Title
Preventing the Spread of Emerging Respiratory Infections through Vaccination, Identifying Predictive Clinical Symptoms, and Targeting Social Networks

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Publication Date
2014

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Preventing the Spread of Emerging Respiratory Infections through Vaccination, Identifying Predictive Clinical Symptoms, and Targeting Social Networks

A dissertation submitted in partial satisfaction of the requirements for the degree Doctor of Philosophy in Public Health (Epidemiology) by Jennifer Michalove Radin

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2014
The Dissertation of Jennifer Michalove Radin is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

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Chair

University of California, San Diego
San Diego State University
2014
DEDICATION

To my loving family and friends who encouraged me along the way and to my cohort of classmates who made it a fun journey.
Disease is not of the body but of the place

*Lucius Annaeus Seneca*
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<td>EID</td>
<td>Emerging Infectious Disease</td>
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<td>FRI</td>
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<td>GDP</td>
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<td>GEE</td>
<td>Generalized Estimating Equation</td>
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<td>GIS</td>
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<td>hMPV</td>
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SARI  Severe Acute Respiratory Infection
SAS  Statistical Analysis Software
SOB  Shortness of Breath
USATC  United States Army Training Center
WHO  World Health Organization
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ACKNOWLEDGEMENTS

I would like to acknowledge Dr. Richard Shaffer, the chair of my committee, who was always available to give me guidance and answer my questions when I stopped by his office. I am also thankful for the encouragement he gave me to pursue my research interests in respiratory infectious diseases by joining the Operational Infectious Disease group.

I would like to thank Dr. James Fowler for giving me support when I first proposed an idea to assess poultry trading and the spread of avian influenza. He has given me invaluable insight on data analysis and data representation and I have learned a lot from our meetings. Additionally, I would like to thank Dr. Suzanne Lindsay, Dr. Maria Rosario Araneta, and Dr. Rema Raman. They gave valuable guidance and feedback on my papers, helped me think of things from different angles, and answered many of my questions when I got stumped.

I would like to thank the Naval Health Research Center for providing me with this opportunity to work on the first two studies. In particular, I would like to thank Anthony Hawksworth who was always available for discussing ideas, carefully reviewed all of the fine details of our study multiple times, and who gave me important insight about the study populations and how the data was collected and analyzed in the laboratory.

I would like to thank my cohort of classmates, Amanda Rondinelli, Corinne McDaniels-Davidson, Carmel Witte and Richard Armenta. We enjoyed enumerable hours of laughter between classes and in the library and they welcomed me to San Diego when I didn’t know anybody else in town. I would also like to thank Dr. Bonnie Tran
who gave me valuable guidance about the dissertation process. They have all become lifelong friends.

Lastly, I would like to thank my husband and my family for their encouragement to pursue this degree in the first place and who gave me their incredible love and support to finish it.

Chapter 2, in full, has been submitted for publication at PLOS ONE. Raman, Rema; Lindsay, Suzanne; Araneta, Maria Rosario; Fowler, James; Shaffer, Richard. The dissertation author was the primary author of this material.

Chapter 3, in full, is a reprint of the materials as it appears in Clinical Infectious Diseases: Radin JM, Hawksworth AW, Blair PJ, Faix DJ, Raman R, Russell KL, Gray GC. (2014) Dramatic decline of respiratory illness among US military recruits after the renewed use of adenovirus vaccines. Clin Infect Dis 59(7):962-8. Published by Oxford University Press. Raman, Rema; Lindsay, Suzanne; Araneta, Maria Rosario; Fowler, James; Shaffer, Richard. The dissertation author was the primary author of this material.

Chapter 4, in full, is currently being prepared for submission for publication of the material to PNAS. Raman, Rema; Lindsay, Suzanne; Araneta, Maria Rosario; Fowler, James; Shaffer, Richard. The dissertation author was the primary author of this material.
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Manual of Clinical Problems in Pulmonary Medicine: Seventh Edition. (pp. 245-

influenza vaccine effectiveness for the 2012-2013 influenza season. MSMR,
20(3):15-16.

Radin JM, Katz M, Tempia S, et al. (2012) Influenza Surveillance in 15 Countries in


ABSTRACT OF THE DISSERTATION

Preventing the Spread of Emerging Respiratory Infections through Vaccination, Identifying Predictive Clinical Symptoms, and Targeting Social Networks

by

Jennifer Michalove Radin

Doctor of Philosophy in Public Health (Epidemiology)

University of California, San Diego, 2014
San Diego State University, 2014

Professor Richard Shaffer, Chair

Background: Emerging respiratory diseases can spread rapidly within closely tied, immune-naive populations through respiratory droplets and aerosols. Many have non-specific symptoms making them especially difficult to identify, differentiate and treat quickly and appropriately. Others have re-emerged as a result of changes in vaccine production and availability. Increased international trade of animals can also impact disease dynamics, making it easier for zoonotic respiratory viruses to spread to new populations.
Methods: Febrile respiratory illness (FRI) and severe acute respiratory infection (SARI) surveillance data was collected among three US populations from 2011 to 2013 to evaluate clinical predictors of pathogen specific respiratory disease, seasonality and co-infection rates. Similarly, FRI surveillance at eight US military recruit training centers from 1996 to 2013 was used to assess the impact of adenovirus vaccines on respiratory disease rates. Finally, a retrospective analysis evaluated the risk of poultry avian influenza (H5N1) infection in an importing country based on the quantity of birds traded, infection in the exporting country, and their statistical interaction.

Results: In the first study, clinical and demographic predictors of rhinovirus, influenza, other pathogen, and no/unknown pathogen were identified. The second study found that military trainees experienced a 100-fold decline in adenovirus disease burden during the two years after adenovirus vaccines reintroduction. Results from the third study suggest that risk of H5N1 poultry infection in an importing country increased by a factor of 1.3 (95% CI: 1.1-1.5) for every 10-fold increase in live chickens imported from countries experiencing at least one case during that year.

Conclusions: Identifying demographic or clinical variables that are predictive of specific respiratory diseases is important for improving timely and accurate treatment, and recognizing the emergence of new pathogens or strains. For respiratory pathogens with existing vaccines, monitoring and evaluating vaccine benefit is important to ensure continued investment in vaccine production and prevent future disease reemergence. Additionally, limiting movement of infected animals by reducing chicken trade during H5N1 epidemic periods or increasing biosecurity measures can also reduce disease spread. Overall, prevention through early identification of cases, vaccination, and
targeting transmission networks is important for preventing epidemics or pandemics of new respiratory infections.
INTRODUCTION

Emerging infectious diseases (EIDs) pose a serious public health risk to humans and animals that often have little or no immunity to them, increasing their ability to spread quickly throughout populations, resulting in significant economic and social costs. Some of the most well-known and worrisome EIDs have been respiratory infections. The transmission route of respiratory infections makes them especially difficult to prevent or control because they can become suspended in the air from a sneeze or a cough or can live on surfaces for extended periods of times. Additionally, the increased movement of both animals and humans means that these viruses can quickly spread around the globe, resulting in disease emergence in previously uninfected regions.

Due to the complexities of emerging infectious diseases, it may be most effective to take an interdisciplinary and multi-angled approach to prevent and treat these diseases. For example, identification of pathogens through predictive models can help improve early detection of outbreaks, especially for respiratory diseases which often have overlapping symptoms. Once an outbreak does emerge, it is important to be prepared by knowing beforehand which populations are at greatest risk. Targeting high risk groups for vaccination is especially important during outbreaks, when supplies are often limited during the initial outbreak period. Finally, it is also important to evaluate the benefit of existing vaccines and communicate their importance in order to maintain vaccine production in the future. In addition to pharmaceutical interventions, non-pharmaceutical interventions such as increasing biosecurity and hygiene or limiting movement of infected animals can further prevent spread of disease.
In an effort to prevent emerging respiratory diseases spread, it is first necessary to know the typical clinical and demographic characteristics associated with individual pathogens. The goal of the first paper was threefold: identify clinical predictors associated with pathogen specific respiratory infection, define seasonality of these pathogens, and investigate co-infection rates. Since disease characteristics may also vary across populations as a result of variances in population exposures, overall health, living conditions, and age, we compared three different groups: US-Military recruits, DoD beneficiaries, and US-Mexico border populations. These results will help diagnosis of cases and identification of outbreaks, improve treatment accuracy, establish baselines of infection and prioritize the development of new vaccines and future treatments.

Without routine surveillance and estimating burden of disease over time, it is impossible to know the degree to which a particular disease is a problem and the effectiveness of prevention measures, such as vaccination. The objective of the second paper was to assess the effectiveness of the new adenovirus vaccines in preventing febrile respiratory illness (FRI) among recruits and determine if new adenovirus strains not covered by the vaccines emerged once the new vaccines were administered. These findings give evidence to the huge burden of disease of adenovirus when no vaccines are administered and the success of the new vaccines. Hopefully, these results will encourage policy makers to continue investment in adenovirus vaccines so reemergence of this virus doesn’t occur in the future.

In some cases, vaccination is not feasible or entirely effective in preventing the spread of emerging diseases. For poultry, vaccination against H5N1 is difficult since there are such large quantities of poultry and high turnover among flocks. Additionally,
the vaccine is not entirely protective and may make symptoms less severe or noticeable while still allowing infection to occur among poultry populations. Consequently, non-pharmaceutical interventions may be the best way to prevent the spread of this emerging respiratory virus. The goal of this study was to determine if the international spread and outbreaks of highly pathogenic H5N1 in poultry have been influenced by the quantity of poultry traded from infected countries. These results quantify how reductions in trade of infected poultry populations can reduce the global spread of this disease.

The findings of this dissertation are based on Naval Health Research (NHRC) surveillance data collected from military recruits, DoD beneficiaries, and US-Mexico border populations, as well as poultry H5N1 case data from the World Health Organization and poultry trading data from the Food and Agriculture Organization. Participants from the first two studies were enrolled at clinic sites if they met the case definition for FRI or Severe Acute Respiratory Illness. A total of 1,444 participants were enrolled from 2011 through 2013. These participants answered questionnaire data about their symptoms and pharyngeal or nasopharyngeal swabs were taken and tested for nine different respiratory pathogens. Additionally, a total of 58,103 pharyngeal swabs were collected and tested for adenovirus from military recruits from 1996 through 2013 to assess the effectiveness of adenovirus vaccines. Finally, poultry trading data and H5N1 poultry case data was collected from 2003 through 2011. Chapters 2 and 3 were approved by the NHRC’s internal review boards (IRB) and were considered exempt from additional review by the IRBs of University of California, San Diego and San Diego State University. Chapter 4 did not include human subject data and used publically available data.
CHAPTER 1
LITERATURE REVIEW

Emerging Respiratory Diseases

Emerging infectious diseases (EIDs) pose a serious public health risk to humans and animals that often have little or no immunity to them, increasing their ability to spread quickly throughout populations, resulting in significant economic and social costs. After adjusting for reporting bias, the number of EIDs in humans has risen appreciably over time since the 1940s [1]. Additionally, 75% of all EIDs have been zoonotic infections [2]. Consequently, greater focus on emergence and spread among animals is highly relevant for improving prevention efforts in humans.

Some of the most well-known and worrisome EIDs have been respiratory infections, such as coronaviruses and influenza viruses. The transmission route of respiratory infections makes them especially difficult to prevent: respiratory droplets, carrying these pathogens, can spread a meter in the air through coughing and sneezing and can also travel through close person-to-person contact, such as a handshake [3]. Some respiratory diseases can also live on fomites or hard surfaces for several days [4]. Additionally, RNA respiratory viruses, such as influenza and coronaviruses, have particularly high mutation rates, which increase the chances for the emergence of new, potentially pandemic strains [5]. Partly because of these factors, the burden of all respiratory infections remains high: lower respiratory infections are the third leading cause of mortality worldwide, causing over three million deaths in 2011 [6]. They also made up 4.65% of all Disability Adjusted Life Years worldwide in 2010 [7]. Therefore,
gaining a better understanding of ways to prevent emerging respiratory infections is an important public health issue.

There are many different factors that impact the emergence and re-emergence of infectious diseases, including changes in public health infrastructure, socioeconomics, environmental, ecological factors, movement of people and animals and genetic mutations [8,9]. In the case of adenovirus, a lack of investment in the late 1990’s resulted in the collapse of the manufacturing facility that made the vaccine, resulting in the reemergence of this previously controlled virus. Other factors, such as population growth and high population density are also associated with a greater number of EIDs [1]. For example, increased population growth has led to greater demands for cheap protein sources, such as poultry. This has impacted the global trade of animals and increased the movement of many species which can facilitate the international spread of zoonotic pathogens. Additionally, infectious diseases are constantly evolving- simple mutations or recombination of genes can reduce the effectiveness of existing vaccines, lower cross-reactivity of pre-existing antibodies, and increase the chances for the emergence of new strains.

Some populations are at greater risk for contracting emerging respiratory infections due to living conditions, access to resources, knowledge and education symptoms and infection, and movement. Military populations are especially at risk for respiratory diseases due to their high density living conditions and physically intense boot camp which make it easy for respiratory pathogens to spread from person to person. Additionally, military populations often move around the globe, coming into contact and regions with different endemic diseases which they sometimes bring back home on their
return [10]. In addition to movement of people, movement of animals can also greatly increase risk for new respiratory pathogens to emerge and spread to new regions of the world, as is seen with avian influenza.

Access to resources and knowledge can greatly influence a population’s ability to diagnose emerging pathogens early with diagnostics. Access to appropriate prevention, such as vaccines, or appropriate treatment, such as antibiotics or antivirals, can help prevent a respiratory outbreak from growing. Additionally, knowledge about the signs and characteristics of respiratory pathogens among clinicians and also the general public is important for early identification of outbreaks and potentially separating sick and non-sick individuals to reduce further transmission.

**Clinical and Demographic Characteristics of Respiratory Viruses**

Acute respiratory infections make up a huge proportion of disease burden in the United States and globally, with an estimated 94 037 000 disability adjusted life years and 3.9 million deaths worldwide each year [11]. Respiratory infections are often difficult to diagnose clinically due to nonspecific and overlapping symptoms. Additionally, diagnostic tests can be time-consuming and costly and often require trained and well-equipped laboratories, making laboratory confirmation of each case impractical. However, laboratory results from various surveillance populations can be paired with clinical, demographic, and seasonality variables to create models that can give timely predictions of disease outcomes. Preventive measures and treatments to reduce respiratory disease burden can also be improved through routine surveillance by gaining a
better understanding of the percent positivity of pathogens among acute respiratory cases, seasonality, and coinfection occurrence.

Currently, limited respiratory disease etiology studies have been done in the United States [12,13], despite many being done in other countries [14,15,16,17,18,19,20]. Additionally, few viral etiology studies have collected clinical signs and symptoms and assessed their association with different pathogens [14,18]. Most predictive models for respiratory diseases have focused on identifying influenza using clinical signs and symptoms [21,22,23,24,25,26], but have ignored predictions for the many other pathogens that occur. Understanding US-specific disease burden and seasonality across multiple populations is important, since disease incidence, distribution, and seasonality may vary between populations, regions, and climates.

Overall, identifying pathogens through clinical and demographic characteristics can improve timely and more accurate diagnosis, inform treatment plans, establish baselines of infection, identify outbreaks, and help prioritize the development of new vaccines and future treatments.

**Adenovirus Burden and Vaccines**

Adenovirus infections are very common among military trainees. This is thought to be due to the trainees close living quarters, minimal time for personal hygiene, persistence of adenoviruses in the environment [27,28], and the vigorous physical and environmental stressors of training camp. Before the adenovirus vaccines were routinely available, it was estimated that 80% of recruits became infected during recruit training, 40% had a significant illness, and 20% required hospitalization [29]. Due to the high
burden of adenovirus disease, the Naval Health Research Center (NHRC) has conducted ongoing adenovirus surveillance among recruits at eight recruit training centers since 1996, as part of the Department of Defense (DoD) Febrile Respiratory Illness (FRI) surveillance program.

After a number of other vaccine constructs were tested, live oral vaccines against adenovirus types 4 (Ad4) and 7 (Ad7) were introduced in 1971 and were successful in greatly reducing respiratory morbidity at recruit training centers. In 1996, adenovirus vaccine manufacturing was halted when the sole manufacturer, Wyeth Pharmaceuticals, declined to continue production. When a military order rationed the remaining adenovirus vaccine stockpile to be used only during winter months from 1997 to 1999, adenovirus rates increased dramatically [30,31]. Rates continued to escalate when vaccine supplies were depleted in 1999, after which the vaccines were no longer administered. The increased number of adenovirus infections cost approximately $10–26 million per year in terms of medical care and lost recruit training time [32,33]. During the period from 1999 to 2010, eight adenovirus-infected recruits died [34,35]. However, it is difficult to compare the vaccines and post-vaccines mortality rates due to changes in surveillance and laboratory capabilities [34].

As it became clear that adenovirus infections had again become highly endemic in US recruit training centers, the US Army contracted with Barr Pharmaceuticals in 2001 to resume production of the Ad4 and Ad7 vaccines. Phase III, double-blind, placebo-controlled clinical trials of the two vaccines were conducted by the US Navy and Army at recruit training sites in Fort Jackson, South Carolina, and the Recruit Training Command (RTC) at Naval Station Great Lakes, Illinois. Results from this trial demonstrated that
Ad4 vaccine had a very high efficacy (99%), and Ad4 and Ad7 vaccines had high seroconversion rates and excellent safety profiles [36]. Following US Food and Drug Administration approval in March 2011, universal administration of Ad4 and Ad7 vaccines at all US recruit training sites resumed in October 2011.

**Avian Influenza (H5N1) and Poultry Trading**

Highly pathogenic avian influenza H5N1 was first identified in Chinese poultry in 1996 and has spread to 53 countries as of July 2014 [37]. Previous studies suggest that both poultry trade and bird migration are the main drivers spreading this virus to previously uninfected countries [38]. However, despite the evidence indicating that trade likely plays a role spreading this emerging virus, countries have continued to import millions of live poultry each year from countries experiencing at least one H5N1 poultry case. Unfortunately, due to limited or poor infectious disease surveillance, identified and reported H5N1 cases are likely to only represent the tip of the iceberg of the true number of cases occurring in a particular country. Consequently, limiting trade for a longer period of time after a case is identified or increasing prevention efforts during this time may have greater impacts on reducing the spread of this disease.

The zoonotic nature and pandemic potential of avian influenza viruses, especially H5N1 [39], makes their introduction into new countries especially troublesome. As of January 2014, there have been a total of 650 identified human cases, and 386 deaths, giving a case fatality rate in humans of 59% [40]. Although it is not currently easily transmittable in mammals, and only a couple cases of human-to-human transmission have occurred, there is concern that this could easily be changed by a few genetic mutations
Additionally, H5N1 can have devastating economic impacts from mass culling of sick birds, decreased market demand during an outbreak, and loss of trade [42]

Gaining a better understanding of the drivers that spread avian influenza globally will be important for targeting and improving future prevention efforts and policies, such as vaccination, increased biosecurity and limiting trade during epidemic periods. Identifying the mechanism for H5N1 will also be important for understanding the spread of similar avian influenza viruses of concern, such as H7N9 which recently emerged in China in February 2013 [43].

A previous study found that poultry trading and bird migration, played a role in the introductions of avian flu to new countries, with the magnitude of risk from these two factors differing by region. However, this study was done in 2006 and didn’t assess some of the more recent introductions of avian influenza into new countries and did not assess the specific role of different species. Additionally, they used an algorithm of infectious bird days that was calculated from the number of days a bird sheds avian flu virus to determine spread via trade [38]. This method likely underestimated the role of poultry traded, as many H5N1 poultry cases are not identified or reported.

Other social network analysis models have looked at live bird market (LBM) trade and the spread of H5N1 at the regional or country level [44,45,46]. One study found that counties in China with H5N1 positive birds identified in LBMs had significantly higher centrality measures than counties without H5N1 in poultry [47], indicating that hub areas may be at greatest risk. Another network study found that avian flu disease transmission can be reduced if the network is fragmented through increasing prevention efforts or limiting trade at the hub sites [44]. Additionally, temporal changes in network
connectivity, due to increased demands for poultry consumption, have also been used to identify time periods that may be associated with greater risk [47]. Unlike previous studies that have only looked at the local or county level networks, our study is unique in that it identifies networks of poultry trading at the global level.
References


CHAPTER 2

Clinical and Demographic Variables Associated With Pathogen-Specific Respiratory Infections Among Three US Populations
ABSTRACT

**Background.** Diagnostic tests for respiratory infections can be costly and time-consuming. Identifying respiratory pathogens through signs, symptoms, and demographic characteristics, along with improving our understanding of coinfection rates and seasonality, may improve treatment and prevention measures.

**Methods.** Febrile respiratory illness (FRI) and severe acute respiratory infection (SARI) surveillance was conducted from October 2011 through March 2013 among three US populations: civilians near the US–Mexico border, Department of Defense beneficiaries, and military recruits. Questionnaire data and respiratory swabs were collected from participants and tested by PCR for nine different respiratory pathogens. A multinomial model was created to identify characteristics predictive of influenza, rhinovirus, other pathogens, and no/unknown pathogen. Additionally, seasonality and coinfection rates were described.

**Results.** A total of 1444 patients met the FRI case definition and were enrolled in this study. Study population, season, sore throat, cough, shortness of breath, congestion, body ache, headache, fever, and days to seeking care were predictive of respiratory infections in the multinomial model. Coinfections were found in 6% of all FRI/SARI cases tested and were most frequently seen with rhinovirus infections. Clear seasonality trends were seen for influenza, rhinovirus, and respiratory syncytial virus.

**Conclusions.** These results can help improve timeliness and accuracy of diagnosis, inform treatment plans, establish baselines of infection, identify outbreaks, and help prioritize the development of new vaccines and treatments.
Keywords: respiratory infection, surveillance, coinfection, seasonality, symptoms

Acute respiratory infections make up a huge proportion of disease burden in the United States and globally, with an estimated 94,037,000 disability adjusted life years and 3.9 million deaths worldwide each year [1]. Respiratory infections are often difficult to diagnose clinically due to nonspecific and overlapping symptoms. Additionally, diagnostic tests can be time-consuming and costly and often require trained and well-equipped laboratories, making laboratory confirmation of each case impractical. However, laboratory results from various surveillance populations can be paired with clinical, demographic, and seasonality variables to create models that can give timely predictions of disease outcomes. Preventive measures and treatments to reduce respiratory disease burden can also be improved through routine surveillance by gaining a better understanding of the percent positivity of pathogens among acute respiratory cases, seasonality, and coinfection occurrence.

Currently, limited respiratory disease etiology studies have been done in the United States [2,3], despite many being done in other countries [4,5,6,7,8,9,10]. Additionally, few viral etiology studies have collected clinical signs and symptoms and assessed their association with different pathogens [4,8]. Most predictive models for respiratory diseases have focused on identifying influenza using clinical signs and symptoms [11,12,13,14,15,16], but have ignored predictions for the many other pathogens that occur. Understanding US-specific disease burden and seasonality is important, since disease incidence, distribution, and seasonality may vary between populations, regions, and climates. This study aimed to evaluate predictive characteristics associated with specific respiratory pathogens, as well as the etiology, seasonality, and
coinfection rates among three US populations: military recruits, Department of Defense (DoD) beneficiaries, and civilians living near the US–Mexico border.

A total of 1444 samples from febrile respiratory illness (FRI) and severe acute respiratory infection (SARI) cases collected from 2011 to 2013 were tested for influenza A and B, adenovirus, rhinovirus, respiratory syncytial virus (RSV), coronaviruses (CoV229E, CoVOC43, CoVNL63), human metapneumovirus (hMPV), *Bordetella pertussis*, *Chlamydophila pneumoniae*, and *Mycoplasma pneumoniae*. These laboratory results were then paired with clinical signs and symptoms, and demographic and seasonality variables to create a predictive model for multiple pathogens. The results of this study can help improve timely and more accurate diagnosis, inform treatment plans, establish baselines of infection, identify outbreaks, and help prioritize the development of new vaccines and future treatments.

**METHODS**

**Participants**

FRI and SARI surveillance was conducted between October 2011 and March 2013 among three surveillance groups in the United States: civilians near the US–Mexico border, DoD dependents, and military recruits. Recruits are typically young and healthy adults who enter into an 8–12 week “boot camp” training program, which involves strenuous, and physically demanding activities and living in high-density barracks. During the first week of training, recruits receive a series of vaccinations, including influenza (seasonal) and adenovirus. Most training centers also administer at least 1 dose of bicillin to incoming trainees as prophylaxis against *Streptococcus* bacteria. The DoD
dependent population is made up of the families of active duty and retired military personnel. This population consists of all ages and has good access to health care through the TRICARE health care program. The border sites are not associated with the military and typically capture underserved populations living near the US–Mexico border in California and Arizona. The border sites are part of a broader Border Infectious Disease surveillance program run by the US Centers for Disease Control and Prevention and San Diego and Imperial counties, for which the Naval Health Research Center (NHRC) serves as the testing laboratory.

The FRI recruit sites included Marine Corps Recruit Depot (MCRD) San Diego, California; Air Force Basic Military Training center, Lackland Air Force Base, Texas; Naval Training Command, Great Lakes, Illinois; US Army Training Center (USATC) Fort Leonard Wood, Missouri; USATC Fort Jackson, South Carolina; USATC Fort Benning, Georgia; MCRD Parris Island, South Carolina; and Coast Guard Training Center, Cape May, New Jersey. The DoD beneficiaries sites included Naval Medical Center San Diego, California; TRICARE Clairemont Mesa, San Diego, California; and TRICARE Great Lakes, Illinois. FRI and SARI border sites included clinics and hospitals in San Ysidro, California; Calexico, California; Brawley, California; El Centro, California; Chula Vista, California; Selles, Arizona; and Tucson, Arizona.

**Ethics Statement**

This research was conducted in compliance with all applicable federal and international regulations governing the protection of human subjects in research. The research conducted in the recruit and DoD beneficiary populations underwent NHRC
IRB approval (NHRC protocols 31230 and NHRC.1999.0002) and written consent or parental guardian consent for minors was obtained for all participants. The data collected from the border population was part of a surveillance program run by the Centers for Disease Control and Prevention and was considered non-research by the NHRC IRB. NHRC staff members only provided diagnostic support and only received non-personally identifiable data from this population.

**Procedures**

Case definitions were slightly different for each of the 3 populations. In the recruit population, an FRI case was defined as a person who sought medical care and had an oral temperature ≥38.0°C (100.5°F) and either cough or sore throat. For the beneficiary population and border populations, the same FRI case definition was used; however, a fever ≥37.8°C (100.0°F) or subjective fever was used. Additionally, inpatients who met SARI case definition at select border sites were also sampled. The SARI case definition included people who presented with either fever ≥37.8°C (100.0°F) or feeling feverish/chills, in addition to cough, and hospital admission, with onset in the last 10 days. Additionally, children under age 5 were included if they were admitted to the hospital with clinical suspicion of pneumonia.

Nasal or combination nasal/pharyngeal swabs and questionnaire data were collected from a convenience sample of up to 20 patients per week per site who sought medical attention, met the FRI or SARI case definition, and provided written informed consent. Specimens were placed in universal transport medium, preserved at −80°C, and later transported on dry ice to the reference laboratory at the NHRC every 1 to 2 weeks.
for testing. Additionally, the following demographic and clinical signs and symptoms were collected from each FRI and SARI case: sex, age, study population, month of illness, pneumonia, sore throat, cough, nausea, shortness of breath, congestion, pink eye, body ache, headache, temperature, number of days of symptoms before seeking care, and date of seeking care.

Samples were extracted using the Roche MagNA Pure LC extraction system following manufacturer’s instructions. Samples were then tested for a broad-spectrum panel consisting of gel tests: hMPV (single plex), CoVNL63 (single plex), and CoV229E and CoVOC43 (multiplex). Real-time polymerase chain reaction (PCR) assays were run on the Applied Biosystems 7500 Fast Real-Time PCR System testing for influenza A, influenza B, adenovirus, RSV, rhinovirus, and a bacterial multiplex consisting of *M. pneumoniae*, *C. pneumoniae*, and *B. pertussis*.

**Statistical Analysis**

The percent positive for each pathogen among the three populations was calculated. Chi-square tests for categorical variables and analysis of variance (ANOVA) tests for continuous variables were used to identify signs, symptoms, seasonality, and demographic variables associated with each pathogen group (influenza, rhinovirus, other virus or bacteria, and no/unknown pathogen). Variables that were univariately associated with the pathogens (*p* < 0.15) were investigated further in a multinomial logistic regression model. Variables with a *p* < 0.05 were considered in the final adjusted model. Age and sex were collinear with study group and were dropped from the final model. Coinfections were coded as “other pathogen” even if 1 pathogen was influenza or
rhinovirus. Season was coded as follows: winter (December through February), spring (March through May), and summer/fall (June through November). Border and DoD dependent populations were combined for modeling because they showed similar characteristics.

Statistical analysis was conducted using SAS software (version 9.3, SAS Institute, Inc., Cary, North Carolina). PROC LOGISTIC with link= GLOGIT was used for the multinomial modeling and PROC ANOVA and PROC FREQ were used for ANOVA and chi-squared analyses, respectively.

RESULTS

During the 2 years of the study, 1444 patients met the FRI or SARI case definitions and were enrolled in this study, consisting 406 (28%) from the FRI/SARI border, 423 (29%) from the DoD beneficiary, and 615 (43%) from the FRI recruit populations. The percent positivity for each pathogen out of all specimens tested was rhinoviruses (16%), influenza (14%), RSV (6%), adenovirus (2%), coronaviruses (5%), bacterial (2%), hMPV (1%), and coinfections (6%). Influenza A (H3N2) was the most common influenza subtype, making up 54% of influenza isolates, followed by influenza B (31%), and pH1N1 (13%). Among coronaviruses, CoVOC43 was the most commonly identified strain, making up 67% of coronavirus isolates, followed by CoV229E (21%) and CoVNL63 (12%). Additionally, viral culture testing of a systematic sample of specimens not positive for influenza or adenovirus found that 1% (8/571) were positive for parainfluenza viruses.
All descriptive characteristics were significantly different ($p < 0.05$) across all pathogens. Rhinovirus and bacterial infections were more frequently isolated from recruits and men who make up the majority of the recruit population; whereas influenza and RSV were less frequent among these groups compared with no/unknown pathogen cases. Fewer rhinovirus and influenza and more RSV pathogens were identified from SARI cases compared to FRI. Pneumonia and shortness of breath were more frequent among rhinovirus cases and less common among influenza cases. However, time to seeking care was shorter for influenza and hMPV and longest for bacterial infections. Influenza, RSV, adenovirus, and hMPV cases all had significantly higher temperatures compared with no/unknown pathogen, and rhinovirus has significantly lower temperature (Table 1).

The multinomial logistic regression model included all variables except for age and sex, which were collinear with study group, and pneumonia, nausea, and pink eye, which were not significant in the multivariate model. Compared to participants with no or unknown pathogens, participants with influenza had greater odds of sore throat, cough, fever, short time to seeking care, of being a nonrecruit, and the illness occurring in the winter or spring. Participants with rhinovirus had greater odds of cough, congestion, less body ache, and being a recruit with illness in the spring. Finally, participants with other pathogens had greater odds of cough, fever, and illness in the winter, and less shortness of breath and headache. (Tables 2 and 3).

At least 1 pathogen was identified in 51% of all FRI and SARI cases. Coinfections were found in 81 (6%) of all FRI/SARI cases tested, among which were 76 double, 4 triple, and 1 quadruple coinfections. The most frequent pathogen associated
with a coinfection was rhinovirus, followed by RSV and CoVOC43. The top 3 most common coinfections were rhinovirus and RSV (15% of all coinfections, 11 cases), rhinovirus and adenovirus (13% of all coinfections, 10 cases), and rhinovirus and CoVOC43 (12% of all coinfections, 9 cases) (Table 4).

Seasonality patterns were apparent for influenza, rhinovirus, and RSV, whereas underlying low levels of infection were seen for adenovirus and bacterial infections. The recruit population had a more constant number of FRI cases sampled than the other two populations throughout the study period. Overall, they also had a greater percent positivity for rhinovirus compared with the other two populations, but lower percent positivity for influenza and RSV. Rhinovirus appeared to have two peaks: one in spring and one in summer/fall among the border and beneficiary populations and consistently higher levels among the recruit population. In all populations influenza and RSV peaked in the winter (Figure 1). Influenza A typically peaked before influenza B.

**DISCUSSION**

In order to reduce respiratory disease burden, it is necessary to gain a better understanding of the percent positivity, coinfection rates, and seasonality of specific respiratory pathogens. Additionally, a predictive clinical model that uses symptoms, seasonality, and patient demographics can also help improve prevention efforts and patient treatment. Timeliness is especially important for influenza antivirals, which work best if given within the first 48 hours of symptoms. However, rapid diagnostic tests have poor sensitivity [17] and multiplex PCR tests are impractical for most clinical settings. Additionally, treatment with antibiotics can often be incorrectly prescribed for viral
infections, leading to increased antibiotic resistance. Therefore, creating models to better predict the type of pathogen using symptoms and characteristics easily collected by a clinician at the time of visit could improve treatment accuracy and help protect the effectiveness of existing antibiotics and antivirals.

Although several studies have compared clinical characteristics between two or more different respiratory pathogens, [4,18] or used clinical symptoms to create a model to differentiate two pathogens [19], none have created a model that combines clinical, demographic, and seasonality variables into one model that predicts the probability of multiple pathogens. Most existing predictive models for respiratory disease have been done specifically for influenza [11,12,13,14,15]. Our model is unique in that it will predict multiple outcomes (influenza, rhinovirus, other pathogen, no/unknown pathogen).

Many respiratory etiology studies have been done outside the United States among patients with both severe, lower respiratory illness [6,20,21], as well as more mild, upper respiratory disease [6,7,9,10,22]. These studies are important because viral etiologies vary across populations and regions, depending on factors such as population susceptibility, age, circulating strains, climate, comorbidities, and vaccination coverage. Our study adds to the very limited number of etiology studies done in the United States [2,3] by examining viral etiology among three different US populations: military recruits, DoD dependents, and a US–Mexico border civilians. Our study is also unique in that it collected clinical signs, symptoms, and demographics for each case tested with the broad-spectrum respiratory panel. Similar studies have been limited and have used smaller sample sizes, focused on specific age groups, and did not test for as many respiratory pathogens [8]. Identifying population-specific baselines of infection enables us to
identify elevated rates, which may indicate an outbreak or the start of a pandemic. Recognizing associated symptoms can help determine the most likely pathogen, as was seen in 2009 with the pandemic influenza (H1N1) strain first identified in Brawley and San Diego, California, in two of the populations in this study.

There were several key differences of infection among the three populations. The recruit population had consistently higher levels of rhinovirus and bacterial infections than the other groups, which may be reflective of close living conditions and a younger age group, mostly 18–24 years old. This population is also highly vaccinated for influenza and showed the smallest amount of influenza infection compared with the other two populations. Adenovirus, which historically had a large impact on recruits, was also low as a result of resumption of the adenovirus vaccines in October 2011 [23]. RSV, which usually infects young children, was more commonly found in the DoD dependent (48%) and border populations (48%) compared with the recruits (4%). Overall, rhinoviruses were the most common respiratory pathogen identified (236 cases, 16%), followed by influenza (197 cases, 14%), RSV (85 cases, 6%), and coronaviruses (66 cases, 5%), which coincides with other etiology studies [24].

Understanding coinfections can also be useful for preventing respiratory disease. Our study found around 30% to 40% of coronavirus and adenovirus infections occurred as coinfections and they most frequently occurred with rhinovirus. Similarly, other studies have also found the highest ratio of coinfections among adenovirus and coronaviruses [25,26], and have found rhinovirus to be part of the most frequently occurring coinfections [21,25]. These results suggest that infection with some viruses, such as rhinoviruses, could create opportunistic environments for colonization with other
viruses and bacteria. Interestingly, coinfections were most common among the youngest age group, newborn to 4 years, which did not have the highest rhinovirus rate. Targeting rhinovirus infection through creation of new vaccines or treatment could have more far-reaching benefit in protecting a person from other infections.

One limitation of this study is that it only captured people with FRI/SARI who sought medical care. Military recruits may be less likely to seek care than other groups due to concern over losing training time or having to restart the program, and US–border populations may have less access to health care. Therefore, the etiology of more mild infections may be underrepresented for these two groups. Additionally, the case definitions were slightly different for the three populations, which may have influenced which pathogens were identified in each group. Although this study involved three different US populations, the results of this study may be less generalizable to the general US public who are not associated with the military or living on the US–Mexico border. Despite this, signs and symptoms from these pathogens should be similar across other populations in similar age groups and with similar vaccination coverage. Additionally, we found that seasonality of infection for recruits was similar to that of the border and beneficiary populations for several pathogens, but with different intensity. Consequently, illness surveillance in recruits, which made up the largest proportion of our study population, can be beneficial in informing disease trends in the general public.

Although we tested for many pathogens, there are likely still circulating viruses and bacteria for which we did not test, such as parainfluenza viruses or potentially unrecognized viruses; therefore, these cases were likely incorrectly classified as part of the “no pathogen” group. Additionally, the timing of sample collection in the course of
illness could impact whether or not viruses were identified by PCR. Despite this, our study was part of a well-established existing surveillance program that consistently collected and tested a substantial number of specimens at many different sites across the United States. Additional years of surveillance data will help improve our understanding of seasonality in the future.

The multinomial model created in this study can give clinicians the odds that an FRI/SARI patient has a particular pathogen by using the patient’s symptoms, signs, season, and demographics. This model could improve diagnosis and treatment, by informing clinicians on appropriate antiviral and antibiotic treatment during the patient’s visit. This will ultimately reduce the number of lost work days and transmission. Additionally, gaining a better understanding of baseline of disease and seasonality for specific pathogens can improve our ability to detect outbreaks. Identifying the percent positivity of each pathogen, coinfection rates, and risk factors for disease will help inform vaccination programs, and possible investment in the development of future vaccines or treatments.

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Acknowledgments

Chapter 2, in full, has been submitted for publication at PLOS ONE. Raman, Rema; Lindsay, Suzanne; Araneta, Maria Rosario; Fowler, James; Shaffer, Richard. The dissertation author was the primary author of this material.
References


Figure 2.1: Number positive and percent of febrile respiratory illness/severe acute respiratory infection (FRI/SARI) patients positive for respiratory pathogens among US military recruits, DoD beneficiaries, and US–Mexico border populations, 2012–2013.
Table 2.1: Descriptive Characteristics by Pathogen, October 2011-March 2013 (n=1444)

<table>
<thead>
<tr>
<th>Groups in Model</th>
<th>Characteristics of Pathogens in “Other” Group</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RSV (n = 85)</td>
<td>Adenovirus (n = 33)</td>
</tr>
<tr>
<td>Characteristic</td>
<td>N (%)</td>
<td>N (%)</td>
</tr>
<tr>
<td>Sex (% male)</td>
<td>40 (48)</td>
<td>24 (73)</td>
</tr>
<tr>
<td>Age, years</td>
<td>57 (73)</td>
<td>8 (24)</td>
</tr>
<tr>
<td>0–4</td>
<td>15 (19)</td>
<td>20 (61)</td>
</tr>
<tr>
<td>5–24</td>
<td>1 (1)</td>
<td>4 (12)</td>
</tr>
<tr>
<td>25–49</td>
<td>7 (9)</td>
<td>1 (3)</td>
</tr>
<tr>
<td>50+</td>
<td>41 (48)</td>
<td>13 (39)</td>
</tr>
<tr>
<td>Study population</td>
<td>34 (48)</td>
<td>5 (15)</td>
</tr>
<tr>
<td>US–Mexico border</td>
<td>173 (73)</td>
<td>294 (42)</td>
</tr>
<tr>
<td>DoD beneficiary</td>
<td>11 (5)</td>
<td>112 (16)</td>
</tr>
<tr>
<td>Military recent</td>
<td>225 (55)</td>
<td>591 (34)</td>
</tr>
<tr>
<td>Severity</td>
<td>101 (43)</td>
<td>175 (25)</td>
</tr>
<tr>
<td>Outpatient (TPE)</td>
<td>50 (23)</td>
<td>201 (29)</td>
</tr>
<tr>
<td>Inpatient (SARI)</td>
<td>80 (34)</td>
<td>139 (71)</td>
</tr>
</tbody>
</table>
Table 2.1: Descriptive Characteristics by Pathogen, October 2011-March 2013 (n=1444), continued

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Groups in Model</th>
<th>Characteristics of Pathogen in &quot;Other&quot; Group</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rhinovirus (n = 236)</td>
<td>Influenza (n = 197)</td>
<td>No/Unknown (n = 308)</td>
</tr>
<tr>
<td>Characteristic</td>
<td>N (%)</td>
<td>N (%)</td>
<td>N (%)</td>
</tr>
<tr>
<td>Signs and symptoms (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pneumonia</td>
<td>102 (44)</td>
<td>18 (10)</td>
<td>186 (28)</td>
</tr>
<tr>
<td>Sore throat</td>
<td>178 (76)</td>
<td>152 (77)</td>
<td>480 (69)</td>
</tr>
<tr>
<td>Cough</td>
<td>218 (93)</td>
<td>192 (97)</td>
<td>590 (84)</td>
</tr>
<tr>
<td>Nausea</td>
<td>92 (38)</td>
<td>87 (45)</td>
<td>236 (37)</td>
</tr>
<tr>
<td>SOB</td>
<td>127 (54)</td>
<td>65 (32)</td>
<td>298 (41)</td>
</tr>
<tr>
<td>Congestion</td>
<td>203 (87)</td>
<td>154 (79)</td>
<td>468 (69)</td>
</tr>
<tr>
<td>Pink eye</td>
<td>16 (7)</td>
<td>19 (10)</td>
<td>28 (4)</td>
</tr>
<tr>
<td>Body ache</td>
<td>133 (53)</td>
<td>125 (64)</td>
<td>405 (58)</td>
</tr>
<tr>
<td>Headache</td>
<td>153 (65)</td>
<td>127 (65)</td>
<td>444 (64)</td>
</tr>
<tr>
<td>Fever (&gt;100°F)</td>
<td>100 (42)</td>
<td>137 (70)</td>
<td>355 (51)</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature (°F)</td>
<td>99.5 (1.8)</td>
<td>100.9</td>
<td>99.9 (2.0)</td>
</tr>
<tr>
<td>Days to seeking care</td>
<td>6.6 (5.7)</td>
<td>3.6 (3.1)</td>
<td>5.2 (6.1)</td>
</tr>
</tbody>
</table>
Table 2.2: Multinomial Logistic Regression Predicting Influenza, Rhinovirus, Other Pathogen, or No/Unknown Pathogen Using Participant Characteristics (n = 1392)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Influenza (n = 199)</th>
<th>Rhinovirus (n = 285)</th>
<th>Other (n = 223)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study population</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Military recruit</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>US–Mexico border and DoD beneficiary</td>
<td>4.43</td>
<td>2.75–7.12</td>
<td>0.29</td>
<td>0.29</td>
</tr>
<tr>
<td>Season</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Winter (Dec–Feb)</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Spring (Mar–May)</td>
<td>1.07</td>
<td>0.71–1.63</td>
<td>2.21</td>
<td>0.94</td>
</tr>
<tr>
<td>Summer–Fall (Jun–Nov)</td>
<td>0.13</td>
<td>0.06–0.28</td>
<td>0.94</td>
<td>0.94</td>
</tr>
<tr>
<td>Signs and symptoms</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sore throat</td>
<td>1.99</td>
<td>1.29–3.06</td>
<td>1.13</td>
<td>0.89</td>
</tr>
<tr>
<td>Cough</td>
<td>8.18</td>
<td>3.16–21.17</td>
<td>1.99</td>
<td>6.70</td>
</tr>
<tr>
<td>Shortness of breath</td>
<td>0.70</td>
<td>0.48–1.02</td>
<td>1.30</td>
<td>0.62</td>
</tr>
<tr>
<td>Congestion</td>
<td>1.48</td>
<td>0.97–2.28</td>
<td>2.05</td>
<td>1.12</td>
</tr>
<tr>
<td>Body ache</td>
<td>1.27</td>
<td>0.85–1.91</td>
<td>0.66</td>
<td>0.86</td>
</tr>
<tr>
<td>Headache</td>
<td>1.16</td>
<td>0.76–1.77</td>
<td>0.81</td>
<td>0.59</td>
</tr>
<tr>
<td>Fever</td>
<td>2.20</td>
<td>1.50–3.24</td>
<td>0.88</td>
<td>1.59</td>
</tr>
<tr>
<td>Days to seeking care</td>
<td>0.92</td>
<td>0.86–0.97</td>
<td>1.00</td>
<td>1.01</td>
</tr>
</tbody>
</table>

The dependent variable is categorized as control (no/unknown pathogen), influenza, rhinovirus, and other pathogen.

CI, confidence interval; DoD, Department of Defense; OR, odds ratio.
Table 2.3: Symptoms and Characteristics More Likely to Occur in Participants With Influenza, Rhinovirus, and Other Pathogen Compared to Those With No/Unknown Pathogens

<table>
<thead>
<tr>
<th>Influenza</th>
<th>Rhinovirus</th>
<th>Other Pathogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonrecruit</td>
<td>Recruits</td>
<td>Winter</td>
</tr>
<tr>
<td>Winter/ Spring</td>
<td>Spring</td>
<td>Cough</td>
</tr>
<tr>
<td>Sore throat</td>
<td>Cough</td>
<td>Fever</td>
</tr>
<tr>
<td>Cough</td>
<td>Congestion</td>
<td>Less shortness of breath</td>
</tr>
<tr>
<td>Fever</td>
<td>Less body ache</td>
<td>Less headache</td>
</tr>
<tr>
<td></td>
<td>Short time to seeking care</td>
<td></td>
</tr>
</tbody>
</table>
### Table 2.4: Detection of Respiratory Coinfections 2011–2013 (n = 1444)

<table>
<thead>
<tr>
<th>% coinfections</th>
<th>Rhinovirus</th>
<th>Influenza A</th>
<th>Influenza B</th>
<th>RSV</th>
<th>CoV229E</th>
<th>CoVOC43</th>
<th>CoVNL63</th>
<th>Adenovirus</th>
<th>hMPV</th>
<th>M. pneumoniae</th>
<th>C. pneumoniae</th>
<th>Detection Rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhinovirus</td>
<td>296</td>
<td>6</td>
<td>5</td>
<td>11</td>
<td>3</td>
<td>9</td>
<td>4</td>
<td>10</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>20</td>
</tr>
<tr>
<td>Influenza A</td>
<td>152</td>
<td>0</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>11</td>
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<tr>
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<td>CoVNL63</td>
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<tr>
<td>C. pneumoniae</td>
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Four triple and 1 quadruple coinfections were identified but not included in upper portion of the table: rhinovirus/adenovirus/RSV, rhinovirus/influenza A/CoV229E, rhinovirus/influenza A/CoVOC43, rhinovirus/RSV/CoV229E, and rhinovirus/influenza A/adenovirus/RSV.

*C. pneumonia, Chlamydia pneumoniae*; hMPV, human metapneumovirus; *M. pneumoniae*, *Mycoplasma pneumoniae*; RSV, respiratory syncytial virus.
CHAPTER 3

Dramatic Decline of Respiratory Illness Among US Military Recruits After the Renewed Use of Adenovirus Vaccines
Dramatic Decline of Respiratory Illness Among US Military Recruits After the Renewed Use of Adenovirus Vaccines

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Background. In late 2011, after a 12-year hiatus, oral vaccines against adenovirus types 4 (Ad4) and 7 (Ad7) were again produced and administered to US military recruits. This study examined the impact of the new adenovirus vaccines on febrile respiratory illness (FRI) and adenovirus rates and investigated if new serotypes emerged. FRI rates and their associated hospitalizations had markedly risen since vaccine production ceased in 1999.

Methods. From 1996 to 2013, the Naval Health Research Center conducted FRI surveillance at 8 military recruit training centers in the United States. During this period, 58,103 FRI pharyngeal swab specimens were studied, yielding 37,048 adenovirus-positive cases, among which 64% were typed.

Results. During the 2 years after reintroduction of the vaccines, military trainees experienced a 100-fold decline in adenovirus disease burden (from 5.8 to 0.02 cases per 1000 person-weeks, P < .0001), without evidence that vaccine pressure had increased the impact of adenovirus types other than Ad4 and Ad7. Although the percentage of type 14 increased following reintroduction of the vaccination, the actual number of cases decreased. We estimate that the vaccines prevent approximately 1 death, 1100–2700 hospitalizations, and 13,000 febrile adenovirus cases each year among the trainees.

Conclusions. These data strongly support the continued production and use of Ad4 and Ad7 vaccines in controlling FRI among US military trainees. Continued surveillance for emerging adenovirus subtypes is warranted.

Keywords. adenovirus; febrile respiratory illness; military; surveillance; vaccine.

Adenovirus infections are very common among military trainees. This is thought to be due to the trainees' close living quarters, minimal time for personal hygiene, persistence of adenoviruses in the environment [1, 2], and the vigorous physical and environmental stressors of training camp. Before the adenovirus vaccines were routinely available, it was estimated that 80% of recruits became infected during recruit training. 40% had a significant illness, and 20% required hospitalization [3]. Due to the high burden of adenovirus disease, the Naval Health Research Center (NHRC) has conducted ongoing adenovirus surveillance among recruits at 8 recruit training centers since 1996, as part of the Department of Defense (DoD) febrile respiratory illness (FRI) surveillance program.

After a number of other vaccine constructs were tested, live oral vaccines against adenovirus types 4 (Ad4) and 7 (Ad7) were introduced in 1971 and were successful in greatly reducing respiratory morbidity at recruit training centers. In 1996, adenovirus vaccine manufacturing was halted when the sole manufacturer, Wyeth Pharmaceuticals, declined to continue production. When a military order rationed the remaining adenovirus vaccine stockpile to be used only during winter months from...
1997 to 1999, adenovirus rates increased dramatically [4, 5]. Rates continued to escalate when vaccine supplies were depleted in 1999, after which the vaccines were no longer administered. The increased number of adenovirus infections cost approximately $10–$26 million per year in terms of medical care and lost recruit training time [6, 7]. During the period from 1999 to 2010, 8 adenovirus-infected recruits died [8, 9]. However, it is difficult to compare the vaccine and postvaccine mortality rates due to changes in surveillance and laboratory capabilities [8].

As it became clear that adenovirus infections had again become highly endemic in US recruit training centers, the US Army contracted with Burr Pharmaceuticals in 2001 to resume production of the Ad4 and Ad7 vaccines. Phase 3, double-blind, placebo-controlled clinical trials of the 2 vaccines were conducted by the US Navy and Army at recruit training sites in Fort Jackson, South Carolina, and the Recruit Training Command (RTC) at Naval Station Great Lakes, Illinois. Results from this trial demonstrated that Ad4 vaccine had a very high efficacy (99%), and Ad4 and Ad7 vaccines had high seroconversion rates and excellent safety profiles [10].

Following US Food and Drug Administration approval in March 2011, universal administration of Ad4 and Ad7 vaccines at all US recruit training sites resumed in October 2011. The purpose of this study was to assess the effectiveness of the new Ad4 and Ad7 vaccines in reducing FRI among recruits and to determine whether new strains emerged.

METHODS

Participants
FRI surveillance took place at 8 military recruit training facilities across the United States from 1996 to 2013. The sites included RTC Great Lakes; Marine Corps Recruit Depot (MCRD) San Diego, California; MCRD Parris Island, Parris Island, South Carolina; Air Force Basic Military Training Center, Lackland, Texas; Army Basic Combat Training Center, Fort Leonard Wood, Missouri; Army Basic Combat Training Center, Fort Jackson, South Carolina; Army Basic Military Training Center, Fort Benning, Georgia; and the Coast Guard Training Center (CGTC), Cape May, New Jersey. In 1996, the only sites being surveilled were MCRD San Diego and RTC Great Lakes.

Recruit training generally lasts from 6 to 12 weeks, depending on service branch. "Boot camp" is physically demanding and recruits are in close contact throughout the day and night, living in high-density barracks. During the first week of training, recruits are typically given a series of vaccinations. From the 1970s to 1996, Ad4 and Ad7 vaccines were included in year-round vaccinations; from 1997 to 1999 these vaccines were only given during winter months, and from 2000 to 2011 they were not administered at all. Starting in October and November of 2011, adenovirus vaccination was resumed, and by 2012 year-round vaccination was once again given to all incoming recruits.

Procedures
Each week an on-site NHRC research staff member collected data on the number of FRI cases and total recruit population at each recruit training site, with the exception of the relatively small Cape May CGTC, where clinic staff provided the data. An FRI case was defined as a recruit who sought medical care and had an oral temperature ≥38°C (100.5°F) and either cough or sore throat. Pharyngeal or combination nasal/pharyngeal swabs and questionnaire data were collected from a convenience sample of up to 20 recruits per week per site who sought medical attention, met the FRI case definition, and provided written informed consent. Specimens were placed in viral transport medium, preserved at −80°C, and later transported on dry ice to the reference laboratory at NHRC every 1–2 weeks for testing.

From 1996 to 2004, viral culture in ATCC cells was used to identify adenovirus-positive cases, and serotyping was performed on a subset of 10%–30% of positive specimens by microneutralization (MN) assay [4]. Starting in mid-2004 through 2006, initial adenovirus identification was determined by molecular methods, and the MN assay described above was used to serotype approximately 20% of positive specimens. Beginning in 2007, a singleplex, gel-based polymerase chain reaction (PCR) assay [4] was used to test specimens for adenovirus species B, C, and E. Specimens positive for species E were classified as Ad4, and species B and C adenovirus specimens were further tested by type-specific PCR assays to determine serotype. In late 2010, testing for adenovirus was transitioned to a real-time PCR assay, and all positive samples were serotyped by PCR methods as previously described. Despite the different laboratory methods used for our study, our laboratory found 90%–96% agreement when comparing viral culture and PCR results from thousands of FRI samples.

Statistical Analysis
Annual FRI incidence rates were calculated by dividing the number of cases by the total person-weeks at all sites. Person-weeks were computed by summing the number of recruits for each week at all sites. Estimated annual adenovirus incidence rates were calculated by multiplying the percentage of specimens positive for adenovirus by the FRI rate. The estimated number of adenovirus cases was determined by multiplying the adenovirus rate by person-weeks for each site and summing all of the sites to get the total number for each year. In 1998, the estimated number of adenovirus cases was extrapolated from the existing surveillance data from June through December by assuming the number of cases was constant throughout the year. The number of hospitalizations per year was calculated by multiplying the estimated annual number of adenovirus
cases by historical rate estimates for adenovirus hospitalization, which ranged from approximately 8% to 20%. Data were grouped by vaccination period: year-round vaccination (1996), seasonal vaccination in winter months (1997–1999), no vaccination (2000–2011), and year-round vaccination (2012–2013). Differences of FRI and adenovirus rates across vaccination periods were assessed using Poisson regression, and differences of mean estimated number of adenovirus cases were assessed using analysis of variance (ANOVA), with \( P < 0.05 \) considered significant. The PROC GENMOD procedure with a log link was used for the Poisson regression analyses, and the PROC ANOVA procedure was used for the ANOVA analysis. SAS version 9.3 was used.

**Ethics**

This research was conducted in compliance with all applicable federal and international regulations governing the protection of human subjects in research (DoD protocols NHRC 31230 and NHRC.1999.0002).

**RESULTS**

From 1996 to 2013, NHRC collected and tested 58,103 FRI swab specimens, of which 37,048 (64%) were positive for adenovirus. During this time, NHRC typed on average 61% (range: 9%–100% annually) of adenovirus-positive specimens to identify the most prevalent strains. The proportion of adenovirus-positive cases serotyped was lower prior to 2007 due to the use of MN characterization techniques that were labor intensive in comparison with PCR.

As vaccination policies changed throughout the course of surveillance, large fluctuations in both FRI and adenovirus rates were observed. FRI and adenovirus rates followed a similar pattern, peaking during the nonvaccination periods (8.5 FRI cases per 1000 person-weeks; 5.8 adenovirus cases per 1000 person-weeks) and dropping when vaccination resumed (1.2 FRI cases per 1000 person-weeks, 0.02 adenovirus cases per 1000 person-weeks) (Table 1). Annual site-specific adenovirus rates varied across the sites, with peaks during the nonvaccination period between 6.5 and 21.0 adenovirus cases per 1000 person-weeks. During this time, high levels of adenoviral morbidity were observed at all sites, with maximum estimated annual adenovirus cases ranging from 605 at the smallest training center (CGTC Cape May) to 5880 at RTC Great Lakes. From 2012 to 2013, the maximum number of site-specific estimated adenovirus cases was 45 (Table 2). The mean estimated number of adenovirus cases per year was 13,518 during the nonvaccination period, 6,504 during the rationed years, and 60 after resumption.

**Table 1. Characteristics of Adenovirus and Febrile Respiratory Illness as Vaccination Policy Changed, 1996–2013**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Average No. tested per y</td>
<td>201</td>
<td>310</td>
<td>3990</td>
<td>1725</td>
<td></td>
</tr>
<tr>
<td>% Ad positive</td>
<td>11.9</td>
<td>57.2</td>
<td>67.6</td>
<td>4.7</td>
<td></td>
</tr>
<tr>
<td>FRI rate 95% CI</td>
<td>5.8 (5.6–5.9)</td>
<td>8.5 (8.4–8.6)</td>
<td>1.2 (1.1–1.2)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Ad rate 95% CI</td>
<td>3.4 (3.3–3.6)</td>
<td>5.8 (5.8–5.8)</td>
<td>0.02 (0.02–0.02)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Mean (SD) No. estimated Ad cases per y</td>
<td>6504 (7377)</td>
<td>13,518 (2786)</td>
<td>60 (42)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>% Serotyped</td>
<td>100</td>
<td>50</td>
<td>61</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Ad species/type (% of total serotyped)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C1</td>
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<td>0</td>
<td>2</td>
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</tr>
<tr>
<td>C2</td>
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</tr>
<tr>
<td>B/3</td>
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<td>7</td>
<td>2</td>
<td>2</td>
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</tr>
<tr>
<td>E/4</td>
<td>4</td>
<td>65</td>
<td>90</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>C/5</td>
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<td>0</td>
<td>1</td>
<td></td>
</tr>
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<td>20</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>B/14</td>
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<td>0</td>
<td>10</td>
<td>54</td>
<td></td>
</tr>
<tr>
<td>B/21</td>
<td>58</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Other/unknown</td>
<td>4</td>
<td>0</td>
<td>4</td>
<td>30</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: Ad, adenovirus; CI, confidence interval; FRI, febrile respiratory illness; SD, standard deviation.

\( ^{a} \) Marine Corps Recruit Depot San Diego and Great Lakes Recruit Training Command were the only sites in 1996.

\( ^{b} \) P-value is for significant rate or mean difference across 3 time periods: seasonal vaccines (1996–1999), no vaccines (2000–2011), and year-round vaccines (2012–2013).

\( ^{c} \) No. of cases per 1000 person-weeks.

\( ^{d} \) Only includes data from June 1996 to December 1999 (adjusted to estimate full year for 1996).
Table 2. Estimated Adenovirus Cases Annually by Site, 2000-2011 and 2012-2013

<table>
<thead>
<tr>
<th>Site</th>
<th>No Ad Vaccines Administered 2000-2011, Mean (SD)</th>
<th>Year-round Ad4 and Ad7 Vaccines Administered 2012-2013, Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fort Leonard Wood</td>
<td>1453 (513)</td>
<td>5 (6)</td>
</tr>
<tr>
<td>Fort Jackson</td>
<td>2967 (900)</td>
<td>4 (10)</td>
</tr>
<tr>
<td>Fort Benning</td>
<td>1429 (729)</td>
<td>8 (1)</td>
</tr>
<tr>
<td>RTC Great Lakes</td>
<td>3961 (1147)</td>
<td>12 (12)</td>
</tr>
<tr>
<td>MCCD San Diego</td>
<td>1312 (486)</td>
<td>3 (1)</td>
</tr>
<tr>
<td>MCCD Parris Island</td>
<td>942 (480)</td>
<td>27 (26)</td>
</tr>
<tr>
<td>Lackland AFB</td>
<td>1507 (1219)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>CGTC Cape May</td>
<td>177 (153)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

Abbreviations: Ad, adenovirus; AF8, Air Force Base; CGTC, Coast Guard Training Center; MCCD, Marine Corps Recruiting Depot; RTC, Recruit Training Command; SD, standard deviation.

of year-round vaccination (Table 1 and Figure 1). During the nonvaccination period, approximately 1100–2700 adenovirus hospitalizations occurred annually, and in 2012 and 2013 approximately 9–12 hospitalizations occurred with no documented deaths.

In 1996, the last year adenovirus vaccines were given to recruits year-round, Ad21 was the most prevalent type, comprising 58% of typed specimens, whereas Ad4 and Ad7 were only 4% each of all typed viruses. However, when vaccines were rationed from 1997 to 1999, Ad4 and Ad7 reemerged as the most prevalent types, comprising 65% and 20% of all viruses typed, respectively. Ad4 rose even further to 80% of typed viruses during 2000 to 2011, with the remaining 20% comprising the diverse serotypes Ad14, Ad21, Ad5, Ad7, and other/unknown types, when no vaccines were given. However, after vaccines were reintroduced, Ad4 and Ad7 declined to 9% and 1% of typed viruses, respectively (Table 1). During 2012, there were only 5 cases positive for Ad4p (the vaccine strain) and no cases positive for Ad7. The incidence of other adenovirus serotypes has also declined since the vaccine was resumed; for Ad14, the most prevalent nonvaccine adenovirus type, the estimated mean annual number of cases decreased from 610 in 2000-2011 to 44 in 2013.

**DISCUSSION**

With year-round adenovirus vaccination resumption in 2012, adenovirus infections decreased by approximately 100-fold among US military recruit trainees. Besides preventing unnecessary fatalities, adenovirus vaccines in the recruit population can prevent approximately 13 000 acute febrile illnesses a year and the associated lost training time and healthcare costs. As seen in previous years, even a short relapse or reduction in vaccination has resulted in serious adenovirus outbreaks and several deaths among military trainees [8, 9, 12-14]. Reestablishing production of the orphaned Ad4 and Ad7 vaccines was a costly...
and lengthy endeavor—the US government invested approximately $107 million over 10 years to reinstate the 2 vaccines [15]. Ensuring continued vaccine production and sufficient supplies for uninterrupted, year-round vaccination of all recruits should be a public health and fiscal priority for US DoD trainees in the future. Similarly, these vaccines could significantly reduce FRI and adenovirus rates in other foreign military recruits and police trainees who have also experienced adenovirus outbreaks and high burden of disease [16–20]. These vaccines also have the potential to reduce transmission to other geographical locations and to civilians after recruits have completed training and disperse globally [21].

After adjusting 1995 cost estimates associated with adenovirus infection [7] using the US Consumer Price Index for all Urban Consumers (all items and medical care item) [22], we estimated that each adenovirus infection costs approximately $3838 ($3128 for medical costs and $710 for lost training costs) in 2012 (Table 3). With approximately 13,000 clinical adenovirus illnesses prevented annually by year-round vaccination, the DoD would save approximately $50 million per year. During the first few years, the price of the new vaccines ranged from $120 to $150 per person for the 2 doses. Vaccinating approximately 200,000 new recruits a year at $150 per person for the 2 doses would cost $30 million, providing a net savings of approximately $20 million. The annual cost savings of the new adenovirus vaccines is slightly higher than previous estimates of $15–$16 million for the old vaccines [7]. However, this cost savings is still an underestimate, as it does not include the cost associated if a recruit must restart the training program, and it does not include the difficult-to-measure cost and burden of a death.

In contrast to our surveillance findings, 2 cost-benefit studies did not predict an increased benefit of year-round adenovirus vaccination compared with seasonal vaccination during winter months [6, 7]. A study by Hyer et al estimated that year-round vaccination would prevent only an additional 560 cases compared with no vaccination [6]. Similarly, a study by Howell et al estimated that both seasonal and year-round vaccination would prevent approximately the same number of cases [7]. The study by Hyer et al used vaccinative data from 1949 to 1966 that found higher rates in winter months [6]. However, our more recent surveillance data showed that FRI and adenovirus rates were higher in summer and fall months, which have been explained by a correlation with higher recruit populations during this time [5]. Consequently, our data showed that year-round vaccination prevented approximately 6000 and 13,000 additional adenovirus cases compared with seasonal and no vaccination, respectively. These results support the importance of the current year-round vaccination policy and explain the ineffectualness of the previous seasonal vaccination policy that administered vaccines only in winter months.

Our surveillance data had similar burden-of-disease estimates for nonvaccination years and seasonal vaccination years as previous studies predicted. The study by Howell et al estimated that 12,370 cases would occur during years without vaccination and 4570 cases would occur during both seasonal targeted vaccination and year-round vaccination of recruits [7]. We found an estimated 13,318 cases per year during the nonvaccination period, and 6594 cases per year during the seasonal vaccination period. However, unlike Howell et al, we only saw an estimated 680 cases per year during year-round vaccination from 2012 to 2013 (Table 1). This may be a result of the new adenovirus vaccine being a better match to the current circulating strains, at least at present.

After 25 years of year-round Ad4 and Ad7 vaccination, other adenovirus types were present in the recruit training population. NHRC’s limited sampling in 1996 demonstrated the presence of 3 nonvaccine adenovirus types, the majority of which were isolated from the RTC Great Lakes [4], when vaccine became seasonal or nonexistent. Ad4, a vaccine component, emerged as the predominant strain. In 2006, during the nonvaccination period, Ad4 suddenly emerged in 5 recruit training sites and became the most prevalent non-Ad4 subtype [23]. During the next 4 years, Ad4 became the most predominant type for prolonged periods at 4 of the 8 sites (data not shown). When vaccination resumed in 2012, Ad4 decreased significantly, supporting the effectiveness of the vaccine in preventing Ad4. During 2012 to 2013, Ad14 supplanted Ad4 as the most prevalent strain overall, although the estimated annual cases of Ad14 have also decreased markedly (98% reduction) (Table 1 and Figure 1). The decline of estimated annual cases of Ad14 and other types may be reflective of cross-reactive antibodies from the vaccines [24] and overall reduced persistence in the environment or a result of natural variances. As year-round vaccination continues, it will be important to monitor potentially emerging strains through established surveillance systems to see if rates of nonvaccine strains change.
An important strength of this study is that it included >18 years of data from 8 recruit sites throughout the country with continued observation during times of vaccination policy change. The size of the surveillance population, as well as the number of FRI and adenovirus specimens collected and tested, was consistent from year to year, with the exception of 1996 when the surveillance program was started. This consistency made for a robust surveillance system that allowed for comparability across the years. Additionally, our study included type-specific data, allowing assessment of temporal changes in strain predominance.

Despite the large scale of this surveillance program, it is difficult to capture the total number of recruits with FRI as not everyone with symptoms seeks medical attention. Many recruits with symptoms may also avoid seeking medical attention because it may prolong their training or cause them to be removed from service, or because they may be placed in a holding facility [5]. Previous studies have found that approximately one-third of recruits entering training with Ad4 immunity, one-third seroconverted with few symptoms and without a fever, and one-third had a febrile infection [2, 25]. By the end of the sixth week of training, 97% of all recruits had positive adenovirus titers [2]. Therefore, the estimated adenovirus burden and associated costs in this study are likely underestimated. Additionally, changes in healthcare-seeking behavior may also bias results. In 2009, the increase in cases of both adenovirus and FRI (Figure 1) was likely caused in part to more recruits going to clinics when they were sick due to worry about the 2009 H1N1 influenza pandemic. However, adenovirus rates during this year were within the range seen in other years during the no-vaccination period.

Previous studies have shown that adenovirus vaccination is the only dependable prevention measure for adenovirus infection in this population. Hand-washing was found to be protective, but compliance was difficult to enforce [26], and other environmental controls, such as reducing recruit density per barrack, were not effective [27]. The new adenovirus vaccines have an excellent safety profile and high effectiveness [10]; after full resumption of the vaccines in all recruits in 2012-2013, adenovirus rates plummeted from 5.8 to 0.02 cases per 1000 person-weeks. Therefore, year-round vaccination should continue as the first line of defense against adenovirus infection in US military recruits and vaccine production should continue. NHRC’s ongoing surveillance will monitor FRI and adenovirus rates in the future, paying particular attention to any rate increases of adenovirus types not included in the vaccines. This surveillance is important to assess ongoing vaccine effectiveness among military recruits.

Notes

Acknowledgments. We thank Melinda Balansay, Gary Brice, Daisy Cabrera, Johnnie Consolly, Robert Coon, Thomas Cropper, Larihlie Dela Cruz, Julie Fuller, Holly Gallo, Joel Gaydos, Chasity Greer, Christian Hansen, Lesley Henry, Elizabeth Hunt, Marissa Irvine, Kristopher Legge, Mark Lesko, Marietta Malasig, David Metzgar, Chris Myers, Lindsay Navarro, Shelly Oates, Nakia Clemmons, Vinla Paulk, Margaret Ryan, Erica Schwartz, Jennifer Strickler, Dawn Taggert, Miguel Osuna, Susan Varner, Daniel Ventel, Christina West Green, James Whitmer, Sandra Williams, Adriana Kajon, and many other laboratory and surveillance staff for their valuable contributions to this surveillance program. We also thank Michelle LeWark for her role in editing our paper.

Author contributions. J. M. R. and A. W. H. contributed to the literature search, study design, data analysis, data interpretation, creation of the figures, and writing. A. W. H. also contributed to data collection. P. J. B. and D. J. F. contributed to the study design, data collection, data interpretation, and writing. B. R. was involved with data analysis, data interpretation, and writing. K. L. R. and G. C. G. were involved with study design, data interpretation, and writing.

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Financial support. This work was supported by the Department of Defense Global Emerging Infections Surveillance and Response System under Work Unit No. 68005.

Potential conflicts of interest. All authors: No reported conflicts. All authors submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

Acknowledgments

Chapter 3, in full, is a reprint of the materials as it appears in Clinical Infectious Diseases: Radin JM, Hawksworth AW, Blair PJ, Faix DJ, Raman R, Russell KL, Gray GC. (2014) Dramatic decline of respiratory illness among US military recruits after the renewed use of adenovirus vaccines. *Clin Infect Dis* 59(7):962-8. Published by Oxford University Press. Raman, Rema; Lindsay, Suzanne; Araneta, Maria Rosario; Fowler, James; Shaffer, Richard. The dissertation author was the primary author of this material.
CHAPTER 4

International Chicken Trade and Increased Risk for Introducing or Reintroducing Highly Pathogenic Avian Influenza A (H5N1) to Uninfected Countries
Every year billions of chickens and other poultry species are shipped thousands of miles around the globe in order to meet the ever increasing demands for this cheap and nutritious protein source. Unfortunately, transporting chickens internationally can have negative environmental impacts, reduce business for local farmers, and can also increase the chance for introducing zoonotic viruses, such as avian influenza A (H5N1) to new countries. The global spread of this virus is especially worrisome due to its devastating economic impact, its zoonotic nature, and high case fatality rate in both poultry and humans. Since it is primarily an avian virus, humans have little to no natural immunity, increasing the risk for a pandemic if it were to become more easily transmissible from human to human. Our study used a retrospective analysis of poultry trading data to show that the risk of infection in an importing country increased by a factor of 1.3 (95% CI: 1.1-1.5) for every 10-fold increase in live chickens imported from countries experiencing at least one H5N1 poultry case during that year. These results suggest that the risk in a particular country can be significantly reduced if imports from countries experiencing an outbreak are reduced during the year of infection or if biosecurity measures such as screening, vaccination, and infection control practices are increased. These findings show that encouraging consumption of locally produced poultry or increasing infection control practices during infectious periods may be an important step in reducing the spread of H5N1 and other new emerging avian influenza viruses.
Significance Statement

Avian influenza A (H5N1) is often difficult to eradicate once it has been introduced to a new country, as it spreads quickly between poultry and can be easily transmitted to new flocks by water, feed or other infected surfaces. However, our study suggests that this risk may be greatly reduced by limiting chicken imports from infectious countries for a period following the identification of an H5N1 case. Preventing avian flu in poultry also has important implications for preventing avian flu in humans: Although the virus is currently not easily spread to or among humans, greater infection and replication of the virus among birds increases the chance for a mutation that could improve human transmissibility, possibly resulting in a pandemic.
Highly pathogenic avian influenza H5N1 was first identified in Chinese poultry in 1996 and has spread to 53 countries as of July 2014 [1]. Previous studies suggest that both poultry trade and bird migration are the main drivers spreading this virus to previously uninfected countries [2]. However, despite the evidence indicating that trade likely plays a role spreading this emerging virus, countries have continued to import millions of live poultry each year from countries experiencing at least one H5N1 poultry case. Unfortunately, due to limited or poor infectious disease surveillance, identified and reported H5N1 cases are likely to only represent the tip of the iceberg of the true number of cases occurring in a particular country. Consequently, limiting trade for a longer period of time after a case is identified or increasing prevention efforts during this time may have greater impacts on reducing the spread of this disease.

The zoonotic nature and pandemic potential of avian influenza viruses, especially H5N1 [3], makes their introduction into new countries especially troublesome. As of January 2014, there have been a total of 650 identified human cases, and 386 deaths, giving a case fatality rate in humans of 59% [4]. Although it is not currently easily transmittable in mammals, and only a couple cases of human-to-human transmission have occurred, there is concern that this could easily be changed by a few genetic mutations [5]. Additionally, H5N1 can have devastating economic impacts from mass culling of sick birds, decreased market demand during an outbreak, and loss of trade [6].

Gaining a better understanding of the drivers that spread avian influenza globally will be important for targeting and improving future prevention efforts and policies, such as vaccination, increased biosecurity and limiting trade during epidemic periods. Identifying the mechanism for H5N1 will also be important for understanding the spread
of similar avian influenza viruses of concern, such as H7N9 which recently emerged in China in February 2013 [7].

A previous study found that poultry trading and bird migration, played a role in the introductions of avian flu to new countries, with the magnitude of risk from these two factors differing by region. However, this study was done in 2006 and didn’t assess some of the more recent introductions of avian influenza into new countries and did not assess the specific role of different species. Additionally, they used an algorithm of infectious bird days that was calculated from the number of days a bird sheds avian flu virus to determine spread via trade [2]. This method likely underestimated the role of poultry traded, as many H5N1 poultry cases are not identified or reported.

Other social network analysis models have looked at live bird market (LBM) trade and the spread of H5N1 at the regional or country level [8–10]. One study found that counties in China with H5N1 positive birds identified in LBMs had significantly higher centrality measures than counties without H5N1 in poultry [11], indicating that hub areas may be at greatest risk. Another network study found that avian flu disease transmission can be reduced if the network is fragmented through increasing prevention efforts or limiting trade at the hub sites [8]. Additionally, temporal changes in network connectivity, due to increased demands for poultry consumption, have also been used to identify time periods that may be associated with greater risk [10]. Unlike previous studies that have only looked at the local or county level networks, our study is unique in that it identifies networks of poultry trading at the global level.

The goal of this paper is to determine if the international spread and outbreaks of highly pathogenic H5N1 in poultry have been influenced by the magnitude of poultry
traded from infected countries. This study assessed potential covariates on this association such as year, gross domestic product (GDP), and population size of the importing and exporting country and also examined the role of different poultry species, including ducks, chickens, and turkeys, in driving H5N1 global spread. Our final model shows how much risk could be reduced if imports are limited from countries experiencing an H5N1 poultry case or if prevention efforts are increased during these time periods.

RESULTS

The raw data showed that as the number of imports from infected countries increased, the probability of infection also increased, with significantly higher risk in countries importing from one or more infected countries compared to none in 2006. There was a small baseline probability of infection in countries not importing infectious chickens. (Fig. 1)

In 2003 two countries reported an H5N1 poultry case, six years after the last case was identified. It is likely that the virus mutated during this period, becoming more easily transmissible from bird to bird. This number of countries with poultry cases increased and peaked to 37 countries in 2006, and declined to 14 by 2011. The number of countries importing chickens from an infected country followed a similar trend, starting with 7 in 2003, peaking to 87 in 2006, and declining back to 9 in 2011. The percentage of countries with an outbreak in poultry that imported chickens from an infected country also peaked during 2006. The number of chicken imports from any country appears to have slightly declined over time from 2003 through 2011; however the quantity of
chicken imports has steadily increased. Interestingly, H5N1 cases in humans followed a similar trend, with the number of cases and number of countries with a case peaking around the same time as in poultry. (Table 1)

We used statistical models to replicate the pattern seen in the raw data and to assess the relationship between poultry trade and transmission of H5N1 from one country to another (see Materials and Methods). This study found significant and positive interaction between quantity of chickens traded and H5N1 poultry infection in the exporting country, in both the non-adjusted (p=0.0013) and full adjusted models (p=0.0005). For every unit increase in the log of the number of chickens that were imported from an infected country, the poultry infection in the importing country increased by a factor of 1.3 (95% CI: 1.1-1.5) for every 10-fold increase in live chickens imported when adjusting for year, import population and export population. The quantity of chickens traded on its own was not significant (p=0.34), indicating that increased trading of non-infected chickens did not change risk of infection. We also found that human population in the importing and exporting country was positively associated with the outcome. (Tables 1-3) Ducks and turkeys were also assessed in a similar model, but ducks did not have a significant interaction (S1) and turkeys had a significantly negative coefficient for the log of the quantity of turkeys traded (S2).

Using our infection model, we created a map showing the probability of poultry infection in an importing country for 2006, with and without chicken imports from a poultry infected country. This map showed risk reductions as large as 64% in individual countries, if they stopped importing chickens from infected countries during the peak of the epidemic (Fig. 2).
DISCUSSION

The results of this study suggest that the more chickens a country imports from an infected country, the greater the risk for having an H5N1 case in poultry. Unfortunately, it may sometimes be difficult for a country to identify infected chickens in a flock that is being traded. Avian influenza has an incubation period of 3 to 5 days in chickens [12], during which time infected birds may unknowingly be traded before they show illness. Additionally, increased use of avian flu vaccination in poultry may reduce symptom severity and thus delay detection among a flock [13]. H5N1 also has non-specific symptoms in chickens, which may be misdiagnosed as a similar looking infection, such as Newcastle disease, unless laboratory tests confirm H5N1 viruses [12]. Infectious disease surveillance and laboratory resources are also limited in some countries and it is likely that many cases are missed. Consequently, if a country has an identified avian influenza outbreak within its borders, it may be beneficial to limit all exports for that year or longer, as there are likely to be other undetected cases still circulating. If reducing trade is not possible, increased prevention efforts such as vaccination and screening of poultry that is being traded will be important for preventing disease transmission.

Our results also suggest a relatively large risk increase with human population size in the importing country. This likely occurs because more populous countries tend to import larger quantities of chickens and therefore may have a greater risk of importing an infected bird. Additionally, since population size is correlated with GDP, the more populous countries may have better resources to identify imported cases. A small increase in risk was also associated with an increase in export population size (Table 3).
Interestingly, we did not find that the trade of turkeys or ducks significantly impacted H5N1 disease spread across borders (S1 and S2). Although, ducks are commonly asymptomatic carriers of avian influenza and shed the virus for longer [14], the magnitude of duck and turkey trading worldwide was much smaller than that of chickens. Additionally, the demand for and trade of turkeys was especially low in Asia where H5N1 cases predominate. As production and demand for ducks and turkeys increase, it will be important to reassess their role in transmission of avian influenza globally.

One weakness of this study is the timeliness and completeness of reported H5N1 poultry cases. Countries sometimes delay or don’t report H5N1 poultry cases due to fear about the negative impact it could have on their economy. Additionally, public health authorities may not be aware of an outbreak until several weeks later, since many outbreaks occur in people’s backyard where they are unaware of the signs and symptoms of avian flu or may be afraid to report cases to their government. Another weakness of this paper is that it includes ecological level data; therefore we were not able to tie a specific bird trade to the introduction of avian influenza to a new country. We also only knew the year of the poultry trade: consequently, we didn’t know for sure whether the trade happened before or after an identified case. Moreover, it was impossible to know if consecutive yearly H5N1 cases in the same country were the result of continued infections within the country or reintroductions from outside countries. However, by the end of our study period, only a few countries were considered to have endemic H5N1 influenza in poultry [15].
Another potential risk factor that was not accounted for in our model is smuggling of birds across borders through illegal trade [16]. This is not captured by the FAOSTAT database but could have contributed to the spread of H5N1, especially in some regions where borders are not highly regulated. Additionally, since we only looked at poultry trade, we do not know the role that migratory birds played in spreading avian influenza across borders. Avian flu has been found in many migratory birds and other studies have found that both poultry trade and migratory birds likely played a key role in transmission of H5N1 around the globe [2]. The elevated baseline probability of H5N1 poultry infection in countries that didn’t trade infected chickens, as seen in Fig. 1, is likely explained by these other factors.

In an effort to feed the current seven billion people on the globe today, it is necessary to reevaluate the risks of shipping our food thousands of miles around the globe. Gaining a better understanding of how H5N1 spreads internationally is especially important, as poultry has become one of the fastest-growing livestock industries, due to high consumer demand and low price compared to other meat sources [17]. Eating food that is produced locally may greatly decrease the spread of infectious diseases, such as H5N1 which can threaten economies, livelihoods, and human and animal health. If a reduction in trade from infectious countries is not possible, improved screening and infection control practices during epidemic periods can also help reduce transmission across borders.

**MATERIALS AND METHODS**

Data Collection.
A historical prospective network design was used, starting with the first cases of sustained H5N1 transmission in poultry in 2003 and following the spread forward until 2011. There were two earlier cases of H5N1 identified in poultry in 1996 and 1997; however these were not included due to the six year period of no reported poultry outbreaks from 1998 to 2003. Poultry H5N1 cases were identified from the World Health Organization’s avian influenza timeline of events [18]. Binary coding was used for H5N1 infection in each country: countries were considered infected for each year that they reported an H5N1 case in poultry, regardless of outbreak size, production type (backyard, small farm, industrial), or species. Reports of H5N1 amongst wild birds were not included. Human avian influenza data was collected from the World Health Organization’s cumulative case report [4].

Poultry importing and exporting data were collected from the Food and Agriculture Organization’s (FAO) FAOSTAT database [19]. Data was collected from 1996 to 2011 (the most recent year) for live chickens, ducks, and turkeys. This database includes the value and number of live poultry (1,000 heads) that were imported between each country by year. In some instances, there was a trade reported with a value, but no poultry count because the magnitude of the trade was less than 1,000 birds, therefore the exact number of the trade could not be determined as the value per bird varied by country. In these cases, we recoded the trade as 500 poultry heads.

This study included 202 countries, regardless of whether they traded any poultry during the time period of the study. Country population size, density, and GDP in 2011 were collected from the World Bank’s database [20].
Statistical Analysis.

The following Generalized Estimating Equation (GEE) model was used to predict H5N1 poultry infection in an importing country for a given year: \( I_{jt} = \alpha + \beta_1 I_{it} \times C_{ijt} + \beta_2 C_{ijt} + \beta_3 I_{it} + T_{2003...2010} \), where \( I_{jt} \) is H5N1 poultry infection in importing country \( j \) at year \( t \), \( I_{it} \) is H5N1 poultry infection in exporting country \( i \) at year \( t \), \( C_{ijt} \) is thousands of heads of poultry imported from country \( i \) to country \( j \) at year \( t \), and \( T_{2003...2010} \) as year fixed effects. A dummy variable was used to create year fixed effects. This variable was included because reporting of H5N1 cases is likely to have improved over the study period as countries became more aware of this new emerging disease and gained resources for surveillance, identification, and prevention. The GEE model was clustered on import country since trading amongst the same countries was often correlated from year to year. (Table 2) The same model clustering on export country was also evaluated and yielded substantively identical results. The role of ducks and turkeys were also assessed separately in an identical model (S1 and S2)

In our adjusted model we assessed the role of human density and population, since previous studies have found an increased number of poultry H5N1 outbreaks are associated with increased human population density [21]. We also assessed the model controlling for GDP since developing countries usually have less public health resources to investigate outbreaks; however GDP was not included in the full model as it correlates with population and was not significant. Human population, density, and GDP were log-transformed since they were not normally distributed. Our final full model included both import and export population (Table 3).
ArcMap version 10.2.1 was used to create the probability maps in Fig. 1. SAS version 9.3 was used for all statistical analyses: PROC GENMOD was used for the GEE model with a repeated statement for import country, link=logit and a binary distribution. An independent correlation structure was used.

Acknowledgments

Chapter 4, in full, is currently being prepared for submission for publication of the material to PNAS. Raman, Rema; Lindsay, Suzanne; Araneta, Maria Rosario; Fowler, James; Shaffer, Richard. The dissertation author was the primary author of this material.

This work is sponsored in part by the Robert Wood Johnson Foundation Pioneer Grant 67919.
References


Table 4.1: Descriptive Statistics of Chicken Trade and Outbreaks of Avian Influenza A(H5N1) in Poultry and Humans

<table>
<thead>
<tr>
<th>Year</th>
<th>No. of countries</th>
<th>No. of countries with an outbreak</th>
<th>% of countries with an outbreak</th>
<th>No. of chickens imported</th>
<th>% of all chickens that were from cases of H5N1 fatality</th>
<th>No. of human case in poultry</th>
<th>No. of infected countries</th>
</tr>
</thead>
<tbody>
<tr>
<td>2003</td>
<td>2</td>
<td>7</td>
<td>0</td>
<td>702</td>
<td>2180</td>
<td>4</td>
<td>100</td>
</tr>
<tr>
<td>2004</td>
<td>9</td>
<td>7</td>
<td>22</td>
<td>682</td>
<td>35,161</td>
<td>46</td>
<td>70</td>
</tr>
<tr>
<td>2005</td>
<td>9</td>
<td>19</td>
<td>44</td>
<td>672</td>
<td>27,866</td>
<td>98</td>
<td>44</td>
</tr>
<tr>
<td>2006</td>
<td>37</td>
<td>87</td>
<td>68</td>
<td>581</td>
<td>308,001</td>
<td>115</td>
<td>69</td>
</tr>
<tr>
<td>2007</td>
<td>29</td>
<td>80</td>
<td>55</td>
<td>627</td>
<td>374,537</td>
<td>88</td>
<td>67</td>
</tr>
<tr>
<td>2008</td>
<td>22</td>
<td>66</td>
<td>41</td>
<td>598</td>
<td>190,883</td>
<td>44</td>
<td>75</td>
</tr>
<tr>
<td>2009</td>
<td>10</td>
<td>5</td>
<td>20</td>
<td>599</td>
<td>6181</td>
<td>73</td>
<td>44</td>
</tr>
<tr>
<td>2010</td>
<td>15</td>
<td>13</td>
<td>13</td>
<td>597</td>
<td>8544</td>
<td>48</td>
<td>50</td>
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<tr>
<td>2011</td>
<td>14</td>
<td>9</td>
<td>14</td>
<td>581</td>
<td>10,321</td>
<td>62</td>
<td>55</td>
</tr>
</tbody>
</table>
Table 4.2: GEE model regressing H5N1 infection in the importing country on H5N1 infection in the exporting country, thousands of chickens traded, and their interaction. Errors clustered on import country. N = 365,418, QIC= 195,618.3, Null QIC=206,928.9

<table>
<thead>
<tr>
<th>Effect</th>
<th>OR</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Log(chicken)*H5N1 in export country</td>
<td>1.22</td>
<td>1.08, 1.39</td>
<td>0.0013</td>
</tr>
<tr>
<td>Log(chicken)</td>
<td>1.13</td>
<td>0.98, 1.30</td>
<td>0.093</td>
</tr>
<tr>
<td>H5N1 in export country</td>
<td>0.93</td>
<td>0.92, 1.06</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Year</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>2003</td>
<td>0.13</td>
<td>0.03, 0.54</td>
<td>0.0051</td>
</tr>
<tr>
<td>2004</td>
<td>0.63</td>
<td>0.34, 1.15</td>
<td>0.13</td>
</tr>
<tr>
<td>2005</td>
<td>0.63</td>
<td>0.29, 1.34</td>
<td>0.23</td>
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<td>2006</td>
<td>3.03</td>
<td>1.82, 5.05</td>
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<td>2007</td>
<td>2.25</td>
<td>1.35, 3.74</td>
<td>0.0018</td>
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<td>2008</td>
<td>1.65</td>
<td>1.04, 2.59</td>
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<tr>
<td>2009</td>
<td>0.70</td>
<td>0.45, 1.07</td>
<td>0.101</td>
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<tr>
<td>2010</td>
<td>1.08</td>
<td>0.78, 1.49</td>
<td>0.65</td>
</tr>
<tr>
<td>2011</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
**Table 4.3:** Full GEE model* regressing H5N1 infection in the importing country on H5N1 infection in the exporting country, thousands of chickens traded, and their interaction. Errors clustered on import country, $N=365,418$, QIC=150,089.7

<table>
<thead>
<tr>
<th>Effect</th>
<th>OR</th>
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<tr>
<td>Log(chicken)*H5N1 in export country</td>
<td>1.30</td>
<td>1.12, 1.51</td>
<td>0.0005</td>
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<tr>
<td>Log(chicken)</td>
<td>0.93</td>
<td>0.80, 1.08</td>
<td>0.34</td>
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<tr>
<td>H5N1 in export country</td>
<td>0.92</td>
<td>0.91, 0.93</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Year</td>
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<tr>
<td>2003</td>
<td>0.10</td>
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<td>0.0041</td>
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<tr>
<td>2004</td>
<td>0.57</td>
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<td>2006</td>
<td>4.06</td>
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<td>2007</td>
<td>2.77</td>
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<td>2008</td>
<td>1.84</td>
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<td>0.65</td>
<td>0.39, 1.08</td>
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<td>2010</td>
<td>1.09</td>
<td>0.74, 1.62</td>
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<tr>
<td>2011</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Log (export population)</td>
<td>1.01</td>
<td>1.00, 1.01</td>
<td>0.0001</td>
</tr>
<tr>
<td>Log (import population)</td>
<td>7.17</td>
<td>4.14, 12.43</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

*Full model is adjusted for log (export population) and log (import population)
Figure 4.1: Probability of infection (SD) in the importing country in 2006 based on the number of infected countries from which they imported chickens.
Figure 4.2: Probability of H5N1 avian influenza in poultry in 2006 when all chickens were imported (top) and when only chickens from non-infected countries were imported (bottom). Red border outline shows countries that had a poultry H5N1 case in 2006.
Table S1: Full GEE model* regressing H5N1 infection in the importing country on H5N1 infection in the exporting country, thousands of ducks traded, and their interaction. Errors clustered on import country. $N = 365,418$, QIC=150107.2

<table>
<thead>
<tr>
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<th>95% CI</th>
<th>p-value</th>
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<tr>
<td>Log(ducks)*H5N1 in export country</td>
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<td>0.42, 1.17</td>
<td>0.18</td>
</tr>
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<td>Log(ducks)</td>
<td>1.34</td>
<td>0.77, 2.34</td>
<td>0.30</td>
</tr>
<tr>
<td>H5N1 in export country</td>
<td>0.94</td>
<td>0.94, 0.95</td>
<td>&lt;0.0001</td>
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<td>0.004</td>
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<td>2004</td>
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<td>0.27, 1.21</td>
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<tr>
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<td>0.22</td>
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<td>2006</td>
<td>4.10</td>
<td>2.20, 7.46</td>
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<td>2007</td>
<td>2.77</td>
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<td>1.86</td>
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<td>0.39, 1.08</td>
<td>0.10</td>
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<tr>
<td>2010</td>
<td>1.09</td>
<td>0.74, 1.62</td>
<td>0.65</td>
</tr>
<tr>
<td>2011</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Log (export population)</td>
<td>1.01</td>
<td>1.00, 1.01</td>
<td>0.018</td>
</tr>
<tr>
<td>Log (import population)</td>
<td>7.17</td>
<td>4.14, 12.43</td>
<td>&lt;0.0001</td>
</tr>
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</table>

*Full model is adjusted for log (export population) and log (import population)
Table S2: Full GEE model* regressing H5N1 infection in the importing country on H5N1 infection in the exporting country, thousands of turkeys traded, and their interaction. Errors clustered on import country. \( N = 365,418 \), QIC=150075.0

<table>
<thead>
<tr>
<th>Effect</th>
<th>OR</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Log(turkey)*H5N1 in export country</td>
<td>1.52</td>
<td>1.21, 1.90</td>
<td>0.0003</td>
</tr>
<tr>
<td>Log(turkey)</td>
<td>0.77</td>
<td>0.59, 1.00</td>
<td>0.048</td>
</tr>
<tr>
<td>H5N1 in export country</td>
<td>0.93</td>
<td>0.93, 0.94</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Year</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>2003</td>
<td>0.10</td>
<td>0.02, 0.49</td>
<td>0.0041</td>
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<tr>
<td>2004</td>
<td>0.57</td>
<td>0.27, 1.21</td>
<td>0.14</td>
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<tr>
<td>2005</td>
<td>0.57</td>
<td>0.23, 1.40</td>
<td>0.22</td>
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<tr>
<td>2006</td>
<td>4.10</td>
<td>2.20, 7.54</td>
<td>&lt;0.0001</td>
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<tr>
<td>2007</td>
<td>2.77</td>
<td>1.48, 5.21</td>
<td>0.0014</td>
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<tr>
<td>2008</td>
<td>1.84</td>
<td>1.06, 3.19</td>
<td>0.029</td>
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<tr>
<td>2009</td>
<td>0.65</td>
<td>0.39, 1.08</td>
<td>0.10</td>
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<tr>
<td>2010</td>
<td>1.09</td>
<td>0.74, 1.62</td>
<td>0.65</td>
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<tr>
<td>2011</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Log (export population)</td>
<td>1.01</td>
<td>1.00, 1.01</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Log (import population)</td>
<td>7.17</td>
<td>4.14, 12.43</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

*Full model is adjusted for log (export population) and log (import population)
CHAPTER 5

Discussion and Conclusions
Overview

This dissertation was undertaken to identify different ways to prevent the transmission of emerging respiratory diseases. The main questions that were asked included: 1) What characteristics are associated with pathogen specific respiratory viruses and how can these characteristics be used for early identification of cases and initiation of appropriate treatment and prevention measures? 2) What is the disease burden of specific respiratory diseases and how does disease burden change as a result of prevention measures such as vaccination? 3) What are the main routes of transmission of respiratory disease to new regions and how can disease spread be prevented by targeting or reducing these routes? 4) How do the strengths and weaknesses of each surveillance system influence interpretation of these results?

The findings from these studies can be used to prevent disease transmission in several ways. First, they improve early identification of respiratory infections and outbreaks and increase accuracy of treatment. Secondly, they give evidence to the effectiveness and value of prevention measures such as adenovirus vaccination, which will hopefully encourage continued investment in these vaccines by policy makers. And lastly, they identify and quantify the impact of transmission routes which can spread pathogens to previously uninfected regions and suggest ways to reduce further respiratory disease spread. In all cases, knowing the strengths and weaknesses of each surveillance system or data source is necessary to accurately interpret results.

Respiratory Disease Characteristics
In order to prevent emerging respiratory disease transmission, it is necessary to first gain a broad understanding about pathogen specific characteristics such as clinical signs, symptoms, percent positivity, populations affected, risk factors, coinfection rates, and seasonality. Knowing these characteristics can help public health officials identify outbreaks, target high risk populations with important prevention measures, reduce transmission by separating sick and non-sick individuals, and increase biosecurity or sanitation measures.

Unfortunately, treatment cannot always be informed by laboratory confirmation of a pathogen: rapid diagnostic tests have poor sensitivity [1] and multiplex PCR tests are impractical for most clinical settings. However, signs, symptoms, seasonality, and demographics characteristics associated with specific pathogens and predictive models can help inform clinicians about the probability that a sick individual has a particular respiratory infection. This can help inform timely treatment which is especially important for influenza antivirals, which work best if given within the first 48 hours of symptoms. It can also reduce incorrect treatment, such as prescribing antibiotics for a viral infection which can lead to increased antibiotic resistance. Even simply identifying individuals with or without respiratory pathogens can be important for preventing further disease transmission by encouraging contagious individuals to stay at home during the course of their illness.

Understanding seasonality of pathogens is also important for identifying and preventing emerging disease transmission. We found that seasonality of infection for recruits was similar to that of the border and beneficiary populations for several pathogens, but with different intensity. Consequently, illness surveillance in recruits,
which made up the largest proportion of our study population, can be beneficial for informing disease trends in the general public. Identifying seasonality patterns is also important because pathogens identified at high percent positivity outside their normal season may indicate emergence or re-emergence of new strains, as was seen with the 2009 pandemic H1N1 strain, which emerged in the spring [2]. Knowing the correct season is also important for prevention: in the case of adenovirus, it was originally thought that the virus typically occurred predominately in winter. Consequently, when vaccines were rationed in 1997, the vaccine was only administered during the winter months. However, this policy was not effective, because the virus was actually circulating more frequently in summer and fall months. Our first paper added to the literature by describing the seasonality of some respiratory pathogens that have not been studied extensively.

Describing coinfection rates can also be useful for preventing respiratory disease. Our study found around 30% to 40% of coronavirus and adenovirus infections occurred as coinfections and they most frequently occurred with rhinovirus. These results suggest that infection with some viruses, such as rhinoviruses, could create opportunistic environments for colonization with other viruses and bacteria. Targeting rhinovirus infection through creation of new vaccines or treatment could have more far-reaching benefit in protecting a person from other infections. Additionally, if a new respiratory strain emerges, targeting typically occurring co-infections may also be useful for reducing disease transmission.

Disease characteristics, burden, and risk can also vary significantly across populations as a result of climate, population density, vaccination coverage, age,
regionally circulating strains, comorbidities and overall health. Our first study is one of only a few respiratory etiology studies done in the United States [3,4] and found some key differences of respiratory infection among the US military recruits, DoD beneficiaries, and US-Mexico border populations. Our third study also found that population differences influenced risk: We found that as importing and exporting country population sizes increased, so did the odds of having an H5N1 case among poultry. Consequently, it is important to recognize variations in disease characteristics and disease risk across different populations when designing appropriate prevention measures.

**Disease Burden Estimates and Measuring Impact of Vaccines**

Vaccines are one of the key prevention measures for infectious diseases. However, sometimes vaccines work so well, that people may think that a particular disease is no longer a problem. This was the case for the adenovirus vaccines: After years of successfully administering adenovirus vaccines to incoming recruits, very little adenovirus circulated or was identified through routine FRI surveillance. Consequently, when the pharmaceutical company making the vaccines asked for money to repair their facilities, the DoD didn’t see these vaccines as a top priority any more. However, this resulted in sharp increases in adenovirus cases as soon as the vaccine was no longer administered.

Disease burden estimates are essential for influencing policy decisions such as funding for vaccine development or production, investment in new treatment options, moderating trade of goods and investment in screening or biosecurity at borders. In the case of adenovirus, it was especially important to define disease burden among military
recruits and track its fluctuations over time in order to quantify the impact of the new vaccines. Our cost-benefit analysis gives even further evidence to the huge value of these vaccines in preventing lost training time and reducing medical costs.

We found that adenovirus infections decreased by approximately 100-fold among US military recruits following resumption of year-round adenovirus vaccination in 2012. After adjusting 1995 cost estimates associated with adenovirus infection [5] using the US Consumer Price Index for all Urban Consumers (all items and medical care item) [6], we estimated that each adenovirus infection costs approximately $3838 ($3128 for medical costs and $710 for lost training costs) in 2012. With approximately 13 000 clinical adenovirus illnesses prevented annually by year-round vaccination, the DoD would save approximately $50 million per year. During the first few years, the price of the new vaccines ranged from $120 to $150 per person for the two doses. Vaccinating approximately 200 000 new recruits a year at $150 per person for the two doses would cost $30 million, providing a net savings of approximately $20 million. However, this cost savings is still an underestimate, since it does not include the cost associated if a recruit must restart the training program, and it does not include the difficult to measure cost and burden of a death.

In addition to preventing adenovirus disease among US military recruits, these vaccines could significantly reduce FRI and adenovirus rates in other foreign military recruits and police trainees who have also experienced adenovirus outbreaks and high burden of disease [7-11]. These vaccines also have the potential to reduce transmission to other geographical locations and to civilians after recruits have completed training and disperse globally [12]. Our study gives evidence supporting the value of continued
vaccine production and maintaining sufficient supplies for uninterrupted, year-round vaccination of all recruits. Additionally, as year-round vaccination continues, it will be important to monitor potentially emerging strains through established surveillance systems to see if rates of non-vaccine strains change.

**Transmission Routes and Implementing Non-pharmaceutical Interventions**

Transmission of infectious diseases to new regions is often influenced by movement of infected individuals. Due to increasing airline traffic, infectious diseases often spread quickly around the globe, as was seen with pandemic H1N1 which was declared a pandemic in less than three months [13]. Similarly, birds also travel around the globe quickly via bird migration and poultry trade. Non-pharmaceutical interventions (NPIs) are one way to prevent the spread of infectious diseases when pharmaceutical interventions are not feasible, which is usually the case when a new disease emerges. NPIs are aimed at reducing the contact of infected individuals with non-infected individuals by decreasing movement, increasing separation of sick individuals, and decreasing contact with infectious particles by increasing biosecurity and hygiene measures [14]. In the case of avian influenza in poultry, this can be done by reducing trade from infected countries and increasing biosecurity and screening at ports of entry.

The results of our avian influenza and poultry trading study suggest that the more chickens a country imports from an infected country, the greater the risk for having an H5N1 case in poultry. Unfortunately, it can be challenging to identify infected chickens in a flock that is being traded. Avian influenza has an incubation period of 3 to 5 days in chickens [15], during which time infected birds may unknowingly be traded before they
show illness. Use of avian flu vaccination in poultry may also make result in delayed detection because it can reduce symptom severity of infected birds [16]. H5N1 also has non-specific symptoms in chickens, which may be misdiagnosed as a similar looking infection, such as Newcastle disease, unless laboratory tests confirm H5N1 viruses [15]. Additionally, infectious disease surveillance and laboratory resources for identifying H5N1 cases are limited in many countries and it is likely that cases are missed. Consequently, if a country has an identified avian influenza outbreak within its borders, it may be beneficial to limit all exports for that year or longer, as there are likely to be other undetected cases still circulating. If a reduction in trade from infectious countries is not possible, improved screening and infection control practices during epidemic periods can also help reduce transmission across borders.

Gaining a better understanding of the transmission routes which spread H5N1 among poultry is important, as poultry has become one of the fastest-growing livestock industries, due to high consumer demand and low price compared to other meat sources [17]. Although, non-pharmaceutical interventions such as limiting trade during infectious periods may be difficult economically and increased biosecurity and screening will require additional funding, the impact of further H5N1 spread can be also be harmful with culling sick birds, reduced livelihoods for farmers with infected flocks, and increased human and animal health concerns. This study showed that reducing trade from infected countries and eating food that is produced locally may greatly decrease the global spread of H5N1 in poultry.

**Surveillance Systems and Interpretation of Results**
Knowing the strengths and weaknesses of each surveillance system was important for interpreting the results from each study in this dissertation. The diseases and cases captured by surveillance or data collection systems are influenced by health care seeking behavior, stigma associated with reporting a case, case definitions, timing of data collection, length of surveillance period, populations surveyed, and completeness of the data collected or reported.

One limitation of the surveillance system in the first two studies is that it only captured people with FRI/SARI who sought medical care. Military recruits may be less likely to seek care than other groups due to concern over losing training time or having to restart the program, and US–border populations may have less access to health care. Therefore, the etiology of more mild infections may be underrepresented for these two groups and the total estimated adenovirus burden and associated costs in the adenovirus vaccine study are also likely underestimated. Additionally, changes in health care seeking behavior may also bias results. In 2009, the increase in cases of both adenovirus and FRI was likely caused in part to more recruits going to clinics when they were sick due to worry about the 2009 H1N1 influenza pandemic.

Although we tested for many pathogens in our first study, there are likely still circulating viruses and bacteria for which we did not test, such as parainfluenza viruses or potentially unrecognized viruses. Additionally, the timing of sample collection in the course of illness for the first two studies could impact whether or not viruses were identified by PCR. Despite this, the first two studies were part of well-established existing surveillance program that consistently collected and tested a substantial number of specimens at many different sites in the United States. Continued respiratory
surveillance by NHRC will help improve our understanding these respiratory diseases and will help monitor for the emergence of new strains or changes in disease predominance.

One weakness of the avian flu study is the timeliness and completeness of reported H5N1 poultry cases. Countries sometimes delay or don’t report H5N1 cases due to fear about the negative impact it could have on their economy. Additionally, many outbreaks occur in “backyard” flocks where cases may not be reported because people may be unaware of the signs and symptoms of avian influenza or may be afraid to report cases to their government for fear that their birds will be culled. Another weakness of this paper is that it includes ecological level data; therefore it was impossible to tie a specific bird trade to the introduction of avian influenza in a new country. We also didn’t know whether the trade happened before or after an identified case because we only knew the year of a case or trade. Moreover, it was impossible to differentiate continued infections occurring in the same country versus re-introductions from outside. However, only a few countries were considered to have endemic H5N1 influenza in poultry by the end of our study period [18].

Several other variables describing the movement of birds were also not accounted for in our poultry trading data, such as the smuggling of birds across borders through illegal trade [19]. This was not captured by the FAOSTAT database but could have contributed to the spread of H5N1, especially in some regions where borders are not highly regulated. Additionally, since our data only included the movement of birds through poultry trade, we do not know the role that migratory birds played in spreading avian influenza across borders. Avian flu has been found in many migratory birds and
other studies have found that both poultry trade and migratory birds likely played a key role in transmission of H5N1 around the globe [20]. The elevated baseline probability of H5N1 poultry infection in countries that didn’t trade infected chickens is likely explained by these other factors that were not included in our data.

Conclusions

Emerging and re-emerging infectious diseases are pathogens whose incidence has increased in the past two decades or is likely to increase sometime in the near future [21]. EIDs have many potential causes such as microbial adaptation, change in human susceptibility, change in human demographics or trade, breakdown of public health infrastructure, and ecological and environmental changes. EIDs are especially worrisome because populations usually have little or no immunity to them and vaccines and treatment don’t always exist when they first emerge. This can result in rapid spread of EIDs throughout connected populations.

Early identification of infectious diseases is one of the most important measures to reduce the risk of transmission to larger populations. Unfortunately, diagnostic tests can be costly and lengthy to perform, and often require considerable laboratory resources and personnel. Consequently laboratory confirmation is not feasible in most cases, especially in resource poor settings. Having a better understanding of typical signs and symptoms and people at greater risk for specific respiratory pathogens can help clinicians identify pathogens and treat patients. Knowing the baseline and seasonality of infection can also be useful for identifying disease emergence and epidemic periods.
Pharmaceutical interventions and treatment, such as vaccines, antibiotics, and antivirals, are one of the best ways to reduce the spread of infectious diseases if given appropriately and in a timely fashion. Vaccines are especially effective in preventing disease transmission. However, it is necessary to continually evaluate the benefit of vaccination programs in order to maintain political support and funding for future production. Additionally, assessing and comparing different vaccination strategies, such as year round versus seasonal adenovirus vaccine administration, may show that some strategies aren’t as effective as previously thought. Finally, it is important to understand how pharmaceutical interventions may impact surveillance and interpretation of disease trends. For example, increased avian flu vaccination among poultry flocks may make it more difficult to identify circulating cases.

Unfortunately, when new infectious diseases first emerge, pharmaceutical interventions may not be available immediately. Consequently, employing actions other than medicine or vaccination, also known as non-pharmaceutical interventions (NPIs), are necessary to reduce disease spread. Examples of NPIs include social distancing, hygiene and personal protective equipment. Social distancing measures, such as encouraging sick individuals to stay at home or reducing the movement of infected individuals through trade reduction or decreased flying, can also reduce disease transmission and spread. Disease transmission can also be reduced by proper hygiene measures, such as hand washing and cough etiquette. Finally, using personal protective equipment such as face masks and gloves can protect high risk individuals such as health care workers.
Strong surveillance systems are also necessary for preventing emerging infectious respiratory diseases because they enable us to calculate baselines of infection and monitor changes in the magnitude of cases or emergence of new strains. However, there are many factors that may influence surveillance data and reporting of cases such as access to care or fear of negative impacts of seeking care or reporting a case. Additionally, it is important to understand the specificity and sensitivity of the diagnostic tests being used for surveillance, which may be influenced by the timing of specimen collection and different types of laboratory tests. Additionally, availability of diagnostics may vary across regions, and may be limited in resource poor settings which may make comparability challenging. Overall, knowing factors which may impact the consistency of surveillance systems over time or between groups is important when interpreting disease trends over time and comparing different populations.

Since many emerging respiratory infections have the ability to spread between different species, taking a one health approach to preventing disease transmission will also be necessary for preventing the spread of many emerging respiratory pathogens. For example, by limiting the spread avian influenza in poultry, there will be less exposure to humans and fewer opportunities for the virus to replicate and mutate, which may make it more transmissible to humans. Additionally, non-pharmaceutical interventions, such as reducing animal movement by limiting trade and increased biosecurity are important steps for reducing the spread of zoonotic emerging respiratory infections, such as H5N1, to new regions.

In conclusion, this dissertation supports the importance of employing multiple different techniques to prevent the transmission of emerging respiratory infections. Early
identification and quickly containing initial cases before they have the opportunity to spread widely is one of the best means of prevention. However, if pathogens are not contained, vaccination, appropriate treatment, reducing movement of infected individuals, and reducing exposure to non-infected populations by increasing screening and hygiene practices can be used to prevent further spread. In order to monitor the effectiveness of these measures and identify new disease emergence, continued surveillance and monitoring of these pathogens is necessary.
References


