Seasonality in testing and positive respiratory bacterial infections in the Australian Capital Territory, 1997–2007

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Original article

Seasonality in testing and positive respiratory bacterial infections in the Australian Capital Territory, 1997–2007

Xinyi Liu, Aparna Lal, Alice Richardson

Abstract

Background

*Chlamydia pneumoniae* (Cp) and *Mycoplasma pneumoniae* (Myco) bacteria are atypical pathogens that can cause pneumonia and exacerbate underlying conditions such as asthma and chronic obstructive pulmonary disease. In the Australian Capital Territory, there is limited information on how seasonal patterns for positive infections and testing may vary, a gap that has implications for control strategies.

Methods

We examined seasonal patterns of immunoassay results of patients from Canberra Hospital, Australia, who were tested for Cp and/or Myco. Pathology data, collected from August 1997 to March 2007 from 7,275 patients, were analysed with time series additive decomposition and time series regression.

Results

The proportion of positive Cp infections was highest in March and April (autumn) and lowest in June and August (winter). The proportion of positive Myco infections was highest in December and January (summer) and lowest in August (winter), even though testing for the pathogen peaked in winter with a low in summer. Models with a long-term trend and a variable for month were a better fit for the data than the null models for both infections.

Conclusion

We found differences in seasonal patterns of testing and in the proportion of positive infections. These findings suggest that preventative measures for common infections need to account for seasonal testing practices so as to build an accurate picture of temporal changes in these infections.

Keywords: time series, seasonal decomposition, *Chlamydia pneumoniae*, *Mycoplasma pneumoniae*, Australian Capital Territory
Introduction

*Chlamydia pneumoniae* (Cp) and *Mycoplasma pneumonia* (Myco) bacteria are atypical pathogens that can cause pneumonia and exacerbate asthma and chronic obstructive pulmonary disease (COPD). Individuals infected with Cp or Myco are more likely to present with ‘atypical’ general symptoms (e.g. headaches, sweating, and myalgias). Though they differ in their structures and life cycles, *Cp* and *Myco* infections may cause clinically indistinguishable disease patterns among those infected, making laboratory testing essential to identify the underlying pathogens. Deficiencies in testing methods (e.g., commonly-used serological detections are complicated by false negative results in the early acute phase of infection) and the short epidemic peak present challenges for public health: *Cp* and *Myco* infection incidence may increase rapidly, limiting timely control by public health agencies. Identifying seasonal patterns of *Cp* and *Myco* infections is necessary to efficiently allocate public health resources, to guide early prevention, and to establish vaccine priorities.

For both *Cp* and *Myco*, the prevalence of infections varies across time, geographical regions, seasons, study populations, age groups, and possibly bacteria detection techniques. The majority of studies suggest that *Myco* infections exhibit seasonal trends, with weather-related factors such as temperature and humidity cited as possible environmental drivers. A laboratory-based study has found that survival of airborne *Myco* is best at 27 °C and a relative humidity of either less than 25% or greater than 90%, while relative humidity levels between 60% and 80% are most lethal to the pathogen. The extreme sensitivity of *Myco* to relative humidity suggests that control of airborne transmission of *Myco* may be possible in selected spaces. Lenglet et al. found that the majority of *Myco* epidemics in North America begin in summer, peak in autumn, and decrease in winter. Similarly, in Germany, Dumke et al. found that the quarterly incidence of *Myco* peaked in late autumn and early winter.

For *Cp*, seasonality is less clear, with several researchers suggesting that seasonal changes do not affect infection patterns, other studies contradict this. Studies in Suzhou province (China); in Germany; and in Lima (Peru) observed no seasonal differences in the incidence of *Cp*. To date, to the authors’ knowledge, there is little information on how the seasonal patterns for either pathogen vary due to seasonality in testing patterns.

The contradictory nature of existing findings and the limited number of studies utilising Australian data point to a research gap. There is limited information on the seasonal patterns of *Myco* and *Cp* in Australia. Global evidence indicates that the seasonal patterns for infections caused by these two pathogens differ, hence, the *Cp* and *Myco* data were modelled separately. Combining the two pathogens can mask seasonality as well as cyclic epidemic patterns specific to a pathogen and to pathogen-specific biology. Understanding the seasonal patterns can help to identify the drivers of such seasonality, such as attendance at day-care centres, school terms, and ambient temperature. Such knowledge can inform the development of targeted prevention activities and can form the components of an early warning system to help with health resource allocation in areas that may soon experience increases in cases.

This project aims to examine whether respiratory infections caused by *Cp* and *Myco* exhibit seasonality and whether this is related to patterns of testing. The project utilises data collected between 1997 and 2007 in the Australian Capital Territory (ACT).

Methods

Data Sources

The study is based on de-identified data collected from 1997 to 2007 and extracted from ACT Pathology Laboratory databases located in the Canberra Hospital. Variables with missing values were removed, as analysis required complete data. Patients with multiple entries...
(for the same lab test) were removed so as to ensure analysis related solely to single-visit patients. The study sample spanned all age and gender groups.

Pathology data were collected from patients with respiratory diseases who underwent testing for Cp and/or Myco infections. For Myco, a result of < 40 is recorded as negative and ≥ 40 as positive for the infection. For Cp, a result of < 0.9 is recorded as negative, between 0.9 and 1.09 inclusive as indeterminate, and ≥ 1.10 as positive. The immunoassay for the test was carried out in a National Association of Testing Authorities accredited laboratory which also adhered to the Royal College of Pathologists Australasia Quality Assurance Program. We have confidence that the sensitivity (true positive rate) and specificity (true negative rate) of the test are high, and indeed Peeling shows that upper bounds on sensitivity and specificity are 80% and 100% respectively.

The Cp infection series began in October 1998 and the Myco infection series began in July 1997. To begin both series in January, three positive cases of the Cp variable (October 1998 to December 1998) and six positive cases of the Myco variable (July 1997 to December 1997) were removed.

Ethics clearance for this project was obtained through the Australian National University (approval number HREC2017-575).

**Statistical analysis**

Raw data were summarised using time series plots. Two standard statistical methods were then applied to the data: additive time series decomposition and time series regression. Four outcomes were considered: the number of Cp tests; the number of Myco tests; the proportion of positive Cp infection tests; and the proportion of positive Myco infection tests.

Time series regression models were fitted using generalised least squares (GLS) with the best fitting ARMA(p, q) error structure identified in a first stage using Akaike’s Information Criterion (AIC). The full model is:

\[ y_t = \beta_0 + \beta_1 \text{year} + \beta_2 \text{year}^2 + \beta_3 \text{I(month = February)} + \ldots + \beta_{13} \text{I(month = December)} + \epsilon_t \]

where \( y_t \) represents the outcome at time \( t \). The coefficient \( \beta_0 \) denotes the intercept of the line, \( \beta_1 \) represents the long-term linear trend component, and \( \beta_2 \) represents a possible long-term quadratic trend component. Coefficients \( \beta_3, \ldots, \beta_{13} \) represent 11 seasonal components, with January as the baseline. The random error \( \epsilon_t \) is assumed to be normally distributed with an ARMA error structure estimated from the data.

For each outcome, nested models were considered:

1. The linear trend model, which included a nonzero component for the long-term trend represented by the \( \beta_1 \) parameter.

2. The linear trend + seasonal model, which included long-term trend and seasonal components. This model has nonzero seasonal coefficients \( \beta_3, \ldots, \beta_{13} \) with the quadratic long-term trend component \( \beta_2 \) kept at zero.

3. The quadratic trend + seasonal model, which included nonzero linear and quadratic long-term trend components and seasonal components as in the model expression above.

After selecting the trend and/or seasonal components and fitting a model with an appropriate error structure, residuals were checked to ensure model assumptions were satisfied. Autocorrelation function (ACF) and partial autocorrelation (PACF) plots of the normalised residuals were examined to ensure that the selected model included residuals that contained only noise and no systematic components.
In time series data, it is likely that an observed variable’s value in the current period is similar to its value in the previous period or the period prior to that (an autoregressive component); it is also likely that an observed variable’s value in the current period is related to the error component in the previous period or the period prior to that (a moving average component). Therefore, when fitting a regression model to time series data, it is common to include both autoregressive and moving average components in the model.

To compare different models with an autoregressive variance-covariance structure, we used AIC to determine which model best fitted the data. The model with the lowest AIC was chosen. Statistical significance was set at \( p < 0.05 \). Analyses were performed using R version 3.6.1.

Results

Descriptive statistics

The data set comprised tests from 7,275 patients from August 1997 to March 2007. There were 1,633 individuals who tested positive only for Cp infection; 1,880 tested negative, with 3,513 total tests. There were 1,787 individuals who tested positive only for Myco infection; 5,182 tested negative, with 6,969 total tests. Finally, there were 356 individuals who tested positive for both Cp and Myco. These infections were included in both datasets.

Table 1 shows summary statistics of the monthly number of tests and the proportion of positive tests per month. The total number of Cp tests was smaller (\( M = 34.44, SD = 14.71 \)) than the total number of Myco tests (\( M = 59.56, SD = 23.83 \)), though the proportion of positive Cp infections (\( M = 48.02, SD = 13.32 \)) was higher than the proportion of positive Myco infections (\( M = 25.90, SD = 11.30 \)). The proportion of positive tests was very variable: between 19 and 82 percent for Cp and between 8 and 55 percent for Myco.

Time series plots with additive decomposition

The time series for Cp consists of 99 consecutive months, from January 1999 to March 2007, and 111 consecutive months for Myco, from January 1998 to March 2007. The total number of tests (Figure 1A and C, grey lines) is higher for Myco than for Cp. The proportion of positive Cp tests decreases over time, while the proportion of positive Myco tests increases. In 2004, the proportion of positive Cp tests (Figure 1B) drops to the lowest value across to the entire period (1997–2007). Simultaneously, the proportion of positive Myco tests (Figure 1D) reaches its highest peak.

Using a trend + seasonal decomposition (Figure 1, red lines), the existence of a seasonal pattern in all four series is demonstrated.

Figure 1A shows the decomposed time series for the total number of Cp tests: the trend decreases first and then increases. There is a peak normally in August and September, and a trough in February. Figure 1B shows the decomposed time series for the proportion of tests indicating positive Cp infection: the trend shows a sharp decline in 2004 and then rebounds slightly. There is a peak in March and April, and two valleys in June and August.

The highest total numbers of patients tested for Cp infection and Myco infection were noted in the spring and winter periods, respectively. Pneumonia caused by Cp was most often observed in autumn (March and April), while pneumonia caused by Myco was most commonly observed in summer (December and January). The lowest proportion of pneumonia cases caused by these two microorganisms was noted in winter (June and August).

Seasonal patterns for Cp and Myco suggested that both infections occurred throughout the year, with a single peak between March and April for Cp, and a single peak between December and January for Myco.
Figure 1: Time series plot and additive decomposition of observed time series line (grey) with trend and seasonal line (red)

A. Monthly number of Cp tests
B. Monthly proportion of positive Cp tests
C. Monthly number of Myco tests
D. Monthly proportion of positive Myco tests
Table 1: Summary statistics for monthly total number of tests and monthly proportion of positive tests

<table>
<thead>
<tr>
<th></th>
<th>Total number of Cp tests*</th>
<th>Total number of Myco tests*</th>
<th>Proportion of tests positive for Cp (%)b</th>
<th>Proportion of tests positive for Myco (%)b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimum</td>
<td>2</td>
<td>17</td>
<td>18.75</td>
<td>8.33</td>
</tr>
<tr>
<td>Median</td>
<td>32.5</td>
<td>56</td>
<td>47.06</td>
<td>25.42</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>34 (14.7)</td>
<td>60 (23.8)</td>
<td>48.02 (13.32)</td>
<td>25.90 (11.30)</td>
</tr>
<tr>
<td>Maximum</td>
<td>71</td>
<td>144</td>
<td>82.61</td>
<td>54.35</td>
</tr>
</tbody>
</table>

a Monthly total number of patients tested for Cp / Myco.
b Monthly proportion of patients tested and found positive (infected) for Cp / Myco.

### Time series regression

Three models were tested for each time series comprising the null model, the trend only model, and the trend + seasonal model. A quadratic term for the monthly number of tests was also explored. The best fitting ARMA model for the errors was selected using AIC (Table 2). Results from the best fitting model, which in each case was the trend + seasonal model, are shown in Tables 3 and 4.

For the number of Cp tests, the trend + seasonal model is the best fitting model (Table 2) with an AIC of 722.5. The coefficients of year and of the months June to October have \( p < 0.05 \), indicating a clear seasonality (Table 3). The ACF and PACF (Appendix A) indicate a significant autocorrelation, and the autocorrelation estimate is 0.58.

For the proportion of positive Cp tests, the trend + seasonal model is the best fitting model with an AIC of 731.42 (Table 2). The coefficients of year and of the months May, August and October have \( p < 0.05 \) (Table 4), indicating some seasonality. The ACF and PACF (Appendix A) indicate a significant autocorrelation, and the autocorrelation estimate is 0.58.

The total number of Cp tests showed a different seasonal pattern to the model of the proportion of positive Cp infections. The model of the total number of Cp tests had more significant monthly variations (June to October, \( p < 0.05 \)) while the model of the proportion of tests indicating positive Cp infection had weak monthly variations (March, \( p < 0.05 \)).

For the number of Myco tests, the trend + seasonal model is the best fitting model (Table 2) with an AIC of 930.9. The coefficients of year and of the months June to October have \( p < 0.05 \) (Table 4), indicating a clear seasonality. The ACF and PACF (Appendix A) indicate a significant autocorrelation, and the autocorrelation estimate is 0.69.

For the proportion of positive Myco tests, the trend + seasonal model is the best fitting model with an AIC of 738.5 (Table 2). The coefficients of year and of the months May, August and October have \( p < 0.05 \) (Table 4), indicating some seasonality. The ACF and PACF (Appendix A) indicate a significant autocorrelation, and the autocorrelation estimate is 0.58.

### Discussion

We are fortunate that Canberra Hospital allowed us access to the pathology data, which (unlike notification data) record both positive and negative results. These characteristics allowed us to investigate seasonal patterns of respiratory bacteria testing and the proportion of positive infections, with potential implications for public health response and control. We are unaware of other studies within the ACT that have retrospectively investigated seasonality in testing patterns and in positive infection rates. We found considerable inter-annual variability in testing as well as seasonality. In the Australian Capital Territory, testing and detection of Cp and Myco occurs throughout the year. Our analysis found that testing for Cp and Myco peaked during
Table 2: AIC values for AR(1) error structure and best fitting ARMA error structure for trend

<table>
<thead>
<tr>
<th>Infection tested</th>
<th>Data set</th>
<th>Trend</th>
<th>AR(1) model</th>
<th>ARMA model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cp</td>
<td>Number of tests</td>
<td>Linear in year</td>
<td>722.5</td>
<td>ARMA(2,1) 716.97</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Quadratic in year</td>
<td>724.61</td>
<td>ARMA(2,1) 717.92</td>
</tr>
<tr>
<td></td>
<td>Proportion of positive tests</td>
<td>Linear in year</td>
<td>731.42</td>
<td>MA(1) 732.18</td>
</tr>
<tr>
<td>Myco</td>
<td>Number of tests</td>
<td>Linear in year</td>
<td>930.93</td>
<td>AR(1) 930.93</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Quadratic in year</td>
<td>928.49</td>
<td>AR(1) 928.49</td>
</tr>
<tr>
<td></td>
<td>Proportion of positive tests</td>
<td>Linear in year</td>
<td>738.52</td>
<td>ARMA(1,1) 736.57</td>
</tr>
</tbody>
</table>

July–August (winter), with a trough occurring in January–February (summer). Conversely, infections caused by Myco were most frequently observed in December and January (summer), and less frequently in August (winter), despite the peak of testing being in winter. Infections of Cp were most common in March and April (autumn).

The summer peak for Myco infections is supported by several studies. Research by Onozuka et al. demonstrated a significant increase in cases of Myco with increasing average temperature. Tian et al. also found a positive correlation between temperature and Myco infection rates, with the highest incidence recorded in summer and autumn. Tian et al. posited that because Myco grows optimally at 36–37°C, the summer and autumn months, which are the hottest months of the year, are the most conducive to bacterial growth. This is also consistent with the finding of del Valle-Mendoza et al. who found that Myco infections were most prevalent in the summer.

The number of tests for both Cp and Myco was highest from June to November (winter–spring), while the proportion of positive tests was highest in March (autumn) for Cp and in December (summer) for Myco, and lowest in August (late winter) for both Cp and Myco. This suggests a degree of overtesting in late winter, when the number of tests stays high but the proportion of positive tests tends to decrease at this time. Most other research, e.g. that of Du in Shenzhen, China, finds that winter is the season of peak prevalence of Cp infection; this may be because most studies only report positive cases. Even in the present study, the seasonality of positive cases (when considered in isolation) closely correlates with the testing patterns. The current research concentrates on testing rather than on the diagnosis of infection, thus our focus is on workforce planning at the testing end of the disease cycle. For example, if we focussed solely on the modelling results of positive cases, this would suggest increased allocation of resources for the entire period from June to November when testing is at its highest (and, as expected, so are the number of positive infections). By analysing the number of positive cases in proportion to testing we get less clear but different seasonal patterns, with a peak for Myco in December and January and a peak for Cp in March. This can help target resource allocation and identify the underlying mechanisms of seasonality, which are less likely to be an artefact of increased testing. The high proportion of positive Myco infections in summer may be due to behavioural factors or climatic factors such as temperature as shown by Chen et al.

In this study, for both Cp and Myco infections, we show differential seasonal patterns of testing and of the proportion of positive tests, findings which have important implications for public health control of these infections. High testing in certain seasons may dilute the positive rate, since the numerator (actual positive infection patients) is unchanged but the denominator (patients tested for infection) increases. This could cause an incorrect estimation of the actual
peak of infection because the total number of tests will dilute the positive proportion. Often, testing data for infections is not available, with recording only of the number of confirmed positive cases. Consequently, the public health response to the actual peak of infection may be misdirected due to a testing artefact rather than an actual increase in infection. This could potentially lead to misallocation of health responses to controlling the peak of infection, as well as a focus on the wrong potential drivers of the infection.

The proportion of positive Cp and Myco infections may be affected by many other factors not accounted for in this study, for example age and gender. In addition, the analysis was conducted only for patients who were tested; accordingly, not all cases in the community were represented, so there are no estimates of actual community burden. As individuals with Myco infections typically experience mild disease symptoms or no symptoms at all, many may not seek health care, leading to under-ascertainment. However, consistent with Onozuka et al.,9 under-ascertainment is unlikely

<table>
<thead>
<tr>
<th>Model</th>
<th>Number of Cp tests</th>
<th>Proportion of positive Cp tests</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Value</td>
<td>Value</td>
</tr>
<tr>
<td></td>
<td>716.67</td>
<td>731.42</td>
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<table>
<thead>
<tr>
<th>Coefficient</th>
<th>Value</th>
<th>SE(^a)</th>
<th>p</th>
<th>Value</th>
<th>SE(^a)</th>
<th>p</th>
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<tr>
<td>AR(1)</td>
<td>0.44</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AR(2)</td>
<td>0.32</td>
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</tr>
<tr>
<td>MA(1)</td>
<td>-0.03</td>
<td></td>
<td></td>
<td>0.17</td>
<td>0.10</td>
<td>0.0891</td>
</tr>
<tr>
<td>MA(2)</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>-11.78</td>
<td>19.04</td>
<td>0.5376</td>
<td>81.41</td>
<td>7.23</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Year(^c)</td>
<td>2.76</td>
<td>1.42</td>
<td>0.0549</td>
<td>-2.72</td>
<td>0.56</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Yearsq(^d)</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>February</td>
<td>-0.96</td>
<td>3.69</td>
<td>0.7961</td>
<td>-0.2</td>
<td>4.66</td>
<td>0.9657</td>
</tr>
<tr>
<td>March</td>
<td>5.94</td>
<td>3.79</td>
<td>0.1199</td>
<td>10.37</td>
<td>5.13</td>
<td>0.0462</td>
</tr>
<tr>
<td>April</td>
<td>6.22</td>
<td>4.46</td>
<td>0.1663</td>
<td>10.23</td>
<td>5.38</td>
<td>0.0606</td>
</tr>
<tr>
<td>May</td>
<td>7.21</td>
<td>4.65</td>
<td>0.1253</td>
<td>1.86</td>
<td>5.41</td>
<td>0.7300</td>
</tr>
<tr>
<td>June</td>
<td>13.50</td>
<td>4.84</td>
<td>0.0065</td>
<td>-4.75</td>
<td>5.42</td>
<td>0.3827</td>
</tr>
<tr>
<td>July</td>
<td>18.77</td>
<td>4.89</td>
<td>0.0002</td>
<td>1.55</td>
<td>5.42</td>
<td>0.7756</td>
</tr>
<tr>
<td>August</td>
<td>20.17</td>
<td>4.87</td>
<td>0.0001</td>
<td>-6.57</td>
<td>5.42</td>
<td>0.2285</td>
</tr>
<tr>
<td>September</td>
<td>21.65</td>
<td>4.74</td>
<td>&lt;0.0001</td>
<td>-0.69</td>
<td>5.41</td>
<td>0.8991</td>
</tr>
<tr>
<td>October</td>
<td>17.72</td>
<td>4.51</td>
<td>0.0002</td>
<td>-3.77</td>
<td>5.39</td>
<td>0.4862</td>
</tr>
<tr>
<td>November</td>
<td>8.83</td>
<td>4.01</td>
<td>0.0304</td>
<td>-1.36</td>
<td>5.16</td>
<td>0.7931</td>
</tr>
<tr>
<td>December</td>
<td>5.14</td>
<td>3.95</td>
<td>0.1961</td>
<td>3.00</td>
<td>4.70</td>
<td>0.5253</td>
</tr>
</tbody>
</table>

\(^a\) SE: standard error.
\(^b\) Not able to be calculated due to non-positive definite covariance matrix.
\(^c\) Year: years since 1990.
\(^d\) Yearsq: years since 1990 squared.
We provide a population-level view of how testing for respiratory bacterial pathogens has changed across seasons and over time in the ACT. Future studies into the seasonal burden of respiratory infections will need to consider seasonal and other temporal changes in testing patterns.

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Conflict of interest

The authors declare no conflict of interest.

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References


Appendix A: Supplemental tables and figures

1. Time series plots of number of positive tests

Figure A.1: Time series plot and additive decomposition observed time series line (grey) with trend and seasonal line (red)

A  Monthly number of positive Cp tests

B  Monthly number of positive Myco tests
2. Time series regression

Table A.1: Model results for number of positive tests: AR(1) error structure

<table>
<thead>
<tr>
<th>Model</th>
<th>Number of positive Cp tests</th>
<th>Number of positive Myco tests</th>
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*Year = years since 1990.
2.1.1. Total number of Cp tests

Figure A.2: ACF and PACF of normalised residuals from trend + seasonal ARMA(2,1) model of number of Cp tests

2.1.2. Proportion of positive Cp tests

Figure A.3: ACF and PACF of normalised residuals from trend + seasonal AR(1) model of proportion of positive Cp tests

2.1.3. Number of positive Cp tests

Figure A.4: ACF and PACF of normalised residuals from trend + seasonal AR(1) model of number of positive Cp tests
2.2.1. Total number of Myco tests

Figure A.5: ACF and PACF of normalised residuals from quadratic trend + seasonal AR(1) model of number of Myco tests

2.2.2. Proportion of positive Myco tests

Figure A.6: ACF and PACF of normalised residuals from trend + seasonal ARMA(1,1) model of proportion of positive Myco tests

2.2.3 Number of positive Myco tests.

Figure A.7: ACF and PACF of normalised residuals from trend + seasonal AR(1) model of number of positive Myco tests