The Occurrence of Antibody to Bluetongue Virus in New South Wales. II*. Coastal Region and Age Distribution Surveys

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Abstract

Three surveys of cattle for bluetongue (BLU) antibody were conducted over the years 1978-1980 in coastal areas of New South Wales. In each survey the samples were identified by age.

The prevalence of BLU-group antibody, demonstrated in a gel diffusion precipitin test, was highest in the north and decreased progressively to the south. Antibody prevalence increased with age. However, according to variations in prevalence by age and region, it was concluded that the activity of relevant viruses was highly variable between years and was geographically discontinuous.

Evidence is presented that much of the antibody found, especially in animals less than 4 years old, failed to persist from one year to another. Factors likely to contribute to more persistent reactions in older animals are discussed.

Neutralizing antibodies to bluetongue virus serotypes 1 and 21 were demonstrated and prevalence of these was associated with location and age, as was that of group-specific antibody.

Introduction

Two state-wide surveys conducted in 1978 (Burton and Littlejohns 1988) outlined the prevalence of bluetongue (BLU) antibody in sheep and cattle in New South Wales (NSW), and its regional distribution throughout the State. Antibody was very much more common in cattle than in sheep; its prevalence was highest on the north-eastern coastal strip; and antibody in samples from that area was more convincingly indicative of previous BLU virus (serotypes 1 and 21) activity than that found elsewhere. The surveys, together with results (unpublished) of further selective testing which included different age groups, also suggested that antibody prevalence increased with the age of the animals sampled. Three more intensive surveys were conducted in coastal regions during the years 1978–80 to further define the nature and origin of BLU antibody demonstrable in the State.

Materials and Methods

Surveys are described as III to V, for continuity with those reported previously (Burton and Littlejohns 1988). As with the earlier surveys, those reported here are based primarily on a gel diffusion precipitin (GDP) test which is based on a BLU-group antigen derived from serotype 20 (Sharp *et al.* 1988), although limited selective use was also made of virus neutralisation (VN) tests for BLU types 1 and 21. The animals sampled were identified by age (0-1, 1-2 years etc.) up to aged (4 or more years old) and the prevalence of BLU antibody in cattle in coastal regions of NSW, and the distribution of that prevalence according to age, was determined. Survey units used were river systems, represented, from north to south, by the Richmond (Casino and Tweed-Lismore PP districts), Clarence (Grafton), Hastings (Port Macquarie), Manning (Gloucester), Hunter (Maitland), Nepean (Moss Vale), Lower

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Shoalhaven (Braidwood), Upper Shoalhaven (Eden) and Bega/Bemboka (Eden) River systems. Pasture protection districts within which the river systems are contained are given in parenthesis to enable reference to surveys I and II, which were based on those units, and to fig. 1 of Burton and Littlejohns (1988). Upper and Lower Shoalhaven are distinguished from each other because the river transits a considerable length of coast in a south to north direction. The rivers can be arbitrarily grouped as north (Richmond, Clarence and Hastings), central (Manning, Hunter and Nepean) and south (Lower Shoalhaven, Upper Shoalhaven and Bega/Bemboka) coastal.

Survey III

Serum samples were collected within the period August-October 1978. From 116 to 844 samples were taken from individual survey units, making a total of 2836 from the nine river systems. Up to five properties were sampled in each unit. On each property a maximum of 10 animals in each age group was sampled.

Survey III was the most comprehensive of these surveys dealing with the distribution of antibody prevalence according to age and, as it was also the first, the results were analysed for retrospective evidence of relevant events in previous years. The difference between the prevalences of reactors in two age groups is assumed to reflect the incidence of serologically effective events in the period between the dates of the two groups first becoming susceptible to those events. This has initially been taken to be equivalent to the period between their births. If a group of animals, A, is one year older than another group, B, and they have otherwise shared the same environment, then the reactor prevalence in group A, (P_A) , is made up of the incidence of serologically effective events in year A, (I_A) , plus the prevalence of reactors in group B, (P_B) , applied to that part of group A which was not affected in year A, $(1 - I_A)$. That is,

$$P_A = I_A + P_B(1 - I_A)$$

 $I_A = (P_A - P_B)/(1 - P_B)$

Variance of prevalence (var P) was estimated by the conventional method,

$$\operatorname{var} P = P(1 - P)/n \text{ (Swinscow 1978)}$$

while that of I_A , (var I_A), was estimated by

var
$$I_A = [(1 - P_B)^2 \cdot \text{var } P_A + (1 - P_A)^2 \cdot \text{var } P_B](1 - P_B)^{-4}$$
.

(E. A. Roberts, personal communication.)

Note that years to which various I_A estimates are attributed are largely notional values. No allowance has been made for influences which may affect the exact timing of the periods, such as maternal antibody delaying susceptibility to specific viruses.

Two subsets of sera, one from the north coast (Richmond and Clarence Rivers), and one from the central coast (Hunter River) were also examined for antibody to BLU-1 and BLU-21 by VN test.

Survey IV

This was essentially a repeat of Survey III. It differed in detail in that it was not possible to obtain samples as promptly, and sampling extended from August 1979 to January 1980; and only the north and part of the central coastal areas were covered, that is, from Richmond to Hunter Rivers excluding the Manning.

In some cases the same properties that were used in Survey III were sampled but, more commonly, this was not possible and a neighbouring property was substituted. Individual cattle were not identified so that there could be no comparison of results between years for specific animals. Sera were tested only by GDPT test.

Survey V

This had the same design as Survey IV, with the Manning region included. Sampling was completed within the period August-October, 1980, that is, the same months as Survey III.

Results and Interpretations

Survey III

The results of this survey are set out in Fig. 1. A number of generalizations can be made.



Fig. 1. Prevalence of reactors in the gel diffusion precipitin test, by age and region, in survey III.

1 The reactor prevalence increased with age. With some minor qualification, this was apparent in all regions in which antibody was detected.

2 The reactor prevalence was highest on the north coast and decreased moving south. No reactors were detected in the most southern region, Bega/Bemboka. Similarly, the frequency of strong (2 + or 3 +) reactions decreased from north to south.

3 Maximum prevalence values were in the range 75-85%. In the Richmond region the maximum, of 77%, was found in the 3-4 year age group, with only 63% in the aged group. This suggests that figures of the order of the maximum values observed may reflect saturation exposure and that the sensitivity of the GDP test, for the purpose of detecting an antibody response to whatever is the natural immunogen in these regions, is about 80%.

Within these generalizations however, because the rate of increase of reactor prevalence with age is not uniform, either within or between regions, some departures from the general pattern may be noted and conclusions deduced from them.

1 Reactor prevalence of 12-20% was demonstrated in animals up to 1 year of age in three regions (Richmond, Hastings and Hunter). In two of these regions (Richmond and Hunter) this prevalence was greater than that for the 1-2 year age group and, when sufficient information was available, it was noted that reactors were generally less than 6 months old. It was concluded that most, or all, of the reactors detected in this age group were probably carrying antibody of maternal origin.

2 Over the other four age groups, in most areas, the prevalence does not increase at a uniformly diminishing rate, such as should be expected if there were a consistent annual incidence of relevant infections. This implies that there had been a substantial variation, from year to year, in the incidence of serologically relevant events.

3 Several comparisons illustrate gaps or irregularities in the epidemiological continuity of the general trend of viral activity diminishing from north to south.

(a) The prevalences in 1–2 year old animals in the Hastings and Manning regions were comparable (both 19%) and, combined, significantly higher (P < 0.001 by exact test, Swinscow 1978) than were those in the regions immediately to the north (Richmond and Clarence).

(b) Differences between 2-3 year old and 3-4 year old groups were substantial in Richmond and Clarence (differences of $2 \cdot 8$ and $2 \cdot 3$ s.e. respectively) and in the more southerly Hunter region ($3 \cdot 2$ s.e.). However, only negligible differences between the same age groups were found in the intervening Hastings and Manning River regions ($0 \cdot 6$ and $0 \cdot 8$ s.e. respectively).

(c) Convincing differences between 3-4 year old and aged groups were seen only in the widely separated Hastings and Lower Shoalhaven regions ($4 \cdot 4$ and $4 \cdot 6$ s.e. respectively).

The implications of differences in antibody prevalence between different age groups, and between regions, are more apparent when incidence rates are derived from the data. Estimates of the incidence of serologically effective events in notional years 1974 or earlier, 1975, 1976 and 1977 are shown in Fig. 2.



Fig. 2. Estimated incidence of serologically effective events by year, before survey III, and by region. Columns exceeding 2 standard errors of estimate are shaded. Years are shown in reverse order to facilitate visual comparison with Fig. 1.

Results of testing the two subsets of sera from this survey by VN tests for type-specific antibody are given in Table 1. Antibody to BLU-1 and BLU-21 was found in both areas, but at higher prevalence on the north coast. In both areas reactors to BLU-1 were more common than were those to BLU-21. As with group antibody, the trend of increasing antibody prevalence with increasing age was evident for both.

Area	Date of birth of animals	Age group	VN reactors as No. + ve/No. tested (%) BT-1 (%) BT-21 (5)		
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Northcoast ^A	1977/78	0-1	5/11 (45)	3/11 (27)	
	1976/77	1-2	3/17 (18)	0/17	
	1975/76	2-3	9/18 (50)	2/18 (11)	
	1974/75	3-4	10/17 (59)	8/17 (47)	
	1973/74	4 +	17/21 (81)	10/21 (48)	
Central Coast ^B	1977/78	0-1	0/5	0/5	
	1976/77	1-2	0/1	0/1	
	1975/76	2-3	1/12 (8)	1/12 (8)	
	1974/75	3-4	6/28 (21)	1/28 (4)	
	1973/74	4+	62/253(25)	18/253 (7)	

Table 1. VN tests of GDP test positive sera from survey III

^A PP Districts of Grafton and Port Macquarie.

^B PP District of Maitland.

Surveys IV and V

The results of these two surveys are given, together with those for the relevant part of survey III for comparison, in Table 2. To allow comparison of samples from successive years, the age groups from each survey have been converted to year of birth. In almost every group, defined by year of birth and region, there was a marked reduction in prevalence between 1978 (survey III) and 1979 (survey IV). It was only in the aged group that antibody was found consistently in 1979. In 1980 (survey V) reactor prevalences had again increased

Table 2. Comparison of surveys III, IV and V, carried out in 1978, 1979 and 1980 respectively Results are given as percentage positive, with numbers tested in parenthesis. Horizontal lines and vertical drops indicate the fact that the oldest age groups of surveys in 1979 and 1980 are composed of the oldest two age cohorts of the previous survey, though not necessarily the same individual animals

River system	Year of survey	1973/74 1974	Year of /75 1975/76	birth of catt 1976/77	tle tested 1977/78	1978/79	1979/80
Richmond	1978	$\frac{63 (27)^{A}}{4}$ 77 (2	$\frac{22}{404}^{A}$ $\frac{39}{400}$ (23)	5 (20) 2 (49)	20 (30)	6 (51)	
	1980	<u> </u>	$\frac{104}{50} \frac{104}{50}^{\text{A}}$	38 (45)	29 (45)	16 (44)	12 (43)
Clarence	1978 1979 1980	$\frac{74 \ (19)^{A}}{-14 \ (2)} \frac{52 \ (2)}{-14 \ (2)}$	$\begin{array}{c} \underline{25)} & 21 \ (19) \\ \underline{44)^{A}} & \underline{17} \ (42) \\ \hline & \underline{} & 28 \ (60)^{A} \end{array}$	0 (33) 10 (41) 15 (54)	0 (20) 0 (43) 22 (50)	0 (49) 24 (51)	13 (55)
Hastings	1978 1979 1980	$\frac{83 (48)^{A}}{27 (42)}$	$ \frac{72)}{41)^{A}} \qquad \begin{array}{c} 43 \ (42) \\ 0 \ (18) \\ \hline 17 \ (30)^{A} \end{array} $	19 (72) 0 (30) 0 (30)	16 (32) 0 (16) 0 (30)	0 (11) 0 (30)	0 (19)
Manning	1978 1979 1980	<u>46 (530)</u> ^A 40 (1	$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	19 (102) - 0 (44)	- - 0 (21)	0 (10)	0 (8)
Hunter	1978 1979 1980	$\frac{50 (155)^{A}}{46} \frac{46}{18} (155)^{A}$	$ \begin{array}{cccc} 46) & 20 & (49) \\ \hline 197)^{A} & 0 & (33) \\ \hline & 6 & (51)^{A} \end{array} $	3 (31) 2 (44) 2 (39)	14 (20) 0 (56) 9 (45)	5 (86) 0 (20)	0 (5)

^A Includes birth in the year indicated or earlier. Age 4 years or more.

in the two most northern regions, Richmond and Clarence, by comparison with 1979. However, prevalence levels found in similar age groups in 1978 were generally not reached. Further south (Hastings, Manning and Hunter) there was little evidence of new antibody which would indicate virus activity in the previous year (1979–1980).

Discussion

Two previous surveys (Burton and Littlejohns 1988) had established that BLU-20 had not, within the age of the cattle surveyed, occurred in New South Wales but there was evidence that BLU-1 and BLU-21 had occurred and contributed substantially to the pattern of reactors found. Other questions, including whether other BLU serotypes had occurred, and to what extent BLU related viruses were responsible for cross-reactive antibody, remained. The surveys reported in this paper then sought to concentrate on areas of high antibody prevalence, with particular attention to geo-topographical, rather than administrative, areas to recognise differences in prevalence according to age, and to thereby allow retrospective deductions regarding the incidence of serologically effective events in previous years. The surveys also provided data on changes in antibody prevalence within defined cohorts, which could be compared with current events as disclosed by more direct observations on sentinel animals (Littlejohns *et al.* 1988).

The antibody prevalences detected, notably in the Richmond region, allow an estimate of sensitivity of approximately 80%. This estimate is valid only within the context in which it was determined. According to concepts developed here and elsewhere, antibody demonstrable in this test may be due to multiple exposures to related agents which induce various levels of cross-reactivity. Additionally, biotype variation within serotypes, or even antigenic variation in virus proteins other than those responsible for serotypic determination, can be expected to affect the immunogenic efficacy of specific agents with respect to the single antigen of the GDP test used in this work. Caution should therefore be exercised before the estimate is extrapolated or transferred to other contexts.

Precipitating antibody detected in animals less than about 6 months of age may be of maternal origin. Therefore those results should be discounted as evidence of recent virus activity.

From the age distribution of antibody prevalence (Fig. 1) and derived annual incidences (Fig. 2) it can be concluded that there is considerable annual variation in the level of activity of relevant viruses. This is most clearly seen in epidemiologically marginal areas, where there is no apparent activity in some years. In survey III in the lower Shoalhaven, 48% reactors were found in the aged group, but none in younger animals. This would indicate a substantial level of activity in, or before, the year of 1973–74, the last year reflected by the reactor group but not younger animals. There is no serological evidence of virus activity in the following years to 1978. This interpretation may be consistent with other evidence of exceptional activity by an unrelated arbovirus (Akabane) in these marginal areas in the 1974 season (Hartley *et al.* 1977).

Perhaps more importantly, there appears to have been considerable annual variation in virus activity in the more northern areas, and these variations were not consistent between regions. In the most northerly regions, Richmond and Clarence, the negligible prevalence of antibody in the 1–2 year group, and the differences between 1–2 and 2–3, and 2–3 and 3–4 year groups, indicate significant virus activity in 1975 and 1976, but little in 1977. On the other hand, the same analysis applied to the adjacent regions, Hastings and Manning, indicates virus activity in 1976 and 1977, but not in 1975. However, this apparent difference may be artefactual. It has been noted that years to which serologically effective events are related are notional, and are determined on the assumption that a given age group will reflect events which occur in its first year of life. It could also be argued that, if there is a high prevalence of antibody in the dams, and if that antibody is protective against the virus serotypes that are active in the year in question, then the susceptibility of the young

animals could be deferred. Then, across a regional population the relevance of differences in antibody prevalence between age groups to the incidence of events in specific years may be shifted by one year. Whether in 1975 and 1976 there would have been enough difference in adult antibody prevalence between Richmond and Clarence regions on the one hand and Hastings and Manning regions on the other to account for the differences in incidence estimates would seem to be very doubtful, but may depend critically on the serotypes involved at and before that period.

In only the widely separate regions of Hastings and Lower Shoalhaven were there convincing prevalence differences between 3-4 year and aged groups, indicating virus activity in or before 1974. In the north coast regions, especially Richmond, this may be because saturation point has been reached by the age of 3-4 years and demonstrable prevalence is limited by the sensitivity of the test. Also sample sizes are small, of the order of 20-30 animals. In Manning and Hunter regions larger numbers were tested and, assuming the test to have a sensitivity not less than approximately 80%, as demonstrated on the north coast, saturation exposure had not been reached. Therefore there is no evidence of virus activity in those regions, and probably not in Nepean, in or before 1974, as far as ages of animals sampled allows.

The general conclusion is that virus activity on a regional and annual basis may be quite sporadic and not form an epidemic continuum. A corollary is that limits of distribution defined over a short term have very little predictive value.

Comparing surveys III and IV, the low prevalence of reactors in 1979 is quite striking and several possible explanations have been considered.

1 The location of the stock was not comparable. The same herds were not sampled in both years except in a few cases. However, where the same herds were sampled the dramatic fall in antibody prevalence was still evident. Therefore this explanation is rejected.

2 The sampling was not comparable in time. Surveys III (1978) and V (1980) were concluded in October, while Survey IV continued into January of the following year. Studies of sentinel cattle (Littlejohns *et al.* 1988) have shown that new BLU group antibody usually appears in the March-May period, and is often transient. The delay in completing Survey IV could have contributed to the low prevalence in 1979, but as the low prevalence was evident from the beginning of the survey period, this delay does not seem to have been a major factor.

3 There were few effective infections in the 1978–1979 season. This seems to be the simplest and most likely explanation. Note that it also requires that much of the antibody detected in survey III, particulary in the younger animals, must have been of a transient nature.

The fact that group reactivity may be transient is of paramount importance in the proper interpretation of results for either survey or certification purposes. This transiency has been confirmed in direct observations of sentinel animals with antibody being detected for as little as one week (Littlejohns *et al.* 1988). It has also been previously observed in the animal from which the type strain of BLU-21 was isolated (St George 1979) and elsewhere in the world (Sellers 1985).

On the other hand, there is also evidence that some of the reactions, mainly in the aged groups, are much more persistent. This may be due to either quantity of experience, with the number of relevant exposures increasing with age, or quality of experience, depending upon the identity or degree of relatedness of available viruses to the BLU group. In Richmond, Hastings and Hunter, where, between 1978 and 1979, antibody was almost completely eliminated from younger animals, about 50% of reactors persisted in the aged animals. Comparison of reactor prevalence between different age groups in survey III (1978) may reflect the greater experience of the older animals, and imply a quantity of experience accumulated over the years as the likely basis for persistence of antibody. By contrast,

persistent antibody may also be established within one season, as seen in the Lower Shoalhaven in aged animals in 1978 with a 48% prevalence of antibody. The much lower prevalence in younger animals indicates very little effective virus activity since 1974, and evidence presented previously (Burton and Littlejohns 1988) showed that no reactors were detected in this vicinity prior to 1973. It is therefore likely that the reactors among older animals in the Lower Shoalhaven in 1978 are a result of exposures in the summer of 1973–74. Whether the persistent antibody induced is a result of a sufficient number of infections in the one season, or whether viruses of particularly high immunogenic potency, in particular BLU viruses, were responsible, is open to conjecture. Regrettably, VN tests for BLU viruses were not applied to the GDP test reactors in question.

A comparison of surveys IV and V shows that some virus activity had occurred in the year 1979–1980 in the more northern areas. There was little evidence of activity further south in that year.

It is not entirely clear how these data and other information on antibody distribution and prevalence should be finally interpreted. It is unlikely that experimental observations can match the diversity of circumstances, including multiple experiences of variously related agents, by hosts with diverse backgrounds of immune priming and protection, that may occur in the field. Certainly, it can be expected that serial infection by related agents will result in an amplified cross-reactive antibody response because cross-reactivity depends on shared determinants against which an animal will be virtually hyperimmunized by the serial infection. Hence the range of agents to which a test relates will be wider when field samples are examined than would be indicated by experimental infections using single agents, or a limited selection of them. Caution should therefore be exercised in drawing conclusions regarding BLU virus activities from group serological data. This argument relates most obviously to the so-called group tests, but serial infections can also compromise the typespecificity of neutralization tests (Jeggo *et al.* 1983).

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