Ross River virus infection surveillance in the Greater Perth Metropolitan area – has there been an increase in cases in the winter months?

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Abstract

An increase in off-season (June to September) Ross River virus (RRV) notifications from the greater Perth metropolitan area was observed from 2006 to 2009. We investigated the increase to determine whether it is likely to have reflected a true increase in off-season cases. A single positive RRV IgM test result is sufficient for RRV notification but where follow-up testing was performed, the positive predictive value of an IgM test where IgG was negative was very low in the off-season and also in the season when using the only commercially available test kit. The increase in off-season notifications was not associated with an increase in off-season testing. Some Perth laboratories use more stringent notification criteria than the nationally agreed RRV case definition, and the geographical distribution of samples tested varies between laboratories. Our findings make a strong case to change the nationally agreed case definition for RRV to not accept a single IgM positive test result as laboratory definitive evidence where the IgG is negative. Our study also identified a range of challenges in interpreting changes in seasonal patterns and geographical distribution of RRV. Any such observed changes should be investigated through further data analysis and/or mosquito trapping and testing in order to assess validity. *Commun Dis Intell* 2014;38(2):E115–E122.

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Introduction

Ross River virus (RRV) is a mosquito-borne alphavirus infection that occurs throughout Australia and the South Pacific.[1,2] It causes an acute illness characterised by joint pain, arthritis, fatigue and malaise, often accompanied by fever and a rash. While not life-threatening, RRV can cause significant morbidity, with symptoms lasting up to 6 months.[1–3]

RRV is a nationally notifiable disease. Laboratories and treating doctors are required to report to the Western Australian Department of Health (WA Health), all cases of RRV infection meeting the nationally agreed case definition.[4] In Western Australia, notifications are recorded in the Western Australian Notifiable Infectious Diseases Database (WANIDD). The serological component of the case definition is: RRV-IgG seroconversion or a fourfold rise in RRV-IgG between acute and convalescent samples; detection of RRV IgG and IgM in a single sample; or detection of RRV-IgM without IgG when there is no detectable IgM to Barmah Forest virus (BFV).

From 2006 onwards, all laboratories in Western Australia were legislatively required to directly notify cases. Prior to that date, notifiable diseases were notified by doctors based on clinical and/or laboratory diagnoses, and in the early 2000s by the only public sector testing laboratory (PathWest Laboratory Medicine WA) and some private laboratories.

Local governments and the Environmental Health Directorate of WA Health undertake enhanced surveillance of RRV cases notified by treating doctors, to identify the most likely place of exposure and date of onset of symptoms. This information is recorded in the Mosquito Borne Disease Control (MBDC) database.

In the south-west of Western Australia, RRV typically causes outbreaks of varying sizes between October and May. In 2006 it was observed that the number of RRV notifications reported during the off-season (June to September) were higher than expected in the Perth Metropolitan region and the Peel region immediately south of Perth (Figure 1).

In Western Australia, RRV notifications are used to identify areas of high RRV activity to enable additional mosquito control activity and public warnings. Notification data are complemented by mosquito surveillance in areas of historically high activity over summer. A change in the distribution of RRV cases to include the off-season in the south-west is potentially very important as it could flag changes in the ecology of vector mosquitoes and animal hosts. This has implications for human health and for risk mitigation activities such as mosquito control.

This study examined the notification data and laboratory testing data, assessing whether the notifications reflected a real increase in off-season RRV cases or an artefact of testing and/or notification practices.

Methods

This was a descriptive study.

Notification rates

RRV notifications for the Perth Metropolitan and Peel regions by laboratory, month and year of onset from 1 January 1990 to 30 June 2012 were retrieved from WANIDD. Population data at Statistical Local Area level were obtained from the Australian Bureau of Statistics and aggregated into MBDC regions. Notification rates per 100,000 population were calculated by dividing the number of notifications in that time period by the estimated population for that year and multiplying by 100,000, and were annualised by multiplying the rate by 12 divided by the number of months.

Enhanced surveillance data

Notification data by month and year of onset and region of acquisition as documented by enhanced surveillance were retrieved from the MBDC database for the years 2006 to 2009 inclusive. The enhanced surveillance data were compared with the notification data to assess whether having further information about the onset date and likely place of acquisition of infection changed the proportion of off-season notifications in the Perth Metropolitan and Peel regions.

Laboratory testing

Laboratory testing data for the Perth Metropolitan and Peel regions for the period from 1 January 2002 to 31 August 2012 were obtained from PathWest Laboratory Medicine WA (PathWest) and St John of God Pathology (SJGP). PathWest is the state reference laboratory. It uses an in-house immunofluorescence assay (IFA) for the detection of IgM antibodies,[5] and an in-house haemagglutination inhibition (HI) test.[6] The latter detects both IgG and IgM together and is less sensitive than the IFA for detection of IgM.[6] The in-house assays have been validated according to guidelines published by the National Pathology Accreditation Advisory Council, and have been approved for diagnostic use by the National Association of Testing Authorities. The sensitivity and specificity of these tests is not known. Both tests are routinely performed on samples referred for RRV diagnosis. All but one laboratory (which refers their samples to PathWest), including SJGP, use a commercial enzyme immunoassay (EIA); Panbio® RRV IgM ELISA and Panbio® RRV IgG ELISA (Alere, Sinnamon Park, Queensland Australia). The sensitivity and specificity of these tests was estimated in South Australia by testing samples taken in 1996 and 1997 using an HI test as the comparator. The sensitivity of the PanBio ELISA kits was estimated to be 98.5% and 84.6% and the specificity 96.5% and 97.6% for IgM and IgG respectively.[7] These are the only commercial tests currently available in Australia. In response to a doctor's request for RRV serology, all private laboratories test separately for IgM and IgG antibodies.

PathWest routinely requests second samples where the sample is IgM positive within 2 weeks of onset of illness independent of the HI test result. This allows detection of seroconversion or a significant rise in IgG using the HI tests to confirm acute infection. SJGP requests second samples where the initial IgM is positive but the IgG is negative in order to test for seroconversion. The EIA tests do not quantify results and cannot be routinely used to detect rises in IgG, but will detect seroconversion.

A more detailed analysis of positive IgM results was carried out for patients from the Perth Metropolitan and Peel regions. Patients who had results for more than 1 sample were identified and the results were used to assess the interpretation of the first IgM positive sample for each patient. (Table 1).

Notification practices of the laboratories

The three private laboratories (SJGP and Laboratory B and C) and PathWest who, combined, notify the majority of RRV cases, were contacted to ask about their notification practices for RRV.

Table 1: Classification of Ross River virus IgM positive test results following follow-up testing

Classification	PathWest	Private
True positive	Seroconversion from negative IgG (HI <40) to positive OR HI ≥40 and a fourfold or greater rise in HI titre between acute and convalescent samples OR HI ≥40 on first and second samples	IgM with IgG seroconversion OR IgM and IgG positive on initial and a later sample
False positive	HI ≤40 on second sample seven or more days after the first test OR IgM becomes negative within 6 months of the first test OR patient is known to have past Ross River virus infection	IgG negative on repeat testing seven or more days after the first positive IgM test OR IgM becomes negative within 6 months of the first test

The positive predictive value of the test was calculated by the formula: true positive/(true positive + false positive).

Data analysis

Data were analysed for Perth Metropolitan and Peel regions. Comparisons between means were performed using Independent t tests. Analysis was undertaken using Microsoft Excel and SPSS version 21 software.

Results

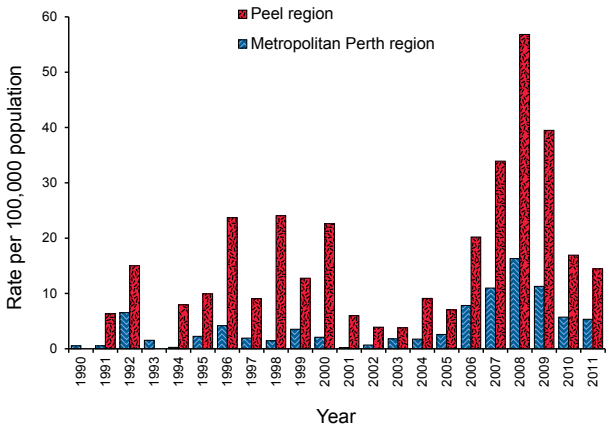
Notification rates

There were 6,337 RRV notifications from Perth Metropolitan and Peel regions from 1 January 1990 to 30 June 2012. The population in the Perth Metropolitan region in 2012 was 1.6 million people and in the Peel region was 236,000. The notification rates in the off-season in Perth Metropolitan and Peel regions were higher in the years between 2006 and 2009 compared to the other years (mean of 11.6 vs 2.4 for the Perth Metropolitan region; 37.6 vs 10.6 for the Peel region ($P<0.05$)), but there was no significant difference in the notification rates during the season for the 2006 to 2009 period compared with the other years (mean of 23.4 vs 18.3 for the Perth Metropolitan region; 88.7 vs 59.6 for the Peel region ($P>0.05$)) (Figure 1 and Figure 2).

Enhanced surveillance

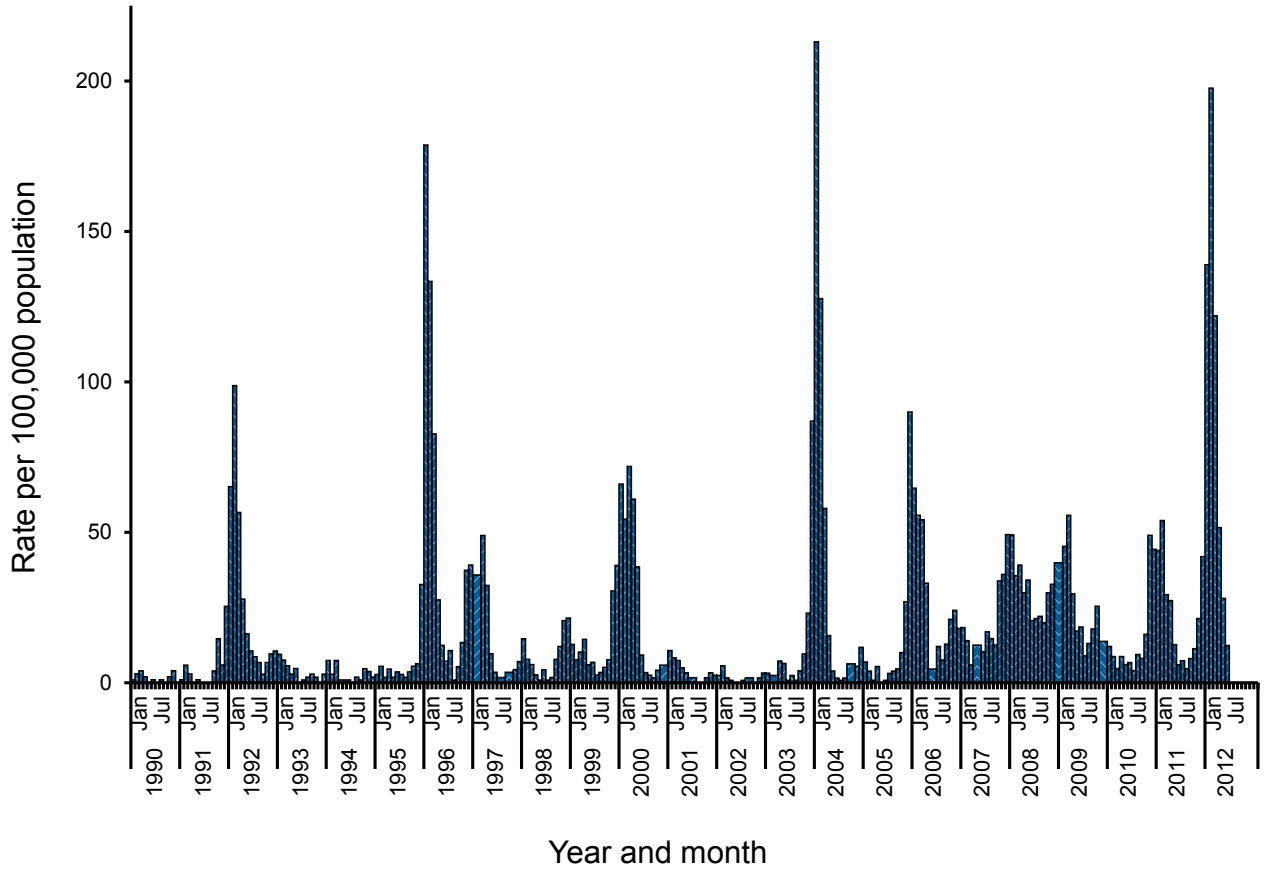
From 2006 to 2009 inclusive, 322 cases of RRV with onset in the off-season in the two regions, were notified on WANIDD. Of these, 67 (20.8%) had enhanced surveillance from which it could be ascertained that 44/67 cases (65.7%) occurred

Figure 2: Notification rates for off-season Ross River virus infections,* Perth Metropolitan and Peel regions, by onset year



* Ross River virus infection notifications occurring in the months of June to September inclusive.

Figure 1: Notification rate Ross River, Perth Metropolitan and Peel regions, by month and year



in the Perth Metropolitan or Peel region in June to September, whilst 23 were reclassified as either seasonal exposure (10), non-Perth Metropolitan/Peel exposure (9) or both (4). Of the 1,416 RRV cases that were notified on WANIDD as seasonal in the two regions from 2006–2009, 455 (32%) had enhanced surveillance. Seventeen (3.7%) of these were reclassified as off-season using enhanced surveillance data; and 5 cases originally classified as non-metropolitan were determined by enhanced surveillance to be in Perth Metropolitan or Peel.

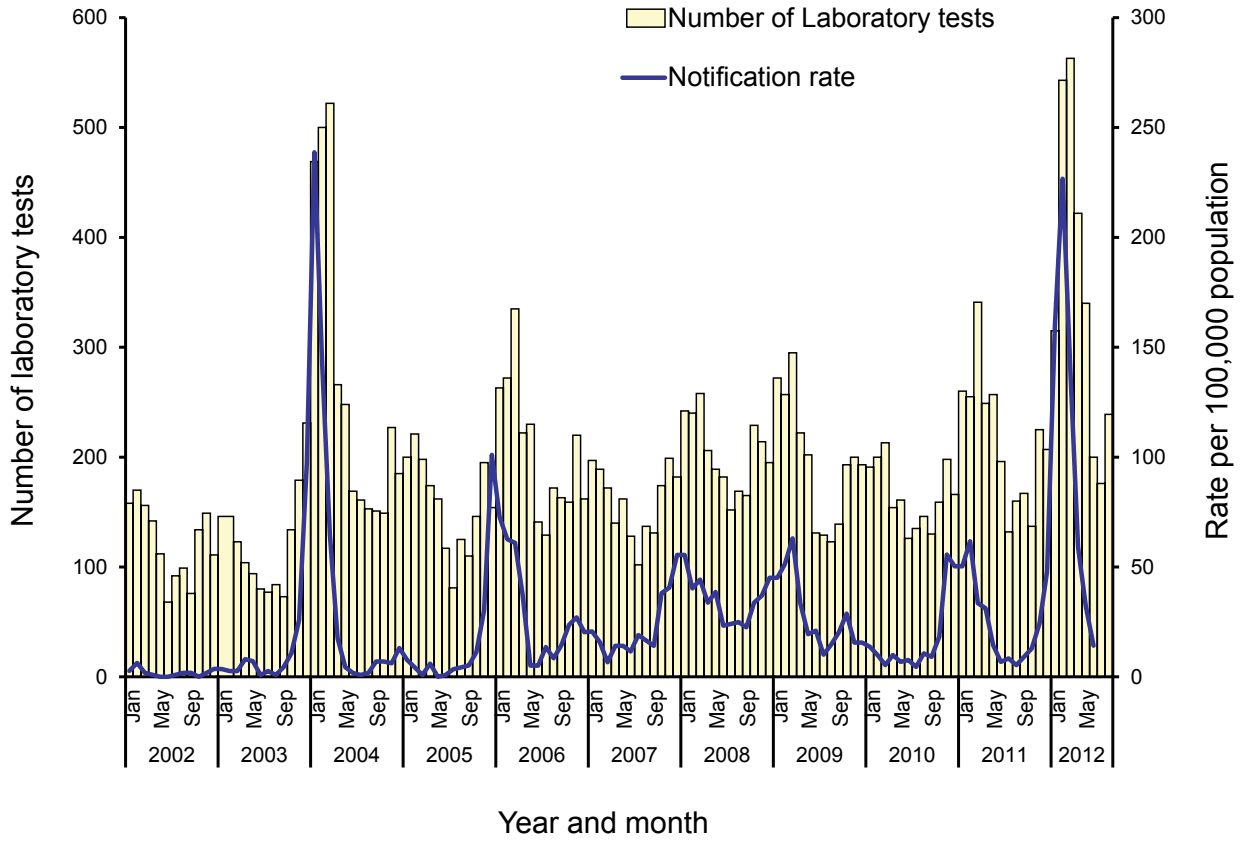
Laboratory testing

Between 1 January 2002 and 30 June 2012, PathWest notified 7.6% of all RRV notifications in the Perth Metropolitan and 14.2% from the Peel region, while 19.7% and 4.3% respectively came from SJGP. While peak periods of testing occurred during the season, testing continued at high numbers during the off-season (Figure 3). A similar pattern of testing occurred for Peel (data not shown). The mean number of off-season RRV tests did not differ significantly between the years of higher off-season notifications (2006–2009) compared with the remainder (573.3 per year vs 484.6 per year, $P=0.25$). Off-season testing increased from 2004 onwards compared with 2002 and 2003 (mean 569.0 per year vs 324.5 per year, $P<0.001$).

In the two regions of interest, the proportion of IgM tests that were positive showed some seasonal variation with peaks in the summer months, while a higher proportion of tests were positive in the off-season from 2007 to 2009, particularly from SJGP (Figure 4). During the off-seasons from 2002 to 2011, the proportion of positive tests from SJGP ranged from 0.4% in 2002 to 15% in 2009. For PathWest the proportion of positive test results ranged from 0% in 2003 to 6% in 2007.

Between 1 January 2002 and 31 August 2012, 8,428 RRV IFA IgM tests were performed at PathWest from the two regions; 1,979 during the off-season and 6,449 during the season. Of these, 142 (7.2%) were positive during the off-season and 1,044 (16.1%) during the season. Sixty-six patients tested during the off-season and 563 tested during the season had more than 1 sample at intervals suitable for patient classification and IgM assessment (Table 2).

Figure 3: Total number of IgM tests performed by PathWest and St John of God Pathology and notification rate for Ross River virus infection, Metropolitan Perth, January 2002 to June 2012, by month and year of specimen collection date



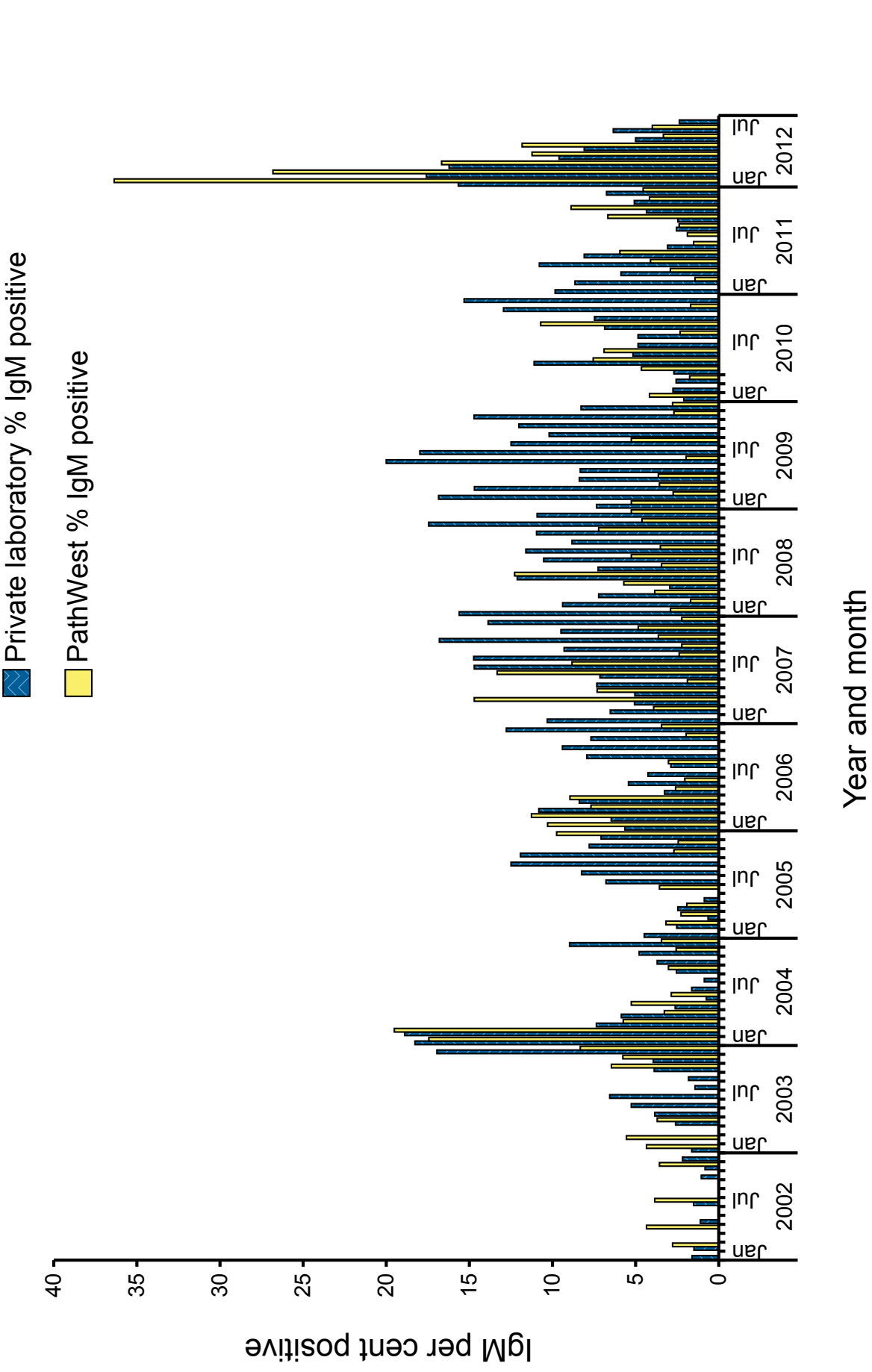


Figure 4: Per cent of Ross River virus IgM tests that were positive, Perth Metropolitan region, January 2002 to June 2012, by month, year of specimen collection date and laboratory

During the off-season the positive predictive value (PPV) for an IgM positive, HI<40 result was 39.3%, and for an IgM positive, HI≥40 result the PPV was 84.2%. During the season the PPV for IgM positive, HI<40 result was 81.1%, and for an IgM positive, HI≥40 result the PPV was 92.7%.

During the off-season, 25 (17.4%) of the 142 PathWest RRV test results meeting the case definition for notification were IgM positive, HI<40. During the season, 177 (17.0 %) of the 1044 PathWest RRV test results meeting the case definition for notification were IgM positive, HI<40.

Between 1 January 2002 and 31 of August 2012, 19,177 RRV EIA IgM tests were performed at SGJP for the two regions; 4,569 during the off-season and 14,608 during the season. Of these, 197 (4.3%) were positive during the off-season and 1,062 (7.3%) during the season. Eighty-one patients tested during the off-season and 366 tested during the season had more than 1 sample at intervals suitable for patient classification and IgM assessment (Table 3).

During the off-season the PPV for an IgM positive, IgG negative result was 4.0%, and 60.0% for an IgM positive, IgG positive result. During the season the PPV for IgM positive, IgG negative result was 24.6%, and 60.7% for an IgM positive, IgG positive result the PPV.

During the off-season, 150 (76.1%) of the 197 SJGP RRV test results meeting the case definition for notification were IgM positive, IgG negative. During the season, 689 (64.9%) of the 1,062 SJGP test results meeting the case definition for notification were IgM positive, IgG negative.

Of the patients from PathWest classified as having genuine IgM, 54.4% had serological evidence of acute infection (either seroconversion or rising HI titres), the remaining having stable HI titres.

Notification practices of the laboratories

SJGP and PathWest contributed data to the study while the others did not, but all pathology laboratories doing their own RRV testing provided information about their notification practices.

PathWest notifies all IgM positive test results. For the period 2006 to 2009, PathWest notified 16.6% of notifications from Perth Metropolitan and Peel regions in the off-season and 30.3% of the notifications for the remaining months.

All private laboratories in Western Australia use the commercial EIA assay, but notification practices varied.

SJGP does not always notify IgM positive test results amongst individuals with known autoimmune disease or viral infections known to cause false positive test results (around one-third of positive IgM results). The approach was variously applied over time. For the period 2006 to 2009, SJGP notified 18.2% of the notifications from the Perth Metropolitan and Peel regions in the off-season and 14.5% of the notifications for the remaining months.

Table 2. Classification of positive IgM tests using immunofluorescence assay and haemagglutination inhibition tests where follow-up testing was performed, 1 January 2002 to 31 August 2012

	Season		Off-season	
Initial test result	IgM positive, HI<40	IgM positive, HI≥40	IgM positive, HI<40	IgM positive, HI≥40
True positive	202	291	11	32
False positive	47	23	17	6
Total	249	314	28	38

Table 3: Classification of positive IgM enzyme immunoassay tests where follow-up testing was performed, 2002 to 31 August 2012

	Season		Off-season	
Initial test result	IgM positive, IgG negative	IgM positive, IgG positive	IgM positive, IgG negative	IgM positive, IgG negative
True positive	82	17	3	3
False positive	252	11	71	2
Total	334	28	74	5

Private laboratory B notifies positive IgM test results only when there is a positive IgG test result either at the time of the initial IgM test or upon seroconversion. For the period 2006 to 2009, this laboratory notified 10.1% of notifications from the Perth Metropolitan and Peel regions in the off-season and 11.2% of notifications for the remaining months.

Private laboratory C notifies all positive IgM test results. For the period 2006 to 2009, this laboratory notified 40.8% of notifications from the Perth Metropolitan and Peel regions in the off-season and 36% of notifications for the remaining months.

Discussion

While we were unable to explain the increase in off-season notifications during 2006 to 2009, we identified a number of challenges in interpreting RRV notification data, particularly in the off-season.

We found that where follow-up tests were performed on patients with their first positive IgM during the off-season, the PPV of an IgM positive test in the absence of IgG was very low regardless of the IgM test used. During the season, the PPV value for the IFA alone rose to 81.1%, while for the EIA it remained low.

Patients who had follow-up serology within the acceptable timeframe may not represent all patients undergoing testing. Furthermore, some patients may have been incorrectly classified, such as those with delayed seroconversion. Despite these limitations, it is clear that detection of IgM in the absence of IgG using the commercial EIA test should be interpreted with caution as there is a high chance that it is a false positive. Similarly the IFA-IgM test alone cannot be reliably used to indicate acute infection during the off-season.

If the HI titre on the initial test is ≥40 the PPV for the IFA/HI is over 80% regardless of the season. For the commercial EIA the PPV of an IgM and IgG positive test was around 60% regardless of the season, which may be acceptable for surveillance purposes, but should be interpreted cautiously for patient diagnosis.

Our findings are consistent with a study that found that 45% of patients with RRV IgM but not IgG failed to seroconvert on follow-up testing.[8] A similar problem has been described with the EIA for IgM to the closely related BFV from the same manufacturer.[9]

Based on these findings, there is a very strong case to remove from the RRV case definition the possibility of laboratory confirmation for RRV IgM positive/ IgG negative test results. This would substantially reduce the number of notifications from the private laboratories; at least for those who notify according to the agreed case definition; but very few genuine positives will be lost. Removing the IFA-IgM only positives detected during the season would remove up to 14% of the genuine cases from PathWest.

RRV IgM antibodies usually appear within a few days of illness onset, so the presence of IgM antibody in sera is considered to be indicative of acute or recent infection.[6] However, in most cases of RRV infection, IgM antibodies remain in sera for 1 year at least.[6,10] Therefore even if IgM is present it may not mean infection or reinfection occurred in the season of sampling, unless it is accompanied by seroconversion or a rise in IgG titre. The latter cannot be easily determined using the EIA tests. Patients with RRV may not present in the acute phase, but rather some time later when their symptoms persist, which may be during the off-season. If the date of symptom onset is not available, as in the majority of cases, the specimen date will be interpreted as the onset date and the cases misclassified as off-season cases.

We also identified variations in reporting practice for single IgM positive results between laboratories and at different times. Some laboratories don't notify these cases, despite them meeting the RRV case definition. Therefore the PPV of notifications from different laboratories differs. This is not problematic if there is consistency in notification practices over time, and if there is no geographical variation in coverage by the different laboratories. However, there are differences in the regional coverage of the laboratories, making the comparison of notification rates between different regions difficult.

Testing data from the two laboratories did not reveal increased RRV testing in the off-season in the years 2006 to 2009. The higher rate of positive results during this time may reflect an actual increase in RRV incidence, but this would usually be accompanied by increased testing. From these data, there is no clear explanation for the increase in off-season notifications in those years.

RRV symptoms can last for up to 6 months.[6] Therefore people with persisting symptoms may be tested after the acute phase. Without enhanced surveillance, a notification of a positive RRV test could be wrongly classified as occurring in the off-season as pathology laboratories do not routinely provide the date of symptom onset. Where enhanced surveillance was undertaken, there were notifications that were thus misclassified in both

the off-season and during the season. This is likely to be due to the long duration of symptoms and the fact that laboratory notifications report the residential address of the case rather than their place of acquisition. Based on our data, if enhanced surveillance was uniformly performed for off-season and seasonal RRV notifications, more RRV notifications in the off-season would be reclassified as seasonal than vice versa.

The change in legislation in Western Australia requiring laboratory notification for notifiable diseases that was introduced in 2006 may explain the observed increase in winter cases from 2006 to 2009. However, as this increase did not continue between 2010 and 2012 it is difficult to make this interpretation until a few more years of notification data become available.

We identified a number of challenges in interpreting RRV notification data. Much of this appears to be due to the low PPV of the RRV IgM test that is used as the basis for most notifications. At peak risk times and in higher risk geographical areas, the PPV of the IgM test results is likely to be sufficiently high to enable identification of the timing and distribution of RRV activity, particularly if the IgM is accompanied by RRV-IgG. Furthermore, the long-term persistence of IgM is likely to be contributing to the over-notification of apparent off-season cases where patients were actually infected during the preceding season. Therefore only large changes in disease notification rates are likely to be able to be interpreted reliably based in IgM results alone.

Finally, enhanced surveillance should remain an important tool for the accurate understanding of RRV epidemiology, particularly when notification data suggests a change in the distribution of RRV, either in time or in place, or when there are unexpected increases in notifications.

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