final report

Project code: B.AHE.0256

Prepared by: PD Kirkland, G Bellis, DS Finlaison, LF Melville, MR Van der Saag
NSW Department of Primary Industries

Date published: 7 December 2014

PUBLISHED BY
Meat and Livestock Australia Limited
Locked Bag 991
NORTH SYDNEY NSW 2059

IVth International Symposium on Bluetongue and Related Orbiviruses

Meat & Livestock Australia acknowledges the matching funds provided by the Australian Government to support the research and development detailed in this publication.

Executive summary

This conference was held in Rome Italy between 5-7 November 2014 and was attended by more 300 delegates representing 38 countries. There was a wide international participation with representatives from most bluetongue affected countries. There were 11 Australian delegates (4 from CSIRO AAHL, 1 from DAAG, Canberra, 4 from EMAI, NSW and 2 from the NT (Dr L. Melville, Berrimah Laboratory and Dr G. Bellis NAQS & NAMP reference entomologist). The travel of 5 of the attendees was supported by travel grants from Livecorp and MLA.

The aims of the conference were:

1. To update the scientific community and policy makers and to disseminate knowledge arising from the research conducted during the past 10 years on bluetongue and the infections caused by orbiviruses.
2. To strengthen the network of diagnostic laboratories under the auspices of the OIE.
3. To capitalize on the advantages produced by the III International Conference on Bluetongue – which substantially induced important changes in the approach to animal movement regulations – and at the same time to define new recommendations for international organisations, the European Union, the OIE member countries

There were 6 scientific sessions over the 3 days. Considering the extent of the program (consisting mostly of invited key note speakers), Australia was extremely well represented on the podium, with 4 of the 5 Australians supported by Livecorp/MLA having been selected for oral presentations (Bellis, Finlaison, Kirkland and Melville) with the other member (van der Saag – EMAI/USyd student) having a poster. Dr Kirkland was also a session chairperson. Other Australian input included the national overview (Dr Daniels, CSIRO, AAHL) and 3 posters from AAHL.

Overall, while the program was lengthy, it was less controversial and considerably more harmonious that the previous 2 international symposia (held in 2003 and 1991). Unlike previous symposia, there were no expert working groups convened to debate scientific issues that should be considered when the OIE Code chapter for bluetongue is reviewed. Indeed, there was no opportunity to discuss the areas in which Australia is seeking to have the BTV Chapter modified. Had there been an opportunity, it is unlikely that direct progress would have been made because few of the attendees are concerned directly with or often even interested in trade-related and zoning issues. However, each of the session chairpersons provided a summary of key areas that require further research or consideration.

Although surveillance and zoning in Australia could be improved, there are many areas where it is obvious that Australia is leading the field or, in BTV research, Australian researchers have made contributions that match other leading research groups. Each of the Australian oral presentations was extremely well received and during the course of the 3 days, received generous acknowledgement from other researchers. The EMAI/USyd student poster (Matt van der Saag) describing a model to assess vector competence received an extremely high level of attention from leading scientists in this field with all seeking further

information on technical detail and requests for advice on the publication of this system. Several commented that it should have been selected for an oral presentation.

Highlights of direct relevance to BTV surveillance and control from an Australian perspective are summarised in the sections that follow.

Epidemiology and risk analysis

Keynote presentations covered the EU experience with bluetongue, a historical overview on bluetongue globally and a review of the molecular epidemiology of bluetongue. The epidemiology of bluetongue viruses in Europe was discussed in detail and the incursion of BTV8 into the UK in 2007 described. The effect of vaccination campaigns in both the UK, where early intervention successfully contained the outbreak, and France, where delay in vaccination resulted in 38,000 cases in 2008, were described. The recent outbreak of BTV4 in Bulgaria was described and the role of reassortant viruses discussed. Work from the USA confirmed the importance of the Culicoides vector as the essential overwintering reservoir of BTV in temperate regions, not ruminant livestock.

Following the keynote speakers, eight papers were presented that covered BTV and other orbiviruses (African Horse Sickness and EHD) in various geographical regions. An outbreak of BTV4 in Brazil was described and an investigation of BTV and potential vectors in Russia discussed. The molecular epidemiology of BTV1 in Italy since 2006 and BTV4 in Europe from 1969 to 2014 were both described and the role of northern vector species discussed. A decade of BTV research in southern India was presented and Lorna Melville described 40 years of monitoring in the Northern Territory at Beatrice Hill Farm. A paper from South Africa described the serotypes of African Horse Sickness virus circulating during the 2013-14 outbreak and the role of vaccination in protection from clinical disease discussed. The final paper in the session was a detailed analysis of the molecular characterization of BTV3, BTV2 and EHDV strains from the USA and BTV11 isolated from canines.

Vector taxonomy and identification/Vector competence

The vector session opened with a review of the progress and knowledge gaps in *Culicoides* ecology and control. The four-fold increase in the number of publications on *Culicoides* biology since the bluetongue outbreaks in Europe in the early 2000's was highlighted. Despite this there has been a global decline in taxonomic research and significant knowledge gaps in the biology of immature stages, noting that the latter probably has the most profound effect on the distribution of populations sufficiently large for pathogen transmission. Similarly, there are significant gaps in the knowledge of host ranges, mechanisms of attraction and adult resting sites. Research into control should aim to control transmission, not just *Culicoides* and that ecological studies need to be conducted in the field rather than in the laboratory. There was a review of developments in genetics and modelling with molecular tools being increasingly used in taxonomic, host range and viral detection studies. Novel work on the genomics of *C. sonorensis* provides a model for studies of other species and holds potential for elucidating many of the cues for the behaviour of midges. Glenn Bellis (DAAG, NT) described a model for assessing the status of species of *Culicoides* using a combination of morphological and molecular techniques, using vector

species from Australasia and Asia as a case study and concluding that Asian and Australian populations of some vectors are not the same species. This was considered by many to be the 'standout' presentation from the invited speakers. There was a presentation on recent developments in midge control strategies used in Europe and in India and research being conducted in the USA.

Of most interest to the Australian situation was the potential to develop sugar-baited saliva collection from field populations of midges for use in viral surveillance. This technique can be used to detect the virus excreted by a single midge. For North American species, UV light was more attractive to midges than green LED but that the relative attractiveness of UV was highly dependent upon its intensity.

Matt Van der Saag (EMAI) presented a poster which received positive feedback from many, together with later requests for additional information. A number of prominent entomologists commented that this work was of great interest and importance and was considered worthy of an oral presentation.

Diagnostics

The session that involved assays and approaches for the diagnosis and surveillance of BTV included an overview of the 'state of the art' and several scientific presentations. This included a presentation from Peter Kirkland on the development of a novel assay for the rapid direct detection of multiple serotypes of BTV. This research was one of the outcomes of the recently finished MLA funded research project. Other aspects of work supported by this project were presented by Deborah Finlaison in a later session on surveillance. It was clear from the content of this session and subsequent discussions that Australia is well placed in this area and in some instances is one of the world leaders in the development of cost-effective rapid diagnostic assays.

Surveillance and control

The invited speakers for this session provided detailed examples of epidemiology, surveillance and control of BTV or AHSV in their regions. Examples included a demonstration of how surveillance that was fit for purpose was used during and after the BTV-8 epidemic in Belgium; a review of the BTV surveillance system in Italy over the last 10 years and the epidemiology and control of AHSV in Southern Africa. While Italy faces different challenges to Australia in relation to BTV (occurrence of clinical disease as well as the impact on trade) it is important to note that the NAMP in Australia clearly provides a cost effective monitoring system. The current annual cost for the Italian sentinel herd program is €4,250,000 which includes monthly sampling of approximately 20-30,000 cattle located across approximately 4,800 farms. Apart from the cost difference, the intensity of monitoring is in stark contrast to what occurs under NAMP and provides an insight into how some countries may view the Australian NAMP. The ASHV presentation showed how control strategies have evolved following the introduction of new diagnostic technologies that are able to more rapidly determine the infecting serotype and hence direct the appropriate choice of vaccine. There were 2 presentations on the use of recombinant live virus vectored vaccines expressing the surface proteins of BTV. While these offer considerable potential to

support a DIVA capability when coupled with an appropriate diagnostic assay there are considerable barriers to be overcome in regard to field safety and community acceptance of GMOs. The presentation by Deborah Finlaison (Use of molecular diagnostic assays to enhance BTV surveillance in animals and insects) was well received. It is clear from presentations by other speakers and poster presentations that the use of real-time PCR (including type specific qRT-PCR assays) is playing an increasing role in surveillance for these viruses. The timeliness with which the infecting serotype can be determined is extremely important and is underpins the selection of an appropriate vaccine. The rapid evolution and global acceptance that has occurred with inactivated vaccines was clearly evident. These have been shown to be highly effective yet completely safe. In contrast, there is pressure to phase out live attenuated vaccines due to issues with vector transmission, reassortment with field strains and foetal infection.

Economic and trade impact

Although most presentations in this section were general in nature, it is clear that recent economic pressures, especially in Europe, appear to have modified attitudes to BTV control. It is unlikely that a vaccination program of the scale adopted in Europe for BTV8 would be readily supported. There were recommendations that control measures and surveillance costs need to be proportional to the impact of the infection or disease. The Australian Bluetongue zone defined my NAMP was also quoted several overseas presenters as the leading example of a surveillance system to support trade.

Conclusions

In conclusion, we believe that Australia's international reputation for producing high quality science has been further enhanced by participation in this conference. We are grateful to the management of Livecorp and Meat and Livestock Australia for their willingness to support our attendance at this important event. The fact that it is only held once every 10 years further underlines the importance of being able to participate on this occasion. A summary of the recommendations that were presented by the conference committee and session chairs is attached (Appendix 1)

Appendix 1:

Concluding remarks of the IV BT and Related Orbiviruses Meeting

1. **Epidemiology and risk analysis.** When considering the epidemiology and the risks posed by BTV, it becomes evident that the genetic evolution of the BT virus needs to be carefully monitored through all available molecular techniques.

   At the same time, it is pivotal to clearly distinguish the implications for:

   - the epidemiology of the disease;
   - the performance of the existing assays; and
   - the effectiveness of vaccination. In this respect, the virus serological typing still represents the golden standard for choosing the most effective vaccine.

2. **Epidemiology and risk analysis.** Overwintering mechanisms, such as the role played by vectors, in temperate zones need to be further investigated taking into account all the components playing a role in BTV persistence and transmission.

   Monitoring, surveillance, and control strategies need to further consider the impact on the movement of exotic strains via long distance means of transport.

3. **Epidemiology and risk analysis.** The reassortment between field strains, vaccine strains, and between field and vaccine strains has generated novel genotypes.

   The potential for these progeny strains to be transmitted more effectively represents significant additional risks for ruminant health. This aspect is still poorly understood and requires further investigation.

4. **Epidemiology and risk analysis.** The determination of both existing and "novel" serotypes by RT-PCR assays and sequence analysis is faster - more sensitive - and more specific than conventional serological methods.

   At the same time, it is important to set quantitative limits for relatedness in Seg2 that defines both serotype and topotype (SI).

5. **Epidemiology and risk analysis.** In view of the sequence variation - in all 10 genome-segments - strain identification by serotype alone only reflects genomic-segment 2 and this is clearly not sufficient to indicate geographic variants and reassortants.

   Full genome sequence analysis should become *'the'* standard for molecular characterisation of novel strains. In this perspective, specific isolates and strain/genotype identification are therefore crucial both for publication and virus identification.

6. **Epidemiology and risk analysis.** The threat of exotic topotypes and evaluation of their ability to cause severe clinical disease in endemic episystems should be considered.

7. **Vectors.** Obstacles to the implementation of developing genomic technologies - such as the production of novel cell lines from vector species, development of blood feeding techniques and colony lines of Culicoides - should be overcome.

   Wherever possible, models should account for the ecosystem of interest.

8. **Vectors.** A strong framework for vector taxonomy should be established across the world on the basis of both morphological and molecular identification and it should be integrated into surveillance strategies.

9. **Vectors.** Surveillance strategies should be developed to better reflect feeding on live hosts and to be used in novel approaches for modelling the spatial and temporal incidence of Culicoides.

10. **Vectors.** There should be a greater emphasis on study of BTV and Culicoides in endemic regions, particularly in South America and Asia. This should be paired with integration of new technologies into diagnostic laboratories in these countries/regions.

11. **Animal-vector-host-virus interactions.** Several aspects of the pathogenesis and diagnosis of orbiviral diseases - other than Bluetongue (notably EHD) - require further study.

12. **Animal-vector-host-virus interactions.** Additional characterization of the biology and significance of newly discovered BTV serotypes with novel properties (BTV 25, 26, 27 and beyond) is a pressing need, and the current OIE Code may need to be re-evaluated in light of these findings.

13. **Animal-vector-host-virus interactions.** Further analysis is also required in order to characterise the genetics and mediators of resistance against animal susceptibility to expression of orbiviral diseases, along with the viral, vectorial and environmental determinants thereof.

14. **Cell/virus interactions.** The presence of pathogenic and non-pathogenic serotypes/strains of BTV creates significant challenges for the design of control strategies for BT.
    There is a need to further understand the molecular determinants of BTV virulence and of transmissibility by vector populations with the objective of defining "pathogenic" and "non-pathogenic (or "low-pathogenic") viral strains, if possible.
    This definition will assist policy makers to put in place control strategies that fit the threat of specific BTV strains/serotypes.

15. **Diagnostic recent developments.** Overall, diagnostics for BTV, EHDV and AHSV have a high standard and both group-specific real-time RT-PCRs and VP7-ELISAs offer a very solid basis. Typing of viruses has further improved by using serotype specific RT-PCRs and real-time RT-PCRs.

16. **Diagnostic recent developments.** Novel technologies – such as next-generation sequencing - allow for the detection of novel orbiviruses and serotypes and enable swift complete genetic characterization.
    Numerous assays are also commercially available; however, especially for AHSV and EHDV, the number of commercial systems is still limited.
    Other new technologies - like isothermal amplification, microsphere-based detection and pen-site testing - are under development. To further optimize and improve the available assays, a continuous consideration of novel viruses and serotypes is necessary.

17. **Diagnostic recent developments.** Fully validated assays for differentiating infected from vaccinated animals by serology are still missing, however there are promising developments e.g. for the detection of NS3-specific antibodies.
    Further work in this area would be welcome.
    The developments will need to be made in parallel with novel vaccine development.

18. **Surveillance and control.** Surveillance strategies should:

    1. be designed according to the required purpose (be fit for purpose);
    2. incorporate diagnostic approaches (sampling and testing) appropriate to those purposes; and
    3. specify the intended responses to findings.

    The expected performance of surveillance should be quantified whenever possible, with the indication of target incidence or prevalence, confidence etc. This is the only way to support trade of animals under known levels of accepted risk.

19. **Surveillance and control.** Given the identified negative issues associated with on-going transmission of genome segments from live attenuated vaccines, the use of these products for the control of orbiviral diseases should be reconsidered. Research on inactivated African Horse Sickness vaccines should be fostered so to reach the same level of acceptance achieved for BT inactivated vaccines.

20. **Surveillance and control.** Disease control measures, particularly at the international level, should be proportional to the severity of the disease, including its usual morbidity and mortality and economic impact.

21. **Surveillance and control.** Sugar traps should be evaluated to detect virus, from insect vector populations in the field.

22. **Economic and trade impact.** From an economic perspective the impact of BT, especially in endemic infected countries, is still an under-investigated field, which requires further attention. Currently available data mostly concern disease incursions in naïve populations such as cost estimates done in some European countries (vaccination costs being one of the most important variable considered in these countries), while information about the rest of the world is still scanty.

23. **Economic and trade impact.** BT impact on the trade in animal products is less important than the impact on the marketing of live animals from both endemically infected countries and countries experiencing outbreaks in naïve populations. Endemically infected countries, in particular in Africa and the Americas, tend to argue for the endorsement and development of measures for safe international trade of live animals.

24. **Economic and trade impact.** The impact of BT can broadly be defined as a mixture of losses in animal production, and includes the costs of surveillance, control and prevention. These measures (or interventions) should ideally be proportionate to the economic losses. All stakeholders should enhance dialogue and take action to provide effective responses to BT.

25. **Economic and trade impact.** Reliable surveillance data on the status of the disease, especially data concerning the circulating serotypes of the BTV, are necessary and should be used to support sanitary guarantees put in place by countries to foster trading and to avoid unnecessary non-tariff trade barriers.