The history of bluetongue and a current global overview

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Summary

Bluetongue (BT) was first reported more than 125 years ago when European breeds of sheep were introduced into southern Africa. BT viruses (BTV) have been identified in many tropical and temperate areas of the world. BT, the disease, is a phenomenon of ruminants in the temperate zones. There is little clinical disease in the tropical and subtropical areas of the world. At least 24 serotypes of BTV have been described. While the viruses are classified antigenically and taxonomically as BTV, each serotype is unique and may not cause BT, the disease. The BTVs are transmitted among ruminants by competent vector species of the genus *Culicoides*, i.e. biting gnats or midges. BTV serotypes exist with vector species of *Culicoides* in predictable, but finite, geographic and ecological cycles or ecosystems around the world. Despite the almost certain movement of livestock and *Culicoides* species between these ecosystems, there is little evidence that introduced BTV serotypes have been established in these ecosystems. Rather, periodic cyclic extensions and remissions of these virus-vector ecosystems permit the viruses and the disease to move into and recede from adjacent non-endemic areas in a pattern characteristic of many other known arthropod-borne viruses (arboviruses).

Earlier publications suggested that a carrier state occurred in cattle infected as foetuses with BTV. No subsequent natural experiences or research support the hypothesis which has not been validated. The conclusions of the research are not accepted by the scientific community. It is logical, therefore, to propose that regulatory restrictions against the movement of cattle from BTV-affected countries be relaxed or eliminated.

Keywords

Bluetongue – *Culicoides* – Epidemiological cycle – Non-tariff trade barriers – Regulatory actions – Vectors – Virus-host interactions.

Bluetongue, bluetongue viruses and bluetongue virus vectors in North America

Bluetongue (BT) was first reported more than 125 years ago with the introduction of European breeds of sheep into southern Africa (12).

Non-native sheep experienced a severe febrile disease with high morbidity and high mortality. The viral aetiology of the disease was demonstrated in 1906. Bluetongue virus (BTV) strains have been identified in many tropical and temperate areas of the world since that time from a latitude of approximately 40°N to 35°S. However, BT, the disease, is a phenomenon of ruminants in the temperate zones. There is little, if any, clinical disease reported in the tropical and subtropical areas of the world, except when non-native ruminants are introduced into a virus-endemic area. At least 24 serotypes of BTV have been described internationally. While the viruses are classified antigenically and taxonomically as BTV, each serotype is unique and may not cause BT, the disease. The BTVs are transmitted among ruminants by competent vector species of the genus *Culicoides*, which are known as biting gnats or midges. With the evolution of improved virological and serological techniques, BTVs were discovered outside Africa. With such discoveries and the extension of clinical BT into temperate areas previously considered to be free of BT, it was presumed that BTVs were emerging from Africa (7). More recently, it has been shown that BTV serotypes exist with vector species of *Culicoides* in predictable geographic and ecological cycles or ecosystems around the world. Despite the

almost certain movement of livestock and *Culicoides* species between these ecosystems, there has been little evidence that BTV serotypes have been moved between these ecosystems and persisted. Rather, periodic cyclic extensions and remissions of these virus-vector ecosystems permit the viruses and the disease to move into and recede from adjacent non-endemic areas in a pattern characteristic of many other known arthropod-borne viruses (arboviruses).

While clinical descriptions compatible with a diagnosis of BT were reported earlier, BTV serotype 10 was first isolated in the United States of America (USA) from sheep in California in 1952 (9). Despite the perception that the disease was emerging from Africa into other countries of the world, it was recognised early in the history of BT that, in fact, BT was primarily a disease problem when susceptible ruminants were introduced into an area with endemic virus activity (7, 12). While there is concern about the movement of BTV via infected animals and via infected insects, there are sufficient examples to suggest that movement of infected *Culicoides*, rather than of infected ruminants, has been the more serious threat (5, 27).

Subsequently, other serotypes of BTV were isolated in North America. Serotypes 2 (1983), 10 and 11 (1955), 13 (1967) and 17 (1962) have been isolated in the USA. *Culicoides sonorensis* is the primary vector of serotypes 10, 11, 13, and 17, while the infrequently reported isolations of serotype 2 have been associated with populations of *C. insignis* that lie at the northernmost limits of the distribution of this southern species, central Florida (31).

In Canada, serotype 11 has been isolated in a discrete focus in the Okanagan Valley of British Columbia, from cattle with mild clinical disease (8). Serotypes 10, 11, 13 and 17 have been identified in Mexico, north of Mexico City to the USA (29). *C. sonorensis* has been shown to be the primary vector of these serotypes (31). *C. occidentalis* has been demonstrated to be a probable vector of North American BTV serotypes in discrete and limited geographic foci in saline environments in the western USA.

Clinical BT has been observed in sheep, cattle, bighorn sheep (*Ovis canadensis*), and white-tailed deer (*Odocoileus virginianus*) through much of the southern range of *C. sonorensis* in temperate climatic zones of the USA. While BTV infection in the USA causes severe and frequently fatal disease in sheep and white-tailed deer which may experience a peracute, lethal, haemorrhagic disease, the clinical disease in cattle is mild and infrequently observed. During the 1970s, severe epizootics of BT involving multiple BTV serotypes circulating in infected flocks at the same time were reported in sheep in the western states annually. In recent decades, for unknown reasons, epizootics have been less frequent and less severe with a decrease in the frequency of multi-serotypic outbreaks.

The predominant species of *Culicoides* found in the eastern provinces of Canada and the north-eastern and New England states of the USA is *C. variipennis*, which is considered to be a poor vector of BTV (31). Therefore, these areas of Canada and the USA are considered to be BT-free and BTV-free because they are vector-free. While the prairie provinces of Canada and the northern tier of the USA as far west as Montana have populations of *C. sonorensis* (previously identified as *C. albertensis* in Canada (10), these areas are considered low risk for, or free of, BTV. Indigenous populations are *C. sonorensis* genetically and morphologically, but there is no overt phenotypic expression of the genetically controlled oral susceptibility to the viruses and the vectorial capacity, e.g. maturation time of eggs and insect densities, which are environmentally influenced, is low. Clinical BT and transmission or isolation of BTV serotypes have not been demonstrated from cattle in these areas.

From Guatemala (and presumably from southern Mexico and Belize) south-east through Central America to Panama, and in the islands of the Caribbean Sea, distinct ecological cycles have been identified with BTV serotypes 1, 3, 4, 6, 8, 12, 14 and 17 (31, 32). The primary vector of these serotypes is *C. insignis*. Clinical BT has not been described in ruminants found in these subtropical and tropical climatic zones. Despite the almost certain movement of livestock and *Culicoides* species between the BTV-*C. insignis* ecosystem of the Caribbean Basin countries and the continental BTV-*C. sonorensis* ecosystem of northern Mexico, the USA and Canada, there has been no indication that BTV serotypes, with the possible exception of serotype 17, have been moved and sustained between the ecosystems.

While there is sparse serological and virological evidence of BTV activity in South America, little co-ordinated research has been published to define the virus-vector situation. It is presumed that *C. insignis* and the Caribbean Basin BTV serotypes are found in South America.

In Australia and Oceania, serotypes 1, 3, 9, 15, 16, 20, 21 and 23 have been isolated. *C. brevitarsis*, and perhaps *C. wadai*, *C. actoni*, and *C. fulvus*, are the presumed primary vectors. There has been no clinical BT reported in cattle but, in Australia, there is evidence that some of the indigenous BTV

serotypes are pathogenic for sheep. Likewise, *C. brevitarsis* appears to be the primary vector of serotypes 1-3, 9, 12, 14-21 and 23 in South-East Asia.

In Africa, *C. imicola* is the reported vector of serotypes 1-15, 18, 19, 22, 24 and 25. While *C. imicola* is distributed in the southern Mediterranean countries of Europe, incursions of BTV have been infrequent and periodic until recently. Past outbreaks through the Middle East into south-eastern Europe, the Iberian Peninsula, and recently, southern Italy and adjacent islands, have occurred. *C. pulicaris* has been reported to be a vector during recent BT outbreaks in Italy. The vector status of naturally occurring populations of a common *Culicoides* species in most of Europe, *C. obsoletus*, has not been established clearly, but historic evidence would suggest that this species is of low vectorial competence and capacity.

It is anticipated that a much clearer picture of the international BTV and vector situations will be painted during the regional presentations.

Vectors and vectorship

The bluetongue chapter of the OIE *Terrestrial animal health code* (1) refers to *Culicoides* by genus, rather than by species, thus conveying the impression that all species of *Culicoides* are competent vectors of BTV serotypes. The logic that follows from this impression is that all countries could be considered at risk for BTV transmission since it appears that only Antarctica can be considered free from *Culicoides*. The fact is that in the absence of confirmed vector status, or competence of indigenous *Culicoides* species, it is illogical to imply that every country with *Culicoides* is at risk if imports of ruminants are permitted from countries in which livestock are considered infected with BTV. In the absence of a competent vector, the importation of infected or viraemic ruminants serves as no threat to an importing country. Restrictions do, however, place a potential trading partner at a competitive disadvantage that is not based upon science. Similarly, seropositivity of a candidate ruminant for proposed import in no way conveys an indication that the animal is viraemic and does not justify legislation against the importation of such animals. Seropositivity simply documents a previous historic experience with a BTV antigen and has no relevance to current infection or infectivity of the host. Furthermore, and perhaps more importantly, these animals are immune to the infecting and closely related viruses.

The presence of a *Culicoides* species, and even isolation of BTV from a species is not evidence of vectorship or the vectorial capabilities of a species. Analogous to Koch's postulates for establishing the relationship of a micro-organism to a disease (23), there are similar basic criteria required to prove vector status of haematophagous insects. Just as finding an organism in a diseased tissue is not sufficient proof that the organism is the cause of that disease, isolation of a virus from an insect is insufficient evidence for differentiating true vectors from those species that are only incidentally infected because of the high titres of virus in the infected host. To prove vector status, four criteria must be met, as follows:

1) the isolation of the disease-producing agent from wild-caught specimens
2) the demonstration of its ability to become infected by feeding upon a viraemic host
3) the demonstration of its ability to transmit by bite
4) the confirmation through field evidence of the association of the infected arthropod with the vertebrate population in which the infection is occurring (30).

Presence of culicoid species of unknown vectorial capability in a country considered BTV-free because it is north of 40°N or south of 35°S is insufficient justification for denying access by the livestock industry of BTV-affected countries to the markets of a BT-free country.

Prior studies in cattle not validated

Publications during the period from 1970 through to mid-1980 suggested that:

- persistent BTV viraemia and a BTV carrier state
- concurrent persistent circulating BTV and antibody
- activation of BTV viraemia by feeding of non-infected *C. sonorensis*
- chronic BTV excretion in semen

occurred in cattle infected as foetuses by feeding of *C. sonorensis* infected with some North American BTV serotypes on pregnant cows (15).

Subsequent studies in the same laboratory and similar studies by internationally recognised scientists at the same and in other laboratories were unable to reproduce these results. No subsequent natural field experiences and no experimental research have supported the original conclusions. The hypothesis has not been validated and the conclusions of the original research are no longer accepted as valid by the scientific community. Acknowledgement that the

previously published results and conclusions were not valid was made at the Second International Symposium on bluetongue, African horse sickness and related orbiviruses (34) in which it was reported that numerous experimental and field studies failed to duplicate and validate the earlier conclusions (35).

During the First International Symposium on bluetongue and related orbiviruses (2), it was confirmed that BTV infections of the bovine and ovine foetuses could produce developmental defects that resulted in death or deformities in offspring and poor viability of the newborn. BTV was not isolated from any foetus or newborn animal and it was considered unlikely that the deformities would be compatible with survival of the young bovid or serve as a threat for BTV persistence and transmission (16).

Transitory excretion of BTV has been demonstrated in the semen of some experimentally infected bulls during the periods of highest viraemia, but BTV was never detected in the semen unless it was present concurrently in the blood (4). It was suggested that the presence of BTV in the semen was the result of infected erythrocytes contaminating the semen at collection rather than infection of cellular components (gametes) in the semen (11). Persistent or chronic BTV excretion was not confirmed and seroconversion was demonstrated in the infected bulls (4). In contrast, however, coincidental excretion of BTV in the semen of a single field study seronegative donor bull (26) and other retrospective, anecdotal field reports of reproductive problems controverted these controlled experimental studies (13, 14).

In their evaluation of the available data, the WHO/FAO Working Team on Pathology at the First International Symposium observed that BTV infections of cattle in many parts of the world did not cause disease and concluded that the role of infected vectors was more important than was vertical transmission by transplacental transfer in the spread and persistence of BTV in a region (37). The WHO/FAO team concluded that:

- the possibility of a carrier state in cattle for BTV infection was not supported by the evidence in the literature
- during viraemia it was not unusual for BTV to be shed in the semen, as is the case with many other blood-borne virus infections
- field and other experimental data did not support the hypothesis that foetal developmental problems and carrier animals occurred naturally.

In the Round-Table discussion on the international regulatory aspects of BT, five principles were used as a framework for the discussions as follows:

1) the movement of livestock and germplasm internationally is in the best interests of mankind
2) it is the first responsibility of regulatory agencies to protect the livestock industries they represent from losses caused by importation of pests or disease agents
3) regulatory policies must be developed uniformly, comprehensively and intelligently
4) regulatory policies work best when they are based upon incontrovertible scientific evidence
5) the scientific method underpinning regulatory decisions demands rigorous proofs (19).

Evaluating the risk of importing BTV to a BTV-free country or of importing new serotypes to a BTV-infected country must be based upon sound science for the benefit of society and humanity rather than upon self-serving, protective attitudes or inaccurate or incorrect information. The latter results in unsupportable regulatory application of non-scientific non-tariff trade barriers to unrestricted international animal movement. The First International Symposium concluded with the recommendation that confirmation of the presence or absence of chronic infections with BTV in cattle and sheep must receive the highest priority (24). It was noted that failure to confirm chronic infections would remove BTV as a major international non-tariff trade barrier. Likewise, it was suggested that if chronic infections in ruminants did not occur, there was no alternative explanation for virus overwintering and virus persistence through adverse environmental conditions, thus requiring a new, more plausible hypothesis. This recommendation was the basis for much of the research published in the Proceedings of the Second International Symposium in which it was concluded that the hypotheses related to persistent infections and carrier cattle were not supported by sound science and, therefore, that the BTV-related regulatory restrictions on international movement of cattle were not valid (35).

A wealth of new knowledge about BTV and other orbiviruses, including the absence of adverse effects of BTV on pregnant cattle and the bovine foetus was presented at the Second International Symposium (35). An explanation for overwintering of BTV was not perceived to be a problem due to the constant renewal of the susceptible host/vector pools (22). It was observed that most amplification of BTV occurred as epizootics when infected vectors were transported by prevailing winds and by movement of infected vectors to areas in which susceptible hosts

and competent arthropod vectors co-existed. The hypothesis was advanced that BTV serotypes evolved independently rather than by evolving from the original viruses first identified in Africa.

Cattle are considered to be the reservoir hosts of BTV because the viraemia is prolonged and the majority of infections are subclinical (17). The hypothesis that persistent infections or a carrier state responsible for BTV persistence in an area was produced in cattle by foetal infection was not substantiated. While BTV may be shed in semen, it is transient in viraemic bulls and, therefore, vertical transmission is unlikely in the epidemiology of BT. In a recent study using a serotype 17 strain from the USA, it was shown that while viraemia in cattle can be prolonged, it was detectable by virus isolation techniques for no more than 49 days and by blood feeding by competent *C. sonorensis* for only 21 days (3). An analysis of the published data on >500 cattle infected with BTVs concluded that there was a >99% probability that detectable viraemia terminated in ≤63 days (28). While viral nucleic acids can be detected by reverse transcriptase polymerase chain reaction techniques for up to 222 days, this signal does not reflect viable virus, but fragments of virus remaining in the metabolically inactive erythrocytes.

Using bulls and pregnant cattle infected naturally in field studies by Australian BTV serotypes, it was demonstrated that BTV was not excreted in the semen of infected bulls, even during viraemia, and there was no evidence of foetal infection in pregnant cows (18). Inoculation of calves *in utero* during the first trimester of gestation with North American BTV isolates produced brain lesions that were not compatible with life and, therefore, the calves could not contribute to the spread of BTV (33). Late gestational infections caused mild lesions and the production of protective antibody to eliminate the infecting virus.

In an extensive study on BTV infection of pregnant cows using North American BTV isolates, all cows became infected, were viraemic, and seroconverted (25). It was concluded that BTV infection of pregnant cows did not produce transplacental infection of the bovine foetus and that induction of immunotolerance and latent or persistent infections did not occur in the calves.

The BTV studies in the Caribbean Basin confirmed that:

- BTV occurs as a ubiquitous inapparent infection in young ruminants of tropical and subtropical countries
- BTV serotypes are isolated frequently from clinically normal ruminants
- there is no evidence to support extension of the Caribbean serotypes to northern Mexico, the USA and Canada or northern serotypes to the Caribbean Basin, despite the movement of livestock and insects (6, 41).

Recognising the important role of insect vectors and sharing of insect habitats across borders and ecosystems, there is still a stable separation of BTV serotypes between the tropical and temperate ecosystems in the Americas. There are no unequivocal examples and there is no confirmed epidemiological evidence of the introduction to and establishment of BTV in a new area that can be attributed to international trade or movement of infected livestock and livestock products (6, 20, 39). It was noted that BTV distribution is consistent with insect habitat, not geopolitical boundaries, and that control of animal movements exceeds the usefulness of this control to prevent distribution of BTV serotypes (6, 21).

Clearly, embryo transfer and the use of semen collected with appropriate mitigations pose no risk for transmission of BTV (38). Movement of seronegative animals or seropositive animals under appropriate mitigations does not pose a risk of introducing BTV into a BTV-free area and there is no evidence that it occurs (40). Persistence of BTV in cattle and the postulated carrier state have not been validated as a threat to importing countries (20). Therefore, it is logical and reasonable to recommend that the epidemiological, virological, vectorial and ecological realities of trading partners must be evaluated before making regulatory policies (36).

The following recommendations for regulatory policy consideration were modified from those presented at the Second International Symposium, but they are as valid today as they were at that time (6):

1) A review of the assignment of BT to List A of the *Terrestrial animal health code* or a modification of the reporting requirements for diseases must be initiated.
2) Special consideration needs to be given in the *Terrestrial animal health code* to the status of arboviruses which are not restrained by international borders and for which animal movements play a minor or no role.
3) A review of the *International animal health code* and the Working Team recommendations from the Second International Symposium will facilitate bilateral or inter-regional discussions between

trading partners and place concerns about BTVs and BTV vectors in the proper scientific perspective.

4) Continued international co-operation and dialogue between scientists and the regulatory community are needed to permit the study and understanding of arbovirus infections which span ecological regions to contribute to promulgation of responsible regulatory policies.

It has come as a surprise to many of us in the scientific community that 12 years after the Second International Symposium appeared to dispel concerns about BTV carrier states in cattle, the international regulatory position and attitudes have not changed to reflect the reality of current science. There is an overwhelming lack of support for the hypothesis of BTV carrier cattle. It is hoped that another 18 years will not pass after this Third International Symposium before the five principles proposed during the First International Symposium by F.A. Murphy (19) and supported by the scientific contributions of the First and Second International Symposia (2, 35) are incorporated into international regulatory policy considerations.

References

1. Anon. (2003). – Bluetongue, Chapter 2.1.9. *In* Terrestrial animal health code, 12th Ed., Office International des Épizooties, Paris.
2. Barber T.L., Jochim M.M. & Osburn B.I. (1985). – Bluetongue and related orbiviruses (T.L. Barber, M.M. Jochim & B.I. Osburn, eds). Proc. First International Symposium, Monterey, California, 16-20 January 1984. A.R. Liss, New York, *Progr. Clin. Biol. Res.*, **178**, 746 pp.
3. Bonneau K.R., DeMaula C.D., Mullens B.A. & MacLachlan N.J. (2002). – Duration of viraemia infectious to *Culicoides sonorensis* in bluetongue virus-infected cattle and sheep. *Vet. Microbiol.*, **88**, 115-125.
4. Bowen R.A., Howard T.H. & Pickett B.W. (1985). – Seminal shedding of bluetongue virus in experimentally infected bulls. *In* Bluetongue and related orbiviruses (T.L. Barber, M.M. Jochim & B.I. Osburn, eds). Proc. First International Symposium, Monterey, California, 16-20 January 1984. A.R. Liss, New York, *Progr. Clin. Biol. Res.*, **178**, 91-96.
5. Braverman Y. (1992). – The possible introduction to Israel of *Culicoides* (Diptera: Ceratopogonidae) borne animal disease by wind. *In* Bluetongue, African horse sickness and related orbiviruses (T.E. Walton & B.I. Osburn, eds). Proc. Second International Symposium, Paris, 17-21 June 1991. CRC Press, Boca Raton, 291-296.
6. Calderon J.E & Homan E.J. (1992). – Policy considerations for bluetongue in the Caribbean Basin. *In* Bluetongue, African horse sickness and related orbiviruses (T.E. Walton & B.I. Osburn, eds). Proc. Second International Symposium, Paris, 17-21 June 1991. CRC Press, Boca Raton, 946-951.
7. Callis J. (1985). – Bluetongue in the United States. *In* Bluetongue and related orbiviruses (T.L. Barber, M.M. Jochim & B.I. Osburn, eds). Proc. First International Symposium, Monterey, California, 16-20 January 1984. A.R. Liss, New York, *Progr. Clin. Biol. Res.*, **178**, 37-42.
8. Dulac G.C., Sterritt W.G., Dubuc C., Afshar A., Myers D.J., Taylor E.A., Jamieson B.R. & Martin M.W. (1992). – Incursions of orbiviruses in Canada and their serologic monitoring in the native animal population between 1962 and 1991. *In* Bluetongue, African horse sickness and related orbiviruses (T.E. Walton & B.I. Osburn, eds). Proc. Second International Symposium, Paris, 17-21 June 1991. CRC Press, Boca Raton, 120-127.
9. Hardy W.T. & Price D.A. (1952). – Sore muzzle of sheep. *J. Am. Vet. Med. Assoc.*, **120**, 23-25.
10. Holbrook F.R., Tabachnick W.J., Schmidtmann E.T., McKinnon C.N., Bobian R.J. & Grogan W.L. (2000). – Sympatry in the *Culicoides variipennis* complex (Diptera: Ceratopogonidae): a taxonomic reassessment. *J. Med. Entomol.*, **37**, 65-76.
11. Howard T.H., Bowen R.A. & Pickett B.W. (1985). – Isolation of bluetongue virus from bull semen. *In* Bluetongue and related orbiviruses (T.L. Barber, M.M. Jochim & B.I. Osburn, eds). Proc. First International Symposium, Monterey, California, 16-20 January 1984. A.R. Liss, New York, *Progr. Clin. Biol. Res.*, **178**, 127-134.
12. Howell P.G. & Verwoerd D.W. (1971). – Bluetongue virus. *In* Virology monographs, Vol. 9 (S. Gard., C. Hallaver & K.F. Meyer, eds). Springer Verlag, New York, 35-74.
13. Jaggers R. & Anderson G. (1985). – Bluetongue in western Nebraska: an area herd study. *In* Bluetongue and related orbiviruses (T.L. Barber, M.M. Jochim & B.I. Osburn, eds). Proc. First International Symposium, Monterey, California, 16-20 January 1984. A.R. Liss, New York, *Progr. Clin. Biol. Res.*, **178**, 121-126.
14. Kvasnicka W.G. (1985). – Reproductive problems associated with bluetongue virus activity in Nebraska. *In* Bluetongue and related orbiviruses (T.L. Barber, M.M. Jochim & B.I. Osburn, eds). Proc. First International Symposium, Monterey, California, 16-20 January 1984. A.R. Liss, New York, *Progr. Clin. Biol. Res.*, **178**, 109-119.
15. Luedke A.J. (1982). – Effect of bluetongue virus on reproduction in sheep and cattle. *In* Bluetongue and related orbiviruses (T.L. Barber, M.M. Jochim & B.I. Osburn, eds). Proc. First International Symposium, Monterey, California, 16-20 January 1984. A.R. Liss, New York, *Progr. Clin. Biol. Res.*, **178**, 71-78.
16. MacLachlan N.J. Osburn B.I., Stott J.L. & Ghalib H.W. (1985). – Orbivirus infection of the

bovine fetus. *In* Bluetongue and related orbiviruses (T.L. Barber, M.M. Jochim & B.I. Osburn, eds). Proc. First International Symposium, Monterey, California, 16-20 January 1984. A.R. Liss, New York, *Progr. Clin. Biol. Res.*, **178**, 79-84.

17. MacLachlan N.J., Barratt-Boyes S.M., Brewer A.W. & Stott J.L. (1992). – Bluetongue virus infection of cattle. *In* Bluetongue, African horse sickness and related orbiviruses (T.E. Walton & B.I. Osburn, eds). Proc. Second International Symposium, Paris, 17-21 June 1991. CRC Press, Boca Raton, 725-736.
18. Melville L.F. & Gard G.P. (1992). – Investigations on the effects of natural infection with orbiviruses on reproduction in cattle. *In* Bluetongue, African horse sickness and related orbiviruses (T.E. Walton & B.I. Osburn, eds). Proc. Second International Symposium, Paris, 17-21 June 1991. CRC Press, Boca Raton, 744-750.
19. Murphy F.A. (1985). – Round-table discussion on current policies regulating the international movement of animals and germplasm. Chairman's comments. *In* Bluetongue and related orbiviruses (T.L. Barber, M.M. Jochim & B.I. Osburn, eds). Proc. First International Symposium, Monterey, California, 16-20 January 1984. A.R. Liss, New York, *Progr. Clin. Biol. Res.*, **178**, 715-717.
20. Osburn B.I. (1992). – Summary of the Second International Symposium on Bluetongue, African horse sickness, and related orbiviruses. *In* Bluetongue, African horse sickness and related orbiviruses (T.E. Walton & B.I. Osburn, eds). Proc. Second International Symposium, Paris, 17-21 June 1991. CRC Press, Boca Raton, 1013-1017.
21. Ozawa Y. (1992). – Summary of the Second International Symposium on Bluetongue, African horse sickness and related orbiviruses. *In* Bluetongue, African horse sickness and related orbiviruses (T.E. Walton & B.I. Osburn, eds). Proc. Second International Symposium, Paris, 17-21 June 1991. CRC Press, Boca Raton, 1009-1012.
22. Parsonson I.M. (1992). – Overview of bluetongue virus infection of sheep. *In* Bluetongue, African horse sickness and related orbiviruses (T.E. Walton & B.I. Osburn, eds). Proc. Second International Symposium, Paris, 17-21 June 1991. CRC Press, Boca Raton, 713-724.
23. Prescott L.M., Harley J.P. & Klein D.A. (1990). – Microbiology. W.C. Brown, Dubuque, Iowa, 10-11.
24. Reeves W.C. (1985). – Perspectives and recommendations for future research. *In* Bluetongue and related orbiviruses (T.L. Barber, M.M. Jochim & B.I. Osburn, eds). Proc. First International Symposium, Monterey, California, 16-20 January 1984. A.R. Liss, New York, *Progr. Clin. Biol. Res.*, **178**, 719-724.
25, Roeder P.L., Taylor W.P., Roberts D.H., Wood L., Jeggo M.H., Gard G.P., Corteyn M. & Graham S. (1992). – Failure to establish congenital bluetongue virus infection by injecting cows in early pregnancy. *In* Bluetongue, African horse sickness and related orbiviruses (T.E. Walton & B.I. Osburn, eds). Proc. Second International Symposium, Paris, 17-21 June 1991. CRC Press, Boca Raton, 760-767.
26. Schultz R.D., Rhodes R., Panangala V.S. & Kaproth M. (1985). – Unusual observations on a serologically negative bluetongue virus infected bull. *In* Bluetongue and related orbiviruses (T.L. Barber, M.M. Jochim & B.I. Osburn, eds). Proc. First International Symposium, Monterey, California, 16-20 January 1984. A.R. Liss, New York, *Progr. Clin. Biol. Res.*, **178**, 97-102.
27. Sellers R.F. (1992). – Weather, *Culicoides* and the distribution and spread of bluetongue and African horse sickness viruses. *In* Bluetongue, African horse sickness and related orbiviruses (T.E. Walton & B.I. Osburn, eds). Proc. Second International Symposium, Paris, 17-21 June 1991. CRC Press, Boca Raton, 284-290.
28. Singer R.S., MacLachlan N.J. & Carpenter T.E. (2001). – Maximal predicted duration of viremia in bluetongue virus-infected cattle. *J. Vet. Diagn. Invest.*, **13**, 43-49.
29. Stott J.L., Blanchard-Channel M., Osburn B.I., Riemann H.P. & Obeso R.C. (1989). – Serologic and virologic evidence of bluetongue virus in cattle and sheep in Mexico. *Am. J. Vet. Res.*, **50**, 335-340.
30. Sudia W.D. (1972). – Arthropod vectors of epidemic Venezuelan equine encephalitis. *In* Venezuelan encephalitis. Proc. Workshop-Symposium on Venezuelan encephalitis virus, Washington, DC, 14-17 September 1971. Pan American Health Organization, Washington, DC, Scientific Publication No. 243, 157-161.
31. Tabachnick W.J. (1996). – *Culicoides variipennis* and bluetongue virus epidemiology in the United States. *Ann. Rev. Entomol.*, **41**, 23-43.
32. Thompson L.H., Mo C.L., Oviedo M.T., Homan E.J. & Interamerican Bluetongue Team (1992). – Prevalence and incidence of bluetongue viruses in the Caribbean Basin: serologic and virologic findings. *In* Bluetongue, African horse sickness and related orbiviruses (T.E. Walton & B.I. Osburn, eds). Proc. Second International Symposium, Paris, 17-21 June 1991. CRC Press, Boca Raton, 106-113.
33. Waldvogel A.S., Anderson G.A. & Osburn B.I. (1992). – Strain-dependent variations in the pathogenesis of fetal infection with bluetongue virus serotype 11. *In* Bluetongue, African horse sickness and related orbiviruses (T.E. Walton & B.I. Osburn, eds). Proc. Second International Symposium, Paris, 17-21 June 1991. CRC Press, Boca Raton, 751-754.
34. Walton T.E. (1992). – Foreword. *In* Bluetongue, African horse sickness and related orbiviruses (T.E. Walton & B.I. Osburn, eds). Proc. Second International Symposium, Paris, 17-21 June 1991. CRC Press, Boca Raton, v-vi.
35. Walton T.E. & Osburn B.I. (1992). – Bluetongue, African horse sickness and related orbiviruses (T.E. Walton & B.I. Osburn, eds). Proc. Second International Symposium, Paris, 17-21 June 1991. CRC Press, Boca Raton, 1 042 pp.

36. Walton T.E., Tabachnick W.J., Thompson L.H. & Holbrook F.R. (1992). – An entomologic and epidemiologic perspective for bluetongue regulatory changes for livestock movement from the USA and observations on bluetongue in the Caribbean Basin. *In* Bluetongue, African horse sickness and related orbiviruses (T.E. Walton & B.I. Osburn, eds). Proc. Second International Symposium, Paris, 17-21 June 1991. CRC Press, Boca Raton, 952-960.

37. WHO/FAO Working Team (1985). – WHO/FAO Working team report: pathology. *In* Bluetongue and related orbiviruses (T.L. Barber, M.M. Jochim & B.I. Osburn, eds). Proc. First International Symposium, Monterey, California, 16-20 January 1984. A.R. Liss, New York, *Progr. Clin. Biol. Res.*, **178**, 657-660.

38. Working Team on Germplasm (1992). – Working team report on germplasm. *In* Bluetongue, African horse sickness and related orbiviruses (T.E. Walton & B.I. Osburn, eds). Proc. Second International Symposium, Paris, 17-21 June 1991. CRC Press, Boca Raton, 994-1000.

39. Working Team on International Impacts (1992). – Working team report on international impacts. *In* Bluetongue, African horse sickness and related orbiviruses (T.E. Walton & B.I. Osburn, eds). Proc. Second International Symposium, Paris, 17-21 June 1991. CRC Press, Boca Raton, 965-970.

40. Working Team on Regulatory Issues (1992). – Working team report on regulatory issues. *In* Bluetongue, African horse sickness and related orbiviruses (T.E. Walton & B.I. Osburn, eds). Proc. Second International Symposium, Paris, 17-21 June 1991. CRC Press, Boca Raton, 1001-1005.

41. Working Team on Vectors (1992). – Working team report on vectors: recommendations for research on *Culicoides* vector biology. *In* Bluetongue, African horse sickness and related orbiviruses (T.E. Walton & B.I. Osburn, eds). Proc. Second International Symposium, Paris, 17-21 June 1991. CRC Press, Boca Raton, 977-981.