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St. George, T.D. and Peng Kegao (ed.) 1996. Bluetongue disease in Southeast Asia and the Pacific. Proceedings of the First Southeast Asia and Pacific Regional Bluetongue Symposium, Greenlake Hotel, Kunming, P.R. China, 22–24 August 1995. ACIAR Proceedings No. 66, 272p.

ISBN 1 86320 187 4

Editorial management and technical editing: E. and R. Beckmann, Canberra
Pre-press production: Arawang, Canberra

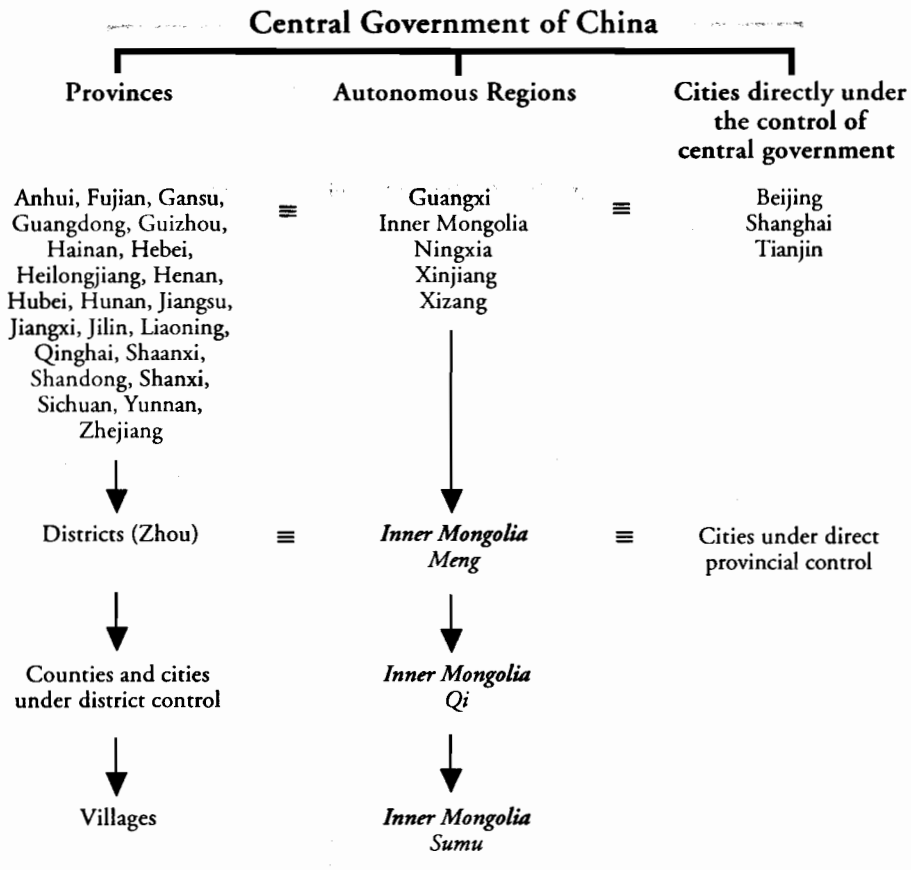
Bluetongue Disease in Southeast Asia and the Pacific

Proceedings of the First Southeast Asia and Pacific Regional Bluetongue Symposium, Greenlake Hotel, Kunming, P.R. China, 22–24 August 1995

Editors: **T.D. St. George and Peng Kegao**

Veterinary Services in China

Some explanation will perhaps enhance the understanding of some of the papers from China where various terms for administrative areas are mentioned. This has a bearing on bluetongue as the responsibility for diagnosis and control is shared at various levels of government and associated veterinary services.



(The Inner Mongolia terms are shown in italics)

Every province and autonomous region has a provincial veterinary station and every district, county and village has a correspondingly titled veterinary station. Each level derives financial support from its own government level with some contribution from the veterinary station of the level above.

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The First Southeast Asia and Pacific Regional Bluetongue Symposium

Welcoming Address

Feng Jinglan*

LADIES and gentlemen—I am very pleased to be the Honorary President of this Conference. It is indeed my great honour, on behalf of the Department of Animal Industry and Veterinary Medicine, Minister of Agriculture, China, and Professor Zhang Nianzu and Dr Peter Kirkland, the Co-Chairs of this Symposium, to welcome all representatives and friends from Australia, China, India, Indonesia, Papua New Guinea, Japan, Malaysia, New Zealand, Philippines, Thailand, United Kingdom, United States of America and Vietnam who are participating in this Symposium.

First of all, we would like to recognise and extend our appreciation for the well thought out preparations and hard work the Organising Committee has done for this event. This group consists of Professor Zhang Nianzu, Peng Kegao, Li Huachun, Dr Toby St. George, Neville Hunt, Dr Lorna Melville and Dr Peter Kirkland. Special thanks are due to Dr Toby St. George and Peng Kegao for the editorial work for the Symposium proceedings. We also appreciate the contribution offered by both the Yunnan Provincial Commission on Science and Technology, the Yunnan Provincial Bureau of Animal Husbandry and the Yunnan Tropical and Subtropical Animal Virus Disease Laboratory. Their input will ensure that this is a successful conference.

Since the first publication of a description of bluetongue by Theiler in South Africa in 1905, numerous studies have been conducted in many countries. A much better understanding of the characteristics and epidemiology of the virus has resulted from this research, particularly in the last 40 years. Much of the knowledge acquired has been directly applicable to the livestock industry. This Symposium provides the opportunity for researchers and scientists to exchange experience. The subjects under discussion will enhance research and control of bluetongue disease which is widely distributed and can have devastating effects. I encourage you to take full advantage of this Symposium, by active participation in formal and informal discussions.

I would like to take this opportunity to briefly describe the current status of animal industries in China. During the last 15 years, since the implementation of the Reform and Opening-up policy, livestock industries have enjoyed rapid growth. In 1994, national meat production amounted to 45 million tonnes. Annual per capita consumption is: meat 33.8 kg, eggs 12.6 kg, milk 5.2 kg and aquaculture 18.3 kg. Milk production, in particular, is far below that of developed countries. China is a vast, populous nation, and offers great potential for further development but at the same time faces enormous challenges. We must continue to develop and protect livestock industries. Obviously, the prevention and control of livestock diseases is extremely important in these endeavours. Since the first isolation of bluetongue virus in the southern province of Yunnan in 1979, China has focused a great deal of effort on the diagnosis and immunology of the disease. At the present time we are vigorously pursuing regional trials of bluetongue vaccines, which we believe will provide a powerful tool for the prevention of disease. We would like to see an increase in cooperation and exchange among all those who are here today.

August is the hottest month of the year in China. However, Kunming, is known as the 'Spring City' because of its pleasant climate. I wish you all a successful and productive conference and an enjoyable stay in China that you will remember for years to come. Thank you.

* Symposium Honorary President; Vice Director of the Department of Animal Industry and Veterinary Medicine, Ministry of Agriculture, Beijing, People's Republic of China.

Epidemiology

Introduction

THE epidemiology of bluetongue involves the interaction of five elements: the bluetongue viruses (24 at present); the vertebrate hosts in which the viruses multiply subclinically; susceptible sheep; the environment; and a range of *Culicoides* vectors capable of being infected and transmitting bluetongue virus (discussed in a later section).

These Proceedings present our current understanding of the epidemiology of bluetongue viruses in the countries represented at the Symposium. Less than 20 years ago, bluetongue viruses were not known to exist east of India. The great majority of the bluetongue viruses discovered since then are serotypes already known. In most of Asia, bluetongue is not an expanding infection and disease but rather an infection which has existed ever since cattle, buffalo and deer have been present. Bluetongue is now coming to our notice for many reasons, including changes in husbandry practices and better detection technology.

The exception to this generalisation applies in the countries to the far southeast of the Asian region, namely Papua New Guinea, Australia and the Pacific Island nations, where ruminants were not present before European colonisation. Proven *Culicoides* vectors, dependent on ruminants, have spread with the bluetongue viruses to occupy the newly created ecological niches. Bluetongue is thus emerging in this area, to the east of Weber's line which marked a geographical barrier for many animal and plant species.

Understanding the epidemiology of bluetongue in southeast Asia, east Asia and the Pacific Islands is impossible without identifying all the virus serotypes circulating in this vast region, as infected insects are blown across national boundaries and very significant ocean gaps.

In the papers in this section, a fine start has been made to bring together the information on bluetongue in China, its neighbouring countries and the region in general. It is essential that this process continues so that, in future, control strategies can be based more fully on objective data.

One of the most interesting epidemiological observations is that, in north China, sheep and goats show higher bluetongue group antibody prevalences than cattle, in contrast to the findings in most other regions of Asia and the Pacific. Another difference is that, in Inner Mongolia, some bluetongue infection continues in winter, suggesting an additional vector system: this finding again contrasts with those in subtropical regions and other temperate areas of the world. So, through this sharing of information, first at the Symposium and now in these Proceedings, we have added yet another new element into our understanding of bluetongue epidemiology.

A Short History of the Discovery of Bluetongue in the World

T.D. St. George*

THE history of bluetongue in the world falls into two distinct phases. The first phase of discovery was based on the diagnosis of clinical bluetongue, mostly in outbreaks in sheep in previously bluetongue-free areas. This produced a picture of a disease which had spread from an original focus in southern Africa to encompass most of Africa, some adjacent parts of Europe, southern Asia and then to north America via ruminant imports. This view was dealt a major blow in 1977, however, when a virus isolated from an insect collected in Australia, a continent with no bluetongue disease, was identified as a bluetongue virus. Since then, large-scale surveys have linked the previously discrete areas of the world into a continuum. Disease is now accepted as only a small manifestation of the presence of this widely distributed group of closely related viruses which infect a wide range of ruminants.

The Emergent Phase: 1900 to 1977

Bluetongue first came to notice when Merino sheep were introduced to southern Africa. The first descriptions of the disease were published there. Bluetongue was a major killer of sheep, with surviving animals suffering a loss of productivity. Much later, research in southern Africa revealed that bluetongue viruses also circulated in cattle and many wildlife species. The disease was soon also recognised in Egypt (1907), Kenya (1909) and West Africa (1927). Outside Africa, bluetongue outbreaks were diagnosed in 1943 in Cyprus, and from then until 1949 in nearby areas of Asia, such as Palestine, Turkey and Syria. In 1956, a major epidemic occurred in Merino sheep in Spain and Portugal. This caused significant alarm, especially in Australia with its vast flock of pure Merino and Merino-based breeds. Around the same time, the isolation of bluetongue virus confirmed its presence elsewhere in the world too; in the USA (1952), in Pakistan (1959) and India (1963).

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In Spain, a combination of movement controls, slaughter out policies and vaccination were applied, and bluetongue appeared to have been eradicated from the country. While the sanitary measures were applied only to sheep, international movement controls or prohibitions were applied to countries where the disease in sheep had been reported, since cattle were known to be subject to subclinical infection by bluetongue viruses.

Endemic Zone Phase

A turning point in understanding bluetongue epidemiology came in 1977, with the recognition that bluetongue virus existed without concurrent disease in Australia, several thousand kilometres away from the nearest known endemic area. Seven years previously, a system of sentinel herds, using healthy cattle as virus indicators, had been developed in Australia. This system had been established to define both the extent of arbovirus infection of ruminants and arbovirus epidemiology that was independent of disease. Once bluetongue was identified in Australia, this existing surveillance technique was used to study the virus's recent history in Australia and to search for additional serotypes of the bluetongue group. The sentinel herd approach has since been applied successfully in other countries, including several descriptions of successes in these Proceedings. Using sentinel cattle, bluetongue viruses of many serotypes have been found in northern Australia, the Caribbean and Central America, Indonesia, Malaysia, China and many other areas. Serological studies, using these newly isolated viruses as well as imported standards, has shown that the tropics, subtropics and some fringing temperate areas of the Americas, Africa, Asia and Australia are bluetongue endemic regions.

Until recently, a country was considered as infected with endemic or epidemic bluetongue according to political boundaries. However, more detailed epidemiological studies have shown that in countries where climate allows the *Culicoides* vector

species to exist only in the hotter areas, there are major zones that are always free of bluetongue virus. For example, this is true of most of the European Community, Canada, southern Australia, northern Japan and parts of far north China. Full acceptance of regional freedom would usher in a new phase for trade and other livestock movements.

How Many Bluetongue Viruses Are There?

Many years ago, apparent failures of vaccines in South Africa led to the realisation that there was more than one antigenic type of bluetongue virus. Cross-protection tests in sheep by W.O. Neitz demonstrated that 10 representative viral isolates from field cases produced solid immunity against the homologous isolate but only partial or no protection against heterologous strains.

This was the first formal classification of bluetongue viruses: based on cross protection tests, this classification established the first 10 different virus types in South Africa. Serological tests, which correlate with cross protection tests, were later used in South Africa to develop the reference system which added other newly discovered bluetongue viruses, and which now divides the bluetongue viruses into 24 serotypes. The World Reference Centre was developed at The Veterinary Research Institute in Onderstepoort, South Africa. New bluetongue viruses are submitted to the Virus Laboratory there for confirmation as an existing type or as a candidate new serotype.

It is well established that bluetongue viruses of the same or separate serotypes can exchange genetic information during dual infections of hosts without affecting their serotypes. The eventual number of serotypes will probably not be much greater than the 24, as the vast regions recently shown to be endemic have usually yielded viruses that fit into the already established serotype classification. Biochemistry and topotyping have produced new parameters for measuring genetic diversity and will, in time, lead to more understanding of regional evolution of the viral complex, but the system of serotypes, based on neutralisation tests, remain the presently accepted scheme of classification.

Vectors

Initial studies on *Culicoides imicola* in South Africa incriminated this species, and the *Culicoides* genus of biting midges, as a bluetongue vector. The number of *Culicoides* species now proven as vectors is limited to five well studied species in Africa, Australia and North America. However, bluetongue viruses have been isolated from six more *Culicoides* species and there is experimental evidence that others can support virus replication. The list of potential vector species is longer still, but there are insufficient resources to test all *Culicoides* species feeding on ruminants for a capacity to transmit bluetongue virus. Indeed, there is strong epidemiological evidence that not all *Culicoides* species which do feed on cattle, buffalo, sheep and goats, are capable of being infected with, and/or transmitting, the viruses.

In practical terms, the northern and southern boundaries of epidemic bluetongue areas in northern Africa, southern Europe, North and South America, Japan, Australia and the Pacific islands are reasonably known to be linked to the distribution of a very few *Culicoides* species. For several of these, little is yet known of important aspects of their biology, such as their breeding sites and larval development. The fluctuations in *Culicoides* populations, caused by season, altitude, winds, rainfall and availability of vertebrate hosts for blood meals, are fundamental in their affects on the epidemiology of bluetongue disease, but are reasonably understood for only a few species.

Control

Bluetongue vaccines evolved to make sheep farming possible in southern Africa in the early part of this century. The use of vaccines has thus been largely targeted at preventing clinical disease in South Africa, with the other major area of vaccine use being the USA. The advantages and disadvantages of the various vaccines attenuated live, killed and inactivated and subunit vaccines have been explored in these Proceedings. Vaccines have not been applied, in an attempt to control virus spread, to those vertebrate species (buffalo, cattle and wild ruminants) in which bluetongue virus multiply but do not cause any clinical signs.

Until recently, quarantine and movement controls were applied rigorously to prevent the spread of bluetongue, as it appeared to be a world-threatening 'emerging disease' being carried from continent to continent by the trade of permanently infected ruminants. Although many of these restrictions remain in place throughout the world, evidence presented in these Proceedings suggest such controls are not necessarily rational. Indeed, there is no evidence that the present distribution of bluetongue viruses has been influenced by these historical actions.

Coordinating Research

The publication of field and research information on bluetongue virus and its vectors, including papers presented at many scientific meetings, has helped the spread of information around the world.

However, effective coordination has been achieved only relatively recently by means of specific symposia:

- Bluetongue and related orbiviruses, Asilomar, California, USA, 16–20 January 1984 (Proceedings edited by T.L. Barber, M.M. Jochim and B.I. Osburn)
- Symposium on bluetongue, African horse sickness and related orbiviruses, Paris, France, 17–21 June 1991 (Proceedings edited by T.E. Walton and B.I. Osburn)
- First Southeast Asia and Pacific Regional Bluetongue Symposium, Kunming, People's Republic of China, 22–24 August 1995 (these Proceedings)

Publication of the proceedings of these meetings, in English and in Chinese, will provide further information on the history of bluetongue. Future meetings will lead to still more knowledge of bluetongue's complexities.

Bibliography

Barber, T.L., Jochim, M.S. and Osburn, B.I. (ed). Bluetongue and related orbiviruses. Progress in Clinical and Biological Research, vol. 178, Alan R. Liss Inc., New York.

Gibbs, E.P. and Greiner, E.C. 1988. Bluetongue and epizootic hemorrhagic disease. In: Monath, T.P., ed., The arboviruses: epidemiology and ecology, vol. II. CRC Press, Boca Raton, Florida, 39–70.

Howell, P.G. 1979. The epidemiology of bluetongue in South Africa. In: St. George, T.D. and French, E.L., ed., Arbovirus research in Australia. Proceedings 2nd Symposium. CSIRO/Queensland Institute of Medical Research, Brisbane, 3–13.

Sellers, R.F. 1981. Bluetongue. In: Gibbs, E.P.J., ed., Virus diseases of food animals. Academic Press, London, 567–584.

Verwoerd, D.W. and Erasmus, B.J. 1994. Bluetongue. In: Coetzer, A.W., Thomson, G.R. and Tudin, R.C., ed., Infectious diseases of livestock. Oxford University Press, Cape Town, 442–459.

Walton, T.E. and Osburn, B.J. ed. 1992. Bluetongue, African horse sickness and related orbiviruses. Proceedings 2nd International Symposium, CRC Press, Boca Raton.

The Global Distribution of Bluetongue

R.A. Hawkes*

Abstract

Bluetongue virus (BLU), earlier thought to be an emergent virus originating in the African region, is now known to be endemic in most tropical regions of the globe, with sporadic incursions into subtropical and temperate regions. The 24 serotypes of the virus are not uniformly distributed throughout the globe, so their distribution has implications for immunisation programs and other aspects of trade in livestock. This paper summarises the known data on the present and past global distribution of bluetongue virus and its serotypes, and briefly discusses the possible factors associated with this distribution.

BLUETONGUE virus (BLU) has the distinction of being the first of the arthropod-borne viruses to be isolated in the laboratory (Karabatsos 1985). For some time bluetongue was considered to be a virus confined to Africa, its continent of initial isolation. However, the more widespread distribution of this virus throughout the world was later acknowledged, and the multiplicity of its serotypes recognised. At present, bluetongue viruses constitute a serogroup with 24 members: these 24 serotypes are differentially present, endemically or transiently, over most of the tropical and subtropical parts of the globe.

The purpose of this review is to present the current and past distribution patterns of BLU serotypes throughout the world. The subject is interesting scientifically and of some importance economically. From the scientific viewpoint, it is apparent that the ability of bluetongue viruses to persist permanently or transiently in a given area is largely dependent on the presence of sufficient populations of the appropriate vectors and hosts. This presence, in turn, depends on climatic variables such as temperature, altitude, moisture availability and wind patterns, as well as on other variables such as land-use, livestock transfer and husbandry practices. Why certain serotypes are found in different countries, but sometimes only in certain parts of such countries, are less well understood.

The distribution of serotypes also has economic implications. Serotypes are delineated by their inability to show significant reciprocal cross-neutralisation with other BLU isolates in cell culture neutralisation tests. This classification is correlated with in vivo cross-protection. Given the lack of significant cross-protection between different serotypes, logic dictates that nations will have a cautious attitude towards importation of potentially infected ruminants from countries possessing exotic serotypes. In addition, the range of serotypes actually or potentially present in a country will influence vaccination policies, where such are practiced.

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Limitations of the Data

Extensive surveys have shown that BLU exists epidemically in most parts of the tropics and subtropics where there are sufficient populations of the appropriate vector species and mammalian (ruminant) hosts (Gibbs and Greiner 1994). At distances further from the equator, the virus tends to occur intermittently, with incursions dependent on the variables mentioned above. In certain countries it is not possible to be sure whether the available data represent a truly epidemic situation, or whether annual reintroduction of the virus from nearby countries is occurring. Before describing the distribution of the serotypes themselves, it is important to discuss the limitations of the existing data.

Imprecision

Where the existence of BLU serotypes is based on virus isolation and identification, a high degree of reliability can be placed on the data. However, if reporting has been based solely on serological testing, less certainty is warranted. Where the agar gel

immunodiffusion (AGID) test has been used, no indication of serotype is possible, because of the group-reactive nature of the test. Furthermore, the results of such surveys are confounded both by insensitivity, leading to an under-estimate of prevalence, and by excessive cross-reactivity with other orbivirus groups, leading to an over-estimate of prevalence. Where the competitive enzyme linked immunosorbent assay (cELISA) with BLU group-reactive monoclonal antibody has been used, the data are representative of genuine BLU infection prevalence but give no indication of serotype.

In those cases where serological testing has been based on virus neutralisation (VN) tests in cell culture, there is a much better prospect of establishing genuine serotype presence. Caution must be exercised even here, however, since a limited but definite cross-reactivity exists between members of different serotypes of the BLU serogroup, even with the VN test. Cross-reactive VN antibodies within an individual animal are accentuated even further when multiple BLU infections occur (MacLachlan 1994). Furthermore, different studies have often employed variations in methodologies (for example, plaque inhibition rather than plaque neutralisation), and used different criteria for evaluation.

For these reasons, the accompanying tables show serotype presence that has been established by virus isolation separately to that established by type-specific serology. Where surveys using only AGID have been carried out in a country, this is noted in the text.

Incompleteness

The data presented here are incomplete for at least two reasons. First, sampling in many countries has been incomplete or even absent. Second, there has sometimes been a reluctance on the part of governments to release data, for trade or other considerations. Indeed, serological evidence supporting virus presence has been formally published for several countries currently registered in the World Animal Health Reports of the Office International des Epizooties (OIE) as never having reported the disease. For these reasons, if a country is not mentioned in the accompanying data, this does not necessarily imply the absence of bluetongue virus.

Freedom from clinical disease is not a good indication of absence of the bluetongue virus, since it may be present in species which rarely exhibit symptoms. However, it is a reasonable assumption that countries at latitudes above those able to sustain vector species are indeed free of bluetongue.

Intra-national variability

Bluetongue viruses are seldom present in equal intensity throughout the whole of a country because of within-country differences in climatic and other factors. Sometimes this differential presence is reflected in the literature, in that the locations of blood and vector samplings are given, but often it is not. This should be kept in mind when interpreting data from countries that embrace, within their borders, large variability in latitude, altitude and precipitation patterns.

Instability

Even in areas where bluetongue viruses are endemic, the predominant serotypes are not always the same ones in successive years. Even in surveillance studies employing consistent methodology, viruses thought to be genuinely persistent in an area may sink to undetectable levels of prevalence from time to time, only to emerge in succeeding years (Gard and Melville 1992). The reasons for this cycling are largely obscure. It is beyond the scope of this review to present these year-by-year changes in serotype for parts of the world where bluetongue is endemic.

In areas where the virus occurs transiently, disease tends to occur in immunologically naive populations of sheep. In such areas, it is not always the same serotype which is involved in successive appearances. For these 'epidemic' areas, an attempt has been made to specify the serotype in each section.

Global Distribution of Serotypes

The following information, summarised graphically in Figure 1, is presented under the continent/country classification adopted by the annual World Animal Health reports of the OIE. The designation 'disease never reported' is based on the OIE's latest available report (Anon. 1993), modified where reports in the scientific literature have indicated the presence of BLU antibodies or BLU isolations.

Africa

Of the 24 known BLU serotypes, only BLU23 has not been shown to be present in Africa (although only serological evidence exists for BLU17, 20 and 21). Most BLU serotypes are probably endemic over most of the continent, with the possible exception of the north western corner (Gibbs and Greiner 1994). Of the 47 mainland and island African countries listed in the OIE reports, only seven (Algeria, Libya, Tunisia, Guinea Bissau, Ethiopia, Comoros and Madagascar) have either never reported the disease or never shown serological evidence of virus presence (Anon. 1993). Fifteen countries have clinical or serological evidence for virus presence, but no data on serotypes: Burkina Faso (Anon. 1993), Ivory Coast (Taylor and McCausland 1976), Ghana (Taylor and McCausland 1976), Guinea (Konstantinov 1990), Mali (Maiga and

Sarr 1992), Niger (Weitzman et al. 1991), Chad (Taylor and McCausland 1976), Zaire (Anon. 1992), Tanzania (Hamblin et al. 1990), Somalia (Hussein-Hag et al. 1985), Botswana (Simpson 1979), Mozambique (Ferriera and Rosinha 1986), and Zambia (Ghirotti et al. 1991). Some countries have presented no data but BLU is probably present in most of these (Ozawa 1985). Eleven African countries (OIE classification) have serotype-specific data (Table 1).

The distribution of serotypes shows no particular pattern throughout the continent, except that, so far as can be determined from the uneven intensity of sampling, the multiplicity of serotypes diminishes in the north, as distance from the equator increases. Both serotypes from Reunion were identified during an investigation of a single outbreak occurring on the island in 1979.

The Americas

The BLU status in the United States of America has been well studied for many years, and the situation in Central America and the Caribbean studied increasingly in recent times. On the South American continent, studies have been more restricted and the southern limits of the virus have yet to be determined.

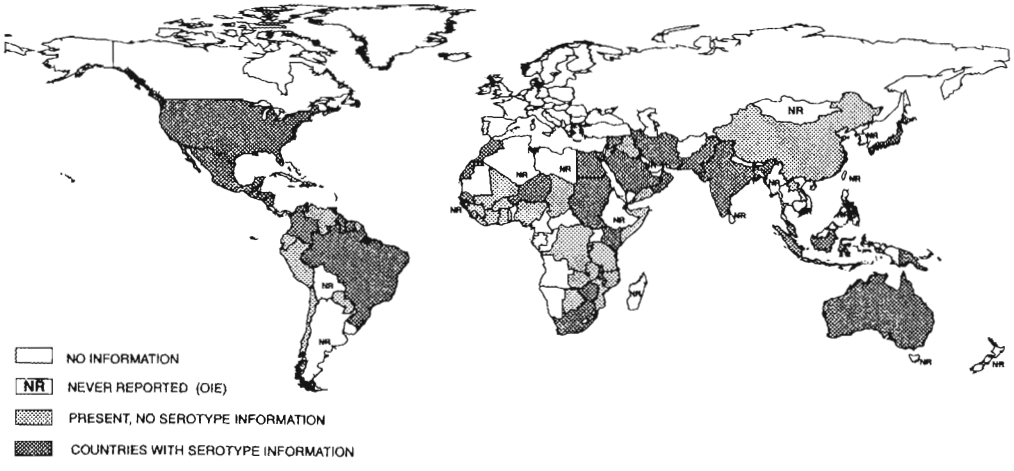

Figure 1. Global distribution of bluetongue.

Table 1. Bluetongue (BLU) serotypes in Africa.

OIE zone	Country	BLU serotypes identified by: virus isolation	BLU serotypes identified by: serology (additional)	Reference
1	Egypt	1, 12,16	4, 10	Barsoum 1992, Hafez and Ozawa 1981
	Morocco		10	Tomori et al. 1992
2	Cameroon	1, 4, 5, 12, 14, 16		Ekue et al. 1985a, 1985b
	Nigeria	1, 2, 3, 5–8, 10, 11, 12, 16, 22	4, 9, 13, 20	Sellers 1984, Herniman et al. 1983, Lee et al. 1974
	Senegal		6, 14	Lefevre and Taylor 1983
4	Kenya	1, 2, 3, 4, 8, 13, +3 untyped	? 9 others	Sellers 1984, Davies 1978
	Sudan	1, 2, 4, 5, 16	6–10, 14, 17, 20, 21, 22	Mohammed and Mellors 1990, Abu-Elzein 1985, Elfatih et al. 1987
5	Malawi		1, 2, 3, 5, 8, 10, 14, 15, 20, 21, 22	Haresnape et al. 1988
	South Africa	1–15, 18, 19, 24		Sellers 1984, Nevill et al. 1992
	Zimbabwe	11		Blackburn et al. 1985
	Reunion	2	4	Barrem et al. 1985

Of the 37 American countries or island groups listed by OIE, five have presented no data and seven (Argentina, Bermuda, Bolivia, Cuba, Grenada, Haiti and Uruguay) have never reported the disease or positive serology (Anon. 1993). The OIE lists a further five countries (Belize, Paraguay, Venezuela, Montserrat and St. Vincents and the Grenadines) as having clinical or serological evidence for the virus (Anon. 1993). Chile (Tamayo et al. 1983), Ecuador (Lopez et al. 1985), French Guiana (Lancelot et al. 1989) and Peru (Rosadio et al. 1984) have untyped virus or group serology only. Other countries have serotype-specific information (Table 2).

The Canadian data represent incursions of BLU11 into the Okanagan Valley in southwestern Canada in 1975 and 1987/88. In the USA the virus is epidemic in the southern and western States (Gibbs and Greiner 1994). BLU10, 13, 14 and 17 may have evolved together in the USA over a long period of time but BLU2, which is apparently confined to the southern States, may be a recent introduction from elsewhere (Heidner et al. 1992). To date, the only serotype in common between those on the continent (Mexico and northwards) and those in the other parts of the Americas is BLU17: with respect to bluetongue, the two regions appear to be distinct ecological zones (Gibbs and Greiner 1994).

Asia

Of the 35 Asian countries listed by the OIE, nine have provided no data on BLU prevalence and ten (Bahrain, Hong Kong, Korea, Mongolia, Sri Lanka, Myanmar, Philippines, Singapore, Taipei China and Vietnam) have reported the absence of past disease or any positive serology (Anon. 1993). Nevertheless, some of these countries probably have one or more BLU serotypes. Five countries have reported the presence of bluetongue disease and/or positive group serology: these are Laos, People's Republic of China (Anon. 1993), Iraq (Hafez et al. 1978), Yemen (Stanley 1990), and Bangladesh (Hakim 1985). Eleven countries have serotype-specific data (Table 3).

Table 2. Bluetongue (BLU) serotypes in the Americas.

OIE zone	Country	BLU serotypes identified by:		References
		Virus isolation	Serology (additional)	
1	Canada	11 (1975, 1977/78)		Dulac et al. 1992, Shapiro et al. 1987, Thomas et al. 1982
	Mexico	10, 11, 13, 17		Stott et al. 1989
	USA	2, 10, 11, 13, 17		Gibbs and Greiner 1994, Heidner et al. 1992
	Costa Rica	1, 3 ,6		Mo et al. 1994
	El Salvador	1, 3, 6		Mo et al. 1994
	Guatemala	1, 3, 6, 17		Mo et al. 1994
	Honduras	1, 2, 6, 17		Mo et al. 1994
	Nicaragua	1, 3, 6		Mo et al. 1994
	Panama	1, 3, 6		Mo et al. 1994
2	Barbados	1, 3		Mo et al. 1994
	Dominican Republic	4, 6, 8		Mo et al. 1994
	Jamaica	3, 12		Mo et al. 1994
	Puerto Rico	3, 4, 17		Mo et al. 1994, Shaw 1992
	Trinidad and Tobago	3		Mo et al. 1994
	Caribbean Islands[a]		1, 6, 12, 14, 17	Mo et al. 1994
3	Brazil	4		Gurgel-da-Cunha 1990, Grocock and Campbell 1982
	Colombia		1, 6, 12, 14, 17	Homan et al. 1992
	Surinam		6, 14, 17	Homan et al. 1992
	Guyana		6, 14, 17	Homan et al. 1992

[a] Antigua, Barbados, Grenada, Jamaica, St.Kitts, St.Lucia, Trinidad and Tobago.

Table 3. Bluetongue (BLU) serotypes in Asia.

OIE zone	Country	BLU serotypes identified by:		References
		Virus isolation	Serology (additional)	
1	Iran		3 (7, 20, 22)	Moakhar et al. 1988
	Israel	2, 4, 6, 10, 16		Hassan 1992, Sellers 1975
	Jordan		6 (2?, 4 ,9 ,13 ,15)	Taylor et al. 1985, Taylor and Mellors 1994
	Oman		3, 4, 6, 14, 15, 17, 19, 20, 21, 22	Al-Busaidy and Mellors 1991, Taylor et al. 1991
	Saudi Arabia		6, 14, 17, 19 ,20	Hafez and Taylor 1985
	Syria	2, 4, 6, 9, 13, 15		Taylor et al. 1985, Taylor and Mellors 1994
2	India	1, 2, 3, 4, 8, 9, 12, 16, 17, 18	5, 6, 7, 10, 11, 14, 15, 20	Uppal 1992, Kulkarni et al. 1992
	Pakistan	16		Ritter and Roy 1988
3	Indonesia	1, 7, 9, 12, 21, 23		Sendow et al. 1993
	Japan		1, 12, 20	Miura et al. 1982
	Malaysia	1, 2, 3, 9, 16, 23	5, 20, 21	Hassan 1992, Sharifah et al. 1995

Oceania

There is currently no evidence for the presence of BLU in Fiji, New Caledonia (St George. 1992), New Zealand (Ryan et al. 1991) or Vanuatu (Doyle 1992). Papua New Guinea apparently has at least three serotypes, thought on serological grounds to be BLU1, 21 and 23 (Gard et al. 1985, D.H. Cybinski, pers. comm.). An untyped strain of the virus has been isolated from the Solomon Islands (Doyle 1992). Eight BLU serotypes (BLU1, 3, 9, 15, 16, 20, 21 and 23) have been isolated in Australia (Doyle 1992), but the southern part of the continent is free of the virus, as is Tasmania (Sadler and Witt 1992). Six of the eight serotypes are apparently confined to the northernmost part of the continent, with only BLU1 and 21 having a wider distribution southwards (Ward 1994).

Europe

Of the 33 European countries submitting reports to OIE, 28 have not experienced bluetongue (Anon. 1993). The virus is thought not to be endemic within mainland Europe at present (Gibbs and Greiner 1994), with the possible exception of Turkey (Sellers 1984). Most outbreaks in Europe appear to have been introduced from elsewhere and the virus has tended to die out after epidemics. Five European countries have identified serotypes (Table 4).

Discussion

The global distribution of BLU serotypes can be expected to be in a state of continuous flux as climatic changes, especially those relating to temperature, rainfall and wind patterns affect the distribution of vectors and hosts. In addition, changes in land use, especially those relating to the geographical distribution of ruminants, influence the areas where new BLU serotypes can intrude and perhaps persist.

The current situation, as presented above, is subject to the limitations previously mentioned, especially

Table 4. Bluetongue (BLU) serotypes in Europe.

OIE zone	Country	BLU serotypes identified by:		References
		Virus isolation	Serology (additional)	
2	Cyprus	3 (pre-1965)	10, 12	Ritter and Roy 1988, Polydorou 1978
		4 (1969, 1977)		
	Greece (Lesbos, Rhodes)	4 (1979)		Papadopoulos 1992
	Portugal	10 (1957–59)		Sellers 1984
	Spain	10 (1957–60)		Sellers 1984
	Turkey	4 (1977–80)	2 (1981)	Burgu et al. 1992

the one of non-uniform sampling across the whole globe. Nevertheless, an overall global analysis, based conservatively on virus isolation alone, reveals some interesting trends which can be discussed tentatively, pending the acquisition of further data.

With reference to bluetongue, Central America (south of Mexico) is grouped with the Caribbean and South American countries (Gibbs and Greiner 1994). Using this modified OIE country classification, it is interesting to compare the distribution of serotypes in the five endemic zones (Fig. 2). Of the 24 BLU serotypes, 20 have been isolated in Africa, 16 in Asia, eight in Australia/Oceania, seven in Central/Caribbean/South America, and five in North America. (In the present discussion, Europe is best regarded as an 'incursion zone', of little significance in BLU epidemiology.) On the basis of multiplicity of serotypes, it probably remains reasonable to postulate that the original source of BLU was in Africa.

Six serotypes have been isolated only from one continent; BLU5, 14, 19, 22 and 24 from Africa, and BLU20 from Australia. Six serotypes have been isolated only from two of the OIE groupings: BLU7 and 18 have been recovered from both Africa and Asia, BLU11 and 13 have been isolated from both Africa and North America, and BLU21 and 23 have been recovered from both Asia and Australia. Ten serotypes (BLU2, 4, 6, 8, 9, 10, 12, 15, 16 and 17) have been recovered from three of the five geographical groupings. Finally, two serotypes (BLU1 and 3) are very widespread in their distribution, having been found in all continents and island groupings with the exception of northern America. At present, no serotypes have been recovered from all five geographical groupings.

Whether these trends reflect the real state of affairs, or whether they represent grossly incomplete sampling, remains for future research to determine. Progress on defining the situation in the People's Republic of China is reported in these Proceedings.

References

Abu-Elzein, E.M.E. 1985. Bluetongue in the Sudan. Revue Scientific and Technique, Office International des Epizooties, 4, 795–801.

Al-Busaidy, S.M. and Mellors, P.S. 1991. Isolation and identification of arboviruses from the Sultanate of Oman. Epidemiology and Infection, 100, 403–413.

Anon. 1992. World animal health 1992. Office International des Epizooties, 12 rue de Prony, 75017 Paris, France.

—1993. World animal health 1993. Office International des Epizooties, 12 rue de Prony, 75017 Paris, France.

Barrem, N., Erasmus, B.J., Gautier, A., Reme, A. and Valin, R. 1985. Bluetongue, a new disease of sheep on Reunion (Indian Ocean). Revue d'Elevage et de Medecine Veterinaire des Pays Tropicaux, 38, 16–21.

Barsoum, G.W. 1992. Bluetongue and African horse sickness situation in Egypt. In: Walton, T.E. and Osburn, B.I., ed., Bluetongue, African horse sickness and related orbiviruses. Proceedings 2nd International Symposium. CRC Press, Boca Raton, 42–43.

Blackburn, N.K., Searle, L. and Phelps, R.J. 1985. Viruses isolated from *Culicoides* (Diptera: Ceratopogonidae) caught at the veterinary research farm, Mazowe, Zimbabwe. Journal of the Entomological Society of Southern Africa, 48, 331–336.

Burgu, I., Urman, H.K., Akca, Y., Yonguc, A., Mellors, P.W. and Hamblin, C. 1992. Serologic survey and vector surveillance for bluetongue in southern Turkey. In: Walton, T.E. and Osburn, B.I., ed., Bluetongue, African horse sickness and related orbiviruses. Proceedings 2nd International Symposium. CRC Press, Boca Raton, 168–174.

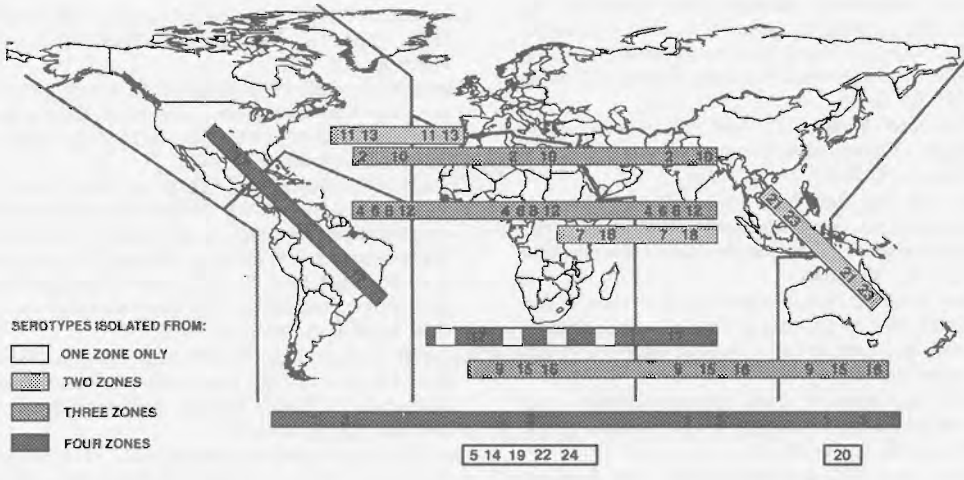

Figure 2. Global distribution of bluetongue in the OIE zones.

Davies, F.G. 1978. Bluetongue studies with sentinel cattle in Kenya. Journal of Hygiene, 80, 197–204.

Doyle, K.A. 1992. An overview and perspective on orbivirus disease prevalence and occurrence of vectors in Australia and Oceania. In: Walton, T.E. and Osburn, B.I., ed., Bluetongue, African horse sickness and related orbiviruses. Proceedings 2nd International Symposium. CRC Press, Boca Raton, 44–57.

Dulac, G.C., Sterritt, W.G., Dubuc, C., Afshar, A., Myers, D.J., Taylor, E.A., Jamieson, B.R. and Martin, M.W. 1992. Incursions of orbiviruses in Canada and their serologic monitoring in the native animal population between 1962 and 1991. In: Walton, T.E. and Osburn, B.I., ed., Bluetongue, African horse sickness and related orbiviruses. Proceedings 2nd International Symposium. CRC Press, Boca Raton, 120–127.

Ekue, F.N., Nfi, A.N., Tsangue, P., Taylor, W.P. and Gumm, I.D. 1985a. Bluetongue in exotic sheep in Cameroon. Tropical Animal Health and Production, 17, 187–188.

—1985b. Prevalence of bluetongue virus antibodies in ruminants in Cameroon. Tropical Animal Health and Production, 17, 189.

Elfatih, M., Mohammed, H. and Taylor, W.P. 1987. Infection with bluetongue and related orbiviruses in the Sudan detected by the study of sentinel calf herds. Epidemiology and Infection, 99, 533–545.

Ferriera, M.L. and Rosinha, A. 1986. Preliminary analysis of data from 'Operation Buffalo' (*Syncerus caffer*) in Mozambique. Repositorio de Trabalhos do Laboratorio nacional de Investigacao Veterinaria, 18, 83, 85–100.

Gard, G.P. and Melville, L.F. 1992. Results of a decade's monitoring for orbiviruses in sentinel cattle pastured in an area of regular arbovirus activity in Northern Australia. In: Walton, T.E. and Osburn, B.I., ed., Bluetongue, African horse sickness and related orbiviruses. Proceedings 2nd International Symposium. CRC Press, Boca Raton, 85–89.

Gard, G.P., Shorthose, J.E., Cybinski, D.H. and Zakrzewski, H. 1985. The isolation from cattle of 2 bluetongue viruses new to Australia. Australian Veterinary Journal, 62, 203.

Ghirotti, M., Semproni, G., Meneghi, D-de, Mungaba, F.N., Nannini, D., Calzetta, G., Paganico, G. and De-Meneghi, D. 1991. Sero-prevalences of selected cattle diseases in the Kafue flats of Zambia. Veterinary Research Communications, 15, 25–36.

Gibbs, E.P.J. and Greiner, E.C. 1994. The epidemiology of bluetongue. Comparative Immunology, Microbiology and Infectious Diseases, 17, 207–220.

Grocock, C.M. and Campbell, C.H. 1982. Antibodies to bluetongue viruses in animals imported into United States zoological gardens. Canadian Journal of Comparative Medicine, 46, 160–164.

Gurgel-da-Cunha, R. 1990. Neutralizing antibodies for different serotypes of bluetongue virus in serum samples from cattle and goats in Brazil. Revista Brasileira Medicina Veterinaria, 12, 3–7.

Hafez, S.M. and Ozawa, Y. 1981. Antigenic types of bluetongue virus prevalent in Egypt. Tropical Animal Health and Production, 13, 49–54.

Hafez, S.M., Pollis, E.G. and Mustafa, S.A. 1978. Serological evidence of the occurrence of bluetongue in Iraq. Tropical Animal Health and Production, 10, 95–98.

Hafez, S.M. and Taylor, W.P. 1985. Serotypes of bluetongue virus present in Saudi Arabia. In: Barber, T.C., Jochim, M.S. and Osburn, B.I. ed., Bluetongue and related orbiviruses. Progress in Clinical and Biological Research, vol. 178, Alan R. Liss Inc, New York, 531–537.

Hakim, M.D.A. 1985. Bangladesh. In: Della-Porta, A.J., ed., Veterinary viral diseases, their significance in South-East Asia and the Western Pacific. Academic Press, Sydney, 168–173.

Hamblin, C., Anderson, E.C., Jago, M., Mlengeya, T. and Hirji, K. 1990. Antibodies to some pathogenic agents in free-living wild species in Tanzania. Epidemiology and Infection, 105, 585–594.

Haresnape, J.M., Taylor, W.P. and Lungu, S.A.M. 1988. The epidemiology of bluetongue in Malawi. Epidemiology and Infection, 100, 493–499.

Hassan, S. 1992. Status of bluetongue in the Middle East and Asia. In: Walton, T.E. and Osburn, B.I., ed., Bluetongue, African horse sickness and related orbiviruses. Proceedings 2nd International Symposium. CRC Press, Boca Raton, 38–41.

Heidner, H.W., Iezzi, L.G., Osburn, B.I. and MacLachlan, N.J. 1992. Genetic variation and evolutionary relationships among bluetongue viruses endemic in the United States. Virus Research, 21, 91–109.

Herniman, K.A.J., Boorman, J.P.T. and Taylor, W.P. 1983. Bluetongue virus in a Nigerian dairy cattle herd. 1. Serological studies and correlation of virus activity to vector population. Journal of Hygiene, 90, 177–193.

Homan, E.J., Gibbs, E.P.J., Walker, J.S., Walton, T.E., Yuill, T.M., Gonzalez, J., Garreto, G.H. and Greiner, E.C. 1992. Central American and Caribbean regional bluetongue epidemiologic studies: antecedents and geographic review. In: Walton, T.E. and Osburn, B.I., ed., Bluetongue, African horse sickness and related orbiviruses. Proceedings 2nd International Symposium. CRC Press, Boca Raton, 99–105.

Hussein-Hag, A., Gallo, C., Caracappa, S. and Vesco, G. 1985. Serological survey for antibodies to abortion-producing agents in zebu in Somalia. Atti della Societa Italiana de Buiatria, 17, 573–575.

Karabatsos, N. 1985. International catalogue of arboviruses 1985. American Society of Tropical Medicine and Hygiene.

Konstantinov, O.K. 1990. Ticks of the family Ixodidae as arbovirus reservoirs in the Republic of Guinea. II. The arboviruses. Revue-d'Elevage et de Medecine Veterinaire des Pays Tropicaux, 43, 15–22.

Kulkarni, D.D., Bannalikar, A.S., Karpe, M.B., Gujar, M.B. and Kulkarni, M.N.1992. Epidemiological observations on bluetongue in sheep in the Marathwada region of Maharashtra State in India. In: Walton, T.E. and Osburn, B.I., ed., Bluetongue, African horse sickness and related orbiviruses. Proceedings 2nd International Symposium. CRC Press, Boca Raton, 193–196.

Lancelot, R., Calvez, D., Waller, J., Dremer, M., Sanite, L. and Lefevre, P.C. 1989. Epidemiological observations on bluetongue in French Guiana. Epidemiologie et Sante Animale, 15, 103–116.

Lee, V.H., Causey, O.R. and Moore, D.L. 1974. Bluetongue and related viruses in Ibadan, Nigeria: isolation and preliminary identification of viruses. American Journal of Veterinary Research, 35, 1105–1108.

Lefevre, P.C. and Taylor, W.P. 1983. Epidemiological situation of bluetongue in Senegal. Revue d'Elevage et de Medecine Veterinaire des Pays Tropicaux, 36, 241–245.

Lopez, W.A., Nicoletti, P. and Gibbs, E.P.J. 1985. Antibody to bluetongue virus in cattle in Ecuador. Tropical Animal Health and Production, 17, 82.

MacLachlan, N.J. 1994. The pathogenesis and immunology of bluetongue infection of ruminants. Comparative Immunology, Microbiology and Infectious Diseases, 17, 197–206.

Maiga, S. and Sarr, J. 1992. Epidemiological survey of the main respiratory viruses of small ruminants in Mali. Revue-d'Elevage et de Medecine Veterinaire des Pays Tropicaux, 45, 15–17.

Miura, Y., Inaba, Y., Tsuda, T., Tokuhisa, S., Sato, K. and Akashi, H. 1982. Seroepizootiological survey on bluetongue virus infection in Japan. National Institute of Animal Health Quarterly (Japan), 22, 145–158.

Mo, C.L., Thompson, L.H., Homan, E.J., Oviedo, M.T., Greiner, E.C., Gonzalez, J. and Saenz, M.F. 1994. Bluetongue virus isolations from vectors and ruminants in Central America and the Caribbean. American Journal of Veterinary Research, 55, 211–215.

Moakhar, R.K., Taylor, W.P., Ghaboussi, B. and Hessami, M. 1988. Serological survey of sheep in Iran for type specific antibody to bluetongue virus. Archives de l'Institut Razi, 38/39, 92–99.

Mohammed, M.E.H. and Mellors, P.S. 1990. Further studies on bluetongue and bluetongue-related orbiviruses in the Sudan. Epidemiology and Infection, 105 (3), 619–632.

Nevill, E.M., Erasmus, B.J. and Venter, C.J. 1992. A six-year study of viruses associated with *Culicoides* biting midges throughout South Africa (Diptera: Ceratopogonidae). In: Walton, T.E. and Osburn, B.I., ed., Bluetongue, African horse sickness and related orbiviruses. Proceedings 2nd International Symposium. CRC Press, Boca Raton, 314–319.

Ozawa, Y. 1985. Bluetongue and related orbiviruses: overview of the world situation. In: Barber, T.C., Jochim, M.S. and Osburn, B.I. ed., Bluetongue and related orbiviruses. Progress in Clinical and Biological Research, vol. 178, Alan R. Liss Inc, New York, 13–20.

Papadopoulos, O. 1992. Bluetongue and epizootic hemorrhagic disease in Europe and the European community. In: Walton, T.E. and Osburn, B.I., ed., Bluetongue, African horse sickness and related orbiviruses. Proceedings 2nd International Symposium. CRC Press, Boca Raton, 34–37.

Polydorou, K. 1978. The 1977 outbreak of bluetongue in Cyprus. Tropical Animal Health and Production, 10, 229–232.

Ritter, D.G. and Roy, P. 1988. Genetic relationships of bluetongue virus serotypes isolated from different parts of the world. Virus Research, 11, 33–47.

Rosadio, R.H., Evermann, J.F. and DeMartini, J.C. 1984. A preliminary serological survey of viral antibodies in Peruvian sheep. Veterinary Microbiology, 10, 91–96.

Ryan, T.J., Frampton, E.R., Motha, M.X.J. and Horner, G.W. 1991. Arbovirus and arbovirus vector surveillance in New Zealand. Surveillance, Wellington, 18, 24–26.

Sadler, E.C. and Witt, D.J. 1992. Survey of cattle in Tasmania for antibody to bluetongue virus. Australian Veterinary Journal, 69, 262.

Sellers, R.F. 1975. Bluetongue in Cyprus. Symposium on Bluetongue, Adelaide, May 1974. Australian Veterinary Journal, 51, 198–203.

—1984. Bluetongue in Africa, the Mediterranean region and Near East—disease, virus and vectors. Preventive Veterinary Medicine, 2, 371–378.

Sendow, I., Daniels, P.W., Soleha, E., Erasmus, B., Sukarsih and Ronohardjo, P.1993. Isolation of bluetongue virus serotypes new to Indonesia from sentinel cattle in West Java. The Veterinary Record, 133, 166–168.

Shapiro, J.L., Wiegers, A., Dulac, G.C., Bouffard, A., Afshar, A., Myers, D.J., Dubuc, C., Martin, M.W. and Koller, M. 1987. A survey of cattle for antibodies against bluetongue and epizootic hemorrhagic disease of deer viruses in British Columbia and southwestern Alberta in 1987. Canadian Journal of Veterinary Research, 55, 203–204.

Sharifah, S.H., Ali, M.A., Gard, G.P. and Polkinghorne, I.G. 1995. Isolation of multiple serotypes of bluetongue virus from sentinel livestock in Malaysia. Tropical Animal Health and Production, 27, 37–42.

Shaw, J. 1992. Sentinel herd study of bluetongue in Puerto Rico. Preliminary results, January 1991. Annals of the New York Academy of Sciences, 653, 194–196.

Simpson, V.R. 1979. Bluetongue antibody in Botswana's domestic and game animals. Tropical Animal Health and Production, 11, 43–49.

St. George, T.D. 1992. Occupation of an ecological niche in Pacific Ocean countries by bluetongue and related viruses. In: Walton, T.E. and Osburn, B.I., ed., Bluetongue, African horse sickness and related orbiviruses. Proceedings 2nd International Symposium. CRC Press, Boca Raton, 76–84.

Stanley, M.J. 1990. Prevalence of bluetongue precipitating antibodies in domesticated animals in Yemen Arab Republic. Tropical Animal Health and Production, 22, 163–164.

Stott, J.L., Blanchard-Channell, M., Osburn, B.I., Riemann, H.P. and Obeso, R.C. 1989. Serologic and virologic evidence of bluetongue virus infection in cattle and sheep in Mexico. American Journal of Veterinary Research, 50, 335–340.

Tamayo, R., Alonso, O. and Schoebitz, R. 1983. Antibodies to bluetongue virus in cattle—first report from Chile. Archivos de Medicina Veterinaria, Chile, 15, 49.

Taylor, W.P., Al-Busaidy, S.M. and Mellors, P.S. 1991. Bluetongue in the Sultanate of Oman, a preliminary epidemiological study. Epidemiology and Infection, 107, 87–97.

Taylor, W.P. and McCausland, A. 1976. Studies with bluetongue virus in Nigeria. Tropical Animal Health and Production, 8, 69–173.

Taylor, W.P. and Mellors, P.S. 1994. Distribution of bluetongue virus in Turkey, 1978–81. Epidemiology and Infection, 112, 623–633.

Taylor, W.P., Sellers, R.F., Gumm, I.D., Herniman, K.A.J. and Owen, I. 1985. Bluetongue epidemiology in the Middle East. In: Barber, T.C., Jochim, M.S. and Osburn, B.I. ed., Bluetongue and related orbiviruses. Progress in Clinical and Biological Research, vol. 178, Alan R. Liss Inc., New York, 527–530.

Thomas, F.C., Skinner, D.J. and Samagh, B.S. 1982. Evidence for bluetongue virus in Canada: 1976–1979. Canadian Journal of Comparative Medicine, 46, 350–353.

Tomori, O., Baba, S., Adu, F. and Adeniji, J. 1992. An overview and perspective on orbivirus disease prevalence and occurrence of vectors in Africa. In: Walton, T.E. and Osburn, B.I., ed., Bluetongue, African horse sickness and related orbiviruses. Proceedings 2nd International Symposium. CRC Press, Boca Raton, 23–33.

Uppal, P.K. 1992. Present position of African horse sickness and bluetongue in India. In: Walton, T.E. and Osburn, B.I., ed., Bluetongue, African horse sickness and related orbiviruses. Proceedings 2nd International Symposium. CRC Press, Boca Raton, 175–180.

Ward, M.P. 1994. The epidemiology of bluetongue virus in Australia a review. Australian Veterinary Journal, 71, 3–7.

Weitzman, G.L., Stem, E.C., Gilfillan, R.S. and Lindenmayer, J.M. 1991. Preliminary serological survey for bluetongue and toxoplasmosis in sheep in Niger. Tropical Animal Health and Production, 23, 258.

Bluetongue Viruses in India: a Review

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Abstract

After the first outbreak in 1964, bluetongue has become endemic even in native Indian sheep. Antibodies were found by AGID in 45.56% of native sheep and goats respectively, with 5.25% more positives detected by cELISA. Initially, the morbidity among native sheep was 80%. There is no evidence of clinical bluetongue in cattle and goats despite a high incidence of seroconversion. The pattern of disease in native sheep was studied during 1983–1994. Morbidity was cyclical and ranged from 2% to 27.7%, while the rate of case fatalities ranged from 1% to 27.6%. Bluetongue occurred in India between June and October, after the onset of rains. In Andhra Pradesh (south India), the disease occurred during September to October when the temperature was moderate, between 25° to 32°C. Several bluetongue virus (BLU) serotypes have been identified among exotic and crossbred sheep and, recently, BLU has been isolated and identified from native sheep. The clinical nature and severity in natural and experimental bluetongue infection among native sheep differed slightly from that among exotic and crossbreeds.

BLUETONGUE has become one of the important diseases of sheep in India, being reported annually from the states of Andhra Pradesh, Tamil Nadu, Karnataka and Maharashtra. The disease is considered to be a serious problem in native sheep in southern India.

Clinical Signs and Symptoms

Exotic breeds of sheep, like Corriedale, Merino and Rambouillet, exhibit classical symptoms of bluetongue (Uppal and Vasudevan 1980; Vasudevan 1982; Jain et al. 1986) and a similar clinical picture was noticed in crossbreeds. Swollen ears hanging while the sheep were grazing in the hot sun was usually the first symptom observed by shepherds, with affected sheep trying to seek shade.

Native sheep, however, showed less conspicuous swelling of the face and lips. The mucocutaneous borders of lips do appear to be very sensitive to touch and bleed easily upon handling, but cyanosis of the tongue and reddening of the coronary band is not evident in many cases. The inconspicuousness of these signs can mainly be attributed to the general appearance of the native sheep, as some of the breeds have pigmented skin, mucous membranes and tongue.

Morbidity and Mortality

In 1961, bluetongue outbreaks were recorded among native sheep and goats in Maharashtra, with a morbidity of 16 to 20% and a case fatality of 20% (Sapre 1964). During 1981, bluetongue was widespread in southern states. In Maharashtra, morbidity and mortality rates were 9.7% and 1.1% respectively, with a case fatality rate of 11.5% (Harbola et al. 1982). During subsequent outbreaks, the severity of the disease increased with an overall morbidity of 32%, mortality of 8% and case fatality of 25% (Kulkarni et al. 1992).

Bluetongue epidemics occurring in Andhra Pradesh in 1981 were more severe than in Maharashtra, with

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morbidity and mortality rates of 30% and 6.5% respectively. In 1983 bluetongue outbreaks were reported from all over Andhra Pradesh with a case fatality rate of 21.9%. The increased case fatality rates could be ascribed to an increase in virus virulence. Bluetongue was subsequently noticed regularly in this state with case fatality rates ranging from 2.4% to 22.2%. A cyclical pattern in the disease was observed with variations in severity of infection (Table 1). The severity of bluetongue was also greater in Karnataka during the 1981 epidemic (Srinivas et al. 1982). From 1986, a total of 77 outbreaks were reported from Tamil Nadu over a period of five years, with morbidity rates from 3.3% to 22.8% and mortality rates from 0% to 6.1% (Saravanabava 1992).

In north India, the first episode of bluetongue was recorded in Haryana in 1972, with a morbidity of 2%. Subsequently, outbreaks were noticed regularly with severe clinical disease during 1975 and 1982. The morbidity rate was 10% in adults and 20% in weaners with mortality rates of 0.5% and 1.3% in adults and weaners respectively (Vasudevan 1982). Bluetongue again appeared in 1985 affecting 48 Rambouillet sheep, resulting in 15 deaths among 565 animals. In 1986 and 1988 the disease occurred with 7.7% morbidity and a case fatality rate of 38.9% (Mahajan et al. 1991). Bluetongue was also noted in Russian Merinos in Himachal Pradesh in 1973: of 96 sheep, 41 were affected and eight died (Uppal and Vasudevan 1980).

The most recent data show India's bluetongue situation in 1992/93 (Table 2). Andhra Pradesh experienced severe outbreaks in September, October and November 1993. The available data suggest that bluetongue is endemic in southern India, in contrast to the absence of reports from northern India.

Table 1. Bluetongue outbreaks in sheep in Andhra Pradesh, 1983–1994.

Year	Organised farms			Village flocks			
	Morbidity (%)	Mortality (%)	Case fatality (%)	Outbreaks	Attacks	Deaths	Case fatality (%)
1983	7.2	1.2	16.4	1103	246737	54042	21.9
1984	2.0	–	–	–	–	–	–
1985	6.7	1.8	27.6	311	13093	1652	12.6
1986	2.8	0.1	4.8	35	2225	99	4.4
1987	4.8	–	–	101	6609	157	2.4
1988	11.1	1.6	14.5	225	53293	6036	11.3
1989	5.3	–	–	112	7959	523	6.6
1990	27.8	0.5	1.9	119	3719	405	10.9
1991	2.4	–	–	284	19975	1056	5.3
1992	8.1	0.6	7.3	55	929	111	11.9
1993	3.1	–	–	168	6543	306	4.7
1994	na	na	na	283	41717	9261	22.2

na =data not available.

Table 2. Bluetongue situation in India in 1992 and 1993 (from Agricultural Informatics Division, Department of Animal Husbandry and Dairying, Indian Ministry of Agriculture).

State	1992			1993		
	No. of outbreaks	No. of cases	No of. deaths	No. of outbreaks	No. of. cases	No. of deaths
Andhra Pradesh	55	929	111	168	6543	306
Karnataka	7	126	15	2	2093	5
Maharashtra	4	279	49	9	271	24
Tamil Nadu	16	690	190	2	140	15
Total	82	2024	365	181	9047	350

Species and Breeds

Antibodies to bluetongue virus (BLU) have been detected in sheep, goats, cattle and buffalo sera but not in horse and camel sera (Prasad et al. 1987). No information is available on infections in wild animals in India. However, elephants appear to show seroconversion (Mehrotra and Shukla 1990). Although Sapre (1964) reported clinical disease in sheep and goats, only sheep have been observed to exhibit characteristic clinical symptoms in all subsequent outbreaks of the disease. Cattle, goats and buffalo have not shown any clinical signs, even when they have been in close association with affected sheep and seroconversion has been recorded.

Severe clinical disease was noticed in Dorset sheep on a farm in Andhra Pradesh in 1974. However, native sheep maintained in close proximity on the same farm did not show any symptoms. The available farm data indicated that the disease did occur in native sheep from 1985 onwards. Though clinical signs of bluetongue were observed in exotic breeds (eg. Merino, Rambouillet, Corriedale, Suffolk and Dorset), another exotic breed, Karakul, maintained on the same farm did not show any symptoms (Sharma et al. 1985). Rambouillet and Merinos and their crosses were found to be more susceptible than Dorset and Suffolk breeds and their crosses. Native sheep maintained together with exotics and their crossbreds were also reported to suffer from bluetongue (Harbola et al. 1982; Sharma et al. 1985; Mullick 1988).

Age

Our investigation in Andhra Pradesh revealed that sheep aged 6 to 12 months of age were more susceptible than adults. Disease has not been noticed in lambs. Similar observations have also been reported from Maharashtra and Haryana (Uppal and Vasudevan 1980; Harbola et al. 1982). In contrast, severe infections in adult sheep have been reported from Tamil Nadu.

Vectors

The primary vectors of BLU viruses are midges of the genus *Culicoides*. Various species of *Culicoides* fed on viraemic sheep differed in their susceptibility to infection. The *Culicoides* species that transmit the disease vary from country to country. Jain et al. (1988) isolated BLU from *Culicoides* midges but the species involved was not identified. Information on the vector species that transmit infection in India is lacking.

Seasonality

Maximum numbers of outbreaks in Andhra Pradesh were recorded during the south-west monsoon period (June to September), whereas in Tamil Nadu outbreaks were more frequent during north-west monsoon periods (October to December). In south India, the monsoon season (June to December), with its temperatures ranging from 21.2 to 35.6°C, appears to be a favourable period for the multiplication of *Culicoides* resulting in more outbreaks. In Haryana State in north India, outbreaks were reported between April and October. In Rajasthan, outbreaks were mostly confined to September and November (Mahajan et al. 1991; Sharma et al. 1985). The available data indicate that outbreaks were not recorded in north India during winter (December to March).

Seroprevalence

Serological studies conducted in Andhra Pradesh using agar gel immunodiffusion (AGID) revealed the presence of antibodies in 45.7% and 43.6% of sheep and goats respectively. When a competitive enzyme linked immunosorbent assay (cELISA) was used, 5.2% more positives were detected. Prasad et al. (1987) showed the presence of BLU antibodies in 82.2% of exotic sheep which had aborted and 36.6% of apparently healthy exotic sheep in Haryana. Sodhi et al. (1981) noted antibodies in 6.6% of sheep and 1.44% of goats in Punjab State, whereas Bandopadhyay and Mullick (1983) reported 3% prevalence of BLU antibodies in goats in Uttar Pradesh.

Our investigations have revealed the presence of antibodies in 23% of native cattle and 71.9% of exotic cattle in Andhra Pradesh. Oberoi et al. (1988) demonstrated the presence of BLU antibodies in 37.5% of buffalo and 70% of cattle sera in Punjab. In Gujarat, 13.4% of buffalo and 15.6% of cattle sera were positive for BLU antibodies (Tongaonkar et al. 1983). Jain et al. (1992) noticed that the incidence of BLU antibodies was higher in buffalo (10.6%) than in cattle (4.2%). Mehrotra and Shukla (1990) reported that prevalence of BLU antibodies ranged between 16.2% and 62.2% in different states. Information is lacking on the seroprevalence of antibodies in eastern and north-eastern India.

Bluetongue Virus Serotypes

BLU serotypes 2 and 12 were identified on the basis of neutralising antibodies in serum samples collected from experimentally inoculated sheep and cattle which, after inoculation with clinical material, seroconverted during the disease outbreak in 1993 in Andhra Pradesh. Field sera revealed neutralising anti-

bodies to BLU4, 12, 13, 14, 17, 18 and 19, indicating the circulation of multiple serotypes. Studies on the prevalence of BLU serotypes in India (Table 3) have shown a total of eight serotypes, and have identified neutralising antibodies to 17 serotypes (Vasudevan 1982; Tongaonkar et al. 1983; Janakiraman et al. 1991; Mehrotra and Misra 1993).

Table 3. BLU serotypes in India.

State	Species	Basis for serotyping	
		Virus	Neutralising antibodies
Tamil Nadu	sheep	3, 16	1, 4–7, 11, 12, 14–17, 19, 20
Andhra Pradesh	sheep	2	4, 12, 13, 14, 17, 18, 19
	cattle	–	6, 12
Karnataka	cattle	–	1, 14, 16
Maharashtra	sheep	1, 2, 3, 4, 9, 16, 18	–
Gujarat	buffalo	–	1, 15, 17
	cattle	–	2, 12, 20
Madhya Pradesh	sheep	18	–
Uttar Pradesh	sheep	9, 18	–
Haryana	sheep	1, 4	14
	cattle	–	1, 2, 8, 12, 16
Himachal Pradesh	sheep	3, 9, 16,17	4
Jammu & Kashmir	sheep	18	–

References

Bandopadhyay, S.K. and Mullick, B.B. 1983. Serological prevalence of bluetongue antibodies in India. Indian Journal of Animal Sciences, 53, 1355–1356.

Harbola, P.C., Chaudhary, P.G., Lal Krishna, Siriguppi, B.S. and Kole, R.S. 1982. Incidence of bluetongue in sheep in Maharashtra. Indian Journal of Comparative Microbiology, Immunology and Infectious Diseases, 3, 121–127.

Jain, N.C., Gupta, Y. and Prasad, G. 1992. Bluetongue virus antibodies in buffaloes and cattle in Haryana State of India. In: Walton, T.E. and Osburn, B.I. ed., Bluetongue, African horse sickness and related orbiviruses. Proceedings 2nd International Symposium. CRC Press, Boca Raton, 188–192.

Jain, N.C., Prasad, G., Gupta, Y. and Mahajan, N.K. 1988. Isolation of bluetongue virus from *Culicoides* species in India. Revue Scientifique et Technique Office International des Epizooties, 7, 375–378.

Jain, N.C., Sharma, R. and Prasad, G. 1986. Isolation of bluetongue virus from sheep in India. Veterinary Record, 119, 17–18.

Janakiraman, D., Venugopalan, A.T., Ramaswamy, V. and Venkatesan, R.A. 1991. Serodiagnostic evidence of prevalence of bluetongue virus serotypes among sheep and goats in Tamil Nadu. Indian Journal of Animal Sciences, 61, 497–498.

Kulkarni, D.D., Bannalikar, A.S., Karpe, A.G., Gujar, M.B. and Kulkarni, M.N. 1992. Epidemiological observations on bluetongue in sheep in Marathawada region of Maharashtra State in India. In: Walton, T.E. and Osburn, B.I., ed., Bluetongue, African horse sickness and related orbiviruses. Proceedings 2nd International Symposium. CRC Press, Boca Raton, 85.

Mahajan, N.K., Prasad, G., Jain, N.C., Dhanoea, J.S. and Gupta, Y. 1991. Epizootiological studies on bluetongue at an organised sheep breeding farm near Hissar, Haryana. Indian Journal of Animal Sciences 61, 1–5.

Mehrotra, M.L. and Misra, S. 1993. Clinical symptoms and cellular reactions of bluetongue virus serotypes of Indian sheep. In: Indian Veterinary Research Institute, Proceedings of the international symposium on virus-cell interaction: cellular and molecular responses. Indian Veterinary Research Institute, Bangalore, 103 p.

Mehrotra, M.L. and Shukla, D.C. 1990. Seroprevalence, diagnosis and differential diagnosis of bluetongue virus disease in India. Indian Journal of Virology, 1, 98–104.

Mullick, S.G. 1988. Some epidemiological observations on the occurrence of bluetongue in organised farms in Andhra Pradesh. Indian Journal of Comparative Microbiology, Immunology and Infectious Diseases, 9, 201–207.

Oberoi, M.S., Singh, G. and Kwatra, M.S. 1988. Serological evidence of bluetongue virus activity in cattle and buffalo population. Indian Journal of Virology, 4, 50–51.

Prasad, G., Jain, N.C., Mahajan, N.K. and Vasudevan, B. 1987. Prevalence of bluetongue precipitating antibodies in different domestic animals. Indian Journal of Animal Sciences, 57, 522–524.

Sapre, S.N. 1964. An outbreak of bluetongue in goats and sheep. Veterinary Review, 15, 78–80.

Saravanabava, K. 1992. Studies on bluetongue virus in sheep. Ph.D. Thesis, Tamil Nadu Veterinary and Animal Sciences University, Madras, India.

Sharma, M.M., Lonkar, P.S., Srivastava, C.P., Dubey, S.C., Maru, A. and Kalra, D.B. 1985. Epidemiology of bluetongue in sheep at an organised farm in semiarid part of Rajasthan, India. Indian Journal of Comparative Microbiology. Immunology and Infectious Diseases, 6, 188–192.

Sodhi, S.S., Oberoi, M.S., Sharma, S.N. and Baxi, K.K. 1981. Prevalence of bluetongue precipitating antibodies in sheep and goats in Punjab, India. Zentralblatt für Veterinär medizin, B., 28, 421–423.

Srinivas, R.P., Moogi, V.M., Ananth, M., Gopal, T. and Keshavamurthy, B.S. 1982. Bluetongue disease outbreaks among sheep and goats of Karnataka. In: Central Sheep Wool Research Institute, Abstracts of national seminar on sheep and goat disease. Central Sheep Wool Research Institute, Avikanagar, Rajasthan, 14–20.

Tongaonkar, S.S., Ayangar, S.K., Singh, B.K. and Kant, R. 1983. Seroprevalence of bluetongue virus in Indian buffalo (*Bubalus bubalis*). Veterinary Record, 112, 32.

Uppal, P.K. and Vasudevan, B. 1980. Occurrence of bluetongue in India. Indian Journal of Comparative Microbiology, Immunology and Infectious Diseases, 1, 18–20.

Vasudevan, B. 1982. A note on bluetongue incidence among sheep in India. In: Indian Veterinary Research Institute, Proceedings of the National Workshop on Bluetongue. Indian Veterinary Research Institute, Izatnagar, 129–144.

Serological Study for Bluetongue in Thailand

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Abstract

Serological testing for bluetongue in Thailand began in 1991. Agar gel immunodiffusion (AGID) was used to test sera from 522 imported and domestic cattle, native sheep and goats, for which positive rates were 28.6%, 39.4% and 73.0% respectively. Though a high seropositive rate was found in native sheep, the tested animals showed no clinical disease. Bluetongue has not yet posed any significant or detectable animal health problem for ruminant farming in Thailand.

THAILAND is located on the mainland of Southeast Asia: its borders adjoin Myanmar to the west and north, Laos to the north and east, Cambodia to the east, and Malaysia to the south. Thailand's area of 513,115 km^2 can be divided into the fertile central plain, the mountainous north, the semi-arid northeast and the topographically diverse south. There are two distinct climates: a tropical savanna climate from the Gulf of Thailand to the north and a tropical monsoon climate in the south. The year generally has three seasons: hot, rainy, and cool, except in the south where there is no distinct cool season.

The livestock industry in Thailand is expanding both to meet the demands of the increasing domestic population and to supply world markets. Beef and dairy cattle populations are increasing, and provide an important source of cash income for villagers. Recently large numbers of breeding animals (beef and dairy cattle breeds, i.e. Brahman and Friesian) have been imported into Thailand, mainly from USA, Australia and New Zealand, and draught animals have also been imported from neighbouring countries (Table 1). In 1994, Thailand's livestock population was estimated at about 5.6 million cattle, 4.6 million buffaloes, 162 000 sheep and 120 000 goats (Table 2).

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Thailand has not experienced any clinical bluetongue disease in sheep or cattle. Thailand has regulations for the importation of animal and genetic materials: the imported animals must be tested for the disease within the month before export if they are from bluetongue-infected areas, or be certified as having originated from bluetongue-free areas. However, evidence of bluetongue in Thailand has been determined by the serological testing that began in 1991.

Methods

Serum samples were collected from 297 imported cattle, 128 native cattle, 60 sheep and 37 goats. The imported cattle were bled two to five days after arrival. All serum samples were tested for antibody to bluetongue (BLU) by agar gel immunodiffusion (AGID). The antigen was prepared from BLU1 at National Institute of Animal Health, Bangkok (Apiwatnakorn et al. 1994).

Results

AGID tests for BLU antibody were carried out on sera collected between 1991 and 1994 from 522 imported cattle, native cattle, sheep and goats: seropositive rates were 28.6%, 59.4%, 75% and 73% respectively (Table 3).

Table 1. Number of imported animals/semen by export country, 1994

Export country	No. of cattle			No. of buffalo	No. of horses	Dose of cattle semen
	Beef	Dairy	Draught			
Australia	–	998	–	–	135	11750
Canada	–	4	–	–	–	31600
Denmark	–	–	–	–	9	–
France	–	–	–	–	–	20000
Germany	2	–	–	–	–	20055
Japan	–	–	–	–	–	10000
Laos	–	–	95	2970	–	–
Malaysia	–	9	–	–	34	–
Myanmar	–	–	1353	500	–	–
Netherlands	–	–	–	–	2	–
New Zealand	4667	–	–	–	–	5815
USA	530	–	–	–	84	34468
Total	5199	1011	1448	3470	264	133 688

(Source: Division of Planning, Department of Livestock Development, Bangkok).

Table 2. Livestock population in Thailand.

Year	Cattle	Buffalo	Sheep	Goats	Horses
1990	5 668 530	4 694 290	162 496	120 519	19 758
1991	6 626 971	4 805 071	166 102	136 035	20 331
1992	7 121 479	4 728 271	176 229	159 642	18 852
1993	7 472 573	4 804 146	110 465	151 860	18 047
1994	7 637 350	4 224 791	90 508	141 076	14 032

Table 3. Results of serological testing by AGID for bluetongue virus antibodies, Thailand 1991 to 1994.

Animals	Total no. of animals tested	No. positive (%)
Imported cattle	297	85 (28.7%)
Native cattle	128	76 (59.4%)
Sheep	60	45 (75.0%)
Goats	37	27 (73.0%)

Discussion

Although BLU antibodies were detected in Thailand in sera from native cattle, sheep and goats, clinical signs have not been seen nor has virus been isolated. In many countries, the prevalence of infection in ruminants has been reported as greater than 50% even though clinical disease has never been recorded (Gibbs and Greiner 1988). This study suggests that BLU may occur in Thailand, which is a tropical country suitable for BLU vectors. Bluetongue infections are common in sheep, cattle and other ruminants in most tropical, subtropical and temperate zones of the world (Eaton et al. 1989). The distribution of BLU is focal, with the focality depending primarily on the range of the host reservoir, vector and domestic animals. As the range of vectors depends on temperature and water availability, BLU will spread more rapidly at warm temperatures as vectors develop to maturity more rapidly (Shope 1992).

Despite Thailand's regulations for importing animals and genetic materials (including BLU testing within one month of shipment), some imported cattle have shown positive reactions to BLU. Although this study focuses only on serological studies, our results are expected to warn all those concerned with importing animals and/or genetic materials into Thailand to prevent the introduction of exotic viruses.

Acknowledgments

The National Institute of Animal Health, Japan, provided BLU1 seed for antigen preparation, and the Department of Livestock Development provided resources and facilities.

References

Apiwatnakorn, B., Supcharoen, A., Ekgatat, M. and Gagornsombat, D. 1994. Bluetongue antigen preparation for agar gel immunodiffusion test. Kasetsart Animal Hospital Journal, 5 (1–2), 1–5.

Eaton, B.T., Hyatt, A.D., Gould, A.R. and White, J.R. 1989. The structure of bluetongue virus. Biennial Report 1987–1989 CSIRO Australian Animal Health Laboratory, 65–70.

Gibbs, E.P.J. and Greiner, E.C. 1988. Bluetongue and epizootic hemorrhagic disease. In: Monath, T.P., ed., The arboviruses: epidemiology and ecology, vol. II. CRC Press, Boca Raton, 39–70.

Shope, R.E. 1992. Health implications of arboviral diseases resulting from global changes. In: Walton, T.E. and Osburn, B.I. ed., Bluetongue, African horse sickness and related orbiviruses. Proceedings 2nd International Symposium. CRC Press, Boca Raton, 9–13.

Recent Studies on Bluetongue in Peninsular Malaysia

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Abstract

Bluetongue is endemic in Malaysia. Bluetongue virus infection is widespread among local cattle, buffalo, sheep and goats, but the clinical form of the disease is inapparent in indigenous sheep. The Veterinary Research Institute and CSIRO collaborated to investigate bluetongue in Malaysia. Sentinel groups of cattle and sheep were established at four sites throughout the country. Blood samples from these sentinels were collected over a nine-month observation period. Sixteen viruses isolated from heparinised blood samples were typed by the plaque-reduction test into six different serotypes: BLU1, 2, 3, 9, 16 and 23. The pathogenicity of five bluetongue virus serotypes was studied by intravenous inoculation of 0.5 mL heparinised blood of the original isolates into progenies of imported commercial Merino–Border Leicester crossbred sheep, shown free of bluetongue antibodies by AGID and competitive ELISA. Although virus titres in their blood ranged from $10^{2.4}$ to $10^{1.9}$ ECE LD_{50}/0.5 mL, the sheep did not show any clinical signs.

THE first documented evidence of bluetongue in Malaysia came in 1977 when antibodies were detected by agar gel immunodiffusion (AGID) tests in healthy local and imported ruminants (Anon. 1978). However, despite the serological evidence of bluetongue infection, local animals seemed naturally resistant as no overt clinical signs were reported.

In October 1987, an outbreak of bluetongue occurred in Malaysia in a batch of sheep imported from South Australia (Chiang 1989). Bluetongue virus (BLU) serotype 1 was recovered from one of the dead sheep and serological evidence also indicated presence of BLU3 and BLU8 (serotyping by Pirbright Laboratories, U.K.). During the outbreak, the local in-contact goats and sheep did not suffer from the disease. In 1990, following the outbreak, another comprehensive serological survey involving 16 340 ruminant animals was conducted using AGID (Della-Porta et al. 1983). In that survey, virus replication appeared to be highly active among cattle and buffalo in comparison to recently imported Poll Dorset sheep and local goats. These results add to the evidence that BLU infection is widespread and endemic in Malaysia.

The incidence of bluetongue disease has caused great concern to the sheep industry, as Malaysia's aim to increase the genetic potential of its breeding stock now faces a major problem in the susceptibility of imported animals to bluetongue. A collaborative research program to investigate bluetongue in West Malaysia was therefore developed between Malaysia and the Bureau of Rural Research, Australia. The main aim was to accumulate more data on the disease status in Malaysia in order to formulate suitable and effective control measures for BLU infections in sheep. As there are 24 BLU serotypes known worldwide, the preliminary project was targeted at the isolation, identification and characterisation of BLU serotypes causing clinical disease in Malaysia. This paper outlines current research findings on the epidemiology of BLU infections in Malaysia and discusses prospects for the control of the disease, especially in imported temperate breeds.

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Current Research Findings on the Epidemiology of Bluetongue

Occurrence of *Culicoides* species

Bluetongue viruses, like many other orbiviruses, are dependent on *Culicoides* for their natural transmission. *Culicoides brevitarsis*, the main BLU vector in Australia, also occurs in Papua-New Guinea, Solomon Islands and Indonesia (Doyle 1992). However, in a survey of *Culicoides* species collected in five sheep sheds in West (peninsular) Malaysia, *C. brevitarsis* was collected from only two farms and in very low numbers. On the basis of observed abundance, distribution and host preference, *C. peregrinus, C. orientalis* and *C. shortii* are considered the important BLU vectors in Malaysia (Cheah and Rajamanickum 1991).

Vector competence studies need to be conducted on these three species, and on several others collected near livestock. Studies of the genetic and environmental factors controlling the complex traits of vector capacity, vector–virus–host interactions and vector ecology will be of considerable importance in achieving a full understanding of bluetongue disease, epidemiology and use of vaccines. However, there is a current lack of expertise and facilities in these various disciplines in Malaysia to support such research.

Sentinel herds

In the absence of clinical bluetongue disease in local ruminants, the use of sentinel herds was the most effective approach to obtaining BLU isolates. In March 1991, three herds of sentinel calves, tested negative for BLU antibodies by AGID, and an antibody-negative flock of adult sheep were established on four government farms in West Malaysia. Heparinised and clotted blood samples were collected weekly or biweekly from each individually-identified sentinel animal over a nine-month observation period. During this study, BLU infections of sentinels were found to be associated with periods of higher rainfall, with the incidences of seroconversions being markedly higher in cattle than in sheep (Sharifah et al. 1995).

Virus isolation and serotyping

The sera of the sentinel animals were examined by AGID for antibodies to bluetongue and the heparinised bloods of seroconverting animals were tested for the presence of virus (Gard et al. 1988). Briefly, virus recovery was attempted in an isolation system involving passage through embryonated chicken eggs (ECE), the C6/36 cell line of *Aedes albopictus*, and BHK21 cell cultures. Virus was detected by the microscopic observation of cytopathic effect (CPE) in the BHK21 cell monolayers. Cytopathic agents were grouped as BLU by an indirect immunospot peroxidase test using a monoclonal antibody to VP7 of BLU1 (Gard and Kirkland 1993). Viruses serogrouped as BLU were then serotyped in a plaque reduction test (Gard and Kirkland 1993) against reference antisera (supplied by Dr B. Erasmus, Veterinary Research Institute, Onderstepoort, Republic of South Africa) to each of the 24 internationally recognised BLU serotypes. Sixteen bluetongue viruses were recovered from the heparinised bloods of seroconverting sentinels: one was from a sheep in Pusat Pembiakan Kambing, Kuala Pah, and the remainder were from cattle from all three sites (Table 1). From the 16 isolates, 6 serotypes BLU1, 2, 3, 9, 16 and 23 (Table 1; Sharifah et al. 1995) were identified, with serotype identities confirmed by Dr B. Erasmus.

At the Veterinary Research Institute, Perak, work is continuing on isolating BLU, especially from sick and aborting sheep as isolations from these sheep

Table 1. Bluetongue virus serotypes isolated from heparinised blood collected from sentinel livestock at four sites in Peninsular Malaysia.

Location	Month of blood collection				
	May 1991	June 1991	October 1991	December 1991	January 1992
Batu Arang		BLU2		BLU1	BLU1
		BLU2		BLU3	BLU1
					BLU3
Jelai Gemas	BLU1	BLU1			BLU23
	BLU9	BLU2			
		BLU16			
Kuala Pah	BLU1				
Sungai Siput	BLU1		BLU16		

may reflect the virulence of the virus. To date, three bluetongue viruses have been isolated from sick sheep in Institute Haiwan, Kluang, although these viruses have not yet been serotyped.

Pathogenicity of Malaysian BLU viruses in crossbred sheep

Sharifah et al. (1996) studied the pathogenicity of five serotypes of BLU isolates. The trial was conducted on the progeny of commercial Merino–Border Leicester crossbreds in a *Culicoides*-proof room, using twelve sheep tested as free of BLU antibodies by AGID and cELISA (Gard and Kirkland 1993). Heparinised blood (0.5 mL) containing viruses of each of the original isolates from the sentinels (BLU1, 2, 3, 9 and 23) was administered intravenously to two sheep (i.e. each virus serotype was inoculated into two sheep). Two sheep were not inoculated and kept as controls. The viruses were titrated by the intravenous inoculation of infected blood into 11- to 13-day-old ECE. As calculated by the method of Reed and Muench (1938), the titres of the viruses were low ($10^{2.4}$ ECE LD_{50}/0.5 mL to $10^{1.9}$ ECE LD_{50}/0.5 mL). The progeny of the imported crossbred sheep did not show any clinical signs of bluetongue (Table 2) except for a mild rise in body temperature (1–2°C) in two sheep (one inoculated with BLU9, the other with BLU23 at 5 days post-infection). However, as one sheep in the control group also showed a rise in body temperature this response may not be indicative of BLU infection. In this trial, all the viruses were reisolated from the sheep's heparinised blood, collected on days 5 and 8 (i.e. at peak virus titres; Uren and Squire 1982), indicating the presence of virus in peripheral blood. The lack of clinical signs suggested some degree of tolerance or resistance to the disease in the sheep. However, because they did not produce disease in crossbreds, the virulence of these viruses cannot be determined until susceptible purebred sheep are used in pathogenicity trials.

Isolation of BLU viruses from pneumonic lungs

BLU isolations were attempted on 200 pneumonic lung samples submitted to the laboratory by three government farms with problems of pneumonia in sheep. Virus isolations were conducted in ECE as previously described. Although BLU antigen-capture ELISA (Meecham 1992) was conducted to detect any bluetongue viruses from the harvested and homogenised liver and heart of the egg embryos, none were isolated from any of the samples.

Table 2. Responses of sheep to five Malaysian bluetongue virus serotypes.

Virus serotypes	Titres ECE LD_{50}/0.5 mL	Sheep no.	Rectal temperature (dpi[a])	AGID		Competitive ELISA[b]	Antigen capture[c] ELISA on egg embryos
				21 dpi	35 dpi	21 dpi	
1	$10^{2.2}$	L101T	–	–	–	low +ve	positive
		L1100	–	–	–	low +ve	positive
2	$10^{2.0}$	L989	–	–	–	low +ve	positive
		L1030	–	–	–	low +ve	positive
3	$10^{2.0}$	L1480	–	–	–	low +ve	positive
		L702	–	–	–	low +ve	positive
9	$10^{1.9}$	L1497	5th dpi	–	1+	+ve	positive
		L1500	–	2+	2+	+ve	positive
23	$10^{2.4}$	L984	–	–	2+	+ve	positive
		L985	5th dpi	–	–	low +ve	positive
Control		1486	–	–	–	–	negative
		1478	5th dpi	–	–	–	negative

[a] dpi = days post-infection
[b] % inhibition >50%+
% inhibition < 40%–
negative controls O.D. 1.1–1.6.
[c] OD > 0.25+

Proposed Measures for Control

Import regulations

As both bluetongue viruses and disease have been identified in Malaysia, overemphasis on importation of seronegative, susceptible animals will result in losses from infections contracted after arrival in this country. Provision of vaccines should be part of all national livestock development programs which involve the introduction of exotic breeds. One of the problems with BLU vaccination is that immunity afforded by one serotype does not protect against others. Identification of pathogenic BLU serotypes present in the country is extremely valuable in enabling the efficient immunisation of susceptible sheep before importation, thus avoiding the uncontrolled introduction of BLU vaccine viruses which may be inappropriate for protection against local serotypes.

However, due to genetic differences even within similar serotypes, there is concern that vaccine virus may reassort with Malaysian viruses resulting in more virulent strains. One way to minimise this risk is to hold sheep for at least 14 days after vaccination in the exporting country to allow virus clearance before importing them into Malaysia. This is useful because viraemia in sheep increases rapidly to a peak on the 5th to 8th day after infection, decreasing rapidly to be scarcely detectable on the 11th to 12th day (Luedke 1969; Uren and Squire 1982; Jeggo et al. 1985). Further surveillance of these sheep after importation should include regular clinical and serological monitoring.

Embryo transfer

For a country with endemic bluetongue, reproductive technology such as embryo transfer has been suggested as a cheaper and safer means of animal introduction (Doyle and Howard 1992). The passive immunity transferred to exotic offspring from local recipient dams could result in their protection, a control mechanism unavailable in introduced adult animals. Embryo transfer using local recipients avoids the risk of pregnancy wastage that may occur if pregnant animals are imported. However, the use of embryo transfer technology for this purpose in Malaysia would require the capability and the resources to perform it successfully.

Development of Vaccines from Local Isolates

With the availability of Malaysia's own BLU isolates, the possibility of the local production of vaccines could be considered. Vaccination produces solid immunity against homologous challenge but variable protection against heterologous infection. The development of any vaccine in Malaysia would have to be from local strains to avoid the possibility of gene reassortment among exotic strains and endemic Malaysian strains. Polyvalent vaccines would have to be produced against all pathogenic serotypes. However, limited demand is foreseen as vaccines are recommended only for susceptible imported temperate sheep. Moreover, the perception and expectation that a new generation of genetically engineered or synthetic vaccines or immunogens may soon be forthcoming could be additional disincentives to the development of local vaccines.

Conclusion

Bluetongue virus research in Malaysia will be partly influenced by the breeds of sheep imported into the country. However, it is imperative that continued support and resources be allocated to the development of systematic national surveillance using sentinels and diagnostic programs.

Acknowledgments

Dr Nor Aidah A.R. critically reviewed this paper. Tan C.L. and Liew S.W. prepared the visuals.

References

Anon. 1978. Report to the Director General of Veterinary Services on the serological survey for bluetongue in Peninsular Malaysia. Department of Veterinary Services, Malaysia, 1–15.

Cheah, T.S. and Rajamanickam, C. 1991. Occurrence of *Culicoides* spp. in sheep sheds and their relevance to bluetongue in Peninsular Malaysia. Tropical Animal Health and Production, 23, 63–65.

Chiang, B.L. 1989. An outbreak of bluetongue in imported sheep. In: Veterinary Association Malaysia, Proceedings, First Congress, 40–52.

Della-Porta, A.J., Sellers, R.F., Herniman, K.A.J., Littlejohns, I.R., Cybinski, D.H., St.George, T.D., McPhee, D.A., Snowdon, W.A., Campbell, J., Cargill, C., Corbould, A., Chung, Y.S. and Smith, V.W. 1983. Serological studies of Australian and Papua New Guinean cattle and Australian sheep for presence of antibodies against bluetongue group viruses. Veterinary Microbiology, 8, 147–162.

Doyle, K.A. 1992. An overview and perspective on orbivirus disease prevalence and occurrence of vectors in Australia and Oceania. In: Walton, T.E. and Osburn, B.I., ed., Bluetongue, African horse sickness and related orbiviruses. Proceedings 2nd International Symposium. CRC Press, Boca Raton, 44–57.

Doyle, K. and Howard, T. 1992. Working Team Report on international impacts. In: Walton, T.E. and Osburn, B.I., ed., Bluetongue, African horse sickness and related orbi-

viruses. Proceedings 2nd International Symposium. CRC Press, Boca Raton, 965–970.

Gard, G.P. and Kirkland, P.D. 1993. Bluetongue; virology and serology. In: Bagust, T. and Corner, L. ed., Australian standard diagnostic techniques for animal diseases. CSIRO, Melbourne, 1–17.

Gard, G.P., Weir, R.P. and Walsh, S.J. 1988. Arboviruses recovered from sentinel cattle using several virus isolation methods. Veterinary Microbiology, 18, 119–125.

Jeggo, J.M., Corteyn, A.H., Taylor, W.P., Davidson, W.L. and Gorman, B.M. 1985. Virulence of bluetongue virus for British sheep. Research in Veterinary Science, 42, 24–28.

Luedke, A.J. 1969. Bluetongue in sheep: viral assay and viraemia. American Journal of Veterinary Research, 30, 499–509.

Meecham, J.O. 1992. Detection of bluetongue viruses by an antigen-capture enzyme-linked immunosorbent assay. In: Walton, T.E. and Osburn, B.I., ed., Bluetongue, African horse sickness and related orbiviruses. Proceedings 2nd International Symposium. CRC Press, Boca Raton, 666–670.

Reed, L.J. and Muench, H. 1938. A simple method for estimating fifty percent end-points. American Journal of Hygiene, 27, 493–497.

Sharifah, S.H., Ali, A.R., Aziz, H., Maizan, M., Aminahkadariah, A.L. and Zainal, M. 1996. Failure of 5 Malaysian bluetongue virus serotypes to induce clinical disease in crossbred sheep. Journal of Veterinar Malaysia.

Sharifah, S.H., Ali, A.R., Gard, G.P. and Polkinghorne, I.G. 1995. Isolation of multiple serotypes of bluetongue virus from sentinel livestock in Malaysia. Tropical Animal Health and Production, 27, 37–42.

Uren, M.F. and Squire, K.R.E. 1982. The clinico-pathological effect of bluetongue virus serotype 20 in sheep. Australian Veterinary Journal, 58, 1, 11–15.

Bluetongue Virus Research in Indonesia

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Abstract

Sentinel herds were established in West Java, Bali, Nusa Tenggara Timur and Irian Jaya to determine the presence and seasonality of bluetongue virus (BLU) infection in Indonesia. Insects were collected near sentinel sites. BLU isolates were obtained from cattle blood and insects. Serological results indicated that seroconversion mostly occurred at the end of the wet season and that large ruminants had a higher prevalence of antibody than small ruminants. BLU serotypes 1, 3, 7, 9, 12, 16, 21 and 23 were successfully isolated from blood from apparently healthy cattle: BLU21 was also isolated from a pool of *Culicoides fulvus* and *C. orientalis*. The pathogenicity of BLU isolates was determined in local and imported sheep.

BLUETONGUE disease does not affect Indonesian livestock. However, an outbreak of clinical disease suspected as bluetongue was reported where imported Suffolk sheep from Australia were raised in West Java (Sudana and Malole 1982). It has since been recognised that in many tropical countries bluetongue viruses (BLU) may be circulating inapparently, without causing disease in local sheep (Gibbs et al. 1989; Sendow et al. 1992). The outbreak in West Java appears analogous to that reported in Cameroon in which imported sheep succumbed to bluetongue disease while local sheep were unaffected (Ekue et al. 1985).

A serological survey indicated that several BLU serotypes were widespread on the main islands of Indonesia (Sendow et al. 1986, 1991a). A program to study bluetongue viruses in Indonesia in greater detail was therefore implemented. Since bluetongue disease did not appear to be causing financial loss to farmers, the bluetongue work was incorporated into a broader plan to study several arboviruses of veterinary significance (Daniels et al. 1991, 1995).

Components of the Indonesian Bluetongue Research Program

The first objective was to obtain local BLU isolates, for without these the presence of bluetongue would remain unconfirmed. The Australian model of monitoring well-placed sentinel herds of cattle (St. George 1980) was adopted. The development of the sentinel program in Indonesia has already been described by Sendow et al. (1988, 1989, 1992). The initial serological survey confirmed the experiences of other countries, i.e. that large ruminants had higher prevalences of exposure than small ruminants (Sendow et al. 1986). Cattle were therefore used in sentinel groups monitored for virus isolation: these animals were introduced to the program at a young age to allow sampling of naive animals during the course of primary viral infections. Cattle were sampled weekly; blood was processed for virus isolation and held for serology and further virological studies.

Sentinel groups were placed in areas with significant livestock populations, at locations considered suitable for vector, and hence virus, activity. Major sampling sites were Depok, West Java; Denpasar,

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Bali; Kupang in Nusa Tenggara Timur (NTT) province; and Jayapura and Merauke in Irian Jaya (Fig. 1; Sendow et al. 1992). From time to time, sentinels were established at other sites to fulfil specific objectives, for example at Cisarua in West Java to allow comparison between this high altitude site and the low altitude site at Depok.

To identify potential vectors and their seasonal abundance, insects were collected at sentinel sites using light traps (Sukarsih et al. 1993). As Depok and Cisarua were near the laboratory, attempts were made to isolate viruses from freshly caught insects.

Isolation and characterisation of isolates to serotype allowed comparisons to be made with the situations in neighbouring countries, with the aim of developing a regional perspective of these BLU infections (Daniels et al. 1995; Daniels and Melville 1996). This approach is now being extended through molecular studies of the isolates (Daniels et al. 1995; Sendow et al. these Proceedings). Isolation of local BLU strains has allowed the start of pathogenicity tests to study aspects such as the virulence of local strains and the apparent resistance of local sheep.

Viruses Isolated from Sentinel Cattle

Initially, virus isolation was attempted by inoculation of samples into baby hamster kidney cell cultures (BHK21). However, a protocol based on intravenous inoculation of embryonated chicken eggs (ECE) followed by blind passage in *Aedes albopictus* cell cultures (C6/36) before passage to BHK21 was soon adopted (Sendow et al. 1993a, 1993b). Subsequent identification of viruses to groups used tests based on the BLU group-specific monoclonal antibody 20E9b762 (Lunt et al. 1988; Sendow et al. 1993a), with serotyping being requested at international reference laboratories.

Isolation of BLU viruses has been confirmed only from the Depok and Jayapura sentinel sites, although further isolates remain to be characterised (Table 1). The first serotypes reported were BLU7 and BLU9 (Sendow et al. 1991b), with BLU1, 12, 21 and 23 subsequently identified at Depok (Sendow et al. 1993a) and BLU1, 21 and 23 from Jayapura (Sendow et al. 1993b): two further serotypes have since been identified, BLU3 from Depok and BLU16 from Jayapura (Table 2).

Table 1. Results of viral isolation from heparinised sentinel cattle blood in Indonesia, 1989–1993.

Area	No. of specimens processed	Isolates	BLU group isolates
Bali	221	10	10
Irian Jaya	1327	46	11
West Java	1584	49	18
West Timor	1536	24	32
Total	4668	129	61

Confirmed BLU isolations have been made from January to June (Table 2), which is the period from the middle to the end of the wet season (Sendow et al. 1992). At Jayapura isolates were obtained throughout the wetter months, from November to May (Table 2; Sendow et al. 1992). At both locations, however, ade-

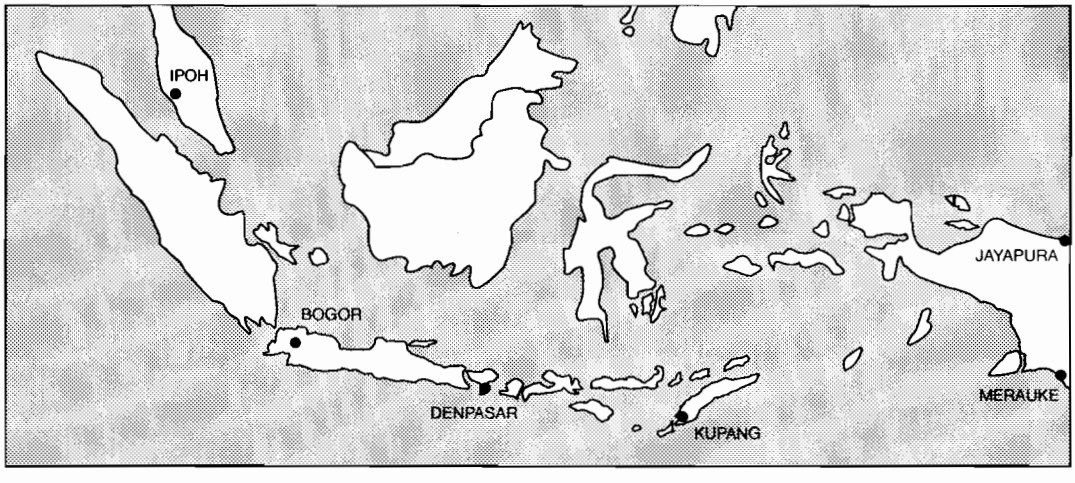

Figure 1. Map of Indonesia showing sentinel herd sampling sites.

quate rainfall to maintain vector populations occurs throughout the year (Sukarsih et al. 1993). The isolations to date probably identify peaks of activity rather than an absolute presence or absence of BLU infections.

Table 2. Bluetongue viruses isolated from sentinel cattle in Indonesia, 1988–1990.

Province and site	Animal no.	Date sampled	Bluetongue (BLU) serotype
Depok, West Java	D15	9 March 88	9
	D54	6 June 88	7
	D124	20 January 90	9
	D153	19 March 90	3
	D150	2 April 90	21
	D155	9 April 90	21
	D154	9 April 90	12
	D163	29 May 90	1
	D165	25 June 90	21
Jayapura, Irian Jaya	1166	11 November 89	23
	1169	16 December 89	16
	1159	23 January 90	23
	1164	13 February 90	21
	1163	29 May 90	1

Vector Studies

To confirm the vector status of an insect for an arbovirus must involve experimentally proving the insect's ability to transmit the virus biologically from mammalian host to mammalian host after a period of virus replication within the insect. Since such studies have not yet been possible in Indonesia, studies of potential vectors have been based on the assumption that insects implicated as vectors in neighbouring countries may also be vectors in Indonesia. Attention has thus focused on certain species of *Culicoides* shown to be vectors in Australia (Standfast et al. 1985). *Culicoides* populations adjacent to sentinel cattle have been sampled, and much is now known of the *Culicoides* fauna of livestock-producing areas in Indonesia (Sukarsih et al. 1993).

Insects collected at Depok were identified to species, pooled and processed for virus isolation (Sendow et al. 1993c). From more than 1000 pools processed, 16 isolates have been yielded, of which five were identified as BLU in grouping tests. Emphasis was given to *Culicoides* of the subgenus *Avaritia*, which contains the known Australian vector species. Other *Culicoides* species shown to support virus replication (Standfast et al. 1985) were also processed, as were pools of mosquitoes to isolate other arboviruses. The confirmed BLU isolates from insects (Table 3) include an isolate from a pool of *Aedes* mosquitoes (Sendow et al. 1994).

Table 3. Bluetongue viruses isolated from insects at Depok, West Java.

Insect pool	Date	Bluetongue (BLU) serotype
Culicoides fulvus and *C. orientalis*	May 91	21
Anopheles spp.	May 91	21
C. fulvus	April 92	1
C. perigrinus	April 92	21

Pathogenicity Studies

The pathogenicity studies were aimed at determining the virulence of Indonesian BLU isolates. Since naturally-occurring clinical disease had been reported in imported sheep but not in local sheep, perhaps because of resistance factors, local sheep were considered unsuitable for experiments. Instead sheep known to be susceptible were used, with aged Merino sheep from north-western Queensland being donated by the Queensland Department of Primary Industries: such animals have been reported more susceptible in pathogenicity trials in Australia (Johnson et al. 1992).

Collection protocols from sentinel animals provided for the storage of heparinised whole blood at each sampling, so as to have aliquots of viraemic blood for further studies in the event of a viral isolate being obtained at any sampling. Natural virulence of BLU strains can only be assessed using virus not passaged in eggs or cell cultures (Johnson et al. 1992). Preliminary inoculations of susceptible Merino sheep were conducted to propagate adequate stores of infected blood for the trials. Pairs of sheep were inoculated with cattle blood from which BLU1, 9, 21 and 23 had been isolated. Sheep inoculated with viraemic blood of BLU1, 9 and 21 responded clinically and seroconverted. Blood for further transmissions was collected during the febrile period.

Groups of Merino and local Indonesian sheep were then inoculated with infected sheep blood. Most sheep showed some clinical signs, had detectable viraemias and seroconverted. However, the clinical signs were mild and mainly limited to mild oedema of facial tis-

sues, mild hyperaemia of mucous membranes and, in a few cases, coronary bands. It was concluded that, under the experimental conditions, the isolates tested were of low pathogenicity.

Discussion

The bluetongue research program in Indonesia has yielded eight BLU serotypes recognised by international reference laboratories BLU1, 3, 7, 9, 12, 16, 21, and 23. With potential for a still greater yield of isolates, the program has thus had considerable success in defining the bluetongue status of Indonesia. Serological studies in association with sentinel monitoring confirm the widespread prevalence of animals exposed to these viruses.

As further serotypes become available, further pathogenicity tests should be conducted. The inclusion of local sheep in such studies should be continued, for this has led to the first observations of clinical signs associated with bluetongue infections in local sheep. Although only mild responses were observed, it would be useful to extend the range of observations.

Perhaps more importantly, further trials should also address the apparent discrepancy between the observation of natural disease in imported sheep but not in experimental infections of such animals. Mild disease may not be a feature of infections with all Indonesian serotypes or strains. Commercial interests periodically consider establishing farming systems based on imported Australian sheep. A full understanding of factors leading to disease is important to support such international trading initiatives, which would benefit both countries.

Acknowledgments

Dr Eha Soleha's major role in the sentinel program , including active participation in field work and serological and virological studies, is acknowledged respectfully. Technical assistance was provided by Sdr Iman Salihin, Risa Indriani, Ace Endang, M. Sulaiman, Hannipah Aryana, Maria Goretti, Ekon, Kordir, Anna, Saepudin, Edi Satria, Suminta, Cornelia Halim and Neville Hunt. Field work was assisted by the provincial offices of the Dinas Peternakan and by the Disease Investigation Centre, Denpasar. Dr G.P. Gard advised on the design of pathogenicity studies. The work was substantially assisted by the Australian Quarantine and Inspection Service under the Northern Australia Quarantine Surveillance program.

References

Daniels, P.W. and Melville, L. Arboviruses of livestock in the Northern Territory. CSIRO and Northern Territory DPIF. 1996

Daniels, P. W., Sendow, I., Soleha, E., Jennings, J. and Sukarsih. 1991. A veterinary arbovirus monitoring program. In: Martin, S.W., ed., Proceedings 6th Congress of the International Society for Veterinary Epidemiology and Economics. ISVEE, Ottawa, 207–209.

Daniels, P.W., Sendow, I., Soleha, E., Sukarsih, Hunt, N.T. and Bahri, S. 1995. Australian–Indonesian collaboration in veterinary arbovirology. A review. Veterinary Microbiology, 46, 151–174.

Ekue, F.N., Nfi, A.N., Tsangue, T., Taylor, W.P. and Gumm, I.D. 1985. Bluetongue in exotic sheep in Cameroon. Tropical Animal Health and Production, 17, 187–188.

Gibbs, E.P.J., Greiner, E.C., Homan, E.J., Yuill, T.M., Mo, C.L. and Walton, T.E. 1989. Bluetongue and ecosystems a New World perspective. In: Uren, M.F., Blok, J. and Manderson, L.H., ed., Arbovirus research in Australia. Proceedings 5th Symposium. CSIRO/Queensland Institute of Medical Research, Brisbane, 293–297.

Johnson, S.J., Hoffmann, D., Flanagan, M., Polkinghorne, I.G. and Bellis, G.A. 1992. Clinico-pathology of Australian bluetongue virus serotypes for sheep. In: Walton, T.E. and Osburn, B.I., ed., Bluetongue, African horse sickness and related orbiviruses. Proceedings 2nd International Symposium. CRC Press, Boca Raton, 737–743.

Lunt, R.A., White, J.R. and Blacksell, S.D. 1988. Evaluation of a monoclonal antibody blocking ELISA for the detection of group specific antibodies to bluetongue virus in experimental and field sera. Journal of General Virology, 69, 2729–2740.

Sendow, I., Daniels, P.W., Cybinski, D.H., Young, P.L. and Ronohardjo, P. 1991a. Antibodies against certain bluetongue and epizootic haemorrhagic disease viral serotypes in Indonesian ruminants. Veterinary Microbiology, 28, 111–118.

Sendow, I., Daniels, P.W., Soleha, E., Erasmus, B., Sukarsih and Ronohardjo, P. 1993a. Isolation of bluetongue virus serotypes new to Indonesia from sentinel cattle in West Java. Veterinary Record, 133, 166–168.

Sendow, I., Daniels, P.W., Soleha, E., Hunt, N., Bale, A. and Ronohardjo, P. 1989. Studies on orbiviral infections of sentinel ruminants in Indonesia. In: Uren, M.F., Blok, J. and Manderson, L.H. ed., Arbovirus research in Australia. Proceedings 5th Symposium. CSIRO/Queensland Institute of Medical Research, Brisbane, 311–315.

Sendow, I., Daniels, P.W., Soleha, E., Hunt, N. and Ronohardjo, P. 1991b. Isolation of bluetongue viral serotypes 7 and 9 from healthy sentinel cattle in West Java, Indonesia. Australian Veterinary Journal, 68, 405.

Sendow, I., Daniels, P.W., Soleha, E. and Sukarsih. 1992. Epidemiological studies of bluetongue viral infections in Indonesian livestock. In: Walton, T.E. and Osburn, B.I. ed., Bluetongue, African horse sickness and related orbiviruses, Proceedings 2nd International Symposium. CRC Press, Boca Raton, 147–154.

Sendow, I., Hunt, N., Bale, A., Bartz, C., Tarmudji, Ronohardjo, P. and Daniels, P.W. 1988. Studies of arboviral infections of sentinel cattle in Indonesia. In: Sastradipradja, D. and Sigit, S.H., ed., Proceedings 6th FAVA Congress, Denpasar, Indonesian Veterinary Association, Jakarta, 367–373.

Sendow, I., Soleha, E., Daniels, P.W., Sebayang, D., Achdiyati, J., Karma, K. and Erasmus, B.J. 1993b. Isolation of bluetongue virus serotypes 1, 21 and 23 from healthy cattle in Irian Jaya, Indonesia. Australian Veterinary Journal, 70, 229–230.

Sendow, I., Sukarsih, Soleha, E. and Daniels, P.W. 1994. Isolation of bluetongue virus serotype 21 from mosquitoes in West Java, Indonesia. Penyakit Hewan, 26 (48), 21–25.

Sendow, I., Sukarsih, Soleha, E., Erasmus, B.J. and Daniels, P.W. 1993c. Isolation of bluetongue virus serotype 21 from *Culicoides* spp. in Indonesia. Veterinary Microbiology, 36, 349–353.

Sendow, I., Young, P., and Ronohardjo, P. 1986. Preliminary survey for antibodies to bluetongue-group virus in Indonesian ruminants. Veterinary Record, 119, 603.

Standfast, H.A., Dyce, A. L. and Muller, M.J. 1985. Vectors of bluetongue in Australia. In: Barber, T.C., Jochim, M.S. and Osburn, B.I. ed., Bluetongue and related orbiviruses. Progress in Clinical and Biological Research, vol. 178, Alan R. Liss Inc., New York, 177–186.

St. George, T.D. 1980. A sentinel herd system for the study of arbovirus infections in Australia and Papua-New Guinea. Veterinary Science Communications, 4, 39–51.

Sudana, I.G. and Malole, M. 1982. Penyidikan penyakit hewan bluetongue di desa Caringin, Kabupaten Bogor. In: Annual report of disease investigation in Indonesia during the period of 1975–1981, Direktorat Kesehatan Hewan, Direktorat Jenderal Peternakan, Jakarta, 110–121.

Sukarsih, Daniels, P.W., Sendow, I. and Soleha, E. 1993. Longitudinal studies of *Culicoides* spp. associated with livestock in Indonesia. In: Uren, M.F. and Kay, B.H. ed., Arbovirus research in Australia. Proceedings 6th Symposium. CSIRO/Queensland Institute of Medical Research, Brisbane, 203–209.

The History of Bluetongue in Australia and the Pacific Islands

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Abstract

Between 1959 and 1977 in Australia there was a long period of development of virology and vector expertise in preparation for bluetongue. Nevertheless, the initial discovery of bluetongue virus (BLU) in 1977 near Darwin was unexpected. Near that location, eight BLU serotypes have been discovered in Australia since 1977 although only two are continually present. Five of the serotypes are highly virulent but they have not persisted as silent infections of cattle and buffalo for more than a few years, nor have they spread far from their initial focus in the far north of the Northern Territory. Serotype 1 has spread beyond Australia and Papua New Guinea to the Solomon Islands but not yet to other island nations of the Pacific, although a suitable vector, *Culicoides brevitarsis*, has reached as far east as Noumea, Fiji and Tonga. The establishment of bluetongue in countries east of Weber's line has depended completely on the previous introduction of ruminants and the presence of cattle-reliant *Culicoides* species which can spread the virus. This sequence has occurred in Australia, Papua New Guinea and the Pacific Islands, where cattle did not exist before being introduced by European settlement.

A change in the interest of Australian veterinary authorities toward bluetongue followed a major epidemic in Merino sheep in the Iberian peninsula in 1956. As the disease existed in the eastern Mediterranean and the United States, it was clear that bluetongue was present close to all the shipping routes from countries that were Australia's sources of improved breeding cattle, sheep and goats. Thus all commercial vessels would pass close to infected regions or call at ports in countries where the disease was endemic, whether they came via the Suez Canal, around Africa or via the Americas. Air transport of ruminants was not a commercial proposition in the 1950s. Reliable laboratory screening tests did not then exist to detect bluetongue virus (BLU) in sheep or in the inapparent cattle host. Australia was not alone in expecting severe consequences from bluetongue, which gave an appearance of continual spread as more countries reported bluetongue as a new disease, or recognised it as the cause of an existing condition.

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Australian Reaction to the Apparent Expansion of Bluetongue

The Australian reaction to this apparent expansion of bluetongue took several forms. The import of cattle, sheep and other ruminant species, including zoo herbivores, was stopped from most regions of the world for many years. These sanctions were extended to donor animals when methods were developed for the long-term storage of semen (Gee 1975). The restrictive effects on sources of gene plasm for Australia were severe.

Assistance from Abroad

Preparation was wide-ranging. Steps were taken to familiarise Australian veterinarians with bluetongue disease, to supplement those few who had experience with the disease in Africa. Some were sent to courses on exotic diseases conducted at Grosse Isle, Canada. This meant that experienced diagnostic teams could be sent to any possible outbreak. This precautionary approach has continued up to the present although now training is done within Australia. An extensive

tour of Australia by a group of the world's principal bluetongue experts was sponsored by the Australian Bureau of Animal Health in 1974, culminating in a symposium at the Australian Veterinary Association Conference in Adelaide. Inactivated reagents for the complement fixation test and vaccine seed stocks were prepared in South Africa, and held under secure restrictions in case of an outbreak of bluetongue in Australia.

Australian Virus and Vector

The major long-term strategy was the development of an Australian capability to isolate viruses and to define the *Culicoides* vector species in Australia. This was undertaken by CSIRO Division of Animal Health and Production as a deliberate long-term policy instituted in the mid-1950s by Drs L.B. Bull and T.S. Gregory. They recognised that there was no significant laboratory capability to deal with the laboratory aspects of exotic virus disease. Foot-and-mouth disease was the more pressing reason for the establishment of modern virology with the appointment of Dr E.L. French and W.A. Snowdon to the CSIRO Parkville Laboratory in Melbourne, but the threat of vector-borne bluetongue was the basic reason for developing the *Culicoides* studies.

A vector study group was created with the object of identifying the vectors of ephemeral fever, a serious arthropod-borne virus infection of cattle. The necessary taxonomy developed for this research was, in fact, preparation for bluetongue or other exotic vector-borne disease. The entomology was established within CSIRO by M.D. Murray, A.L. Dyce, H.A. Standfast and M.J. Muller (Muller 1995). By 1977, this team had defined most of the major and minor species of *Culicoides* feeding on cattle and sheep in Australia. The most important of the potential vectors of bluetongue were found to be species linked biologically to cattle as a food source and to cattle dung for breeding sites. These species had become established in Australia from Asia after cattle and buffalo were introduced to northern Australia.

A multidisciplinary team based in Sydney and Brisbane made headway in defining the biology of *Culicoides* species later found to be vectors of bluetongue and related viruses. Vector control plans by aerial and ground disinsection were produced in case of an outbreak of bluetongue but were never used.

In 1968, a network of sentinel cattle had been established (St. George 1980) so that recently collected serum samples were available from cattle in most major regions of Australia, as well as those stored as a library at CSIRO Long Pocket Laboratories since the inception of the system in 1969. By 1977, a system of co-located insect vector traps to monitor populations was being developed (St. George and Standfast 1983) and mechanisms were in place for rapid expansion. As is amply demonstrated in these Proceedings, this team approach has since served as a model for studying vector-borne viruses of livestock in Southeast Asia.

The final and precipitating event in the bluetongue preparatory period was a continuous study over several months in the subcoastal plains region of the Northern Territory at Beatrice Hill (Standfast et al. 1984). Although the aim of this study was to find the maintenance focus of ephemeral fever virus, techniques for the isolation of viruses from solely mouse brain injection were altered in April 1975 to cater for the entry of bluetongue.

Discovery of Bluetongue in Australia

Infection and disease

Despite all the preparation described above, the recognition of bluetongue in Australia in October 1977 (St. George et al. 1978) was only accepted with difficulty by some individuals and organisations. Transmission studies in sheep at CSIRO Long Pocket Laboratories were carried out promptly in a newly-commissioned arthropod-proof building. The fever, disability and lesions observed were typical of bluetongue, though there were no fatalities (St. George and McCaughan 1979; Uren and Squire 1982). However, only laboratory-adapted virus was available so the true potential could not be tested.

An enormous cooperative effort between CSIRO and the respective Departments of Agriculture or Primary Industries of the States and Territories of Australia and Papua New Guinea placed the discovery in context within a few months. The first bluetongue virus (BLU20) had a very limited distribution in far northern Australia, with a probable time of entry into Australia of 1973. The existence of additional bluetongue serotypes was suspected in early November 1977. The evidence was strengthened when seroconversion to BLU1 was demonstrated at Pirbright Laboratory, England, using suitable paired sera from sentinel cattle (Snowdon and Gee 1978). The BLU group antibody, detected by agar gel immunodiffusion (AGID), was much more widely distributed than the neutralising antibody specific to BLU20: it extended into Western Australia, Queensland and New South Wales (Della-Porta et al. 1983), approximating the distribution of *Culicoides brevitarsis*.

Most of the bluetongue group antibody activity was explained by the isolation in 1979, after a deliberate search by sentinel herd techniques, of two further bluetongue viruses (BLU1 and 21). These viruses were used for retrospective serology (Figs 1 and 2) and, as shown in Table 1, their presence was

traced back to at least 1958 (St. George et al. 1980). However, the AGID test was found not to be limited to BLU antibodies: the isolation of five viruses of the epizootic hemorrhagic disease (EHD) group enabled many more of the anomalous serological reactions to be explained (St. George et al. 1983).

The southern limits of the distribution of antibodies detected in surveys using Australian bluetongue and related orbiviruses were approximately reciprocal to those of the Australian commercial sheep flock. This also approximated the distribution of *Culicoides brevitarsis*, which was later found to be an inefficient BLU vector (St. George and Muller 1984; Standfast et al. 1985, 1992). Although six other species of *Culicoides* were found experimentally to be possible BLU vectors (Standfast et al. 1985), *C. brevitarsis* is the species that most closely impinged on the sheep raising areas. BLU1 has been isolated from 'wild caught' *C. brevitarsis* and *C. fulvus* (Standfast et al. 1979; St. George and Muller 1984).

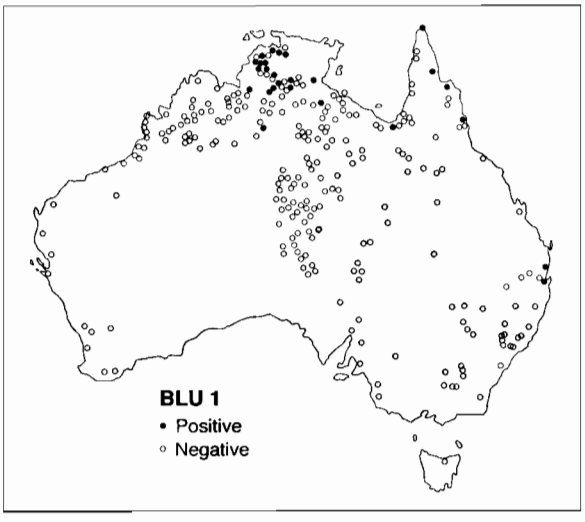

Figure 1. The distribution of neutralising antibodies to BLU1 in sera collected from cattle between November 1977 and January 1978.

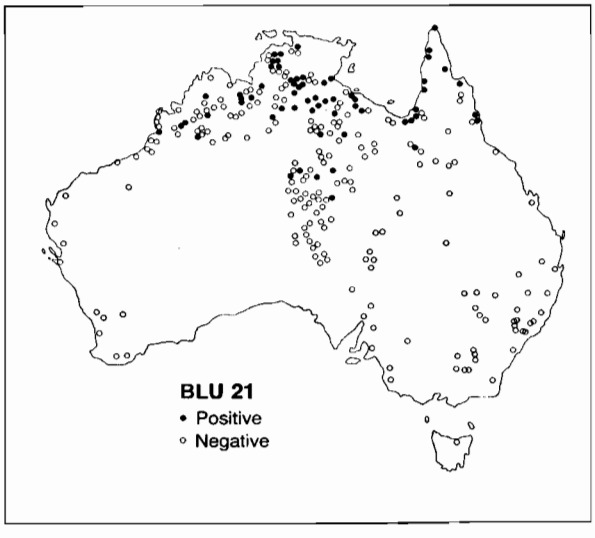

Figure 2. The distribution of neutralising antibodies to BLU21 in sera collected from cattle between November 1977 and January 1978.

Table 1. Chronology of discovery and retrospective serology of bluetongue viruses (BLU) in Australia.

BLU Serotype	Year of first isolation	Most recent isolation	Year when antibody first detected in cattle	Test in sheep
20	1975	1995	1973	virulent
1	1979	1994	1958	mild
21	1979	1994	1958	mild
15	1982	1986	1980	virulent
23	1982	1989	1974	virulent
9	1985	1986	1985	non-pathogenic
3	1986	1991	1986	virulent
16	1986	1986	1984	virulent

Van Kammen and Cybinski (1981) found BLU antibodies in cattle in Papua New Guinea. In a further search for the source of the new BLU, ruminant sera were obtained from Indonesia and Malaysia in cooperation with those countries where AGID testing had shown the presence of bluetongue group antibody (Geering and Gard 1989). However, using the combined evidence of both virus isolations and *Culicoides* species biology and distribution, Indonesia was the most probable source of Australia's bluetongue viruses (St. George 1986). This conclusion is supported by the finding of bluetongue viruses and antibodies in Indonesian cattle (Sendow et al. 1989, 1993)

By 1981, after the initial delineation of the distribution of BLU and their vectors, it was widely assumed that a steady state existed. *Culicoides brevitarsis* was endemic mainly in the north of Australia and on the east coast, and infested the main sheep-raising areas only marginally and irregularly (Murray 1986). Direct field evidence to confirm that *C. brevitarsis* was infected with BLU did not emerge until later (St. George and Muller 1984).

Under experimental conditions, the first three BLU serotypes had produced moderate illness but no fatalities (Uren and Squire 1982). The isolation of further serotypes between 1982 and 1986 (Table 1), in a new virological unit in Darwin under G.P. Gard, did not attract much attention, despite serological evidence of recent entry (Gard et al. 1985, 1987a, 1987b; Gard and Melville 1989, 1992). The rapid southward expansion of *C. wadai*, a more efficient BLU vector than *C. brevitarsis* (Standfast et al. 1983) was checked by a long succession of dry summers (Muller 1995). CSIRO funds and staff were redirected to other research as the threat of vector expansion was not considered important.

Subsequently, two events altered this perception. First, an experimental demonstration in 1988 showed that four Australian serotypes could cause disease (Johnson et al. 1989). Second, there was a severe clinical case due to natural disease acquired in the first few days of February 1989 in a sentinel sheep near Darwin (L.F. Melville, pers. comm.). Together with the demand for up-to-date information by customer countries to verify the freedom of much of Australia from bluetongue, these events stimulated a new approach. There was a need to reinstate the surveillance of the distribution of known BLU serotypes and vectors to give warning of threat. Data were also needed on the vaccine potential of Australian BLU, and their persistence in blood and semen.

The capacity to detect both new BLU incursions into Australia and extensions of its range within Australia have changed considerably since 1977, when the delay was some 31 months. Now various state laboratories carry out serology and can isolate BLU. The fatal clinical case near Darwin was diagnosed on 9 Feb 1989, the provisional identity of the causative virus was known on 13 February, and formal identification of BLU23 was made at Berrimah Laboratory on 11 March (L.F. Melville, pers. comm.). The development of molecular techniques means that the initial identification process can be carried out even more quickly in future, using field material directly and with a result in one to two days. These advances mean the protracted delays in the identification of the original Australian BLU isolate need not occur again.

Control strategies from 1977 to 1996

Geering (1975) described the anticipated strategies to control bluetongue if it entered Australia. The planned actions included a slaughter out policy of ruminants within a five mile radius, movement controls and disinsection by air and ground application of insecticides to suppress *Culicoides*.

The information available to the regulatory authorities at their first meeting was confined to where and when the insects were caught that had yielded the first BLU isolates. Office International des Epizooties (OIE) and customer countries were advised immediately of the presence of bluetongue in Australia.

The research program progressively provided more information. The planned slaughter out policy and disinsection were never put in place as they were completely inappropriate.

Movement controls on cattle, sheep and buffalo were imposed by various states: these either prohibited or restricted cattle movements north of latitude 18°S. The necessity to protect overseas markets for live animals and products overrode detached scientific judgement on the possible effectiveness of such controls. Prohibitions by importing countries affected not only live cattle, sheep and other ruminants but also germplasm, meat hides and wool, either from bluetongue infected areas or from the whole of Australia. Of necessity, the emphasis changed gradually from detecting infection to defining which parts of Australia were not infected with BLU. This attitude applies even more strongly in 1995, when proof of regional freedom from a disease is becoming an international marketing tool.

Movement controls within Australia, which did not begin to be relaxed until late 1978, took some time to disappear, and even longer for the export trade (Geering and Gard 1989). Rapidly developed serological tests provided data on which to base decisions about movements. Unfortunately, at that time the presence of BLU antibodies was equated to lifelong infection, and many uninfected animals were excluded. Even the accumulating evidence to the contrary is still slow to change this attitude. There is no evidence that movement controls had any beneficial effect whatsoever on limiting the spread of bluetongue to vector-free regions.

In 1981, Australian live sheep shipments became infected with bluetongue after arrival in Indonesia (Sendow et al. 1989) and in 1987 Malaysia (Geering and Gard 1989). These sheep originated from bluetongue-free areas. In the early 1990s sheep were also diagnosed as having bluetongue on arrival in Middle-East countries. However, again the available data indicated that these sheep had originated from bluetongue-free areas within Australia and could not have had bluetongue.

The first three Australian BLU serotypes differed biochemically from the South African ones, indicating genetic diversity (Gorman et al. 1982). Extensive studies at CSIRO Australian Animal Health Laboratories have since confirmed that Australian BLU are more closely related to each other than to South African or American BLU, though structurally identical (Gould et al. 1986, 1989). The earlier decision not to release new BLU genes into the Australian environment by using South African live virus vaccines was thus justified. Vaccine seed stocks of Australian origin (BLU1, 20 and 21) were prepared under security (Wark et al. 1982); subsequently these have included all five serotypes.

Two further events, however, demonstrated that bluetongue was still a threat. The first was a series of experiments in 1988 at Oonoonba Laboratory, Townsville, using unadapted bluetongue field or 'wild' viruses. These experiments clearly showed that strains of BLU3, 15, 16 and 23 were highly virulent to Merino sheep, giving clinical signs equivalent to those seen with the most severe bluetongue and causing 8–32% mortality (Johnson et al. 1992a, b). D. Hoffmann and S.J. Johnson developed one of the most reliable procedures for producing severe clinical bluetongue in the world, which is also used by L.F. Melville. The second event was the occurrence of a naturally-acquired fatal bluetongue disease in a sheep near Darwin in 1989.

Bluetongue research management

A Bluetongue Research Management Committee, headed by G.I. Alexander, was established by the Australian Agricultural Council and reported on research from 1989 to 1994 (Alexander 1990, 1991). The achievements in this era clarified the different effects of 'wild' bluetongue virus in blood from naturally infected animals and laboratory-adapted virus.

In contrast to the unmodified 'wild' virus, virus adapted in tissue cultures to a vaccine standard became teratogenic to first and second trimester pregnant ewes (Johnson et al. 1992b). Also 'wild' virus, whether naturally acquired (Melville et al. 1993) or injected experimentally, was not excreted in the semen of bulls. However, adapted virus was excreted in the semen of older bulls, possibly due to contamination of semen with red blood cells in the viraemic period. Contamination of semen with BLU during natural infection seems to be a very uncommon event. This has implications for the use of live virus vaccines. The modified virus, which has acquired the undesirable characteristics of teratogenesis and excretion in semen of bulls and rams, has the potential to become established in nature. H.A. Standfast (pers. comm.) and I have shown that even low titre, tissue culture-adapted virus $10^{2.3}$/mL can be taken up by *C. brevitarsis*.

In field trials, Muller and Harris (1993) found that local populations of *C. brevitarsis* could be suppressed by injecting cattle with Ivermectin. This approach is more effective than treating sheep as there is suppression of the larval stage of the life cycle by rendering the cattle dung toxic.

The vaccine options live attenuated, killed whole virus and subunit vaccines were examined by the management committee. The advantages, disadvantages and cost options for each type of vaccine, and for a vaccine bank, were examined. The time frames to produce each type of vaccine were also delineated (Alexander et al. 1993). Further development of

whole virus, chemically-inactivated, and sub-unit vaccines were deemed necessary before definitive recommendations could be justified.

Indonesia, Papua New Guinea, Solomon Islands, New Caledonia, Fiji and Tonga

The source of BLU that periodically enter the north-west of the Northern Territory is now certainly Indonesia, from the evidence of the isolation of BLU serotypes and the vector identifications presented in these Proceedings. Evidence of the presence of many other arboviruses found in the same vectors is steadily accumulating. Probably all BLU serotypes that have been isolated in northern Australia will be found in Indonesia in due course. *Culicoides brevitarsis* carries many other arboviruses that infect cattle. Of the 17 viruses of the Simbu, Palyam and ephemeral fever group, of which *C. brevitarsis* is the proven or suspected vector in Australia, many are known from Indonesia from virus isolation or serological surveys, eight are present in New Guinea, seven in the Solomon Islands, three in New Caledonia and one in Fiji.

The cattle in Papua New Guinea have fewer BLU serotypes than cattle in Australia and there is only a single serotype in the Solomon Islands. New Caledonia and Fiji had no bluetongue in 1987. *C. brevitarsis* has colonised these islands and Tonga, so the potential remains for spread if bluetongue viruses were introduced in infected *Culicoides* blown in on the wind. The range of *Culicoides* species linked to cattle is greatest in Indonesia, with fewer in Papua New Guinea and Australia, and probably only a single species in Noumea and Fiji (Dyce 1982). New Zealand is presently free of both *C. brevitarsis* and the viruses it can spread.

There is thus a gradient in the number of arboviruses carried by *Culicoides* from west to east and in the number of arbovirus-carrying species that feed on ruminants. Large distances across the sea seem to be less of a barrier to the passive movement of insects than was thought in the past.

Lessons from History

The announcement that bluetongue was present in Australia caused a severe economic impact by halting interstate movement of cattle and live exports for months, even though the virus did not kill a single sheep. Also, it had been implicitly assumed that other countries had the same knowledge of how to limit the spread of an arthropod-borne virus as had been developed in Australia as a result of systematic preparation. Any initial announcement regarding bluetongue must be followed up by relaying information as it becomes available, preferably in the language of importing countries, in case scientific data are not available there.

Before bluetongue was discovered in Australia its threat was considered to be linked to the importation of animals and germplasm from Europe and North America. It is now known that BLU and its vectors are merely part of the movement of a wide range of arboviruses and insect vectors expanding from the islands of Southeast Asia to the Pacific (St. George 1986, 1992). In contrast to most of the tropical and subtropical world, bluetongue is an emerging disease in Australia, Papua New Guinea and the Pacific islands.

The current Australian Veterinary Plan for bluetongue, developed in 1994, depends heavily on continued surveillance to give warning, but the capacity to deal with an outbreak or an epidemic is limited to suppression of *Culicoides* vectors by treatment of cattle and sheep with Ivermectin (Muller and Harris 1993). Until a non-living, broad spectrum vaccine is available, the Australian commercial sheep flock remains as vulnerable as it was in 1977.

References

Alexander, G.I. 1990. Bluetongue in Australia. Australian Veterinary Journal, 67, 277.

— 1991. Bluetongue research in Australia 1990. Australian Veterinary Journal, 68, 125–126.

Alexander, G.I., St. George, T.D. and Gard, G.P. 1993. Report on bluetongue vaccines to Australian Meat Corporation, Sydney.

Della-Porta, A.J., Sellers, R.F., Herniman, K.A.J., Littlejohns, I.R., Cybinski, D.H., St. George, T.D., McPhee, D.A., Snowdon, W.A., Campbell, J., Cargill, C., Corbould, A., Chung, Y.S. and Smith, V.W. 1983. Serological studies of Australian and Papua New Guinean cattle and Australian sheep for the presence of antibodies against Australian bluetongue group viruses. Veterinary Microbiology, 8, 147–162.

Dyce, A.L. 1982. Distribution of *Culicoides Avaritia* spp (Diptera: Ceratopogonidae) west of the Pacific Ocean. In:St. George, T.D. and Kay, B.H. ed., Arbovirus research in Australia. Proceedings 3rd Symposium. CSIRO/Queensland Institute of Medical Research, Brisbane, 35–43.

Gard, G.P. and Melville, L.F. 1989. The evolution of bluetongue in northern Australia. In: Uren, M.F., Blok, J. and Manderson, L.H., ed., Arbovirus research in Australia. Proceedings 5th Symposium. CSIRO/Queensland Institute of Medical Research, Brisbane, 303–305.

— 1992. Results of a decade's monitoring for orbiviruses in sentinel cattle pastured in an area of regular arbovirus activity in northern Australia. In: Walton, T.E. and Osburn, B.I, ed., Bluetongue, African horse sickness and related orbiviruses. Proceedings 2nd International Symposium. CRC Press, Boca Raton, 85–92.

Gard, G.P., Shorthose, J.E., Cybinski, D.H. and Zakrzewski, H. 1985. The isolation from cattle of 2 bluetongue viruses new to Australia. Australian Veterinary Journal, 62, 203.

Gard, G.P., Shorthose, J.E., Weir, R.P. and Erasmus, B.J. 1987a. The isolation of a bluetongue serotype new to Australia. Australian Veterinary Journal, 64, 87–88.

Gard, G.P., Weir, R.P., Melville, L.F. and Lunt, R.A. 1987b. The isolation of bluetongue virus type 3 and 16 from northern Australia. Australian Veterinary Journal, 64, 388.

Gee, R.W. 1975. Bluetongue certification Australian policy. Australian Veterinary Journal 51, 213–215.

Geering, W.A. 1975. Control of bluetongue in an epizootic situation. Australian Veterinary Journal 51, 220–224.

Geering, W.A. and Gard, G.P. 1989. Bluetongue infection of sheep in Malaysia. In: Uren, M.F., Blok, J. and Manderson, L.H., ed., Arbovirus research in Australia. Proceedings 5th Symposium. CSIRO/Queensland Institute of Medical Research, Brisbane, 310–311.

Gorman, B.M., Taylor, J., Finnimore, P.M., Bryant, J.A., Sangar, D.V. and Brown, F. 1982. A comparison of bluetongue viruses isolated in Australia with exotic bluetongue virus serotypes. In: St. George, T.D. and Kay, B.H., ed., Arbovirus research in Australia. Proceedings 3rd Symposium. CSIRO/Queensland Institute of Medical Research, Brisbane, 101–109.

Gould, A.R. and Eaton, B.T. 1986. Genotype variants of Australian orbiviruses. In: St. George, T.D., Kay, B.H. and Blok, J.,ed., Arbovirus research in Australia. Proceedings 4th Symposium. CSIRO/Queensland Institute of Medical Research, Brisbane, 253–258.

Gould, A.R., Hyatt, A.D., Eaton, B.T., White, J.R., Hooper, P.T., Blacksell, S.D. and Le Blanc Smith, P.M. 1989. Current techniques in rapid bluetongue diagnosis. Australian Veterinary Journal, 66, 450–454.

Johnson, S.J., Hoffmann, D., Flanagan, M., Polkinghorne, I.G. and Bellis, G.A. 1989. Recent experience with bluetongue in Queensland. In: Uren, M.F., Blok, J, and Manderson, L.H., ed., Arbovirus research in Australia. Proceedings 5th Symposium. CSIRO/Queensland Institute of Medical Research, Brisbane, 307.

Johnson, S.J., Polkinghorne, I.G., Flanagan, I.M. and Townsend, W.L. 1992a. Clinico-pathology of Australian bluetongue virus serotypes for sheep. In: Walton, T.E. and Osburn, B.I., ed., Bluetongue, African horse sickness and related orbiviruses. Proceedings 2nd International Symposium, CRC Press, Boca Raton, 737–743.

— 1992b. The Australian experience: results of a bluetongue vaccination program. In: Walton, T.E. and Osburn, B.I., ed., Bluetongue, African horse sickness and related orbiviruses. Proceedings 2nd International Symposium, CRC Press, Boca Raton, 868–873.

Melville, L.F., Kirkland, P.D., Hunt, N.T. and Williams, C.F. 1993. Excretion of bluetongue virus serotypes 1 and 23 in semen of bulls. In: St. George, T.D., Kay, B.H., and Blok, J., ed., Arbovirus research in Australia. Proceedings 6th Symposium. CSIRO/Queensland Institute of Medical Research, Brisbane, 185–187.

Muller, M.J. 1995. Veterinary arbovirus vectors in Australia a retrospective. Veterinary Microbiology, 46, 101–116.

Muller, M.J. and Harris, E.J. 1993. The use of a systemic insecticide to control the arbovirus vector *Culicoides brevitarsis*. In: Uren, M.F. and Kay, B.H., ed., Arbovirus research in Australia. Proceedings 6th Symposium. CSIRO/Queensland Institute of Medical Research, Brisbane, 23–27.

Murray, M.D. 1986. The influence of abundance and dispersal of *Culicoides* on the epidemiology of arboviruses of livestock in New South Wales. In: St. George, T.D., Kay, B.H. and Blok, J., ed., Arbovirus research in Australia. Proceedings 4th Symposium. CSIRO/Queensland Institute of Medical Research, Brisbane, 232–234.

Sendow, I., Daniels, P., Soleha, E., Hunt, N.T., Bole, A. and Ronohardjo, P. 1989. Studies on orbiviral infections of sentinel ruminants in Indonesia. In: Uren, M.F., Blok, J. and Manderson, L.H., ed., Arbovirus research in Australia. Proceedings 5th Symposium. CSIRO/Queensland Institute of Medical Research, Brisbane, 311–315.

Sendow, I., Soleha, E., Daniels, P.W. and Sukarsih. 1993. Serological studies of bluetongue virus in Indonesia. In: St .George, T.D., Kay, B.H. and Blok, J., ed., Arbovirus research in Australia. Proceedings 4th Symposium. CSIRO/Queensland Institute of Medical Research, Brisbane, 271–273.

Snowdon, W.A. and Gee, W. 1978. Bluetongue virus in Australia. Australian Veterinary Journal, 54, 505.

Standfast, H.A., Dyce, A.L. and Muller, M.J. 1985. Vectors of bluetongue virus in Australia. In: Barber, T.C., Jochim, M.S. and Osburn, B.I. ed., Bluetongue and related orbiviruses. Progress in Clinical and Biological Research, vol. 178, Alan R. Liss Inc., New York, 177–186.

Standfast, H.A., Dyce, A.L., St. George, T.D., Cybinski, D.H. and Muller, M.J. 1979. Vectors of a bluetongue virus in Australia. In: St. George, T.D. and French, E.L. ed., Arbovirus research in Australia. Proceedings 2nd Symposium. CSIRO/Queensland Institute of Medical Research, Brisbane, 20–28.

Standfast, H.A., Dyce, A.L., St. George, T.D., Muller, M.J., Doherty, R.L., Carley, J.G. and Filippich, C. 1984. Isolation of arboviruses from insects collected at Beatrice Hill, Northern Territory of Australia, 1974–1976. Australian Journal of Biological Sciences, 37, 351–366.

Standfast, H.A., Muller, M.J. and Dyce, A.L. 1983. A recent extension of the range of *Culicoides wadai* to south-east Queensland. Australian Veterinary Journal, 60, 383–384.

— 1992. An overview of bluetongue virus vector biology and ecology in the oriental and Australasian regions of the western Pacific. In: Walton, T.E. and Osburn, B.I., ed., Bluetongue, African horse sickness and related orbiviruses. Proceedings 2nd International Symposium. CRC Press, Boca Raton, 253–261.

St. George, T.D. 1980. A sentinel herd system for the study of arbovirus infections in Australia and Papua New Guinea. Veterinary Science Communications, 4, 39–51.

— 1986. Arboviruses infecting livestock in the Australian Region. In: St. George, T.D., Kay, B.H. and Blok, J., ed., Arbovirus research in Australia. Proceedings 4th Symposium. CSIRO/Queensland Institute of Medical Research, Brisbane, 23–25.

— 1992. Occupation of an ecological niche in the Pacific Ocean countries by bluetongue and related viruses. In: Walton, T.E. and Osburn, B.I., ed., Bluetongue, African horse sickness and related orbiviruses. Proceedings 2nd International Symposium. CRC Press, Boca Raton, 76–84.

St. George, T.D., Cybinski, D.H., Della-Porta, A.J., McPhee, D.A., Wark, M.C. and Bainbridge, M.H. 1980. The isolation of two bluetongue viruses from healthy cattle in Australia. Australian Veterinary Journal, 56, 562 - 563.

St. George, T.D., Cybinski, D.H. and Standfast, H.A. 1983. The isolation of five different viruses of the epizootic hemorrhagic disease of deer. Australian Veterinary Journal, 60, 216–217.

St. George, T.D. and McCaughan, C.J. 1979. The transmission of the CSIRO19 strain of bluetongue virus type 20 to sheep and cattle. Australian Veterinary Journal 55, 198–199.

St. George, T.D. and Muller, M.J. 1984. The isolation of a bluetongue virus from *Culicoides brevitarsis*. Australian Veterinary Journal 61, 95.

St. George, T.D. and Standfast, H.A. 1983. Bovine ephemeral fever in north Queensland 1974–1977. Queensland Department of Primary Industries Bulletin, Brisbane, QB83001, 9.

St. George, T.D., Standfast, H.A., Cybinski, D.H., Dyce, A.L., Muller, M.J., Doherty, R.L., Carley, J.G., Filippich, C. and Frazier, C.L. 1978. The isolation of a bluetongue virus from *Culicoides* collected in the Northern Territory of Australia. Australian Veterinary Journal, 54, 53–54.

Uren, M.F. and Squire, K.R.E. 1982. The clinico-pathological effect of bluetongue virus serotype 20 in sheep. Australian Veterinary Journal, 58, 11–15.

Van Kammen, A. and Cybinski, D.H. 1981. A serological survey for antibodies to bluetongue virus in Papua New Guinea. Australian Veterinary Journal, 57, 253–255.

Wark, M.C., Shepherd-Clark, M.B., Smith, H.V. and Collins, W.D. 1982. Laboratory evaluation of a living attenuated vaccine against bluetongue type 20 virus. Australian Veterinary Journal, 59, 6.