Barmah Forest virus serology; implications for diagnosis and public health action

Patrick Cashman, Linda Hueston, David Durrheim, Peter Massey, Stephen Doggett, Richard C Russell

Abstract

Barmah Forest virus (BFV) is a commonly occurring arbovirus in Australia. Notifications of Barmah Forest infections diagnosed by a single positive IgM serology test have been increasing in coastal New South Wales north of Newcastle. We report on a 6 month prospective review of all routine notifications of BFV from the Lower Mid North Coast of New South Wales. Sera from 37 consecutive cases were sent for confirmatory testing by ELISA and neutralisation assays and 32 cases were interviewed. On confirmatory testing, 7 patients' sera (19%) was found to contain no BFV antibodies and 6 (16%) had BFV IgG only. Only 4 cases had antibody levels compatible with recent infection. A clinical presentation of fever with either rash or joint pain was associated with confirmation of recent BFV infection. On the basis of these findings, caution is advised in the interpretation of a single positive IgM for Barmah Forest disease and the clinical picture is an important factor in the diagnosis. Serological notifications of BFV alone should not prompt public health action such as public warning and targeted vector control in endemic areas. *Commun Dis Intell* 2008;32:263–266.

Keywords: Barmah Forest, arbovirus, serology, false positive

Introduction

Barmah Forest virus (BFV) isan alphavirus transmitted by mosquitoes that was first isolated in Australia in 1974.[1] Human infections have since been reported from all states and territories.[2] Symptoms of BFV infection include acute onset of fever, arthralgia and a florid maculopapular erythematous rash, after an incubation period of 5–21 days.[3–5] The disease has a seasonal occurrence with the highest incidence during the summer and autumn for most of Australia except south-east Western Australia where peak incidence is in spring.[6] There is no specific treatment available and so management is primarily directed at alleviation of symptoms.

The arthralgia, myalgia and accompanying lethargy, may be incapacitating. As 10% of cases remain symptomatic for more than 6 months, there would be attendant economic consequences yet to be quantified.[7]

BFV is most commonly transmitted in coastal New South Wales by the salt-marsh mosquito, *Aedes* (*Ochlerotatus*) *vigilax*.[8] As many breeding sites are in environmentally sensitive locations and occur over extensive geographical areas, source reduction is often not acceptable or practical, and opportunities for vector control are usually limited. This species' extensive flight range, which often exceeds 5 kilometres, also complicates vector control. The urban mosquito, *Aedes (Finlaya) notoscriptus,* which can use domestic containers for breeding, has also been implicated in transmission. *Aedes* (*Ochlerotatus*) *procax*, a freshwater species that breeds in forested ground-pools, has produced many isolates of BFV over recent years from the New South Wales Arbovirus Surveillance and Mosquito Monitoring Program and may also have a significant role in BFV transmission.[9]

The natural reservoir of BFV is yet to be determined. Circumstantial evidence suggests a more mobile (perhaps avian) host since epidemics spread rapidly, genetic analysis has found that isolates are homogeneous (a feature typical of bird-associated viruses), and studies on placental and marsupial mammals have shown these groups to be incompetent reservoirs.[10–13]

Of the nearly 2,500 BFV cases notified in the past 11 years in New South Wales, 80% occurred in the coastal region north of Newcastle.[14] BFV appears to be of increasing public health importance in this area, which is experiencing rapid population growth. In the first decade of BFV notification in New South Wales, outbreak years were followed by 2–3 seasons of low activity.[15] In the past 5 seasons, however, there have been sustained higher notification rates without a return to the previous baseline.[14]

BFV cases are notifiable in New South Wales under the *NSW Public Health Act, 1991*. A confirmed case requires only laboratory evidence either by:

- isolation of BFV; or
- detection of BF virus by nucleic acid testing; or
- IgG seroconversion or a significant increase in antibody level or a fourfold or greater rise in titre to BFV; or
- detection of BFV- specific IgM.[16]

As a precursor to a possible case control study to explore the effectiveness of personal protective measures, we embarked on a 6 month prospective review of all routine notifications of BFV from the Lower Mid-North Coast (LMNC) of New South Wales. This region north of Newcastle encompasses 3 Local Government Areas roughly defined from Hawkes Nest in the South to Taree in the North along the coast and inland to the mountains west of Gloucester. The Mid-North Coast area of New South Wales has had the highest rate of BFV infection in Australia (67.5 cases per 100,000 population in 2005).[17] The purpose of the review was to investigate how many notified BFV cases during this 6 month period were actually recent infections.

Methods

All notifications of BFV in the LMNC area of New South Wales between 1 January and 30 June 2007 were investigated to describe the clinical presentation and confirm laboratory diagnosis.

Cases were interviewed by structured questionnaire for clinical presentation, particularly for onset date, prior medical history, exposure details and clinical features at presentation; specifically fever, rash, lethargy, arthralgia and myalgia.

Sera from the notifying laboratories were forwarded to the Centre for Infectious Diseases and Microbiology, Institute of Clinical Pathology and Medical Research (CIDMLS-ICPMR), Westmead Hospital for confirmatory testing as follows:

- BFV IgG (in-house indirect IgG ELISA standardised against neutralisation) sensitivity 98%, specificity 97.8% (personal communication, Linda Hueston, CIDMLS-ICPMR)
- BFV IgM (in-house antibody class capture ELISA standardised against neutralisation) sensitivity 97.9%, specificity 98.9% (personal communication, Linda Hueston, CIDMLS-ICPMR)
- neutralisation (micro-neutralisation using a 90% end-point).

Neutralisation titres of greater than or equal to 640 on single serum samples were considered diagnostic of recent infection. A seroconversion or fourfold or greater rise in neutralising antibody titre between acute and convalescent sera were considered to be diagnostic of recent infection.

Results

There were 37 BFV notifications from 1 January to 30 June 2007 for the LMNC. Five patients could not be contacted despite repeated attempts, and therefore 32 people were interviewed. All 37 cases were notified by laboratories following routine diagnosis as BFV IgM positive using the PanBio kit. Sera from all 37 BFV cases was sent for confirmatory testing.

On confirmatory testing, 7 patient's sera (19%) was found to contain no BFV antibodies and 6 (16%) had BFV IgG only (which was confirmed by neutralisation). The remaining notifications had both IgM and IgG antibodies (confirmed by neutralisation) but only 4 had antibody levels/titres compatible with recent infection. The antibody level/titre in 20 cases indicated prior infection.

Of the 32 patients who were interviewed, all were symptomatic with: 13/32 (41%) having fever, 5/32 (16%) having rash, 24/32 (75%) reporting fatigue, 16/32 (50%) arthralgia and 10/31 (32%) myalgia on initial presentation.

Self-reported fever was the only symptom statistically significantly associated with laboratory evidence of recent BFV infections (Table). All 4 cases that were laboratory-compatible with recent infection had fever and either rash or arthralgia at presentation. Of the 24 cases who presented with fatigue, only 3 were confirmed to have had recent BFV infection (Table).

Table. Clinical presentation and likelihood of confirmed recent Barmah Forest infection in this cohort of recent Barmah Forest virus notifications, Lower Mid North Coast, 1 January to 30 June 2007

Symptoms reported	ICPMR laboratory confirmed cases of recent BFV infection (n=4)		Other cases notified as BFV infection on initial laboratory result (n=28)		p-value (Two-tailed Fisher's Exact)
	n	%	n	%	
Fever	4	100	10	36	0.028
Rash	2	50	3	11	0.105
Fatigue	3	75	21	75	1.000
Arthralgia	2	50	14	50	1.000
Myalgia	0	0	10 (n=27)	37 (n=27)	0.277

Discussion

The high false positive rate of 19% (7/37) in this series of notifications would suggest that caution is advisable in interpreting a single positive BFV IgM result as diagnostic of recent BFV infection.

Our findings suggest that a clinical picture of fever with either rash or joint pain would be of assistance in the application of BFV serology in areas where BFV is endemic. Testing on the basis of a single symptom of fatigue would seem to be less reliable as a clinical criteria of recent infection. However it is important to distinguish between the typical macular rash of BFV disease and rashes with fever that could herald more ominous diagnoses, including measles and invasive meningococcal disease.

Amongst this small cohort of patients, a relatively large proportion had evidence of previous BFV infection, and thus clinicians need to be cautious in ascribing the 'classic' symptoms to recent BFV infection in an endemic area on the basis of a single positive test in the absence of fever.

The majority of BFV infections are subclinical or inapparent.[8,18] As BFV virus appears to be endemic in the LMNC, testing needs to be guided by the appropriate group of symptoms for BFV, as false positive results could lead to misdiagnosis and other necessary clinical investigations erroneously omitted.

Laboratory reporting of results may be aided by the treating clinician providing details of onset date and symptoms. Optical density readings, cut-off ratios or titres may assist clinicians and public health authorities in determining whether an infection is recent.

Obvious limitations of currently available commercial arboviral tests must be borne in mind when interpreting the results of epidemiological studies and understanding the distribution and trends of BFV notifications.[19] Sero-surveillance has the potential for directing and catalysing public health action, which could include public warning and targeted vector control in affected areas.[20] However, these LMNC findings make it advisable to conduct confirmatory testing before committing resources for responding to suspected incursions or increases in BFV disease.

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