

Influenza D virus in cattle

Research was conducted this year at the Central Veterinary Research Laboratory, Department of Agriculture, Food, and Marine Laboratory Services to determine whether influenza D virus was present in cattle in Ireland and to investigate epidemiologic factors that might be related to this virus. Orla Flynn, a scientist at the Central Veterinary Research Laboratory, outlines the process and outcomes

ABSTRACT

We detected influenza D virus in 18 nasal swab samples from cattle in Ireland that were clinically diagnosed with respiratory disease. Specimens were obtained from archived samples received for routine diagnosis during 2014-2016. Sequencing showed that virus from Ireland clustered with virus sequences obtained in Europe within the D/swine/ OK/1334/2011 clade.

Influenza D virus is a recently characterised addition to the family *Orthomyxoviridae*. This virus was originally detected in pigs in the United States¹; however, cattle are now believed to be the main reservoir species.² Evidence suggests that this virus plays a role in bovine respiratory disease, although experimentally, it caused only mild disease by itself.³ Influenza D virus has been found to be associated with respiratory disease in feedlot cattle.⁴ The zoonotic potential of influenza D virus remains unclear; this virus can replicate in ferrets (a model for human influenza infection), and a seroprevalence of 91% was found in persons working closely with cattle.⁵ However, a study of 3,300 human respiratory samples from Scotland did not detect any influenza D virus-positive samples.⁶ This virus has been detected in bovine samples in several other countries, including France⁷, Italy⁸, Japan, and China.⁹ Cattle are a major part of the economy in Ireland, where there are ≈7 million.¹⁰ To determine whether influenza D virus was present in cattle in Ireland and to investigate epidemiologic factors that might be related to this virus, we conducted a cross-sectional study by using 320 nasal swab specimens from cattle with respiratory disease that were submitted

to the Central Veterinary Research Laboratory for routine bovine viral pathogen testing during 2014-2016. We tested swab specimens by using real-time PCR for influenza D virus as described.¹ We selected samples with a cycle threshold (Ct) ≤25 for further molecular characterisation by using three primer sets⁷ that are specific for the seven virus gene segments. We performed cDNA synthesis by using qScript cDNA SuperMix (Quantabio, Beverly, MA, USA) and PCR amplification by using AccuStart II PCR ToughMix (Quantabio). We processed PCR products by using Illustra ExoProStar 1-Step (GE Healthcare, Little Chalfont, UK) according to the manufacturer's instructions before Sanger sequencing. We analysed sequence data by using DNASTAR Lasergene 12 SeqMan Pro (DNASTAR, Madison, WI, USA) and performed sequence alignment by using ClustalW in MEGA 5.01 (<http://www.megasoftware.net/>). We constructed phylogenetic trees by using the maximum-likelihood method in MEGA 5.01. Herd information for 2015 was available for 84 herds of origin of these nasal swab specimens. Data was obtained from the Animal Identification and Movement System database of the Department of Agriculture, Food, and Marine of Ireland (www.agriculture.gov.ie/animalhealthwelfare/animalidentificationmovement/cattle/irishbovineanimalidentificationssystem-overview/). We performed univariate statistical analysis by using Stata/SE14¹ (StataCorp LLC, College Station, TX, USA). Herd factors investigated for a possible association with influenza D virus herd status were herd size, numbers of stillbirths, dairy cows in herd, beef cows in herd, inward movements from markets, inward movements to farm, and carcasses moved to knackeries.

FINDINGS

A total of 18/320 samples were positive for influenza D virus by PCR. Of the 18 positive samples, 13 were also positive by PCR for one or two other viral pathogens (bovine herpesvirus 1, parainfluenza 3 virus, bovine coronavirus, bovine respiratory syncytial virus, bovine viral diarrhoea virus). Seven of the influenza D virus-positive specimens were from calves, two from weanlings, and one from a cow; other specimens were not described by animal age. Nine of the influenza D virus-positive samples had a Ct \leq 25 and were selected for sequencing.

We obtained partial sequences for five samples and deposited the sequences in GenBank (accession nos. KY992090–KY992103). Phylogenetic analysis (online Technical Appendix Figure 1, <https://wwwnc.cdc.gov/EID/article/24/2/170759-Techapp1.pdf>) showed that the five influenza D virus isolates from Ireland clustered with viruses from Europe in the D/swine/OK/1334/2011 clade. We also determined the distribution of positive and negative samples at county level in Ireland (online Technical Appendix Figure 2). Herd information was available for 10 of the influenza D virus-positive herds and for 74 comparison herds (for which the nasal swab specimens were negative for influenza D virus but which had clinical respiratory disease outbreaks). We found no associations between herd characteristics and influenza D virus status; this finding was determined by evaluating mean values with 95% CIs for infected herds and non-infected herds.

CONCLUSION

This study confirms the emergence of influenza D virus in Ireland. Presence of the virus in nasal swab specimens submitted from routine respiratory disease cases supports the hypothesis that this virus plays a role in the bovine respiratory disease complex. Analysis of herds for infected cattle did not show any epidemiologic differences between influenza D virus infection and infection with other common respiratory viral pathogens. This finding is consistent with the hypothesis that influenza D virus might have limited effect by itself but can potentiate effects of other respiratory pathogens in causing respiratory disease.^{3,4} Detection of two virus lineages in Ireland clustering with viruses isolated in Europe within the D/swine/OK/1334/2011 clade raises the issue of how influenza D virus might spread internationally. Surveillance efforts could be targeted for data on trade of live cattle, which is extensive within Europe. Further research is planned to investigate the seroprevalence of influenza D virus in cattle in Ireland and to determine the effect of this virus in a cattle farming context in this country.

Orla Flynn is a scientist at the Central Veterinary Research Laboratory, Department of Agriculture, Food, and Marine Laboratory Services, Celbridge, Ireland. Her research interests include avian and mammalian pathogens affecting farmed animals.

REFERENCES

1. Hause BM, Ducatez M, Collin EA, Ran Z, Liu R, Sheng Z, et al. Isolation of a novel swine influenza virus from

Originally published: This article was originally published in *Emerging Infectious Diseases*, February 2018.

Authors: Orla Flynn, Clare Gallagher, Jean Mooney, Claire Irvine, Mariette Ducatez, Ben Hause, Guy McGrath and Eoin Ryan.

Author affiliations: Department of Agriculture, Food, and Marine Laboratory Services, Celbridge, Ireland (O. Flynn, C. Gallagher, J. Mooney, C. Irvine, E. Ryan); École Nationale Vétérinaire de Toulouse, Toulouse, France (M. Ducatez); Kansas State University College of Veterinary Medicine, Manhattan, Kansas, USA (B. Hause); University College Dublin, Dublin, Ireland (G. McGrath)
DOI: <https://doi.org/10.3201/eid2402.170759>

Address for correspondence: Eoin Ryan, Department of Agriculture, Food, and Marine Laboratory Services, Virology Division, Backweston Campus, County Kildare, Celbridge; eoin.ryan@agriculture.gov.ie

- Oklahoma in 2011 which is distantly related to human influenza C viruses. *PLoS Pathog.* 2013;9:e1003176. <http://dx.doi.org/10.1371/journal.ppat.1003176>
- Hause BM, Collin EA, Liu R, Huang B, Sheng Z, Lu W, et al. Characterization of a novel influenza virus in cattle and swine: proposal for a new genus in the Orthomyxoviridae family. *MBio.* 2014;5:e00031-14. <http://dx.doi.org/10.1128/mBio.00031-14>
 - Ferguson L, Olivier AK, Genova S, Epperson WB, Smith DR, Schneider L, et al. Pathogenesis of influenza D virus in cattle. *J Virol.* 2016;90:5636-42. <http://dx.doi.org/10.1128/JVI.03122-15>
 - Ng TF, Kondov NO, Deng X, Van Eenennaam A, Neiberghs HL, Delwart E. A metagenomics and case-control study to identify viruses associated with bovine respiratory disease. *J Virol.* 2015;89:5340-9. <http://dx.doi.org/10.1128/JVI.00064-15>
 - White SK, Ma W, McDaniel CJ, Gray GC, Lednicky JA. Serologic evidence of exposure to influenza D virus among persons with occupational contact with cattle. *J Clin Virol.* 2016;81:31-3. <http://dx.doi.org/10.1016/j.jcv.2016.05.017>
 - Smith DB, Gaunt ER, Digard P, Templeton K, Simmonds P. Detection of influenza C virus but not influenza D virus in Scottish respiratory samples. *J Clin Virol.* 2016;74:50-3. <http://dx.doi.org/10.1016/j.jcv.2015.11.036>
 - Ducatez MF, Pelletier C, Meyer G. Influenza D virus in cattle, France, 2011–2014. *Emerg Infect Dis.* 2015;21:368-71. <http://dx.doi.org/10.3201/eid2102.141449>
 - Chiapponi C, Faccini S, De Mattia A, Baioni L, Barbieri I, Rosignoli C, et al. Detection of influenza D virus among swine and cattle, Italy. *Emerg Infect Dis.* 2016;22:352-4. <http://dx.doi.org/10.3201/eid2202.151439>
 - Murakami S, Endoh M, Kobayashi T, Takenaka-Uema A, Chambers JK, Uchida K, et al. Influenza D virus infection in herd of cattle, Japan. *Emerg Infect Dis.* 2016;22:1517-9. <http://dx.doi.org/10.3201/eid2208.160362>
 - Department of Agriculture, Food, and Marine Laboratory Services. Age profile for dairy and beef animals, 2017 [cited 2017 Sep 20]. <https://www.agriculture.gov.ie/animalhealthwelfare/animalidentificationmovement/cattle/bovinebirthandmovementsmonthlyreports/>