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SOCIAL NETWORK ANALYSIS (SNA) OF CLARK COUNTY, NEVADA

TUBERCULOSIS (TB) CASE AND CONTACT INVESTIGATION

DATA TO DETERMINE PEDIATRIC RISK FACTORS

FOR DISEASE TRANSMISSION

By

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> A dissertation submitted in partial fulfillment of the requirements for the

Doctor of Philosophy - Public Health

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> University of Nevada, Las Vegas December 2014



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Social Network Analysis (SNA) of Clark County, Nevada Tuberculosis (TB) Case and Contact Investigation Data to Determine Pediatric Risk Factors for Disease Transmission

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December 2014

ABSTRACT

Social Network Analysis (SNA) of Clark County, Nevada Tuberculosis (TB) Case and Contact Investigation Data to Determine Pediatric Risk Factors for Disease Transmission

by

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Within the spectrum of childhood (infancy, early childhood, middle childhood, and adolescence), TB is a priority disease, and preventive care is recommended by the American Academy of Pediatrics (AAP)/Bright Futures initiative (AAP, 2008). Within the early childhood stage (15 months to 4 years), TB is classified as "HR2" (high risk 2), behind lead poisoning, which is "HR1" (AAP, 2008), the highest risk within this age range. Within the middle childhood stage (5 to 10 years), TB becomes an HR1. TB is preventable, and targeted screening is the best prevention method. The AAP/Bright Futures initiative further specifies the risk based on pediatric contacts, such as household members/close contacts.

From 2008 to 2012, Nevada led the nation in pediatric TB with 5.7 cases per 100,000 in the 1 to 4 year-old age range (OTIS, 2014). In 2010, the Nevada State Health Division recognized the following pediatric TB risk factors (Paulson, 2010): many of these cases are children of young mothers, or are young mothers themselves; individuals

who have spent time in jails, detention centers and prisons have been identified as contacts to these pediatric TB cases; most of the cases (especially less than 5 years of age) had recent interactions with healthcare providers prior to being diagnosed with TB; and country of birth of pediatric contacts is a risk factor.

The objective of this study was to create a social network model (Hanneman, 2005) and perform associated social network analysis to evaluate tuberculosis case and contact investigation data in Clark County, Nevada. The social network model was then used to assess pediatric disease transmission based on network metrics and individual risk factors. Social network analysis was used to assess pediatric TB transmission based on links between pediatric cases and contacts in Clark County, Nevada for the years 2010, 2011, and 2012. Network metrics were used to establish locational properties of cases and contacts, and through incorporation of individual risk factors disease transmission potential was established. Whole-network, group network and individual network metrics and risk factors provided areas of focus for prevention, treatment, prophylaxis, control, and case management of pediatric TB cases.

The Wilcoxon signed-rank test, Kruskal-Wallis test, logistic regression, and logistic regression with bootstrapping were used to calculate the significance of the risk factors and network metrics. The TB network, as a whole, was stable and relatively static from 2010 to 2012 based on density and clustering coefficient; however, at the individual and group levels there were focal areas that were more dynamic. The risk factors identified by the Nevada State Health Division varied in terms of significance, with significance demonstrated only by logistic regression and logistic regression with bootstrapping. Although social network analysis has limitations it can be useful as a complementary method for on-going surveillance of pediatric TB that can improve health outcomes with targeted and cost-effective interventions, and can influence public health policy. Some advantages of TB infectious disease modeling are: better resource allocation, improved contact investigation efficiency, prioritized treatment, education, and improved Directly Observed Treatment Short-Course (DOTS) therapy.

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CHAPTER 1 - INTRODUCTION

Tuberculosis (TB) is a disease that changed our world (Sherman, 2007) and can be broadly defined as a "social disease with medical implications" (Mandalakas, 2005). The study of TB transmission must include a social aspect where individual interactions, group interactions and network theory provide the best representation of disease transmission within a community. The Centers for Disease Control and Prevention (CDC) is the agency that has authority over the national TB program and has recognized the importance of social networks in disease transmission by including social network methodology into guidelines for infectious disease case and contact investigations, specifically relating to TB. The CDC has defined the following social network terms relating specifically to TB (CDC, 2005, 2006):

Social network – linkage of persons or places where TB is spread through shared air space and common ties (e.g., social) amongst the persons and settings involved.

Social network analysis – investigation of routinely collected interview data to find common links amongst cases and infected persons; these links may be persons or places.

Figure 1 shows the structural components of a TB network. Although the aforementioned definitions classify a TB network as a social network, it is more accurately referred to as a transmission network (Luke, 2007) because TB is an infectious disease. Several CDC studies specifically involving TB refer to "transmission network analysis" (Cook, 2007; McKenzie, 2007). For the purposes of this research, social network, transmission network, and network are synonymous and refer to the Clark County, Nevada TB

networks in 2010, 2011, and 2012.



Figure 1. Structural Components of a Tuberculosis Network Source: adapted from Hanneman, 2005; Borgatti, 2013; Valente, 2010

PURPOSE OF THE STUDY

The primary purpose of this study is to use a social network model (Hanneman, 2005) and associated social network analysis to evaluate tuberculosis case and contact investigation data in Clark County, Nevada. The model will act as a descriptive model to show the current burden of pediatric TB in Clark County by highlighting individual case and contact risk factors. Because a TB network is highly dynamic and heterogeneous, the model will also be used as an analytical tool to calculate individual, group and whole-network metrics, which can be statistically analyzed to determine disease transmission risks. The following network metrics will be calculated for the related research questions: betweenness (research question 2), clustering coefficient (research question 3A) and density (research question 3B). While a primary advantage of this model is on-going surveillance of pediatric TB in Clark County, the ultimate goal is for

the model to act as a public health management tool to identify and assess the pediatric subgroup of the network and apply focused preventive measures, such as targeted screenings, intervention, expedient treatment, public education, case management, and Directly Observed Therapy, Short-Course (DOTS). Focused preventive measures are the most cost-effective and crucial for the pediatric population as it is a highly vulnerable group. For example, isolating a pediatric subgroup can provide more expedient treatment to prevent progression from latent TB to active TB in hopes of lowering the pediatric case rate by preventing disease transmission within the network. It is expected that theoretical concepts of social epidemiology can provide insight into the evolution of a pediatric TB network in light of research challenges associated with TB genotyping and epidemiological links. Finally, an evidence-based, empirical and real-world model can help implement health policy change at the state and local level where it is needed the most.

SIGNIFICANCE OF THE STUDY

It is universally accepted within the field of public health and medicine that the pediatric population represents a highly vulnerable subgroup where delays in diagnosis and treatment can be life-threatening. This is perfectly exemplified by TB, where rapid progression can occur from initial infection to the two most fatal forms of childhood TB: miliary (systemic) and meningeal (Marais, 2011). Within the spectrum of childhood (infancy, early childhood, middle childhood, and adolescence), TB is a priority disease, and preventive care is recommended by the American Academy of Pediatrics(AAP)/Bright Futures initiative (AAP, 2008). The Tuberculin Skin Test

(TST) is the recommended screening method during all stages of childhood with appropriate follow-up care as necessary, and using Social Network Analysis (SNA) can complement this method by focusing screenings cost-effectively within the highest risk groups. Within the early childhood stage (15 months to 4 years), TB is classified as "HR2" (high risk 2), behind lead poisoning, which is "HR1" (AAP, 2008), the highest risk within this age range. Within the middle childhood stage (5 to 10 years), TB becomes an HR1. TB (and lead poisoning) is preventable, and targeted screening is the best prevention method. The AAP/Bright Futures initiative further specifies the risk based on pediatric contacts: household members/close contacts, recent immigrants with high TB rates, migrant workers, residents of homeless shelters, and persons with underlying medical conditions where these risks are of special importance (AAP, 2008). This research is consistent with the mission of the Institute for Quantitative Health Sciences as part of the University of Nevada, Las Vegas (UNLV) Knowledge Fund Proposal which states:

"The mission of the Institute is to support the health and medical economy of Southern Nevada by improving health outcomes and providing quantitative analysis that enhances medical decision-making and education programs" (UNLV, 2013).

Network analysis is a novel quantitative analytical method intended to improve health outcomes of pediatric TB by enhancing medical-decision making. This research also attempts to address the need for epidemiological and statistical applications for understanding trends in hospital and database registries (SNHD TB case and contact database), as well as chronic and infectious disease incidence and prevalence

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management and prevention efforts (UNLV, 2013). Empirical data from network analysis can provide the basis for a screening intervention by focusing on pediatric subgroups. A proposed longitudinal network model can determine yearly trends which can determine the success or failure of an intervention. This research incorporates a longitudinal design by analyzing yearly TB network trends from 2010 to 2012, and can be considered a quantitative method by the use of network metrics. Furthermore, the UNLV Institute for Quantitative Health Sciences has one goal particularly relevant to this research:

"To develop data analytic capabilities for use of health information technology

to improve health services and health outcomes." (UNLX, 2013).

Network analysis can provide the overall benefit of an on-going disease surveillance system that can improve health outcomes with targeted and cost-effective interventions, and can influence public health policy. Some advantages of TB infectious disease modeling are: better resource allocation, improved contact investigation efficiency, prioritized treatment, education, and improved Directly Observed Treatment Short-Course (DOTS) therapy.

PUBLIC HEALTH PROBLEM

GLOBAL PEDIATRIC TB

The epidemiology of TB requires special consideration towards the pediatric population, where CDC classifies a pediatric case of TB as a child less than 15 years of age (CDC, 2014). Probably the two biggest TB risk factors are age and host immunity, and it is widely accepted that the within these two categories, children under 5 years of age and persons with HIV/AIDS represent the populations with the greatest risk of

developing active TB disease once becoming infected (Schaaf, 2009; Marais, 2011). The simple explanation is these two risk factors result in a compromised immune response, therefore a pediatric case of TB is a sentinel event; it represents a recent transmission (Blake, 2013). Globally, pediatric TB is not evenly distributed, developed countries, such as the U.S., have much fewer cases of pediatric TB. Children less than 15 years of age make up about 40-50% of the entire population in developing countries, whereas in developed countries this percentage is about 5, and young adults (having the highest prevalence) in developing countries are exposing a larger pool of children, so as the prevalence increases in young adults, the pediatric cases increase exponentially (Schaaf, 2009). With a much smaller percentage of pediatric population, childhood cases are much less in developed countries. Disease prevalence surveys are important for understanding the burden of disease; however, they have limitations, especially when studying the epidemiology of pediatric TB (Schaaf, 2009):

- TB is rare in the U.S.; it must be studied in relation to a whole country to provide enough statistical power. It is difficult to study pediatric TB because there is additional stratification which lowers the 'n';
- Study tools are limited in children: questionnaires, chest x-ray (CXR), bacteriological analysis
- 3) Rapid progression of TB occurs in children, thus the need for early identification and treatment;
- 4) Most TB programs focus on targeting adult cases, however, it may be advantageous to also focus on children themselves (Heymann, 2000);

5) Current contact investigation procedures might be missing cases (Heyman, 2000). The World Health Organization (WHO) estimated that there were 530,000 new global pediatric TB cases (range 510,000 – 550, 000) in 2013 with 74,000 deaths (HIV-negative children), with pediatric cases comprising about 6% of the total new cases of TB (WHO, 2013). This does not include HIV-positive children; therefore, these TB rates are drastically underestimated. The more urgent issue is the disparity of pediatric TB cases among various countries where pediatric cases encompass a wide range of the total cases. Ranges of pediatric TB case percentages of total TB cases vary from Thailand at 3% and the United States at 6% to Afghanistan, Brazil, and Pakistan with greater than 20% (Nelson, 2004). Furthermore, WHO data are further severely underestimated because only smear-positive cases are reported. WHO provides reasonable justification for only reporting smear-positive cases because this method is affordable, provides a fair degree of specificity, and smear-positive cases represent the most infectious cases. In some developing countries, sputum smear testing may be the only method because it only requires a microscope, slides, stains and the ability to identify the organism. A sputum smear is a microbiological method that involves placing a small amount of sputum on a microscope slide and spreading it into a thin layer using a cover slip. Appropriate stains are then added to easily identify the organism, Mycobacterium tuberculosis. This represents the most advanced diagnostic technique for some developing countries. The severe underestimation of pediatric TB cases results from the fact that 95% of children less than 12 years of age are smear-negative, and children less than 5 years of age are usually not smear-positive. (WHO, 2013). WHO estimates that for every smear-positive case there is a smear-negative case which could be a false negative (WHO, 2013), thus

for every smear-positive case that is detected there is one that is being missed.

A major question often posed in public health is: has the case rate actually increased or has the disease simply been underreported from previous years? Or, in the case of (pediatric) TB, does a large disparity of cases actually exist among the different countries, or do differences in surveillance methods account for much of the difference? With pediatric TB especially, there is actually a disparity, however, surveillance methods most likely overestimate the difference. The case definition of TB is not globally consistent (WHO, 2013; Nelson, 2004) which can be a cause of the disparity. Other causes of global pediatric TB disparity are lack of a definitive diagnosis, missing data, and lack of age stratification (WHO, 2013; Nelson, 2004). Clearly a future research challenge for pediatric TB involves globally consistent case surveillance and reporting. Alternatively, the disparity of pediatric TB may actually exist between countries because many countries do not conduct contact investigations which are crucial for preventing the progression of TB from the latent to active stage (Hsu, 1963; AAP, 2004). The pediatric TB disparity also exists simply because the pediatric population in many countries represents a larger percentage of the total population which provides more viable hosts for the pathogen. Overcrowding, poverty, and malnutrition are other risk factors that play a role in the global disparity of pediatric TB (Nelson, 2004).

PEDIATRIC TB IN THE UNITED STATES

The pediatric TB rate in the United States is one of the lowest in the world, where pediatric TB cases comprise approximately 6% of the total TB cases nationally (OTIS, 2014). This is due, in part, to improved diagnostic methods, case detection, and

treatment; however local health districts must get most of the credit based on rapid case detection resulting from systematic contact investigations. From the 1950s to the present date, pediatric TB cases have steadily declined due mostly to the invention of chemotherapeutic agents, such as rifampin (Marais, 2006, 2011). The exception to this decline occurred from 1984-1992 due to the HIV/AIDS epidemic (CDC, 2014). In the United States, in past years, three states, Texas, California, and New York comprise over 50% of the pediatric TB cases, and from 1993 to 2010 the age group of less than 5 years of age represented the highest risk within the pediatric age range of less than 15 years old. (CDC, 2014). Figure 2 shows pediatric TB case rates from 1993-2010 stratified by age. In this same time period, pulmonary tuberculosis was the most common site of disease comprising over 70% of pediatric TB cases (CDC, 2014). Of the remaining 30%



Figure 2. Pediatric TB Case Rates by Age Groups, 1993-2010. Source: CDC, 2014

involving extrapulmonary TB, the majority were lymphatic in nature (CDC, 2014). A positive aspect of this statistic is that meningeal and miliary TB, the most fatal forms of pediatric TB, only comprised 3.3% and 1.5% of extrapulmonary cases, respectively (CDC, 2014). As low as these percentages seem for meningeal and miliary pediatric TB they can be improved because pediatric TB cases can be located using network analysis before reaching these fatal stages. In 2012, the pediatric age group (less than 15 years old) had the lowest TB case rate among all age groups (0-15, 15-24, 25-44, 45-64, 65+ years old) at 0.8 cases per 100,000 (CDC, 2013a).

PEDIATRIC TB IN NEVADA

In 2010, the Nevada State Health Division recognized significant specific pediatric TB risk factors that presented missed opportunities for prevention, early detection, and timely management of pediatric TB (Paulson, 2010):

- 1) Many of these cases are children of young mothers, or are young mothers themselves.
- Individuals who have spent time in jails, detention centers and prisons have been identified as contacts to these pediatric TB cases.
- 3) Most of the cases (especially less than 5 years of age) had recent interactions with healthcare providers prior to being diagnosed with TB. Most notably, these cases are presenting in emergency departments and urgent care centers with respiratory or unresolved pediatric issues (e.g., ear infections, gastric symptoms, enlarged lymph nodes without an established infection, or are being seen as part of well-baby exams without screening for TB as recommended by the American

Academy of Pediatrics).

4) Country of birth of pediatric contacts is a risk factor.

Due to the rapid progression of TB in children, early identification and treatment are crucial (Nevada State Health Division, 2011), but most TB programs focus on targeting adult cases, and it may be advantageous to also focus on children because current contact investigation procedures might be missing cases (Heyman, 2000).

According to the CDC Online Tuberculosis Information System (OTIS), from 2008 to 2012 Nevada led the nation in pediatric TB rate for the stratified age range of 1-4 years of age, with a rate of 5.70 cases per 100,000 (OTIS, 2014). This case rate was also the highest rate among all pediatric age ranges (less than 1 year old, 1-4 years old, and 5- 14 years old) as stratified by CDC. From 2006 to 2010, the Nevada pediatric TB case rate was 5.17 cases per 100,000, which was second to Alaska in the 1-4 year old age range and third among all pediatric age ranges (OTIS, 2014). Thus, the pediatric TB case rate has increased from the period of 2006-2010 to 2008-2012 from 5.17 cases to 5.70 cases per 100,000 (OTIS, 2014). Another disconcerting trend is the 5-14 year old age range where Nevada ranked eighteenth with 0.84 cases per 100,000 from 2006 to 2010, but then moved up to third nationally with 1.04 cases per 100,000 from 2008 to 2012 (OTIS, 2014). The almost indistinguishable increase from 0.84 cases to 1.04 cases seems insignificant, however, the more important fact is that most other states lowered or kept case rates the same, while the case rate in Nevada increased. Another important point is that Nevada had pediatric case rates that exceeded the highest-risk border states of California and Texas (OTIS, 2014). Pediatric TB in Nevada for the age range of less than 1 year of age was not in the top-10 nationally (OTIS, 2014).

The CDC also divides the nation into Metropolitan Statistical Areas (MSA) that have populations greater than 500,000, and from 2008-2012 the Las Vegas-Paradise MSA accounted for almost 4% of the national cases within the 1-4 year old age range, which ranked 5th nationally (OTIS, 2014). Clearly, the 1-4 year old age range requires detailed analysis, but it presumptively appears as if the risk factors identified by the State of Nevada Health Division (Paulson, 2010) have not been addressed and require further analysis. The 5-14 year old age range also requires detailed analysis based on the Nevada rank increase from 18th to 3rd nationally from 2008-2012 (OTIS, 2014). Table 1 shows a comparison between the national average and Nevada pediatric TB rates from 2008 to 2012 stratified by age which shows the biggest age range of concern is 1-4 years. The proposed network model will compare the years 2010, 2011, and 2012, which encompass the time period from the first identification of TB risk factors for pediatric patients, 2010, to the most current statistics per CDC, 2012. Based on these current statistical trends, it is highly improbable that Nevada will meet the National TB Program Objectives and Performance Targets for 2015 (CDC, 2009) in which CDC set a goal for a case rate of less than 0.4 per 100,000 for children less than 5 years old.

Table 1: Stratified Pediatric Case Rates: Nevada vs. the National Average 2008-2012 (Table created from OTIS, 2014)

Age range	National Average per 100,000	Nevada per 100,000
< 1 year old	2.08	*
1-4 years old	1.80	5.70
5-14 years old	0.61	1.04

* Results are suppressed for counts less than four when data are not national and do not represent the total count for a state

RESEARCH QUESTIONS AND HYPOTHESES:

RESEARCH QUESTION 1

Are the risk factors identified by the State of Nevada Health Division significant?

HYPOTHESIS 1

H_o: There is no association between pediatric TB cases and identified risk factors.

H_a: There is an association between pediatric TB cases and identified risk factors.

Based on the aforementioned specific risk factors of pediatric TB identified by the Nevada State Health Division (Paulson, 2010), it is hypothesized that there is a significant association between pediatric contacts with these identified risk factors and pediatric TB in 2010, 2011, and 2012, where p < 0.05 demonstrates a significant (nonrandom) association by bootstrapping. Bootstrapping (Efron, 1979) and node-level regression, a method of regression analysis applicable to social networks (Hanneman, 2005), will be the statistical methods used where the pediatric contacts with identified risk factors are the independent variables and age is the dependent variable (where a pediatric TB case is defined as a case less than 18 years of age). The Southern Nevada Health District defines a pediatric case as less than 18 years of age; therefore, this age is used as opposed to the CDC definition of a pediatric TB case, which is less than 15 years of age. It is predicted that network permutations created by bootstrapping will demonstrate that the association is non-random. Because it is predicted that these risk factors are still significant, further case management and targeted prevention will be necessary.

RESEARCH QUESTION 2

2A) Is the Southern Nevada Health District (SNHD) prioritizing pediatric contact investigations based on the most likely transmission risks within the entire TB network?

2B) Do pediatric TB contacts with the highest betweenness scores (a network metric related to potential disease transmission) (McKenzie, 2007) match the risk factors identified by the Nevada State Health Division (Paulson, 2010)?

HYPOTHESIS 2(A)

- H_o: There is no difference between SNHD contact prioritization in 2010, 2011, and 2012 when using the betweenness metric.
- H_a: There is a difference between SNHD contact prioritization in 2010, 2011, and 2012 when using the betweenness metric.

HYPOTHESIS 2(B)

- Ho: There is no association between pediatric TB cases and identified risk factors of contacts with the top-20 betweenness scores.
- Ha: There is an association between pediatric TB cases and identified risk factors of contacts with the top-20 betweenness scores where p < 0.05 demonstrates a significant (non-random) association by bootstrapping.

Based on network theory of cases, contacts, and links, the betweenness centrality metric can be used to prioritize contacts for investigation purposes (McKenzie, 2007). Contacts with high betweenness scores denote an increased risk of disease transmission because they act as bridges between otherwise isolated cases and clusters. SNHD prioritizes contacts based on the following scale: high, medium, low, and marginal. It is predicted that the SNHD contact prioritization in 2010, 2011, and 2012 will differ significantly from prioritization using the betweenness metric because this metric only prioritizes based upon case and contact connections, not individual risk factors. Further analysis of individual risk factors will be required; however, the betweenness metric can focus prevention and investigative resources because it identifies the associated cases and clusters with the highest risk. There are no generally accepted ranges for betweenness scores, therefore: the top-20 scores will be calculated and assigned a score of 1 (high priority). Using a Likert-scale comparison, these scores will then be compared to the SNHD prioritization scale where:

- 1=high prioritization
- 2=medium prioritization
- 3=low prioritization
- 4=marginal prioritization

Prioritizing contacts can help identify high-risk pediatric areas, where upon further case analysis, it may be determined that the contact investigation area needs to be expanded, which may lead to the identification of new cases. High-risk areas equate to increased risk of disease transmission, so it is more cost effective to locate pediatric cases in high risk areas in conjunction with associated case and contact risk factors than to evaluate the entire network solely on case and contact risk factors.

RESEARCH QUESTION 3(A)

Based on the clustering coefficient, has the 2010 to 2012 Nevada pediatric TB network expanded from a local network to a small-world network?

HYPOTHESIS 3(A)

H_o: There is no difference between clustering coefficients in data analyzed from 2010, 2011, and 2012.

H_a: There is a difference between clustering coefficients in data analyzed from 2010, 2011, and 2012.

RESEARCH QUESTION 3(B)

Has the network density increased from 2010 to 2012?

HYPOTHESIS 3(B)

H_o: There is no difference in Nevada pediatric overall density from 2010-2012.

H_a: There is a difference in Nevada pediatric overall density from 2010-2012.

Individual risk factors of the pediatric contacts with the top-20 betweenness scores were analyzed using node-level regression as stated in Hypothesis 1. It is predicted that there will be a significant association between these risk factors (Paulson, 2010) and pediatric TB contacts with the top-20 betweenness scores. Clustering coefficient and density are common group-level metrics that determine potential for disease

transmission within a network (Hanneman, 2005). Because weak ties (Granovetter, 1973) are predicted to exist in the network, it has expanded from a local network (e.g., close, household contacts) to a small world network. A small world network (Watts, 1998) is made of casual, less common contacts. An example of a contact for a pediatric TB case in a small-world network would be a health care clinician, who is not generally considered a close contact. A small-world network can include specific locations as well, such as hospitals and day care centers. It is hypothesized that network clustering coefficient and density will be indicators of disease transmission and it is predicted that the clustering coefficient will decrease from 2010-2012 indicating network expansion from a local network to a small-world network, and thus increased potential for disease transmission. It is predicted that density has increased from 2010 to 2012 due to higher contact rates, which increases the risk of disease transmission. Clustering and density can help determine if cases are being missed, and can help isolate highrisk areas based on pediatric subgroups and connections to high-risk nodes. Nodelevel clustering coefficients will also be calculated. High-risk areas and pediatric clusters can be targeted for prevention thus providing a more cost-effective and rapid response. Although the entire network is analyzed, node-level pediatric clustering coefficients and connections between high-risk nodes and pediatric subgroups are the focus of metric analysis. Clustering coefficient and density are two of the most commonly used metrics in network analysis, especially networks involving infectious disease transmission (Kiss, 2005; Klovdahl, 1985) because they can demonstrate the stability of a network, and they can be predictors of a potential outbreak based on connections within a network.

CHAPTER 2 - REVIEW OF SCIENTIFIC LITERATURE

ORIGIN OF NETWORK THEORY

Network theory, and more specifically social network theory, has its origins in the field of sociometry (the measurement of interpersonal relations in small groups), as termed by Jacob J. Moreno (Moreno, 1932; Freeman, 2000). Moreno created the first formal graphic representation of a social network using a sociogram (Appendix A), however, the first public health application of network theory was demonstrated by William L. Munson, M.D., New York State Health Officer, who conducted case and contact investigations of syphilis and gonorrhea in the early 1930s (Munson, 1933)(Appendix A). Dr. Munson's visual diagrams and contact tracings provide succinct insight into individual risk factors and disease transmission. However, the first modern application, and arguably the most substantial, of transmission network theory Immunodeficiency was during the initial Human Virus (HIV)/Acquired Immunodeficiency Syndrome (AIDS) outbreak investigation (Auerbach, 1984) (Appendix A). At the time this study was done, the infectious agent, the HIV virus (a retrovirus), had not been isolated. If the infectious agent is not known the only other method of investigation possible is the social aspect. If the infectious agent node is removed from the epidemiological triangle the other two remaining nodes are host and environment. The CDC aided in the investigation and was responsible for identifying the mode of transmission without knowing the infectious agent. HIV/AIDS is an infectious disease, but also had a social aspect so the investigation combined social network and transmission network theories. Cases and contacts were interviewed regarding risk factors thought to be the most substantial such as sexuality and intravenous drug use. Appendix A

(Auerbach, 1984) shows the network diagram with nodes labeled with patient number and city. Patient '0' is centrally located which is common during an outbreak. Calculating network metrics used today would demonstrate Patient '0' as the source of the outbreak due to high centrality measures such as betweenness. Patient '0', either indirectly or directly, can be linked to all the other presumed cases of HIV/AIDS. Or it may be that patients were interviewed and links were established that demonstrated Patient '0' as the outbreak because of his high centrality score. Because Appendix A (Auerbach, 1984) not only shows the links but also the cities, related diseases (by shading), and patient numbers this network diagram can be considered the first application of social network analysis. The fact that Patient '0' linked so many patients within different cities was consistent with his occupation, flight attendant. The network diagram also shows the sequential onset of disease shown by the number after the city. The diagram is an excellent example of social network analysis in that, through simple visualization, many important clues can be immediately identified. Klovdahl (1985) then proposed the use of network theory as a method of supporting the infectious agent hypothesis. He theorized that complementing the infectious agent hypothesis with network theory can provide an early indicator of a transmissible agent during an outbreak. Klovdahl further demonstrates the usefulness of network theory as a method of disease containment through ring vaccination and quarantine, where ring vaccination involves vaccination of those persons closest to patient zero, and quarantine involves isolation of patient '0'.

Centrality is a major component of networks where disease containment involves focusing on persons who maintain the most central positions in the network where centrality can be measured by certain metrics such as betweenness and reach. Social network theory has core concepts that make it ideal for application to public health research (Wasserman, 1994). Actors and their actions are viewed as interdependent rather than independent, autonomous units.

- Relational ties (linkages) between actors are channels for transfer or "flow" of resources (either material or nonmaterial); where transfer of resources can be altered to mean transmission of disease.
- 2) Network models focusing on individuals view the network structural environment as providing opportunities for, or constraints on, individual action; where opportunities are equivalent to Directly Observed Treatment-Short Course (DOTS) therapy, targeted interventions and screenings, education, and case management, and constraints can be quarantine.
- Network models conceptualize structure (social, economic, political, so forth) as lasting patterns of relations among actors, where emphasis is on social interaction (a primary component of disease transmission).

Network theory has been applied extensively within the core areas of public health based on the value it provided during the original HIV/AIDS outbreak investigation (Auerbach, 1984; Klovdahl, 1985). Public health network studies can be classified as: conceptual models, descriptions of an existing real-world structure or system, mathematical models, or simulations (Luke, 2007), and are often used as a descriptive tool to provide basic demographic information regarding disease cases and contacts (McElroy, 2003). In addition, networks and network analysis have four primary components (Luke,2007):

• Structural approach that focuses on patterns of linkages between actors

- Grounded in empirical data
- Makes frequent use of mathematical and computational models
- Highly graphical

Empirical data are derived from observation and/or experimentation. In this study, observational data will be derived from TB case and contact investigations conducted by the Southern Nevada Health District (SNHD), as opposed to theoretical and probabilistic data. Network studies are divided into three main categories: transmission networks, social networks, and organizational networks. Network studies have been applied to all areas of public health (Figure 3).





As previously mentioned, infectious disease network studies are more accurately referred to as transmission network studies, as opposed to social network studies. Examples of applicable research studies are listed in the respective categories in Figure 3. Because this research topic is tuberculosis, it is classified as a transmission network study and, thus, a more detailed review of transmission network studies is necessary.

Transmission network studies involving infectious diseases are dominated by Sexually Transmitted Diseases (STDs) and HIV/AIDS (Luke, 2007); however, other infectious diseases have been subjects of research, such as pertussis (Munene, 2013) and Severe Acute Respiratory Syndrome (SARS) (Ancel-Meyers, 2005). The pertussis study was under outbreak conditions involving secondary data, case and contact investigation, network metrics, and respiratory infection. Network parameters were degree, closeness, betweenness, eigenvector and hub centrality. However, this research (as with many transmission network studies) was in response to an outbreak as opposed to an existing network which would allow implementation of preventive measures in an effort to prevent an outbreak. Ancel-Meyers, et al. (2005) applied network theory to estimate the basic reproductive number (R_0), which is the number of new cases of SARS resulting from a single initial case. Transmission networks were created based on real-life contacts which allowed for epidemiological predictions and interventions. Interventions were divided into transmission interventions (e.g., handwashing) and contact interventions (e.g., avoiding public places). Two advantages of this study were minimal mathematical simulation and no *a priori* (predictive) assumptions about the network structure. Network metrics, such as centrality, were not emphasized. In addition to the aforementioned studies involving

pertussis and SARS, the following public health studies use network theory as a main analytical tool to study infectious disease transmission, some in an outbreak context:

- 1) Rabies (Hirsch, 2013)
- 2) Pneumonia (outbreak) (Meyers, 2003)
- 3) Foot-and-Mouth Disease (outbreak) (Kiss, 2005)
- 4) Avian influenza (Poolkhet, 2013)
- 5) Malaria (Huang, 2013)

Given the global morbidity and mortality of TB, there are surprisingly very few studies that have specifically examined tuberculosis network analysis, and virtually all of those have applied transmission network theory in the context of an outbreak response. McKenzie (2007) proposed the use of transmission network analysis as a complementary method to standard contact investigations. Network metrics, such as reach, degree, and betweenness were calculated, which provided a means of prioritizing contacts. Benefits from the study were the ability to understand variables on the individual level and help focus TB control. It was concluded, though, that staff training and time involved with data management and case/contact linking were major limitations due to the amount of data collected during case and contact investigations.

Two studies explored tuberculosis and transmission networks as models not specifically related to outbreaks. Cook (2007) used interviewing, demographics and clinical data from patient medical records to determine common locational links between cases and contacts in three separate locations: Contra Costa County, California; DeKalb County, Georgia; and Vancouver, Canada. Tuberculin Skin Tests (TST) were

incorporated into the model, and it was concluded that a correlation between TST positive individuals and density is valuable for determining location, and thus is an important method of contact prioritization. Klovdahl (2001) presented the most related research involving tuberculosis and transmission networks using secondary data analysis and calculating the centrality metric. TB network model studies have also been done in the context of an outbreak where the network model included secondary data analysis, was simply descriptive, and with no metrics being calculated (McElroy, 2003). McKenzie, et al. (2007) developed a model of an ongoing outbreak where metrics were calculated, and a CDC-funded study (Cook, 2007) was done that involved interviewing, demographics, clinical records, TSTs, metrics and genotyping, where the study was more cross-sectional in nature and not under outbreak conditions. This proposed research incorporates many of the aforementioned elements, but attempts to address research needs that are specific to the Nevada pediatric TB population. The following specific elements were studied with elements that address research needs shown in bold: ongoing surveillance system (not outbreak conditions), empirical model, focus on risk factors specifically identified by the Nevada State Health Division. targeted prevention, network metrics, secondary data analysis, longitudinal, pediatric TB, major urban area with a highly transient and heterogeneous population, network cluster and density yearly comparison.

TB transmission modeling is progressing towards network analysis (Klovdahl, 2001; McKenzie, 2007; Cook, 2007; Ancel-Meyers, 2005), where modeling connections of people within a population has become one of the central ideas. Dividing a population into groups with different risk factors and determining how

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these groups are connected can have stronger effects on infection levels than considering how many people within the population have the risk factor (Koopman, 2004). This is the core concept of transmission networks, interactions among individuals and groups within a population. While it is advantageous to know the disease prevalence, social interactions are more important because infectious disease transmission is based on exposure to an infected individual. A compartmental model (Dimitriv, 2010), also commonly known as a Susceptible-Infectious-Recovered (S-I-R) model, (Sattenspiel, 2009) considers the outcome of one individual independent of the outcome of another individual with regard to susceptibility, infectiousness, and recovery. This is in direct contrast to network modeling, where the population is interdependent based on connections between people (cases and contacts) and particular groups (e.g., clusters of pediatric cases). The complexities of TB infection and disease are best modeled as a network rather than a compartmental model. A compartmental model ("infectious") is linear, which is in direct contrast with TB infection and disease, which can be cyclical and have many stages (Ernst, 2012).

INFECTIOUS DISEASE MODELING

Communities are dynamic and perhaps none more so than southern Nevada, more specifically Las Vegas. The transient nature of the Las Vegas population makes it ideal for transmission network modeling. The study and analysis of infectious disease transmission requires methods that incorporate population dynamics, as this will provide the most realistic picture of the burden of disease at a point in time and will also provide predictions about transmission. Numerous models exist, and the use of a specific model depends on the purpose of the research. For example, outbreaks are often modeled for the purpose of determining who will become infected and how long it will take for an entire population to become infected. This is considered a predictive mathematical simulation model that uses complex mathematics and algorithms based on a hypothetical population. This is also considered a stochastic model (Hurd, 2008) where the purpose is predictive and is based in probability theory. Stochastic models are commonly employed for risk assessments (Hurd, 2008) that use dose-response data for input variables. Public health is based on the concept of prevention science, and the most effective method for determining prevention is the use of deterministic models which are based on empirical data. Empirical data are data obtained from observation and/or experimentation, as opposed to hypothetical simulation and prediction (Hurd, 2008). For the purposes of this study the empirical data are the case and contact investigation data based on interviewing, medical evaluation, and test results such as Tuberculin Skin Tests (TST) which help establish the individual risk factors. TB has many stages and cycles (Figure 4) (Ernst, 2012) based on risk factors and social interactions that are best addressed by an empirical model that considers case and contact attributes and risk factors. Figure 4 is based on clinical, epidemiological, and immunological studies, where the question marks represent hypothetical situations and the central stages denoted in the green boxes represent evidence-based studies demonstrating bacteria at distinct stages of the immunological life cycle. If the purpose is to determine preventive measures, the rate of transmission which uses probabilities is not necessary, especially with a real-world structural model. A longitudinal/cross-sectional/empirical study is usually based on existing cases, and while the probability of transmission is undoubtedly important, knowing case and contact



Figure 4. The Stages in the Immunological Cycle of Tuberculosis. PAMP, pathogenassociated molecular pattern; TB, tuberculosis; TNF, tumor necrosis factor. Source: Ernst, 2012

attributes and risk factors and the connections are more important. Population system epidemiology (Koopman, 1999) is the analysis of how population characteristics and patterns of exposure affect disease levels and transmission. The cases and contacts are existing so there is no temporality, and determining causation is not the purpose. The cases and contacts are associated based on the contact investigation process through interviewing.

The Susceptibility-Infected-Resistant (S-I-R) model has been the conventional model for infectious disease modeling (Sattenspiel, 2009). This model is also known as

Susceptibility-Exposed-Infected-Resistant (S-E-I-R) model. The S-I-R model is a compartmental model with the following health states:

1) Susceptible: individual has never had the disease and is susceptible to infections

2) Infected: individual currently has the disease and can infect others

 Resistant: individual does not have the disease, cannot infect others, and cannot be infected.

The principal output parameter for a compartmental model is R_o , the basic reproduction number (Ancel-Meyers, 2005). R_o is the expected number of new infections created by an infected individual under the most favorable conditions for transmission. The formula is $Ro=\beta/\gamma$, where $\beta=$ infectivity parameter and $\gamma=$ infectious period parameter. If $R_o > 1$ an epidemic is present. If one individual creates 2 infected individuals during his/her infectious period, those 2 will create 4, etc. This compartmental model assumes (Koopman, 2004):

- (a) Everyone is identical,
- (b) Contact is an instantaneous event with no duration in time,
- (c) Mixing is instantaneously thorough so that the chances of meeting an individual are independent of having met them in the past,
- (d) The population of individuals in each stage of infection is large so that further division of each compartment in the model is always possible,
- (e) Every infection event is identical to every other infection event,
- (f) Contagiousness is constant over the entire course of infection, and

(g) The rate of recovery from infection is constant over the entire course of infection.

This is not a realistic model of population dynamics because not everyone is identical, and everyone has different risk factors, especially for TB. In addition, contact is not instantaneous, nor is mixing. Children and household contacts (for example) do not mix instantly, and chances of meeting are not necessarily independent. Exposure period (time) is a direct indicator of TB transmission, and compartmental models do not account for incubation periods which are especially confounding with TB where the incubation period is 2 weeks to 3 months (Heymann, 2008). This is not instantaneous contact and does have a specific time duration. Finally, infection events are not identical especially with pediatric TB, and other risk factors such as HIV/AIDS and contagiousness and rate of recovery are not constant. Too many assumptions are required for compartmental models that would make it unrealistic, especially during an outbreak. Upon executing the S-I-R model an individual is randomly chosen, and this individual is placed in one of the three health states susceptible, infected, or resistant. Because the model is discrete an individual can only occupy one health state at a time, and progression must follow from susceptible, to infectious, to resistant. What about a TB case where an individual is infected (LTBI) but is healthy and thus cannot infect others? This TB state represents 90% of individuals who become infected. Where does this person fit in the model? This individual is infected and does not infect others because disease has not progressed to the active stage. Compartmental models are always linear, and TB is not a linear disease. Figure 5 shows the compartmental model in comparison with the network model which is more indicative of TB transmission. Latency, reactivation, and secondary infections are

not addressed in compartmental models. The network model takes into account personal risk factors, personal attributes and population mixing. Populations are heterogeneous (made of many types of individuals with specific risk factors) which may make them more or less susceptible to disease, and network model incorporates social interactions, population dynamics, and heterogeneous populations which provides a more realistic representation of TB infection and disease. The S-I-R model is a complex mathematical model that, ultimately, applies health state probabilities to groups (not individuals). For example, the probability of transition from 'susceptible' to 'infected' may be 0.4, but the entire 'susceptible' population is treated equally without consideration for individual risk factors. Modeling TB using an S-I-R model could be done, however there would be



Figure 5. Graphical Comparison between a Compartmental Model and a Network Model. Source: Ancel-Meyers, 2007

severe limitations because TB is not a linear disease as shown by the complex immunological transmission cycle in Figure 4. For example, if three individuals have

been randomly chosen as susceptible what is the probability of linear transition to the infected state? What if one person is 2 years old, one has HIV/AIDS, and the third person is healthy? The transition probability is not equal because it has been established that children 5 years of age or younger and HIV/AIDS co-morbidities are the two biggest risk factors for developing TB (Schaaf, 2009, 2010; Schlossberg, 2011). The S-I-R model must also address co-morbidities, however it treats all three individuals with equal transition probability simply based on susceptibility only, but susceptibility is also based upon individual risk factors which are not considered in the S-I-R model. S-I-R models are commonly used for outbreak scenarios to determine who will develop the disease and how long it will take for everyone in the population to become infected. Infectious diseases are inherently difficult to model, without limitations, because they are caused by microorganisms which produce feedback loops (Philippe, 1998) on a cellular level, especially during an immune response. For example, puberty and menopause induce qualitative changes which cause the emergence of new disease states. Epigenesis (Philippe, 1998) is the term used to describe the formation of these new disease states which are specifically based on structural non-linearity at the cellular/ biochemical/ immunological level. Epigenesis can be considered the micro level of feedback loops where feedback loops can be positive or negative. Because microorganisms produce feedback loops which are cyclical in nature, an S-I-R model would not be the most appropriate model for infectious disease modeling due to their linear nature. Dimitrov, et al. (2010) mention contact network models which is essentially what this research is. Models which represent a more practical approach to population dynamics are ones that employ contact networks. Stochastic simulation methods then can be applied to contact

networks for outbreak investigations. This is probably the best representation of a dynamic population model because it predicts disease based on transmission algorithms and probabilities and it also incorporates network theory. For example, Meyers, *et al.* (2003) have modeled contact patterns of respiratory disease in a psychiatric hospital from the distribution of the patients and caregivers within wards of the hospital where the nodes were health care workers and entire wards filled with patients.

As previously mentioned, TB transmission modeling is progressing towards network analysis (Klovdahl, 1985, 2001; Andre, 2007; Cook, 2007) where modeling connections of people within a population has become one of the central ideas. Compartmental models consider the outcome of one individual independent of the outcome of another individual with regard to susceptibility, infection, and resistance. This is in direct contrast to network modeling, where the population is interdependent based on connections between people (cases and contacts) and particular groups (clusters of pediatric cases, for example).

A big advantage of models is the ability to influence public health policy. Some specific advantages of TB infectious disease modeling are: providing better resource allocation, improving contact investigation efficiency, prioritizing treatment, education, and DOTS therapy by identifying clusters (high-risk areas in Clark County), high risk individuals and groups. Models ensure that public health policy makers and stakeholders are effectively influenced because they (Koopman, 2004):

1) <u>Improve the intuitions of policy makers with regard to the ways that their decisions</u> <u>affect the behavior of the transmission system.</u> Social network models are more intuitive, and preventive measures can be implemented, and results are direct and immediate. Social networks can be visualized which makes them a more intuitive model. This model is based on risk factors/comparisons which have been shown to be critical by Nevada State Health Division (Paulson, 2010).

- Deal explicitly with all the issues that the policy makers see as important to making their decision; and
- Allow the policy makers to locate reality as they see it within the model structure so that they feel confident that the results they are looking at are relevant to their decision.

This transmission network model represents the current real burden of TB, and policy makers can be more confident with this model as opposed to a stochastic model that is based on probability theory with multiple assumptions.

A BRIEF INTRODUCTION: MYCOBACTERIUM TUBERCULOSIS

Mycobacterium tuberculosis, often referred to as the "tubercle bacilli", is the causative agent of tuberculosis, and was first identified by Robert Koch in 1882 (Sherman, 2007). Much of the accrued knowledge about *M. tuberculosis* can be attributed to his research, for which he won the Nobel Prize in Physiology or Medicine in 1905 (Robert Koch-facts, 2014). The organism is a slightly curved or straight rod which appears pink or red under a microscope when stained. Figure 6 shows *M. tuberculosis*

(circled) that has been isolated in a sputum smear, and is readily identifiable by its contrasting pink color.



Figure 6. Photomicrograph of *M. tuberculosis* (circled) in a Sputum Smear Isolated Using the Ziehl-Neelsen Staining Technique. Source: CDC, Public Health Image Library (Ronald Smithwick, 1971)

Although classified as Gram-positive, the very thick peptidoglycan layer creates a varied result when using the Gram stain, therefore an acid-fast stain (Ziehl-Neelsen or modified Kinyoun) is commonly used for identification purposes (Southwick, 1971). In addition, the cell wall contains lipomannan and arabinogalactin, which are pathogenic determinants (Bloom, 1994). For example, lipomannan has been shown to induce apoptosis in macrophages (Dao, 2004). Figure 7 shows a schematic cross-section of the cell wall of *M. tuberculosis*. The organism is an intracellular pathogen and an obligate aerobe that is non-spore forming, non-encapsulated, and non-motile (Vasanthakumari,

2007). It is 1-4 μ m long, and 0.3 to 0.6 μ m thick (Vasanthakumari, 2007). Because *M*. *tuberculosis* is an obligate aerobe, the lungs are a perfect growth environment and the organism has a viability of 8 to 10 days in droplet nuclei and 6 to 8 months in culture at room temperature (Vasanthakumari, 2007).



Figure 7. A Schematic Cross-Section of the Cell Wall of *M. tuberculosis*. Source: Kleinnijenhuis, 2011

TAXONOMY

Mycobacteria belong to the Order Actinomycetales, Family *Mycobacteriaceae*, Genus *Mycobacterium*, and *Mycobacterium* means fungus-bacterium because of the growth characteristics on liquid media which resemble mold pellicles (USEPA, 1999). Early techniques to classify mycobacteria were biochemical testing and culture, and today there are 71 species of mycobacteria described, with over 20 species known to cause disease in humans (USEPA, 1999), with two of the most common being tuberculosis and leprosy. The lipid content comprises about 60% of the cell wall, which creates a cell wall with very low permeability; therefore, the organism is extremely resistant to disinfection (USEPA, 1999).

THE M. TUBERCULOSIS COMPLEX

Mycobacteria not identified as tuberculosis or leprosy complex are identified as: atypical mycobacteria, Mycobacteria Other Than Tubercle bacilli (MOTT), environmental mycobacteria, or Non-Tuberculous Mycobacteria (NTM) (Wolinsky, 1979). MOTT and NTM are the two most used classifications. Many mycobacterial species that cause disease are also classified as follows (Heymann, 2008):

- Mycobacterium tuberculosis complex: M. tuberculosis, M. bovis, M. bovis-BCG, M. africanum, M, canetti
- 2) Mycobacteria other than tuberculosis (MOTT); or Non-tuberculous *Mycobacterium* (NTM): *M. microti, M. pinnipedii, M. caprae*

Differentiation between the *Mycobacterium tuberculosis* complex and MOTT is vital when determining a clinical case of tuberculosis. The CDC case definition (APPENDIX B) lists isolation from a clinical specimen using Nucleic Acid Amplification (NAA) of any species from the *M. tuberculosis* complex as meeting the case definition for *M. tuberculosis*. For example, if *M. bovis* is isolated from a patient, the case definition of tuberculosis using the laboratory criteria for diagnosis has been met. DNA probes cannot distinguish the *M. tuberculosis* complex organisms, and the use of the *M. tuberculosis* complex organisms. A confirmed case of TB can be established by meeting the laboratory criteria **OR** the clinical definition. Realistically, diagnosis of TB will involve a medical (clinical)

evaluation and laboratory testing, especially if Multi-Drug Resistant (MDR) and Extensively-Drug Resistant (XDR) strains are present. All other species are referred to as MOTT or NTM which are sometimes loosely referred to as environmental mycobacteria. Transmission of the organism can occur via several routes including inhalation and ingestion of unpasteurized dairy products (Heymann, 2008); however, for the purposes of this research, the inhalation route is the focus, and active cases, or latent infection, of tuberculosis are limited to those which have a pulmonary origin.

Figure 8 shows the anatomy of the respiratory system. Respiratory pathogens, in the form of droplet nuclei, that are less than or equal to $5 \,\mu$ m in diameter are most likely



Figure 8. Anatomy of the Respiratory System;

(A) Anterior view of the respiratory System (B) Alveoli, bronchioles, and blood supply (C) Gas exchange region.

Source: adapted from NIAID, 2014

to reach the alveoli in substantial number (Macher, 1999). Because tubercle bacilli have a maximum diameter of 0.6 μ m, they can easily reach the alveoli and impede gas exchange, and ultimately respiration.

Without question, *M. tuberculosis* is an organism of interest in public health based on the global morbidity and mortality statistics. Figure 9 shows the various stages of TB, and thus the difficulty in diagnosing the disease. Of particular note are the terms endogenous reactivation and exogenous reinfection (Bloom, 1994). Endogenous





reactivation refers to an existing infection with a particular strain of *M. tuberculosis* that has been reactivated from a dormant state within the body. Exogenous reinfection refers to infection from a new strain of *M. tuberculosis* not previously dormant within the body.

The key is that there are two different strains of the organism. Until the advent of TB genotyping, TB in an existing patient would most likely be assumed endogenous reactivation; however, with the ability to differentiate strains using genotyping, it has been shown that exogenous reinfection could be the cause (Cronin, 2002).

Understanding the many stages of TB, such as LTBI, active disease, and reactivation necessitates a discussion of the immune response to *M. tuberculosis*. Because *M. tuberculosis* is an intracellular pathogen it elicits a cell-mediated immune (CMI) response (Dannenberg, 1991, 1994). It has also been demonstrated that a Type IV Hypersensitivity (Delayed-Type Hypersensitivity, DTH) response plays a role in the immune response (Kindt, 2007). T lymphocytes and macrophages are the primary immune cells that mediate the response to *M. tuberculosis*. Eosinophils, neutrophils, monocytes (inactivated macrophages) and natural killer cells have been shown to play a role in vivo (Bloom, 1994). TB CMI response is comprised of two classes of effector cells (Kindt, 2007):

- 1) T-cells having direct cytotoxic ability
 - a) Cytotoxic T Lymphocytes (CTL)(CD8+)
 - b) Alveolar macrophages
- 2) T-cells that mediate DTH
 - a) Helper T-cell (Th1)(CD4+)
 - b) Helper T-cell (Th2)(CD4+)

CELL-MEDIATED IMMUNITY (CMI)

In general, CMI requires activation of T-cell populations through antigen processing and presentation. With TB, alveolar macrophages ingest and destroy the tubercle bacilli using lytic enzymes, then present the peptide antigen (tuberculin) to Th1 cells for activation (Kindt, 2007). Th1 cells then release chemokines that stimulate monocyte production and activation through cellular signaling. This is but one example of the CMI response to TB which is based on recognition, and activation of T-cell populations by tuberculin, a protein in the cell wall of *M. tuberculosis*. Tuberculin also activates the DTH response which is the basis for the TST. Cellular activation is the key component of the TB cell-mediated immune response which can be specifically defined as (Dannenberg, 1994):

the immune process that results in the accumulation of large numbers of *activated* macrophages around (solid) caseous tuberculous foci. These macrophages ingest the live bacilli escaping from the edge of the caseum, inhibit their intracellular multiplication, and eventually destroy them.

DELAYED-TYPE HYPERSENSITIVITY (DTH)

In certain instances the immune response can be hypersensitive. Technically hypersensitivity is defined as an increased, or heightened, response but it can also be an inappropriate response to an antigen (Kindt, 2007). Hypersensitive immune responses can result from a humoral response such as anaphylaxis which results from the antigenantibody complex; or with CMI response which is the case with TB where the response is to the *M. tuberculosis* antigen tuberculin. Anaphylaxis is an immediate hypersensitivity that can become fatal quickly, whereas tuberculin produces a DTH where symptoms develop days after exposure (Kindt, 2007). Gell and Coombs developed the classification system for the four types of hypersensitivities in which the immune response to tuberculin, or tuberculin-like products, is classified as a Type IV, Delayed-Type Hypersensitivity (DTH) (Figure 10). This classification is listed as "cell-mediated hypersensitivity" in the figure to differentiate it from the other three types which involve the humoral (antigen-antibody complex) response. A more descriptive definition is provided specific to the immune response to *M. tuberculosis* (Dannenberg, 1994):

DTH, as defined herein, is the cytotoxic immune process that results in the killing of non-activated macrophages that have permitted the multiplication of tubercle bacilli within them. Since the surrounding tissues frequently are killed in the process, DTH to the antigens of the tubercle bacillus actually causes most, if not all of the tissue damage that characterizes the disease. The tubercle bacillus itself is rather non-toxic; only the host's reaction to its tuberculin-like products destroys the lung.

Allergen Fc receptor for IgE Allergen- specific IgE Degranulation Type I	ADCC ADCC Fc receptor Cyto- toxic Surface cell Target antigen cell Complement activation Immune complex Type II	Immune complex Complement activation	Antigen Sensitized T _H 1 Cytokines Activated macrophage Type IV
IgE-Mediated Hypersensitivity	lgG- or lgM-Mediated Cytotoxic Hypersensitivity	Immune Complex–Mediated Hypersensitivity	Cell-Mediated Hypersensitivity
Ag induces cross-linking of IgE bound to mast cells and basophils with release of vasoactive mediators.	Ab directed against cell surface antigens meditates cell destruction via complement activation or ADCC.	Ag-Ab complexes deposited in various tissues induce complement activation and an ensuing inflammatory response mediated by massive infiltration of neutrophils.	Sensitized T _H 1 cells shown above release cytokines that activate macrophages or T _C cells that mediate direct cellular damage. T _H 2 cells and CTLs mediate similar responses.
Typical manifestations include systemic anaphylaxis and localized anaphylaxis such as hay fever, asthma, hives, food allergies, and eczema.	Typical manifestations include blood transfusion reactions, erythroblastosis fetalis, and autoimmune hemolytic anemia.	Typical manifestations include localized Arthus reaction and generalized reactions such as serum sickness, necrotizing vasculitis, glomerulonephritis, rheumatoid arthritis, and systemic lupus erythematosus.	Typical manifestations include contact dermatitis, tubercular lesions, and graft rejection.

Figure 10. The Four Types of Hypersensitivities. Type IV-DTH is associated with *Mycobacterium tuberculosis* and the associated Tuberculin Skin Test (TST). Source: Kindt, 2007

Dannenberg's definitions of CMI and DTH are based on TB pathogenesis, and he specifically differentiates these two responses, and this differentiation is critical to understanding disease progression in immunocompetent and immunosuppressed persons which becomes evident in the following discussion on TB pathogenesis and stages of disease.

The ability of tuberculin to elicit an immune response was first discovered by Robert Koch during his pioneering work with *M. tuberculosis*. He believed that this immune response might lead to a cure for tuberculosis and he was not successful in demonstrating this. His research led to the Tuberculin Skin Test (TST) which is a commonly used screening tool for TB (Sherman, 2007). **DTH has two specific phases: (1) sensitization phase and (2) effector stage** (Kindt, 2007). Figure 11 shows the detailed cellular response of these two phases.



Figure 11. The Two Phases of the Tuberculin Skin Test (TST) Response: (a) sensitization phase and (b) effector phase. Source: Kindt, 2007

Approximately 2 weeks after initial contact with the antigen, Helper-T (T_h) cells are activated by tuberculin peptides (antigen) by means of antigen-presenting cells (macrophages) that have engulfed the tubercle bacilli, processed the antigen, and presented it for binding and activation. T_h cells have now been activated and are now technically labeled as " T_h1 " to indicate activation. Any subsequent exposure to tuberculin (as with an injection during a TST) results in the effector phase where Th_1 cells release cytokines that activate resting macrophages as well as recruiting nonspecific inflammatory cells. The peak DTH response is about 24-72 hours after the second contact with the antigen, or injection of the tuberculin for the TST (Kindt, 2007). The delay results from recruitment of non-specific cells that are not local and are transported through the bloodstream to the injection site, as well as macrophage activation. The erythema around the TST induration is part of the localized inflammatory response where blood vessels have dilated to increase inflammatory cell migration.

TB PATHOGENESIS AND THE ROLE OF CMI/DTH

Resistant and susceptible strains of inbred rabbits have been used as animal models for early human TB research, and accurately represent the various stages within newborn infants, immunosuppressed individuals and immunocompetent adults (Lurie, 1964). The initial infection of approximately 3 tubercle bacilli that forms the Ghon focus within the alveoli is contained by previously activated alveolar macrophages. Alveolar macrophages have three functions in response to tuberculosis: ingest the tubercle bacilli, produce specific cytokines that mediate the immune response, and process and present mycobacterial antigens (Bloom, 1994). During this **onset (first stage)** (Dannenberg,

1991) CMI has not been activated as the tubercle bacilli are too low in number. If the tubercle bacilli are not contained, the alveolar macrophage will lyse and release them, and this release attracts monocytes (non-activated macrophages) from the bloodstream by the release of complement component C5a and MCP-1, a monocyte chemoattractant protein (Bloom, 1994). The inability of the initial alveolar macrophage to contain the organism leads to a **second stage: symbiosis** (Lurie, 1964) where the monocytes have engulfed the tubercle bacilli, and a symbiotic relationship exists where the organism is contained, but still viable as the monocytes have not been activated and cannot produce lytic enzymes. At this stage, the monocytes are not destroyed because the DTH has not yet developed. This symbiotic stage occurs 7 to 21 days after the initial infection, and this lesion grows as a result of the accumulation of macrophages and bacilli. The monocytes provide an ideal environment for logarithmic bacilli growth, thus resulting in an early primary tubercle (Figure 12). The third stage starts with initial caseous necrosis (Bloom,



Figure 12. Granuloma (Tubercle) from the Lung of a Minipig. Central Necrosis has been Formalin-Fixed and Stained with Hematoxylin-Eosin. Source: Guirado, 2013

1994) where DTH becomes activated, and the patient is tuberculin positive 2-3 weeks after inhalation of the original bacilli that formed the Ghon focus. At the end of stage two, resistant and susceptible host immune responses have inhibited bacillary growth equally even though susceptible hosts have 20-30 times more bacilli in the lungs (Bloom, 1994). Activation of DTH results in the destruction of monocytes eliminating the favorable growth environment for the bacilli and resulting in contained and uncontained and fragmented and unfragmented bacilli within the caseum. Bacilli within this formed solid caseum are viable, but cannot reproduce. Local lymphokine production is highly stimulated during this stage due to the presence of tuberculin antigen. Stage four (Bloom, 1994) begins at about 3 weeks where bacillus multiplication is well controlled in susceptible and resistant hosts, but by different methods. Susceptible hosts control multiplication by using the DTH response because CMI is weak, and because DTH is inflammatory in nature further lung tissue destruction is observed. Numerous inactivated macrophages allow bacillary growth in susceptible hosts which results in a weak CMI response, thus the DTH is needed to destroy the inactivated macrophages, progressing the cycle of lung tissue destruction and the increased potential for the bacilli to be released from the tubercle, become systemic, and form the more fatal forms of TB (miliary and meningeal) which are seen in immunosuppressed hosts such as children and HIV/AIDS patients (Bloom, 1994). Stage 4a has been observed in susceptible animal models, and Stage 4b has been observed in resistant animal models in which a strong CMI is observed in resistant animal models and does not rely on destroying non-activated macrophages thus ultimately limiting tissue necrosis (Lurie, 1964; Dannenberg, 1991). Tcell populations are expanded through stimulation caused by antigen processing and

presentation by macrophages which, in turn, cause T-cells to release Interferon Gamma and lymphokines which activate more macrophages. Immunocompetent persons have a strong CMI which is the more effective method of immune response because activated macrophages ingest bacilli, destroy them with lytic enzymes, and present antigen to $T_h 1$ cells for further macrophage activation (Kindt, 2007). This not only efficiently destroys bacilli, but greatly reduces tissue damage. Immunosuppressed persons rely heavily on a DTH response which is inflammatory in nature, causing monocytes to ingest bacilli without destruction. Monocytes do not produce lytic enzymes and release viable bacilli when the DTH response is forced to be activated causing tissue damage and the release of bacilli to the periphery of the tubercle which may result in systemic TB (Bloom, 1994). Pediatric TB cases are at highest risk for systemic TB because the CMI has not fully developed. HIV/AIDS cases are defined by a low T-cell count and are at the greatest risk because the CMI response to TB is specific to T-cell populations such as T_h1, T_h2, and Cytotoxic T Lymphocytes (CTL) (Bloom, 1994). Disease progression in healthy individuals is limited or even prevented because activated macrophages are always present around the caseous areas to ingest and destroy bacilli that may reach the periphery of the lesion. Stage 5: Liquefaction and Cavity Formation (Bloom, 1994), liquefaction and cavity formation can cause the progression of TB, even in immunocompetent individuals (Lurie, 1994). Liquefaction is the formation of liquified material resulting from the immune response to tuberculin antigen and lytic enzyme release which causes bronchial rupturing and necrosis which forms a cavity. This liquified material is highly oxygenated and provides a perfect growth medium for the bacilli (Canetti, 1955) which is easily aerosolized and spread via droplet nuclei to other

parts of the lung and the breathing zone of the patient. Liquefaction is the process that perpetuates the disease in humans because the large number of bacilli in the liquified material can give rise to MDR and XDR strains which is why multiple treatments are given simultaneously (Bloom, 1994).

M. tuberculosis defense mechanisms

The ability of *M. tuberculosis* to be engulfed by alveolar macrophages, yet remain viable in a latent state results from the *M. tuberculosis* cell wall proteins that prevent the fusion of lysosome and phagosome to form the phagolysosome within the macrophage (Kindt, 2007). Without formation of phagolysosome, the lytic enzymes cannot be released leaving the tubercle bacilli trapped in the phagosome, viable but not destroyed. Lipoarabinomannan (LAM) is an *M. tuberculosis* cell wall protein that disrupts biochemical signaling and blocks phagosomal maturation by the following mechanisms: inhibits protein kinase, blocks trafficking pathway from trans-Golgi network to phagosomes, inhibits Ca2⁺ in macrophages, and blocks lytic enzymes (Rajni, 2011). Cord factor (Trehalose dimycolate-TSM) is another cell wall protein that is toxic to immune cells (Rajni, 2011). It inhibits phagosome and lysosome fusion, causes weight loss in organisms (cachexis), and is toxic to polymorphic neutrophils. It also helps maintain the granulomatous response.

KOCH'S POSTULATES

Understanding the complexities and associated theories of infectious disease transmission requires at least a basic understanding of the germ theory of disease (Sherman, 2007) which has its basis within the fields of microbiology and immunology. This understanding not only provides valuable knowledge about host-agent interactions,

but it also helps explain the various TB risk factors. Knowing individual risk factors helps determine preventive measures which are the goal of public health. The research of Robert Koch helped establish the germ theory of disease which subsequently provided the foundation for modern theories of infectious disease transmission. Koch's postulates (Sherman, 2007), the basis for the germ theory of disease, were derived from his work with *Mycobacterium tuberculosis*. To identify the causative agent for a microbial disease, four conditions (Koch's postulates) must be met. First, it must be demonstrated that the agent is present in every case of the disease. Second, the agent must not be present in any other diseases. Third, after isolation and repeated growth in pure culture, the agent must produce the same disease when introduced into a healthy animal. Fourth, the agent must be reisolated from the experimentally infected animal. Having satisfied all of Koch's postulates it can be concluded that the microbe is the causative agent. Even though Koch's postulates apply to an individual host response it is these individual host responses that determine the potential for the disease to be transmitted to others. There is evidence that host immune response promotes disease transmission in TB (Ernst, 2011), and knowing individual immune responses can aid in diagnosis, treatment and prevention of tuberculosis.

Three core concepts of public health, more specifically infectious disease transmission, have their basis in social network theory as it relates to individuals and populations:

1) HERD IMMUNITY (NIAID, 2014)

2) EPIDEMIOLOGICAL TRIANGLE (CDC, 2014a)

3) QUARANTINE (Schlossberg, 2011)

Herd immunity involves vaccinating a critical portion of the population against infectious diseases which, in turn, reduces the amount of viable hosts thus reducing the potential for an outbreak. Figure 13 clearly shows how the social networks are applicable to public health, especially given the intricate population mixing patterns. The epidemiological triangle (Figure 14) is a well-known basic model of disease transmission where relational links exist between agent, host, and environment where the "flow" is an infectious



Figure 13. Graphical Representation of Herd Immunity; none of the population is immunized (top); some of the population is immunized (middle); most of the population is immunized (bottom). Source: NIAID, 2014

disease. In this research the agent is *M. tuberculosis*, the hosts are represented by the "actors" in the network (cases and contacts), and the environment can be a household, hospital, daycare center, etc. The environment is the social factor. As in the epidemiological triangle, triads will exist in the tuberculosis network where opportunities for prevention are present within the network.



Figure 14. The Epidemiological Triangle Source: CDC, 2014a

Quarantine is perhaps one of the oldest concepts of public health, and is based on the principle of isolating the infected individual from the population which eliminates exposure, and thus transmission because there is no other viable host present (Sherman, 2007). Without social interaction between the case and other contacts there are no links. By analyzing network structure, TB cases can be quarantined which puts constraints on individual actions (concept 3, above) and limits links and connections, and ultimately the spread of infectious diseases. Early 20th-century methods of TB control, subsequent to Koch's discovery of the tubercle bacilli, involved TB patient isolation in sanatoriums (isolation wards) for quarantine and treatment, although ultimately treatment was neglected and patients became wards of the state (Sherman, 2007).

EPIDEMIOLOGY AND NATURAL HISTORY OF TB

The origins of TB pre-date human writing with Pott's Disease (spinal TB) (Turqut, 2001) being observed in ancient Egyptian mummies from the period of 3700 to 1000 B.C. (Sherman, 2007). 'Hunter-gatherer' civilizations were not affected by TB due to their transient nature and lack of human contact and congregation. However, around 8000 B.C. agrarian societies developed, where humans and animals congregated, and TB was introduced into the human population through contact with cattle and infection with M. bovis (Schaaf, 2009). This was the beginning of the epidemic cycles of spread and decline within the human population that is based on the complex relationship between the human host and the organism, M. tuberculosis. One widely accepted theory used to explain the epidemic cycles of spread and decline of TB within the human population is the genetic development of herd immunity (Stead, 1992) (Figure 13). Parasites and humans develop mutations based on environmental stressors; however, because the lifespan is much shorter for a parasite, infection of a large percentage of a human population can occur before humans can develop immunity. Although a large percentage of the human population is eliminated during this initial phase there are some survivors that develop immunity, and this trait can be passed on to their progeny. Each successive generation of progeny then develops resistance, causing a decline in TB epidemics; however, M. tuberculosis can mutate in response to environmental stressors causing a new epidemic where infection and disease occur within human populations that are no

longer resistant to the mutated strain of *M. tuberculosis*. To a lesser degree, the ability of *M. tuberculosis* to mutate as an adaptation to an environmental stressor can be observed by multi-drug resistant (MDR) and Extensively-Drug Resistant (XDR) strains of *M. tuberculosis* (Stead, 1992).

In addition to genetic precursors, TB epidemic cycling can also be attributed to personal, environmental, and sociological factors such as nutrition, overcrowding, and population density, respectively (Nelson, 2004). More specifically, colonization of people in Europe and the United States in the 1700s and 1800s combined with the industrial revolution, extreme population growth, and immigration, created megacities where a large amount of people were confined to a small area in which a respiratory disease such as TB is easily spread. With respect to a modern day global population, air transport has allowed for frequent travel among developing and industrialized countries and cities, thus eliminating natural geographic barriers of disease, and causing an increase in TB cases (Huang, 2013). Perhaps nowhere is this more evident than in the Las Vegas-Henderson-Paradise area of Nevada where approximately 75% of TB cases are foreignborn versus approximately 25% which are U.S.-born (CDC, 2013a). From the initial introduction of *M. tuberculosis* into the human population of the first agrarian societies, the sociological aspect of the host-agent interaction and TB transmission cannot be ignored. This is because diagnosis, treatment, and epidemic cycling of TB mandates a detailed and analytical approach. This approach must encompass population dynamics, interpersonal contacts, and relationships in conjunction with host factors that increase or lessen the likelihood of TB disease such as age and host immune status (Koopman, 1999, 2004).

From an infectious disease and microbiological viewpoint, it was Robert Koch who first isolated *M. tuberculosis* in 1882, and this represents a vital period in public health history where the transmission, diagnosis, treatment, and prevention of TB could be studied using the scientific method based on causation. Shortly after the discovery of the bacterium *M. tuberculosis*, Wilhelm Roentgen discovered the X-ray in 1895 (American Physical Society, 2001) which allowed visualization of the presence and growth of *M. tuberculosis* and progression of the disease within the human body, rapidly advancing the ability to diagnose TB disease. The discovery of the causative agent of TB and X-rays led to the optimum time period for TB's natural history research, 1920-1950. Four criteria made this the optimum research period (Marais, 2011):

1) Robert Koch's identification of the bacterium M. tuberculosis

- 2) Wilhelm Roentgen's X-ray discovery
- 3) Lack of chemotherapeutic agents
- 4) Lack of confounding comorbidities, namely HIV/AIDS

Chemotherapeutic agents were first developed for TB around 1950 (Schaaf, 2009). This coupled with the discovery of HIV/AIDS ended the ability to study the natural history of TB disease as chemotherapeutics provided treatment that could not ethically be denied, and HIV/AIDS confounded research studies as a comorbid condition (Marais, 2011). Much of the research within this time period provided valuable information regarding the stages of TB disease, risk factors, and disease progression relative to age. Figure 15 represents research conclusions developed during this time period that are still applicable today (Wallgren, 1938; Wallgren, 1948; Marais, 2011). The top timeline is a general timeline of tuberculosis and the various stages of pathogenesis

from initial infection to adult-type disease. For example, adult type disease (Stage IV), which Wallgren defined as an individual greater than 10 years of age, can develop approximately within 6 months to 3 years of initial infection. The research of Wallgren is still valuable today because at the time of his research chemotherapeutics were not



Adapted from the original time-line of tuberculosis described by Wallgren.(5)

Hypersensitivity responses and tuberculin skin test conversion

- Ghon focus and/or Disseminated (Miliary) disease Ш
- Ш Lymph node disease (<5yrs of age) / Pleural or pericardial effusion (>5yrs of age) IV
- Adult-type disease (>10yrs of age)





Adapted from the age-related manifestations of pulmonary tuberculosis reported in immunecompetent children.(5)

- Complicated Ghon focus, Disseminated miliary disease and TB meningitis
- Uncomplicated and complicated lymph node disease 11
- III Pleural effusion
- IV Adult-type disease

Figure 15. General Timeline of Tuberculosis Progression (top) and Age-based Timeline of Tuberculosis Progression (bottom).

Source: adapted from (Wallgren, 1938; Wallgren, 1948; Marais, 2011).

developed, and he could follow natural disease progression. The bottom timeline is an age-based timeline of tuberculosis and the various stages of progression. For example, children ages 0 to 4 years are at the highest risk for developing miliary and meningeal TB. This figure also demonstrates the difficulty in diagnosis of pediatric TB because many of the stages overlap. It was from these research studies of 1920-1950 that three common concepts arose for identifying and addressing current and future challenges of tuberculosis transmission, prevention, control, and diagnosis (Marais, 2011):

- 1) Need for accurate case definitions
- 2) Importance of risk stratification
- Diverse spectrum of disease pathology, requiring accurate disease classification

The importance of TB risk stratification is vital, and population dynamics must be considered because individuals are stratified into risk categories based on age, immune status, country of origin, etc. which address questions regarding disease progression, treatment, prevention, and control. The use of network theory incorporates population dynamics and complex personal interactions, as well as aiding in addressing the importance of risk stratification by not just considering TB cases, contacts, and links, but also individual risk factors (categories), primarily age, associated with TB cases and contacts.

PATHOGENESIS OF PEDIATRIC TB

While the mode of infection is generally consistent among all persons, containment and progression to disease represent a more severe risk to children, especially 5 years of age or younger, with progressively increasing risk below 5 years of age. Primary infection (Marais, 2006) is the term used to denote the very first exposure to the tubercle bacilli. Inhalation of droplet nuclei (Houk, 1968) results in an initial infection of the alveoli where less than 3-5 tubercle bacilli are necessary for infection. This

localized infection within the alveoli is referred to as the Ghon focus (Marais, 2006, 2011). For 4-6 weeks, replication occurs within the Ghon focus, and the tubercle bacilli then drain into the lymph nodes via the lymphatic system causing swelling of the lymph nodes (lymphadenopathy). Primary infection is so rapid in pediatric cases that cell-mediated immunity has not been activated and cultures may be positive without clinical disease being present. Age is the most important factor in progression to disease following primary infection (Marais, 2006, 2011), and greater than 95% of children who progress to disease do so within 12 months of primary infection (Marais, 2006, 2011). Although pediatric TB is diagnosed as a single disease it has many stages that can develop from the primary infection if treatment is not initiated immediately. The stages of progression of pediatric TB are based on dissemination from the lungs into other areas of the body.

TB can occur *in utero* or during the birth of a baby, however it can be difficult to differentiate congenital TB from postnatal TB. Congenital TB occurs by hematogenous spread via the umbilical vein or ingestion of amniotic fluid during birth, and postnatal TB occurs via inhalation of bacilli from a mother or other source case with infectious pulmonary TB (Schaaf, 2010). Congenital TB has most likely occurred if an infant has a TB lesion, and one or more of the following: present within the first week of life, a primary hepatic complex or caseating hepatic granuloma, TB infection of the placenta or endometrial TB in the mother, exclusion of postnatal TB through exclusion of TB in other contacts (Cantwell, 1994). Mothers with recent TB infection (with pleural effusion) and meningeal TB or miliary TB with a bacillaemic phase are most likely to have transmitted in utero, whereas mothers with cavitating disease are most likely to have transmitted

postnatally (Schaaf, 2010). Perinatal tuberculosis is the preferred term for TB in which congenital and postnatal transmission is difficult to distinguish (Schaaf, 2010).

Pulmonary TB includes both intrathoracic lymphadenopathy and parenchymal disease, and is more common in adolescents than in children 5 to 10 years of age (Cruz, 2010), and is most likely to cause respiratory problems due to the small size of the airways (Marais, 2006, 2011). Intra-thoracic lymph node disease (Marais, 2006, 2011) occurs as the primary infection disseminates and causes lymphadenopathy within the intra-thoracic cavity causing alveolar collapse and caseation. Caseation may cause caseating pneumonia which then leads to cavitation where lung tissue is destroyed. This stage is most common in children less than 5 years of age and is most likely to cause respiratory problems due to the small size of the airways. Primary pulmonary disease has three distinct time frames: primary parenchymal, progressive primary, and reactivation (Cruz, 2010).

Pleural TB (Andreu, 2004) is a complication from primary TB where the Ghon focus ruptures into the pleural lining of the lungs causing TB antigen release which stimulates a Delayed-Type Hypersensitivity (DTH) with infiltrate observed. Pleural TB is a complication observed in young adults and adolescents caused by primary infection, or in general with postprimary TB 6-12 weeks after primary infection.

Tuberculosis associated with the central nervous system is rare, only developing in about 2% of cases with 50% of the cases being younger than 2 years of age (Cruz, 2010). Meningitis is the most common form of central nervous system tuberculosis, has a peak incidence in the 0 to 4 year age group, and occurs with greater frequency in HIV-infected persons (Bloom, 1994). Proliferation of tubercle bacilli occurs from an existing pulmonary focus, with spreading into the arachnoid space with the meningitis typically showing in the brain stem (Bloom, 1994). Cerebrospinal fluid analysis usually shows lymphocytes, low glucose concentration, and a high protein value (Cruz, 2010). As with all types of tuberculosis, multiple methods of diagnosis are required. Because a TST is only positive 33% of the time and isolation of acid-fast bacilli from cerebrospinal fluid is unlikely, chest radiographs, which are abnormal approximately 90% of the time, can help identify a miliary pattern of disease spread (Cruz, 2010).

Miliary TB presents shortly after primary infection and is caused by lymphohematogenous spread in younger or immunocompromised children (Cruz, 2010). This type is systemic, bloodborne and often fatal (Andreu, 2004), and children with miliary disease should always be evaluated for meningeal TB as well. Miliary, as well as meningeal, TB is responsible for the majority of TB-related mortality in infants (Schaaf, 2010). Figure 16 shows three different images of miliary TB, (a) chest x-ray (CXR), (b) CXR, and (c) High-Resolution Computed Tomography (HRCT). In image (a) the budding diffusion is noticeable in the lungs, a consistent finding with miliary TB. In image (b) nodules are noticeable, and image (c) shows random distribution of the miliary nodules. The benefits

of HRCT, in addition to CXR, are evident in that the cross-sectional spread of miliary TB is readily observable which gives a more complete picture of the extent of the disease.

Skeletal TB is more common in older children, around 20 years of age with the exception of Pott's disease which is specific to the spinal cord (Cruz, 2010). Pott's disease is common in young children where multiple systemic lesions are present in immunocompromised children and local symptoms of inflammation predominate (Cruz,



Figure 16. Diagnostic Images of Miliary TB: (a) CXR , (b) CXR , and (c) HRCT; TB=tuberculosis; CXR=chest x-ray; HRCT=high-resolution computed tomography Source: Andreu, 2004

2010). TST results are positive in most pediatric cases and Acid-Fast Bacilli (AFB) cultures of bone are positive in up to 75% of cases (Cruz, 2010). In areas where pediatric TB is endemic, high rates of transmission are sustained by high case density and prolonged diagnostic delay (Nelson, 2004). It is not just a large number of cases, but density refers to a large number of cases based on a particular area. Transmission network analysis allows allocation of resources and can provide more accurate areas for targeted screening and prevention which can shorten diagnosis and expedite treatment. Three crucial criteria differentiate pediatric TB from adult TB, where the risk increases with children under the age of 5 years (Marais, 2006, 2011):

- 1) Reduced incubation period
- 2) More rapid progression to active TB disease
- More rapid progression to fatal forms of TB, specifically miliary and meningeal

Immature cellular immunity (Kindt, 2007) is the main cause for these three crucial criteria, therefore it is mandatory that all pediatric cases be investigated immediately.

DIAGNOSIS OF PEDIATRIC TB

Two of the biggest diagnostic challenges associated with pediatric tuberculosis are identification of untreated infection with a high degree of sensitivity and specificity in immunocompromised children, and identification of symptomatic disease as early as possible, especially in immunocompetent children over 3 years old (Marais, 2006). Ninety percent of persons infected with *M. tuberculosis* show no symptoms and are classified as having Latent Tuberculosis Infection (LTBI); however, 10% of persons infected with *M. tuberculosis* develop disease and are classified as active tuberculosis patients (CDC, 2013). Table 2 shows a clinical comparison between LTBI and active TB. As observed in the table, diagnosis is usually by multiple methods, and the CDC case

Table 2. Clinical comparison between LTBI and Active TB; TST=Tuberculin Skin Test; IGRA=Interferon Gamma Release Assay; CXR=Chest X-Ray Source: CDC 2013

<u>Boulee: CDC, 2013</u>		
Latent TB Infection (LTBI)	Active	
1) No symptoms	1) Symptoms: fatigue, cough, chest pain	
2) TST or IGRA usually positive	2) TST or IGRA usually positive	
3) CXR normal	3) CXR usually abnormal	
4) Respiratory specimens:	4) Respiratory specimens:	
smear, culture negative	smear, culture positive	
5) Non-infectious	5) Infectious	
6) Treatment recommended to	6) Treatment needed	
prevent disease		

definition (Appendix B) allows diagnosis by either clinical or laboratory methods. The following are the most widely accepted methods of TB diagnosis, and usually multiple methods are used for confirmation: TST (Marais, 2006; Starke, 2004; CDC, 2013; AAP,
2004) and epidemiological information such as medical history (AAP, 2004) and exposure to a known source case (Marais, 2006; Starke, 2004). Clinical diagnosis usually consists of a CXR and/or High-Resolution Computed Tomography (HRCT) (Marais, 2006; Starke, 2004) where HRCT provides a cross-sectional anatomical view especially useful for diagnosis of pleural and miliary TB. Bacterial culture analysis is a common clinical method, however it is rarely used as the sole method of diagnosis. A sputum smear involves direct examination of sputum that is thinly spread on a microscope slide with specific dyes added. Bacterial culture involves spreading the sputum sample on a medium specific to mycobacteria, and observing the growth of colonies after an appropriate incubation time. With suspected pediatric TB cases, sputum and culture diagnosis is limited, and usually confirmed by another method such as CXR. Only 30-40% of suspected pulmonary cases are culture-positive in children (Starke, 2004), and with a sputum smear only 10-15% of suspected cases are positive (Marais, 2006; Nelson, 2004); however higher yields may be present at greater than 10 years of age (Marais, 2006). Children have a paucobacillary load, and the small amount of sputum collected for testing usually has a low yield (Marais, 2006: Nelson, 2004). Gastric (stomach) aspirates are sometimes tested, but even these samples have a low yield of mycobacteria, less than 20% positive on smears and less than 50% positive with culture (Nelson, 2004).

TUBERCULIN SKIN TEST (TST)

The TST is the most commonly used test for preliminary TB diagnosis because it is cost-effective, good for screening, and easy to train a clinician to administer it (AAP, 2004). It involves injection of tuberculin (or purified protein derivative) just under the skin which, if positive, elicits a Delayed-Type Hypersensitivity (DTH) type IV response within 48-72 hours. The most common method of administration is the Mantoux method, and reading of the TST is done within 48-72 hours to correspond with a potential DTH response (AAP, 2004). An induration of the skin is observed at the injection site which is measured by a trained clinician. Table 3 lists pediatric induration cut-off ranges recommended by the American Academy of Pediatrics (AAP, 2004).

Table 3. Tuberculin Skin Test (TST) Induration Cut-Off Ranges. Source: adapted from AAP, 2004

1) Induration greater than or equal to 5 mm

- Children or adolescents in close contact with a known or suspected infectious TB case
- Children or adolescents with suspected TB disease:
 - Finding on chest radiograph consistent with active or previously active TB Clinical evidence of TB disease
 - -Children or adolescents who are immunosuppressed (receiving immunesuppressive therapy or with immunosuppressive conditions such as HIV/AIDS)

2) Inducation greater than or equal to 10 mm

- Children or adolescents of increased risk of disseminated disease:
 - Those less than 4 years old
 - Those with concomitant medical conditions such as Hodgkin's disease,
 - lymphoma, diabetes mellitus, chronic renal failure, or malnutrition
- Children or adolescents with increased risk of exposure to cases of TB disease:
 - Those born in a country with a high prevalence of TB cases
 - Those who travel to a country with a high prevalence of TB cases
 - Those with parents born in a country with a high prevalence of TB cases
 - Those frequently exposed to adults with high risk factors for TB disease, such as adults with HIV/AIDS or homeless, users of illicit drugs, those who are incarcerated, or migrant farm workers

3) Induration greater than or equal to 15 mm

- Children greater than or equal to 4 years of age with no known risk factors

The TST has limitations, and while adequate as a screening tool is never used as

the sole method of TB diagnosis. An individual is subject to a boosting effect where the

TST induration size increases with repetitive testing in individuals previously sensitized to mycobacterial antigens (AAP, 2004). Boosting can be misinterpreted as conversion which occurs when a negative TST converts to a positive TST, however boosting can be eliminated if multiple TSTs are done less than 1 week apart (AAP, 2004). Conversion can be determined by following a negative TST with another TST around 3 months later, which is the maximum incubation period of *M. tuberculosis* (Heyman, 2008). If the follow-up TST is positive, conversion has occurred. A medical history must be obtained from each patient when evaluating TST results. Table 4 shows examples of conditions which can lead to false-positive and false-negative TST results. The Interferon-Gamma Release Assay (IGRA), the new gold standard, and Enzyme-Linked Immunospot (ELISPOT) (AAP, 2004) are two promising diagnostic bioassays which are more sensitive because they can differentiate T-cell response among the mycobacterial species.

THE BACILLE CALMETTE-GUERIN (BCG) VACCINE

The BCG vaccine was created by Albert Calmette and Camille Guerin of the Pasteur Institute in the early 20th century (Sakula, 1983). Technically called the "Mycobacterium bovis bacillus Calmette-Guerin" vaccine (Doherty, 2005, Sakula, 1983) it was administered to infants in 1921 with a 90% success rate (Doherty, 2005). Given the high success rate it nonetheless remains a controversial vaccine where the efficacy varies between 0% and 80% based on differences in BCG strains, age of vaccination, and methodological differences (Brandt, 2002). The neonatal vaccination seems to have very high efficacy against military and meningeal TB, but wanes 10-15 years later, and pulmonary TB can occur (Brandt, 2002), thus it has short-term pediatric benefits, but no long-term benefits (ASPH, 1946). It is contraindicated for immunosuppressed individuals

such as HIV/AIDS and pregnancy (CDC, 1996). Up until 1996, the Tice strain was the

Factors	False-Negative Reactions	False-Positive Reactions
Infections	 -Viral illnesses (HIV, measles, chicken pox) -Bacterial (typhoid fever, typhus, leprosy) -Early TB infection (less than 12 wk.) -TB disease (meningeal, miliary, pleural) -Fungal (Blastomycosis) 	Exposure to NTM (<i>M</i> . <i>marinum</i>)
Live virus vaccines	-Measles, polio, smallpox	BCG vaccine
Concomitant medical conditions	 -Metabolic abnormalities (chronic renal failure) -Malignancies (Hodgkin's disease, lymphoma, leukemia) -Sarcoidosis -Poor nutrition 	Transfusion with whole blood from donors with known positive TST
Drugs and technical factors	 -Corticosteroids, chemotherapy -Newborns and < 2 years of age -Material: poor quality, inadequate dose; expired, exposed to light -Administration: too long in syringe -Reading: biased, inexperienced too early/late 	Inexperienced or biased reader
Interpretative	Decreasing mm of induration	Increasing mm of induration

Table 4. Factors Associated with False-Negative and False-Positive TST Reactions Source: adapted from AAP, 2004

only FDA-approved BCG vaccine, produced by Organon, Inc. of West Orange, New Jersey, the only FDA-approved company to produce this vaccine. The BCG vaccine is not routinely given in the United States, however the CDC provides the following conditions for pediatric use (CDC, 1996):

1) An infant or child who has a negative tuberculin skin test result;

2) Continuous exposure to an untreated or ineffectively treated patient who has

infectious pulmonary TB, and the child cannot be separated from the presence of the infectious patient or given long-term primary preventive therapy;

3) Continuous exposure to a patient who has infectious pulmonary TB caused by *M. tuberculosis* strains resistant to isoniazid and rifampin, and the child cannot be separated from the presence of the infectious patient.

TREATMENT OF PEDIATRIC TB

The primary goal of treating pediatric TB is to prevent the emergence of drugresistant organisms, and this goal is achieved with 3 specific objectives: a rapid reduction of organism load, effective eradication of dormant and persistent bacilli, and minimal adverse effects on the pediatric patient (Marais, 2006). Treatment is specific and depends on the following factors: disease classification, anatomical location of the disease, route of administration, medication adverse effects and interactions, and isolate susceptibility (Cruz, 2010).

TB exposure

While TB infection and disease are the two primary disease classifications that are generally considered, TB exposure is also a classification for pediatric TB. This category is defined as asymptomatic children who have had contact with persons suspected of TB disease and in whom the TST result and chest radiograph are normal (Cruz, 2010). Due to

the potentially rapid progression of TB disease, children under 5 years of age who meet the classification for TB exposure should be started on medication even with a negative TST (Starke, 2004). If a second TST is negative at 3 months after separation from the known TB case, the treatment, usually isoniazid (INH), can be discontinued. However, if the TST is positive, the child should be placed on a 9-month course of INH (Starke, 2004).

TB infection (LTBI)

It is recommended that children with a positive TST be placed on a 9-month course of INH, or a 6-month course of rifampin if side effects from INH exist (Cruz, 2010).

TB disease

Normally, children have a small bacillary load; however, with TB disease the bacillary load is higher, which justifies the use of combination therapy using the Directly Observed Therapy Short-course (DOTS) system. The four most commonly used anti-TB medications used to treat TB disease are INH, rifampin, pyrazinamide, and ethambutol. In selecting medications, drug toxicity, interactions (synergism, etc.), duration, MDR/XDR, combinations, dosages, site of disease, and co-morbidities such as HIV/AIDS must be considered.

INFECTION CONTROL AND PREVENTION OF PEDIATRIC TB

Chemoprophylaxis is given to prevent progression of LTBI to active disease (Cruz, 2010). Infection control of healthcare associated transmission involves typical Standard Operating Procedures (SOPs) such as isolation of patients in negative-pressure

rooms, evaluation of caregivers for signs and symptoms of TB, and the use of N-95 masks (Cruz, 2010).

Contact investigations (Cruz, 2010, Lobato, 2008), source case investigations (AAP, 2004), and targeted screenings are common methods of prevention within the community. Programs targeting children have little short-term influence on disease rates, but are critical for long-term control of the disease (Hsu, 1963), and the contact investigation examining persons close to a suspected case of pulmonary tuberculosis— is the activity that identifies exposed children (Hsu, 1963). Children do not transmit TB because they have a paucobacillary (very low number of organisms) load and they do not produce a forceful enough cough to expel the organism in an infective dose (Cruz, 2010). However, a source case investigation must be done to prevent further pediatric exposure within the network. A network may be a household network or a community network such as a daycare. The CDC recommends source case investigations for 2-year olds and under with LTBI, and 4-year olds and under with active disease, with a high prioritization based on these sites of disease: pleural, laryngeal, and pulmonary (CDC, 2005, 2006). These sites of diseases are high-priority because they are the most infectious types of TB via the respiratory route.

PEDIATRIC CASE AND CONTACT INVESTIGATIONS

Because a pediatric TB case indicates a sentinel, or recent event, the contact investigation has always proceeded on the basis that the index case was due to close contact with an adult who has infectious pulmonary TB. This is a rational and logical approach given that child-to-child transmission is not possible because children do not produce a forceful enough cough to expel organisms in an amount that would cause infection (Cruz, 2010). The assumption though is that the close contact with the adult represented a household contact (Schaaf, 2003). This seems logical as well, but considering the State of Nevada Health Division has identified risk factors that may be associated to non-household contacts (Paulson, 2010), it may be beneficial to investigate contacts that expand beyond household contacts. As an example, the Nevada State Health Division has identified healthcare providers and prisoners as potential contacts. Social network analysis can possibly link pediatric TB cases to these potential contacts that may have been overlooked.

Other studies are consistent with the Nevada State Health Division recommendations that alternate contact sources, other than household contacts, are justified for contact investigations (Schaaf, 2003; AAP, 2004). Other potential locations for investigations are churches, dance halls, physician offices/waiting rooms, day care centers, schools; and even mobile sources such as taxis (Schaaf, 2003). Researchers used Restriction Fragment Length Polymorphism (RFLP) to differentiate the strains of *M. tuberculosis* as proof that the strains of the cases were identical to the strains of the contacts, and thus distinct from household contacts (Schaaf, 2003). Table 5 shows that, of 35 children whose strains were typed, 19 (54%) lived in a house where at least one other household member had bacteriologically-confirmed TB. Of these 19 pediatric cases, 12

were RFLP-linked to another household member with 6 cases being identified as a strain found in a community cluster. Another important point is that of the 35 cases, 16 had no household members with known TB which further reinforces the idea that pediatric contact investigations beyond the household level must be conducted. Twelve (34%) of

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	Household members with confirmed TB n = 19 (%)	No known household members with TB n = 16 (%)
RFLP analysis identified source case	12 (63) household members	3 (19) community source cases
RFLP community cluster, but no source case identified	6 (32)*	8 (50)
RFLP unique strains	1 (5)	5 (31)

Table 5. Culture-Confirmed Childhood TB Cases. Contact Tracing Results (n = 35) Source: adapted from Schaaf, 2003

*Two children had probable household source cases, but their RFLP results were not

available

the children were greater than or equal to 6 years of age and 23 (66%) were less than 6 years of age. This supports network theory because community cluster identification can present opportunities for prevention and targeted screening that are missed when cases and contacts are not linked with respect to the entire TB transmission network.

A pediatric case of TB is considered a sentinel event, an event that indicates recent transmission. Therefore, detecting cases (and contacts) is crucial; thus a contact investigation model must provide the most (cost) effective method:

 Programs targeting children have little short-term influence on disease rates, but are critical for long-term control of the disease (Hsu, 1963).

 2) The contact investigation—examining persons close to a suspected case of pulmonary tuberculosis— is the activity that identifies exposed children (Hsu, 1963).

3) TB infection in children is diagnosed by the TST (AAP, 2004). This

exemplifies the importance of contact investigation which aids in preventing progression from infection to disease.

Based on a 1999 study of 28 United States health jurisdictions (Jereb, 1999), contact investigations identified approximately 1% of active TB cases and 23% of LTBI cases. Other studies have shown similar results, with identification of 1-2% of active TB cases, and 31-36% of LTBI cases (Reichler, 2002; Marks, 2000). The high percentage of LTBI case identification is encouraging because up to 5% of newly acquired LTBI cases will develop active TB within 2 years (Ferebee, 1970), thus treatment can be initiated immediately to prevent these LTBI cases from progressing to active TB.

Targeted screenings are useful and usually involve tuberculosis skin testing (AAP, 2004). Screenings are only useful if they are cost-effective, therefore it is advantageous to focus on high-risk groups such as adult immigrants (Lobato, 2008). In general, schoolbased screenings are not cost-effective because they only have a percentage of LTBI detection of less 0.02% with a rate of active disease detection less than 2% (AAP, 2004). Foreign-born adolescents are particularly high-risk; therefore, a targeted screening program can be beneficial for this group, as well as middle school and high school students in some communities (AAP, 2004). The use of a risk factor questionnaire as a screening tool can be useful prior to beginning a TST screening program (AAP, 2004) with the following risk factors associated with pediatric TB: HIV/AIDS, over-crowding, poverty, malnutrition, non-Hispanic black, Hispanic, American Indian/Alaska native, foreign born children in U.S., U.S.-born children born into immigrant families, and overseas travel (Nelson, 2004).

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From a global perspective, the following are specific pediatric case reduction and surveillance strategies: use of consistent case definitions for research and surveillance, use of globally stratified data (at least 0-4 years old and 5-14 years old), report all pediatric TB cases (regardless of smear status), more complete data collection similar to the CDC RVCT form (i.e., site of disease, co-morbidities (HIV), drug resistance, method of detection and treatment outcomes, better surveillance to improve case detection, contact investigations in low-income countries) in order to find LTBI cases and prevent progression to active TB, interventions targeted specifically at children, a consistent global case definition, treatment completion data, analysis of co-morbidities (not just HIV/AIDS), and MDR/XDR research specific to children (Nelson, 2004).

DIRECTLY OBSERVED TREATMENT SHORT-COURSE (DOTS)

DOTS was originally created in 1993 when WHO declared TB a global emergency, when it is estimated that 49 million cases of infectious TB were prevented and 5 million deaths averted (Schlossberg, 2011). The primary purpose of the original DOTS program was to prevent transmission through detection of the organisms by smear microscopy and the cure of infectious TB through short course chemotherapy (Schlossberg, 2011). While the original program was successful, it had to be reassessed because it did not effectively address issues such as the HIV/AIDS, MDR-TB, and universal access of health care. As part of the new WHO "Stop TB Strategy" the new DOTS goals are to reduce prevalence and deaths from TB by 50% by the year 2015 compared to 1990 baseline, and to eliminate TB as a public health problem by the year 2050 (Davies, 2003). Component 1 of the Stop TB strategy involves DOTS expansion and enhancement (WHO, 2002, Davies, 2003):

1. Pursue high-quality DOTS expansion and enhancement

- a) Secure political commitment, with adequate and sustained financing
- b) Ensure early case detection, and diagnosis through quality-assured bacteriology
- c) Provide standardized treatment with supervision, and patient support
- d) Ensure effective drug supply and management
- e) Monitor and evaluate performance and impact

In the United States, DOTS has become associated solely with direct supervision of therapy, however this is just one of the 5 components. For DOTS enhancement and expansion to be successful all of the components are crucial. A time-series cross-sectional analysis using empirical data has shown that element 'c' involving standardized treatment has been successful (Obermeyer, 2008), however it is not known which area of standardized treatment has been successful. The authors (Obermeyer, et al.) believe, based on a review of the literature, that HIV-TB patients have received an 18% increase in standardized treatment success rates. It was also concluded that national adoption of the new expanded and enhanced DOTS program did not result in an increase in case detection or case notification rates. The authors state that this study is limited by the lack of sensitivity of their methods to detect the impact of DOTS enhancement on case detection. A related limitation is a potential change in reporting methods. In other words, the methods used may not differentiate between an actual increase in case rates or a change in reporting methods, especially since the study is global, and countries have different reporting criteria which may vary year-to-year.

MULTI-DRUG RESISTANT (MDR) AND EXTENSIVE-DRUG RESISTANT (XDR) TB

MDR-TB is defined as a mycobacterial strain that is resistant to at least isoniazid and rifampicin (NIH, 2014). The World Health Organization (WHO) estimated about 650,000 global cases of MDR-TB in 2010 (WHO, 2014), where approximately 20% are pediatric cases (Pandian, 2013). XDR-TB is defined as a mycobacterial strain that is resistant to isoniazid and rifampicin plus any fluoroquinolone and at least one of three injectable second-line drugs (such as amikacin, kanamycin, or capreomycin) (NIH, 2014). The number of pediatric XDR-TB cases is unknown, however it is estimated that overall 9% of MDR-TB cases are also XDR-TB cases (WHO, 2014).

The following is the recommended pediatric treatment regimen (IOM, 2011) that has 80-95% cure, or probable cure, rates (Al-Dabbah, 2011):

- Group 1—Remaining first-line drugs: a combination of a high dose of isoniazid and ethionamide can create one effective drug. Although ethambutol and pyrazinamide are used, they are not regarded as reliable, and 50 percent of cases of MDR-TB in children are resistant to ethambutol.
- Group 2—Second-line injectables: kanamycin, amikacin, and capreomycin. The reason for using amikacin is that it causes fewer side effects in children, and the doses are relatively easy to administer.
- Group 3—Fluoroquinolones: although said not to be suitable for children, maximum doses are used. These are very important drugs in MDR-TB therapy.
- Group 4—Second-line oral bacteriostatic drugs: split into two doses per day initially to alleviate any adverse effects.

Group 5—Drugs that have an unclear role in the treatment of drug-resistant TB.

Linezolid and clarithromycin are sometimes used, although they are very expensive and difficult to obtain.

THE NATIONAL TB PROGRAM

Tuberculosis is a nationally reportable disease (CDC, 2013). The Centers for Disease Control and Prevention (CDC) is the federal agency, under the U.S. Department of Health and Human Services, that has jurisdiction over the national TB program. This authority was transferred from the United States Public Health Service (USPHS) in 1960 (Schlossberg, 2011). Two major functions of CDC are to aid in outbreak investigations at the request of local health districts and to compile statistics on incidence, prevalence, risk factors, demographics, etc. at the national and state level (and certain metropolitan areas) (CDC, 1995; ACET, 1995). The CDC maintains the Online Tuberculosis Information System (OTIS, 2014) which is a valuable public health resource that provides graphs, tables, and summary statistics for health research.

Although the CDC is the national governing agency, it is the state and local health districts that provide TB control and prevention services to communities. State health departments fund local health districts, provide outbreak support, and compile state statistics. In addition, states correspond with other states during outbreaks. Local health districts are the agencies responsible for the seven core components of a TB prevention and control program as listed by the Advisory Council for the Elimination of Tuberculosis (ACET, 1995):

- 1. Conducting overall planning and development of policy
- 2. Identifying persons who have clinically active TB
- 3. Managing persons who have or are suspected of having disease
- 4. Identifying and managing persons infected with *Mycobacterium tuberculosis*
- 5. Providing laboratory and diagnostic services
- 6. Collecting and analyzing data
- 7. Providing training and education

The CDC establishes guidelines and reference materials as models for TB prevention and control programs, but local health districts establish the methods and Standard Operating Procedures (SOPs). A health department program may have a mission statement to prevent the transmission of tuberculosis to reduce the overall morbidity and mortality within a community/population with the following goals established to meet the mission statement (CDC, 1995; ACET, 1995; Schlossberg, 2011):

- I. Identify and treat TB disease
- II. Finding persons exposed to TB, evaluate them for infection and disease, and treat if necessary
- III. Screen populations at highest risk for LTBI and progression to active disease, and provide treatment for progression to active disease

THE STATE OF NEVADA TB PROGRAM

The statutory authority for tuberculosis control in the State of Nevada is Nevada Revised Statute (NRS) Chapter 441A-Infectious Diseases; toxic agents: Tuberculosis: 441A.340-.400 (NRS, 2014). Statutory authority is general and does not provide regulations on the operation of a TB program. The Nevada Division of Public and Behavioral Health (previously known as the Nevada State Health Division) is the state agency under which the TB program is regulated. Ultimately, the CDC mandates TB case reporting by each state, however states rely on local health districts for case reports, mostly to compile public health statistics. The State of Nevada has 3 Health Districts (DHHS, 2014):

- Southern Nevada Health District (SNHD), whereas, the Southern Nevada Health District has been established by the County of Clark and the cities of Las Vegas, North Las Vegas, Henderson, Mesquite, and Boulder City as the Public Health Authority for those entities, pursuant to Nevada Revised Statutes 439 (NRS, 2014).
- 2) Washoe County District Health Department
- 3) Carson City Health and Human Services

All other counties, cities, and municipalities are under the jurisdiction of the Nevada Division of Public and Behavioral Health. State public health laboratories provide TB diagnostic services and are vital for rapid identification during outbreaks. In Nevada, the Southern Nevada Health District has a public health laboratory. The Northern Branch of the Nevada state public health laboratory is on the campus of the University of Nevada, Reno (UNR), while the Southern Branch is on Shadow Lane. Health districts and state laboratories, however, cannot do this alone: clinics, hospitals, laboratories, private physicians are integral, thus the reason TB is reportable. If a patient has TB he/she is more likely to go to a hospital, clinic, or private physician rather than the health district.

TB PLANNING AND POLICY DEVELOPMENT

TB planning and policy development is a systematic process which can be divided into 3 groups that must involve all the stakeholders of a community (WHO, 1998):

- Those served or affected by a program such as patients, advocacy groups, elected officials, and community members;
- Those involved in program operations such as management, program staff, funding agencies, and coalition members;
- Users of developed health policies such as decision makers, partners, coalition members, and the general public (taxpayers).

All states differ with respect to TB morbidity and mortality, so it is logical that planning and policy development is based on the existing burden of disease within that state, or even specifically at the local level. Although the CDC provides guidelines, all state and local health agencies must plan and develop policies that specifically address the existing burden of TB infection and disease. Nevada provides an excellent example of the need to develop TB planning and policy regionally. Well over 80% of TB cases in Nevada were in Clark County in 2010 to 2012 (Nevada State Health Division, 2013), thus the planning and policy development is much different than in more rural Nevada counties such as Washoe County. Funding, program staff, and resource allocation will all vary among the various health districts of Nevada. For example, in 2012, Washoe County had 8 cases of TB and Clark County had 70 cases (Nevada State Health Division, 2013), therefore treatment and DOTS, for example, will require more funding, program staff and resource allocation in Clark County than Washoe County simply based on the number of cases. Not only is case management more varied, but contact investigations must be considered also. Because Las Vegas is in Clark County, a highly transient population is always present, and given the population difference between Clark County and Washoe County, the risk of TB transmission is greater in Clark County simply because more people are present within a smaller geographic area, with the largest population density in Las Vegas.

Analysis of current data showing recent morbidity trends (total cases and case rates) is the first step of TB planning and policy development (Schlosser, 2011) as this provides the best indication of resource requirements. Analysis of these data not only provides optimal resource allocation for case management, but also aids in predicting resources necessary for contact investigations, TSTs, and LTBI case management because these are directly associated with TB morbidity.

Epidemiological analysis is the next step of TB planning and policy development (Schlosser, 2011). Foreign-born person, HIV/AIDS patients, and race/ethnicity are known risk factors for TB, however these risk factors vary with location and must be evaluated as such. For example, as previously stated, Nevada had the highest pediatric TB case rate in the nation in 2012 (OTIS, 2014) so this must be an area of focus. This risk group is the focus of this dissertation. Program evaluation is then necessary to determine if goals and

objectives are being met, and six key steps are necessary for a detailed analysis of a TB program within a local or state health agency (Schlosser, 2011):

1) Engage stakeholders

- 2) Describe the program
- 3) Focus the evaluation design
- 4) Gather credible evidence
- 5) Justify conclusions
- 6) Ensure use and share lessons learned.

Social network analysis (SNA) provides an analytical method that can aid in the analysis of a TB program as mentioned above. For example, a TB network can engage stakeholders such as elected officials through simple visualization of cases, contacts, and links. Highlighting high-risk areas using colors and symbols provides a non-technical representation of TB that is easily understandable. SNA is a validated method of analysis of TB cases, contacts, and links as a means of demonstrating the current burden of disease (morbidity) which, as previously mentioned, is the first step in TB planning and policy development. CDC recognizes the value of SNA as a research method as noted in the guidelines for TB network case and contact investigation where CDC recommends the complementary use of SNA with the concentric circle method (CDC, 2005). CDC has even funded pilot studies for TB case and contact evaluations in 3 areas of North America (Cook, 2007). Although CDC used SNA for an outbreak investigation, it was concluded that a need exists for an ongoing systematic approach that could periodically analyze a health department's contact investigation data for the existence of transmission patterns with the benefit of contact prioritization (McKenzie, 2007).

SNA provides a method to show this existing burden of TB disease which can provide optimum resource allocation for various systems of a TB program such as targeted screenings and treatment programs based high-risk subgroups, in this case the pediatric population. SNA can also aid in addressing steps 4 and 5 of a TB program as previously listed. SNA can be thought of as an empirical model, one that is based on observations, specifically observations through TB case and contact investigations, and interviews that help create valuable information regarding risk factors for disease transmission and most importantly demonstrating the current burden of disease. TB case and contact investigations conducted by SNHD provide credible evidence, however SNA can help justify conclusions during program evaluation by showing specific links, clusters, high-risk areas, and high risk populations (in this case pediatric TB because of the increased rate of disease as well as the noted risk factors by the State Health Department) as noted by the CDC recommendation for an "ongoing systematic approach" (McKenzie, 2007).

Collecting and analyzing data is an ACET core component (ACET, 1995). SNA can provide a means of data analysis of collected (secondary) data, specifically case and contact investigation data by SNHD, thus as an ongoing systematic model it can also aid in active surveillance of TB. (McKenzie, 2007). Said data analysis can provide targeted LTBI testing, population demographics, and treatment outcomes.

Identification and treatment of persons with clinically active TB is the first priority of TB programs in the United States (ACET, 1995). These persons represent the biggest risk of transmission. Identification and treatment of persons with Latent Tuberculosis Infection (LTBI) presents a slightly lower risk because persons with LTBI are less likely to infect others. These are the core components with the universal consensus that case and contact investigations are vital protocols for identification and treatment of persons with clinically active TB and LTBI.

In general, there are 2 basic methods of case detection (Schlossberg, 2011):

1) ACTIVE

- Contact investigations: locate recent exposure
- Outbreak investigations: epidemiology and molecular methods
- Screening of high-risk populations: targeted testing for LTBI

2) PASSIVE

 Reporting by: hospitals/emergency rooms, physician's offices, clinics, laboratories using a CDC Report of a Verifiable Case of Tuberculosis (RVCT) form or similar

Health districts, especially at the local level, rely on both methods. Arguably, case and contact investigations are the most vital component of a TB program because they are the best way to locate source cases, index cases, active TB cases, and Latent TB infectious cases. The main issues are: which cases require investigation? How are contacts prioritized? When does a contact investigation end, or when do we know we have investigated enough contacts to determine the spread of the disease from the source case?

All health districts conduct case and contact investigations, however there is not one universal method. To date, there are 3 models for case and contact investigation:

- 1) Concentric Circle Analysis (CCA) (Veen, 1992)
- 2) Social/Transmission Network Model (CDC, 2005, 2006)
- 3) Contact Priority Model (CPM) (Psu, 2002; Bailey 2002; Gerald, 2002)

A cursory review of each state health department was conducted regarding model types with a summary shown in Table 6. This was a simple comparative review based on information contained in each state website and is not intended to be a complete evaluation of contact investigation models for each state. Of the states where a specific systematic protocol could be determined, the CDC guidelines were listed. These states specifically referenced CDC guidelines. Many states also still continue to use the Concentric Circle Model (CCM). A question mark indicates there was no direct reference to CCA, CDC, SNA, or CPM. CDC guidelines (CDC, 2005, 2006) only mention two types of models, CCA and SNA, so it is presumed that the states that directly mention CDC guidelines use CCA and/or SNA although this could not be determined. Tennessee uses social networks analysis (Holt, 2014), however the SNHD considers social networks. Tennessee uses visualization and standard social network analytical techniques whereas SNHD only mentions social networks (Blake, 2013), with no specific application of analytical techniques such as determining metrics (closeness, density, etc.). SNHD, though, has recently applied basic network theories to a TB outbreak investigation among a social network of people engaged in the sale and use of methamphetamine (Mitruka, 2014). A major purpose of this research is to apply analytical techniques to determine specific risk factors for TB disease transmission, as well as prevention methods, using the social networks considered by SNHD.

The State of Alabama uses a unique model where prioritization of contact investigations is determined by risk factors most common to Tuberculin Skin Test (TST) results. This model was developed as a collaborative effort between the Alabama State Health Department and the University of Alabama, Birmingham (Bailey, 2002; Gerald, 2002; Psu, 2009), and will be discussed in detail after the SNA method.

Source: adapted from state TB program websites, 2014; ?=unknown; CDC=Centers for Disease Control and Prevention: CCA=Concentric Circle Analysis: SNA=Social Network

Analysis; SN	Social Netv	vork (only, no ana	alysis); CPM=	Contact Priority Mod	lel
State	<u>Model</u>	State	Model	State	Model
Alabama	CPM	Kentucky	CDC	North Dakota	CDC
Alaska	CCA	Louisiana	CCA	Ohio	CDC
Arizona	CCA	Maine	?	Oklahoma	CCA
Arkansas	CDC?	Maryland	CCA	Oregon	CDC
California	?	Massachusetts	CCA	Pennsylvania (Phil)	CDC
Colorado	?	Michigan	CCA	Rhode Island	CDC
Connecticut	?	Minnesota	CDC	South Carolina	?
Delaware	?	Mississippi	CCA	South Dakota	CDC
Dist. of Col.	?	Missouri	?	Tennessee	CCA,SNA
Florida	CCA	Montana	?	Texas	?
Georgia	CDC	Nebraska	CCA	Utah	CCA
Hawaii	?	Nevada C	CDC(SN)	Vermont	CDC
Idaho	CDC	New Hampshire	?	Virginia	CCA
Illinois	?	New Jersey	?	Washington	CDC
Indiana	CDC	New Mexico	?	West Virginia	CDC
Iowa	CDC	New York	CCA	Wisconsin	CCA
Kansas	CCA	North Carolina	CDC	Wyoming	CDC

Table 6. Summary of State Case and Contact Investigation Models.

COMPARISON OF THREE ANALYTICAL MODELS USED FOR TUBERCULOSIS CONTACT INVESTIGATIONS

(1) CONCENTRIC CIRCLE ANALYSIS (CCA)

CCA is the most common model, used for TB contact investigations, by states that list a specific model (Table 6). CCA was first formally demonstrated in the early 1990s

by J. Veen (1992) who considered previous research by Shaw (1954) and Geuns (1975) when he created the concentric circle model. Shaw had shown that household contacts of sputum smear-positive cases had the highest risk of infection, and Geuns acknowledged the bacteriological aspect, but added another risk factor, intimacy of contact. This model is valuable because it has a social aspect and it is based on infectious disease concepts that consider sputum smear results. The model prioritizes contacts based on risk factors and exposure to the index case. As an example, a 3-year old child with a mother who is sputum-smear positive is a high-priority contact, actually the highest priority contact based on age and exposure. Practical application of this model is simple, interview the source case and work outward to locate the contacts considering the various social settings. The limiting question is how far out from the index case should investigations stop? Figure 17 shows the concentric circle model again with a hypothetical calculation for contact investigation determination. This hypothetical calculation is based on a case study by the CDC (1999). Table 7 shows Individuals A through K who have been evaluated as contacts of an infectious TB case. The skin test indurations are listed along with the category of the result. A 5-mm induration or greater is considered to be a positive TST. Contact conversions are individuals who initially had negative skin test results, but a subsequent test showed conversion to a positive result. The following is a summary table of the results:

Table 7. Hypothetical Calculation of Infection Rate Using the Concentric Circle Model. Source: adapted from CDC, 1999

Contact conversions	Negative reactions	Initial positive reactions
Individual A (11 mm)	Individual D (4 mm)	Individual J (11 mm)
Individual B (10 mm)	Individual E (2 mm)	Individual K (13 mm)
Individual C (8 mm)	Individual F (0 mm)	
	Individual G (3 mm)	
	Individual H (0 mm)	
	Individual I (4 mm)	



Figure 17. Graphical Representation of the Concentric Circle Model Approach to Tuberculosis Contact Investigations. Source: adapted from Etkind, 1993.

Using a 5-mm induration as the minimum TST positive result, the infection rate is $(5/11) \ge 100\% = 45\%$. Five of the eleven individuals exceeded the 5-mm cut-off. Initial TST results and TST conversions from negative to positive must be considered. This exceeds the background infection rate of 12%, therefore contact investigations are expanded outward to medium priority contacts and further if necessary until the 12% infection rate is reached. This case study used an example background infection rate of 12%. The presence of contact conversions also necessitates further contact investigation. The major limitation to the CCA model is determining the background rate of LTBI which is difficult to determine and usually varies from 5-10% in the U.S.; however, frequently screened groups such as health care workers can raise the background rate to 20% (Webb, 2003). Active TB is reportable, and incidence and prevalence rates can be determined, but LTBI is not reportable. Therefore, it is much more difficult to determine the infection rate as opposed to the disease rate. The CDC (2005) considers this model valuable and practical, but limited based on this principle. Other limitations cited by the CDC are: surrogates for estimating exposure (household contacts, for example) do not predict infection; and susceptibility and vulnerability of contacts is not considered. CDC states that CCA does have simple and intuitive value and it is cost-effective because the contact investigation is not continued until evidence of transmission exists which presents unnecessary interviewing and skin testing.

(2) SOCIAL NETWORK ANALYSIS

The theories of social networks have been shown to be effective when applied to public health, specifically through transmission network analysis of infectious diseases.

It is becoming more common for infectious diseases such as tuberculosis, but normally for outbreak investigations. In fact, the majority of studies using SNA involve outbreak investigations (STD, SARS, HIV/AIDS, influenza). The CCA model is simple and easy to use, and health districts are not fully aware of the benefits of SNA, therefore the CCA model is the most common. CDC does mention SNA in its guidelines (CDC, 2005, 2006), but only in the context of an outbreak with more prospective studies being necessary. CDC states that SNA is an effective way to list TB contacts and assign priorities. The following are the major limitations of SNA (McKenzie, 2007):

1) Training of staff in software use and output interpretation

- 2) Resource, time, and labor intensive
- 3) Strict data management

(3) CONTACT PRIORITY MODEL (CPM) (Bailey, 2002: Gerald, 2002; Psu, 2009)

The CPM is a collaborative effort specific to the State of Alabama. The Alabama Department of Health and the University of Alabama, Birmingham developed a predictive model to identify positive TSTs during contact investigations. This predictive model uses case, contact, and environmental exposure variables to develop a practical decision tree for use by health professionals during contact investigations. From January to October 1998 demographic and interview data were obtained for 292 consecutive TB cases and 2941 associated contacts. Using a Generalized Estimation Equation (GEE), a type of regression analysis common with longitudinal analysis of non-independent variables, specific risk factors were derived which were most associated with a positive TST. These factors were sputum-positive smears, cavitation, and hours of exposure to the

contact per month. With a false-negative rate of less than 10% and a reduction in administered TSTs by 40%, health care professionals were able to provide more costeffective skin testing without compromising disease control. The predictive model had to be applicable and readily usable to health department staff when conducting contact investigations so a decision tree was created. Classification and Regression Tree (CART) analysis was used to develop the decision tree because it provided the ability to incorporate the previous predictive model with categorical values. This allowed for a stepwise progression of simple 'yes' and 'no' answers to determine whether to administer the TST. Demographic and clinical decision trees were created to further simplify the investigation process. The decision trees had high sensitivities (87-94%) while maintaining a false-negative rate approximately equal to the background rate of LTBI.

A full cost-effectiveness analysis of the predictive model was conducted and compared to the conventional CCA model. A computer simulation was run using 1000 healthy adults with a background LTBI rate of 10% to determine long-term costs and benefits of the model. As a result of conducting 40% fewer TSTs, the CPM saved \$45,000 over the lifetime of the cohort, but only led to 0.5 additional TB cases detected with 0.24 fewer years of life. The CCA, while more effective, cost \$92,934 to prevent one additional case of TB, and \$185,920 to gain one additional life year, which proved to be more costly than the CPM.

TB GENOTYPING

BASICS

An elaborate discussion of tuberculosis genotyping is beyond the scope of this research, however a basic discussion is warranted especially as it relates to the

epidemiology of tuberculosis. SNHD recognizes the importance of tuberculosis genotyping, and a future research need might be determining the association between genotyping and contact investigations based on locations (Cronin, 2002) in Clark County, Nevada.

Tuberculosis genotyping combined with traditional case and contact investigations is under the broad field of molecular epidemiology (Cronin, 2002; Wootton, 2005) and is becoming more common since the introduction of Polymerase Chain Reaction (PCR) (CDC, 2014b; Cronin, 2002).

CDC recognizes three methods of tuberculosis genotyping: spoligotyping, Mycobacterial Interdispersed Repetitive Units (MIRU) analysis, and IS (Insertion Sequence) 6110-based (Van Embden, 1993) Restricted Fragment Length Polymorphism (RFLP) analysis. Spoligotyping and MIRU are PCR-based methods, and RFLP is generally used as a more definitive test if isolates have matching genotypes based on spoligotyping and MIRU analysis (CDC, 2014b). Genotyping has the following advantages (CDC, 2014b):

- 1) Outbreaks will be detected earlier and controlled more rapidly.
- Incorrect TB diagnoses based on false-positive culture results will be identified more easily.
- Unsuspected relationships between cases, and new and unusual transmission settings will be discovered.
- Transmission that occurs between patients who reside in different jurisdictions will be detected more readily.

5) TB programs will be able to evaluate completeness of routine contact investigations and progress toward TB elimination by monitoring surrogate measures of recent TB transmission

Advantages 3, 4, and 5 have provided insight into the case and contact investigation process and have confirmed the need to reevaluate the contact investigation process to include social determinants of tuberculosis transmission (CDC, 2014b; Cronin, 2002). Social network analysis can identify personal risk factors as well as risk factors based on locations, more importantly locations that extend beyond a local and regular TB contact network that has always been considered the traditional network: household, close relative, and close friend (Cronin, 2002). This is especially important for pediatric cases where transmission has always been assumed to be in a household setting with the source case being an infectious adult (Schaaf, 2003). Table 8 shows a comparison of a traditional TB network to a nontraditional TB network based on contact investigations and RFLP cluster investigations (Cronin, 2002). The value of RFLP is shown in this table based on an analysis of traditional and non-traditional networks. For example, of 28 total patients in a traditional household setting, 25 were identified by routine contact investigations, whereas RFLP only established a household setting in 3 of the 28 patients. The most surprising result is the nontraditional bar setting where 10 patients were known to be associated with this setting, but contact investigations only identified one patient within this setting, whereas cluster investigations involving RFLP identified 9 of the 10 patients. Standard contact investigations seem to be missing common social settings, and investigators may be presuming a traditional setting when the setting may be a nontraditional setting. Other advantages of genotyping are (CDC, 2014b):

- 1) Identifies genetic links between *Mycobacterium tuberculosis* isolates from different TB patients
- 2) Aids in confirming that two TB patients having isolates with non-matching genotypes are not involved in the same chain or recent transmission
- 3) For pediatric cases genotyping can help refute or confirm household transmission which can aid in source case and contact investigations.

Table 8. Identified Transmission Settings for 114 Patients with Recently Acquired Tuberculosis (TB).

Source:	ada	pted	from	Cronin,	2002

Settings	Total patients with known settings (%)	Setting identified by routine contact investigation (%)	Setting identified by DNA cluster investigation (%)
Traditional			
Household	28 (24.6)	25 (34.7)	3 (7.1)
Close relative	13 (11.4)	13 (18.1)	0
Close friend	17 (14.9)	11 (22.2)	6 (14.3)
Nontraditional			
Hospital (24,28)	10 (8.8)	5 (6.9)	5 (11.9)
Other workplace	6 (5.3)	6 (8.3)	0
Social club (26)	11 (9.6)	7 (9.7)	4 (9.5)
Homeless	5 (4.4)	0	5 (11.9)
Bar	10 (8.8)	1 (1.4)	9 (21.4)
Prison/jail (26)	5 (4.4)	3 (4.2)	2 (4.8)
Store (27)	2 (1.8)	0	2 (4.8)
Church	2 (1.8)	0	2 (4.8)
Nursing home	2 (1.8)	0	2 4.8)
School	1 (0.9)	0	1 (2.4)
Ship	1 (0.9)	1 (1.8)	0 (2.4)
Mortuary (29)	1 (0.9)	0 (1.4)	1 (2.4)
Total	114 (100.0)	72 (100.0)	42 (100.0)

Figure 18 shows that cases 3, 5, 6, 9, and 10 have the same RFLP pattern and thus are considered to be epidemiologically linked (CDC, 2014b). TB genotyping is best done in conjunction with case and contact investigations because there are limitations to genotyping such as laboratory contamination, DNA-sequence mutations, lack of discriminatory power of the genotyping test (PCR vs. RFLP), endogenous reactivation versus exogenous reinfection, and transmission of common endemic strains in relatively closed populations (CDC, 2014b). For example, in Figure 20 cases 3, 5, 6, 9, and 10 are considered to be epidemiologically linked, however a false-positive could occur as a result of a laboratory error when a specimen containing *M. tuberculosis* contaminated a non-*M. tuberculosis* specimen falsely showing that they are a match.



Figure 18. TB Genotyping Showing an Epidemiological Link Between Cases 3, 5, 6, 9, and 10 Source: CDC, 2014b

CHAPTER 3 - METHODOLOGY

STUDY DESIGN

The study design was longitudinal (years 2010, 2011, and 2012) involving secondary data analysis of TB cases and contacts based on an empirical social network model that represents the current burden of disease (cross-sectional) in Clark County, Nevada. Data were collected analyzed retrospectively based on TB case and contact links and connections. This TB network represents the theoretical TB population (not sample) in Clark County. Empirical data are data obtained from actual observations and experimentation based on a systematic investigation process (Rychetnik, 2002), and thus best represent the current burden of disease in a population.

For the purposes of this research, data were obtained from interviewing and medical evaluations during TB case and contact investigations conducted by the SNHD. A starting year of 2010 was chosen because the risk factors identified by the Nevada State Health Division occurred during this year. An ending year of 2012 was chosen to provide a means of comparison to national TB statistics, where 2012 data are the most recent published statistics by CDC that provide multi-year state and national comparisons through the Online Tuberculosis Information System (OTIS). The following are specific study design criteria based on network theory:

A) Description of an existing real-world structure or system (Luke, 2007) where the proposed network model represents a real-world structure that shows links between TB cases and contacts based on empirical data.

- B) Whole-network (Hanneman, 2005) that shows all TB case and contacts for 2010, 2011, and 2012 in Clark County, Nevada.
- C) Two-mode network where TB cases are one mode and TB contacts are the second mode (Wasserman, 1994).
- D) Network boundary (Hanneman, 2005): Clark County, NV which includes the following municipalities: Las Vegas, North Las Vegas, Henderson, Mesquite, and Boulder City.

NETWORK ATTRIBUTE DATA

Conventional epidemiological studies analyze attributes at the individual level; however, network analysis requires the creation of a network based on a matrix (Borgatti, 2013; Valente, 2010). Table 9 shows attribute data used in conventional epidemiological research in comparison to network matrix data. With conventional epidemiological data, a sample population is chosen based on specific attributes and this sample population is then followed through time, for example, to determine if a specific exposure will lead to a specific disease. Attribute data are N-by-k with N subjects being measured on k attributes, whereas network data are N-by-N (Luke, 2007) where subjects are compared for linkage and connections. Table 9 is a simple example of a friendship network using binary data in which 1 = friend and 0 = not a friend. In this example, individual A is only friends with individual D. Data in this format are called matrices, which are the foundation of network theory.

Through the thorough and systematic process of TB case and contact investigations conducted by SNHD, the cases and contacts have already been determined, therefore, a

network research study is often cross-sectional in nature (Hanneman, 2005). Because the State of Nevada has identified specific risk factors, as previously mentioned (Paulson, 2010), network analysis is ideally suited to determine disease transmission potential based on whether a case or contact is connected, directly or indirectly. This is the important social

				ATTRIBUT	Έ		
ID	Age	Gender		Education	# pa	artners	Diagnosed
1	25	Μ		low	_	7	Y
2	32	F		low		3	Ν
3	33	F		high		4	Ν
4	34	Μ		medium		4	Y
				NETWORF	X		
		ID	A	В	С	D	
		А	0	0	0	1	
		В	0	0	1	1	
		С	0	1	0	1	
		D	1	1	1	0	

Table 9. Conventional Epidemiological Attribute Data vs. Network Matrix Data. Source: Luke, 2004

aspect of disease transmission. The focus is common social setting, such as a school or daycare center, or is there a household relationship? Ultimately, forming these links and connections between cases and contacts allows comparison of attribute data, which can lead to more effective case management and treatment. If there is a common pediatric risk pattern for children at daycare centers, it can be found using network analysis; targeted prevention, education, and case management can be conducted, which allows for a more effective use of limited resources. The network data used for this research are defined as complete or bounded data (Luke, 2004) because they are based on prior identification of network members (TB cases and contacts); thus, they are also secondary data.

MANAGEMENT OF PROTECTED HEALTH INFORMATION (PHI)

Social network research encompasses many types of data (qualitative, quantitative, categorical, etc.) and these data are inherently descriptive and fall under the Health Information Portability and Accountability Act (HIPAA) (45 CFR 164.514) as being sensitive data that can be used to identify research subjects. Any such research, particularly that which is based in infectious disease transmission, must provide a benefit to society. The benefits of the research as a whole must be greater than any risks or consequences to the subjects. Secondary data are existing data collected, and the assumption is made that collection of these data adheres to HIPAA. Analyzing secondary data requires deidentification of specific descriptive data, and transmission network research requires the use of TB case and contact individual data, not just the connections between the cases and contacts. Identified data must be used to determine personal attributes and risk factors that may contribute to the increased potential for infectious disease transmission. Knowing how these personal risk factors and attributes are associated with infectious disease transmission will provide local health agencies with the best and most effective systematic approach for diagnosis, treatment, prevention, and control of TB in all its particular phases, such as latent infection, active disease, primary pulmonary, post-primary pulmonary, reactivation, MDR, and XDR. Social network analysis was first applied to the initial HIV/AIDS epidemiological investigation in the early 1980s (Auerbach, 1984) and played a vital role in determining the mode of
transmission, as well as prevention and control measures, even without knowledge of the causative agent of the disease.

To disclose a limited data set, a covered entity (SNHD) must enter into a data-use agreement with the recipient (researcher), who agrees to use or disclose the PHI for limited purposes. Disclosure of a limited data set is not subject to the accounting requirement, but must meet the minimum necessary standards of the Privacy Rule/HIPAA (45 CFR 164.514).

As determined through the literature review, identified data are necessary to establish TB case and contact links. Multiple cases and contacts may be associated as determined by SNHD tuberculosis case and contact investigations. To determine if a TB contact is common to multiple TB cases, linking (matching) must be done, and using the most demographic data (first name, last name, age/DOB, and country of origin) will provide the highest matching percentage. Age is particularly important as this research is mainly focused on pediatric TB. Although pediatric TB is the main focus, all cases and contacts must be evaluated to provide the most representative TB network in Clark County, Nevada. Tuberculosis case and contact data collected by SNHD during the course of an investigation are considered PHI, and many elements of these data are private and confidential. Although these data have already been collected, HIPAA is still applicable. Because the research involves existing data from TB cases and contacts over a 3-year period, it is not feasible to obtain authorization for data use from each case and contact, of which there are over 200 cases and 3000 contacts; therefore, a waiver of authorization was obtained. A data management plan, as follows, was approved by the UNLV Institutional Review Board that will protect the identifying data (first name,

last name, age/DOB, etc.) from improper use and disclosure. A unique identification number was assigned to each tuberculosis (TB) case and contact, and all references to cases and contacts utilized this numbering system. Data receipt, storage, and analysis was done within the confines of the SNHD TB Annex Facility located at 400 Shadow Lane, Suite 104, Las Vegas, Nevada, 89106. A dedicated laptop computer was used for data receipt, storage, and analysis during the study. All necessary word processing and statistical programs were pre-loaded with a full antiviral scan performed prior to data receipt from SNHD. The dedicated laptop computer was never connected to the internet, and always remained inside the SNHD TB Annex Facility. The unique identification number will be used for public presentations and manuscript preparation to avoid PHI disclosure. For UNLV review/audit purposes, the data, deidentified using the unique identification number, will be stored in the Principal Investigator's (PI) office located in the Bigelow Health Sciences (BHS) building, room 516 for a period of 3 years. Final disposition or destruction of all data will involve storage of de-identified data at UNLV in the office of the PI as mentioned above. If necessary, electronic data will be deleted from the hard drive by Office of Information Technology staff, and hard copies will be shredded. A HIPAA training course as mandated by SNHD was completed, and written assurances of protection of applicable protected health data were provided.

CREATING THE TB NETWORK

Five distinct steps are necessary for creating the TB network: defining the network boundary, case and contact matching, creating the matrix, linking the attributes, and visualization (Hanneman, 2005; McElroy, 2003; Borgatti, 2013; Valente, 2010; Wasserman, 1994; McKenzie, 2007):

DEFINING THE NETWORK BOUNDARY

In theory, the network boundary represents the entire population, defined as all TB cases and contacts in Clark County, Nevada. It is important to analyze all cases and contacts (not just pediatric) because indirect contacts provide a more thorough representation of potential disease transmission. Clark County has well over 80% of the total cases of TB in Nevada (Nevada State Health Division, 2013) and thus provides the best representation of a TB network. As per the study design, the network boundary is defined as locations under which SNHD has authority: Clark County, which includes the following municipalities: Las Vegas, North Las Vegas, Henderson, Mesquite, and Boulder City (Nevada Revised Statute 439).

CASE AND CONTACT MATCHING (McElroy, 2003)

SNHD stores TB cases and contacts on Microsoft Excel spreadsheets, where one file contains case information along with an associated line list of contacts with demographics. The primary intent of creating the network is to determine if multiple contacts are associated with cases, specifically pediatric cases. Pediatric cases represented no more than 16% of the entire number of TB cases from 2010 to 2012 (Nevada State Health Division, 2013) in the entire state and creating a network will help isolate these cases for further case management. A longitudinal analysis can help determine if the same cases are present each year and if a pediatric subgroup is expanding or connections are changing. Matching contacts and cases was done using first name, last name, age/DOB, gender, and country of birth. Three separate networks

were created: 2010, 2011, and 2012, and each network had all cases and contacts for that specific year with the pediatric subgroup(s) isolated. Once matched, creation of the network matrix was the next step.

CREATING THE NETWORK MATRIX

The matrix is the foundation of a network. Whereas Table 8 shows data arranged in a binary (0, 1) adjacency matrix, where A, B, C, and D represent nodes, and 0 = noconnection and 1 = connection, a TB network matrix is better exemplified by Table 10, where A, B, and C represent cases, and D, E, and F represent contacts. This is a two- mode matrix that can be visualized as shown in Figure 17. The TB network matrix was created using the social network analysis software Ucinet version 6 for Windows (Borgatti, 2002). This program allows data entry in a more user-friendly nodelist format. This is the preferred method in a TB network because contacts will greatly outnumber cases, and "0" is not required for each cell where a case and contact are not linked.

Table 10. Nodelist Format for TB Case and Contact DataSource: Borgatti, 2002

Nodelist1 = case, contact 1, contact 2 (row x column format)

Case AContact DCase BContact DCase CContact F

LINKING THE ATTRIBUTES

The Ucinet social network analysis program (Borgatti, 2002) allowed a separate attribute file to be imported and linked to the matrix. The goal of this step is to apply

pertinent case and contact attributes and analyze the pediatric cases with respect to the attributes of their contacts. The State of Nevada has identified specific risk factors listed in the "public health problem" section, and the idea is to determine if these risk factors are still present, and if so, how targeted interventions can be applied in a cost-effective manner. Linking attributes also provides insight into potential emerging risk factors where a longitudinal analysis can help identify trends.

VISUALIZATION

Based on the matrix created in step 3, graphical representation of the data was created using the Netdraw function of Ucinet (Borgatti, 2002). An example of this visualization is shown in Figure 19, where nodes A and D, nodes B, D, and E; and nodes C and F are connected based on the line list. Appendix A shows several examples of network diagrams, including an actual TB transmission network graphical representation of a CDC outbreak investigation in Oklahoma City in 2002 (McKenzie, 2007).



Figure 19. Network Diagram Created from Nodelist Data in Table 9. Source: Borgatti, 2002

NETWORK METRICS

Network metrics are measurements of structural and locational properties of a network (Wasserman, 1994). Networks have been used as descriptive models for TB (McElroy, 2003), where attributes of cases and contacts, such as age and gender, have been used to show the current burden of TB within a community. While basic demographics are vital for determining current TB burden, the proposed methodology of this dissertation attempts to explain not only the current burden of pediatric TB, but also the risk factors for disease transmission as it relates to pediatric TB cases and contacts, thus network metrics are incorporated. Essentially, determining the risk factors of individual TB cases and contacts is the first half of a network transmission model. Network metrics provide quantitative measures of analysis, which is the second half of a network transmission model. Structural and locational properties of a network can be analyzed at the individual, group, and network level. The following network metrics were calculated for the related research questions: betweenness (research question 2), clustering coefficient (research question 3A) and density (research question 3B).

The concept of centrality is the basis for all the individual metrics within a network, and can be extrapolated to group metrics. Individual and group metrics were determined in this study, and these are further described below. Centrality is defined as "the extent to which a person inhabits a prestigious or critical position in a network" (Valente, 2010). The reach, degree, closeness, and betweenness are all types of centrality measures because having a central position can relate to a personal contact, a group contact, multiple group contacts, and entire network contacts. Depending on the application of the network, centrality can have different meanings. In the context of

infectious disease transmission, a central position within a group, groups, or networks often represents the source of disease transmission. Transmission networks involving infectious disease transmission will have the index case at the central position. In information networks, the position is critical for the flow of information; however, in infectious disease transmission the central position is critical for the spread of the disease. Thus eliminating the most central positions will fragment the network and prevent further disease transmission. Therefore, centrality is therefore a valuable metric overall to determine locations for vaccination and quarantine, and more specifically DOTS, education, and contact prioritization for TB. Locating a central figure in a pediatric TB cluster can provide clues for case management and follow up because a central figure may have risk factors for potential disease transmission within a pediatric population. The concept of centrality has also been applied to the concentric circle analysis method (Veen, 1992) of TB contact investigation, where the case is the central figure and all contacts are determined based on proximity to the index (central) case.

A TB transmission network is consistent with Granovetter (1973), many cases with strong direct ties to contacts that are most likely friends, family, and occupational with a few weak ties that stretch beyond a "local" network. Auerbach (1984) and Mackenzie (2007) show infectious disease transmission networks which have similarities and differences. Auerbach shows the social network created during the initial HIV/AIDS investigation where Patient '0' is at the center of the network that resembles a star graph. Extending out from the star graph shape is the dendritic "branching" structure seen commonly in Sexually Transmitted Diseases (STDs). In Mackenzie (2007), the network is a TB transmission network in which the star graph pattern is dominant, where each TB case is centrally located, and the contacts radiate outward from each case. All the cases and associated contacts seem to be in isolated clusters, which is consistent with Granovetter (1973). Networks are dominated by strong local ties between cases and contacts, and held together by weak contacts. It is these weak contacts that tie the entire network together.

INDIVIDUAL METRICS

DEGREE (CENTRALITY)

Degree is simply the number of links to and from a person, or node, in a directed network. TB transmission networks are usually considered undirected (Klovdahl, 2001). The CDC/Oklahoma State TB outbreak investigation also used undirected ties (McKenzie, 2007) because only a simple connection between a case and a contact was necessary (Appendix A). If a case has a degree of 1, the case has 1 contact.

REACH (CENTRALITY)

Although degree is an important network metric, it only measures an individual connection to another node (or a connection between a case and contact). A node may have a high degree (large number of individual connections), but a lesser reach. For this reason, reach is a better predictor of the spread of a disease.

Reach is the general term for indirect, or secondary, connections (McKenzie, 2007). As a metric, 2-step reach calculates the number of connections beyond the direct contact. In other words, how many nodes can a case or contact "reach" in two steps. Figure 20 shows a comparison between degree and reach. Diagram (a) shows a connection between a case and a contact. Diagram (b) shows the two-step reach that extends beyond a direct connection and includes indirect connections of a case. Two-step reach is a better indicator of disease transmission because it includes indirect connections. This metric is also normalized, so an increased score means that an individual can reach a larger number of cases or contacts within the entire network in two steps. A larger 2-step reach increases the potential for disease spread if the individual has infectious TB simply because he or she has a larger number of indirect contacts. The



Figure 20. Graphical Representation of (a) Degree and (b) 2-Step Reach Metrics Source: Hanneman, 2005: Borgatti, 2013

2-step reach metric is also normalized. This metric is commonly referred to as 'k'-step reach where k=the number of steps, in this case two. Ucinet allows calculation of different k values, however only k=2 was calculated in this study. Whereas, betweenness is related to being a common contact, reach is not necessarily related to having common contacts. However, individuals with a higher betweenness will also have higher reach scores. Because there were very few common contacts in the 2010, 2011, and 2012 networks, 2-step reach may be a better predictor of disease transmission within a network.

BETWEENNESS (CENTRALITY)

Betweenness centrality "is a measure of how often a given node falls along the shortest path between two other nodes" (Borgatti, 2002). Betweenness is a measure of

centrality. In Figure 21, diagram a, the case and contact make a dyad. For the case and contact to reach each other, there is no node in between; however, in diagram b there is a central contact that will have a high betwenness score because, for the other contact to reach the case, he/she must pass through the central contact; however, the case and contact on the end will have a zero score because they have no other connections. Cases and contacts with high betweenness scores are usually bridges between individual network clusters. In McKenzie (2007) (Table 11) the top-20 scores are shown, as are the lowest 5, for TB cases and contacts of a TB outbreak in Oklahoma City. Many of the scores are identical and are ranked sequentially when the scores start to repeat. Because TB cases and contacts are either connected or not, the metrics are simple proportions between 0 and 1 based on the binary code entered in the spreadsheet. When all cases and contacts are assigned a binary value, each case and contact can be analyzed relative to all others. For example, node 1, the index case has a betweenness score of 0.849 which means that it can be linked, directly or indirectly, to 84.9 % of all cases in the network. Node 1 has a much smaller degree (i.e., 0.385), which means it has many indirect connections relating perhaps to a specific social setting. Node 9 only has 1 connection, node 1 the index case, so its degree score is very minimal. However, its one connection is the index case so it has a much wider reach. A reach will be much greater if a connection is to a TB case, as opposed to a contact, because the risk of disease transmission is greater. The betweenness score is more intuitive. TB case 9 has only one connection; therefore, it is a terminal contact of the index case, thus it has no centrality. Many of the metrics are proportions, or percentages, because of the simple binary nature of the data. Case 1 is the index case so it is logical that it has the highest reach, degree, and

betweenness scores. McKenzie, et al. (2007) used high betweenness scores to prioritize contacts during a TB outbreak; however, in this research, the scores were used to prioritize contacts for case management.

Table 11. Degree, Reach, and Betweenness Scores of a Tuberculosis Outbreak Investigation. Source: adapted from McKenzie, 2007. Highest 20 Scores

Hignest 20 Scores										
	Reach		Deg	gree	Betwee	Betweenness				
Score/Rank	Node	Score	Node	Score	Node	Score				
1	1	0.830	1	0.385	1	0.849				
2	1135	0.538	8	0.253	8	0.289				
3	1268	0.538	14	0.110	12	0.208				
4	1777	0.538	33	0.099	2	0.187				
5	1793	0.538	19	0.071	14	0.179				
6	1797	0.538	18	0.066	1833	0.179				
7	1799	0.538	22	0.060	33	0.128				
8	1800	0.538	29	0.038	19	0.118				
9	1813	0.538	35	0.038	5	0.104				
10	1861	0.538	12	0.033	17	0.095				
11	1868	0.538	13	0.027	2034	0.064				
12	1869	0.538	17	0.022	18	0.062				
13	1889	0.538	21	0.022	35	0.054				
14	1905	0.538	3	0.016	1239	0.043				
15	1910	0.538	1135	0.016	13	0.033				
16	1924	0.538	1268	0.011	22	0.033				
17	1925	0.538	1777	0.011	29	0.018				
18	1929	0.538	1793	0.011	30	0.011				
19	8	0.538	1797	0.011	6	0.011				
20	2	0.516	1799	0.011	7	0.011				
Lowest 5 Scores										
5	1935	0.022	25	0.005	3	0.000				
4	25	0.022	34	0.005	34	0.000				
3	1253	0.011	37	0.005	37	0.000				
2	15	0.011	4	0.005	4	0.000				
1	1854	0.011	9	0.005	9	0.000				

GROUP AND WHOLE-NETWORK METRICS

DENSITY

Network density is a common metric that can provide critical information regarding disease transmission (Hirsch, 2013). It is simply the number of ties within a network divided by the total number of possible ties and can be calculated for an egonetwork or a whole network (Retrum, 2013). An ego-network can be defined as an individual, or personal, network where TB contacts surround a common central source case and the source case represents a focal node (ego) (Hanneman, 2005). Density is commonly reported as a metric for other infectious diseases, namely STDs (Doherty, 2005) where denser networks represent higher contact rates, increasing the risk of transmission. This same principle of density and transmission risk can also be applied to TB. A logical assumption might be that as pediatric TB cases have increased, the TB transmission network has increased from 2010 to 2011, and 2011 to 2012 resulting in density increases within these time periods. An obvious limitation to the density metric is that the network is affected by incidence, and an increase or decrease in new cases will not provide an adequate comparison year-to-year. Hypothetically, if 2010 density is 8 connections per 10 total (density = 80%), 2011 density is 8 connections per 15 total (density = 53%) and 2012 density is 8 connections per 20 total (density = 40%) a valid decrease in density is observed, but there are still eight connections. If those eight connections are between TB cases, the risk may not necessarily be reduced simply because density is reduced. A more complete analysis was conducted as part of research question 3 to calculate and compare the cluster coefficient with the density to determine whether high risk clusters(pediatric population) have increased or decreased (Hanneman,

2005). Clustering coefficient and density are described in more detail below.

CLUSTERING COEFFICIENT

Clustering can be defined as "the tendency towards dense local networks", and in network theory the clustering coefficient is the measure of the average of the densities of the neighborhoods of the TB cases and contacts (Hanneman, 2005). Clustering coefficient and density provide several pieces of critical information based on the method of calculation. Being able to locate and define clusters using density and cluster coefficient metrics allows maximum allocation of resources for targeted screening and prevention programs, such as TSTs, DOTS, and education. Knowing the attributes of the cases and contacts that form the clusters can help determine high-risk areas of pediatric TB within the entire network. The ideal function of cluster analysis is to target high-risk cases and contacts for public health intervention(s). The importance of clustering was demonstrated during the Foot-and-Mouth disease outbreak in the United Kingdom in 2001 (Kiss, 2005) where a clustering coefficient of 0.5 limited the final spatial spread of the epidemic in comparison with an initial clustering coefficient of 0 even when the average number of connections stayed the same at 10. A higher clustering coefficient will limit the spread of disease within the entire network.

Recall McKenzie, et al. (2007) where the TB case was in the center of the star pattern and the contacts usually radiated outward from the center. Although many of these related cases and contacts appear scattered, contacts with high betweenness scores can act as bridges which may form clusters.

Ultimately, it is not just the number of connections, but which nodes are connected. With respect to TB, one must consider cases and contacts, the various demographics (age, race/ethnicity, etc.) of each case and contact, as well as the health state of each case and contact (LTBI, active, no disease/infection, reactivated, DOTS-treated, etc.). Figure 21 provides a realistic scenario regarding two separate networks, a and b, and the relationship between clustering and density, and the importance of who is connected. Consider these two networks as representing two different populations. The density is the same for both, 9 connections divided by 13 total possible connections for each network. It would seem that both networks have the same potential for disease transmission because the node numbers and locations are exactly the same. But, it is not just the overall network density, node numbers and connections; it is also who is connected. Now consider an infectious disease that enters these two networks from the left node at step 1. In two steps, 7 of 8 nodes are infected in the (b) network, where the lone uninfected node is the far right node. In the (a) network, in those two identical steps, only 4 of 8 nodes are infected. One might think that disease clusters increase the spread of disease, but this is not necessarily the case (Kiss, 2005). The (a) network can be



Figure 21. Structural Differences between Two Networks, a and b. Source: Klovdahl, 2005

considered two linked clusters, whereas the (b) network is not as extensively clustered. Disease progression, although not prevented, is slowed because a third step is needed to link the left cluster to the right cluster in the (a) network. Disease transmission in clusters limits the number of viable hosts, and with no other connections to viable hosts, disease transmission is limited within the cluster. This is the basis of herd immunity and the epidemiological triad. In the (b) network, two new transmission routes are provided at the top and bottom of the network which allow transmission to two new hosts.

TRANSMISSION NETWORK DATA

TB case and contact investigations are systematic, complex, and involve the collection of many different types of data. A common template for TB case and contact investigations is the CDC Report of a Verifiable Case of Tuberculosis (RVCT) form. A cursory review of this form provides a good perspective of the detail involved with data collection. Much of the form is divided into clinical data, patient data (demographics, contact information, risk factors, etc.), and test data. SNHD manages case and contact data on a Microsoft Excel spreadsheet, but much of the data are similar to the RVCT form. Case and contact demographic data are collected which provides the basis for determination of risk factors. Much of the data are categorical (sex, race/ethnicity, etc.) with some quantitative data such as TST induration in millimeters.

Transmission network data can take many forms with the most simple form being binary. Binary data simply denote the presence or absence of a connection, link, or tie. These binary data are then used to create the adjacency matrix. For example, if during a case investigation, a case lists a contact during the interview, they form a dyad within the transmission network (2 linked nodes). When creating the adjacency matrix a "1" is placed in the cell within the Excel spreadsheet to denote a connection. A "0" denotes no connection. The network data in Table 9 are set up in an adjacency matrix format. These connections are what create the transmission network. Linking attribute data to the cases and contacts allows comparisons of risk factors based on these attributes. For example, a pediatric contact may be connected to an individual, whose country of origin is not the United States, with active TB disease. This not only provides clues for direct transmission risk to the pediatric case, but also clustering and the spread of disease within the cluster. Transmission network data can also be valued, for example, the number of times two people meet during the week (Hanneman, 2005).

STATISTICAL METHODS IN NETWORK ANALYSIS

Network data analysis requires statistical measures that are non-parametric (Hanneman, 2005; Krackhardt, 1988; Snijders, 1999; Mantel, 1967) for two major reasons. First, network data are autocorrelated (Krackhardt, 1988), and thus interdependent, which is in direct contrast to most public health research that attempts to compare independent and dependent variables. The systematic process of TB case and contact investigation aids in determining which cases and contacts are associated. This systematic process results in a network where all cases and contacts are correlated to some degree. Referring to attribute data versus network data, individuals are not chosen based upon age, gender, etc., the network already establishes the population and links between cases and contacts. Network studies attempt to determine such demographics that provide insight into disease transmission. The connections have already been

established. The goal is to identify individual, group, and network characteristics based on these connections to determine TB transmission risks and more importantly preventive measures and interventions. Second, the systematic process of case and contact investigation provides a network that is theoretically not random, so standard inferential statistical procedures cannot be used (Hanneman, 2005). Public health statistical methods in transmission network analysis are not consistent with conventional public health statistics, which are based on inferential statistics. Inferential statistics involve deriving a sample population from a population and drawing statistical conclusions about the entire population based on this sample population; however, a network represents the theoretical population, not a sample.

BOOTSTRAPPING AND PERMUTATIONS

Bootstrapping (Efron, 1979) can be applied to a transmission network to obtain common inferential statistical parameters (i.e., bootstrap regression). Bootstrapping is a method of constructing artificial data sets from the observed data sets within the entire network. Conducting many permutations (approximately 1000) creates artificial data sets, which can then be statistically analyzed using inferential statistics. For example, the degree metric is a common network parameter and is basically the number of direct connections a node has. In a TB transmission network, it may be beneficial to know the average number of contacts that are listed by a case. The mean degree can then be calculated using permutations to create the artificial data sets, analyzed by bootstrapping with replacement. Permutations simply rearrange the cases, contacts, and links randomly, where each permutation acts as a population sample thus each permutation is a separate sample drawn from a new population, creating independent variables for comparison. Bootstrapping (Efron, 1979) was incorporated into node-level regression, a method of regression analysis applicable to social networks (Hanneman, 2005), which will be the statistical method used for hypothesis 1 where the pediatric contacts with identified risk factors are the independent variables and age (where a pediatric TB case is defined as a case less than 18 years of age) is the dependent variable.

CHAPTER 4 - NETWORK ANALYSIS, HYPOTHESIS TEST RESULTS, AND DISCUSSION

NETWORK DESCRIPTIVE STATISTICS

Table 12 is a summary of the TB network descriptive statistics for 2010, 2011, and 2012. Only the pediatric cases for which contact investigations were done were included in the networks. When connecting cases and contacts, the connections were only based on data as recorded during the case and contact investigations. No assumptions were made

Descriptive Statistics	Year			
	2010	2011	2012	
Total cases	97	85	70	
Total cases with contact investigations	60	64	58	
Total pediatric cases (< 18 years old)	19	11	13	
Total pediatric cases with contact investigations	4	6	6	
Total contacts	1373	1111	1098	
Total pediatric contacts	190	204	221	
Average age of cases	41	45	42	
Average age of contacts	34	33	37	
Gender (% male)	62	53	59	
Number of pediatric subgroups, n (%)	43 (72)	44 (69)	40 (69)	
Foreign country of birth – % total cases	68	73	76	
Foreign country of birth – % pediatric cases	21	36	54	

Table 12. Tuberculosis Network Descriptive Statistics: 2010, 2011, and 2012

as to indirect connections. In other words, transitivity was not implied. For example, if two contacts were listed by a source case, these two contacts were connected to the source case, but not to each other. Transitivity is based on the algebraic principle where if a=b and b=c then a=c. This is commonly visualized as an egocentric layout where the source case is the ego, or centrally located, and the contacts are the altars (non-central nodes). The egocentric layout is common in a TB network model (McKenzie, 2007). The purpose of conducting contact investigations for pediatric cases is to locate the source case, and many of the pediatric cases were previous contacts, therefore when these previous pediatric contacts developed TB the source cases were already established through common network connections and identification of individual TB risk factors. Cases in 2010 were assigned unique identification numbers 1-100, and the contacts were assigned numbers 1000-2099. Cases in 2011 were assigned numbers 200-299, and the contacts were assigned numbers 3000-4999. Cases in 2012 cases were assigned numbers 300-400, and the contacts were assigned numbers 5000-6000. For many of the pediatric cases, contact investigations were not conducted because young children cannot transmit TB, therefore there is no exposure risk. However, some children were close to 18 years of age and presented an increased risk of transmission so they were investigated for contacts. Pediatric TB cases also require source case investigations which are not necessarily the same as contact investigations. Locating a source case may require contact investigations; however, most likely it is a close household contact (e.g., mother, father, etc.) that is the source case, especially for very young children. In these instances, the source case is located and evaluated with no further contact investigations conducted due to the very young age of the child and the lack of exposure risk. Although not

included in the year-end TB case numbers, several children with LTBI were included in the network because reverse contact investigations were conducted that can provide valuable information regarding risk factors of contacts. With pediatric LTBI (as opposed to active TB) the source case is not readily identifiable, therefore, a true contact investigation was necessary to locate the source case. The term "reverse contact investigation" is used because a standard contact investigation begins with the source case and proceeds outward from close contacts (e.g., mother, father, etc.) to more casual contacts; however, with an unknown source case the process must be reversed. The limited number of pediatric TB contact investigations is a limiting factor, however, network bootstrapping is incorporated to reduce this limitation. Pediatric TB cases for which contact investigations were not conducted were not included in the network because at least a dyad must be present in order to provide valuable metrics. An example of a dyad would be a connection between a case and contact. For example, clustering coefficient cannot be calculated for a single node. Individual cases, however, were incorporated into data analysis when a network connection was not necessary. To assess mother's age as a risk factor for pediatric TB, a connection to a contact was not necessary, with the obvious exception of the mother-to-child connection.

Numerical ranges for case and contact identifiers were created to differentiate the years as well as differentiating cases from contacts. These ranges exceeded the actual number of cases and contacts, and this was purposeful to prevent potential overlap due to data entry errors and to allow cases or contacts to be entered retroactively if necessary. A master spreadsheet was created which contained each year as a separate workbook with all the cases and contacts numbered. The unique identification numbers can then be

traced back to a specific case or contact. Table 13 shows network level metrics as calculated by Ucinet for Windows version 6.503 (Borgatti, 2002). Yearly network metrics were calculated for the whole network, pediatric network (ped), and non-pediatric network. In network analysis, whole network metrics are typically used because they provide the best representation of the network, especially when conducting logistic regression with bootstrapping. However, the pediatric networks and non-pediatric

2010				2011			2012		
			non-			non-			non-
metric	whole	ped	ped	whole	ped	ped	whole	ped	ped
Avg.									
Degree	1.918	1.849	1.847	1.87	1.883	1.92	1.883	1.921	1.87
Density	0.001	0.003	0.009	0.002	0.003	0.003	0.002	0.002	0.009
Connect									
edness	0.193	0.038	0.111	0.044	0.04	0.126	0.057	0.074	0.248
Fragmen									
tation	0.807	0.962	0.889	0.956	0.96	0.874	0.943	0.926	0.752

Table 13: Network Metrics: 2010, 2011, and 2012

networks were calculated for comparative purposes. Consider the 2010 network as an example. A total of 60 cases compose the network; only 60 cases are used because they are the cases with contact investigations. Each of these 60 cases can be considered personal networks that collectively create the entire network. These personal networks can be household networks that contain close contacts such as mothers or fathers. Pediatric networks are simply networks that have a pediatric case or contact, and non-pediatric networks do not contain a pediatric case or contact.

The metrics of interest are average degree, density, connectedness, and fragmentation. The average degree is the average number of connections per case or contact. The networks are arranged in an ego-centric format (Hanneman, 2005) where the

cases are central to the contacts. The density is the number of connections divided by the total number of possible connections. The density metric will be further discussed as part of hypothesis #3. Connectedness and fragmentation metrics were not included in the analysis of the hypotheses, however, they provide alternative methods of understanding networks. Network connectedness is low in comparison to fragmentation because there is only one common contact in the 2010 network. This is the case in all of the networks because there are very few common contacts. Because network matrices are based on 0 =no connection and 1 = connection, the fragmentation and connectedness sum to 1. With respect to disease transmission, a 2010 fragmented network represents a network that is made up of 60 personal networks that are in essence quarantined. All individual and network metrics were normalized, and the average degree metric was calculated to ignore the direction of ties. Incorporating direction of ties implies disease transmission which may or may not occur. The intent of the proposed networks is to simply connect cases and contacts based on contact investigations conducted by the Southern Nevada Health District, therefore the direction of ties is ignored. When ignoring direction of ties, a dyad has a density of 1. When considering ties, a dyad has a density of 0.5 because direction is considered, and a reciprocating tie is not present. Other studies have used a nondirectional Tb network where ties are not considered (McKenzie, 2007; Klovdahl, 2001).

STATISTICAL ANALYSIS

SPSS v.22 (IBM, 2013) and Ucinet v.6 (Borgatti, 2002) were used to calculate advanced statistics, and Microsoft Excel v.2010 was used to calculate basic statistics, such as averages, sums, etc. For analysis in SPSS, a spreadsheet was created in Excel and imported into SPSS. The Excel spreadsheet was set up in a linelist format where the first

column was a list of all the contacts associated with an individual for whom a contact investigation was performed. A second column contained a binary value for each contact. If the contact was a contact of a pediatric case, the value was recorded as "yes" and if the contact was a contact of a non-pediatric case, the value was recorded as "no". This column represented the dependent variable. A third column contained "dummy" variables where "no" from the second column was assigned a dummy variable of "0" and "yes" was assigned a dummy variable of "1". Dummy variables are necessary for bootstrapping analysis, which requires numerical values. A fourth column contained the risk factors to be analyzed, such as country of birth, which represent the independent variables, or predictors. The goal was to determine if a risk factor is more likely to be associated with a contact being connected to a pediatric case. For example, a specific country of birth of a contact may be more associated with a contact being connected to a pediatric case (a "yes" value in the second column). For logistic regression bootstrapping within SPSS, the permutations were set to 1000 and the data were stratified when the independent variable had an outcome that was not binary. For example, country of birth was stratified because multiple countries of birth are listed for cases and contacts, whereas history of incarceration was not stratified because it had a binary outcome of yes or no. Because several analyses were stratified the estimated multinomial logistic regression coefficients (B, in SPSS) were also analyzed. If B was greater than 0 and p was less than 0.05 for a specific risk factor, then that risk factor was considered significant. Bootstrapping, due to a much more conservative estimation, required B to be analyzed. Logistic regression (without bootstrapping), did not require B to be analyzed.

In 2010, 97 cases of TB were reported by SNHD. Of the 60 individuals for which contact investigations were conducted, 57 individuals were counted as Nevada cases in 2010. Three cases were classified as active TB patients, but these individuals did not reside in the USA (cases 4, 41, 47). Because contact investigations were conducted for these 3 patients, they were included in the analysis. Of the 57 individuals who resided in the USA, 12 were diagnosed with clinical tuberculosis and 45 were diagnosed as tuberculosis cases. Pediatric TB cases totaled 19 with 4 cases involving contact investigations (cases 5, 10, 21, and 1067). Case 1067 has a unique number for a contact because this case was a previous contact. Two of these 4 cases had progressed to extrapulmonary TB (cases 10 and 21) and 2 of the 4 (Cases 5 and 1067) had either sputum or culture positive laboratory tests. SNHD defines a TB case as one in which the organism, *M. tuberculosis*, was isolated either through culture or sputum smear. A clinical diagnosis is based on methods other than sole isolation of the organism including, but not limited to, CXR, HRCT, medical history, etc. Pediatric cases are defined as "clinical" or "pediatric", rather than case, because it is very rare for a child to be culture or smear positive due to a paucobacillary load.

In 2011, 85 cases of TB were reported by SNHD. Of the 64 individuals for which contact investigations were conducted, 63 individuals were counted as Nevada cases in 2011. One case of active TB was reported, but the individual did not reside in the USA (case 210), therefore it was not included in the final 2011 statistics. Contact investigations were conducted for this case, therefore they were included in the analysis. Of the 63 individuals who resided in the USA, 16 were diagnosed with clinical tuberculosis and 47 were diagnosed as tuberculosis cases. Pediatric TB "cases" (defined as clinical or

pediatric) totaled 11, with 6 cases involving contact investigations (cases 213, 248, 250, 252, 255, and 256). Reverse contact investigations were conducted for 3 of these cases (all one year of age or less) with LTBI (cases 248, 252, and 255). Because the children did not have active TB, a reverse contact investigation was conducted to find the source case because a child is not a source case. A standard contact investigation begins with the source case to locate active TB cases or LTBI, reverse contact investigations aim to locate the source case. One pediatric contact investigation was incidental because the case was reported at the time of death of the child (case 250).

In 2012, 70 cases of TB were reported by SNHD. Contact investigations were conducted for 58 of these cases, not all were active cases. Three of the contact investigations involved pediatric LTBI with reverse contact investigations being done (cases 300, 315, and 355). One contact investigation involved a suspect case, non-pediatric (case 305), and one contact investigation involved a case that was reported to SNHD in late 2012, but officially counted as a case in 2013 (case 347). Because of the report date and the fact that the contacts were tested in 2012, this was included in the network analysis. Pediatric TB cases totaled 13 with 6 cases involving contact investigations. Three contact investigations were associated with active TB cases (cases 335, 357, and 358) and 3 were reverse contact investigations as previously mentioned (cases 300, 315, and 355). These 3 reverse contact investigations involved children who were 2 years of ages or less at the time of LTBI diagnosis.

It is advantageous to include suspect and LTBI cases into the network model because they can provide insight into potential disease transmission. For example, a subgroup of LTBI cases can be given treatment and monitored over time to determine if clustering coefficient, betweenness, and density are affected within the entire network.

HYPOTHESIS TEST RESULTS (Appendix C)

RESEARCH QUESTION 1

Are the risk factors identified by the State of Nevada Health Division significant? HYPOTHESIS 1

H_o: There is no association between pediatric TB cases and identified risk factors.

H_a: There is an association between pediatric TB cases and identified risk factors.

Risk factor 1:

Many of these cases are children of young mothers, or are young mothers themselves. Table 14 shows descriptive statistics of mothers of pediatric TB cases. For the purposes of this analysis a young mother is considered to be less than 25 years of age. Of the pediatric cases where the mother's age was known and a contact investigation was done, 9 out of 14 total mothers were young mothers in 2010. In 2011, of the 8 total there were no young mothers, and in 2012 there was one young mother out of 6 total mothers. The limitations of this analysis are the definition of a young mother, the small sample size of pediatric cases, and many mothers where the age was unknown.

Table 14: Descriptive Statistics of Age of Mothers of Pediatric Tuberculosis Cases: 2010, 2011, and 2012

				Age					
Year	Ν	Range	Min.	Max.	Mean	Median	St. Dev.	Variance	
2010	14	17	17	34	23.3	25.5	4.5	20.2	-
2011	8	18	25	43	33.6	27	6.5	42.8	
2012	6	20	23	43	33.2	32.5	9.7	95.0	

From 2010 to 2012, mothers' ages were significantly different (p < .05) with the median age increasing each year using the SPSS Median test. The Kruskal-Wallis test demonstrated a significant difference (p < .05) in the distribution of mothers' ages from 2010 to 2012 as well.

For the logistic regression analysis, each TB case was line-listed as a pediatric or non-pediatric case. The contact investigation spreadsheet was searched for cases that had mothers identified as contacts, but many of the cases did not have the mother identified as a contact. The age of the mother of the contact was then placed in the line list. Dummy variables were created for bootstrapping where "no" equals 0 and "yes" equals 1. If the case was a pediatric case, it had a dummy variable of 1 and a non-pediatric case had a dummy variable of 0. An arbitrary age of less than 25 years was used as the definition of a young mother. If a mother was less than 25 years of age, the dummy variable of 1 was used. If not, the dummy variable 0 was used. Using these numerical dummy variables, bootstrapped samples were created. Both logistic regression and logistic regression with bootstrapping were used for comparison.

In 2010, there were 80 cases out of 100 total cases that did not have the mother identified as a contact during the contact investigation. The 100 total cases include the 97 SNHD cases plus the additional 3 cases that were included because they had contact investigations done. Logistic regression did not show a significant association between a young mother and a pediatric case; however, logistic regression with bootstrapping did show a significant association.

In 2011, there were 70 cases out of 89 total cases that did not have the mother identified as a contact during the contact investigation. The 89 total cases include the 85

SNHD cases plus the one non-USA cases and the 3 pediatric LTBI cases because they had contact investigations done. None of the pediatric cases had mothers that were under 25 years of age; therefore, neither logistic regression nor logistic regression with bootstrapping were performed.

In 2012, there were 58 cases out of 75 total cases that did not have the mother identified as a contact during the contact investigation. This includes the 70 SNHD cases, the 3 pediatric LTBI cases, the one suspect case, and the one 2013 case for which contact investigations were done. Logistic regression did not show a significant association between a young mother and a pediatric case; however, logistic regression with bootstrapping did show a significant association (p < .05).

In 2010, 2011, and 2012, no female pediatric TB cases were young mothers. There were seven female pediatric cases in 2010, and all cases were well below child-bearing age (less than 5 years of age). There were six female pediatric cases in 2011, and one female was of child-bearing age; however, a contact investigation revealed that this female had no children. There were six female pediatric cases in 2012, and three females were of child-bearing age; however, through case and/or contact investigations it was determined that none of these females had children.

In summary, the age of a mother was a not a predictor of a pediatric TB case in 2010, 2011, or 2012 using logistic regression, but it was a predictor in 2010 and 2012 using logistic regression with bootstrapping. Logistic regression with bootstrapping resulted in p < .05; therefore, the null hypothesis was rejected, demonstrating a significant association. From a network perspective, in the years 2010 and 2012, bootstrapping rearranged the connections between cases and contacts, and the resulting bootstrapped

samples show a connection between a young mother and a pediatric case that would not result from chance. In other words, a young mother, defined as less than 25 years old, was significantly associated with a pediatric TB case in 2010 and 2012 using logistic regression with bootstrapping. The second part of the risk factor states that there are pediatric cases who are young mothers themselves; however; the female pediatric TB cases in 2010, 2011, and 2012 were found to have no children. There are several limitations to the analysis. First, a large number of cases could not be analyzed because the mother was not identified as a contact during the contact investigation process. Second, the Nevada State Health Division does not define the age of young mothers' when listing them as a risk factor; therefore a young mother was defined as a mother less than 25 years of age. Increasing or decreasing this value would likely change the results. Third, these mother-related risk factors may have been identified prior to 2010 which predates this network analysis. Fourth, these risk factors may exist for cases and/or contacts in other counties of Nevada and although Clark County contains the vast majority of the TB cases within the state, they would not be identified using this network analysis. For risk factor 1 identified by the Nevada State Health Division, these statistical results, in part, support the expected results of a significant association using only logistic regression with bootstrapping.

Risk factor 2

Individuals who have spent time in jails, detention centers, and prison have been identified as contacts to these pediatric cases.

For risk factor 2, history of incarceration is the independent variable, and the dependent variable is a pediatric case. Under the risk category of history of incarceration within the

SNHD case and contact investigation spreadsheet, a case or contact could be listed as the following: Booking, CCDC (Clark County Detention Center), CLVDC (City of Las Vegas Detention Center), juvy (juvenile), classification review, medical clvdc, search, or transport. There was one pediatric contact with LTBI who had a history of incarceration as a juvenile (contact 1574). According to the SNHD categories, cases and/or contacts in CCDC, CLVDC, and listed as juveniles were listed as having a history of incarceration. If the individual was listed as: classification review, medical clvcd, search, or transport, they were listed as not having a history of incarceration. If the case or contact had a blank data cell and none of the aforementioned categories were listed in the comment section of the contact investigation spreadsheet, then history of incarceration was listed as no. This was discussed with Ms. Haley Blake of the SNHD TB clinic (personal communication), and the presumption is that if the data cell is blank AND there are no comments regarding history of incarceration it is acceptable to use "no" for history of incarceration. In 2010, there was a significant association between pediatric cases being connected to contacts with a history of incarceration, using logistic regression and logistic regression with bootstrapping. This association was significant because almost half of the contacts in the network had a history of incarceration. In 2010, there was also a significant association between non-pediatric cases being connected to contacts with a history of incarceration with both logistic regression and logistic regression with bootstrapping (p < .05). In 2012 as well, there was a significant association between pediatric and non-pediatric cases being connected to contacts with a history of incarceration using logistic regression with bootstrapping only. Given these results, history of incarceration does, to some degree, appear to be a good predictor of whether a case will be pediatric. There was an expected

significant association between pediatric cases and contacts with a history of incarceration based on this risk factor being identified by the Nevada State Health Division and statistical analysis demonstrates that this prediction was correct for 2010. The p-value was greater than .05; therefore, the null hypothesis failed to be rejected, demonstrating that this particular contact risk factor was not associated with a pediatric case. History of incarceration for 2010 had a p-value less than .05; therefore, the null hypothesis was rejected, demonstrating a significant association. As with risk factor 1, this analysis is limited because these cases and/or contacts may have been identified prior to 2010 which pre-dates this network analysis, and these risk factors may exist for cases and/or contacts in other counties of Nevada; though Clark County contains the vast majority of the TB cases within the state, they would therefore not be identified using this network analysis.

These results demonstrated the importance of history of incarceration of a contact as a risk factor for pediatric TB cases. A benefit of network analysis is the ability to analyze the network at the group level. When the 2010 network is analyzed at the group level where contacts with a history of incarceration represent a subgroup, there is a common contact with a history of incarceration. This contact (as determined by a contact investigation) is also Case 12, which acts as a bridge between the history of incarceration subgroup and another subgroup that contains pediatric cases and contacts. As a result, Case 12 is a weak tie (Granovetter, 1973) with the highest betweenness score in the entire network. The high betweenness score is also a result of the extremely large number of contacts with a history of incarceration.

Risk factor 3

Most of the cases (especially < 5 years of age) had recent interactions with healthcare providers prior to being diagnosed with TB. Most notably, these cases are presenting in emergency departments and urgent care centers with respiratory or unresolved pediatric issues (e.g., ear infections, gastric symptoms, enlarged lymph nodes without an established infection, or are being seen as part of well baby exams without screening for TB as recommended by the American Academy of Pediatrics).

The SNHD contact investigation form contains a column for "relationship" of contact to the case, and this was used to establish the independent variable of healthcare provider. However, multiple relationships other than healthcare provider are possible within the entire network so the bootstrapping analysis is stratified. Healthcare provider is the independent variable and thus it is expected that it will be a predictor of the dependent variable, type of TB case, pediatric (as opposed to non-pediatric). Stratified bootstrapping may demonstrate other significant relationships in addition to healthcare providers.

In 2010, based on cases in which contact investigations were done, pediatric cases were not significantly associated with health care providers using standard logistic regression or logistic regression with bootstrapping. In other words, having contact with a healthcare provider was not a predictor of whether a case would be pediatric. However, bootstrapping resulted in a significant association between a health care provider and a non-pediatric case. In addition, a relationship denoted as "other" during contact investigations was significantly associated with a TB case being a non-pediatric case.

This significant association was observed with standard logistic regression as well as bootstrapping. Although this is a significant association with a non-pediatric case it demonstrates the value of stratified analysis.

In 2011, based on cases in which contact investigations were done, pediatric cases were not significantly associated with healthcare providers for both standard logistic regression and logistic regression with bootstrapping. In other words, having a network connection with a healthcare provider was not a predictor of whether a case would be a pediatric case. Having a connection between a health care provider and a pediatric case would be solely based upon chance. Non-pediatric cases, however, were significantly associated with healthcare providers using both logistic regression and logistic regression with bootstrapping.

In 2012, based on cases in which contact investigations were done, pediatric cases were not significantly associated with health care providers using standard logistic regression or logistic regression with bootstrapping. However, there was a significant association between non-pediatric cases and network connections to healthcare providers using logistic regression with bootstrapping. In other words, having contact with a healthcare provider is a predictor of non-pediatric case.

In summary, for the years 2010, 2011, and 2012, logistic regression and logistic regression with bootstrapping did not show a significant association between pediatric TB cases and contacts who are healthcare providers. A stratified analysis of case and contact relationships using logistic regression with bootstrapping is a future research recommendation because healthcare providers appear to be significantly associated with non-pediatric cases. Various other relationships such as friends, significant others, etc.

may prove to be significant as well. A common contact is present in the 2011 network (contact 3850) who is a healthcare provider. This contact is a weak-tie who connected 2 networks, however disease spread was not observed because the common contact was evaluated by SNHD multiple times using a TST, and the common contact did not have infectious TB during this time. A common contact can act as a bridge between 2 networks, thus increasing the potential for disease transmission. However, for contact 3850, adequate TB contact management was initiated, therefore, disease transmission did not occur.

It was expected that a contact who is a healthcare provider would be a significant predictor of a pediatric TB case, however, this specific contact risk factor is not a significant predictor of a pediatric TB case. The p-value was greater than .05, resulting in failure to reject the null hypothesis. The limited number of pediatric cases for which contact investigations were done can be an explanation for this result. Incomplete contact investigations may also be an explanation, however this is unlikely because network metrics demonstrate adequate contact investigations. Missing data may also play a role in the statistical results because in 2010, 2011, and 2012 there were 14, 66, and 27 contacts, respectively, for which the relationship to the case was not listed. The spreadsheet data cell was left blank, as opposed to being listed as "unknown". The pediatric cases had recent prior interactions with healthcare providers prior to being diagnosed so it is possible that these interactions took place in 2009. Because only case and contact investigation data for years 2010-2012 were analyzed these interactions could have been missed. Future research could involve analyzing the 2009 case and contact investigation data. Future research could also involve analysis of pediatric contacts, as opposed to

cases, to determine risk factors based on specific connections to healthcare providers. This has two benefits: an increased sample size and early prevention. If an association exists, pediatric contacts can be identified and given prophylaxis to prevent progression to active disease if LTBI is present. Although more conservative and less robust, bootstrapping results are accepted for network statistical analysis (Hanneman, 2005; Borgatti, 2002).

Risk factor 4

Country of birth

TB contacts had a diverse range of countries of birth. Table 15 shows the countries of birth of TB contacts for 2010, 2011, and 2012. Because this analysis was stratified the estimated multinomial logistic regression coefficients (B, in SPSS) were also analyzed. If B was greater than 0, and p was less than 0.05, for a specific country of birth, then that country of birth was considered significant. In 2010, all countries had p-values < 0.001 (logistic regression with bootstrapping only); however, only two countries, the Republic of Congo and USA had B values greater than 0.

In 2010, based on cases for which contact investigations were done, logistic regression shows that no countries of birth of contacts are predictors of pediatric cases; however, the Republic of Congo and USA were predictors of pediatric cases when using logistic regression with bootstrapping. Contacts with countries of birth of the Republic of Congo and the USA were significantly connected to pediatric cases.

In 2011, based on cases for which contact investigations were done, logistic regression shows that no countries of birth of contacts are predictors of pediatric cases; however, Belize, Guam, Northern Mariana Islands, Philippines, Tanzania, and USA were

predictors of pediatric cases using logistic regression with bootstrapping. Contacts with

these countries of birth were significantly connected to pediatric cases.

Table 15. Countries of Birth of Tuberculosis Contacts: 2010, 2011, and 2012; OR = Odds Ratio; CI = Confidence Interval

2010

Bhutan, Bosnia and Herzegovina, Canada, China, Colombia, **Congo (Republic)** (**OR=2.610x10¹⁸, CI=2.610x10¹⁸, 2.610x10¹⁸)**, Cuba, Dominican Republic, El Salvador, Ethiopia, Gambia, Georgia, Germany, Guatemala, Honduras, Hong Kong, Ireland, Italy, Lebanon, Mexico, Nepal, Philippines, Romania, Senegal, South Korea, Spain, Taiwan, United Kingdom, Unknown, USA (OR=1.224x10⁸, CI= 7.705x10⁷, 1.705x10⁸), Vietnam

2011

Afghanistan, Albania, Argentina, Bangladesh, **Belize** (**OR=2.610x10¹⁸**, **CI= 2.610x10¹⁸**, **2.610x10¹⁸**), Bhutan, Bosnia and Herzegovina, Bulgaria, Cambodia, Canada, China, Cuba, Dominican Republic, El Salvador, Eritrea, Ethiopia, Ghana, Greece, **Guam** (**OR=8.078x10⁹**, **CI=1.616x10⁹**, **2.610x10¹⁸**), Guatemala, Holland, Honduras, Hong Kong, Japan, Kenya, Laos, Lebanon, Liberia, Malaysia, Mexico, Micronesia, Nepal, Northern Mariana Islands (**OR=2.610x10¹⁸**, **CI=2.610x10¹⁸**, **2.610x10¹⁸**), Pakistan, People's Republic of China, Peru, Philippines (**OR= 7.515x10⁷**, **CI=1.815x10⁷**, **7.830x10⁷**), Poland, Puerto Rico, Romania, Russia, Slovak Republic, South Korea, Sudan, Taiwan, **Tanzania** (**OR=2.610x10¹⁸**, **CI= 2.610x10¹⁸**, **2.610x10¹⁸**), Thailand, Uganda, Unknown, **USA** (**OR=1.850x10⁸**, **CI= 1.360x10⁸**, **2.369x10⁸**), Venezuela, Vietnam

<u>2012</u>

Afghanistan, Argentina, Armenia, Austria, Canada, China, Colombia, Congo (Republic), Cuba, Czech Republic, El Salvador, Ethiopia, Guatemala, Honduras, India, Indonesia, Iran, Korea, Laos, Macau, Malaysia, Mexico (OR=4.487x10⁷, CI=0.000, 1.087x10⁸), Nicaragua, Northern Mariana Islands, Peru, Philippines (OR=2.937x10⁷, CI=0.000, 7.553x10⁷), Puerto Rico, Romania, Russia, South Korea (OR=3.231x10⁷, 0.000, 2.610x10¹⁸), Sri Lanka, Sudan (OR=2.610x10¹⁸, CI=2.610x10¹⁸, 2.610x10¹⁸), Taiwan, Thailand, Unknown, USA (OR=8.337x10⁷,CI=4.586x10⁷, 1.284x10⁸), Vietnam (OR=6.461x10⁷, CI=0.000, 4.038x10⁹)

In 2012, based on cases for which contact investigations were done, logistic regression shows that no countries of birth of contacts are predictors of pediatric cases;
however, Mexico, Philippines, South Korea, Sudan, USA, and Vietnam were predictors of pediatric cases when using logistic regression with bootstrapping. Contacts with these countries of birth were significantly connected to pediatric cases.

In summary, only logistic regression with bootstrapping showed a significant association between pediatric cases and contacts with specific countries of birth. The highlighted countries of birth in Table 15 are significant. A p-value of less than .05 resulted in rejection of the null hypothesis, demonstrating a significant association between the risk factor of country of birth of a contact and a network connection with a pediatric case. USA was a significant country of birth of contacts in 2010, 2011 and 2012, while Philippines was a significant country of birth of contacts in 2011 and 2012. These results must be cautiously interpreted, even more so than the logistic regression with bootstrapping for the other risk factors, due to the large number of contacts for which there was no country of birth identified. Many contacts had either a country of birth recorded as "unknown" or the data cell in the spreadsheet was left blank. In 2010, there were 118 blank data cells and 539 unknown countries of birth. In 2011, there were 187 blank data cells and 86 unknown countries of birth, and in 2012 there were 349 blank data cells and 6 unknown countries of birth. It was expected that country of birth of contacts would be a significant risk factor for pediatric TB cases, and logistic regression with bootstrapping demonstrates a significant association for certain countries, however the results are limited by the large number of contacts for which no country of birth was listed, or with a country of birth listed as "unknown".

Country of birth has always been a significant risk factor, in general, for TB. Foreign countries with high incidence of TB disease can play a major role in increasing

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the case rate in the USA. The percentage of foreign-born cases in Clark County, Nevada has risen from 68% to 73% to 76% in 2010, 2011, and 2012, respectively. Pediatric cases within this same period have increased from 21% to 36% to 54%. Country of birth as a risk factor combined with genotyping would provide a productive area for future research. Genotyping could determine if a pediatric cluster is linked to a contact with a country of birth other than the United States. This risk factor is discussed more in detail in 'Class B designations'.

When assessing the aforementioned risk factors, as a whole, identified by the Nevada State Health Division, it was expected that they would be significant, and it has been demonstrated that some are significant. Table 16 summarizes the year, risk factor, and the significance of the risk factor based on both logistic regression and logistic regression with bootstrapping.

Odds Ratios (OR) and Confidence Intervals (CI) were also calculated for the risk factors (Appendix D). ORs and CIs for the countries of birth are shown in Table 15. In SPSS v.22, Beta (B) is the regression coefficient, where "Exp (B)" represents the OR (Laerd, 2014). The OR is a measure of association between an independent and dependent variable based on a reference variable (Laerd, 2014). For example, the reference variable for C.O.B. in 2010 is Bhutan (independent variable), where the value is 1.000. Any OR greater than 1.000 demonstrates increased odds of association with the dependent variable which is a pediatric TB case. An OR less than 1.000 demonstrates no association (or an inverse association) with the dependent variable, a pediatric TB case. Therefore, a contact having a C.O.B. of the Republic of Congo, is 2.610x10¹⁸ (Table 15) more likely to be associated with a pediatric TB case than a contact with a C.O.B. of

Bhutan. The reference C.O.B. in 2011 and 2012 was Afghanistan.

Extremely large ORs and associated CIs, as shown in Table 15, are due to "zero cell count": where the dependent variable is invariant (unchanging) for one or more values of a categorical independent variable (Menard, 2002). For example, the small number of pediatric TB cases seemed to be exclusively associated with contacts with

Year	Risk Factor	Logistic regression	p-value	Logistic regression with bootstrap.	p-value
		-	-	*	*
2010	Mother's age	no	1.000	yes	0.002
	History of incarceration	yes	0.016	yes	0.007
	Healthcare provider	no	1.000	no	0.153
	Country of birth	no	1.000	yes	0.001
2011	Mother's age	*	*	*	*
	History of incarceration	no	0.918	no	0.871
	Healthcare provider	no	1.000	no	0.626
	Country of birth	no	1.000	yes	0.001
2012	Mother's age	no	1.000	ves	0.001
	History of incarceration	no	0.998	ves	0.001
	Healthcare provider	no	1.000	no	0.442
	Country of birth	no	1.000	yes	0.001

Table 16. Summary of Significance of Risk Factors for Pediatric Tuberculosis Cases: 2010, 2011, and 2012

* In 2011, no mothers were less than 25 years of age

countries of birth of the Republic of Congo, and the U.S.A. in 2010. This resulted in a large number of zero cells, for the other countries of birth. Logistic regression showed no association due to the very small number of pediatric cases. However, logistic regression with bootstrapping showed significant associations. The zero cells caused the statistical estimation procedure to fail, which resulted in extremely large ORs for the Republic of Congo, and the U.S.A. (IDRE, 2014). Logistic regression with bootstrapping,

demonstrates a significant association more often because artificial data sets are created, which increase the sample size; however, this also subsequently lowers the statistical power. The zero cells also cause highly fluctuating CIs, where the upper limit for the 95% CI can be infinity. Also, the CI can match the OR exactly, as seen in the Republic of Congo and the U.S.A. in 2010.

Zero-cell counts are not uncommon with binomial logistical regression, and do not necessarily mean there is anything wrong with the model, or the theory underlying the model (Menard, 2002). Often, the empirical patterns in the data, indirectly, cause the extremely large ORs and fluctuating CIs (Menard, 2002). Specific empirical patterns in this research are: the disproportionately large number of zero cells and blank data cells, and the disproportionately small number of pediatric cases for which contact investigations were done. Because these specific empirical patterns are present, they represent limitations, which necessitate cautious interpretation of the results.

RESEARCH QUESTION 2

A) Is the Southern Nevada Health District (SNHD) prioritizing pediatric contact investigations based on the most likely transmission risks within the entire TB network?

B) Do pediatric TB contacts with the highest betweenness scores (a network metric related to potential disease transmission) (McKenzie, 2007) match the risk factors identified by the Nevada State Health Division (Paulson, 2010)?

HYPOTHESIS 2(A)

H_o: There is no difference between SNHD contact prioritization in 2010, 2011, and 2012 when using the betweenness metric.

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H_a: There is a difference between SNHD contact prioritization in 2010, 2011, and 2012 when using the betweenness metric.

Where 1 = high prioritization

2 = medium prioritization

3 = low prioritization

4 = marginal prioritization

Tables 17, 18, and 19 show the top-20 calculated betweenness scores (column 2) for the 2010, 2011, and 2012 networks, respectively.

The original intent of this hypothesis was to compare TB contact prioritization based on betweenness scores with prioritization established by SNHD which uses primarily individual risk factors and not locational properties, however the networks contained very few non-zero scores which were all cases (except in 2012) as opposed to contacts. The remaining betweenness scores of zero in the table were simply listed sequentially. The cases with the highest scores were either common contacts or had a large number of contacts within their personal network. The non-zero scores were given high prioritization, and the remaining scores of zero were given marginal prioritization which is the lowest rank. It was expected that network contacts would have the highest prioritization scores, however the highest scores were cases, and the additional 14 scores were cases for consistency. The number of non-zero betweenness scores increased from 2010 to 2012 because more common contacts were observed or cases had a larger number of contacts. The 2012 network does have four betweenness scores in the top-20 that were contacts (5549, 5552, 5553, and 5689). A pair-wise comparison can still be done, however the results will be limited because all TB cases (as opposed to contacts)

inherently have, in general, high prioritization. The following prioritizations have been established for cases:

- 1) Pediatric case = high prioritization
- 2) Smear positive, culture positive = high prioritization
- 3) Smear or culture positive with cavitation = high prioritization
- 4) Clinical, suspect = medium

Table 17. The To	p-20 Betweenness	Scores for the	2010 Tuberculosis	s Network

case	Between score	degree	2-step reach	Between priority	Between priority rank	SNHD priority	SNHD priority rank
12	0.179	0.408	0.424	high	1	high	1
21	0.013	0.016	0.424	high	1	high	1
26	0.004	0.064	0.064	high	1	high	1
31	0.003	0.055	0.055	high	1	high	1
27	0.001	0.027	0.027	high	1	high	1
38	0.001	0.031	0.031	high	1	high	1
1	0	0.005	0.005	marginal	4	high	1
2	0	0.003	0.003	marginal	4	high	1
3	0	0.008	0.008	marginal	4	med	2
4	0	0.009	0.009	marginal	4	high	1
5	0	0.011	0.011	marginal	4	high	1
6	0	0.001	0.001	marginal	4	high	1
7	0	0.005	0.005	marginal	4	high	1
8	0	0.003	0.003	marginal	4	high	1
9	0	0.006	0.006	marginal	4	high	1
10	0	0.005	0.005	marginal	4	high	1
11	0	0.003	0.003	marginal	4	med	2
13	0	0.004	0.004	marginal	4	high	1
14	0	0.003	0.003	marginal	4	med	2
15	0	0.016	0.016	marginal	4	high	1

Case	Between score	Degree	2-step reach	Between priority	Between priority rank	SNHD priority	SNHD priority rank
205	0.010	0.101	0.101	high	1	high	1
208	0.009	0.095	0.095	high	1	high	1
225	0.005	0.072	0.072	high	1	high	1
244	0.005	0.068	0.068	high	1	high	1
203	0.003	0.051	0.051	high	1	high	1
207	0.002	0.048	0.048	high	1	high	1
217	0.002	0.047	0.047	high	1	high	1
220	0.002	0.045	0.045	high	1	high	1
237	0.001	0.033	0.033	high	1	high	1
250	0.001	0.025	0.025	high	1	high	1
251	0.001	0.014	0.015	high	1	high	1
200	0	0.013	0.013	marginal	4	high	1
201	0	0.003	0.003	marginal	4	high	1
202	0	0.003	0.003	marginal	4	high	1
204	0	0.002	0.002	marginal	4	high	1
206	0	0.005	0.005	marginal	4	high	1
209	0	0.004	0.004	marginal	4	medium	2
210	0	0.008	0.008	marginal	4	high	1
211	0	0.004	0.004	marginal	4	high	1
212	0	0.003	0.003	marginal	4	high	1

Table 18. The Top-20 Betweenness Scores for the 2011 Tuberculosis Network

Case/ contact	Between score	Degree	2-step reach	Between priority	Between priority rank	SNHD priority	SNHD priority rank
324	0.018	0.133	0.133	high	1	high	1
304	0.012	0.111	0.111	high	1	high	1
325	0.005	0.048	0.049	high	1	high	1
344	0.004	0.063	0.063	high	1	high	1
352	0.004	0.036	0.037	high	1	high	1
343	0.003	0.058	0.058	high	1	high	1
312	0.002	0.043	0.043	high	1	high	1
313	0.002	0.043	0.043	high	1	high	1
336	0.002	0.025	0.026	high	1	high	1
354	0.002	0.041	0.041	high	1	high	1
308	0.001	0.029	0.029	high	1	high	1
338	0.001	0.021	0.022	high	1	high	1
5549	0.001	0.002	0.082	high	1	high	1
5552	0.001	0.002	0.082	high	1	high	1
5553	0.001	0.002	0.082	high	1	high	1
5689	0.001	0.002	0.046	high	1	high	1
300	0	0.002	0.002	marginal	4	high	1
301	0	0.009	0.009	marginal	4	high	1
303	0	0.001	0.001	marginal	4	high	1
305	0	0.003	0.003	marginal	4	high	1

Table 19. The Top-20 Betweenness Scores for the 2012 Tuberculosis Network

For comparative purposes, all cases were analyzed with high priorities as well. Table 20 shows the results of a Wilcoxon signed-rank test of paired betweenness scores with SNHD prioritization scores for each network. The Wilcoxon signed-rank test is commonly used for network pair-wise comparison (Cook, 2007). The null hypothesis was rejected (p < .05) in the 2010 and 2011 networks, which demonstrates a significant difference between prioritization based on network betweenness scores (location) and prioritization based on SNHD prioritization (individual risk factors). In 2012, the null hypothesis failed to be rejected which demonstrates no difference in prioritization. The

 Table 20. Significance of a Pair-Wise Comparison between SNHD Risk Factor

 Prioritization and Betweenness Metric Prioritization

		Network	
	2010	2011	2012
Z-score _b	-3.494	-2.887	-1.890
Asymp. Sig. (2-tailed)	0.000	0.004	0.059
^{b.} Based on positive ranks.			

results of these tests are limited and must be interpreted carefully. The 2012 network shows equal prioritization most likely based upon the large amount of non-zero scores of TB cases which automatically receive high prioritization because they are the top-20 scores. When compared to SNHD prioritization, which assigns high priority to cases, the top-20 scores are also cases (except for 4 contacts) so there is no difference statistically.

An additional analysis of prioritization scores using the Wilcoxon signed-rank test was conducted with SNHD prioritization for all TB cases changed to 'high' priority. The results are unchanged for the 2010 and 2011 networks, however the p-value for the 2012 network was lowered to 0.046 which results in rejection of the null hypothesis demonstrating a difference in prioritization. Increasing 'n' will likely not change the results, as the remaining betweenness scores are all zero and the distribution is extremely skewed.

Given the statistical results, betweenness score is a metric best suited for an outbreak network with degree and 2-step reach being the more applicable metrics to on-going surveillance networks such as this. Because network contacts have degree and 2-step reach scores, a better pair-wise comparison can be made with SNHD contact prioritization scores, which was the original intent of the hypothesis. The large number of betweenness scores equal to zero indicates a fragmented, low-density whole network with few common contacts which results in very limited disease propagation. In conclusion, prioritization of contacts based on individual risk factors, as done by SNHD, is the preferred method. Betweenness scores provide limited value in an on-going surveillance network, however these scores can complement prioritization during outbreak conditions (McKenzie, 2007) because there will be a large amount of common contacts within the network.

In summary, it was expected that contact prioritization based on betweenness scores would be significantly different than SNHD contact prioritization. While the statistical analysis seems to support this, the results could be due to chance and, therefore, are limited. The original expectation was based on 2 assumptions. First, there would be at least 20 non-zero scores for adequate pair-wise comparison. Second, these 20 non-zero scores would all be contacts, not cases. These 2 assumptions were invalid, thus limiting

the statistical results. As previously mentioned, the betweenness metric seems to be a metric best suited to an outbreak network, whereas the 2-step reach and degree metrics would provide more value to this TB network which is not an outbreak network.

The previous results compared betweenness prioritization scores and SNHD prioritization scores using the Wilcoxon signed-rank test. The following section involves an *a posteriori* analysis (Oleckno, 2008) in which no study hypothesis has been previously developed and an "after the fact" statistical comparison is done based on the previous results to determine if network metrics (degree, 2-step reach, and betweenness) can be used as predictors of a pediatric TB case. Logistic regression and logistic regression with bootstrapping are used as the statistical tests, and the two aforementioned assumptions are valid.

Network metrics as predictors for pediatric cases

In the 2010 network, there was no significant association between degree, 2-step reach, or betweenness and pediatric cases. The individual scores of these metrics associated with contacts in the network did not predict an association with pediatric cases using logistic regression, or logistic regression with bootstrapping. The 2-step reach scores of the contacts, however, were adequate predictors of connections to non-pediatric cases using both logistic regression and logistic regression with bootstrapping.

In the 2011 network there was no significant association between degree, 2-step reach, or betweenness and pediatric cases. The individual scores of these metrics associated with contacts in the network did not predict connections with pediatric cases using logistic regression, or logistic regression with bootstrapping. The 2-step reach scores of the contacts, however, were adequate predictors of connections to non-pediatric

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cases using both logistic regression and logistic regression with bootstrapping. The degree scores of the contacts were adequate predictors of connections to non-pediatric contacts using logistic regression with bootstrapping, but were not adequate predictors using just logistic regression.

As in the 2011 network there was no significant association between degree, 2-step reach, or betweenness and pediatric cases in the 2012 network. The individual scores of these metrics associated with contacts in the network did not predict connections with pediatric cases using logistic regression, or logistic regression with bootstrapping. The 2-step reach scores of the contacts, however, were adequate predictors of connections to non-pediatric case using both logistic regression and logistic regression with bootstrapping. The degree scores of the contacts were adequate predictors of connections to non-pediatric contacts using logistic regression with bootstrapping. The degree scores of the contacts were adequate predictors of connections to non-pediatric contacts using logistic regression with bootstrapping, but were not adequate predictors using just logistic regression.

In summary, degree, reach and betweenness were not significant predictors for pediatric cases using both logistic regression and bootstrapped logistic regression. In other words, a contact with an increased degree, reach, and betweenness score would not significantly be associated with a pediatric case. Any connection would be due to random chance. Even though this was an *a posteriori* analysis, the null hypothesis is similar to the null hypothesis for research question 1, an association between the degree, reach, and betweenness scores of contacts and pediatric cases (as opposed to an association between risk factors of contacts and pediatric cases). The null hypothesis is rejected because p is greater than .05, resulting in a non-significant association between degree, reach, and betweenness scores of contacts and pediatric cases. Two-step reach scores of contacts,

however, were adequate predictors of non-pediatric cases using both logistic regression and logistic regression with bootstrapping. The network as a whole was fragmented so the lack of total connections within the network could explain this. In addition, the small number of pediatric cases, especially compared with non-pediatric cases, could offer a possible explanation. Betweenness, as a predictor using logistic regression as well as a pair-wide comparison for prioritization, is a metric best suited for an outbreak network, and the vast majority of the betweenness scores were zero due to the small number of common contacts which is consistent with a fragmented network. Because betweenness was not an adequate predictor, degree and 2-step reach were also compared. As general metrics, degree and 2-step reach are better suited for on-going surveillance networks because they can describe the current conditions of a network and can be used as predictors for outbreaks. Degree is simply the number of contacts a node (case or contact) has, and normalized degree is used where an increased degree means a node has more contacts. Degree is a valuable metric, however it only provides information about direct connections, and disease transmission within a network involves assessment of indirect contacts, thus 2-step reach can be calculated.

RESEARCH QUESTION 2(B):

2) Do pediatric TB contacts with the highest betweenness scores (a network metric related to potential disease transmission) (McKenzie, 2007) match the risk factors identified by the Nevada State Health Division (Paulson, 2010)?

HYPOTHESIS 2(B)

H_o: There is no association between pediatric TB cases and identified risk factors of contacts with the top-20 betweenness scores

 H_a : There is an association between pediatric TB cases and identified risk factors of contacts with the top-20 betweenness scores where p < .05 demonstrates a significant (non-random) association by bootstrapping

Based on the results of Hypothesis 2(A) an overall statistical analysis would be of no value. Therefore, research question 2B cannot be answered. In the 2010 and 2011 networks, the individuals with the top-20 betweenness scores were cases, not contacts. In 2012, however, four of the individuals with the top-20 betweenness scores were contacts. It is beneficial to examine each of these four contacts individually as three of the four are common contacts that connect two separate networks. Two of the four contacts are female (5549 and 5689), however they are not young mothers. None of the four contacts have a history of incarceration. All four contacts were listed as an "other" relationship to their respective cases, and all four contacts are foreign-born with three having a country of birth of Mexico.

RESEARCH QUESTION 3(A)

Based on the clustering coefficient, has the 2010 to 2012 Nevada pediatric TB network expanded from a local network to a small-world network?

HYPOTHESIS 3(A)

H_o: There is no difference between clustering coefficients in data analyzed from 2010,

2011, and 2012

H_a: There is a difference between clustering coefficients in data analyzed from 2010,

2011, and 2012

Clustering coefficient can be defined as the interconnectivity of a network (Valente, 2010). The clustering coefficients of each yearly network were calculated using Ucinet 6 for

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Windows (Borgatti, 2002). Each TB case for which a contact investigation was conducted represents a personal network containing a source case with associated contacts. The 2010 network had 60 cases for which contact investigations were completed. The 2011 network had 64 cases for which contact investigations were completed, and the 2012 network had 58 cases for which contact investigations were completed. The yearly network clustering coefficient is comprised of the clustering coefficients of each TB case. Because a TB network is usually egocentric where the source case is the "ego" and the contacts are the altars (Hanneman, 2005) with the associated contacts connected radially in a "wheel-and-spoke" arrangement, the clustering coefficient will likely be zero (Mckenzie, 2007).

The Kruskal-Wallis test is a common non-parametric test applied to network data to determine differences in network parameters (Cook, 2007). Table 21 shows the SPSS results of the Kruskal-Wallis test and the median test for the distribution of the clustering coefficient. There was no difference between clustering coefficients in data analyzed from 2010, 2011, and 2012, therefore the null hypothesis is retained.

Table 21. Kruskal-Wallis Test Summary for Network Clustering Coefficients: 2010, 201	1,
and 2012	
Source: adapted from SPSS v.22	

Null hypothesis	Test	Significance	Decision
The medians of clustering coefficient are the same across categories of year	Independent- Samples Median Test	0.340	Retain the null hypothesis
The distribution of clustering coefficients is the same across categories of year	Independent- Samples Kruskal-Wallis Test	0.342	Retain the null hypothesis

RESEARCH QUESTION 3(B)

Has the network density increased from 2010 to 2012?

HYPOTHESIS 3 (B)

Ho: There is no difference in Nevada pediatric density from 2010-2012

Ha: There is a difference in Nevada pediatric density from 2010-2012

Density is defined as the number of case-contact connections divided by the total number of possible case-contact connections. Table 22 shows the densities of various networks. The very low densities indicate multiple fragmented and isolated personal networks within the whole network. Density is also the average of the individual network clustering coefficients. There is no difference in Nevada pediatric density from 2010-2012 using the same statistical tests used for clustering. It was predicted that density would increase due to higher contacts rates within the network which would aid in explaining the increase in pediatric TB cases, however density was unchanged. The whole network contains all the cases for which contact investigations were conducted. The pediatric network includes any individual network that contains a pediatric case or contact. The non-pediatric network contains any individual network that contains no pediatric cases or contacts.

Density	2010	2011	2012	p-value
whole network	0.001	0.002	0.002	0.368
pediatric network	0.003	0.003	0.002	0.368
Non-pediatric network	0.009	0.003	0.009	0.368

Table 22. Tuberculosis Network Densities for 2010, 2011, and 2012

It was predicted that the clustering coefficient would decrease from 2010 to 2011 and 2011 to 2012, however the statistical results show that there was no change within these time periods. Therefore the null hypothesis failed to be rejected because the p-value was 0.368 which is greater than 0.05. A decrease in yearly clustering coefficients would indicate expansion of the TB network which would provide a plausible explanation for the increase in pediatric TB cases from 2008 to 2012, however, the increase in pediatric cases appears to be less related to network properties and more related to individual network properties, as well as individual risk factors. Clustering coefficient is a metric best suited for an outbreak network (Kiss, 2005), however it could be used during ongoing surveillance as a predictor metric for an outbreak.

A less dense (fragmented) network discovered during on-going surveillance indicates multiple local personal networks that are isolated, however it takes only one weak tie to connect the networks. The 2010 network has a weak tie (Case 12) that connects a pediatric subgroup to a subgroup with a history of incarceration (Figure 23). This weak tie acts as a bridge between the networks and increases the potential for disease spread. A fragmented network indicates multiple local networks. These multiple local networks that create the entire yearly networks have not caused a small-world network to develop (Watts, 1998), but network randomness may be slightly increasing. Figure 22 shows the evolution of a small-world network (Watts, 1998) where a random network has more contacts and the path length between the nodes is shortened. A small-world network is more random than a regular local network; however, it is less random than a network that is completely random (p=1) where p=1 could indicate outbreak conditions.



Figure 22. Network Evolution with Respect to Randomness. Source: Watts, 1998

The Clark County TB personal networks with the common contacts (not the entire network) appear to be expanding beyond a regular network into a small-world network (Figure 24), but have not reached the random stage where p=1. The 2010, 2011, and 2012 whole networks, with all the case and contacts considered, are stable and static in comparison with these personal networks with the common contacts.

A fragmented yearly network indicates thorough contact investigations are being conducted because common contacts that may act as weak ties are being identified and/or treated as necessary which prevents network expansion and disease transmission potential. Fragmented networks and the lack of numerous common contacts also demonstrate adequate case management, treatment, and education. It is best to maintain these fragmented, or isolated, networks because case management is much easier within local personal (household) networks. These types of local networks are more predictable and less random. Whole-network metrics such as clustering coefficient and density provide minimal insight into the increase of pediatric TB for the TB networks in 2010,

2011, and 2012. These results and conclusions are only applicable to the TB networks during 2010, 2011, and 2012.

The value of network theory is not only the ability to analyze the whole network, but also to analyze cases and contacts at the group and individual levels. The expectations were that clustering coefficient would decrease and density would increase which would then provide a plausible explanation for the increase in pediatric cases. However, these two metrics were unchanged from 2010 to 2012. The argument could be made based on the clustering coefficients and density that while pediatric cases did increase from 2010 to 2012, based on state statistics, this increase did not reach an epidemic level as proven by the static state of the network within this time period. Within the entire 2010 TB network there were two groups that were the most dynamic. Figure 23 is a network visualization showing a group containing cases and contacts with a history of incarceration, and a second group which is made of local, household contacts. These two groups are connected by multiple common contacts. Case 12 is the central node in the history of incarceration network and is the common contact to multiple cases and contacts in the local household network. Because common contacts can act as bridges between networks, it is important to locate them during case and contact investigations to limit the spread of disease. Case 12 is a weak tie (Granovetter, 1973) in the sense that this case provides a connection that is not a local household connection but a more casual and random connection related to a social setting (e.g., prison, jail, etc.). The clustering coefficient of this network is 0.596, much higher than the network as a whole because the interconnectivity of the cases and contacts is increasing within these two network groups. Recall that a disease spreads faster in a network with an increased clustering coefficient,



Figure 23. TB Network Visualization Showing Common Contact(s), 2010

assuming the same number of average contacts (Kiss, 2005). Case 12, who also has other TB risk factors besides history of incarceration linked these two otherwise isolated networks leading to 4 pediatric cases and one non-pediatric case in the local, household network. The disease spread more quickly than if the two networks were isolated, but yet the density of the whole network was extremely low because the cases were more manageable. This provides further support for the theory that an epidemic was likely prevented especially considering none of the 500+ contacts within the history of

incarceration network developed active TB. The multiple connections were starting to form a small-world network (Watts, 1999) where the local, household network was starting to merge with the history of incarceration network. This particular network group reinforces the importance of analyzing network metrics (density, clustering coefficient, etc.) as a complement to individual risk factors (age, history of incarceration) when assessing disease transmission. Even though the whole network appears stable and static, groups within the network may be dynamic where adequate screening, treatment, and case management can limit the spread of TB within the whole network.

Figure 24 shows 2 distinct networks in 2011 that are connected by a common contact. Both networks are personal, local networks that do not have a common social setting like a jail, detention center, etc. as seen in 2010. The common contact (3850) is a healthcare provider which demonstrates, to a certain extent, the importance of healthcare providers as a risk factor mentioned by the Nevada State Health Division. However, the networks that are linked by the healthcare provider have no pediatric cases, but they do have pediatric contacts. Having a healthcare provider as a common contact provides a network bridge which increases the exposure risk, but not necessarily disease transmission. This healthcare provider has had 2 consecutive negative TSTs so the two cases that are linked (241 and 251) by the healthcare provider are not due to exposure to the healthcare provider. Again, this reinforces the importance of individual risk factors in addition to network metrics. There is no statistical significance to the lack of pediatric cases within these two networks; in fact; it could be random chance that there are none. These two combined personal networks demonstrate the importance of the role of the healthcare provider and the need for screening and testing. So while it may appear that

healthcare providers are not statistically significant in the whole network, they play a vital role in disease prevention at the group (and individual) level. This is another



example of the weak tie theory (Granovetter, 1973) where the healthcare provider is more of a casual and random contact, seemingly unimportant, but yet acts as the tie to 2 otherwise unconnected network groups.

Figure 25 shows 2 distinct networks that are connected by 3 common contacts. One network is a personal, local network made of friends or relatives and the second network is also a personal network of friends and relatives also with a large number of healthcare providers. Although both personal, local networks in 2012 have neither pediatric cases, nor pediatric contacts, they are shown to reinforce the importance of individual risk factors. All three common contacts have a country of birth of China and are elderly so they are classified as high risk. Contact 5549 does not have active disease or LTBI. Contacts 5552 and 5553 both have LTBI but not active TB. Contact 5552 is being treated to prevent active TB and Contact 5553 is not being treated to prevent active TB. Although SNHD can mandate treatment for active TB, LTBI treatment is not mandatory. These 2 networks, as

in the 2011 networks, also show the importance of the healthcare provider as a risk factor.

Due to the large number of healthcare providers within the networks, this network has the potential to spread to a small-world network from a local, friendship network with close contacts.

To elaborate on individual risk factors as mentioned above, it is beneficial to examine each of these four contacts individually as three of the four are common contacts that connect two separate networks. Two of the four contacts are female (5549 and 5689), however they are not young mothers. None of the four contacts have a history of incarceration. All four contacts were listed as an "other" relationship to their respective

cases (5549, 5552, 5553, and 5689) and all four contacts are foreign-born with three having a country of birth of China and one having a country of birth of Mexico. Therefore, network analysis again demonstrates the importance of risk factors at the individual and group level. In 2012, China was not a significant country of birth using logistic regression, or logistic regression with bootstrapping, of the whole network (hypothesis 1). However, at the individual and group levels the three contacts (5549, 5552, and 5553), with a country of birth of China, acted as weak ties between two otherwise isolated networks

(Figure 25). As such, they increase the potential for disease transmission, because they form bridges between the two personal and local networks. These 3 contacts did not have active TB; therefore, they were not viable routes of disease transmission, even though they were bridges. This reinforces the importance of assessing individual risk factors.

2012 TB NETWORK



- Non-pediatric contact local network (friend or relative)
- Healthcare provider
- High-risk non-pediatric contact, relationship "other"
- Non-pediatric case

Figure 25. TB Network Visualization Showing Common Contact(s), 2012

PEDIATRIC TB AND CLASS B DESIGNATION

SNHD operates a very proactive TB treatment and control clinic that provides public services such as case management (education, treatment, and DOTS), Class B immigrant/refugee evaluation, contact investigations, and education.

The U.S. Department of State requires all refugees and immigrants to have a preimmigration medical exam for active pulmonary TB (MDH, 2014). Persons 15 years old or older are required to have a CXR, and children ages 2 to 15 years of age are required to have a TST. A negative pre-immigration exam requires no follow-up, a pre-exam that is positive for infectious TB requires full treatment before traveling. If the preimmigration TB exam shows positive results for disease other than infectious TB then the individual is given a "Class B" designation as follows (MDH, 2014):

- Class B1: The individual has an abnormal CXR with evidence of TB and/or a history of treatment for active TB disease.
- Class B2: The individual was diagnosed with LTBI (generally children whose TST was positive, but the CXR was normal).
 - Class B3: The individual is a recent contact of an infectious TB case; an individual can have this designation along with another Class B designation.

The SNHD TB treatment and control clinic evaluates abnormal CXRs of Class B immigrants in Clark County. Class B immigrants are required to submit CXRs to CDC upon arrival into the USA, and when the immigrant arrives in Clark County, CDC sends

the CXRs to the SNHD treatment and control clinic. As a condition of immigration, Class B immigrants must immediately visit the SNHD treatment and control clinic. Class B immigrants cannot work in Clark County until the CXR is reviewed and cleared by the SNHD TB treatment and control clinic, and if the Class B immigrant has TB, treatment guidelines must be followed with a course of treatment being prescribed until the individual is negative for TB infection or disease.

In this study, several mothers of pediatric TB cases were listed as Class B immigrants from 2010 to 2012. Future research could involve analyzing Class B immigrant status of pediatric TB cases and/or parents and contacts of pediatric TB cases. Table 23 shows the number of pediatric cases in Clark County from 2010 to 2012 with a country of birth (COB) other than the USA. The increase in pediatric TB cases in Clark County, and therefore Nevada, can be attributed, at least in part, to a child and/or parent

Table 23	Table 23 Nevada Pediatric TB Cases with a Country of Birth other than the USA						
Year	Number of pediatric cases with a COB other than the USA	Total number of pediatric cases in Clark County	%				
2010	4	19	21				
2011	4	11	36				
2012	7	13	54				

or guardian having a COB other than the USA. Pursuant to the U.S. Department of State guidelines, a child less than 2 years of age does not receive a Class B designation, and thus is not required to get a TST, and several of these pediatric cases were children less than 2 years of age. The important question is when were these pediatric TB cases diagnosed, prior to arrival in the USA, upon arrival in the USA, or after arrival into the USA? These questions provide important additional areas of future TB research in Clark County, Nevada.

CONCLUSIONS

Communities are dynamic and perhaps none more so than southern Nevada, more specifically Las Vegas. The transient nature of the Las Vegas population makes it ideal for transmission network modeling. The study and analysis of infectious disease transmission requires methods that incorporate population dynamics as this will provide the most realistic picture of the burden of disease at a point in time and also predictions about transmission.

This study demonstrates the importance of analyzing networks at all levels: whole-network, group, and individual. Whole-network metrics, such as clustering coefficient and density, provide valuable information that can be applied to outbreaks. Individual analysis proves that while a connection may exist between a case and a contact, personal risk factors aid determining actual disease transmission. The Nevada State Health Division also recognizes the importance of individual risk factors in the transmission of pediatric TB. Although individual risk factors may have been shown to be insignificant at the network level, group-level analysis has provided the greatest benefit because it identified individual networks that are connected by common contacts. Focusing on network groups can provide focused treatment and prevention that is more manageable because the spread of disease is contained within the group networks and thus has not spread to the whole network.

In the 2010 network, two groups, the group where the vast majority of the contacts have a history of incarceration and the local network with multiple cases and contacts, were connected by multiple common contacts. The clustering coefficient of these two combined groups was 0.596 which shows an increase in interconnectivity

among cases and contacts. Kiss (2005) proved that, given the same number of average connections, disease will propagate slower within the whole network as clustering coefficient increases (Figure 26). The connected groups in our 2010 network are consistent with this conclusion. While the disease propagated quickly within the two 2010 connected groups, networks where the clustering coefficient was 0.596, the network as a whole was unaffected; therefore there was no outbreak. Because the spread of TB was contained within these two connected networks, testing, prevention, and treatment



Average connections = 10Clustering coefficient = 0

Average connections = 10Clustering coefficient = 0.5

Figure 26. The Spread of Disease in a Network. Source: Kiss, 2005

could be expedited, and it is speculated that this rapid response allowed containment. This research has also shown that through network models, the fundamental public health concept of quarantine is an effective method for preventing disease transmission. While quarantine is often associated with an individual, it is also effective for groups, which can limit the spread of disease within a network.

Social Network Analysis (SNA) can provide the overall benefit of an on-going disease surveillance system that can improve health outcomes with targeted and costeffective interventions, and can influence public health policy. Social network analysis (SNA) provides an analytical method that can aid in the analysis of a TB program as mentioned above. For example, a TB network can engage stakeholders such as elected officials through simple visualization of cases, contacts, and links. Highlighting high-risk areas using colors and symbols provides a non-technical representation of TB that is easily understandable. SNA is a validated method of analysis of TB cases, contacts, and links as a means of demonstrating the current burden of disease (morbidity) which, as previously mentioned, is the first step in TB planning and policy development. Some advantages of TB infectious disease modeling are: better resource allocation, improved contact investigation efficiency, prioritized treatment, education, and improved Directly Observed Treatment Short-Course (DOTS) therapy. Finally, an evidence-based, empirical and real-world model can help implement health policy change at the state and local level where it is needed the most. It is recommended that a social network model and associated metrics be implemented at the local level. Creation of a baseline network is necessary, in which future cases and contacts can be added sequentially, which will reduce the burden of data management and analysis.

STUDY LIMITATIONS

Secondary data

Secondary data analysis has inherent limitations because data collection methods cannot be verified and could have associated bias, such as recall and interviewer bias. The data collected by SNHD were comprehensive and thus provided adequate risk factors for analysis. Self-reporting by TB cases and contacts can limit the data validity. The perceived stigma associated with TB and the unwillingness to begin a lengthy treatment regimen may influence responses. The coding and definitions were used exactly as denoted on the SNHD spreadsheets with no revisions being made. For example, blank data cells were left blank in the network analysis spreadsheet. It was not known whether these data were forgotten, unknown (possible language barrier), or simply not collected.

Generalizability

The statistical results and associated conclusions apply only to the Clark County TB network and are not generalizable to other counties or Nevada as a whole. Even though Clark County was chosen because it contains well over 80% of the cases in Nevada, results and conclusions cannot be generalized to the entire state. In addition, the results and conclusions are only applicable to the years 2010, 2011, and 2012.

Network statistical methods

Bootstrapping is an accepted method of network analysis (Borgatti, 2013; Hanneman, 2005) due to the interdependent structure of networks. Because the network is the theoretical population, bootstrapping provides the samples necessary for analysis. Bootstrapping provides results that may be conservative and less robust than logistic regression alone. For comparative purposes, statistical results from both logistic regression and logistic regression with bootstrapping were provided.

Network theoretical population

All the TB cases and contacts in Clark County from 2010 to 2012 represent the theoretical TB population, however it is possible that cases and/or contacts present in the population are not in the network. This limitation is minimized (but not eliminated) by the systematic case and contact investigation process. The network does contain all the TB cases on record with the SNHD, and TB is a nationally reportable disease; therefore, it would be more likely that TB contacts may not present in the network.

Perhaps the biggest limitation pertaining to network analysis is resource commitment, where the resources required exceed the capacity of a typical TB program (McKenzie, 2007; Cook, 2007). TB programs focus on case and contact investigations and have limited resources for network data analysis that requires substantial time and training to connect the cases and contacts and conduct metric analysis. Also, completeness of data collected during TB case and contact investigations (interviewing procedures) (Borgatti, 2013) can limit the effectiveness of network analysis. Case and contact matching will not be exact due to missing demographic information during the interviewing process and the use of aliases, with incomplete data for contacts usually occurring (Cook, 2007). Data management errors can also occur, such as connection/node attribute error where non- existent cases, contacts, or links are included in the network (commission errors); or cases, contacts, or links are missing from the network (omission errors) (Borgatti, 2013).

FUTURE RESEARCH

The following are recommended topics for future research:

1. A longitudinal study comparing Class B status (B1, B2, or B3) of contacts as predictors of pediatric TB, using a longer time period such as 5 to 10 years.

2. Comparison of social network analysis with concentric circle analysis to assess completeness of pediatric TB contact investigations.

3. Analysis of pediatric contacts, as opposed to cases, to determine risk factors based on specific connections to healthcare providers. This has two benefits: an increased sample size and early prevention. If an association exists, pediatric contacts can be identified and given prophylaxis to prevent progression to active disease if LTBI is present.

4. Network analysis involving other network metrics that pertain to public health such as hub centrality and eigenvector (Munene, 2013), or modularity (Valente, 2013).

5. Restriction Fragment Length Polymorphism (RFLP): compare genotyping cluster data with epidemiological data to refute or confirm social connections.

6. Application of the Contact Priority Model (Bailey, 2002; Gerald, 2002; Psu, 2009) to Nevada. This model uses TST results to create a flowchart for TST prioritization based on risk factors.

7. Calculation of network metrics (density, clustering coefficient, density, etc.) for the 2013 Summerlin TB outbreak that resulted in the death of a mother and her premature twins. Creation of a network model for comparison of results (metrics) to a network

surveillance model, and combination of this network model with a stochastic model to predict future outbreaks.

8. Create a hospital network model as a preventive model of pediatric TB. Analyze relationships between cases and contacts to determine associations between pediatric cases and contacts based on relationships other than "healthcare provider". Determine if contact relationships involve close, local contacts (mother, father, etc.) or more random, casual contacts such as daycare providers. Assign weighted values for connections where a close contact has a higher weighted connection because disease transmission potential is increased due to increased time of exposure.

9. A stratified analysis of case and contact relationships using logistic regression with bootstrapping is a future research recommendation because healthcare providers appear to be significantly associated with non-pediatric cases. Various other relationships such as friends, significant others, etc. may prove to be significant as well.

APPENDIX A: EXAMPLES OF NETWORK DIAGRAMS



State Health Department TB outbreak investigation (McKenzie, 2007)



APPENDIX B: CDC CASE DEFINITION OF TUBERCULOSIS

Tuberculosis (TB) (*Mycobacterium tuberculosis*) :2009 Case Definition CSTE Position Statement(s)09-ID-65

Clinical Description A chronic bacterial infection caused by Mycobacterium tuberculosis, usually characterized pathologically by the formation of granulomas. The most common site of infection is the lung, but other organs may be involved.

Clinical Criteria: a case that meets all the following criteria:

- A positive tuberculin skin test or positive interferon gamma release assay for M. tuberculosis
- Other signs and symptoms compatible with tuberculosis (TB) (e.g., abnormal chest radiograph, abnormal chest computerized tomography scan or other chest imaging study, or clinical evidence of current disease)
- Treatment with two or more anti-TB medications
- A completed diagnostic evaluation

Laboratory Criteria for Diagnosis

- Isolation of M. tuberculosis from a clinical specimen,* OR
- Demonstration of M. tuberculosis complex from a clinical specimen by nucleic acid amplification test,** OR
- Demonstration of acid-fast bacilli in a clinical specimen when a culture has not been or cannot be obtained or is falsely negative or contaminated.

Confirmed

A case that meets the clinical case definition or is laboratory confirmed

Comment(s)

A case should not be counted twice within any consecutive 12-month period. However, a case occurring in a patient who had previously had verified TB disease should be reported and counted again if more than 12 months have elapsed since the patient completed therapy. A case should also be reported and counted again if the patient was lost to supervision for greater than 12 months and TB disease can be verified again. Mycobacterial diseases other than those caused by M. tuberculosis complex should not be counted in tuberculosis morbidity statistics unless there is concurrent tuberculosis.

*Use of rapid identification techniques for M. tuberculosis (e.g., DNA probes and mycolic acid high-pressure liquid chromatography performed on a culture from a clinical specimen) are acceptable under this criterion.

** Nucleic acid amplification (NAA) tests must be accompanied by culture for mycobacteria species for clinical purposes. A culture isolate of M. tuberculosis complex is required for complete drug susceptibility testing and also genotyping. However, for surveillance purposes, CDC will accept results obtained from NAA tests approved by the Food and Drug Administration (FDA) and used according to the approved product labeling on the package insert, or a test produced and validated in accordance with applicable FDA and Clinical Laboratory Improvement Amendments (CLIA) regulations.
Research Question	Alternative Hypothesis	Statistical test and reference	Signif level	Result
Question 1) Are the risk factors identified by the State of Nevada Health Division significant?	Hypothesis There is an association between the risk factors of TB contacts and ped. TB cases	and reference Logistic regress. and logistic regress. with bootstrapping Hanneman, 2005 Borgatti, 2002	level 0.05	Significance with logistic regression, and logistic regression with bootstrapping: 2010 history of incarceration Significance with logistic regression w/bootstrapping: 2010, 2012: mother's age p = 0.001 and 2010: country of birth Congo and USA $p = 0.001$ 2011: country of birth Belize, Guam, North. Mariana Isl, Philippines, Tanzania, USA $p = 0.001$ 2012: country of birth Mexico, Philippines,
				South Korea, Sudan, USA, Vietnam $p = 0.001$
2(A) Is SNHD prioritizing pediatric contact investigations based on the most likely transmission risks within the entire TB network?	There is a difference between SNHD contact prioritization in 2010, 2011, and 2012 when using the betweenness metric for the top-20 scores	Wilcoxon Signed-Rank test for pair- wise comparison, Cook, 2007	0.05	2010: reject null 2011: reject null 2012: fail to reject null (results are very limited due to extremely skewed betweenness scores)

APPENDIX C: SUMMARY OF HYPOTHESIS TEST RESULTS

Research Questions	Alternative Hypothesis	Statistical test and reference	Signif. level	Result
2(B) Do pediatric TB contacts with the highest betweenness scores match the risk factors identified by the Nevada State Health Division	There is an association between pediatric TB cases and identified risk factors of contacts with the top-20 between- ness scores	Logistic regress. and logistic regress. With bootstrapping Hanneman, 2005 Borgatti, 2002	0.05	Inconclusive due to lack of applicable top- 20 betweenness scores of contacts
3(A) Based on the clustering coefficient, has the 2010 to 2012 Nevada pediatric TB network expanded from a local network to a small-world network?	There is a difference between clustering coefficients in data analyzed from 2010, 2011, and 2012	Median test and Kruskal-Wallis test, Cook, 2007	0.05	Median test p = 0.340; fail to reject null hypothesis, no change in median clustering coeff. Kruskal-Wallis p = 0.342; fail to reject null hypothesis, no change in distribution of clustering coeff.
3(B)Has the network density increased from 2010 to 2012?	There is a difference in Nevada pediatric density from 2010-2012	Median test and Kruskal-Wallis Test, Cook, 2007	0.05	Median test p = 0.368 fail to reject null hypothesis, no change in median clustering coeff. Kruskal-Wallis p = 0.368; fail to reject null hypothesis, no change in distribution of clustering coeff.

		Logistic Reg	gression	Logist with b	ic Regression ootstrapping
Year	Risk Factor	OR	CI	OR	CI
2010	Mother's age History of incarceration	1.346 x 10 ⁹ 2.579	(0.000, *) (1.193, 5.575)	1.346 x 10 ⁹ 2.579	$(3.591 \times 10^8, 4.309 \times 10^9)$ (1.355, 7.531)
	Healthcare provider	1.000	(0.000, *)	1.000	(1.000, 1.000)
	Country of birth	**	**	**	**
2011	Mother's age	***	***	***	***
	History of incarceration	1.065	(0.322, 3.526)	1.065	(0.000, 2.702)
	Healthcare provider	1.000	(0.000, *)	1.000	(1.000, 1.000)
	Country of birth	**	**	**	**
2012	Mother's age	3.554 x 10 ⁹	(0.000, *)	3.554 x 10 ⁹	$(1.257 \text{ x } 10^9, 1.131 \text{ x } 10^{10})$
	History of incarceration	4.519 x 10 ⁷	(0.000, *)	$4.519 \ge 10^7$	$(3.025 \text{ x } 10^7, 6.527 \text{ x } 10^7)$
	Healthcare provider	1.000	(0.000, *)	1.000	(1.000, 1.000)
	Country of birth	**	**	**	**

APPENDIX D: ODDS RATIOS AND CONFIDENCE INTERVALS FOR RISK FACTORS

* Unknown

** See Table 15, p. 132*** In 2011, no mothers were less than 25 years of age

OR = Odds Ratio

CI = Confidence Interval

APPENDIX E: CITI CERTIFICATION

COLLABORATIVE INSTITUTIONAL TRAINING INITIATIVE (CITI) HUMAN RESEARCH CURRICULUM COMPLETION REPORT Printed on 02/09/2014

Anna 194

LEARNER DEPARTMENT PHONE EMAIL INSTITUTION EXPIRATION DATE Darin Cozati (ID: 2565322) Community Health Sciences 702-855-1419 cozatibiti univ.nevada.edu University of Nevada, Las Vegas 02/06/2019

GROUP 1.BIOMEDICAL RESEARCH INVESTIGATORS AND KEY PERSONNEL COURSEISTAGE Back Course!1 PASSED ON: 02/09/2014 REFERENCE ID: 12/315/297

REQUIRED MODULES	DATE COMPLETED
ntroduction	02/07/14
listory and Ethics of Human Subjects Research	02/07/14
Sasic Institutional Review Board (IRB) Regulations and Review Process	02/07/14
nformed Consent	02/07/14
ocial and Behavioral Research (38R) for Biomedical Researchers	02/07/14
lecords-Based Research	02/07/14
Senetic Research in Human Populations	(32/98/14
esearch With Protected Populations - Vumerable Subjects: An Overview	02/06/14
ulnerable Subjects - Research Involving Prisoners	02/06/14
ulnerable Subjects - Research Involving Children	02/09/14
ulnerable Subjects - Research Involving Pregnant Women, Human Fetuses, and Neonates	02/09/14
volding Group Hanns - U.S. Research Perspectives	02/05/14
DA-Regulated Research	02/09/14
esearch and HIPAA Privacy Projections	02/09/14
ulnerable Subjecta - Research Involving Workers/Employees	02/09/14
onflicts of Interest in Research Involving Human Subjects	02/09/14
NLY	11/19/11

For this Completion Report to be valid, the learner listed above must be affiliated with a CITI Program participating institution or be a paid independent Learner. Faitilised information and unauthorized use of the CITI Program course site is unethical, and may be considered research missconduct by your institution.

Paul Braunschweiger Ph.D. Professor, University of Miami Director Office of Research Education CITI Program Course Coordinator

APPENDIX F: UNLV IRB APPROVAL



Biomedical IRB – Expedited Review Approval Notice

NOTICE TO ALL RESEARCHERS:

Please be aware that a protocol violation (e.g., futilire to submit a modification for <u>any</u> change) of an IRE approved protocol may result in mandatory remedial education, additional audits, re-consenting subjects, researcher probation, suspension of any research protocol at issue, suspension of additional existing research protocols, invalidation of all research protocol at issue, suspension of additional issue, and further appropriate consequences as determined by the IRB and the Institutional Officer.

DATE:	March 14, 2014
TO;	Dr. Patricia Cruz, Environmental & Occupational Health
FROM:	Office of Research Integrity - Human Subjects
RE:	Notification of IRB Action Protocol Title: Social Network Analysis (SNA) of Clark County, Nevada Tuberculosis (TB) Case and Contact Investigation Data to Determine Pediatric Tuberculosis Risk Factors for Disease Transmission Protocol #: 1401-4698M Expiration Date:March 13, 2015

This memorandum is notification that the project referenced above has been reviewed and approved by the UNLV Biomedical Institutional Review Board (IRB) as indicated in Federal regulatory statutes 45 CFR 46 and UNLV Human Research Policies and Procedures.

The protocol is approved for a period of one year and expires March 13, 2015. If the above-referenced project has not been completed by this date you must request renewal by submitting a Continuing Review Request form 30 days before the expiration date.

Should there be any change to the protocol, it will be necessary to submit a Modification Form through ORI-Human Subjects. No changes may be made to the existing protocol until modifications have been approved by the IRB. Modified versions of protocol materials must be used upon review and approval. Unanticipated problems, deviations to protocols, and adverse events must be reported to the ORI – HS within 10 days of occurrence.

If you have questions or require any assistance, please contact the Office of Research Integrity - Human Subjects at IRB/multy edu or call 895-2794.

> Office of Passearch Invegrity - Human Subjects 4505 Maryland Parkway - Ban 451047 - Las Vegas, Nerada 89154-1047 (702) 895-2794 - PAX. (702) 895-0805

APPENDIX G: UNLV IRB MODIFICATION APPROVAL



NOTICE TO ALL RESEARCHERS-

Please be aware that a protocol violation (e.g., fullure to submit a modification for <u>any</u> change) of an IRB approved protocol may result in mandatory remedial education, additional audits, re-consenting subjects, researcher probation, suspension of any research protocol at sume, suspension of additional existing research protocols, invalidation of all research conducted under the research protocol at issue, and forther appropriate consequences as determined by the IRB and the Institutional Officer.

TO: Dr. Patricis Cruz, Environmental and Occupational Health

FROM: Office of Research Integrity - Human Subjects

RE: Notification of IRB Action Protocol Title: Social Network Analysis (SNA) of Clark County, Nevada Tuberculosis (TB) Case and Contact Investigation Data to Determine Pediatric Tuberculosis Risk Factors for Disease Transmission Protocol # 1401-4598M Expiration Date: March 13, 2015

The modification of the protocol named above has been reviewed and approved.

Modifications reviewed for this action include:

Data receipt, storage, and analysis will now be done at SNHD TB Annex instead of the SNHD Main Facility.

This IRB action will not reset your expiration date for this protocol. The current expiration date for this protocol is March 13, 2015.

Should there be any change to the protocol, it will be necessary to submit a Modification Form through ORI-Human Subjects. No changes may be made to the existing protocol until modifications have been approved by the IRB. Modified versions of protocol materials must be used upon review and approval. Unanticipated problems, deviations to protocols, and adverse events must be reported to the ORI – HS within 10 days of occurrence.

Should the use of human subjects described in this protocol continue beyond March 13, 2015, it would be necessary to submit a Continuing Review Request Form 30 days before the expiration date.

If you have questions or require any assistance, please contact the Office of Research Integrity - Human Subjects at IRB@univ.edu or call \$95-2794.

Office of Hassarch Integrity - Hammi Subjects 4505 Maryland Parkway - Ban 451047 - Las Vegas, Necada 89154-1047 (702) 895-2764 - EAX. (702) 895-0805

DATE: May 30, 2014

APPENDIX H: SOUTHERN NEVADA HEALTH DISTRICT HIPAA TRAINING CERTIFICATION

SNHD HIPAA TRAINING

Mr. Darin Cozatt

Completed the Southern Nevada Health District HIPAA Training program And successfully passed the competency examination provided by the

SNHD HUMAN RESOURCES DEPARTMENT



PRESENTED	Richard W. Cichy
BY:	Richard W. Cichy

ON THIS DAY: April 4, 2014

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