
Peter E. Kammerer
Sonia Montiel
Paula Kriner
Ietza Bojorquez
Veronica B. Ramirez
Martha Vazquez-Erlebeck
Eduardo Azziz-Baumgartner

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Naval Health Research Center
140 Sylvester Road
San Diego, California 92106-5321
Influenza-like illness surveillance on the California-Mexico border, 2004–2009

Peter E. Kammerer,a Sonia Montiel,b Paula Kriner,c Ietza Bojorquez,d Veronica B. Ramirez,e Martha Vazquez-Erlbeck,f Eduardo Azziz-Baumgartner,b Patrick J. Blaira

aNaval Health Research Center, San Diego, CA, USA. bCenters for Disease Control and Prevention, Atlanta, GA, USA. cImperial County Public Health Department, CA, USA. dDepartment of Population Studies, El Colegio de la Frontera Norte, Tijuana, Baja California, Mexico. eISESALUD, Baja California, Mexico. fCalifornia Department of Public Health/WIDS Program, CA, USA.

Correspondence: Peter Kammerer, Department of Respiratory Diseases Research, Naval Health Research Center, 140 Sylvester Road, San Diego, CA 92106-5122, USA. E-mail: peter.kammerer@med.navy.mil

In this surveillance project, influenza virus was the most commonly identified pathogen in influenza-like illness specimens from both sides of the border from 2004 through 2009.

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Background Since 2004, the Naval Health Research Center, with San Diego and Imperial counties, has collaborated with the US Centers for Disease Control and Prevention to conduct respiratory disease surveillance in the US-Mexico border region. In 2007, the Secretariat of Health, Mexico and the Institute of Public Health of Baja California joined the collaboration.

Objectives The identification of circulating respiratory pathogens in respiratory specimens from patients with influenza-like illness (ILI).

Methods Demographic, symptom information and respiratory swabs were collected from enrollees who met the case definition for ILI. Specimens underwent PCR testing and culture in virology and bacteriology.

Results From 2004 through 2009, 1855 persons were sampled. Overall, 36% of the participants had a pathogen identified. The most frequent pathogen was influenza (25%), with those aged 6–15 years the most frequently affected. In April 2009, a young female participant from Imperial County, California, was among the first documented cases of 2009 H1N1. Additional pathogens included influenza B, adenovirus, parainfluenza virus, respiratory syncytial virus, enterovirus, herpes simplex virus, Streptococcus pneumoniae, and Streptococcus pyogenes.

Conclusions The US-Mexico border is one of the busiest in the world, with a large number of daily crossings. Due to its traffic, this area is an ideal location for surveillance sites. We identified a pathogen in 36% of the specimens tested, with influenza A the most common pathogen. A number of other viral and bacterial respiratory pathogens were identified. An understanding of the incidence of respiratory pathogens in border populations is useful for development of regional vaccination and disease prevention responses.

Keywords Bacterial infections, human, influenza, respiratory tract infections, sentinel surveillance.

Introduction

Acute respiratory infections (ARIs) are the most common illnesses among persons of all ages.1 Approximately 2 million deaths occur globally each year from ARI, mostly among the elderly and young children.2 The burden of disease is greatest in low-income countries where ARIs are the cause of up to 25% of all pediatric deaths.3 In Mexico, ARIs are the leading cause of disease (http://www.dged.salud.gob.mx/contenidos/evaluacion_programas/descargas/CUARTO_INFORME.pdf). In the United States, annual influenza epidemics result in projected lost earnings due to illness and loss of life of $16.3 billion annually and a total economic burden (using projected statistical life values) of $87.1 billion.4 Respiratory illnesses cause more disease and death than any other infection in the United States.5 The annual Northern and Southern hemisphere trivalent inactivated or live-attenuated influenza vaccines are the best way to prevent the spread of influenza and reduce disease related morbidity and mortality in the communities. Nevertheless, limited availability and use of these vaccines in under-resourced settings put a large proportion of the world’s population at risk.6

The migration of persons and products across national borders contributes to the spread of infectious diseases.7 The US-Mexico border region has been defined as the area
of land 100 km (62.5 miles) north and south of the actual international border. This land area has an estimated population of approximately 12 million inhabitants (http://www.borderhealth.org/border_region.php). With 300 million two-way crossings estimated in 2001, the US-Mexico border is one of the busiest in the world. Incidence rates for infectious diseases, such as diphtheria, hepatitis A, measles, mumps, rabies, rubella, and salmonellosis, have been found to be significantly higher in the United States along the Mexican border than in non-border regions. This surveillance program was initiated to identify the respiratory pathogens responsible for illness near the border region and to detect emerging respiratory pathogens in this area, allowing a more timely public health response.

The Centers for Disease Control and Prevention (CDC) Border Infectious Disease Surveillance (BIDS) program and the CDC/California Department of Public Health Early Warning Infectious Disease Surveillance (EWIDS) program, in collaboration with the Naval Health Research Center (NHRC), County of San Diego Health and Human Services Agency and the Imperial County Public Health Department have conducted influenza-like illness (ILI) surveillance since 2004. In 2007, the Mexico Secretariat of Health and the Institute of Public Health of Baja California joined the collaboration. Here, we describe the etiologies associated with ILI in participants who had a specimen collected from 2004 through 2009.

Materials and methods

Site selection and enrollment

Surveillance investigators selected sites along the US-Mexico border following discussion with local health officials. Sites selected were local health clinics, chosen due to their proximity to the border region. (Figure 1). Eligible patients, of all ages, with ILI were voluntarily enrolled. Surveillance was conducted August through June in 2004–2006 and year-round beginning August, 2006 through September, 2009; The NHRC institutional review board reviewed this study protocol and deemed it public health surveillance.

Specimen collection

Surveillance personnel obtained two nasal swabs and one throat swab from all patients with ILI (oral temperature ≥100°F (37.8°C) and presence of either cough or a sore throat in the absence of a known cause other than influenza). This ILI case definition was used at all sites. Specimens that did not meet the case definition (n = 6) were removed from the data. For throat swabs, both tonsils and the posterior pharynx were swabbed, and specimens were stored in tryptic soy broth with 15% glycerol (TSB; Remel, Lenexa, KS, USA). For nasal swabs, the nares with observed discharge were swabbed. If no discharge was observed, one side was chosen. The nares were swabbed twice, with one swab placed in viral transport media (VTM) (M4RT; Remel) and the other tested on site by using a rapid influenza diagnostic test (Quidel Corporation, San Diego, CA, USA). Specimens in TSB and VTM were frozen at −70°C within 4 hours of sample collection and stored in an ultra-low freezer or on dry ice. Frozen specimens were either shipped on dry ice to NHRC or collected by NHRC personnel, who maintained the cold chain.

Extraction

Extraction of RNA was performed using the QIAamp 96 DNA Blood and Body Fluid Kit (Qiagen, Valencia, CA, USA) for conventional polymerase chain reaction (PCR) or the QIAamp Viral RNA Mini Kit (Qiagen) for real-time PCR reaction, according to manufacturer’s instructions.

PCR

PCR was used to detect influenza A and adenovirus (AdV) only. Samples were tested for influenza A as previously described using universal primers made against the M gene. Adenovirus PCR was conducted using AdV universal
β primers\textsuperscript{11} and the BCE multiplex as previously described.\textsuperscript{12} Reactions were performed using an iCycler PCR machine (Bio-Rad Laboratories, Inc., Hercules, CA, USA) or a DNA Engine machine (Bio-Rad).

**Influenza subtyping**

Samples determined to be positive by comparison to influenza A universal primers were subtyped for H1 and H3 on the LightCycler 2.0 (Roche, Basel, Switzerland) using primers that target the hemagglutinin gene. Primers and probe used for H1 subtyping were H1F (5'-GYAGTCTTCC TTTCAGAATGT-3'), H1R (5'-AGTCCTGTAACCATCC TTAATT TG-3'), and H1P (5'-6FAM-TAGGAGAGTTT TCCAAGTATGTCAGGA-TAMRA-3'). Primers and probe used for H3 subtyping were H3F (5'- TGTCTCCAGC GAATAAGC ATCT-3'), H3R (5'-CCCACCTTGATTTTT GAAGTAAACC-3'), and H3P (5'-6FAM-TGGACAAATGT AAAACGGGAGACATACTTTTG-TAMRA-3') (primers developed in house by Luke Daum). Real-time reactions were performed using the RNA UltraSense One-Step Quantitative RT-PCR System (Invitrogen Corporation, Carlsbad, CA, USA), according to manufacturer's instructions with the modification of a final concentration of probe to 0.3 μM per 25 μl reaction.

**pH1N1 testing**

Samples were assayed for pH1N1 by using the Emergency Use Authorized CDC Swine Influenza Virus Real-Time RT-PCR Detection Panel as previously published (http://www.who.int/csr/resources/publications/swineflu/realtimetypepcr/en/)

**PCR/Mass spectrometry assay**

PCR amplicons representing internal gene segments were generated with tandem mass spectra analysis conducted on 15 μl aliquots of purified products by using protocols described previously.\textsuperscript{13} Nucleotide base compositions were derived from the exact mass measurements of the complementary single-stranded oligonucleotides. Relationships were determined by comparing profiles with published sequences (Abbott Laboratories, Abbott Park, IL, USA).

**Virus isolation and identification**

We isolated respiratory viruses following co-culture in rhesus monkey kidney (RMK) and A549 cells. RMK cells were used to isolate influenzas A and B, and parainfluenza viruses (PIVs) 1, 2, and 3. A549 cells were used to isolate AdV, respiratory syncytial virus (RSV), and enterovirus. Cultures exhibiting cytopathic effect were identified and tested using an immunofluorescence assay with type-specific monoclonal antibodies for viral identification. Hemagglutination inhibition was used to serotype influenza isolates using the World Health Organization Influenza Reagent Kit for Identification of Influenza Isolates for that year.

**Bacteriologic testing**

Throat swabs collected in TSB were plated onto Blood Agar and Chocolate plates (BAP, CHOC; Hardy Diagnostics, Santa Maria, CA, USA), and Regan-Lowe plates [BBL; Becton, Dickinson and Company (BD)]. Blood Agar and Chocolate plates were observed daily for growth of group A streptococcus, S. pneumoniae, Neisseria meningitidis, Haemophilus influenza, and Staphylococcus aureus. Regan-Lowe plates were incubated for 7 days and observed daily for growth of Bordetella pertussis. Suspicious colonies were Gram stained, catalase tested, and placed into the BD Phoenix (Becton, Dickinson and Company) for identification. The BD BBL Crystal was used to identify Neisseria and Haemophilus species.

**Statistical analysis**

All data were double-entered into a Microsoft Access database. We used Pearson Chi-square analysis to obtain P values for comparison of laboratory results by age group, season, and country. To identify which pathogens contributed to any observed differences among the study populations, 95% confidence intervals for pathogen proportions among subgroups were calculated using the Wilson\textsuperscript{14} procedure. P values were calculated to compare proportions between two independent groups.\textsuperscript{15}

**Results**

The number and location of sites varied from 2004 through 2009 (Table 1). At study onset, one site was selected near the border in San Ysidro, California. Calexico was an influenza surveillance site in 2004–2005 for the Imperial County Public Health Department, as part of California’s sentinel site program. Calexico joined our collaboration as a second site in 2005–2006. In 2007, at the request of the Mexico Secretariat of Health, two sites in Mexico were added, one in Mexicali and one in Tijuana.

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**Table 1. Number of specimens per site**

<table>
<thead>
<tr>
<th>Year</th>
<th>No. sites USA</th>
<th>No. sites Mexico</th>
<th>Enrollees USA</th>
<th>Enrollees Mexico</th>
</tr>
</thead>
<tbody>
<tr>
<td>2004–2005</td>
<td>1</td>
<td>0</td>
<td>122</td>
<td>0</td>
</tr>
<tr>
<td>2005–2006</td>
<td>2</td>
<td>0</td>
<td>224</td>
<td>0</td>
</tr>
<tr>
<td>2006–2007</td>
<td>3</td>
<td>0</td>
<td>207</td>
<td>0</td>
</tr>
<tr>
<td>2007–2008</td>
<td>4</td>
<td>2</td>
<td>243</td>
<td>143</td>
</tr>
<tr>
<td>2008–2009</td>
<td>12</td>
<td>2</td>
<td>506</td>
<td>410</td>
</tr>
<tr>
<td>Total</td>
<td>1302</td>
<td>553</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
At the start of the 2008–2009 influenza season, enrolling sites included two in California (San Ysidro and Brawley) and two in Mexico (Mexicali and Chihuahua) (Figure 1). As more collaborating surveillance sites were trained, the number of specimens collected rose (Table 1). Overall, the San Ysidro Health Center had the highest number of specimens submitted (725), followed by the Mexicali clinic (543). Although the number of specimens collected per year varied among all sites (average of 373 per year), the largest increase in specimen collection was seen when sites in Mexico were added and with the onset of the 2009 pandemic, when the number of sites was temporarily expanded to 14 (Table 1).

From 2004 through September 2009, a total of 1855 participants (1302 from the US, 553 from Mexico) were enrolled with laboratory samples (Table 2). Among 1677 participants with known gender, 960 (57%) were female. The majority (70%) of participants reported living in the United States (1222), with 99% of US participants reporting US residency and 100% of Mexico clinic participants reporting Mexico residency. Of those reporting age (n = 1826), the most frequent age range represented was 16–54 years (38%), followed by 6–15 years (24%), and 1–5 years (22%). There was a higher percentage of the 16–54 age group in Mexico (P < 0.001) and the >54 (P < 0.001) and 1–5 (P < 0.05) age groups in the US. No significant difference was seen in the two other age groups. The majority of participants were Hispanic (1677; 94%). White non-Hispanic participants represented 2%, and Asian, African American, and Native American study subjects accounted for the remaining 3%. Influenza vaccination status was self reported for 98% of ILI cases and the proportion vaccinated was 28% at US clinics and 19% at Mexico clinics (P < 0.05; data not shown). In the US, 19% of the vaccinated and 32% of the unvaccinated were positive for influenza A or B. In Mexico, 15% of the vaccinated and 17% of the unvaccinated were positive for influenza A or B. The vaccination rate for 0–5 years old was higher in the US (34%) than that for 6 years and older in the US (26%) (P < 0.05; data not shown). The 0–5 year old vaccination rate was higher in Mexico (24%) than that for 6 years and older in Mexico (19%), but the difference was not significant (P = 0.20; data not shown). The difference in the 0–5 year old vaccination rate between the two countries was significant (P < 0.05; data not shown). Of the 1855 participants, 663 (36%) had a pathogen identified. The most frequent pathogen identified was influenza A, with 363 (20%) cases positive by either PCR or viral culture (Table 3). Additional pathogens identified were influenza B (5%), AdV (4%), PIVs 1–3 (1%), RSV (0.6%); other viral pathogens included enterovirus and herpes simplex virus (0.4%). Bacterial pathogens identified included S. pneumoniae, S. pyogenes, Haemophilus influenzae, β-hemolytic streptococcus (not group A), group C streptococcus, and Moraxella catarrhalis, which made up the remaining 7% of diagnoses.

No pathogen was identified in 1192 (64%) of participants. A significantly higher proportion of US clinic cases than Mexico clinic cases were positive for influenza A (22% versus

### Table 2. Demographics of participants

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>USA n = 1302 (70%)</th>
<th>Mexico n = 553 (30%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. (%) male</td>
<td>532 (43)</td>
<td>185 (41)</td>
</tr>
<tr>
<td>No. (%) female</td>
<td>696 (57)</td>
<td>264 (59)</td>
</tr>
<tr>
<td>&lt;1 year</td>
<td>49 (4)</td>
<td>22 (4)</td>
</tr>
<tr>
<td>1–5 year</td>
<td>300 (23)</td>
<td>101 (19)</td>
</tr>
<tr>
<td>6–15 year</td>
<td>322 (25)</td>
<td>120 (22)</td>
</tr>
<tr>
<td>16–54 year</td>
<td>438 (34)</td>
<td>254 (47)</td>
</tr>
<tr>
<td>&gt;54 year</td>
<td>181 (14)</td>
<td>39 (7)</td>
</tr>
<tr>
<td>% Hispanic</td>
<td>1167 (92)</td>
<td>510 (99)</td>
</tr>
<tr>
<td>% White/non-Hispanic</td>
<td>40 (3)</td>
<td>3 (0.6)</td>
</tr>
<tr>
<td>Other</td>
<td>64 (5)</td>
<td>2 (0.4)</td>
</tr>
</tbody>
</table>

### Table 3. Disease etiologies by location

<table>
<thead>
<tr>
<th></th>
<th>Inf A n (%) (95% CI)</th>
<th>Inf B n (%) (95% CI)</th>
<th>AdV n (%) (95% CI)</th>
<th>RSV n (%) (95% CI)</th>
<th>Other n (%) (95% CI)</th>
<th>Neg n (%) (95% CI)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>US</td>
<td>288 (22) (20.0–24.5)</td>
<td>72 (5) (4.4–6.9)</td>
<td>56 (4) (3.3–5.5)</td>
<td>9 (0.7) (0.4–1.3)</td>
<td>106 (8) (6.6–9.6)</td>
<td>799 (60) (58.7–64.0)</td>
<td>1330 (28 co-inf)</td>
</tr>
<tr>
<td>Mexico</td>
<td>75 (13) (11.0–16.7)</td>
<td>15 (3) (1.7–4.4)</td>
<td>16 (3) (1.8–4.6)</td>
<td>2 (0.3) (0.1–1.3)</td>
<td>62 (11) (8.7–13.9)</td>
<td>393 (70) (67.2–74.7)</td>
<td>563 (eight co-inf, 1 tri-inf)</td>
</tr>
<tr>
<td>Total</td>
<td>363 (19)</td>
<td>87 (5)</td>
<td>72 (4)</td>
<td>11 (0.6)</td>
<td>168 (9)</td>
<td>1192 (63)</td>
<td>1893</td>
</tr>
</tbody>
</table>

AdV, adenovirus; CI, confidence interval; Inf, influenza; Neg, negative; PIV, parainfluenza virus; RSV, respiratory syncytial virus.

*Other pathogens – enterovirus, herpes simplex 1, PIV 1–3, Streptococcus pneumoniae, S. pyogenes, Haemophilus influenzae, β-hemolytic streptococcus (not group A), group C streptococcus, Moraxella catarrhalis."
the pandemic) to 44% in 2005–2006 (ranging from 13%
versus 52%, \(P < 0.05\); Table 4). With specimens collected during
the pandemic separated from pre-pandemic specimens, the
higher proportion of influenza A in the 6–15 year age
group was still significant (\(P < 0.05\); data not shown) when
compared with the 1–5, 16–54, and >54 age groups. The
rate of influenza A in the 6–15 year age group was not sig-
nificantly different from that of the <1 year age group.
These relationships were seen in both the pre-pandemic
and pandemic specimens. RSV was only isolated from the
1–5 and >54 years age groups, whereas PIV was identified
in all but those younger than 1 year. The percentage of
samples in which no pathogen was identified was higher
in all but those younger than 1 year. The percentage of
infected cases separated from pre-pandemic specimens, the
proportion of influenza A was higher (32% versus 15%,
\(P < 0.05\); data not shown) when compared with those between
1 through 15 years old (73% among those aged under 1 year and
16 years and older
vs 52%, \(P < 0.05\)). The proportion of influenza-positive cases varied by year,
ranging from 13% in 2008–2009 (prior to the onset of
the pandemic) to 44% in 2005–2006 (Table 5). Type and
subtype also varied by year, with influenza A predominant
in every year except 2004–2005, when influenza B was
2008 seasons, whereas influenza A/H3N2 was predominant
during 2006–2007, and pandemic influenza A/H1N1 was
predominant during 2008–2009 (Table 6). There was no
significant difference (\(P = 0.056\)) in influenza A subtypes
isolated on either side of the border (data not shown).
On March 30, 2009, a specimen was collected from a
9-year-old female participant from Brawley, California,
meeting the ILI case definition. When standard reverse
transcriptase (RT-PCR) assays found infection with an un-
typed influenza A virus, these samples were analyzed by
RT-PCR and electrospray ionization mass spectrometry
assay on an Ibis T5000 platform (Ibis Biosciences, Inc.,
Carlsbad, CA, USA). This analysis showed a novel reassortant
influenza virus, with swine, human, and avian com-
ponents as the highest probability match. Subsequent
characterization at CDC determined that the isolated
virus from the participant was identical to the pandemic
influenza A/H1N1 virus collected 2 days later from a
10-year-old boy from San Diego. These were the first two
laboratory identified cases captured and recorded during
the initial outbreak (16).

### Discussion

Acute respiratory infections contribute significantly to mor-
bidity and mortality, especially in persons younger than
5 years of age. Mexico, Central, and South America have
some of the highest ARI rates. By elucidating the causes
of ILI, public health responses can be implemented to mini-
mize the effect of disease. In this study, we describe the eti-
ologies associated with ILI in participants sampled at clinic
sites on the US-Mexico border from 2004 through 2009. The
most common etiology diagnosed was influenza (25%).

<table>
<thead>
<tr>
<th>Age (year)</th>
<th>Inf A n (%) (95% CI)</th>
<th>Inf B n (%) (95% CI)</th>
<th>AdV n (%) (95% CI)</th>
<th>RSV n (%) (95% CI)</th>
<th>Other n (%) (95% CI)</th>
<th>Neg n (%) (95% CI)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1</td>
<td>9 (12) (6.8–22.4)</td>
<td>1 (1) (0.3–7.6)</td>
<td>4 (6) (2.2–12.6)</td>
<td>0 (0) (0.0–5.1)</td>
<td>6 (8) (3.9–17.0)</td>
<td>52 (72) (62.0–82.2)</td>
<td>72 (one co-infection)</td>
</tr>
<tr>
<td>1–5</td>
<td>74 (18) (15.0–22.5)</td>
<td>21 (5) (3.5–7.9)</td>
<td>25 (6) (4.3–9.0)</td>
<td>9 (2) (1.2–4.2)</td>
<td>54 (13) (10.2–16.8)</td>
<td>228 (55) (52.0–61.6)</td>
<td>411 (10 co-infections)</td>
</tr>
<tr>
<td>6–15</td>
<td>144 (32) (28.4–37.1%)</td>
<td>30 (7) (4.8–9.5)</td>
<td>18 (4) (2.6–6.3)</td>
<td>0 (0) (0.0–0.9)</td>
<td>42 (9) (6.9–12.3)</td>
<td>220 (48) (45.1–54.4)</td>
<td>454 (10 co-infections; one triple co-infection)</td>
</tr>
<tr>
<td>16–54</td>
<td>107 (15) (13.0–18.3)</td>
<td>23 (3) (2.2–4.9)</td>
<td>16 (2) (1.4–3.7)</td>
<td>0 (0) (0.0–0.6)</td>
<td>51 (7) (5.6–9.4)</td>
<td>505 (72) (69.6–76.2)</td>
<td>702 (12 co-infections)</td>
</tr>
<tr>
<td>&gt;54</td>
<td>24 (11) (7.4–15.7)</td>
<td>10 (4) (2.5–8.2)</td>
<td>8 (4) (1.9–7.0)</td>
<td>2 (0.9) (0.3–3.3)</td>
<td>12 (5) (3.1–9.2)</td>
<td>166 (75) (69.4–80.7)</td>
<td>222 (two co-infections)</td>
</tr>
</tbody>
</table>

AdV, adenovirus; CI, confidence interval; Inf, influenza; Neg, negative; PIV, parainfluenza virus; RSV, respiratory syncytial
virus.

*Other pathogens – enterovirus, herpes simplex 1, PIV 1–3, Streptococcus pneumoniae, S. pyogenes, Haemophilus influenzae, β-hemolytic streptococcus (not group A), group C streptococcus, Moraxella catarrhalis.*

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We did not find differences in the types of pathogens isolated from clinics on each respective side of the US-Mexico border, but we found significant differences in the frequency at which influenza A and bacterial pathogens were isolated across the border. The finding of a higher proportion of bacterial pathogens from the Mexico clinics is unexpected, and we do not have a ready explanation for this difference. Continuing surveillance in these populations will allow tracking of this difference, if it persists. The higher proportion of influenza A seen at US clinics could reflect a focus in Mexico as well as the fact that the pandemic occurred in the second year of Mexico’s participation, for which that year’s influenza vaccine was not well matched.

Most studies of ARI in Mexico have focused on children, but very few have conducted testing to determine their precise etiologies. Those studies that have performed viral testing have shown varying results, as expected. Furthermore, most of the studies have investigated RSV, PIV, and *Mycoplasma pneumoniae*, especially in children during the first 2 years of life.\(^{23–25}\) Cabello *et al.*\(^{26}\) studied Mexican children under the age of 5 years and found RSV was the

### Table 5. Specimen test results by year**

<table>
<thead>
<tr>
<th>Year</th>
<th>Influenza A n (%) (95% CI)</th>
<th>Influenza B n (%) (95% CI)</th>
<th>Other n (%) (95% CI)</th>
<th>Negative n (%) (95% CI)</th>
<th>Total n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2004–2005</td>
<td>7 (6) (2.9–11.7)</td>
<td>26 (22) (16.2–31.2)</td>
<td>15 (12) (7.2–18.9)</td>
<td>72 (60) (50.3–67.8)</td>
<td>120</td>
</tr>
<tr>
<td>2005–2006</td>
<td>91 (40) (35.1–47.9)</td>
<td>9 (4) (2.1–7.4)</td>
<td>29 (13) (8.8–17.4)</td>
<td>98 (43) (36.0–48.8)</td>
<td>227</td>
</tr>
<tr>
<td>2006–2007</td>
<td>33 (16) (11.6–21.6)</td>
<td>11 (5) (3.0–9.3)</td>
<td>52 (24) (18.9–30.6)</td>
<td>116 (55) (47.6–61.0)</td>
<td>212</td>
</tr>
<tr>
<td>2007–2008</td>
<td>56 (14) (11.6–18.7)</td>
<td>38 (10) (7.5–13.6)</td>
<td>60 (15) (11.9–19.0)</td>
<td>237 (61) (54.9–64.7)</td>
<td>391</td>
</tr>
<tr>
<td>2008–2009 (through 20 April)</td>
<td>44 (13) (10.3–17.8)</td>
<td>3 (0.9) (0.3–2.7)</td>
<td>61 (18) (14.5–22.9)</td>
<td>223 (67) (63.7–73.8)</td>
<td>331</td>
</tr>
<tr>
<td>2009 (21 April through 16 September)</td>
<td>132 (22) (19.3–26.0)</td>
<td>0 (0) (0.0–0.6)</td>
<td>34 (6) (3.9–7.6)</td>
<td>446 (73) (69.7–76.7)</td>
<td>612</td>
</tr>
<tr>
<td>Total</td>
<td>363 (19)</td>
<td>87 (5)</td>
<td>251 (13)</td>
<td>1192 (63)</td>
<td>1893</td>
</tr>
</tbody>
</table>

**CI, confidence interval.

*Starting in 2005–2006, bacteriological testing was added.

**Other pathogens – adenovirus, enterovirus, herpes simplex 1, parainfluenza virus 1–3, respiratory syncytial virus, *Streptococcus pneumoniae*, *S. pyogenes*, *Haemophilus influenzae*, β-hemolytic streptococcus (not group A), group C streptococcus, *Moraxella catarrhalis*.

### Table 6. Influenza A subtype by year*(

<table>
<thead>
<tr>
<th>Year</th>
<th>H1N1 (seasonal) n (%) (95% CI)</th>
<th>H3N2 n (%) (95% CI)</th>
<th>pH1N1 n (%) (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2004–2005</td>
<td>0 (0) (0.0–39.0)</td>
<td>6 (100) (61.0–100)</td>
<td>0 (0) (0.0–39.0)</td>
</tr>
<tr>
<td>2005–2006</td>
<td>0 (0) (0.0–5.8)</td>
<td>63 (100) (94.3–100)</td>
<td>0 (0) (0.0–5.8)</td>
</tr>
<tr>
<td>2006–2007</td>
<td>25 (86) (69.4–95.0)</td>
<td>4 (14) (5.5–30.6)</td>
<td>0 (0) (0.0–11.7)</td>
</tr>
<tr>
<td>2007–2008</td>
<td>19 (37) (24.8–50.1)</td>
<td>33 (63) (49.9–75.2)</td>
<td>0 (0) (0.0–6.9)</td>
</tr>
<tr>
<td>2008-2009</td>
<td>29 (16) (11.5–22.3)</td>
<td>20 (11) (7.4–16.6)</td>
<td>130 (73) (65.7–78.6)</td>
</tr>
</tbody>
</table>

*(CI, confidence interval.

References:

22. Kammerer et al.

23. Cabello et al.
most prevalent cause of ARI. Another group studied Mexican children with rhinopharyngitis and a history of asthma and wheezing. They found RSV was the predominant cause in preschool-aged children, and influenza A was the main cause among school-aged children and adolescents. A study in a cohort of 100 Mexican children who were aged 6–12 years, in the same school, with ARI, determined significant PCR evidence that AdV C was the leading cause of ARI, with 23% of cases being AdV positive. Another group studied Mexican children with rhinopharyngitis and a history of asthma and wheezing. They found RSV was the predominant cause in preschool-aged children, and influenza A was the main cause among school-aged children and adolescents. A study in a cohort of 100 Mexican children who were aged 6–12 years, in the same school, with ARI, determined significant PCR evidence that AdV C was the leading cause of ARI, with 23% of cases being AdV positive.28 ARI has a different case definition than ILI (ARI – presence of two or more of the following symptoms: fever, cough, sore throat, sneezing, congestion, aphony, or rhinorrhea) and this may result in different rates for pathogens identified compared with this surveillance, which used the ILI case definition.

In our surveillance, we found influenza was the most common cause of ILI in all age groups, but especially so in the 6–15 years age group. This trend was seen in both pre-pandemic and pandemic specimens. We found lower prevalence of RSV (0–6%) and AdV (4%) than earlier studies. RSV tends to be seasonal, causing localized outbreaks mainly affecting young children, older adults, and immunocompromised patients. Sample collection technique is critical in RSV testing. The best and most frequently used sample is a nasal aspirate or wash. Our study used nasal swab as the specimen type, which may partially explain the low levels of RSV we found. However, the RSV-positive cases we did find were in the expected 1–5 and >54 years age groups.

Adenovirus infections occur worldwide in humans and are common in all age groups, causing both hospital- and community-acquired epidemics. AdV probably accounts for 3% of the infections in the civilian population (http://www.who.int/healthinfo/global_burden_disease/en/index.html). In children younger than age 5 years, AdV causes approximately 5% of upper tract respiratory infections and are probably responsible for approximately 10% of the pneumonias in childhood. By age 6, 95% of children are seropositive for AdV. The prevalence of AdV infection in our study (4%) was similar to those in these studies.

Limitations of this surveillance may include collection from a limited number of sites and the clinic population possibly not being representative of the border population as a whole. In addition, at the clinics, not all patients with ILI may be sampled. Self-reported data collected on the case report form may introduce a recall bias. The clinics may vary on the quality of the data collected on the case report form and collection, handling, storage, and shipping of the specimens. During the pandemic, many new sites started to collect specimens, often with limited or no training, which could have affected the quality of the specimens and/or data. The data from the sites in Mexico was collected over 2 years with the majority of specimens (410 of 553) collected in a single surveillance year. Differences between the US and Mexico may reflect this shorter period of collection in Mexico and not an overall trend.

Ongoing knowledge of the circulating pathogens in this region contributes to public health preparedness. In <2 weeks after being received at NHRC, an ILI specimen was found to be unsubtypeable, was analyzed on the IBIS T-5000 and sent to the CDC. The identification of a second influenza case with a nearly, if not identical, previously unseen influenza virus was a harbinger of the coming 2009 pandemic. The discovery of one of the first identified cases of pandemic H1N1 influenza in this population illustrated the importance of surveillance in border regions. Timely transport, testing, and communication allowed health officials to act quickly to diagnose and treat patients, and initiate epidemiologic studies to define groups at risk for severe disease. Across the border region, the number of surveillance sites were increased and severe acute respiratory illness surveillance has been initiated in local hospitals. In summary, this surveillance system met its primary objectives – to identify the respiratory pathogens responsible for ILI and to detect emerging respiratory pathogens in the border region.

This collaborative research has fostered cooperation, joint training, and communication between the participating entities. Mexico has responded to the challenge of pandemic H1N1 and made great strides in strengthening its viral respiratory laboratory diagnostic capacity in state health departments. These binational collaborative relationships will continue to be important in the event of other public health emergencies.

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**Additional authors’ addendum**

A. W. Hawksworth (Naval Health Research Center, San Diego, CA, USA): conceived program, data analysis, write manuscript; D. J. Faix (Naval Health Research Center, San Diego, CA, USA): conceived program, write manuscript; M. L. Nava (ISESALUD, Baja California, Mexico): conceived program; L. Wong Lopez (ISESALUD, Baja California, Mexico): conceived program; E. Palacios (Department of Population Studies, El Colegio de la Frontera Norte, Tijuana, B. C., Mexico): conceived program; R. Flores (National Institute of Epidemiological Reference, Secretariat of Health, Mexico): conceived program, write manuscript; M. Fonseca-Ford (Centers for Disease Control and Prevention, Atlanta, GA, USA): conceived program, data analysis; A. Phippard (Centers for Disease Control and Prevention, Atlanta, GA, USA): conceived program, write manuscript; K. Lopez (Imperial County Public Health Department, CA, USA): conceived program; J. Johnson (County of San Diego Health and Human Services Agency, CA, USA): write manuscript; J. G. Bustamante Moreno (ISESALUD, Baja California, Mexico): conceived program; S.H. Waterman (Centers for Disease Control and Prevention, Atlanta, GA, USA): conceived program, write manuscript.

**Biographical sketch**

Dr. Kammerer works at the Naval Health Research Center in San Diego on a number of respiratory illness surveillance projects. He received his Doctor of Medicine degree from Hahnemann University in Philadelphia and his Master of Public Health degree from San Diego State University.

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Peter E. Kammerer, Sonia Montiel, Paula Kriner, Ietza Bojorquez, Veronica B. Ramirez, Martha Vazquez-Erlbeck, Eduardo Azziz-Baumgartner & Patrick J. Blair

Region-spanning national borders are conducive to the migration of infectious diseases. Since 2004, the Naval Health Research Center, in concert with health officials of San Diego and Imperial counties, has collaborated with the Secretary of Health, Mexico and the US Centers for Disease Control and Prevention to conduct respiratory disease surveillance in the US-Mexico border region. Demographic and symptom information and respiratory swabs were collected from enrollees who met the case definition for influenza-like illness (ILI). Between 2004 and 2009, 1855 individuals were sampled at clinics near the US-Mexico border in California and Baja California. The most frequent pathogen identified was influenza (25% of ILI cases), with those aged 6–15 years the most frequently affected. In April 2009, a young female participant from Imperial County, California, became among the first documented cases of pandemic influenza A/H1N1. A number of other viral and bacterial respiratory pathogens were identified from submitted samples, including adenovirus, parainfluenza viruses, respiratory syncytial virus, *Streptococcus pneumoniae*, *S. pyogenes*, *Haemophilus influenzae* and beta-hemolytic streptococci. These findings illustrate the importance of disease surveillance in areas near national borders.