Epidemiology of bluetongue and epizootic haemorrhagic disease in wildlife: surveillance methods

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Summary

Bluetongue (BT) and epizootic haemorrhagic disease (EHD) are the most important viral diseases that affect wild ungulates, especially white-tailed deer, in the United States of America (USA). For this reason, considerable surveillance has been conducted. Surveillance has relied upon standard serological and virus detection methods, and both passive and active surveillance strategies have been employed effectively. These efforts have led to an improved understanding of the epidemiology of these diseases in wild ungulate populations, specifically the recognition and understanding of geographically predictable disease patterns ranging from enzootic stability to sporadic epizootics. The utilisation of wildlife in BT and EHD surveillance may be unique to the USA where these diseases are important to both wildlife and livestock interests.

Keywords

The bluetongue (BT) viruses (BTV) and epizootic haemorrhagic disease (EHD) viruses (EHDV) can infect ruminant species of wildlife as well as domestic animals. Clinical disease in wildlife, however, is common only in North America where mortality and morbidity have been documented in white-tailed deer *(Odocoileus virginianus)*, mule deer *(O.hemionus)*, pronghorn *(Antilocapra americana)*, elk *(Cervus elaphus)*, mountain goat *(Oreamnos americanus)* and bighorn sheep *(Ovis canadensis)* (14). Of all of these species, white-tailed deer have been the most affected, and mortality has been associated with all of the serotypes of BTV and EHDV that have been isolated in North America (BTV-10, BTV-11, BTV-13 and BTV-17 and EHDV-1 and EHDV-2) except BTV-2 (1, 13, 21, 26). Both BT and EHD are clinically indistinguishable in these wildlife species and BT and EHD in wild ungulates are often collectively referred to as haemorrhagic disease (HD). The first confirmed case of HD was documented in white-tailed deer in New Jersey in 1955 with the isolation of EHDV-1 (21). Mortality events consistent with HD have been reported as early as 1901 and, since 1955, have occurred consistently within the United States of America (18).

Initial interest in understanding the epidemiology of HD in North American wildlife started with the observation of extensive mortality in white-tailed deer in the USA during the 1950s and 1960s (17). At that time, significant efforts were being made by state conservation agencies to rebuild remnant white-tailed deer populations and re-establish them in areas of their former range. The detection of HD-related mortality in these growing populations led to immediate concerns that BT, EHD, or both, would have an impact on these conservation efforts. At present, there are no indications that HD will eliminate, regulate, or limit a wild ungulate population, but severe population reductions capable of affecting short-term management goals can and have occurred (25). HD currently is considered as the most important viral disease affecting white-

tailed deer and for this reason significant surveillance has been directed at wildlife populations and continues to take place.

Surveillance components and methods

Surveillance methods directed at wildlife in the USA have relied on standard serological and virus detection methods, and both passive and active surveillance strategies have been employed effectively. Surveillance strategies successfully used to date include the following:

- virus isolation and polymerase chain reaction (PCR)-based diagnostic support for clinical submissions
- questionnaire-based surveillance
- cross-sectional serologically based studies of selected wild ungulate populations
- outbreak investigations.

White-tailed deer and other wild ungulates routinely enter animal disease diagnostic channels within the USA. In addition, there are increasing numbers of wildlife disease oriented laboratories with diagnostic capabilities. The isolation of BTV or EHDV, especially from clinical submissions from domestic animals, has relied on a combination of egg inoculation and tissue culture (19). Although these same techniques have application to wild ungulate samples, the virus isolation protocols used by the authors rely exclusively on tissue culture, specifically the inoculation of CPAE (cattle pulmonary artery endothelial) and BHK-21 (baby hamster kidney) cells. Of the two cell lines, CPAE cells are most sensitive. This system works in the absence of egg inoculation for several reasons. First, the reported difference in sensitivity of embryonated chicken eggs and CPAE cells for BTV is less than 1 log of virus (27). Secondly, in experimentally infected deer viraemia has been detected with BTV-10 and EHDV-2 by virus isolation in these cell lines for up to 12 and 56 days, respectively (20). This provides an extended window of opportunity to isolate these viruses. Thirdly, because case submissions consist almost entirely of white-tailed deer dying of acute BT or EHD, viral titres in blood and tissue are close to their peak. In experimentally infected deer, peak titres exceeding 10^4 and 10^6 TCID$_{50}$/ml of blood have been observed for BTV-10 and the EHDV (both EHDV-1 and EHDV-2), respectively (9, 20). Finally, these viruses are very durable and viral titres are not greatly reduced due to minor delays in clinical submissions. PCR protocols are readily available for both BTV and EHDV and their use in diagnostics is increasing. Although quicker and potentially more sensitive than virus isolation, results

are usually limited to identification of viruses at the serogroup level. The primary disadvantages associated with clinical submissions relate to the need for 'detected' mortality or morbidity. Because infections with both BTV and EHDV in wild ungulates often do not result in clinical disease, infection rates cannot be estimated from such data. Wildlife diseases also have an inherent problem with case detection resulting in under-reporting. In a recent outbreak of HD in deer in Missouri that was detected in radio-monitored animals, a mortality rate of 8% was estimated (2). However, not a single report of deer mortality or morbidity was received from public sources during this period.

Questionnaire-based surveillance for HD in wild ungulate populations has been used effectively in the USA since 1981 (16). Information relating to HD in free-living wildlife is requested annually from State wildlife management agencies by the Southeastern Cooperative Wildlife Disease Study. This survey is based on four criteria as follows:

1) sudden and unexplained deer mortality that occurs during late summer and early autumn
2) necropsy-based diagnosis of HD based on clinical signs
3) isolation of EHDV or BTV
4) detection of deer with sloughing hooves.

This system has been used effectively to map the distribution of HD in the USA. Although most data (criteria 1, 2 and 3) relate to detected mortality, criteria 4 provides an estimate of morbidity, which is applicable to geographic areas where mortality seldom occurs. The major advantages of this system relate to simplicity, continuity and a national scope. The major disadvantages relate to reporting bias and a lack of confirmatory diagnostics associated with some of the criteria. This problem is improving, however, as diagnostic submissions and laboratory confirmation have become more available.

Cross-sectional serologically based studies have primarily relied on agar gel immunodiffusion (AGID)-based serology and serum neutralisation (22, 23). Competitive enzyme-linked immunosorbent assay (c-ELISA)-based serological tests also have potential application to such studies especially with BTV. The primary advantage of serologically based surveillance lies in the capacity to detect evidence of previous infection. This is especially important for areas where infections are subclinical or result in mild disease. An additional advantage to serologically based surveillance includes the ability to use hunter-killed animals, greatly reducing cost and time associated with sample collection. The primary disadvantage relates to generating reliable prevalence

rates for specific viruses, as problems with specificity are common with both the AGID (between serogroups) and serum neutralisation (between serotypes) (19). Although not a problem in the USA, increased serotype diversity or incomplete knowledge of serotype diversity would also make serum neutralisation cumbersome and possibly inaccurate.

Outbreak investigations are not performed on a routine basis during HD outbreaks in the USA, but can be extremely valuable in attempts to understand impacts on populations. Difficulties associated with outbreak investigations are reflected in the paucity of information available in the scientific literature. To date, there have been few studies that have attempted to measure or even estimate population impacts in free-living white-tailed deer populations (2, 5, 6, 7, 15, 21). The restraints of such work relate to reliability and availability of both pre- and post-outbreak epidemiological and population data.

Wildlife-based surveillance: understanding epidemiology

Work related to BT and EHD in wildlife has not only led to a better understanding of potential disease impacts but also has served to better define the epidemiology of these diseases within the USA. Based on long-term questionnaire-based data, it is clear that patterns of clinical disease in these wild ungulate populations (especially white-tailed deer) are spatially and temporally predictable (25). The distribution of HD extends throughout the south-eastern USA, extending as far north as New Jersey and as far west as eastern Texas. From this area, the range extends in a north-westerly direction through the central USA to eastern Montana. Reports also are relatively common from California, Oregon and Washington, primarily from black-tailed deer *(O.hemionus)*. There are few reports from states in the north-east and south-west and from those states bordering the Great Lakes. Except for the south-western USA, this distribution of HD follows the known distribution of *Culicoides sonorensis* (12); this explains the lack of disease in the north-eastern USA.

Clinical disease with HD is extremely variable ranging from death to subclinical infection. Expected clinical patterns, however, can be predicted based on the occurrence of endemic or epidemic disease patterns (4, 17). Endemic areas include the coastal plains of the south-east, and in this area, most reported cases of HD represent the chronic form of the disease. These 'chronic' cases are characterised by hoof and rumen lesions; the disease may affect condition but most infected animals survive. In contrast, in certain areas of the central USA, and in

the Piedmont and Appalachian Mountain physiographical regions of the south-east, a pattern of epidemic HD occurs where high levels of mortality are common. A third pattern exists in Texas and possibly other areas of the south-west and mid-west. In these areas, infections do not result in clinical disease. In Texas, for example, there are very few reported cases of HD even though infection rates, as determined by the presence of antibodies to these viruses, approach 100% (24). Based on few clinical reports of HD (16) and a high antibody prevalence for both the EHDV and BTV (22), a similar situation of enzootic stability may occur in southern Florida. This clinical variation relates to variation in herd immunity, specifically the combined effects of maternal antibody transfer (10), acquired immunity through previous challenge (9, 11, 20), and innate resistance within specific host populations (8).

Currently, there is no evidence to suggest that this observed regional variation is related to variation in EHDV or BTV virulence, either associated with individual EHDV or BTV serotypes or between strains within these serotypes. Virulent strains of EHDV and BTV do occur in areas of enzootic stability as indicated by the high mortality rates observed in naive penned deer that have been moved to these areas. In fact, all of the virus isolations collected for this study from clinically infected deer in Texas have been associated with mortality in penned deer that have been moved into this state or have originated from such animals.

In wild ungulates, HD is seasonal, occurring from mid-summer through to late autumn, and usually peaks in September (3). From 1990 to 2002, over 220 isolations of EHDV and BTV were made from deer throughout the south-east and mid-west and all have come from clinical submissions within this same seasonal period. Seasonal distribution is most likely related to seasonal patterns in vector abundance.

Annual variation is more difficult to understand. In endemic areas, HD appears to occur in a two- to three-year cycle (3). In epidemic areas, disease occurs in a longer eight- to ten-year cycle (3, 17). These cycles cannot be explained at this time but probably relate to combined effects of herd immunity and natural or weather-induced fluctuations in vector populations. This is further complicated by the possibility that these short- and long-term cycles may occur concurrently.

Although surveillance directed at wildlife has provided much insight into the epidemiology of these diseases, it is important to emphasise that these findings would not be possible with any one

surveillance system or without supportive experimental work.

Application of wildlife surveillance to other geographic areas

Wildlife-based surveillance for BT and EHD is effective in the USA for three reasons. First, the presence of disease and mortality has resulted in interest from wildlife management organisations resulting in both funding and co-operation in obtaining research and diagnostic samples and mortality and morbidity reports on a national level. Secondly, the availability of samples from hunter-killed deer provides for very cost and time-efficient sampling of these populations. Finally, the broad distribution and abundance of these species, especially white-tailed deer, provides national and regional coverage for such surveillance activities. Wildlife-based surveillance of BT and EHD has led to a better understanding of the epidemiology of these diseases, especially EHD, which has little significance to livestock production. This situation, however, may be unique to the USA where these diseases have relevance to both wildlife and livestock interests.

References

1. Barber T.L. & Jochim M.M. (1975). – Serotyping bluetongue and epizootic hemorrhagic disease virus strains. *Proc. Am. Assoc. Lab. Diag.*, **18**, 149-157.

2. Beringer J., Hansen L.P. & Stallknecht D.E. (2000). – An epizootic of hemorrhagic disease in white-tailed deer in Missouri. *J. Wildl. Dis.*, **36**, 588-591.

3. Couvillion C.E., Nettles V.F., Davidson W.R., Pearson J.E. & Gustafson G.A. (1981). – Hemorrhagic disease among white-tailed deer in the Southeast from 1971 through 1980. *Proc. US Anim. Hlth Assoc.*, **85**, 522-537.

4. Davidson W.R. & Doster G.L. (1997). – Health characteristics and white-tailed deer population density in the southeastern United States. *In* The science of overabundance: deer ecology and population management (W.J. NcShea, H.B. Underwood & J.H. Rappole, eds). Smithsonian Institution Press, Washington DC, 164-184.

5. Fay L.D., Boyce A.P. & Youatt W.G. (1956). – An epizootic in deer in Michigan. *Trans. North Am. Nat. Res. Conf.*, **21**, 173-184.

6. Fischer J.R., Hanson L.P., Turk J.R., Miller M.A., Fales W.H. & Gosser H.S. (1995). – An epizootic of hemorrhagic disease in white-tailed deer (*Odocoileus virginianus*) in Missouri: necropsy findings and population impact. *J. Wildl. Dis.*, **31**, 30-36.

7. Gaydos J.K. (2001). – Evaluation of white-tailed deer host resistance factors to epizootic hemorrhagic disease viruses. PhD thesis. Department of Medical Microbiology and Parasitology, University of Georgia, Athens, 146 pp.

8. Gaydos J.K., Davidson W.R., Elvinger F., Mead D.G., Howerth E.W. & Stallknecht D.E. (2002). – Innate resistance to epizootic hemorrhagic diseases in white-tailed deer. *J. Wildl. Dis.*, **38**, 720-728.

9. Gaydos J.K., Davidson W.R., Howerth E.W., Murphy M., Elvinger F. & Stallknecht D.E. (2002). – Cross-protection between epizootic hemorrhagic disease virus serotypes 1 and 2 in white-tailed deer. *J. Wildl. Dis.*, **38**, 713-719.

10. Gaydos J.K., Stallknecht D.E., Kavanaugh D., Olson R.J. & Fuchs E.R. (2002). – The dynamics of maternal antibodies to hemorrhagic disease viruses (*Reoviridae*: *Orbivirus*) in white-tailed deer. *J. Wildl. Dis.*, **38**, 253-257.

11. Hoff G.L. & Trainer D.O. (1974). – Observations on bluetongue and epizootic hemorrhagic diseases in white-tailed deer: (1) distribution of virus in blood (2) cross challenge. *J. Wildl. Dis.*, **10**, 25-31.

12. Holbrook F.R. (1996). – Biting midges and the agents they transmit. *In* The biology of disease vectors (B.J.Beaty & W.C. Marquardt, eds). University Press of Colorado, Niwot, 110-116.

13. Howerth E.W., Greene C.E. & Prestwood A.K. (1988). – Experimentally induced bluetongue virus infection in white-tailed deer. Coagulation, clinical pathologic, and gross pathologic changes. *Am. J. Vet. Res.*, **49**, 906-913.

14. Howerth E.W., Stallknecht D.E. & Kirkland P.D. (2001). – Bluetongue, epizootic haemorrhagic disease, and other orbivirus-related diseases. *In* Infectious diseases of wild mammals (E.S. Williams & I.K. Barker eds). Iowa State University Press, Ames, 77-97.

15. Karstad L., Winter A. & Trainer D.O. (1961). – Pathology of epizootic hemorrhagic disease of deer. *Am. J. Vet. Res.*, **22**, 227-235.

16. Nettles V.F., Davidson W.R. & Stallknecht D.E. (1992). – Surveillance for hemorrhagic disease in white-tailed deer and other wild ruminants. *Proc. Southeast. Assoc. Fish Wildl. Agencies*, **46**, 138-146.

17. Nettles V.F., Hylton S.A., Stallknecht D.E. & Davidson W.R. (1992). – Epidemiology of epizootic hemorrhagic disease viruses in wildlife in the USA. *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, Press, 238-248.

18. Nettles V.F. & Stallknecht D.E. (1992). – History and progress in the study of hemorrhagic disease of deer. *Proc. North Am. Wildl. Nat. Res. Conf.*, **57**, 499-516.

19. Pearson J.E., Gustafson G.A., Shafer A.L. & Alstad A.D. (1991). Diagnosis of bluetongue and epizootic hemorrhagic disease. *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, 533-546.

20. Quist C.F., Howerth E.W., Stallknecht D.E., Brown J., Pisell T. & Nettles V.F. (1997). – Host defense responses associated with experimental hemorrhagic disease in white-tailed deer. *J. Wild. Dis.*, **33**, 584-599.

21. Shope R.E., MacNamara L.G. & Mangold R. (1960). – A virus-induced epizootic hemorrhagic disease of Virginia white-tailed deer (*Odocoileus virginianus*). *J. Exp. Med.*, **111**, 155-170.

22. Stallknecht D.E., Blue J.L., Rollor E.A, Nettles V.F., Davidson W.R & Pearson J.E. (1991). – Precipitating antibodies to epizootic hemorrhagic disease and bluetongue viruses in white-tailed deer in the southeastern United States. *J. Wildl. Dis.*, **27**, 238-247.

23. Stallknecht D.E., Nettles V.F., Rollor E.A. & Howerth E.W. (1995). – Epizootic hemorrhagic disease virus and bluetongue virus serotype distribution in white-tailed deer in Georgia. *J. Wildl. Dis.*, **31**, 331-338.

24. Stallknecht D.E., Luttrell M.P., Smith K.E. & Nettles V.F. (1996). – Hemorrhagic disease in white-tailed deer in Texas: a case for enzootic stability. *J. Wildl. Dis.*, **32**, 695-700.

25. Stallknecht D.E., Howerth E.W. & Gaydos J.K. (2002). – Hemorrhagic disease in white-tailed deer: our current understanding of risk. *Trans. North Am. Wildl. Nat. Res. Conf.*, **67**, 75-86.

26. Thomas F.C., Willis N. & Ruckerbauer G. (1974). – Identification of viruses involved in the 1971 outbreak of hemorrhagic disease in southeastern United States white-tailed deer. *J. Wild. Dis.*, **10**, 187-189.

27. Wechsler S.J. & McHolland L.E. (1988). – Susceptibilities of 14 cell lines to bluetongue virus infection. *J.Clin. Microbiol.*, **26,** 2327-2342.