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SHORT COMMUNICATION

Genotypic analysis of a localised hotspot of *Pestivirus A* (BVDV-1) infections in Northern Ireland

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Abstract

Background: Bovine viral diarrhoea (BVD) is caused by *Pestivirus A* and *Pestivirus B*. Northern Ireland (NI) embarked on a compulsory BVD eradication scheme in 2016, which continues to this day, so an understanding of the composition of the pestivirus genotypes in the cattle population of NI is required.

Methods: This molecular epidemiology study employed 5′ untranslated region (5′UTR) genetic sequencing to examine the pestivirus genotypes circulating in samples taken from a hotspot of BVD outbreaks in the Enniskillen area in 2019.

Results: Bovine viral diarrhoea virus (BVDV)-1e (*Pestivirus A*) was detected for the first time in Northern Ireland, and at a high frequency, in an infection hotspot in Enniskillen in 2019. There was no evidence of infection with BVDV-2 (*Pestivirus B*), Border disease virus (pestivirus *D*) or HoBi-like virus/BVDV-3 (pestivirus *H*).

Limitations: Only 5′UTR sequencing was used, so supplementary sequencing, along with phylogenetic trees that include all BVDV-1 genotype reference strains, would improve accuracy. Examination of farm locations and animal movement/trade is also required.

Conclusions: Genotype BVDV-1e was found for the first time in Northern Ireland, indicating an increase in the genetic diversity of BVDV-1, which could have implications for vaccine design and highlights the need for continued pestivirus genotypic surveillance.

INTRODUCTION

Bovine viral diarrhoea virus (BVDV) is a member of the genus *Pestivirus* within the family Flaviviridae and exists in the distinct species *Pestivirus A* (previously, and more commonly, known as BVDV-1) and *Pestivirus B* (BVDV-2). Other pestiviruses include *Pestivirus D* (Border disease virus) and *Pestivirus H* (BVDV-3 or HoBi-like pestivirus).[1,2] The 5′ untranslated region (5′UTR) of the BVDV genome has a highly conserved nature,[3] and variation in this sequence can be used to classify pestiviruses by species and subsequently by genotypes.[4,5]

Infection with BVDV causes bovine viral diarrhoea (BVD), which has both animal welfare and substantial economic impacts.[6,7] Clinical signs of acute infection include fever, diarrhoea and reproductive issues.[8,9] Fetuses infected in utero between 30 and 125 days of gestation may be born persistently infected (PI) and, since they shed large quantities of the virus throughout their lives, constitute a significant threat to other animals and disease control.[8]

Guelbenzu-Gonzalo et al.[10] conducted a genotypic survey of BVDV in cattle in Northern Ireland (NI) from 1999 to 2011, which showed that the genotype BVDV-1a predominated and detected the presence of genotype BVDV-1b for the first time. Since March 2016, NI has been engaged in a compulsory BVDV eradication programme (BVDep), whereby all calves must be tested at birth (for programme overview and spatial prevalence, see Strain et al.[11] and Charoenlarp et al.[12], respectively). It is recommended, although not compulsory, that PI animals are destroyed.

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The aim of this study was to use 5′UTR sequencing to examine the pestivirus species and genotypes circulating in a hotspot of infections in the Enniskillen area of NI in 2019.

METHODS

Ear tissue and blood samples taken as part of the BVDep are submitted to one of the designated laboratories, such as the Agri-Food and Biosciences Institute (AFBI).[13] The BVDep is managed by Animal Health and Welfare Northern Ireland (AHWNI), who, in 2019, as part of continuous BVDV-positive sample monitoring, noticed a potential hotspot of BVDV infection on farms around the NI Department of Agriculture, Environment and Rural Affairs divisional veterinary office based in Enniskillen. These cases generated 25 samples from 13 different herds over 8 months. Since many of these samples had been previously tested at the AFBI by reverse transcriptase real-time PCR (RT-PCR), the extracted product was sequenced.

Sample preparation and RNA extraction

Upon receipt by AFBI, ear tissue samples were soaked in 250 µL of phosphate-buffered saline, and 50 µL of the eluted sample was taken for RNA extraction. For blood samples, 1 mL of serum from each sample was centrifuged at more than 16,000 *g* and 500 µL of the supernatant was removed for extraction. RNA was the extracted from the samples, a Border disease virus (BDV) reference strain (accession number U65023.1) and a BVDV-1a reference strain (accession number PP374763) using the ThermoFisher Magmax 96 viral RNA isolation kit (Thermo Fisher Scientific) and the Kingfisher Flex (96 extractions/run) magnetic particle processor (Thermo Fisher Scientific). Then, Indical Bioscience VIROTYPE BVDV pan-pestivirus RT-PCR was performed (that detects all strains of BVDV) according to the manufacturer's instructions (INDICAL).[14] Extracted RNA was preserved at −80°C until subsequent 5′UTR sequencing.

5′UTR sequencing

A 288 base pair sequence of each sample and reference strain was amplified by RT-PCR for 40 cycles using primers (after Vilček et al.[3]) (Thermo Fisher Scientific) at 0.5 µM, superscript IV Platinum Superfi Mastermix and enzyme (Thermo Fisher Scientific). The PCR product was cleaned using an AMPure magnetic bead protocol (Beckman Coulter Life Sciences). Single reads of the sequencing reaction of the PCR product utilised BetterBase and the forward primer for 25 cycles in a thermocycler. The post-sequencing clean-up was performed using a CleanSeq magnetic bead protocol (Beckman Coulter Life Sciences). The sequencing products were suspended in formamide Hi-Dye (Thermo Fisher Scientific) and sequenced using an ABI 3130xl Genetic Analyser following the instrument protocol. The results were viewed using Chromas, and a codon code aligner was used to determine base quality. The selected sequences were identified according to pestivirus genotype by nBLAST on the National Center for Biotechnology Information (NCBI) database.[15]

Sequence quality checks

Sequences from 25 BVDV RT-PCR-positive samples from the 2019 Enniskillen hotspot were derived from a single read; therefore, quality checks were required. One sample sequence was excluded for producing no sequence data and another was excluded for having a low-percentage high-quality base pair score (<70%). The remaining 23 samples were subjected to further downstream analyses. The highest quality sequence isolate (2019-03-035253) was submitted for nBLAST to the NCBI database,[15] and returned a high-scoring match for a UK BVDV-1a isolate (LT902241). The 23 sample sequences were trimmed of low-quality end sequences and indels were removed, resulting in an alignment of 242 base pairs (percentage high-quality range 78.9%–99.6%). From these processed/aligned sequence files and the UK BVDV-1a isolate (LT902241), a neighbour-joining algorithm was used to construct a phylogenetic tree in Geneious 2023.1.1.[16] The number of variant loci and short sequence lengths did not support a maximum likelihood tree. All sequences have been uploaded to NCBI GenBank,[17] and accession numbers are detailed in Table 1.

Bias

There is a potential source of bias in the sampling because AFBI is not the sole BVDep designated laboratory, so it is possible that not all samples taken in the Enniskillen area were included in the present study.

RESULTS

The BDV reference strain (accession number U65023.1) produced a 100% match for BDV, and the BVDV-1a reference strain (accession number PP374763) produced a 98% match for BVDV-1a.

A total of 39.1% (n = 9) of the 2019 Enniskillen hotspot samples were BVDV-1e, and the remaining 60.9% (n = 14) were BVDV-1a. Both the BVDV-1a and BVDV-1e samples were mixtures of ear tissues and blood samples. However, it should be noted that BVDV-1e samples 2019-05-13842, 2019-05-13849, 2019-05-13853 and 2019-05-13857 were blood samples from the same farm. BVDV-1e samples 2019-08-11268 and 2019-08-11269 were also blood samples from the same farm (Table 1). Cluster 3 of the phylogenetic tree (Figure 1) shows the BVDV-1e samples in a distinct cluster.

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TABLE 1 Sample type, bovine viral diarrhoea virus (BVDV) genotype and accession numbers uploaded to National Center for Biotechnology Information GenBank[17] for samples obtained from the 2019 Enniskillen hotspot

Sample ID	Sample type	BVDV genotype	Accession no.
2019-03-003095	Blood	BVDV-1a	PP330739
2019-02-030889	Blood	BVDV-1a	PP330740
2019-08-012005	Ear	BVDV-1a	PP330741
2019-05-009895	Ear	BVDV-1a	PP330742
2019-03-003094	Blood	BVDV-1a	PP330743
2019-03-014273	Blood	BVDV-1a	PP330744
2019-05-010262	Ear	BVDV-1a	PP330745
2019-11-001595	Ear	BVDV-1a	PP330746
2019-03-035253	Ear	BVDV-1a	PP330747
2019-01-019237	Ear	BVDV-1a	PP330748
2019-03-009967	Ear	BVDV-1a	PP330749
2019-06-039976	Ear	BVDV-1a	PP330750
2019-05-001824	Blood	BVDV-1a	PP330751
2019-03-000832	Ear	BVDV-1a	PP330752
2019-08-011268	Blood	BVDV-1e	PP330753
2019-05-013842	Blood	BVDV-1e	PP330754
2019-02-013036	Blood	BVDV-1e	PP330755
2019-05-013857	Blood	BVDV-1e	PP330756
2019-05-014232	Ear	BVDV-1e	PP330757
2019-08-011269	Blood	BVDV-1e	PP330758
2019-04-011917	Ear	BVDV-1e	PP330759
2019-05-013849	Blood	BVDV-1e	PP330760
2019-05-013853	Blood	BVDV-1e	PP330761

FIGURE 1 Neighbour-joining phylogenetic tree of pestivirus isolates obtained from the 2019 Enniskillen bovine viral diarrhoea hotspot

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DISCUSSION

Analysis of the 2019 Enniskillen hotspot data found that 39.1% (n = 9) of infections were BVDV-1e, suggesting that BVDV-1e was established in NI, at least in the Enniskillen area. This finding contrasts with Guelbenzu-Gonzalo et al.,[10] who found that BVDV-1a predominated throughout NI. Interestingly, O'Brien et al.[9] found BVDV-1e to be circulating throughout the Republic of Ireland, with which Enniskillen shares a border. BVDV-1a dominates infections in England, Wales and Scotland[18]; however, BVDV-1b and BVDV-1e dominate infections in France and Italy.[19,20] In fact, Rivas et al.[19] did not find any evidence of BVDV-1a in France, and Ebranati et al.[20] suggested that BVDV-1e originated in Lombardy, Italy. Therefore, it is possible that the Enniskillen hotspot of predominately BVDV-1e infections is due to movement of animals from continental Europe into the area. Booth et al.[18] also used phylogenetic analysis to examine disease transmission, so this approach may be useful in NI.

Current UK vaccines are inactivated BVDV-1 or live-modified BVDV-1 and BVDV-2 viruses; however, the efficacy of these vaccines has been questioned,[21,22] and Sozzi et al.'s[23] work has shown that four vaccines, composed of inactivated and live-modified BVDV-1a and 1b, did not generate virus neutralising antibodies against BVDV-1e in any vaccinated animals. However, this does not necessarily mean that these animals were not immune to BVDV-1e infection. Therefore, continued surveillance of circulating pestiviruses is essential to avoid vaccine escape. This is of particular importance considering the high mutation rate of BVDV and the recent emergence of *Pestivirus H* or 'HoBi-like viruses'.[24,25] HoBi-like virus is thought to have emerged in India,[25] made its way to Europe in fetal bovine serum imported from Brazil[24] and has since been detected in other species.[26,27] Infection with HoBi-like virus causes clinical signs similar to those of BVDV infection,[25] but detection and differentiation of infections are limited.[28] Most molecular methods do not detect HoBi-like viruses or do so with low frequency.[29] Therefore, because the BVDep involves RT-PCR testing of newborn calves, the absence of phylogenetic screening may mean that HoBi-like virus-infected animals will go undetected, which could have a detrimental impact on the BVDep. It should also be noted that BVDV can infect species other than cattle, which could constitute infection and mutation reservoirs.[30,31]

This study revealed that BVDV-1e was present in NI, and at a high frequency, in a hotspot of BVDV infection in the Enniskillen area in 2019, and it should be noted that four BVDV-1e samples were found on a single farm and two on another farm. This warrants further investigation. The continued evolution of the virus through high mutation rates and recombination with genetically related viruses[32] could lead to novel virus strains that may impair infection diagnosis,[28,29] control and possibly reduce vaccine efficacy.[23]

The work presented here illustrates the value of sequencing data in BVD outbreak investigations, the need to incorporate it into conventional investigations of infection transmission networks and cluster analyses and its utility in surveilling emerging new strains and virus threats such as HoBi-like virus.

Further work should focus on how animal movement and trade contribute to the spread of pestiviruses. A comparison of all sequenced data with published 5′UTR sequences of all known *Pestivirus A* genotypes would be required, and an examination of infection clusters and locations would be of particular interest. As part of this study, the extracted RNA from 298 samples, which were the first BVDV-positive ear tissue samples taken from farms throughout NI, were sequenced to determine the pestivirus genotype. Preliminary and unpublished nBLAST analysis data suggest that the composition of pestiviruses in NI is similar to that found in previous studies,[10] but examination of phylogenetic trees is required. Lastly, the study is limited by only using 5′UTR sequencing. Npro sequencing, and others, should also be considered.

AUTHOR CONTRIBUTIONS

James McConville, Adrian Allen, Asa Moyce, Aoibheann Donaghy, Maria Guelbenzu-Gonzalo and Emma Holmes made substantial contributions to the conception and design, or acquisition of data, or analysis and interpretation of data. James McConville, Adrian Allen, Asa Moyce, Joe Clarke, Maria Guelbenzu-Gonzalo, Andrew W. Byrne, Sharon Verner, Sam Strain, Barry McInerney and Emma Holmes were involved in drafting the manuscript or revising it critically for important intellectual content. All the authors agreed to be accountable for all aspects of the work and ensured that questions related to the accuracy or integrity of any part of the work were appropriately investigated and resolved.

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CONFLICT OF INTEREST STATEMENT

The authors declare they have no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data available on request due to privacy restrictions.

ETHICS STATEMENT

The data were gathered from samples already taken by farmers and submitted to AFBI for testing as part of the BVDep. Approval to use these data was given by AHWNI. Therefore, the project was not subject to the UK Animals (Scientific Procedures) Act 1986.

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