Introduction

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Introduction

The timely need for a third international symposium on bluetongue (BT) was emphatically emphasized by the unexpected and unprecedented recent occurrence of the disease throughout much of the Mediterranean Basin. Furthermore, international understanding of BT clearly has not kept pace with scientific developments since the last symposium in 1991, and it also now is nearly 10 years since the Uruguay Round of negotiations of the General Agreement on Tariffs and Trade; these negotiations lead to the introduction of the Sanitary and Phytosanitary regulations of the World Trade Organization that now guide international trade of animals and animal products.

Intense international interest in BT and BTV was reflected in the some 300 individuals who attended the symposium, and in the 45 scientific oral presentations and over 90 posters in which relevant information was presented. In conjunction with the symposium, international experts were assigned to various working groups that were charged with providing constructive, transparent and science-based recommendations pertaining to the understanding and international regulation of BT.

Critical conclusions and findings from the symposium

Global occurrence of bluetongue virus episystems.

Several researchers elegantly confirmed the original concept pioneered by P. Gibbs, A. Gould and others at the second BT symposium in 1991 that distinct strains of BTV (virus topotypes) vectored by different species of *Culicoides* vectors occur in specific regions of the world. It was further shown that the topotypes of BTV and the vector species that occur within each episystem are relatively stable, despite extensive and ongoing trade and movement of ruminants between individual episystems. Much remains to be learned about the ecological, climatic and environmental factors that lead to expansion of BTV episystems, as recently occurred in the Mediterranean Basin for example, but it is increasingly evident that an understanding of these factors is prerequisite to defining what limits the boundaries of individual BTV episystems.

It was shown that the northern distribution of BTV in Asia and Europe is similar to that in North America, and far beyond the 400 N limit that traditionally was proposed. Specifically, BT recently has occurred to approximately 450 N in Europe, and BTV infection of ruminants has been documented as far as 500 N in Asia. Much remains to be understood about these northern Eurasian BTV episystems, in terms both of their species of insect vector as well as the specific strains of BTV that occur within each. Similarly, the strains of BTV and the relative importance of different potential vector species awaits adequate characterization in variable portions of the extensive BTV episystems that occur in South America, Africa, the Middle East and Asia.

Although further refinement and sophistication is ongoing, existing diagnostic technology is adequate for comprehensive global surveillance and monitoring of the distribution and activity of BTV. Indeed, there has been remarkable international acceptance and adoption of virus-detection assays based on the polymerase chain reaction (PCR) since the second symposium, and the widespread use of PCR technology also has enhanced our understanding of the global ecology of BTV infection because it has facilitated sequence analysis of the strains of BTV that infect the insect vectors and ruminants that reside within each of the various BTV episystems. A potential disadvantage of the PCR technology is that it is so exquisitely sensitive that it can detect BTV nucleic acid in the tissues of previously infected ruminants in the absence of infectious virus, an issue that is relevant to the regulation of animal movement from BTV-endemic areas. Clearly, however, the available diagnostic technologies specifically and sensitively can

identify BTV infection of the insect and animal hosts of the virus. Thus, the global and regional distribution of BTV can now comprehensively be determined using appropriate surveillance and monitoring. Furthermore, the collation of such data should be an issue of the highest priority to the international community given that BTV has been identified on every continent except Antarctica, and that little information currently is available from many areas of the world. An integrated, comprehensive network of surveillance, monitoring and reporting is required to establish the global limits of the distribution of BTV and of competent *Culicoides* vectors.

Lifecycle of bluetongue virus infection

Several studies confirmed conclusions of the first and second symposia that BTV infection of ruminants is transient, whereas infection of the *Culicoides* insect vector is persistent. Detailed and elegant studies by Australian workers who evaluated large numbers of naturally infected cattle have unequivocally shown that BTV infection of these animals does not persist more than a few weeks. Thus, international trade policies must increasingly reflect the reality that BTV infection of ruminants is transient, and that seropositive animals are resistant to reinfection with the homologous BTV serotype and can be safely moved. Attention should now be focused on the climatic, ecological and environmental factors that determine the range of the insect vectors that persistently harbour BTV within each episystem, because detailed understanding of these factors, and not unwarranted restrictions on animal movement, is prerequisite to the ultimate control of BT.

Vaccines and vaccination

Inactivated, live-attenuated (modified live), and subunit vaccines all have been developed to protectively immunize ruminants against BTV infection. Each of these different vaccines types has perceived inherent advantages and disadvantages, including their ease of production and cost, number of immunizations required, availability, efficacy, duration of immunity, and potential adverse side-effects. However, only live-attenuated BTV vaccines currently are commercially available in the quantities that are required to confront major outbreaks of BT; thus, these vaccines will continue to be utilized until such time as viable substitutes are produced in sufficient quantity. Given the enormous scope of recent outbreaks of BT in the Mediterranean Basin and elsewhere, there is a clear need to develop and evaluate all potential vaccine strategies to both protect animals and to facilitate trade from endemically infected areas. Provocative data also was provided suggesting that strategic vaccination of all susceptible animals reduced virus circulation during the recent incursion of BTV into the European episystem, an observation that clearly warrants further study.

Summary

The third symposium showcased the remarkable progress that has been made on the understanding of BT and BTV since the first and second international symposia that were held in 1984 and 1991. Attention has now shifted from ruminants to *Culicoides* insects as the primary host of BTV, meaning that animals can safely be moved between and within BTV episystems using transparent, science-based criteria. Current diagnostic technology provides the tools for very accurate surveillance and monitoring within BTV episystems, and to better predict incursion of BTV into previously unaffected areas and to guide the safe movement of animals. Critical deficiencies persist in regard to our understanding of the global ecology of BTV and its episystems, however, including the lack of detailed understanding of the environmental factors that precipitated the recent expansion of the range of competent insect vectors and/or BTV in the Mediterranean Basin for example. Similarly, some global BTV episystems are yet to be defined in any detail at all, including those in South America, portions of Africa and Asia, and at the northern margins of the virus' range in Eurasia. Lastly, viable options (choices) of vaccines that can be produced in the quantities needed to confront an extensive BT outbreak currently are limited to live-attenuated vaccines, meaning that efforts should continue to evaluate all potential strategies to minimize the economic impact of BTV when it incurs into previously unaffected regions.

Summary of the OIE Third International Symposium on Bluetongue

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Scientists, regulatory officials and livestock producers met at the Third International Symposium on Bluetongue (BT) to discuss current scientific advances, issues and policies as well as to identify areas needing additional research related to policy matters.

The symposium addressed:
1) epidemiology and global distribution;
2) monitoring and surveillance;
3) biology of BT and its vectors;
4) diagnostics;
5) vaccines;
and 6) strategies for intervention.

Epidemiology and Global Distribution

Significant changes in our understanding of BT became evident during the course of the symposium when we learned that the global distribution has changed. As recently as our previous symposium, the distribution was thought to occur between the latitudes of 40 degrees north and 35 degrees south. Since 2000, BT appears to have become established at 45 to 50 degrees north latitude. These new observations of distribution have expanded our perceptions of BT.

At the Second International Symposium on BT, the epidemiology of BT viruses (BTV) was categorized into zones: endemic, epidemic and incursion zones. The endemic zone lies in tropical climates where competent *Culicoides* spp. are actively spreading BTVs all year. BT disease is rarely observed in this zone. The epidemic zone is located in temperate climates where competent *Culicoides* spp. appear during the warm season, and some disease is observed seasonally. The incursion zones are those where BT appears every decade or so, associated with climatic changes. The competent *Culicoides* spp. appear for one to two years, and outbreaks disease occur as long as competent vectors are in the area.

Maps depicting the distribution of BT are historic records of BT's occurrence. Boundaries move with the vectors, which do not respect political boundaries. Instead, vector distribution is based on climatic and environmental conditions. We realized that we must now approach BT, not as a disease of countries, but one of continents.

Monitoring and surveillance

The symposium highlighted the critical role of vectors as the principal means of spreading BTVs.

Not all *Culicoides* spp. transmit BTV. When seeking to determine potential distribution of BTVs, regulatory agencies need only consider those *Culicoides* spp. that are competent for transmission of BTV.

In the absence of competent *Culicoides* spp. vectors, BTV will not survive in an area.

There is no evidence that BTV persist in cattle, a clear indication that ruminants are of no importance in the movement of BTV from one geographic region to another.

Symposium participants acknowledged the importance of competent *Culicoides* spp. vectors in the distribution of BTV in Europe.

Biology of Bluetongue and its Vectors

BTVs are gastrointestinal viruses of *Culicoides* spp. Domestic and wild ruminants are the amplifying hosts for the insect vectors of BTV. One gene controls BTV competency in *Culicoides* spp. The phenotypic expression of the gene is influenced by temperature, rainfall, soil pH, and other factors. The role of these vectors in overwintering of BTV in *Culicoides* spp. appears to be based on temperature. If the environmental temperature is not sufficient for complete viral protein assembly, incomplete virus will remain in the intestinal cells of the vector until the critical temperature for virus assembly is reached.

Identifying the *Culicoides* spp. vectors in Europe and Central Asia will assist in better understanding the distribution of BTV. The genotyping of viruses based on Non-structural protein 3 (NS-3) has led to the concept of "topotyping" and topotyping makes a significant difference in determining the limitations of the virus serotypes in various locations around the world. For example, BTV 2, 10, 11, 13 and 17 occur in North America.. BTV 2 is only described in Florida and adjacent states in the United States (U.S.). The vector for BTV 2 is *Culicoides insignis* (*C. insignis*), whereas the other North American serotypes are transmitted by *C. sonorensis*. BTV 2 has not adapted to *C. sonorensis*, even though this vector is in Florida.

Scientists have also made remarkable progress in characterizing the BTV structure and function since the Second International Symposium on BT. Phenomenal advances have taken place with the BTV model, which has helped define serology, virulence, cell biology, and viral assembly.

Topotyping strategies have led to important advances in our understanding of the biology of BTV. The topotyping procedures of BTVs in Australia, Southeast Asia, and South-Central Asia have led to the recognition of regionally distinct viral groupings classified as Australia A, Java A, Java C and Malaysia A. Classifying these viral isolates is important for evaluating whether new groupings will move into defined geographical areas. Experimental evidence was presented to demonstrate that BTVis a quasi-species virus.

Understanding the pathogenesis of BTV infection in ruminants helps define the pathogenic characteristics of these viruses in sheep and cattle. BTV infection is capable of causing hemorraghic lesions. BTV in sheep causes vascular damage resulting in disseminated intravascular coagulopathy with secondary effects include hemorrhage, edema and vascular thrombi leading to skeletal and cardiac muscle necrosis. Endothelial damage does not occur in cattle and therefore clinical disease is rare.

Studies undertaken to follow viremias in experimentally infected cattle revealed that the virus that can be recovered by virus isolation techniques for as long as 45 to 50 days. In contrast, viral RNA can be detected by polymerase chain reaction (PCR) for as long as 220 days after infection. The significance of this observation is that careful consideration of the clinical signs and PCR results is critical for appropriate diagnosis.

Diagnostics

Researchers have also developed improved viral diagnostics by applying molecular techniques to PCR assays for the identification of viral RNA in tissues of infected animals. The potential for application of new sophisticated technologies could greatly enhance diagnostic capabilities for virus identification and differentiation in the near future. Serological tests can be used in a variety of ways to evaluate BTV infections and epidemiology.

Vaccines

Information derived from molecular studies of viral assembly have led to the development of subunit viral proteins that can be recombined to create efficacious and safe vaccines. These newer vaccine types may ultimately replace attenuated and inactivated vaccine products which have been associated with fetal malformation and contamination of semen.

The South African attenuated virus vaccine strategies used on ruminants on Corsica and Italy were described. The sophisticated epidemiological studies will provide the relevant information as to the effectiveness of the vaccines in controlling infection, mortalities and distribution of BTV in Southern

Europe. The vaccine strategies used in South Africa were described where 3 different vaccinations containing 5 serotypes of virus are administered over a 3 week period. This strategy has proven to be an effective means of controlling disease in ruminants in South Africa.

Control and Trade Issues

A review of the OIE International Standards for BT set the stage for reports of regulatory procedures in North America, South America and in the European Union. The movement of animals in North America bridges all of the epizones that BT is known to occur. Cattle movement from Mexico with similar and different serotypes of virus found in the U.S. was confined by the vector species. Cattle movement did not influence the distribution of virus beyond the vector boundaries. Similarly, the movement of cattle from the epizootic and incursion zones of the U.S. into the non-BT Northeastern U.S. and Canada has not resulted in the establishment of BTV infection in those zones. Again, *C. sonorensis* is not present in Northeastern U.S. or Canada thereby limiting the distribution of BTV to those areas. BTV infection was described in Argentina, Brazil and Chile. The virus was confined to the more temperate climates of these South American countries.

Monitoring and Surveillance - Group 1

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Committee charge:

Consider what monitoring and surveillance practices might be developed to address all of the animal, vector and virus factors associated with the potential risk of spread of BTV, and how these practices would be interfaced with the current OIE Terrestrial Animal Health Code. Also, consider innovative ways to evaluate risk pertaining to movement of animals from BTV-endemic areas, including the risk associated with the movement of immune versus non-immune animals.

Prior to consideration of a review of the requirements for surveillance and monitoring for BTV, the group was briefed (AC/AG) on a planned OIE Chapter on General Guidelines for Surveillance and Monitoring. The key features of the draft of the proposed General Surveillance and Monitoring Chapter are:

- Compared to the surveillance guidelines in the current Bluetongue Chapter, the proposed chapter on surveillance and monitoring is not prescriptive. If adopted, it would be acceptable to use a number of different sources of data and the merits of each different source could be taken into account. Data sources also could be derived on a random or non-random (structured/planned) basis.
- The analysis of data must be scientifically sound. The proposed chapter recognises the merits of merging data from different sources. Though different data sets may be complex, they may enhance each other.
- The aim of surveillance and monitoring is to generate data for use in risk-based assessments to support trade and usually aims to demonstrate freedom from infection, or the presence of an agent and define areas of low risk. The approach in the proposed chapter is intended to be output oriented, not method oriented.
- The Working Group recommends that OIE convene an ad hoc Group to review the current Bluetongue Chapter. The current BT Chapter is too prescriptive and confusing. In particular, there are a number of issues that require attention. They are listed in the order in which they appear in the Code and not in any order of priority. Those that need to be addressed are:
- The infective period - currently defined as 100 days but there is no data to support a period of longer than 60 days. Consideration could be given to risk assessments based on probabilities determined from the distribution of the duration of viraemias.
- Reference to northern and southern limits in terms of latitude.
- In view of the changing distribution of BTV, specifying actual northern or southern latitude is not appropriate. In the absence of confirmed disease, when a country lies within the latitude of the current distribution of BTV, or is adjacent to an infected country or region, a surveillance and monitoring program should be conducted.
- Use of the term "*Culicoides*" on its own is misleading because most countries have one or more species of midges from this genus. The taxonomic term should be clarified to indicate midges from the genus *Culicoides* that have been shown or are suspected to be vectors of BTV.
- Methods of surveillance and levels of sampling needed to achieve the required degree of confidence need not be specified, rather that surveillance complies with the provisions of the proposed general chapter. Nevertheless, some examples of appropriate surveillance systems that provide guidance to the intensity and frequency of surveillance could be of benefit.
- The extent of a surveillance program in countries adjacent to a country that does not have free status. A distance of 100 km is specified but a lesser distance could be acceptable if there are relevant geographical features that interrupt the transmission of BTV.

- When a country is proven to be free, consideration should be given to less frequent surveillance if the country is not immediately adjacent to a bluetongue zone where the situation is unstable.
- The term "surveillance zone" is confusing because surveillance also occurs within the free zone. The purpose of this zone is to acknowledge a degree of uncertainty in the exact limits of BTV activity and to increase confidence in the status of the free zone. The term "buffer zone" is more appropriate though it is acknowledged that this term is defined in the Code as a zone that is used to prevent spread of a disease or agent into a free zone. Depending on geographical features, this zone may not actually prevent spread of BTV, though it does provide additional assurance for the safety of the free zone. While the width of such a zone has been suggested as 50 km, this may need to be narrower or wider, depending on local circumstances that are relevant to BTV transmission.
- It would be of benefit if the Manual of Diagnostic Tests in future specifies measures of sensitivity and specificity to assist the design of surveillance programs. In the absence of these measures in the Manual or when different tests are used, when a surveillance program is designed the performance characteristics of the test should be described.
- When surveillance is conducted, the species and age of animals needs to be considered to ensure that there is appropriate sensitivity for that surveillance. While cattle are usually more readily infected, other species may be used if they have been shown to be infected at a higher incidence.
- The presence of ecological zones for BTV in different parts of the world warrants recognition. Factors pertaining to vectors and hosts in one system may not be relevant to another.
- In consideration of the movement of live animals and germplasm between countries or zones within a country, it is suggested that a risk-based approach be adopted. Persistent infection with BTV does not occur. Further, the occurrence of virus in semen is rare and confined to the early period of viraemia. Consequently, appropriate strategies can be developed to allow the safe movement of animals (including those that are seropositive either as a result of natural infection or vaccination) and semen from animals in zones where BTV infection may occur. These movement controls should reflect the finite period of viraemia in both natural infections and after vaccination with live vaccines.

Research Needs.

The following research activities would be of benefit to surveillance and monitoring activities:

- For surveillance purposes, tests that distinguish between vaccinated and naturally infected animals will be of value;
- Detailed studies of viruses, vectors and their relationships at the boundaries of continental episystems;
- Improved type-specific serology;
- Enhanced methods for antigenic and genetic analyses of viruses;

The group also endorses the recommendations for research on vectors.

Vectors - Group 2

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Committee charge;
To develop specific recommendations that address issues pertaining to assessment of:
Vector competence
Vector capacity
Vector speciation and systematics
Vector ecology and control

Vector systematics and taxonomy

A clear understanding of *Culicoides* systematics and taxonomy is crucial to virtually all bluetongue virus (BTV) vector studies. Most important *Culicoides* vectors exist as species complexes and the members of these complexes may occur together or in different regions. Since individual members may differ widely in vector capacity it is vital that they are able to be distinguished.

Recommendation 1

Better tools to identify and distinguish members of these complexes are urgently required. Tools to be developed should be both morphological and molecular, with the one informing the other.

At least one important *Culicoides* vector, *C. imicola*, appears to be spreading rapidly in Europe. The pattern of spread is not known. There is evidence that *C. imicola* in Europe occurs as several haplotypes.

Recommendation 2

Molecular tools to identify haplotypes and other specific traits should continue to be developed as a priority to enable vector population movement to be identified and monitored.

In many parts of the world, especially Europe, Asia and South America, the systematics and taxonomy of *Culicoides* are in need of revision. Identification of related species may facilitate the discovery of novel vectors and should significantly improve our ability to assess disease risk.

Recommendation 3

The systematics and taxonomy of *Culicoides* in Europe, Asia, South America and other parts of the world should be addressed. Phylogenetic analysis of the sequences of multiple genes should be used to identify the relationships between known and novel vector species.

Worldwide, there are few competent *Culicoides* taxonomists.

Recommendation 4

Consideration should be given to capacity building in the systematics and taxonomy of *Culicoides*.

Vector Competence

Vector competence is under genetic and environmental control, and varies inter- and intra-specifically. In refractory species or individuals, barriers to infection may occur at several steps in the infection and transmission processes. These barriers are poorly understood, and consequently, no methods currently exist for predicting whether species or populations are competent.

Recommendation 5

Barriers to the infection and dissemination of BTV within individual *Culicoides* should be characterised, and molecular genetic tools developed that permit prediction of vector competence.

Vector competence is difficult to measure, as field-caught *Culicoides* do not survive well in captivity and rarely feed. Consequently, transmission from field-caught *Culicoides* to hosts can rarely be demonstrated. There is some recent preliminary evidence suggesting that vertical transmission of BTV might occur in vector *Culicoides* species.

Recommendation 6

Methods to improve laboratory survival and feeding of field-caught *Culicoides* should be investigated. Direct and indirect methods of recording transmission, or transmission potential, should be evaluated. Possible vertical transmission of BTV in vector *Culicoides* should be further investigated.

Relatively little is known about the competence of *Culicoides* vectors in many parts of the world, especially Europe, Asia and South America. Work to date indicates complex relationships between vector species and their competence for different orbiviruses and/or viral genotypes as well as intraspecific variability in vector competence.

Recommendation 7

The vector competence of *Culicoides* species and populations should be measured, where possible using field viruses. Candidate species can be prioritised on the basis of epidemiological evidence, feeding preference for hosts and level of abundance.

Epidemiological analysis (serosurveys, vector surveys, ecological analysis, study of outbreaks) can provide guidance for the selection of candidate species for vector competence studies, and can be used to assess the likely significance of results.

Recommendation 8

Future and historical data sets should be analysed to investigate the possible role played by different vector species in the transmission of BTV.

Vectorial capacity

Vectorial capacity provides a measure of disease risk, incorporating vector competence, abundance, biting rates, survival rates and extrinsic incubation period. Many of these remain to be determined. Methods and tools for measuring some components remain to be developed, particularly in a field context. Interactions of these variables with the environment remain to be characterised.

Recommendation 9

Standard techniques for measuring the variables of vectorial capacity should be developed and adopted, to facilitate comparison of data and data sharing.

Trapping methods should be evaluated against a 'gold standard' (e.g. drop-trap over animal, and the Onderstepoort-type light trap).

Biases in trapping methods should be measured.

Improved methods for reliably aging *Culicoides* should be developed.

Improved methods for recording host preferences should be developed.

The effects of the environment, host demography and climate on vectorial capacity should be investigated.

Measures of vectorial capacity should be correlated with other indicators of disease risk, such as host disease status.

Ecology

The ecology of the major and minor *Culicoides* vectors is poorly understood and their breeding sites are largely uncharacterised. Means and rates of adult dispersal, both local and long distance are unknown. The comparative value of sentinel herds or wild-caught *Culicoides* as an aid to the early detection of virus activity has not been fully investigated. Adult overwintering in temperate zones has been little studied, but could play a part in the persistence of BT.

Recommendation 10

Larval microhabitats and diets should be characterised as an aid to colonisation and to the identification of breeding sites. Means and rates of dispersal of adult *Culicoides*, both local and long distance, need to be defined. Rates and times of virus or viral RNA detection in sentinel herds and vector surveillance systems should be compared. The possibility of adult overwintering in temperate and cool zones needs to be investigated. Development of vector population-simulation models is a long-term goal.

Control

Vector control methods are often used in the event of BT disease outbreaks, but there has been little quantitative work on short and long-term efficacy. Other means of reducing virus transmission that have lower environmental impact (e.g. physical and chemical barriers, husbandry modification), have received little attention.

Recommendation 11

Specific methods for the long and short-term suppression of *Culicoides* populations (adults and immatures) should be evaluated and quantified, and clear recommendations given to veterinary authorities. Alternative methods of interrupting the transmission cycle, such as the use of repellents, housing, breeding site destruction or modification, should be investigated. These measures should be

evaluated in the context of existing arthropod control efforts. Control success should be judged in terms of disease reduction and/or seroconversion.

Diagnostics working group - Group 3

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Committee charge:

To develop specific recommendations that address issues pertaining to the perceived advantages and disadvantages of existing and new virologic and serologic diagnostic procedures for detection of BTV infection of insects and animals and how these interface with the OIE Manual.

Specifically address the issue of the role of the polymerase chain reaction (PCR) assay in the regulation of animal movement.

Existing procedures in the Manual

Virus isolation

Intravenous inoculation of embryonated chicken eggs (ECE) is the most sensitive technique for isolation of BTV. However, it is a slow procedure, compounded by the need for subsequent virus identification steps. Some ECE-propagated viruses may not readily replicate in cell culture

Virus identification

Serogrouping

A number of techniques such as anti-antigen capture ELISA and immunofluoresence that take advantage of the availability of serogroup-specific monoclonal antibodies work well.

The use of serogroup-reactive PCR increases the speed of identification. Precautions must be taken to prevent cross-contamination while doing PCR.

Serotyping

The neutralisation test is biologically relevant and has a number of successful formats such as plaque reduction and microtitre neutralisation. Virus cross-relatedness may make interpretation of results difficult. Maintaining serotyping reagent uniformity is difficult, particularly on a world-wide basis. Such reagents are also costly to make.

'Typing' by PCR-sequencing is a novel and welcome addition to the repertoire of typing tests. It is very rapid and highly information (see new procedures).

Serological tests

The AGID assay while easy and cheap to perform do lacks sensitivity and manifests cross reactions with EHDV. The C-ELISA is now standard technology.

New procedures

Typing instead of serotyping

PCR/sequencing provides information on 'type', genotype and topotype very rapidly. Segments coding for VP2, VP5, VP3, NS1 and NS3 are currently relevant.

Successful identification of BTV around the world depends on availability of relevant sequence data for primer development

Every effort should be made to send viruses or PCR products to all OIE reference labs or other competent laboratories to be sequenced and primer information made available (via the web) to facilitate characterization at the source laboratory

An excellent start has been made in the process of collecting relevant sequence data

http://www.iah.bbsrc.ac.uk/dsRNA_virus_proteins/

http://www.iah.bbsrc.ac.uk/dsRNA_virus_proteins/btv_sequences.htm provides phylogenetic tree analysis of BTV isolates based on RNA2

Real time versus nested PCR?

Real time PCR technology is faster and more expensive than traditional PCR methods but is less susceptible to contamination problems. There may be problems attempting to identify new isolates with already-existing 'real time' probes. The technology requires expensive equipment.

IgM ELISA

An IgM ELISA would provide information on recent infection status and offer an opportunity to determine if the presence of IgM antibodies was correlated with the duration of viraemia.

Future trends

Possibilities include multiplexed flat and bead DNA and protein technologies and biosensing technologies

Recommendations

That the AGID test remain in the manual but not be a prescribed test for international trade

That research into novel diagnostic methods continues with tests showing promise being subject to validation by collaborating OIE laboratories and other competent national laboratories.

That the genetic characterisation continues of BTV isolates from diverse regions of the world with the aim of:

- compiling sequence data and identifying new viruses and their genetic relationships
- sharing sequence information thereby increasing the size of the data bases
- facilitating establishment of PCR technology and use of appropriate primers in the submitting country
- validating the technology by reference to the 'gold standard' neutralisation test

That, following extensive validation by collaborating laboratories, the current neutralisation-based virus serotyping system be replaced by a genetic typing system

That an IgM ELISA or similar test be investigated to determine if they would provide a simple test that correlates with viraemia in infected animals and could be used to facilitate trade.

That use of the PCR to differentiate between wild-type and vaccine virus continue.

Vaccines & vaccinations - Group 4

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Specific recommendations in regard to vaccines and vaccination strategy:

- Encourage the development and transfer of complementary and alternative vaccine materials and strategies that provide safe and efficacious inactivated or subunit BTV vaccines, and further encourages that vaccine companies adopt these products and make them available to producers.
- Vaccine strains should be fully sequenced and the data made available to the FAO/OIE Reference database as well as other databases such as the EMBL data base.
- Encourage the development and validation of technologies that will distinguish vaccinated from infected animals, both for current vaccines and the vaccines that are likely to be available in the foreseeable future
- Encourage countries applying current or future vaccine technologies and strategies to make all data on monitoring of vaccination programs, and the surveillance of control programs, available to OIE for addressing future disease outbreaks.
- Animals receiving vaccines produced by culture in embryonated chicken eggs shall not be moved internationally.
- Update and keep current the OIE Manual on research information and data on the efficacy of both subunit and inactivated BT vaccines.

Impact of interventional strategies on virus spread, disease and regulation - Group 5

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Committee charge:
Address issues pertaining to the impact of interventional strategies on monitoring and surveillance practices and the risk of spread of BTV.

Conclusions:
Considering the potential movement of bluetongue seropositive animals from an infected to a free zone or country:

- animals may move at any time without posing a risk of virus spread if they have been vaccinated with a licensed or authorised attenuated, inactivated, sub-unit or genetically manipulated vaccine at least one month prior to movement, provided that the vaccine used covers all serotypes which would be expected from adequate surveillance to be present at origin and that the animals are identified as vaccinates in the accompanying certification;
- in the case of healthy, non-vaccinated animals, animals (whether seropositive from natural infection or seronegative) may move at any time without posing a risk of virus spread provided that an adequate surveillance system has been in place in the source population for a period of 60 days immediately prior to dispatch without detecting evidence of bluetongue virus circulation

Pursuant to the above recommendations, the working group invites the OIE to review the relevant chapters of the Terrestrial Animal Health Code to bring them in line.

The working group recommends the OIE to back up safe trade in bluetongue seropositive animals by ensuring the existence of an adequate network of reference laboratories which shall inter alia ensure the archiving of viral strains and derived sequence data to provide a comprehensive database to be made available for research, surveillance and trade purposes.

The working group recommends that animals vaccinated with attenuated vaccines produced by culture in embryonated eggs shall not be moved.

Control and Trade - Group 6

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Committee Charge:

To address the potential impact of issues raised by the other 5 working groups on international trade and movement of animals; specifically, to address issues pertaining to the movement of seropositive as well as potentially viremic animals.

Specific conclusions of the Working Group:

A. In considering the potential movement of BTV seropositive animals from an infected to a free zone or country, the Working Group concludes that animals may move at any time without posing a risk of virus spread if they have been vaccinated with a licensed or authorized attenuated, inactivated, subunit, or genetically manipulated vaccine at least one month prior to movement provided that the vaccine used covers all serotypes which would be expected to be present at origin from adequate surveillance and that the animals are identified as vaccinates.

B. In the case of healthy, non-vaccinated animals, animals (whether seropositive from natural infection or seronegative) may move at any time without posing a risk of virus spread provided that an adequate surveillance system has been in place in the source population for a period of 60 days immediately prior to dispatch without detecting evidence of bluetongue virus circulation.

C. The committee endorses the recommendations of Working Group 5 (Impact of Interventional Strategies on Virus Spread, Disease and Regulation) that the OIE should reevaluate the Terrestrial Animal Health Code in light of conclusions of the 3rd symposium. Further, that the OIE can further ensure the continued safe movement of ruminants that are seropositive to BTV by supporting the network of reference laboratories that will archive BTV strains and derived sequence data to ensure that a comprehensive database is available for research, surveillance and trade purposes.

D. The committee encourages the OIE to ensure that periodic surveillance for BTV occurs in zones with no previous evidence of virus activity; and, that any new evidence of virus activity in these zones be immediately reported to OIE.

E. The committee considers that the agar gel immunodiffusion (AGID) test assay lacks the requisite sensitivity and specificity (because of potential cross reactions with other viruses, particularly EHDV). The C-ELISA is now considered the standard and appropriate technology for serological diagnosis of previous exposure to animals to BTV.

F. The committee endorses the use of polymerase chain reaction (PCR)-based technologies for detection of BTV nucleic acid in animals and insects. The "real time" PCR technology is faster than traditional PCR methods, and is less susceptible to the problems of contamination that compromise nested PCR assays in particular. However, further validation is required as there may be problems in the identification of new strains of BTV with existing "real time" probes.

G. The Working Group recommends that OIE convene an ad hoc Working Group to address the current Bluetongue Chapter and the guidelines for bluetongue surveillance and monitoring, as it is agreed that the current Chapter is both prescriptive and confusing.

H. Issues to be addressed, as detailed by the working group (Working Group 1):

- Infective period based on current scientific information and technologies, i.e., vector capabilities and competence, cell culture and PCR information, etc.
- The recent information on the distribution of BTV makes the current BTV limits based on latitudes obsolete. Consider that BTV distribution is based on continental ecological zones or episystems with associated defined parameters. Adjacent zones should have surveillance and monitoring practices for BTV presence. Evidence of BTV in the adjacent zone should be immediately reported to OIE.
- Reconsider the broad use of the term "*Culicoides*" to indicate midges from the genus *Culicoides* spp. that have been shown or are suspected to be probable vectors of BTV. In other words, be specific as to the species involved.
- Consider broad guidelines addressing the intensity and frequency of surveillance, which will compliment the provisions of the general chapter.
- The extent of a surveillance program in countries (zones) adjacent to a country (zone) that does not have free status. (Leave as stands)
- When a surveillance program is designed, the predictive value of the tests used in the program should be described as part of the study.
- When surveillance is conducted, the species and age of animals needs to be considered to ensure that there is appropriate sensitivity for that surveillance.
- The presence of ecological zones for BTV in different parts of the world warrants recognition. Factors pertaining to vectors and hosts in one system may not be relevant to another.
- Tests that distinguish between vaccinated and naturally infected animals will be of value to surveillance programs.

I. Specific recommendations in regard to vaccines and vaccination strategy:

- Encourage the development and transfer of complementary and alternative vaccine materials and strategies providing safe and efficacious inactivated or sub unit vaccines and further encourages that vaccine companies adopted these products and make them available to producers.
- Vaccine strains should be fully sequenced and the data are made available to a reference database(s).
- Encourage the development of technologies, which will distinguish vaccinated from infected animals.
- Encourage countries applying current or future vaccine technologies and strategies to make all data on monitoring and surveillance of control programs available to OIE for addressing future disease outbreaks.
- Animals receiving vaccines produced by culture in embryonated chicken eggs shall not be moved internationally.
- Update and keep current the OIE Manual on research information and data on the efficacy of both subunit and inactivated bluetongue vaccines.