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Isolation of bluetongue virus serotypes new to Indonesia from sentinel cattle in West Java

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THE bluetongue viruses are arboviruses of ruminants that cause economically important disease in sheep. Certain *Culicoides* species are the vectors (Erasmus 1975). Recent epidemiological observations have shown that bluetongue infections are endemic in tropical areas of the world but without associated disease in the local sheep populations (Gibbs and others 1989, Sendow and others 1986, 1991a). An interpretation of the history of bluetongue (Erasmus 1985) and its worldwide distribution (Ozawa 1985) is that breeds or species of ruminants susceptible to disease are those that have evolved in temperate climates, or areas where bluetongue viruses are not endemic (Gibbs and others 1989). In Indonesia, bluetongue disease has been observed only in sheep imported from southern Australia (Sudana and Malole 1982) and a similar situation has been observed in the neighbouring country of Malaysia (Geering and Gard 1989). However, variations in the pathogenicity of strains of bluetongue virus have also been reported among some serotypes (Johnson and others 1989). A prerequisite for a full epidemiological understanding of the bluetongue status of any country and the pathogenicity of the serotypes present is the isolation and characterisation of the viruses. This paper reports the isolation of viruses obtained as part of a national investigation of arboviruses of livestock in Indonesia (Daniels and others 1991).

The bluetongue viruses isolated previously in Indonesia were serotypes 7 and 9 which were isolated from blood samples

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TABLE 1: Bluetongue viruses isolated from sentinel cattle in West Java in 1990

Bluetongue virus serotype	Isolate	Passage history	Date of collection
9	RIVS 74	B5	January 20
21	RIVS 60	E1 A1 B9	April 2
	RIVS 23	E1 A1 B3	April 9
12	RIVS 67	B5	April 9
1	RIVS 46	E1 A1 B4	May 29
23	RIVS 41	E1 B7	June 4
21	RIVS 63	E1 D1 B5	June 25

E Number of passages in embryonated chicken eggs
A Number of passage in C6/36 cells
B Number of passage in BHK-21 cells

from healthy sentinel cattle near Bogor, West Java, in 1987 (Sendow and others 1991b). The sentinel programme, including virus isolation, was continued in subsequent years at the same site with some modification of methods to yield more viruses. A method of isolation based on that of Gard and others (1988) using a primary passage in embryonated chicken eggs was adopted. For the identification of the viruses isolated, an immuno-dot blotting test based on the method of Afshar and others (1987) was used.

Heparinised blood samples were collected from sentinel cattle in West Java at Depok, which has an altitude of 95 m and an average annual rainfall of 2500 mm (Sendow and others 1989). From samples collected in 1990, 253 were diluted 1:10 in phosphate buffered saline (PBS) containing antibiotics (100 iu/ml of kanamycin, 100 iu/ml penicillin and 100 mg/ml streptomycin) then sonicated for two periods of 10 seconds. The sonicated samples were inoculated in 0·1 ml volumes in quadruplicate into 11-day-old embryonated chicken eggs which were then incubated for five days at 33·5°C. Any embryos found dead on the first day after inoculation were discarded but embryos dying during the remainder of the incubation period were harvested. The hearts of the embryos inoculated with the same sonicate were ground together in a sterile glass tissue grinder in Eagle's minimum essential medium containing antibiotics but without fetal bovine serum. The resultant tissue suspension was clarified by centrifugation at 1000 *g* for 10 minutes and the supernatant fluid passed through a 450 nm filter (Sartorius) before inoculation into cell cultures; either directly into baby hamster kidney (BHK-21) cell cultures or first into *Aedes albopictus* C6/36 cells before passaging into BHK-21 cells. Cultures in BHK-21 cells were observed for a cytopathic effect, indicative of a viral infection. BHK-21 cultures not showing any cytopathic effect were passaged three times before being discontinued.

The isolates were identified to a viral group by the immuno-dot blotting test using a bluetongue monoclonal antibody (20E9b762) specific for viruses of the bluetongue group (Lunt and others 1988) and supplied by the Australian Animal Health Laboratory, Geelong, Australia. Forty isolates have been recovered from the sentinel cattle blood collected during 1990 and seven that reacted in the test for bluetongue were subsequently identified to serotype. The results are presented in Table 1.

This report brings the number of confirmed serotypes of bluetongue isolated in Indonesia to six, serotypes 1, 7, 9, 12, 21 and 23. Serological surveys have shown that reactors to the bluetongue group are widespread (Sendow and others 1986, 1989, 1991a) and bluetongue viruses may thus be considered endemic in this tropical country. In West Java, where the isolations were made, 77 per cent of the buffaloes, 73 per cent of the cattle, 28 per cent of the goats and 19 per cent of the sheep tested reacted in the agar gel immunodiffusion test for bluetongue (Sendow and others 1986). However, only 1 per cent of the cattle and 7 per cent of the buffalo sera, and no goat or sheep sera, had antibodies against bluetongue serotype 1, and 2 per cent of the cattle tested had antibodies against bluetongue serotype 12 (Sendow and others 1991a). The serological data indicate that there may be a sizeable population of sheep naive for bluetongue infections. This raises the question of why the disease is not reported. Pathogenicity studies in both local and imported sheep should now be possible with the bank of confirmed isolates.

Little comparative information on bluetongue viruses in the region is available from other countries except Australia, where eight bluetongue serotypes have been reported, serotypes 1, 3, 9, 15, 16, 20, 21 and 23. Most of these viruses are confined to the north of Australia adjacent to Indonesia (Gard and Melville 1989). Such observations raise the possibility of a common pool of viruses in the region, and suggest that the full range of bluetongue viruses in Indonesia may not yet have been isolated. Since studies of stored sera show many of the Australian isolates to be recent introductions, the epidemiological situation in the region appears to be evolving (Gard and Melville 1989). Unfortunately Indonesia's bank of stored sera (Young and others 1985) has been established only recently, so that historical studies seeking evidence of infections by these viruses cannot be conducted. There is a need to establish a collection of sera representative of all regions of Indonesia so that the national distribution of each new serotype of bluetongue isolated can be determined (Daniels and others 1991).

It can be seen from the dates of collection of the specimens yielding isolates (Table 1) that there was a tendency for different serotypes to be circulating in the environment at different times. For example, there was a 10-week interval between the isolation of serotype 9 in January and serotype 21 in April, although in April both serotypes 21 and 12 were isolated at the same time from different cattle. Similarly there was an interval of seven weeks between the isolation of serotypes 12 and 21 in April and serotype 1 in May. A week later, in early June, serotype 23 was isolated. However, three weeks later serotype 21 was again isolated, suggesting that this virus had persisted in the presumed endemic area. These isolates were all from different cattle, rather than sequential infections in the same animals. Further intensive study of such sentinel groups of cattle is needed to determine the patterns of natural exposure to bluetongue viruses and the serological responses associated with the infections, so that more can be learned of the epidemiology of bluetongue virus infections in a heavily infected region.

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