Isolation of bluetongue virus serotypes 1, 21 and 23 from healthy sentinel cattle in Irian Jaya, Indonesia

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Twenty-four serotypes of bluetongue (BLU) virus have been reported world wide. BLU serotypes 7 and 9 have been isolated from healthy sentinel cattle in West Java (Sendow *et al* 1991a), but isolates have not been reported previously from other provinces of Indonesia. We report isolations of BLU viruses in Irian Jaya province on the western part of the island of New Guinea, in the eastern part of Indonesia.

As part of a broader study of arboviruses infecting livestock in Indonesia (Daniels *et al* 1991), sentinel cattle were established at several locations including a low altitude site in Irian Jaya (Sendow *et al* 1991b), in the transmigration village of Koya Timur (2° 41'S 140° 52'E) in the Jayapura district on the north coast of the province near the border with Papua New Guinea. Jayapura has an average annual rainfall of 2750 mm, and average monthly falls of not less than 150 mm (Anon 1973) in the drier months. A group of 15 Bali cattle (*Bos javanicus*) of average age 6 months were identified individually and monitored for 12 months, then replaced annually with a similar group. Monitoring of sentinel cattle, including collection and storage of specimens, was conducted by staff of the provincial veterinary services. Heparinised blood samples for viral isolation were collected weekly, stored in liquid nitrogen and sent about every 3 months to the Research Institute for Veterinary Science (RIVS) in Bogor for testing.

The viral isolation procedures were adapted from those of Gard *et al* (1988), and involved an initial passage in embryonated chicken eggs (ECE) followed by passages in cell cultures. Blood samples collected before 1990 were processed directly from eggs to baby hamster kidney (BHK-21) cell cultures, while samples from 1990 were passaged from eggs to cultures of *Aedes albopictus* (C6/36) cells before inoculation into BHK-21 cells. Inocula were prepared by diluting thawed whole blood one in ten in phosphate buffered saline containing antibiotics (100 IU/mL of kanamycin, 100 IU/mL of penicillin and 100 mg/mL of streptomycin). Diluted blood samples were sonicated and a 0.01 mL sample was inoculated into each of four 11-day-old ECE. Inoculated eggs were examined daily for 5 days. Embryos that died on the first day after inoculation were discarded. Hearts from embryos that died after the first day were ground together in a glass tissue grinder in 2 mL of Eagle's Minimum Essential Media with antibiotics but without foetal bovine serum. The resulting suspension was centrifuged at 1000 *g* for 10 min. Supernatant fluid was then passed through a 450 nm filter and used as inoculum for blind passage in C6/36 cells followed by passaging in BHK-21 cell cultures, which were observed daily for cytopathic effect (CPE). Three passages in BHK-21 cultures were performed and these cultures discarded if CPE was not observed. BHK-21 cell cultures showing CPE were considered to contain viral isolates.

Preliminary identification of isolates was by immuno dot blotting (IDB) using a modification of the method of Afshar *et al* (1987), with a monoclonal antibody (20E9/B7/G2) specific for the BLU group of orbiviruses (Lunt *et al* 1988) obtained from the Australian Animal Health Laboratory, Geelong. Isolates reacting with the monoclonal antibody in the IDB test were submitted to the World Reference Centre for Bluetongue Virus at the Veterinary Research Institute, Onderstepoort, Republic of South Africa, for serotyping.

During the period September 1989 to October 1990, 870 blood samples were tested and 45 viruses were isolated. Four of these have been confirmed as BLU viruses, which included one isolate of BLU serotype 1, one isolate of BLU serotype 21 and 2 isolates of BLU serotype 23 (Table 1).

BLU serotypes 1, 21 and 23 have also been isolated from sentinel cattle in West Java (Sendow I unpublished). BLU serotypes 1 and 21 (St George *et al* 1980) and 23 (Gard *et al* 1985) have been isolated in Australia and BLU serotype 1 has been isolated in Malaysia (Geering and Gard 1989). It is interesting to note that these BLU serotypes have now been isolated from widely separated areas of the Australian and South East Asian region. The 'geotypes' (Gould 1988) of these viruses from eastern and western Indonesia and from Australia and Malaysia should be compared to indicate whether BLU viruses in the region have evolved as part of the same genetic pool, or separately. Such information could be expected to have epidemiological significance in helping to interpret apparent movements of viruses within the region, and in predicting future changes in distribution.

Antibody against BLU serotype 1 has been detected in goat and cattle sera collected in the vicinity of the Jayapura sentinel sites with prevalences of 11% and 8%, respectively (Sendow I unpublished). A previous serological survey of cattle, buffaloes, sheep and goats from several provinces in Indonesia found reactors to BLU serotype 1 only in the provinces on Java island, with prevalences up to 21% in buffaloes and lower prevalences in other species (Sendow *et al* 1991c). In another but smaller survey of 82 cattle from Java and Bali, 54% were reactors to BLU serotype 1 (Muira *et al* 1982).

Previous serological surveys of BLU serotype 21 have shown it to have wider distribution than serotype 1, with reactors detected in Java, South Sumatra, South Kalimantan, South Sulawesi and West Timor, in cattle, buffaloes and goats (Sendow *et al* 1991a). The antibody status of Indonesian ruminants to BLU serotype 23 has not been evaluated. The present finding of BLU serotype 1, 21 and 23 in Irian Jaya extends

TABLE 1
Serotypes of bluetongue viruses isolated from sentinel cattle at Jayapura, Irian Jaya

Isolate	Collection date	Serotype
RIVS 105	11 November 1989	BLU 23
RIVS 90	23 January 1990	BLU 23
RIVS 113	13 February 1990	BLU 21
RIVS 92	29 May 1990	BLU 1

the known range of these viruses and confirms they may be spread widely throughout Indonesia.

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A comparison of two preparations of 'swormlure-2' in a bait for screw-worm flies

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Predicted economic losses to the Australian livestock industry from the establishment of screw-worm fly (SWF) vary from $100m per annum to $430m per annum (Anon 1990). Predictions from a simulation model give estimates of production losses of $72 m if SWF spread throughout its natural range in Australia (Mayer *et al* 1991). There are 2 species of SWF; *Cochliomyia hominivorax* (Coquerel), the New World SWF, which exists in the Americas, and *Chrysomya bezziana* (Villeneuve), the Old World SWF, which exists in Africa, India, South East Asia and Papua New Guinea. SWF do not occur in Australia although both species have been intercepted; live *C bezziana* were trapped in a returned livestock vessel in Darwin in 1988 (Rajapaksa and Spradbery 1989) and *C hominivorax* larvae were found in a lesion on the neck of a woman who had returned from a visit to South America, at Wagga in 1992 (Searson *et al* 1992). However, the closely related species, *Chrysomya megacephala, C saffranea, C rufifacies* and *C varipes* are present and are caught in large numbers when trapping for SWF in northern Australia. Since 1980 when the trapping program began, SWF have not been found in any traps, even on Saibi and Boigu islands, close to the Papua New Guinea mainland (Bock and Tanzer 1985). Monitoring is based on the use of the CSIRO SWF sticky trap described by Spradbery (1981) baited with a pungent mixture of 11 chemicals, called 'swormlure-2' (SL-2) (Coppedge *et al* 1977; Brown and Mackley 1983). In a survey over 12 Texas counties, prevalence estimates from as few as 5 SL-2 traps in a county were about equal to the number of cases reported by ranchers in detecting screw-worm flies (Coppedge *et al* 1978). However, studies in Papua New Guinea have shown that a wounded sentinel steer is about 5 times more attractive to SWF than an SL-2 trap, and that a wounded steer with an active SWF infection is more than twice as attractive as a non-infested animal (Spradbery *et al*, unpublished). The current method of presenting lure is by means of a 100 mL glass pomade jar containing 25 to 50 mL of SL-2, screwed onto a lid fastened to the underside of the trap. Odour escapes through matching holes in the jar lid, trap base and gel-coated tray. The use of 4 propylene coated polyester fibre wicks inserted through the holes to control odour release, as described by Broce *et al* (1979) and Spradbery (1981), has been discontinued for reasons unknown to the authors.

Researchers involved with tsetse fly trapping have successfully baited traps with 50 mm square sachets made of polythene sheets 120 or 150 microns in thickness, each containing 4 mL of lure. Polythene sachets are currently being used to transport 25 mL aliquots of SL-2 and are packed in stainless steel, wide-mouthed vacuum flasks to meet Australian air freight regulations. The sachets are cut open to dispense the lure into each jar when setting the trap. The disadvantages of the standard technique include the expense of transporting the toxic and corrosive liquid in specially made flasks, the risk of contamination of operators, the comparatively large volume of SL-2 used in the trap, and the difficulty of disposing of used lure when the trap is serviced. These disadvantages would be greatly reduced or avoided if unopened sachets containing SL-2 were shown to be as effective as the jar method. This paper describes 4 field trials carried out in the Torres Strait that compared the attractiveness of polythene sachets containing SL-2 with the jar method. Flies trapped were endemic secondary blowflies of *Chrysomya* sp (not SWF).

Trial 1 — Comparative Efficacy of Sachet and Jar — Six sites for traps were selected at locations at least 200 m apart in a crosswind direction to avoid overlap of odour plumes. Three traps were assigned randomly to the jar method, and 3 to sachets. Sachets were 50 mm by 90 mm, made of 120 micron polythene sheet, and contained 25 mL of SL-2. The sachet was placed on the adhesive gel in the centre of the plate. After one week, the number of Chrysomya sp flies on each trap were counted, the types of lure were reversed, and the trial repeated. The numbers of flies per trap were subjected to an analysis of variance (after a log transformation was applied) in which the factors: trap type, week, and site, were tested for significance. The analysis showed no significant effects for any of these factors, but there were only 4 degrees of freedom available from the small data set on which to estimate an error term. The means for daily catches from jars and sachets were 217 and 358 flies, respectively.

Trial 2 — Longevity of Attraction — To assess the comparative longevity of SL-2 in jar and sachet presentations, 2 trap sites were selected 150 m apart, with one trap of each presentation. Sachets and jars contained 25 mL of SL-2. For 2 weeks adhesive gel plates were changed each day at sunset and flies counted. The type of lure at each site was then reversed, and the trial continued for a