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Regional overview of bluetongue viruses in South-East Asia: viruses, vectors and surveillance

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Summary

Structured epidemiological studies based on sentinel herds in Indonesia and Malaysia have provided much information regarding the bluetongue (BT) viruses (BTV) and their likely vectors in South-East Asia. Serotypes 1, 2, 3, 7, 9, 12, 16, 21 and 23 have been isolated. Molecular analyses show all group within the Australasian topotype, with four genotypic sub-groupings identified to date. There are relationships to isolates from both India and Australia. Strains of BTV in South-East Asia do not appear to be highly virulent, since BT disease is not seen in local sheep. Known vector species identified include *Culicoides fulvus*, *C. actoni*, *C. wadai* and *C. brevitarsis*. *C. imicola* has not been identified in Malaysian or Indonesian studies. Molecular analyses indicate movement of South-East Asian strains of BTV into northern Australia, and the gradation in observations between India and eastern Australia regarding serotype, genotype, virulence and vector species suggests movement along a conceptual gradient through South-East Asia.

Keywords

Bluetongue virus – Molecular epidemiology – Sentinel herd – Serotype – Topotype – Transect – Vector – Virulence.

Since sheep are not a major livestock species in many countries of South-East Asia, studies of bluetongue (BT) have not had the priority given to some other diseases. However, in Malaysia and Indonesia there is interest in the production of sheep meat, and in these countries imports of European sheep breeds have been attempted from time to time. On two occasions this has led to the diagnosis of BT in the imported sheep (1, 33). These outbreaks led to subsequent studies of the epidemiology of bluetongue viruses (BTV) in these countries, from which much of our knowledge of BTV in the region derives.

Surveillance programmes

For a number of years during the 1990s there was extensive collaboration between Australia and Indonesia (3) and between Australia and Malaysia (29) in BTV epidemiological studies.

At the Research Institute for Veterinary Science in Bogor, Indonesia, arbovirology was developed as a discipline, with early work based on serological surveys (17, 18). This progressed to epidemiological studies based on sentinel herd technology (2, 4, 21). The resulting knowledge of arboviruses in Indonesia has been reviewed (3), and the information regarding BTV is summarised again in this paper together with comparisons with data from elsewhere in South-East Asia as well as India to the north-west and Australia to the south-east.

Sentinel herd technology was also the basis of BTV studies in peninsular Malaysia (29), where a laboratory capacity was developed at the Veterinary Research Institute in Ipoh. Similar studies have not been reported from neighbouring countries such as Thailand, Vietnam and the Philippines, and little seems to be known of the BTV fauna in these places. It is noted that sentinel herd studies in Southern China, in the Yunnan Province, have also resulted in numerous isolations of BTV over the three-year period 1995 to 1997 in a geographical area in close proximity to South-East Asia (10).

In Indonesia, collections of *Culicoides* were routinely made in conjunction with the sampling of sentinel

animals, and extensive data is available regarding the species present in various parts of the country (34, 35).

Unfortunately, for those interested in the natural history of BTV in the region, other animal health issues have taken precedence in all countries in the region, and the information from the mid-1990s is in many cases the most recent.

Serological surveys

Serological surveys for BTV have been conducted in different areas in Indonesia from cattle, buffalo, goats and sheep. Sera were screened with group reactive tests such as the agar gel immunodiffusion (AGID) test and the competitive ELISA (c-ELISA) (11). Reactors were evaluated by the serotype-specific serum neutralisation (SN) test (19).

Large ruminants had a higher prevalence of exposure than small ruminants. Reactors to BTV serotypes 1, 12, 20 and 21 were widespread on the main islands of Indonesia (17, 19) and, as the studies were broadened, antibodies to BTV serotypes 2, 3, 5, 6, 7, 9, 15, 16 and 23 were also detected. Multiple BTV serotypes were hypothesised to be circulating. Reactors to BTV serotypes 1, 15, 16, 20, 21 and 23 were most prevalent.

Elsewhere in the region, in Papua New Guinea to the east, early serological studies detected seroconversions to BTV-1 and other BTVs not identified at that time (6). Subsequently antibodies to BTV-20 and BTV-21 were reported (16). Serological surveillance conducted from 2000 to 2002 identified cattle with antibodies to BTV-16 and BTV-21, and in 2003 antibodies to BTV-3, BTV-20 and BTV-23 were detected (R.A. Lunt, J. Lee, I. Puana and P.W. Daniels, personal communication). Similarly there is serological evidence of BTV infections in East Timor.

Isolation of viruses

Virus isolation from sentinel cattle in Indonesia has yielded eight serotypes of bluetongue (20, 22, 26). All were recovered in West Java with the exception of serotype 16 which has been isolated to date only in the Province of Papua, formerly known as Irian Jaya, some 4 000 km-4 500 km to the east of the West Java site. Serotypes 1, 21 and 23 have also been isolated from Papua (23). These BTV serotypes are widely distributed in Indonesia. It is noted that serotypes 1 and 21 are the two serotypes that are also widely distributed in Australia.

Isolation attempts from sentinel cattle in Malaysia have yielded serotypes 1, 2, 3, 9, 16 and 23 (29). Table I compares published information on the BTV serotypes isolated in Malaysia and Indonesia with data from India and northern Australia. BTV 2 was the only serotype found in Malaysia but not Indonesia. Some serotypes were common to all four countries in the comparison (BTV-1, BTV-3, BTV-9 and BTV-16), while BTV-4, BTV-8, BTV-17 and BTV-18 have not been identified further east of India. BTV-2, BTV-7 and BTV-12 were reported in South-East Asia but not Australia, and BTV-20, BTV-21 and BTV-23 have been isolated in South-East Asia and Australia, but not further west in India.

Other serotypes may exist there that have not yet been isolated. For instance, the serological survey of Sendow and colleagues (19) showed reactors to serotype 20 were prevalent (23%) in Indonesia, although this virus has not yet been isolated there.

Interestingly, the serotypes isolated in southern China a few years later were essentially similar, namely: serotypes 1, 2, 3, 4, 9, 11, 12, 15, 16, 21 and 23 (10). BTV-11 was the only serotype identified in the study conducted in China that had not already been reported in the region (Table I).

Genotyping of bluetongue virus isolates from South-East Asia

The virus of BT contains 10 double-stranded ribonucleic acid (RNA) segments, or genes. Seven code for structural proteins. RNA3 codes for one of

Table I
Serotypes of bluetongue viruses isolated in India, South-East Asia and Australia

BTV serotype	1	2	3	4	7	8	9	12	15	16	17	18	20	21	23
India	■	■	■	■		■	■	■		■	■	■			
Malaysia	■	■	■				■			■					■
Indonesia	■		■		■		■	■		■				■	■
Northern Australia	■		■				■		■	■			■	■	■
Eastern Australia	■													■	

the proteins of the inner core and is relatively conserved among the BTVs. Early molecular epidemiological studies showed that the pattern of nucleotide changes in this gene allow groupings of BTV, irrespective of serotype, that correspond with the geographic origin of the isolates. Hence BTV can be identified to the global region of origin through molecular analyses of RNA3 (7). Within those topotypes, more defined subgroupings can also be recognised, and on this basis, the BTVs of Indonesia and Malaysia have been analysed and compared (15). This latest report further refines previous observations (27).

In summary, from the Depok sentinel site in West Java, four genotypes of RNA3 were identified. All were grouped within the Australasian topotype of Gould (7) and, for the purposes of discussion, these subgroupings are now designated as Java A, Java C, Malaysia A and Australia A. Over the 25 years since the first detection of BTV in Australia, a stable genotype has predominated. This is now designated as Australia A in these studies, and some of the isolates from West Java group to this genotype (15). The Java C grouping includes isolates from India. The Malaysia A grouping includes the isolates from Malaysia analysed to date as well as the isolates from the Province of Papua in eastern Indonesia (15).

Hence, in Indonesia, genetic groupings of BTV have been identified that overlap with genetic groupings from India to the north-west as well as with those long identified in Australia to the south-east. These relationships are illustrated in Table II.

Vector studies

Major studies of the distribution of *Culicoides* spp. have been conducted in Indonesia and extensive lists of collected insects published (34, 35). Four species are proven vectors in Australia, namely *C. actoni*, *C. brevitarsis*, *C. fulvus* and *C. wadai*; they are also present and widely distributed in Indonesia. There are other closely related *Culicoides* spp. that could perhaps be vectors and which are not as widely distributed in the region, being exotic to Australia for instance. *C orientalis* and *C. nudipalpis* are considered of particular interest. Specific studies of vector competence have not been conducted but there has been a programme of isolation of viruses from insects caught in the wild that has yielded an isolate of BTV serotype 21 from a mixed pool of *C. fulvus* and *C. orientalis* (24). An isolate of serotype 21 has also been recovered from a pool of *Anopheles* mosquitoes (25), but it would be incorrect to consider mosquitoes as vectors without experimental confirmation.

Some 42 species of *Culicoides* have been identified in Malaysia, and it was suggested on the basis of their abundance in insect collections, geographical distribution and host preference, that the possible vector species in that country include *C. peregrinus*, *C. orientalis* and *C. shortti* (8). However, more rigorous studies of vector competence have not been reported.

Throughout South-East Asia, there is a need for such objective studies and, at present, the most conservative course of action may be to extrapolate from studies in other regions that have conclusively implicated certain species as vectors. The four species implicated as vectors in Australia occur throughout the region and have been reported in India.

It is interesting to note that *C. imicola*, the dominant vector species in Africa and the Middle East, has not been reported in much of South-East Asia. It is known to occur at least as far east as India and has been reported in Thailand and Vietnam (36).

Pathogenicity of bluetongue virus in South-East Asia

Bluetongue has been reported in the region only in imported European breeds of sheep (1, 33). There have been no reports of BT in local breeds of sheep, in spite of serological evidence that BTV infections of these breeds has been quite common (8, 19). Experimental infections of Merino sheep with some Indonesian BTV strains maintained by animal

Table II
Bluetongue virus genotypes identified in South-East Asia showing relationships with isolates from India and eastern Australia (15)

Genotype	India	Malaysia	Western Indonesia	Eastern Indonesia	Northern Australia	Eastern Australia
Java C	■		■			
Malaysia A		■	■	■		
Java A			■			
Australia A			■		■	■

transmission did not elicit clinical disease (28), indicating that these strains were of low virulence.

This contrasts with the situation in India where BT is a serious disease of local sheep, resulting in mortalities and pathology, such as myocarditis as well as the usual changes associated with vascular permeability (14, 31). The observations suggest that European breeds of sheep may be more susceptible to BT than Asian breeds, and that the BTV strains in South-East Asia are not as virulent as some strains in India.

To the south-east, in northern Australia, the BTV strains are considered to be of only moderate virulence. Certainly they are not as virulent as some South African strains as assessed experimentally (9). Strains in eastern Australia are considered to be essentially non-virulent. This perceived variation in virulence potential of the BTV strains from India through to eastern Australia is illustrated in Table III.

Patterns in the ecology of BTV in South-East Asia

Variability occurs in the serotypes, genotypes, virulence and vectors of the BTV strains across the South-East Asian region. To give a framework for these observations, it is helpful to consider a conceptual transect from India across South-East Asia, through northern Australia to eastern Australia (Fig. 1). Such transects are used in ecological studies to give rigor to comparative observations of related phenomena in adjacent geographical areas, particularly, where changes may be expected as distance increases from a nominated point of origin (5).

Patterns can be described along this transect. At the western end, in India, a greater number of serotypes have been described, including serotypes that do not occur further east. Conversely, serotypes 20, 21 and 23 that are present to the east have not been reported in India.

BTVs are considered recent introductions to the viral fauna of northern and eastern Australia, at the eastern end of the transect (30, 32). They are parasites with ruminants as the vertebrate host, and would not have been established in Australia prior to the introduction of ruminants in the last 200 years. The most likely mode of introduction is via infected insects carried on wind movements from infected areas to the north and north-west. The presence of BTV serotypes in northern Australia indicates movement from the west to the east along the transect.

In the molecular epidemiological analyses of isolates from South-East Asia, four genotypic subgroupings have been described. The first includes isolates from both India and western Indonesia, the second isolates only from western Indonesia, the third isolates from both western and eastern Indonesia as well as Malaysia, and the fourth isolates from western Indonesia and northern and eastern Australia (Fig. 1).

Importantly, genotypes isolated in Indonesia in 1988 and 1990 have appeared in northern Australia in 1992, 1994 and 1995 (5, 13, 15), further evidence of movement of BTV from north-west to south-east along the transect. Most recent molecular analyses have detected more *de novo* appearances of South-East Asian genotypes in northern Australia, such as the Malaysia A genotype in 2001 (15).

At the western end of the transect, highly virulent strains of BTV occur, whereas those at the eastern end are considered essentially non-virulent. Midway along the transect in South-East Asia and northern Australia, some strains occur that show moderate virulence, being moderately pathogenic for European breeds of sheep but non-pathogenic for Asian breeds. Other strains in these areas are also non-virulent.

Similarly, it has been noted that species of *Culicoides* identified as vectors of BTV in northern Australia also occur throughout South-East Asia and in India. Differences between the western and eastern ends of the transect are that *C. imicola* occurs in India as well as the other vector species, whereas in eastern Australia the main vector species has been reduced to *C. brevitarsis*, although *C. actoni* and *C. wadai* also occur in the more northern areas.

Table III
Observed variability in virulence of bluetongue viruses from India through South-East Asia to eastern Australia

Bluetongue virus	India	Malaysia	Indonesia	Northern Australia	Eastern Australia
Virulent		No	No	No	No
Mild to moderate virulence	?				No
Non-pathogenic	?	?			

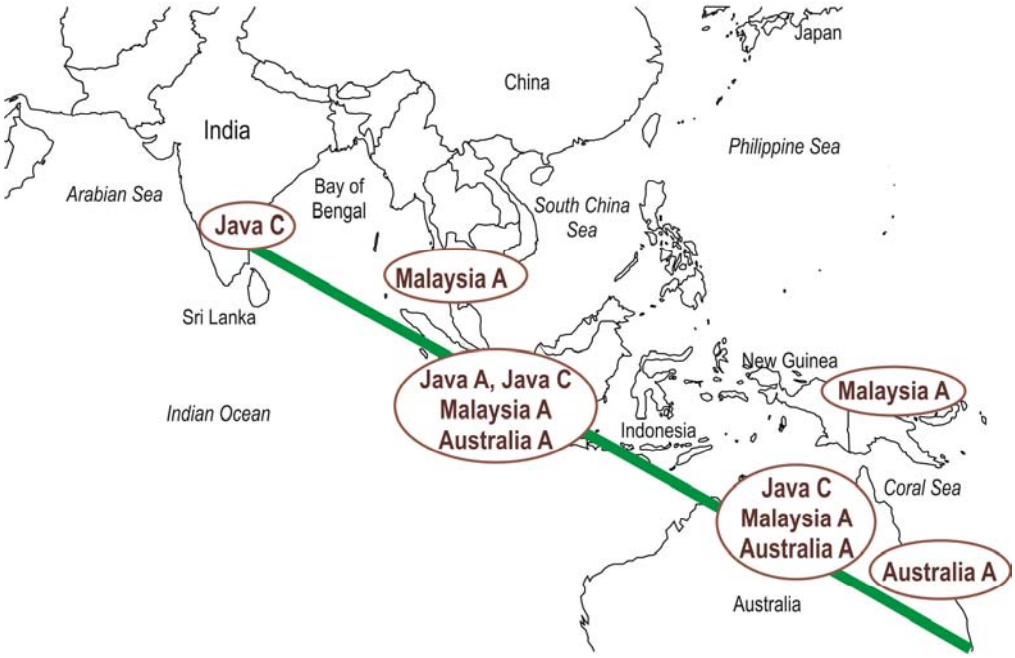

Figure 1
Patterns of change in genotype of bluetongue virus isolates along a transect from South Asia through South-East Asia into northern and eastern Australia, based on analyses of RNA 3

Hence, in addition to the observed patterns along the transect that suggest a continuum of BTV activity, there are specific observations for which the most biologically plausible explanation is movement of strains along the transect from west to east.

Will future movements from west to east result in the arrival of more virulent strains than currently occur in some areas? *C. imicola* is recognised as a vector in countries where virulent BTV occurs. Would extension of the range of *C. imicola* further eastwards be an event of concern for animal health in the region? Its presence where virulent BTV strains are circulating and absence where BTV is less pathogenic may be an association that should be investigated experimentally.

To balance these concerns, it should be noted that there may be ecological blocks along the transect. For example, although *C. imicola* has been reported in the more northerly countries of South-East Asia, it has not moved into Malaysia and Indonesia. Is it simply a matter of time, or are other unidentified factors involved? Similarly, in northern Australia six of the eight identified serotypes that are maintained in a complex vector system involving *C. fulvus*, *C. actoni*, *C. wadai* as well as *C. brevitarsis* (12) have not moved to the eastern states where serotypes 1 and 21 are maintained in a predominantly *C. brevitarsis* ecosystem. There is a challenge to devise experimental systems to explore the observed associations. In any dynamic biological system there will be checks and balances. These are not understood for BTV in South-East Asia, where the minimum requirement is for more surveillance and monitoring of the BTV ecosystems to provide basic current data.

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