

The Occurrence of Antibody to Bluetongue Virus in New South Wales.

I. Statewide Surveys of Cattle and Sheep

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Abstract

Two State-wide surveys were carried out in 1978 to detect bluetongue (BLU) virus antibody in cattle and sheep sera in New South Wales (NSW). The first survey showed that BLU group antibody in cattle 18-24 months old was confined to the coastal regions (east of the Great Dividing Range) and the Hunter Valley. However, in the second survey, of cattle more than 5 years old, reactors were much more widely distributed over the north-eastern third of the State and into the western division with prevalences up to 85% in some areas. In contrast, very few reactors were detected in sheep in either survey (less than 1% of the sheep sera tested).

In a retrospective study of stored cattle sera, BLU group reactors were detected in the north-east of the State in each year examined since 1968, the earliest year in which samples were available from that region. Areas to the south and west were free of antibody from 1966 until the summer of 1973, but subsequently reactors were common.

Examination of selected area for type-specific antibody indicated that infection of cattle with two of the three Australian BLU serotypes which were known at the time, BLU-1 and BLU-21, had occurred in NSW. No antibody to BLU-20, the original Australian isolate, was detected.

A close association was observed between strong group antibody reactions and type-specific neutralizing activity against BLU-1 and BLU-21. Both were largely confined to that area of the State in which a high (75% or more) prevalence of group antibody was recognised in the older animals. In contrast, reactions from areas with a low prevalence of group antibody were almost all weak and rarely associated with neutralizing activity for BLU-1 or BLU-21. We suggest that weak reactions may be group cross-reactive and induced by BLU-related, rather than BLU, viruses.

Introduction

The first isolation of a bluetongue (BLU) virus in Australia was made from a mixed pool of *Culicoides* midges collected during 1975 in the Northern Territory (St George *et al.* 1978b). This virus was originally designated CSIRO 19, and later identified as a new BLU serotype, BLU-20 (Snowdon 1979). Two additional viruses, recovered during 1979 and designated CSIRO 154 and CSIRO 156, have also proved to be distinct BLU serotypes (St George *et al.* 1980). CSIRO 154 has been identified as a further new serotype, BLU-21, and CSIRO 156 as a member of type 1 (BLU-1) (Erasmus, B., Onderstepoort, personal communication, cited by St George *et al.* 1982). Other BLU serotypes have been isolated since the work reported here was concluded.

There has never been any evidence of BLU disease under field conditions in Australia. Nevertheless, the discovery of BLU-20 prompted intensive investigations throughout Australia (St George and McCaughan 1979; Coackley *et al.* 1980; Della-Porta *et al.* 1983). This paper presents the results of initial serological investigations of BLU virus activity in cattle and sheep over the whole of NSW. Results of more intensive sampling in the areas of higher antibody prevalence are reported in the accompanying paper (Littlejohns and Burton 1988).

Serological Techniques

The gel diffusion precipitin (GDP) test, using group antigen prepared from BLU-20 virus (Sharp *et al.* 1988), was carried out by the standard method of the Bureau of Animal Health (Littlejohns 1981). Positive test reactions were graded from 1+ to 3+.

Virus neutralisation (VN) tests were conducted by conventional methods in microtitre plates from an initial serum dilution of 1/3. Viruses used were derived either by two passes in BHK cells (BLU-1 and BLU-21) or by one pass in Vero cells plus two or three passes in BHK cells (BLU-20) from stocks received from T. D. St George, CSIRO, Long Pocket Laboratories, Indooroopilly. The viruses were received as: BLU-1 and BLU-21, which were plaque-purified CSIRO 156 and CSIRO 154 respectively, and BLU-20 which was a fourth pass in BHK after isolation.

Serological Surveys

NSW is divided into 58 Pastures Protection (PP) districts (Fig. 1) and these were used as basic geographic units for the surveys and for the reporting of results.



Fig. 1. Location of Pastures Protection districts in NSW.

Survey I. January-May 1978

The aim of this survey was to determine if BLU group antibody was present in cattle and sheep in NSW, and if so, to determine the distribution of exposed livestock.

The survey design required sampling from five cattle and five sheep properties in each of the 58 PP districts. This provided sera from approximately 25 cattle and 25 sheep in each district. Stock aged 18–24 months, which were born on the property, were selected.

All samples were initially tested in the GDP test, and sera giving positive results were then tested using the VN test for BLU-20.

When BLU reactors were found supplementary sampling of cattle, irrespective of age, was begun on the properties involved, and on surrounding properties. This testing uncovered more reactors than had been anticipated, particularly in old animals, and consequently the supplementary testing was discontinued and Survey II initiated.

Survey II. June–August 1978

The aim was to determine the prevalence and distribution of BLU group antibody in NSW in animals more than 5 years old.

The same survey design was used as described for Survey I. In coastal PP districts few sheep were sampled, as there is no substantial sheep industry on the coast. In some of these PP districts, however, more than the required 25 cattle were bled.

Ninety-four sera which gave positive GDP reactions, and 111 which gave negative reactions, were tested for the presence of VN antibody against BLU-1 and BLU-21 viruses. These sera were all from 8–10 year old cattle, and all from the eastern half of the State, but included sera from areas of high, intermediate and low GDP test reactor prevalence.

Retrospective Survey of Stored Serums

The aim of this testing was to determine if BLU antibody was present in NSW prior to 1978.

Sera stored at the Regional Veterinary Laboratory, Wollongbar, and the Veterinary Research Station, Glenfield, were tested in the GDP test. The Wollongbar collection consisted of 117 sera collected in the Tweed-Lismore PP district between 1968 and 1976. The Glenfield collection was made up of 223 sera collected between 1966 and 1976. Of these, 62 came from the Dubbo PP district; 89 from animals bled at the research station itself, which is in the Moss Vale PP district; and the remaining 72 from widely scattered areas of central and southern NSW.

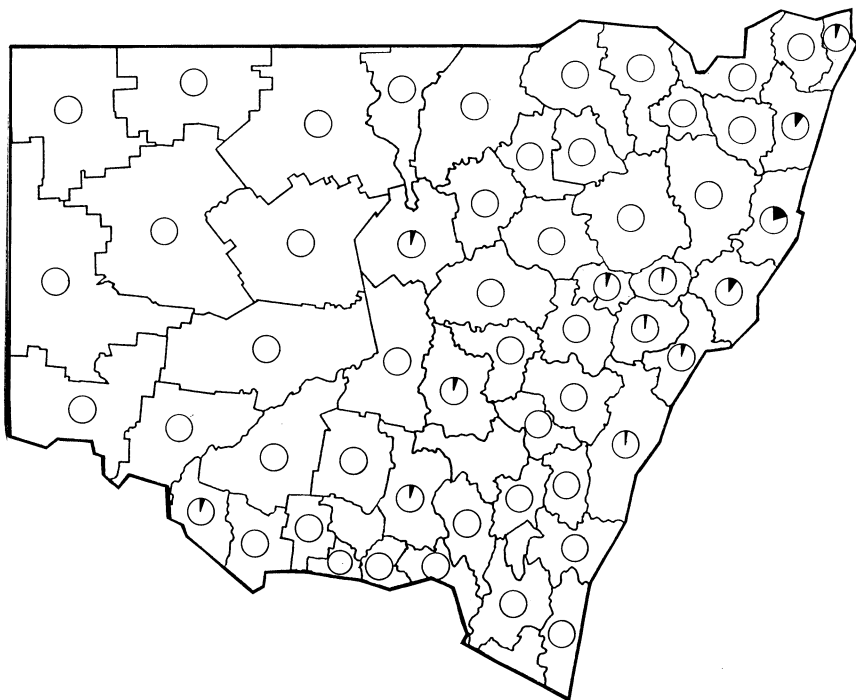


Fig. 2. Results of Survey I, showing the distribution of GDP test antibody in sampled cattle 18–24 months of age by Pastures Protection district. The black sectors within the circles depict the percentage of positive animals in the sample of each PP district.

Results*Survey I*

The results of Survey I, with respect to cattle, are set out in Fig. 2. In total, 1610 sera representing 329 herds were tested, and 52 GDP reactors were detected.

The vast majority of these GDP reactions in cattle were found in the coastal strip as far south as the Moss Vale PP district, with an inland extension following the Hunter Valley, which falls within the PP districts of Gloucester, Upper Hunter, Merriwa, Denman-Singleton and Maitland. GDP reactor prevalence in these PP districts ranged from 2 to 22%. Single reactors were recorded in the PP districts of Canonba, Forbes, Wagga, Moulamein and Cooma/Bombala.

Of 1436 sheep sera tested, only 8 gave positive reactions, and these were all weak (1+). A single reactor was recorded in each of five PP districts: Tweed-Lismore, Port Macquarie, Coonabarabran, Cooma and Deniliquin. Three reactors were found in one flock in the Wagga district.

Where GDP test reactors were found, all sera collected on the property involved were then subjected to the VN test for BLU-20 virus. In all, approximately 1000 sera were tested, and 42 gave weak positive (titres <5) reactions which were not repeatable on retest. There was no recognisable geographic distribution or clustering by herds.

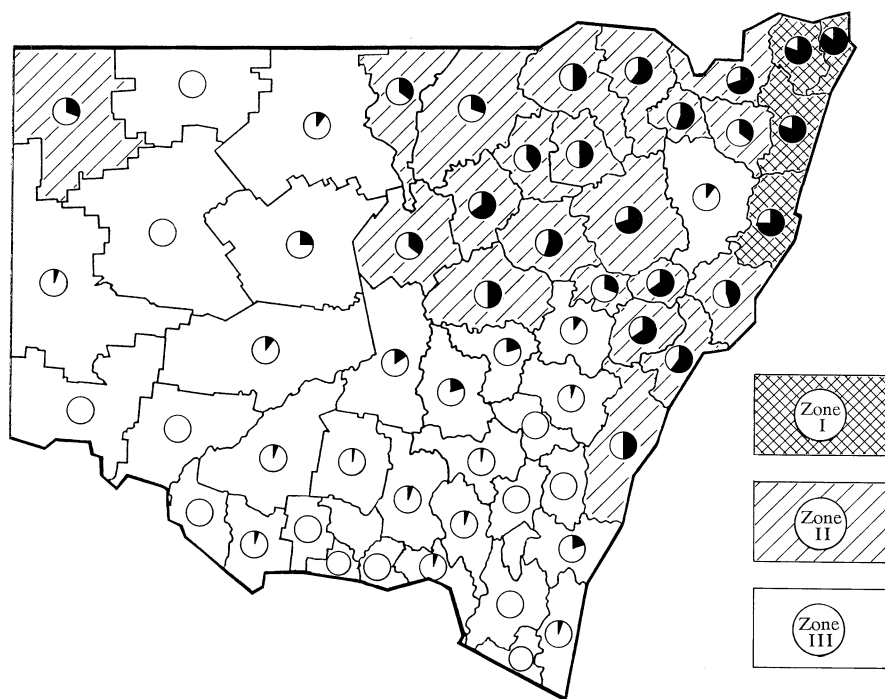


Fig. 3. Results of Survey II, showing the distribution of GDP test antibody in sampled cattle over 5 years of age, by Pastures Protection district. The black sectors within the circles depict the percentage of positive animals in the sample for each PP district. Zone I, GDP reactor prevalences >75%. Zone II, GDP reactor prevalences between 30 and 75%. Zone III, GDP reactor prevalences <30%.

Survey II

The results of Survey II, with respect to cattle, are set out in Fig. 3. A total of 746 GDP reactors were detected from 2176 sera tested.

In cattle over 5 years old the prevalence of reactors was much higher than that seen in the younger cattle sampled in Survey I. The geographic distribution of reactors was also much more extensive. Reactors were concentrated in the north-eastern third of the State, with the notable exception of the comparatively low reactor rate (9%) in the Armidale PP district. The highest prevalence recorded was in the Tweed-Lismore district, with 85% of

tested animals being positive. Significant numbers of reactors also occurred in the more western district of Cobar, Brewarrina and Milparinka.

Very few reactors were detected in sheep over 5 years of age. Of 1221 sera tested only 9 were positive, and these gave only weak (1+) reactions. One reactor occurred in each of the PP districts of Tweed-Lismore, Tenterfield, Moree, Pilliga, Coonamble, Cobar and Broken Hill. Two reactors were recorded in the Coonabarabran PP district. Sheep reactors were found in Tweed-Lismore and Coonabarabran in both Survey I and Survey II.

The geographic distribution of reactors in old cattle could be broadly grouped in three zones (Fig. 3):- a 'high prevalence' zone in the north-east comprising Casino, Tweed-Lismore, Grafton and Port Macquarie PP districts, with prevalences in excess of 75%; a much more extensive 'intermediate' zone, generally covering about 40% of the State in its north-eastern part, together with its north-western corner (Milparinka PP district), with prevalences in excess of 30%; and a 'low prevalence' zone covering the remainder of the State.

All 3+ reactors were confined to the north-east of the State. Reactors of this strength were confined to 'high' and 'intermediate' prevalence zones, and accounted for less than 15% of the reactors in each zone.

Of the 94 GDP test positive sera examined by VN testing, 43 were positive for both BLU-1 and BLU-21. A further 16 were positive for one virus only (eight each) while 34 were negative to both. Titres to both viruses were relatively low and ranged from 3 to 27. As 50% of titres to BLU-1, and 33% of those to BLU-21, were >9 they were considered to include at least some meaningful reactions. However, there was no recognisable threshold

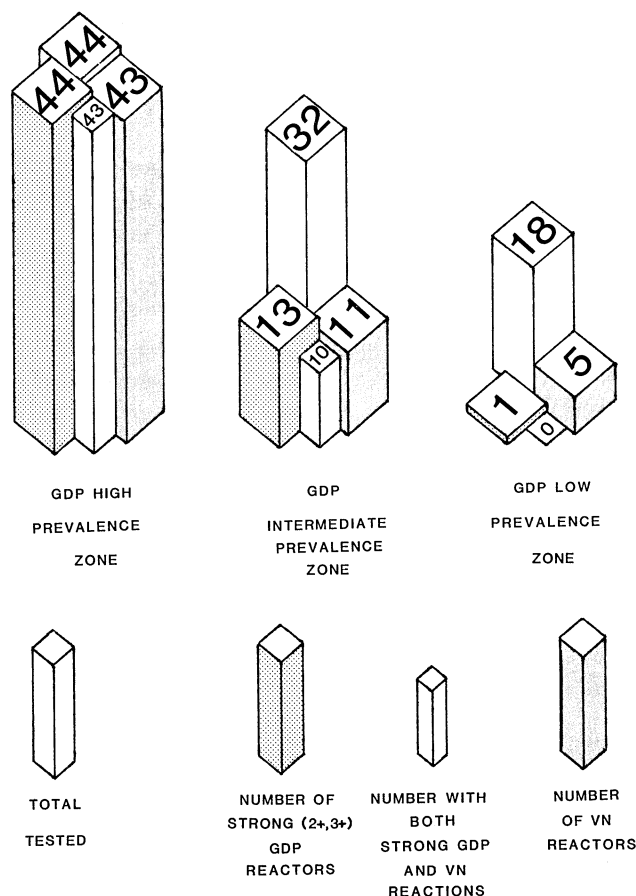


Fig. 4. The association between GDP reactor prevalence zones, as shown in Fig. 3, and the number with strong GDP antibodies, the number with VN activity and the number with both, for selected samples from Survey II.

of significance, and so all were accepted for further interpretation. The close association of these reactors with areas of high prevalence of GDP reactors, and with strong GDP reactions, gives credence to the presumed specificity of these tests.

There was a clear association between strong (2+ or 3+) GDP reactions and VN antibody to either BLU-1 or BLU-21 (Fig. 4). For the *high prevalence* zone, all 44 samples examined were strong GDP reactors and 43 of these had VN antibody to one (5 to BLU-1 and 3 to BLU-21) or both (35 samples) of BLU-1 and BLU-21. Of 32 samples examined from the *intermediate prevalence* zone, 13 were strong GDP reactors and, of these, 10 had VN activity (2 to BLU-1, 3 to BLU-21 and 5 to both). Among the 19 weak (1+) GDP reactors only one had VN activity (to both BLU-1 and BLU-21). However, in this context, the intermediate zone samples were derived from only four PP districts and these could be seen as two subgroups of different character. Of 15 samples from two PP districts (Warialda and Maitland), 12 gave strong GDP reactions. Ten of these 12, plus one of the three weak GDP reactors, had VN activity. Of 17 samples from the other two PP districts (Coonabarabran and Moss Vale) only one was a strong GDP reactor and none had VN activity. Hence, recognising these two subgroups, the association between strong GDP reaction and VN antibody was, as in the high prevalence zone, virtually absolute and both appear to be location-related rather than prevalence-related. From the *low prevalence* zone, of 18 samples examined only one was a strong GDP reactor. Five (28%), including the strong GDP reactor, had VN antibody, one to BLU-1, two to BLU-21 and two to both.

Of 111 GDP test-negative sera tested there were three positives for BLU-1, one each from Grafton, Warialda and Brewarrina, and three positives for BLU-21, all from Grafton and including the BLU-1 reactor. Forty of the 111 sera were from high or intermediate prevalence areas, and these included all of the VN reactors. No VN reactors were found among the 71 sera from low prevalence areas.

Retrospective Survey of Stored Serum

BLU antibody was present in the Wollongbar sera (Tweed-Lismore PP district) in every year tested, i.e. from 1968 to 1976. Yearly prevalence varied from 14% to 95% but the number, class and origin of stock sampled varied considerably from year to year. Of 117 sera tested, 65 (56%) were positive.

In the Glenfield collection 158 samples collected prior to December 1973 were negative. In contrast 32/65 (49%) samples collected after that date were positive. Limiting the comparison to animals resident at the research station itself, 50 sera collected during the period 1966-1973 were all negative, whereas 26/39 (67%) samples collected from mid-1974 to mid-1976 were positive in the GDP test.

Discussion

The weak neutralizing activity for BLU-20, initially found in about 4% of sera from animals in groups which had contained GDP reactors, could be confidently concluded to be false or non-specific since the titres were low and not reproducible. Although titres in VN tests for BLU-1 and BLU-21 were relatively low, they were considered to be meaningful because of the close association between VN and GDP reactivity, both in terms of reaction strength and geographic distribution. In addition, the absence of VN reactors in 71 GDP negative sera from the low prevalence zone suggested that false positives were uncommon.

To put these VN results in perspective, it should be noted that VN tests for BLU-20 were only applied to young cattle (Survey I), while those for BLU-1 and BLU-21 were applied to older animals (Survey II). However, this decision was based on information accumulating at the time which indicated that BLU-20 was confined to the most northern parts of Australia, while BLU-1 and BLU-21 were much more widely distributed (Della-Porta *et al.* 1983).

When this work began BLU-20 was the only known Australian BLU virus. The surveys reported here give no evidence of BLU-20 occurring in NSW. In the 5 years following these surveys, a limited number of samples were tested for BLU-20, all with negative results.

In 1979 BLU-1, BLU-21, and a number of BLU-related orbiviruses had been isolated elsewhere in Australia. Serological testing for BLU-1 and BLU-21 indicated that both serotypes had occurred in NSW. However, reactors in both VN tests were largely confined to the north coastal region, i.e. the area where almost all of the strong (2+ or 3+) GDP test reactors were found. The fact that 34 of the 94 GDP test-positive sera were negative for both BLU-1 and BLU-21 is interesting. It could indicate that other BLU serotypes had been active, or, alternatively, that closely related viruses are capable of inducing strong BLU GDP reactions. The virtual absence of strong GDP reactors, and VN reactors, in the low antibody prevalence areas, suggests that weak BLU GDP antibody is most reasonably explained as group cross reactivity stimulated by BLU related viruses.

This association between BLU virus activity and strong GDP reactions could imply a causative relationship. However, the association has been observed in an area of intense activity of BLU-related viruses and high antibody prevalence (Littlejohns *et al.* 1988). Therefore the strong reactions could also depend on the sheer number, frequency and variety of contributing BLU-related viruses, rather than on the specific activity of BLU-group viruses, although the different character of samples from two subgroups of the intermediate prevalence zone would suggest that this mechanism is not solely responsible.

It seems clear that group antibody can result from more than one experience of relevant viral infection. Early experiences, whether themselves leading to detectable antibody or not, would then have a priming role with respect to the response to later experiences.

BLU viruses are insect transmitted, and insects of the genus *Culicoides* are the only recognised vectors. *C. brevitarsis* has been suggested as a potential vector of BLU virus in Australia (Murray 1975), and has been infected experimentally by feeding on viraemic sheep (Muller *et al.* 1982). The distribution of BLU group antibody in old animals described in this paper is similar to that of *C. brevitarsis* in NSW as defined by Murray (1975). The differences in prevalence and distribution of antibody between young and old cattle could reflect variations in vector distribution, with extensions to the south and west under climatic conditions favourable to *C. brevitarsis*. The striking extension of antibody in the summer of 1973/74, as seen in the Glenfield sera, may be the result of an unusually favourable vector environment. Akabane virus was also active in the same endemically marginal areas in that season (Della-Porta *et al.* 1976) and *C. brevitarsis* is thought to be a vector for this virus (St George *et al.* 1978a).

The comparatively low (9%) prevalence of reactors in the Armidale PP district, compared to surrounding districts (Fig. 2), would be consistent with limited insect activity since the district is located on the New England plateau at an average elevation of 1400 m above sea level.

Since BLU is essentially a disease of sheep overseas, it was especially interesting to note the very low reactor rate for sheep in both surveys. Furthermore, these few reactions were weak and widely scattered, and may be non-specific reactions due to BLU-related viruses. However, some parts of the State with high antibody prevalence in old cattle, e.g. Warrialda, also have large sheep populations. In South Africa the vector of BLU (*C. imicola*) bites cattle much more readily than sheep (Nevill 1978). It is possible that a similar situation could operate with vectors of BLU in NSW, and such a mechanism may go some way towards explaining the rarity of infections in sheep.

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