Supplemental Material for Seasonality of respiratory viruses causing hospitalizations for acute respiratory infections in children in Nha Trang, Vietnam

Highlights
- Hospitalizations for respiratory viruses are seasonal in Vietnam
- Respiratory syncytial virus peaks in the late summer months, and influenza A in April to June
- No clear seasonality is seen for human rhinovirus
- Human parainfluenza 3 and human rhinovirus are positively associated with dew point
- This work can inform the timing of influenza and RSV vaccination and the judicious use of antibiotics in Vietnam
Seasonality of respiratory viruses causing hospitalizations for acute respiratory infections in children in Nha Trang, Vietnam


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Abstract

Background
Acute respiratory infections (ARIs) are the most common causes of death in children under 5 years of age. While the etiology of most pneumonia and ARI episodes is undiagnosed, a broad range of ARI-causing viruses circulate widely in South East Asia. However, the patterns and drivers of the seasonal transmission dynamics are largely unknown. Here we identify the seasonal patterns of multiple circulating viruses associated with hospitalizations for ARIs in Nha Trang, Vietnam.

Methods
Hospital based enhanced surveillance of childhood ARI is ongoing at Khanh Hoa General Hospital in Nha Trang. RT-PCR was performed to detect 13 respiratory viruses in nasopharyngeal
samples from enrolled patients. Seasonal patterns of childhood ARI hospital admissions of various viruses were assessed, as well as their association with rainfall, temperature, and dew point.

**Results**

Respiratory syncytial virus peaks in the late summer months, and influenza A in April to June. We find significant associations between detection of human parainfluenza 3 and human rhinovirus with the month’s mean dew point. Using a cross-wavelet transform we find a significant out-of-phase relationship between human parainfluenza 3 and temperature and dew point.

**Conclusions**

Our results are important for understanding the temporal risk associated with circulating pathogens in Southern Central Vietnam. Specifically, our results can inform timing of routine seasonal influenza vaccination and for when observed respiratory illness is likely viral, leading to judicious use of antibiotics in the region.
Introduction

Acute respiratory infections (ARIs) in South East Asia cause substantial morbidity and mortality, especially in children under 5 years of age [1]. Pneumonia continues to be the number one cause of under 5 death despite effective treatments [2]. A substantial contributor to this is the largely unknown etiology of most pneumonias, with both viral and bacterial origin. The patterns of pneumonia and other ARI hospitalizations serves as a proxy for determining the transmission dynamics of viruses and bacteria contributing to these hospitalizations, and is of key importance in understanding and limiting burden of this childhood killer.

In addition to informing on the relative likelihood of the potential viral etiology of a pneumonia case based on seasonally circulating pathogens, knowledge of seasonal influenza epidemiological dynamics can aid in informing optimal timing for vaccination efforts [3, 4] and judicious use of antivirals [5, 6]. Identification of low incidence seasons would provide a target window for vaccination, with the hope of maximizing population immunity before the onset of the influenza season. Similarly, when respiratory syncytial virus (RSV) vaccination becomes available, knowledge of its seasonality will be useful to maximize benefit [7, 8], and in the potential administration of passive immunoprophylaxis with palivizumab to reduce the number of severe outcomes associated with RSV infection among high-risk infants [9]. Finally, knowledge of seasonal patterns of virus circulation can inform the clinical use of antibiotics, again limiting use when viral circulation is traditionally high to minimize antibiotic resistance [10, 11, 12, 13].

Previous work has identified the potential etiology of pneumonia and other ARIs in Southern Central Vietnam [14, 15]. Adenovirus (AoV), bocavirus (HBoV), coronavirus (CoV), human metapneumovirus (HMPV), human parainfluenza 1–4 viruses (HPIV1–4), human rhinovirus (HRV), Influenza A and B, and respiratory syncytial virus (RSV) all contribute to the disease burden and circulate widely. Information on viral transmission dynamics across seasons in Vietnam is relatively under-explored. Do et al. found seasonality of RSV infections and slight seasonality of HMPV infections in Ho Chi Minh City, but no seasonality of influenza [14]. Several studies have similarly found high variability in Influenza A & B incidence over the year [16, 17, 18]. Yoshida et al. found RSV occurring in the hot months, influenza A in the cool months, and year-round detection of HRV in Nha Trang [15]. Few other studies have examined seasonality of these common viruses
across South East Asia.

Here we examine the seasonal trends of hospitalizations and circulation of multiple viruses in Nha Trang, Vietnam. Using enhanced hospital based surveillance of childhood ARI we identify seasonal patterns in hospitalizations as a proxy for transmission and explore the relationship of hospitalizations associated with virus detection with rainfall, temperature, and dew point, to try and identify contributing factors to observed seasonality.

Methods

Study site

The study site is Nha Trang, central Vietnam, where the study population has been described previously [19, 15, 20]. The hospital based enhanced surveillance of childhood ARI is ongoing. We analyze data from January 29, 2007 to April 26, 2012 at Khanh Hoa General Hospital (KHGH) which is the only tertiary care facility located in Khanh Hoa Province. According to the field site census survey in July 2006, the study catchment area encompassing the 16 non-touristic of the 27 communes in Nha Trang city, had 198,729 residents including 13,952 children less than 5 years of age. An ARI case was defined as any child presenting to KHGH with cough or/and difficulty in breathing. Before study enrollment, informed consent was obtained from parents of children who presented with ARI and lived in the study catchment area. Clinical and demographic information, chest radiographs (CXR), laboratory data, and nasopharyngeal (NP) samples were collected from all enrolled patients. KHGH is the only hospital in Nha Trang, Khanh Hoa province and the only one accessible for residents of the catchment area. Hence for incidence calculations we assume that all children with ARI are eligible to be hospitalized and enrolled into the study and use the population of the catchment area as denominator. Acute respiratory infection patients with normal CXR were categorized as upper respiratory tract infection (URTI). Patients with abnormal CXR were categorized as lower respiratory tract infection (LRTI).

NP samples were collected at the time of admission and viral nucleic acid was extracted using QIA viral RNA minikit (QIAGEN Inc., Valencia, CA). Four multiplex-PCR assays (1: influenza A, influenza B, RSV, hMPV; 2: PIV-1, -2, -3, and -4; 3: rhinovirus, coronavirus 229E, coronavirus OC43; 4: adenovirus and bocavirus) were performed to detect 13 respiratory viruses in each NP
sample. A second confirmatory-PCR was performed for samples positive on the initial PCR test. Samples positive for both PCR assays were defined as positive. Reverse transcription-PCR (RT-PCR) assays were performed using one-step RT-PCR kit from QIAGEN. For the multiplex PCR and hemi-nested PCR assays, TaqDNA polymerase (Promega, San Luis Obispo, CA) was used as previously described [15]. Positive templates were used in each assay for quality control.

Weather data

Three weather variables – rainfall (inches), temperature (F°), and dew point (the temperature to which air must be cooled in order to reach saturation with water) – were collected from the Nha Trang Station (ID: 488770) reported by the US National Oceanic and Atmospheric Administration [21]. We considered monthly averages of all weather variables as well as both the weather variable on the day of admission (t₀) as well as averaged over the previous 7 days (t₋7 to t₋1) assuming up to a week incubation period for the viral infections (see supplementary material) [22]. The weather in Nha Trang central Vietnam is warm throughout the year (between 20 and 30 C°). In terms of temperature, December to February months are cooler (referred to here as the winter months) while June to August months are hottest months (referred to here as the summer months). September, October, November are the wettest months.

Statistical analysis

For each PCR+ for virus a series of log-link Poisson models were fit to assess respective seasonality with calendar month as the main predictor, log-commune population size as an offset term, monthly averaged rainfall, temperature, and dew point, and calendar year as adjusting variables. The outcome was monthly aggregate cases, with resulting coefficients as incidence rate ratios as compared to January. We excluded hospitalizations with more than one virus detected in the nasopharynx to adjust for a potential bias through inclusion of cases who by virtue of being co-infected may otherwise have been asymptomatic [23]. This approach underestimates the true incidence of NP carriage among ARI cases but allows estimation of seasonal patterns that are not biased by other circulating viruses; although co-circulation of bacteria was not accounted for. We assessed the numbers and variety of viruses in ARI hospitalizations using binomial proportion tests for each virus.
**Cross-wavelet transform**

To examine the relationship between monthly average rain, temperature, and dew point and incidence hospitalized childhood ARI infections, we estimated the cross-wavelet transform between the z-standardized time series (we subtracted the mean of the time series and divided by the standard deviation) of weather and viral detections [24]. The cross-wavelet transform identifies regions of high power in phase-space and identifies the relative phases of each time series, i.e., in-phase or out-of-phase [25]. The wavelet transform can be thought of a Fourier transformation over time that can identify what is the dominant frequency composing a time series as the signal changes in time. The cross-wavelet transform allows us to compare how two time signals co-vary: we can identify if the presence of a particular frequency at a given time in the time series of hospitalizations corresponds to the presence of that same frequency at the same time in a weather covariate [26]. Additionally, we can identify the magnitude by which weather precedes or follows hospitalizations through the phase angle of the two time series. Finally, we can identify the statistical significance of the identified constituent frequencies over time by comparing the observed frequencies to a red-noise process.

**Sensitivity analyses**

Sensitivity analyses, presented in the supplementary materials, were performed as follows: 1) case counts of less than 70 per virus over the whole study period were deemed too low for robust statistical inference; 2) alternative Poisson regression models where the reference category is July; 3) logistic regression models were formulated as an alternative to the Poisson regressions above with detection of a virus by PCR (yes/no) as the outcome, with month as the main predictor, adjusted for weather, commune of residence, age, sex, smoking indoors, socioeconomic status (SES), and calendar year, with weather variables on the day of admission ($t_0$) as well as averaged over the previous 7 days ($t_{-7}$ to $t_{-1}$); and 4) additional wavelet analyses of viral isolations not presented in the main text.
Results

The study enrolled 3431 children between 2007 to 2013. Among those, 374 (11%) had multiple viruses detected in their NP swabs, for 59 presence of viruses in the NP was not determined, and were excluded from the analyses, thus the total study population was 2998. Among all cases with a virus detected, HRV, RSV, and Influenza A were the most frequently detected viruses, with 569 (33.5% of all viral detections), 455 (26.8%), and 282 (16.6%) detections, respectively (Table 1 and Figure 1). Counts of bocavirus (HBoV), coronavirus (CoV), and human parainfluenza 1, 2, and 4 viruses (HPIV1–4) were less than 70 and are reported in the supplementary material.

Seasonality

Strong seasonality, as defined by at least three consecutive months with a consistently higher or lower incidence than expected (IRR or OR greater or less than 1, respectively) and at least one of those statistically significantly different from the baseline, was observed for Influenza A, RSV, and the presence of any virus (Figures 2 and 3). RSV peaked in July through November, with August seeing a 15.69 (95% confidence interval [CI]: 3.05, 80.56) times higher risk of identifying RSV as the sole viral agent from the nasopharynx of a childhood case as compared to January. Influenza A peaked in May with an IRR of 6.28 (95% CI: 2.2, 17.89) as compared to January. Estimates of odds ratios from the supplemental logistic regression are qualitatively similar to the Poisson regression, save for HPIV3, which exhibits a consistent yet non-significant peak in the cool months in the primary analysis which becomes significant in the supplemental analysis (see supplemental material).

Association with weather

Weather patterns over the study period were similar to patterns before and after the study period (supplementary material, Figure S3). Monthly average rainfall (in inches), temperature (1° F), and dew point (1° F) correlated with the seasonality of some of our endpoints. Figure 4 shows the incidence rate ratios for the three weather effects from the seasonally-adjusted models. Overall hospitalizations for ARI were negatively associated with temperature (IRR 0.92 per 1 degree increase, 95% CI: 0.87, 0.97, p = 0.003) and positively associated with dew point (IRR 1.08 per 1
degree increase, 95% CI: 1.04, 1.13, p < 0.001). Of the few other significant effects, Influenza A and HRV had a negative association with temperature (IRR 0.85, 95% CI: 0.75, 0.9, p = 0.0116, and IRR 0.86, 95% CI: 0.79, 0.94, p < 0.001, respectively), and HPIV3 and HRV were positively associated with dew point, with IRRs 1.26 (95% CI: 1.04, 1.52, p = 0.0164) and 1.13 (95% CI: 1.05, 1.21, p = 0.0009), respectively. Logistic regression found that RSV was positively associated with the previous week’s rainfall, with an odds ratio of 1.90 (95% CI: 1.21, 2.99, p = 0.0053). Previous week’s temperature was marginally associated with RSV (OR: 1.14 (95% CI: 0.98, 1.33, p = 0.08) (see supplementary material).

Figure 5 shows the cross-wavelet transform of all hospitalizations and the three weather variables in the month of admission. Significant bands of high power around 1 year can be seen for temperature and dew point. This indicates that hospitalizations and temperature and dew point share variability at yearly frequencies over the study period, and the phase relationship indicates that changes in weather slightly precede changes in hospitalizations (arrows point about 45° down).

Figure 6 shows the cross-wavelet transform of RSV and the three weather variables with similar significance bands around 1 year for temperature and dew point. The phase indicates temperature and dew point lead RSV incidence. While not significant, RSV was found to be leading rainfall by 90° (3 months) at the one year period band (see supplementary material). Significant bands of power were seen between temperature and dew point with HPIV3 with an indicated phase relationship of nearly completely out-of-phase (Figure 7). Similar patterns were seen (1 year significant bands between temperature and dew point and virus) for ADV, HBoV, CoV, HPIV1,2&4, HRV, and influenza A, though the phase differences varied across these viruses; HMPV and influenza B had bands lying outside the cone of influence (ie, not statistically significant; see supplementary material). These results in general indicate strong associations between weather covariates and viral hospitalizations at a yearly timescale.

Discussion

Here we have identified seasonal trends of several common respiratory viruses in hospitalized children in Nha Trang, Vietnam. By fitting a series of statistical models to the observed data, we allow the data to identify salient features contributing to the seasonality of these viruses. We evaluated
seasonal patterns and associations with weather of hospitalizations for several respiratory viruses using three lines of evidence: 1) Poisson regression examining the relative incidence across months of virus detections adjusted for weather covariates, 2) cross-wavelet transforms of hospitalizations with viral detections, and 3) a sensitivity analysis with a logistic regression model finding odds ratio of hospitalizations with viral detections and weather variables.

Any viral detection showed distinct seasonality with peaks in May through September, a negative association with temperature, positive association with dew point, and cross-wavelets indicating temperature and dew point leading viral detection. Of commonly detected viruses, RSV, Influenza A, and HPIV3 had significant seasonality. RSV peaked in July through December, was positively associated with the week’s previous average rainfall. Cross-wavelets showed temperature and dew point to lead RSV, rain was found to non-significantly fall behind RSV at 1 year frequencies, and precede RSV at shorter (< 100 day) frequencies. Finally, HPIV3 while not significant, had peaks in January and February, was positively associated with dew point, and was completely out of phase with temperature and dew points in cross-wavelet analyses. These results contribute to the growing body of knowledge on the epidemiology of respiratory pathogens in South East Asia and Southern Central Vietnam.

Using a cross-wavelet transform we evaluated the time-dependence of virus hospitalizations with the weather covariates. We found strong yearly associations with RSV over the study period with temperature and dew point in phase with hospitalizations. This seasonality is opposite to observed seasonality in temperate climates, where RSV typically peaks in winter [27, 28]. Recent reviews of RSV seasonality in tropical regions highlights the uncertainty in the effects of weather on RSV transmission. Studies in Brazil [29], Hawaii [30], India [31], Kenya [32], and Malaysia [33, 34, 35], have shown negative associations between temperature and RSV, while studies in Hong Kong [36], Mexico [37], Singapore [33], and Taiwan [38] have shown positive associations. We find strongly positive associations between temperature and RSV hospitalizations with a slight lead of temperature on RSV. This is evident both from the cross-wavelet transform and the regression results indicating a positive effect of the previous week’s temperature on RSV.

There is less uncertainty in the role of rainfall on RSV transmission in tropical areas, where the majority of work indicates that RSV generally occurs during rainy seasons [28]. Colombia [39], The Gambia [40], Hong Kong [36], Kenya [32], Malaysia [34], and Papua New Guinea [41] all show
positive RSV associations with rainfall. Omer et al. [42] showed significant positive associations between rainfall and temperature in the previous 8 days and RSV incidence in Lombok, Indonesia. We find similar associations, with the mean rainfall and temperature over the previous 7 days having odds ratios for RSV of 1.98 and 1.23, respectively. This association is plausible as the incubation period of RSV is estimated to be between 4 and 5 days [22]. Detailed contact tracing studies, coupled with climatological data could refine this association.

Somewhat surprisingly, the cross-wavelet transform of RSV and rain showed no significant association, though areas of high power were observed in the 1-year and 6-month bands, with phase indicating RSV leading weather. However, none of the viruses studied here showed appreciable associations with rain in the cross-wavelet transform, possibly indicating the dominance of other weather effects (temperature and dew point) on virus hospitalizations.

As with previous studies examining HPIV incidence, we found a predominance of HPIV3 (72 detections) compared to 41, 13, and 3 for HPIV1, 2, and 4, respectively. Typical seasonality for HPIV3 is the spring and early summer months in the temperate regions [43, 44], and has little to no observed seasonality in the tropics and subtropics [33, 45]. We find evidence for winter peaks in HPIV3 hospitalizations when employing both the Poisson regression model as well as the logistic regression model to estimate odds ratios, though the peaks were not statistically significant when controlling for weather.

The cross-wavelet transforms reveals HPIV3 to vary significantly at 1 year periodicity with temperature and dew point across the study. It also shows that HPIV3 hospitalizations are nearly completely out of phase with temperature and dew point throughout the study period. HPIV3 has been associated with low temperature and low relative humidity [46] though there is in general a paucity of data on the transmission routes of HPIV3 [47, 48]. Future work could explore in more depth the epidemiological relationship between HPIV and weather variables.

This study is not without limitations. First, while we have nearly 6 years of data, this is still a relatively short period to assess long-term seasonal trends, or to increase confidence in the estimates of seasonal patterns. However, the length of the analyzed time series is similar to other studies examining seasonal trends in viral respiratory pathogens in the tropics [33, 44], and gives indications for areas of future study. Second, we excluded individuals with more than one virus detected. Examination of changes in the seasonality of other viruses and coinfection over this
period is worthy of study and is outside the scope of this paper. Third, this study used hospital-based surveillance, necessarily presenting the most ill children. We take as an assumption that hospitalizations are a fraction of all transmission and severity of illness is not related to weather. Finally, this study examines the influence of weather on viral hospitalizations and does not address other drivers of seasonal patterns of transmission, such as school closures [49], differences in other social behavior such as contact rates [50, 51], susceptible recruitment through births [52], or possible seasonal changes in host immune responses [53]. Future work examining these drivers in this setting is necessary.

Limitations aside, our study adds to the body of literature on seasonality of common respiratory patterns in tropical regions and will be of use when consideration of the epidemiology of these pathogens is necessary. For example the timing of influenza peaks in the mid-spring months (April, May, June) would indicate routine vaccination in the winter months would be of biggest impact. Similarly, knowing RSV peaks in the late summer/early fall when RCP is at its lowest may help in limiting unnecessary antibiotic [54] or antiviral use [6]. In Vietnam and most of Asia, most antibiotics are acquired from a pharmacist without a formal prescription [10]. This fact makes results like those presented here of high importance to public health decision-makers to inform pharmacists of seasonality of respiratory infection etiologies and urge judicious prescribing practices.

Additional future work could include examination of the effects of contact clustering [55], co-infection [5, 56], and asymptomatic carriers [23] on transmission of the examined viruses all of which may be influenced by weather.

Contributions
Study design: BMA, SF, HH, LMY; Data collection: LMY, LNM, VDT; Data analysis: BMA, SF; Writing first draft: BMA, Writing subsequent drafts: BMA, SF, GR, LMY; Contributed intellectually: all authors

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or preparation of the manuscript.

**Ethical approval**

The study was approved by institutional review boards in the National Institute of Hygiene and
Epidemiology, Vietnam and the Institute of Tropical Medicine, Nagasaki University, Japan.
References


Figure 1: **Monthly hospitalizations by virus in Nha Trang, Vietnam.** Figure shows monthly detections of virus as percentages of all enrolled ARI hospitalizations.
Figure 2: **Seasonality of hospitalizations and any viral detection in Nha Trang, Vietnam.** Figure shows weekly counts of all hospitalizations (top row) and any virus detection (bottom row). Right-hand column shows model-adjusted incidence rate ratios for month of year as compared to January. Red months indicates statistically significant deviations from January.

Figure 3: **Seasonality of common respiratory viruses in Nha Trang, Vietnam.** Figure shows model-adjusted incidence rate ratios for month of year as compared to January. Red months indicates statistically significant deviations from January.
Figure 4: **Weather effects.** Figure shows model-adjusted incidence rate ratios for the main three weather effects: monthly mean rainfall (in inches), temperature (1° F), and dew point (1° F).
Figure 5: Cross-wavelet transform of the z-standardized all hospitalization counts and weather time series. Figure shows the cross-wavelet of all hospitalization counts and rainfall, temperature, and dew point. Colors indicate increasing cross-wavelet power (strength of coherence between the time series) blue to red. The 5% significance level against red noise is shown as a thick contour and the cone of influence (within which the wavelets are not influenced by the edges of the time series) is shown in white shading. The relative phase relationship is shown as arrows (with in-phase pointing right, out-of-phase pointing left, and weather leading hospitalizations by 90° pointing straight down).
Figure 6: Cross-wavelet transform of the z-standardized RSV and weather time series. Figure shows the cross-wavelet of RSV and rainfall, temperature, and dew point. Colors indicate increasing cross-wavelet power (strength of coherence between the time series) blue to red. The 5% significance level against red noise is shown as a thick contour and the cone of influence (within which the wavelets are not influenced by the edges of the time series) is shown in white shading. The relative phase relationship is shown as arrows (with in-phase pointing right, out-of-phase pointing left, and weather leading RSV by 90° pointing straight down).
Figure 7: Cross-wavelet transform of the z-standardized HPIV3 and weather time series. Figure shows the cross-wavelet of HPIV3 and rainfall, temperature, and dew point. Colors indicate increasing cross-wavelet power (strength of coherence between the time series) blue to red. The 5% significance level against red noise is shown as a thick contour and the cone of influence (within which the wavelets are not influenced by the edges of the time series) is shown in white shading. The relative phase relationship is shown as arrows (with in-phase pointing right, out-of-phase pointing left, and weather leading HPIV3 by 90° pointing straight down).
### Table 1: Annual hospitalizations by virus.

Table shows counts of hospitalizations for adenovirus (ADV), human metapneumovirus (HMPV), human parainfluenza virus 3 (HPIV3), influenza A, influenza B, rhinovirus (HRV), and respiratory syncytial virus (RSV). Viruses with less than 70 cases were excluded from this table (I) and are presented in the supplement. Counts of all virus detections (including those presented in the supplement are in the column ‘any virus’).

<table>
<thead>
<tr>
<th>Year</th>
<th>ADV</th>
<th>HMPV</th>
<th>HPIV3</th>
<th>HRV</th>
<th>Influenza A</th>
<th>Influenza B</th>
<th>RSV</th>
<th>Any</th>
</tr>
</thead>
<tbody>
<tr>
<td>2007</td>
<td>21</td>
<td>28</td>
<td>19</td>
<td>137</td>
<td>84</td>
<td>1</td>
<td>149</td>
<td>451</td>
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<tr>
<td>2008</td>
<td>15</td>
<td>10</td>
<td>10</td>
<td>134</td>
<td>51</td>
<td>8</td>
<td>85</td>
<td>330</td>
</tr>
<tr>
<td>2009</td>
<td>20</td>
<td>1</td>
<td>9</td>
<td>129</td>
<td>74</td>
<td>10</td>
<td>68</td>
<td>333</td>
</tr>
<tr>
<td>2010</td>
<td>12</td>
<td>2</td>
<td>4</td>
<td>95</td>
<td>25</td>
<td>24</td>
<td>84</td>
<td>271</td>
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<tr>
<td>2011</td>
<td>13</td>
<td>24</td>
<td>17</td>
<td>61</td>
<td>37</td>
<td>8</td>
<td>69</td>
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<td>6</td>
<td>7</td>
<td>12</td>
<td>13</td>
<td>11</td>
<td>19</td>
<td>0</td>
<td>76</td>
</tr>
</tbody>
</table>

Total (%) 87 (5.1%) 72 (4.2%) 71 (4.2%) 569 (33.5%) 282 (16.6%) 70 (4.1%) 455 (26.8%) 1696 (100%)