Received: 9 May 2019 | Revised: 11 June 2019 | Accepted: 15 June 2019

DOI: 10.1111/tbed.13273

SHORT COMMUNICATION

Transboundary and Emerging Diseases WILEY

Detection of influenza D virus in bovine respiratory disease samples, UK

Hannah Dane[1] | Catherine Duffy[2] | Maria Guelbenzu[2] | Ben Hause[3] | Sean Fee[2] | Fiona Forster[2] | Michael J. McMenamy[2] | Ken Lemon[2]

[1]School of Biological Sciences, Queen's University, Belfast, Northern Ireland

[2]Veterinary Sciences Division, Agri-Food and Biosciences Institute, Belfast, Northern Ireland

[3]Kansas State University College of Veterinary Medicine, Manhattan, KS, USA

Correspondence
Ken Lemon, Veterinary Sciences Division, Agri-Food and Biosciences Institute, Belfast, Northern Ireland.
Email: kenneth.lemon@afbini.gov.uk

Abstract

Influenza D is a newly described virus of cattle, pigs and small ruminants first detected in North America during 2011. Cattle have been shown to be the main viral reservoir and mounting evidence indicates that infection with influenza D may contribute to the development of bovine respiratory disease. The virus has been detected across the United States, Europe and Asia. To date, influenza D has not been reported in the UK. During the winter and spring of 2017/2018, we performed molecular testing of cattle submitted for post-mortem examination where respiratory disease signs were present. We detected influenza D virus in 8.7% of cases, often as the sole viral agent and always in conjunction with bacterial co-infection with one or more agents. Viral RNA was present in both the upper and lower respiratory tract and pathological changes in lung tissues were observed alongside signs of concurrent bacterial infections. Sequencing of one UK isolate revealed that it is similar to viruses from the Republic of Ireland and Italy.

KEYWORDS
influenza D virus, influenza, bovine, respiratory disease

Bovine respiratory disease (BRD) is one of the most economically important diseases affecting cattle worldwide. The aetiology of BRD is complex and includes host, environmental and infectious factors. Infectious causes include viruses (bovine herpesvirus-1 (BoHV-1), bovine respiratory syncytial virus (BRSV), parainfluenza-3 virus (PI3V), bovine coronavirus (BoCV), bovine viral diarrhoea virus (BVDV)) and bacteria (*Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni*, *Mycoplasma bovis*). Primary viral infection is thought to predispose cattle to secondary bacterial infection, in many cases by bacteria already present in the bovine respiratory tract. Despite widespread use of BRD vaccines and antibiotics, deaths attributed to BRD have steadily increased since the mid-1990s (Loneragan, Dargatz, Morley, & Smith, 2001; Snowder et al., 2007).

Influenza D virus (IDV) is a newly described member of the *Orthomyxoviridae* family. Although initially identified during a 2011 outbreak of respiratory disease in North American pigs (Hause et al., 2013), cattle were subsequently shown to be the main reservoir of the virus (Collin et al., 2015; Ducatez, Pelletier, & Meyer, 2015; Ferguson et al., 2015; Hause et al., 2014). IDV has a wide geographic distribution with high seroprevalence in many herds and has been circulating in the United States since at least 2003 (Ferguson et al., 2015; Luo et al., 2017). Accumulating evidence indicates that IDV is associated with BRD complex. A recent metagenomic study has shown that IDV was among the top 3 viruses associated with BRD in dairy calves (Ng et al., 2015). In another study, metagenomics was used to characterize the virome of nasal swab samples collected from beef cattle with acute BRD at feedlot operations in Mexico and the United States (Mitra, Cernicchiaro, Torres, Li, & Hause, 2016). Although 21 different viruses were detected, including those normally associated with

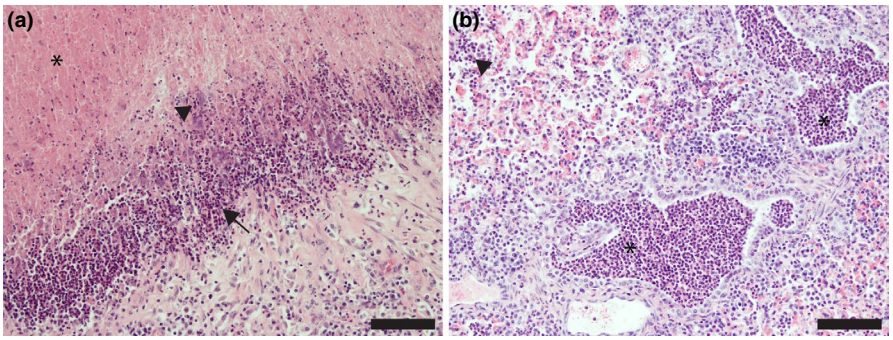

FIGURE 1 (a) H&E stained lung section from case 24280 showing eosinophilic coagulum of caseous necrosis (asterisk) containing bacterial clumps (arrowhead), rimmed by a band of neutrophils (arrow) and further bordered by macrophages, lymphocytes and fibrosis. (b) H&E stained lung section from case 24280 showing neutrophil migration into bronchioles (asterisks) and alveolar spaces (arrowhead). Scale bars 100 μm [Colour figure can be viewed at wileyonlinelibrary.com]

BRD, statistical analysis of the association between virus detection in animals not exhibiting clinical signs and acutely ill animals found that only IDV was a risk factor of significance. Recent experimental infections in cattle using aerosolized virus have confirmed that IDV can induce moderate clinical disease in the absence of other pathogens and has tropism for both the upper and lower respiratory tract (Salem et al., 2019).

During the winter and spring of 2017/2018, cattle from Northern Irish herds submitted to the Agri-Food and Biosciences Institute, Veterinary Sciences Division (Belfast, Northern Ireland) for post-mortem examination with respiratory disease signs were tested for the presence of IDV by real-time reverse transcription PCR as described previously (Hause et al., 2013). We tested nasal swabs, trachea and lung tissues from a total of 104 cattle. The upper respiratory tract of nine animals tested positive for IDV (8.7% prevalence), with cycle threshold (C_t) values ranging from 18.5 to 38.3 (Table 1). The lower respiratory tract of five animals was also positive for IDV, with C_t values in lung tissue ranging from 19.6 to 32.4.

Samples from IDV positive animals were tested for co-infections with other respiratory pathogens. All animals were negative for BRSV, BoHV-1, BPIV-3 and BVDV using either real-time PCR or IFAT. Co-infections with BCoV were detected in three of the nine samples.

TABLE 1 Detection of influenza D virus in bovine respiratory samples

			Average C_t value		
Sample ID	Sex	Age	Nasal swab	Trachea	Lung
23852	F	>24 m	22.6	24.2	19.6
24280	M	1–5 m	18.5	18.6	26.1
25195	F	>18 m	38.3	33.6	32.4
009	M	1–5 m	21.7	N	N
1336	M	1–5 m	28.9	N	N
1561	M	6–8 m	NT	33.4	N
7433	M	6–8 m	28.8	N	N
8421	M	1–5 m	33.2	32.8	28.8
8451	F	6–8 m	28.5	26.9	29.2

N, negative; NT, not tested.

Mycoplasma bovis co-infections were common, occurring in 2/3rd of cases. Bacterial co-infections with *Pasteurella multocida*, *Histophilus somni*, *Mannheimia haemolytica*, *Bibersteinia trehalosi*, *Trueperella pyogenes*, *Proteus* species and alpha-haemolytic streptococci were also detected.

Examination of haematoxylin and eosin (H&E) stained lung sections from IDV positive cases revealed pathological changes associated with concurrent bacterial infection including bronchiocentric foci of coagulative necrosis (Figure 1a) and significant neutrophil infiltration of bronchi, bronchioles and alveolar spaces (Figure 1b).

The sample with the lowest C_t value was selected for further molecular characterization. A 10% homogenate was prepared from tracheal tissue of sample 24280 and used to infect swine testicle cells. Virus was successfully isolated and its full genome amplified by RT-PCR from RNA extracted from swine testicle passage 2 (primer sequences available upon request). Full viral genomic sequence was determined by Sanger dideoxy sequencing and submitted to GenBank (accession numbers MK101118- MK101124). Segment 4 of D/bovine/Northern Ireland/24280/2017 was aligned with all available IDV sequences using MUSCLE in MEGA6 (http://www.megasoftware.net) and a maximum-likelihood tree constructed based on a 597 nucleotide fragment common to most deposited sequences (Figure 2). The strain clustered with European isolates of the D/swine/Oklahoma/1334/2011 lineage, especially Irish and Italian isolates from 2014 to 2016.

Our results demonstrate that IDV is circulating in cattle herds in Northern Ireland. Its detection in animals with confirmed respiratory disease at a prevalence of 8.7% is in broad agreement with similar studies performed in the United States, Europe and Asia (Collin et al., 2015; Ducatez et al., 2015; Ferguson et al., 2015; Flynn et al., 2018; Zhai et al., 2017). For example, Flynn et al. detected IDV at a prevalence of 5.6% in cattle respiratory samples in the Republic of Ireland (Flynn et al., 2018). Due to the high level of movement between herds in Northern Ireland and the Republic of Ireland it is therefore unsurprising that the strain identified in this study is genetically similar to Irish strains. Our finding of high viral loads (indicated by low C_t values) in lung tissue from some animals alongside lung pathology adds to the growing evidence

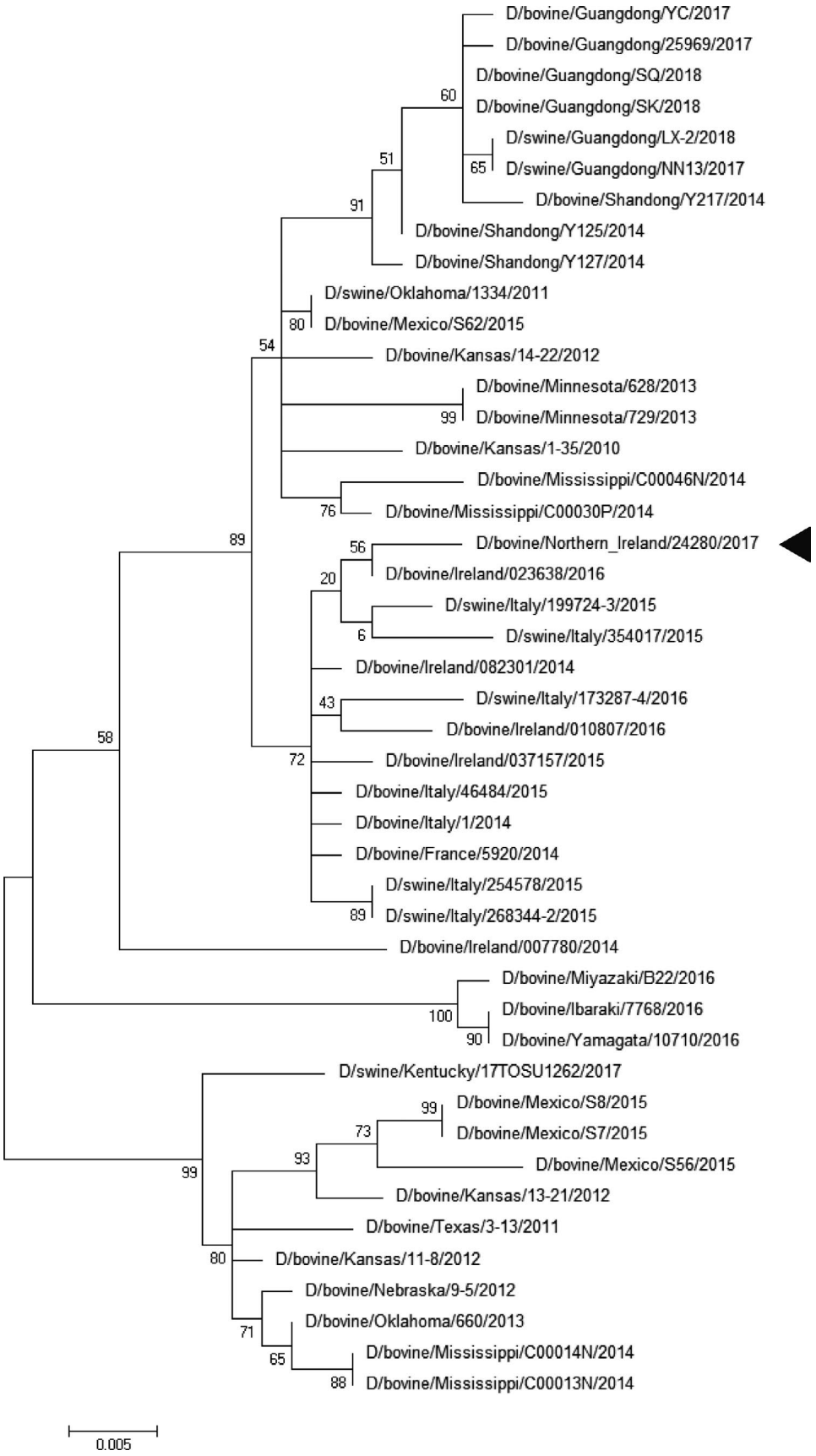

FIGURE 2 Phylogenetic tree of IDV segment 4 (HEF) at the nucleotide level. The gene sequences of D/bovine/Northern Ireland/24280/2017 (arrowhead) were compared to all available IDV sequences on GenBank and maximum-likelihood analysis performed using MEGA6. The bootstrap analysis was performed with 1,000 replicates. Scale bar indicates nucleotide substitutions per site

that IDV plays a role in BRD. Further studies are warranted to conclusively determine the contribution of the virus to the development of BRD.

ACKNOWLEDGEMENTS

We thank Eoin Ryan and Orla Flynn (Department of Agriculture, Food and Marine) and Feng Li (South Dakota State University) for providing advice and reagents.

CONFLICT OF INTERESTS

There are no potential conflicts of interest.

ORCID

Ken Lemon https://orcid.org/0000-0001-9844-1573

REFERENCES

Collin, E. A., Sheng, Z., Lang, Y., Ma, W., Hause, B. M., & Li, F. (2015). Cocirculation of two distinct genetic and antigenic lineages of proposed influenza D virus in cattle. *Journal of Virology*, *89*, 1036–1042. https://doi.org/10.1128/JVI.02718-14

Ducatez, M. F., Pelletier, C., & Meyer, G. (2015). Influenza d virus in cattle, France, 2011–2014. *Emerging Infectious Diseases*, *21*, 368–371. https://doi.org/10.3201/eid2102.141449

Ferguson, L., Eckard, L., Epperson, W. B., Long, L.-P., Smith, D., Huston, C., … Wan, X.-F. (2015). Influenza D virus infection in Mississippi Beef Cattle. *Virology*, *486*, 28–34. https://doi.org/10.1016/j.virol.2015.08.030

Flynn, O., Gallagher, C., Mooney, J., Irvine, C., Ducatez, M., Hause, B., & Ryan, E. (2018). Influenza D virus in cattle, Ireland. *Emerging Infectious Diseases*, *24*, 389–391. https://doi.org/10.3201/eid2402.170759

Hause, B. M., Collin, E. A., Liu, R., Huang, B., Sheng, Z., Lu, W., … Li, F. (2014). Characterization of a novel influenza virus strain in cattle and swine: proposal for a new genus in the Orthomyxoviridae family. *mBio*, *5*, 1–10. https://doi.org/10.1128/mbio.00031-14

Hause, B. M., Ducatez, M., Collin, E. A., Ran, Z., Liu, R., Sheng, Z., … Li, F. (2013). Isolation of a novel swine influenza virus from Oklahoma in 2011 which is distantly related to human influenza C viruses. *PLoS Pathogens*, *9*, https://doi.org/10.1371/journal.ppat.1003176

Loneragan, G. H., Dargatz, D. A., Morley, P. S., & Smith, M. A. (2001). Trends in mortality ratios among cattle in US feedlots. *Journal of the American Veterinary Medical Association*, *219*, 1122–1127.

Luo, J., Ferguson, L., Smith, D. R., Woolums, A. R., Epperson, W. B., & Wan, X.-F. (2017). Serological evidence for high prevalence of Influenza D Viruses in Cattle, Nebraska, United States, 2003–2004. *Virology*, *501*, 88–91. https://doi.org/10.1016/j.virol.2016.11.004

Mitra, N., Cernicchiaro, N., Torres, S., Li, F., & Hause, B. M. (2016). Metagenomic characterization of the virome associated with bovine respiratory disease in feedlot cattle identified novel viruses and suggests an etiologic role for influenza D virus. *Journal of General Virology*, *97*, 1771–1784. https://doi.org/10.1099/jgv.0.000492

Ng, T. F. F., Kondov, N. O., Deng, X., Van Eenennaam, A., Neibergs, H. L., & Delwart, E. (2015). A metagenomics and case-control study to identify viruses associated with bovine respiratory disease. *Journal of Virology*, *89*, 5340–5349. https://doi.org/10.1128/JVI.00064-15

Salem, E., Hägglund, S., Cassard, H., Corre, T., Näslund, K., Foret, C., … Meyer, G. (2019). Pathogenesis, host innate immune response and aerosol transmission of Influenza D virus in cattle. *Journal of Virology*, *JVI.01853-18*, https://doi.org/10.1128/JVI.01853-18

Snowder, G. D., Van Vleck, L. D., Cundiff, L. V., Bennett, G. L., Koohmaraie, M., & Dikeman, M. E. (2007). Bovine respiratory disease in feedlot cattle: Phenotypic, environmental, and genetic correlations with growth, carcass, and longissimus muscle palatability traits. *Journal of Animal Science*, *85*, 1885–1892. https://doi.org/10.2527/jas.2007-0008

Zhai, S.-L., Zhang, H., Chen, S.-N., Zhou, X., Lin, T., Liu, R., … Li, F. (2017). Influenza D virus in animal species in Guangdong province, Southern China. *Emerging Infectious Diseases*, *23*, 1392–1396. https://doi.org/10.3201/eid2308.170059

How to cite this article: Dane H, Duffy C, Guelbenzu M, et al. Detection of influenza D virus in bovine respiratory disease samples, UK. *Transbound Emerg Dis*. 2019;66:2184–2187. https://doi.org/10.1111/tbed.13273