance and incidence in Georgia and determine whether any change in incidence differed amongst demographic groups. I hypothesize that the effect of CIDT use did impact *Salmonella* incidence rates in the state of Georgia after its introduction in 2011 and that CIDT use will have a significant impact on *Salmonella* incidence rates among different demographic groups.

Data Acquisition

The Georgia Department of Public Health (GDPH) uses their State Electronic Notifiable Disease Surveillance System (SendSS) to capture and manage data associated with *Salmonella* cases reported within the state of Georgia. *Salmonella* cases are reported to the GDPH by several methods: manual entry of physical clinical laboratory reports, digital electronic laboratory reporting (ELR) or direct entry by clinical laboratories. The physical laboratory reports are entered manually into SendSS while ELR automatically uploads the digital copies into SendSS. The dataset used in this study was queried from SendSS and includes all *Salmonella* cases reported in Georgia from 2008 to 2018. The data from 2018 are preliminary and is not the official reported numbers from Georgia in 2018. The dataset had a total of 27,793 *Salmonella* cases and 25 variables after the SendSS query was completed.

Data Cleaning and Management

The dataset includes demographic variables such as gender, race, ethnicity, age, and county of residence. For gender, the value was considered missing if the response was 'other' (n = 3). For race and ethnicity, the value was considered missing if the response was 'not available' (race, n = 779 and ethnicity, n = 1,619). There were two cases where age was set to missing as the age reported was deemed invalid. The dataset includes other categorical variables such as hospitalization status and patient outcome. Patient outcome (alive or dead) is considered to be at hospital discharge or seven days after the specimen collection data if the patient was not hospitalized (4). Hospitalization status was considered missing if the response was 'not available' (n = 91). If the response to hospitalization status was 'ER only' it was changed to 'no'. The rest of the variables were either categorical or numerical testing variables such as earliest lab test date, facility name where the test was conducted, type of lab test, result of the lab test, was the specimen identified by culture, was an isolate or specimen sent to the Georgia Public Health Laboratory (GPHL), and the brand name of the PCR test conducted. Any response of 'unknown' for all variables was considered missing within the dataset. No cases were excluded after the initial cleaning of the dataset.

Diagnostic test types reported for each case were reviewed and each case was assigned to one of three test type categories (Figure 1); culture positive only, culture positive and CIDT positive, and CIDT positive only. A case was categorized as culture positive only if the only diagnostic test used to detect *Salmonella* in the clinical sample was through culture. A case was categorized as culture positive and CIDT positive if both culture and PCR diagnostic tests were used to detect *Salmonella* in the clinical sample. Finally, a case was categorized CIDT positive only if the only diagnostic test used to detect *Salmonella* in the clinical sample was a PCR test.

After each case was categorized there were four cases were excluded from the dataset: One case was a duplicate case and three cases had test results that could not be interpreted as the testing results were either unknown or missing and had no isolates sent to the GPHL. After sorting the cases into the correct test type classification variable, the cases had to be sorted based on whether or not the case would be considered confirmed or probable (Figure 1). The 2017 Council of State and Territorial Epidemiologists (CSTE) case definition was used to classify the cases (12). Using this case definition and the four test type categories, there was 26,268 confirmed and 1,521 probable *Salmonella* cases for total case count of 27,789.

A population variable was created using population data from Georgia's Online Analytic Statistical Information System (OASIS). OASIS is a web-based standardized health data repository that contains population-level data on mortality and morbidity, maternal child health, infant mortality, and population characteristics for the state of Georgia from 1994 to 2018. The population variable created within the dataset was populated with data using OASIS' population statistics. Total state population and county population numbers for each year from 2008 to 2018 was entered into the dataset.

Data Analysis

Salmonella incidence rates were calculated by dividing the population data obtained from OASIS for any given year from the total amount cases for any given year. That number was multiplied by 100,000 to achieve the *Salmonella* incidence rate per 100,000 persons. A Poisson regression model was created to identify if the effect of the introduction of CIDTs is associated with an increase or decrease in incidence rate of Georgia's *Salmonella* cases from year to year. In the regression model total case count was the outcome of interest with the year of onset and test type as the predictors while controlling for the change in population. In this regression model

Salmonella incidence rates from 2008 to 2010 were combined to create an average annual *Salmonella* incidence rate to be used as the reference incidence rate as CIDTs were not used to identify *Salmonella* in clinical samples until 2011. Using the regression model, percent change was calculated for every subsequent year after 2010 (23).

Positive culture rate is defined as the number of *Salmonella* cases with a positive culture per 100 *Salmonella* cases while positive CIDT rate is defined as the number of *Salmonella* cases with a positive CIDT per 100 *Salmonella* cases. Positive CIDT rate and positive culture rate were calculated by taking the amount of *Salmonella* cases that had either a positive culture or positive CIDT in a given year, dividing that number by that given years total *Salmonella* case count, and multiplying by 100 to achieve the positive culture rate or positive CIDT rate per 100 *Salmonella* cases. A *Salmonella* case with a positive culture and a positive CIDT was included in both positive culture rate and positive CIDT rate calculations. The purpose of each rate is to show how many *Salmonella* cases had a positive culture or a positive CIDT for any given year relative to that year's total *Salmonella* case count. To calculate the percent change in positive culture and positive CIDT rates for *Salmonella* cases over time, two Possion regression models were used (23). In the regression model either a positive culture or a positive CIDT was the outcome and the year was the predictor while controlling for the change in *Salmonella* case count and for age as an effect modifier because of the differences in CIDT use amongst varying age categories. The positive culture rates for 2008 to 2010 were combined to create an annual average positive culture rate that will be used as the reference period for the positive culture rates regression model. The positive CIDT rates for 2011 to 2013 were combined to create an average annual positive CIDT rate that will be used as the reference period for the positive CIDT rates regression model.

To identify if any significant differences exist between demographic variables and test type used to identify *Salmonella,* a Poisson regression model was created. Within the regression model, the predictors were the demographic variables of interest and outcome of interest was the test type used to identify the *Salmonella* in the clinical sample. Any significant differences were identified via odds ratios. Dummy variables were created for any predictor variable that was nominal such as age and race. The analysis included cases identified from 2011 through 2018 as *Salmonella* was only identified via culture before 2011. The reference period for this analysis was 2011 to 2013 as older age categories had few cases and produced confidence intervals that were too wide and made the result invalid.

RESULTS

From 2008 to 2018, there were a total of 27,789 *Salmonella* cases reported in the state of Georgia. *Salmonella* cases were responsible for a total of 7,839 hospitalizations and resulted in 167 deaths. Among the 27,789 *Salmonella* cases, 24,704 (88.9%) were culture positive only, 1,564 (5.6%) were culture positive and CIDT positive, and 1,521 (5.5%) were CIDT positive only (Table 1) (Figure 2). Of the 1,521 *Salmonella* cases identified via CIDT only, 63 (4.1%) were tested by culture but had a negative result. The most common type of PCR test used were laboratory-developed tests (n = 1,381). Two lab facilities were responsible for 97.4% of all laboratory-developed tests. Diatherix Laboratories conducted a total of 1,235 (89.4%) laboratory-developed tests and Alimetrix conducted a total of 111 (8.0%) laboratory-developed tests. The BioFire FilmArray and Luminex PCR panels were the next most commonly used CIDT (n = 876 and n = 438, respectively). BD MAX Enteric Bacterial Panel was the least commonly used CIDT (n = 210). Incidence rates for *Salmonella* were measured for each year since 2008 (Table 2). The highest total *Salmonella* incidence rate in the study was 28.77 *Salmonella* cases per 100,000 persons in 2010. The lowest total *Salmonella* incidence rate in the study was 22.48 *Salmonella* cases per 100,000 persons in 2017. The highest *Salmonella* incidence rate in the study for probable cases was 3.58 per 100,000 persons in 2018.

When compared to the culture positive only average *Salmonella* incidence rate between 2008 – 2010, 2017 had the largest percent change in confirmed *Salmonella* incidence rate of -39.03% (95%CI: -42.20 to -35.69) (Table 3a). From 2013 to 2018 every year had a substantially lower culture positive only *Salmonella* incidence rate compared to the culture positive only average *Salmonella* incidence rate for 2008 – 2010. When compared to the culture and CIDT positive or CIDT positive only average annual *Salmonella* incidence rate for 2011 – 2013, every

year had a substantial increase in culture and CIDT positive or CIDT positive only *Salmonella* incidence rate (Table 3b).

The percentage of *Salmonella* cases with a positive culture has decreased substantially every year after 2013 when compared to the positive culture rate from 2008 – 2010. In 2018 the positive culture rate was 87.09 positive cultures per 100 *Salmonella* cases; a 12.91 percent decrease (95%CI: -16.74 to -8.9) compared to the positive culture rate before the introduction of CIDTs in 2008 – 2010 (Table 4). The positive CIDT rate for *Salmonella* has increased substantially every year since 2014 when compared to the average positive CIDT rate of 2011 – 2013. 2018 had the highest positive CIDT rate of 49.07 positive CIDTs per 100 *Salmonella* cases; a 1,733 percent increase (95%CI: 1480 to 2024) compared to the average positive CIDT rate of 2011 – 2013 (Table 5).

CIDT use for detecting *Salmonella* in clinical samples varies among different age categories. From 2011 to 2013 the odds for those less than 1 year old and 1 to 5 years old of having a CIDT used to detect *Salmonella* in their clinical sample were 5.0 and 5.2 respectively, while the odds for those 20 to 59 years old and 60 years or older of having a CIDT used to detect *Salmonella* in their clinical sample were 0.59 and 0.49 respectively. In 2018, those 20 to 59 years old or 60 years or older were 51.57 and 69.32 times more likely of having a CIDT used to detect *Salmonella* in their clinical sample when compared to their odds from 2011 – 2013. While those less than 1 year old or 1 to 5 years old were 10.41 and 9.10 times more likely of having a CIDT used to detect *Salmonella* in their clinical sample when compared to their odds from 2011 – 2013 (Table 6). The odds of having a CIDT used to detect for *Salmonella* in a clinical sample did not significantly vary among those of different races nor did it significantly vary among those of different ethnicities.

DISCUSSION

In Georgia, the number of reported CIDT positive *Salmonella* cases has increased almost every year since their introduction. Increases in reported CIDTs were initially subtle from 2011 to 2013 but increased drastically beginning in 2014 as more clinical laboratories adopted CIDTs. Overall, the increased use of CIDTs in *Salmonella* detection in clinical samples does not appear to have influenced the total incidence rate for *Salmonella* in Georgia. This result echoes what earlier FoodNet studies have shown that CIDTs have not had an effect on *Salmonella* incidence rates at a national level. CIDTs have still had an impact on *Salmonella* in Georgia.

The number of cultures used to detect *Salmonella* in clinical samples has decreased substantially since 2014. The decrease in positive culture in diagnosing *Salmonella* may continue as the trend shown in table 4 shows positive culture rate decreasing consistently throughout the study period while the trend in table 5 shows the meteoric increase in CIDT use. CIDT use could be considered one of the reasons why positive culture rate has decreased, because ever since the introduction of CIDTs positive culture rate has decreased. This is important as a loss of cultures results in a loss of isolates. CIDTs do not provide clinical laboratories with bacterial isolates, which are crucial for *Salmonella* surveillance in the state of Georgia. Bacterial isolates are used in variety of ways in *Salmonella* surveillance, such as monitoring trends in *Salmonella* subtypes, detecting outbreaks throughout Georgia, identifying vehicles in outbreaks and testing antimicrobial susceptibility (8, 22, 27). The decreasing number of cultures and the resulting loss of isolates will put additional strain on the Georgia Public Health Laboratory to culture clinical samples that were tested with a CIDT at clinical laboratories and not cultured by the clinical laboratory. The GPHL may have to increase their current testing capabilities, selectively choose

which specimens to culture or stress the importance of reflex culturing to clinical laboratories throughout the state in order to deal with the increased testing load.

In the following years it should be expected that the positive CIDT rate for *Salmonella* will only continue to increase in Georgia. There are multiple factors that will be involved in the increasing proportion of *Salmonella* cases reported as CIDT positive in the following years. CIDTs are easier to prepare, run and reduce overall healthcare costs per patient compared to cultures (15). *Salmonella* alone is responsible for an estimated 365 million dollars in direct medical costs annually (9). Clinicians wanting to improve clinical management of patients is another factor as the decreased test run time will allow them to diagnosis and treat patients faster. The availability of CIDTs is an additional factor. More types of CIDTs are becoming available for use and more clinical laboratories are adopting CIDTs in their routine testing procedures. Relative to culturing, CIDTs have a higher positive and negative predicative value which is highly valued to clinicians as accurate results are critical for correctly diagnosis *Salmonella* in patients (18). Positive CIDT rates are continuing to rise across all age groups; especially among those who are older than five years (Table 6). Initially, CIDT use for *Salmonella* diagnosis was highest amongst those younger than five years of age, but the margin between the age categories has shrunk. This is shown in the data as CIDTs were introduced in 2011 and for the following years 74.88 percent of all the reported cases with a positive CIDT were amongst those younger than five years of age (n = 155). When this is compared to 2018, only 45.32 percent of all reported cases with a positive CIDT were amongst those younger than five years of age (n = 528).

In conclusion, this study was not able to associate CIDT use for *Salmonella* detection in clinical samples with an increase or decrease in *Salmonella* incidence. The increased use of

CIDT in *Salmonella* surveillance will have public health implications and will create challenges for public health surveillance epidemiologists. The simplicity of conducting CIDTs, CIDTs improvement of clinical management of patients and the potential reduction of overall health care costs will be drivers in the continued use of CIDTs in Georgia. Overall, increasing CIDT use affects how surveillance epidemiologists interpret national and local *Salmonella* surveillance data and establish historic trends.

Strengths and Limitations

This study has several strengths that set it apart from other studies currently published in the field. The first strength being that our data includes a large sample size of almost all *Salmonella* cases reported from 2008 to 2018. Many studies conducted on the effect of CIDTs on foodborne disease surveillance are limited to four years or less. It is difficult to prove if a true difference between trends exist with a study period less than four years. Other studies also have not compared current incidence rates to incidence rates before CIDTs existed. This difference allowed us to determine if the incidence rates truly changed once CIDTs were introduced or if it was just due to variance in *Salmonella* cases occurring in any given year. Another strength is that other studies did not look at the effect of CIDTs across different demographic groups. This study looked at the initial effect CIDTs had on *Salmonella* surveillance amongst differing age groups, races and ethnicities. Any differences among demographic groups would be valuable information to know so that those effects could be studied further.

This study is subject to several limitations. The first limitation being that not all *Salmonella* cases were included in the data from 2008 to 2018. The *Salmonella* subtypes of *Typhi* and *Paratyphi* were not included in the analysis. The inclusion of these two subtypes may have changed the results that the data produced. Another limitation is that within the 11 years

included in this dataset public health surveillance, population characteristics, access to health services, and health behaviors might have changed. Any of those changes may have affected the incidence of *Salmonella* for that given year and the years to come afterwards. The last limitation is that all CIDTs used in this study were not introduced at the same time nor in an evenly distributed manner. Each CIDT used in this study was introduced in the state of Georgia at different times and all have different test specifics such as sensitivity and specificity. The incidence rate observed may be different if CIDTs were introduced at a similar time and had similar test specifics.

Future Directions

Future studies will need to be conducted to monitor CIDT use in *Salmonella* surveillance and other foodborne disease surveillance as CIDTs are still a relatively novel testing type. If CIDTs are determined to be affecting *Salmonella* incidence rates or any another foodborne illness incidence rates, additional tools will need to be developed in order to properly manage and evaluate trends in foodborne illnesses (27). This will be of critical importance as foodborne illnesses still pose a major public health concern in the United States. Foodborne illnesses including *Salmonella* are preventable through good surveillance and public health practices. The state of Georgia, in collaboration with FoodNet, will continue to adapt to future developments in diagnostic testing practices used for *Salmonella* and other foodborne illnesses in order to conduct thorough surveillance and contribute to national data that can inform policy makers.

REFERENCES

1. Huang, R. S., Johnson, C. L., Pritchard, L., Hepler, R., Ton, T. T., & Dunn, J. J. (2016). Performance of the Verigene(R) enteric pathogens test, Biofire FilmArray gastrointestinal panel and Luminex xTAG(R) gastrointestinal pathogen panel for detection of common enteric pathogens. *Diagn Microbiol Infect Dis, 86*(4), 336-339. doi:10.1016/j.diagmicrobio.2016.09.013
2. Buss, S. N., Leber, A., Chapin, K., Fey, P. D., Bankowski, M. J., Jones, M. K., . . . Bourzac, K. M. (2015). Multicenter evaluation of the BioFire FilmArray gastrointestinal panel for etiologic diagnosis of infectious gastroenteritis. *J Clin Microbiol, 53*(3), 915-925. doi:10.1128/JCM.02674-14
3. Khare, R., Espy, M. J., Cebelinski, E., Boxrud, D., Sloan, L. M., Cunningham, S. A., . . . Binnicker, M. J. (2014). Comparative evaluation of two commercial multiplex panels for detection of gastrointestinal pathogens by use of clinical stool specimens. *J Clin Microbiol, 52*(10), 3667-3673. doi:10.1128/JCM.01637-14
4. CDC. (2015). Foodborne Diseases Active Surveillance Network (FoodNet). https://www.cdc.gov/foodnet/index.html
5. Gilliss, D. C., Alicia; Cartter Matthew. (2011). Vital signs: incidence and trends of infection with pathogens transmitted commonly through food--foodborne diseases active surveillance network, 10 U.S. sites, 1996-2010. *MMWR Morb Mortal Wkly Rep, 60*(22), 749-755.
6. Iwamoto, M., Huang, J. Y., Cronquist, A. B., Medus, C., Hurd, S., Zansky, S., . . . Prevention. (2015). Bacterial enteric infections detected by culture-independent diagnostic tests--FoodNet, United States, 2012-2014. *MMWR Morb Mortal Wkly Rep, 64*(9), 252-257.

7. Huang, J. Y., Henao, O. L., Griffin, P. M., Vugia, D. J., Cronquist, A. B., Hurd, S., . . . Patrick, M. E. (2016). Infection with Pathogens Transmitted Commonly Through Food and the Effect of Increasing Use of Culture-Independent Diagnostic Tests on Surveillance--Foodborne Diseases Active Surveillance Network, 10 U.S. Sites, 2012-2015. *MMWR Morb Mortal Wkly Rep, 65*(14), 368-371. doi:10.15585/mmwr.mm6514a2
8. Marder, E. P., Cieslak, P. R., Cronquist, A. B., Dunn, J., Lathrop, S., Rabatsky-Ehr, T., . . . Geissler, A. L. (2017). Incidence and Trends of Infections with Pathogens Transmitted Commonly Through Food and the Effect of Increasing Use of Culture-Independent Diagnostic Tests on Surveillance - Foodborne Diseases Active Surveillance Network, 10 U.S. Sites, 2013-2016. *MMWR Morb Mortal Wkly Rep, 66*(15), 397-403. doi:10.15585/mmwr.mm6615a1
9. CDC. (2013). Infection with Salmonella. https://www.cdc.gov/training/SIC_CaseStudy/ Infection _ Salmonella _ptversion.pdf
10. WHO. (2015). WHO Estimates of Global Burden of Foodborne Illnesses. https://apps.who.int/iris/bitstream/handle/10665/199350/9789241565165_eng.pdf?sequence=1
11. CDC. (2013). An Atlas of Salmonella in the United States, 1968 - 2011. https://www.cdc.gov/ salmonella/pdf/salmonella-atlas-508c.pdf
12. CDC. (2017). Salmonella | 2017 Case Definition. https://wwwn.cdc.gov/nndss/conditions/Salmonella/case-definition/2017/

13. Buchan, B. W., Olson, W. J., Pezewski, M., Marcon, M. J., Novicki, T., Uphoff, T. S., . . . Ledeboer, N. A. (2013). Clinical evaluation of a real-time PCR assay for identification of Salmonella, Shigella, Campylobacter (Campylobacter jejuni and C. coli), and shiga toxin-producing Escherichia coli isolates in stool specimens. *J Clin Microbiol, 51*(12), 4001-4007. doi:10.1128/JCM.02056-13

14. Koplan, J. P., Fineberg, H. V., Ferraro, M. J., & Rosenberg, M. L. (1980). Value of stool cultures. *Lancet, 2*(8191), 413-416.

15. System, G. H. (2019). Stool Culturing Procedure for Salmonella https://www.gundersen health.org / app/files/public/6765/Lab-Policies-Culture-Routine-Stool-Lab-3105.pdf

16. Beal, S. G., Tremblay, E. E., Toffel, S., Velez, L., & Rand, K. H. (2018). A Gastrointestinal PCR Panel Improves Clinical Management and Lowers Health Care Costs. *J Clin Microbiol, 56*(1). doi:10.1128/JCM.01457-17

17. BioFire. (2015). FilmArray™ Gastrointestinal Panel. https://www.biofiredx.com/wp-content/uploads/2016/03/IS-MRKT-PRT-0234-07-FilmArray-Gastrointestinal-Panel-Information-Sheet.pdf

18. Besser, J. M. (2018). Salmonella epidemiology: A whirlwind of change. *Food Microbiol, 71*, 55-59. doi:10.1016/j.fm.2017.08.018

19. Cronquist, A. B., Mody, R. K., Atkinson, R., Besser, J., Tobin D'Angelo, M., Hurd, S., . . . Mahon, B. E. (2012). Impacts of culture-independent diagnostic practices on public health surveillance for bacterial enteric pathogens. *Clin Infect Dis, 54 Suppl 5*, S432-439. doi:10.1093/cid/cis267

20. McAdam, A. J. (2017). Unforeseen Consequences: Culture-Independent Diagnostic Tests and Epidemiologic Tracking of Foodborne Pathogens. *J Clin Microbiol, 55*(7), 1978-1979. doi:10.1128/JCM.00678-17
21. Langley, G., Besser, J., Iwamoto, M., Lessa, F. C., Cronquist, A., Skoff, T. H., . . . Harrison, L. H. (2015). Effect of Culture-Independent Diagnostic Tests on Future Emerging Infections Program Surveillance. *Emerg Infect Dis, 21*(9), 1582-1588. doi:10.3201/eid2109.150570
22. Imdad, A., Retzer, F., Thomas, L. S., McMillian, M., Garman, K., Rebeiro, P. F., . . . Woron, A. M. (2018). Impact of Culture-Independent Diagnostic Testing on Recovery of Enteric Bacterial Infections. *Clin Infect Dis, 66*(12), 1892-1898. doi:10.1093/cid/cix1128
23. Henao, O. L., Scallan, E., Mahon, B., & Hoekstra, R. M. (2010). Methods for monitoring trends in the incidence of foodborne diseases: Foodborne Diseases Active Surveillance Network 1996-2008. *Foodborne Pathog Dis, 7*(11), 1421-1426. doi:10.1089/fpd.2010.0629
24. Berenger, B., Chui, L., Reimer, A. R., Allen, V., Alexander, D., Domingo, M. C. . . . Canadian Public Health Laboratory, N. (2017). Canadian Public Health Laboratory Network position statement: Non-culture based diagnostics for gastroenteritis and implications for public health investigations. *Can Commun Dis Rep, 43*(12), 279-281.
25. Fitzgerald, C., Patrick, M., Gonzalez, A., Akin, J., Polage, C. R., Wymore, K., . . . Nachamkin, I. (2016). Multicenter Evaluation of Clinical Diagnostic Methods for Detection and Isolation of Campylobacter spp. from Stool. *J Clin Microbiol, 54*(5), 1209-1215. doi:10.1128/JCM.01925-15

26. Shea, S., Kubota, K. A., Maguire, H., Gladbach, S., Woron, A., Atkinson-Dunn, R., . . . Miller, M. B. (2017). Clinical Microbiology Laboratories' Adoption of Culture-Independent Diagnostic Tests Is a Threat to Foodborne-Disease Surveillance in the United States. *J Clin Microbiol, 55*(1), 10-19. doi:10.1128/JCM.01624-16

27. Gu, W., Dutta, V., Patrick, M., Bruce, B. B., Geissler, A., Huang, J., . . . Henao, O. (2018). Statistical adjustment of culture-independent diagnostic tests for trend analysis in the Foodborne Diseases Active Surveillance Network (FoodNet), USA. *Int J Epidemiol, 47*(5), 1613-1622. doi:10.1093/ije/dyy041

TABLES

Table 1. **Characteristics of a cohort[a] of Georgia *Salmonella* cases according to test type, 2008 to 2018[b]**

	Culture Positive Only (N = 24,704)	Culture and CIDT Positive[†] (N = 1,564)	CIDT Positive Only (N = 1,521)
	No. (%)	No. (%)	No. (%)
Gender			
Male	12,179 (49.5)	784 (50.39)	798 (52.47)
Female	12,426 (50.5)	772 (49.61)	714 (46.94)
Age			
Less than 1	4,030 (16.32)	332 (21.24)	493 (32.41)
1 to 5	5,019 (20.32)	352 (22.52	535 (35.17)
5 to 9	2,059 (8.34)	120 (7.68)	130 (8.55)
10 to 19	1,795 (7.27)	124 (7.93)	119 (7.82)
20 to 59	7,479 (30.28)	378 (24.18)	156 (10.26)
60 or Older	4,319 (17.49)	257 (16.44)	88 (5.79)
Race			
White	15,812 (70.96)	1,034 (69.63)	864 (69.31)
Black or African American	4,993 (22.41)	351 (23.64)	284 (22.07)
American Indian/Alaskan Native	40 (0.18)	6 (0.40)	0
Asian	377 (1.69)	32 (2.15)	34 (2.68)
Hawaiian/Pacific Islander	17 (0.08)	3 (0.20)	1 (0.08)
Multiracial	272 (1.22)	28 (1.89)	19 (1.51)
Other	771 (3.46)	31 (2.10)	55 (4.35)
Ethnicity			
Not Hispanic	17,239 (91.74)	1,305 (93.08)	1,156 (91.30)
Hispanic	1,553 (8.26)	97 (6.92)	108 (8.70)
Hospitalized			
Yes	7,121 (29.57)	608 (39.74)	110 (5.94)
No	16,962 (70.43)	922 (60.26)	1,315 (94.06)
Patient Outcome			
Dead	153 (0.66)	7 (0.46)	7 (0.50)
Alive	23,196 (99.34)	1,521 (99.54)	1,467 (99.50)

Abbreviations: CIDT = culture-independent diagnostic test

[a]Based on Georgia's State Electronic Notifiable Disease Surveillance System (SendSS).

[b]2018 is based on Preliminary Data

[†]CIDT positive is defined as detection of *Salmonella* in a clinical sample using a CIDT

Table 2. **Incidence rates[*] for *Salmonella* in Georgia, 2008 to 2018[a]**

	Confirmed Cases[§]		Probable Cases[†]		Total Cases	
Year	Cases	Incidence	Cases	Incidence	Cases	Incidence
2008	2,298	24.18			2,298	24.18
2009	2,364	24.57	NA		2,364	24.57
2010	2,794	28.77			2,794	28.77
2011	2,623	26.72	27	0.28	2,650	27.00
2012	2,651	26.72	82	0.83	2,733	27.55
2013	2,289	22.91	58	0.58	2,347	23.49
2014	2,242	22.20	176	1.74	2,418	23.95
2015	2,146	21.01	230	2.25	2,376	23.26
2016	2,256	21.88	287	2.78	2,543	24.66
2017	2,061	19.76	284	2.72	2,345	22.48
2018[a]	2,544	24.18	377	3.58	2,921	27.77

[*]per 100,000 population

[a]2018 is based on Preliminary Data

[§]Confirmed cases include all cases with a positive culture result and any case with a positive CIDT result that was confirmed by culture

[†]Probable cases include all cases with a positive CIDT result that was not confirmed by culture

Table 3a. **Percent change in culture only incidence rates for Georgia *Salmonella* cases from 2011 - 2018[a] compared to 2008 - 2010 average annual incidence rate**

	Culture Positive Only[§]		
Year	**IR[*]**	**% Change**	**95% CI**
2008 - 2010	25.85	Ref	
2011	26.68	3.20	-1.29 to 7.9
2012	26.54	2.66	-1.80 to 7.33
2013	22.73	-12.09	-16.13 to -7.87
2014	21.91	-15.27	-19.20 to -11.15
2015	20.26	-21.62	-25.35 to -17.71
2016	19.82	-23.32	-26.98 to -19.48
2017	15.76	-39.03	-42.20 to -35.69
2018 [a]	16.68	-35.47	-38.74 to -32.03

[*]per 100,000 population

[a]2018 is based on Preliminary Data

[§]Culture positive only is defined as detection of *Salmonella* in a clinical sample using culture only

Table 3b. **Percent change in culture and CIDT positive or CIDT positive only incidence rates for Georgia *Salmonella* from cases 2011 - 2018[a] compared to 2011 - 2013 average annual incidence rate**

	Culture and CIDT Positive[†] or CIDT Positive only		
Year	**IR[*]**	**% Change**	**95% CI**
2011 - 2013	0.70	Ref	
2014	2.10	201	149 to 265
2015	3.08	343	271 to 428
2016	4.99	617	510 to 743
2017	6.94	897	754 to 1064
2018 [a]	11.41	1539	1314 to 1800

Abbreviations: CIDT = culture-independent diagnostic test

[*]per 100,000 population

[a]2018 is based on Preliminary Data

[†]CIDT positive is defined as detection of *Salmonella* in a clinical sample using a CIDT

Table 4. **Percent change in positive culture rates for *Salmonella* cases from 2011 - 2018[a] compared to 2008 - 2010 average annual positive culture rate**

	Culture Positive[§]		
Year	**Positive Culture Rate[*]**	**% Change**	**95% CI**
2008 - 2010	100.00	Ref	
2011	98.98	-1.02	-5.33 to 3.48
2012	97.00	-3.00	-7.21 to 1.40
2013	97.53	-2.47	-6.93 to 2.21
2014	92.72	-7.28	-11.55 to -2.80
2015	90.32	-9.68	-13.91 to -5.24
2016	88.71	-11.29	-15.37 to -7.01
2017	87.89	-12.11	-16.29 to -7.72
2018[a]	87.09	-12.91	-16.74 to -8.90

[*]per 100 *Salmonella* cases

[a]2018 is based on Preliminary Data

[§]Culture positive is defined as detection of *Salmonella* in a clinical sample using culture includes *Salmonella* cases also identified by CIDT

Table 5. **Percent change in positive CIDT rates for *Salmonella* cases from 2014 - 2018[a] compared to 2011 - 2013 average annual positive CIDT rate**

	CIDT Positive[†]		
Year	**Positive CIDT Rate[*]**	**% Change**	**95% CI**
2011 - 2013	2.68	Ref	
2014	7.77	190.00	139 to 252
2015	11.20	318.00	250 to 398
2016	21.26	694.00	575 to 833
2017	28.99	983.00	827 to 1164
2018 [a]	49.07	1733.00	1480 to 2024

Abbreviations: CIDT = culture-independent diagnostic test

[*]per 100 *Salmonella* cases

[a]2018 is based on Preliminary Data

[†]CIDT positive is defined as detection of *Salmonella* in a clinical sample using a CIDT includes *Salmonella* cases also identified by culture

Table 6. **Odds ratios for CIDT use in *Salmonella* detection by age from 2014 to 2018 [a] compared to 2011 - 2013**

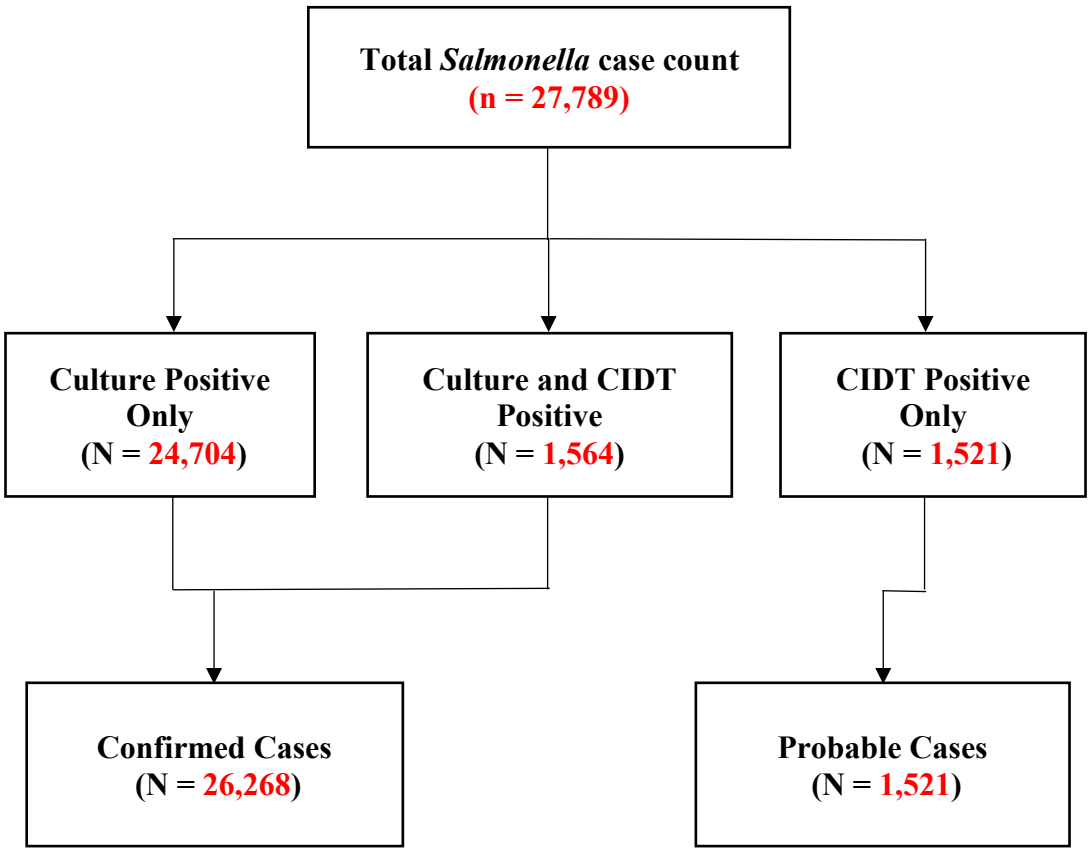
	Age Category					
	Less than 1 yr	**1 to 5 yr**	**5 to 9 yr**	**10 to 19 yr**	**20 to 59 yr**	**60 or Older yr**
Year	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**
2014	3.27 (2.33, 4.57)	2.75 (2.01, 3.75)	3.63 (2.04, 6.47)	7.03 (3.49, 14.16)	3.62 (1.72, 7.62)	NA
2015	5.08 (3.76, 6.86)	4.06 (3.04, 5.41)	3.40 (1.83, 6.34)	7.33 (3.59, 14.96)	9.04 (4.81, 16.97)	5.57 (2.06, 15.07)
2016	6.56 (4.91, 8.76)	6.03 (4.66, 7.81)	8.04 (4.82, 13.42)	13.13 (6.77, 25.46)	14.29 (7.87, 25.94)	14.64 (6.14, 34.95)
2017	9.43 (7.11, 12.53)	7.39 (5.74, 9.52)	12.87 (7.88, 21.02)	17.82 (9.34, 34.01)	34.40 (19.50, 60.69)	35.55 (15.53, 81.35)
2018 [a]	10.41 (7.93, 13.66)	9.10 (7.17, 11.55)	13.84 (8.65, 22.12)	22.80 (12.15, 42.77)	51.57 (29.55, 90.01)	69.32 (30.79, 156.06)

Abbreviations: CIDT = culture-independent diagnostic test

[a]2018 is based on Preliminary Data

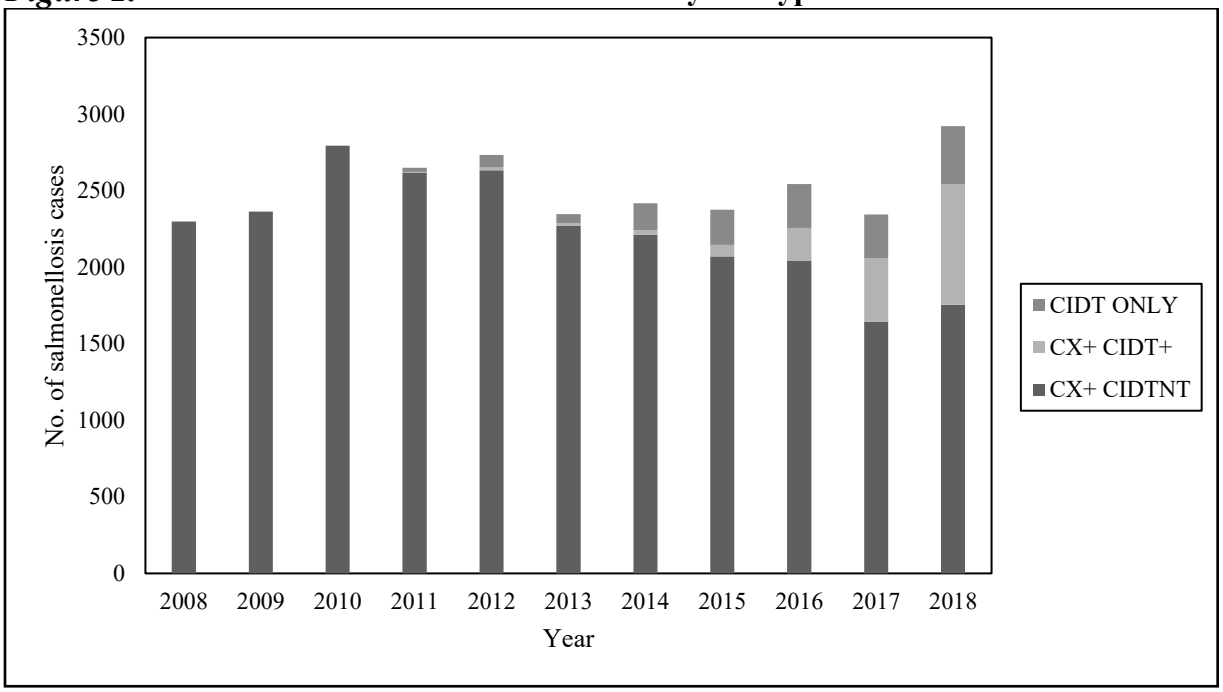
FIGURES

***Figure 1*. Flowchart demonstrating the distribution of *Salmonella* cases from 2008 to 2018[a]**



Abbreviations: CIDT = culture-independent diagnostic test
[a]2018 is based on Preliminary Data

Figure 2. **Number of *Salmonella* cases identified by test type from 2008 – 2018** [a]



Abbreviations: CIDT = culture-independent diagnostic test

[a]2018 is based on Preliminary Data