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Influenza D Virus in Cattle, Ireland

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DOI: <https://doi.org/10.3201/eid2402.170759>

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 characterized addition to the family *Orthomyxoviridae*. This virus was originally detected in pigs in the United States (1); 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 seroprevalance 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 France (7), Italy (8), 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 (Celbridge, Ireland) 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 ($C_t \leq 25$) for further molecular characterization by using 3 primer sets (7) that are specific for the 7 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 analyzed 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 were obtained from the Animal Identification and Movement System database of the Department of Agriculture, Food, and Marine of Ireland (<https://www.agriculture.gov.ie/animalhealthwelfare/animalidentificationmovement/cattle/irishbovineanimalidentificationsystem-overview/>). We performed univariate statistical analysis by using Stata/SE14.1 (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.

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 1 or 2 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, 2 from weanlings, and 1 from a cow; other specimens were not described by animal age. Nine of the influenza D virus-positive samples had a $C_t \leq 25$ and were selected for sequencing. We obtained partial sequences for 5 samples and deposited the sequences in GenBank (accession nos. KY992090–KY992103).

Phylogenetic analysis (online Technical Appendix Figure 1, <https://wwwnc.cdc.gov/EID/article/24/2/17-0759-Techapp1.pdf>) showed that the 5 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 noninfected herds.

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 2 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.

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Novel *Streptococcus suis* Sequence Type 834 among Humans, Madagascar

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DOI: <https://doi.org/10.3201/eid2402.171138>

Two cases of meningitis caused by *Streptococcus suis* occurred in Madagascar, 1 in 2015 and 1 in 2016. We report the characterization of the novel sequence type, 834, which carried the *mrp+/sly+/epf+* virulence marker and a mutation G→T at position 174, leading to a substitution mutS1 to mutS284.

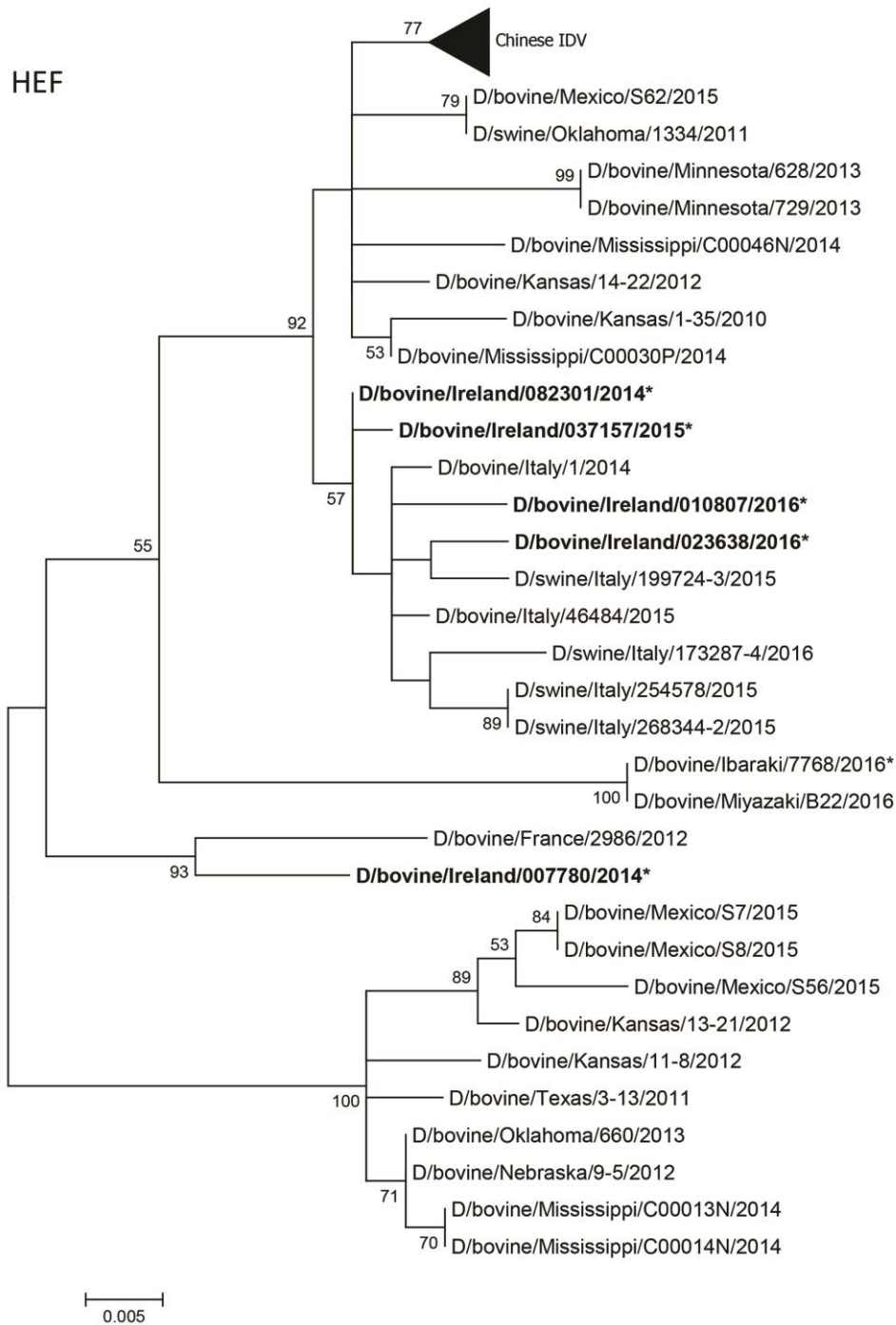
Streptococcus suis is a common pathogen among pigs that can be transmitted to humans, in whom it causes invasive infection. In recent years, cases among humans have been reported worldwide, and a large outbreak occurred in China in 2005 (1). In some areas, *S. suis* appeared as the most common etiology of adult meningitis (2). Data from Africa were scarce until the recent report of 15 cases in Togo (3). We report 2 cases of *S. suis* meningitis in Antananarivo, Madagascar.

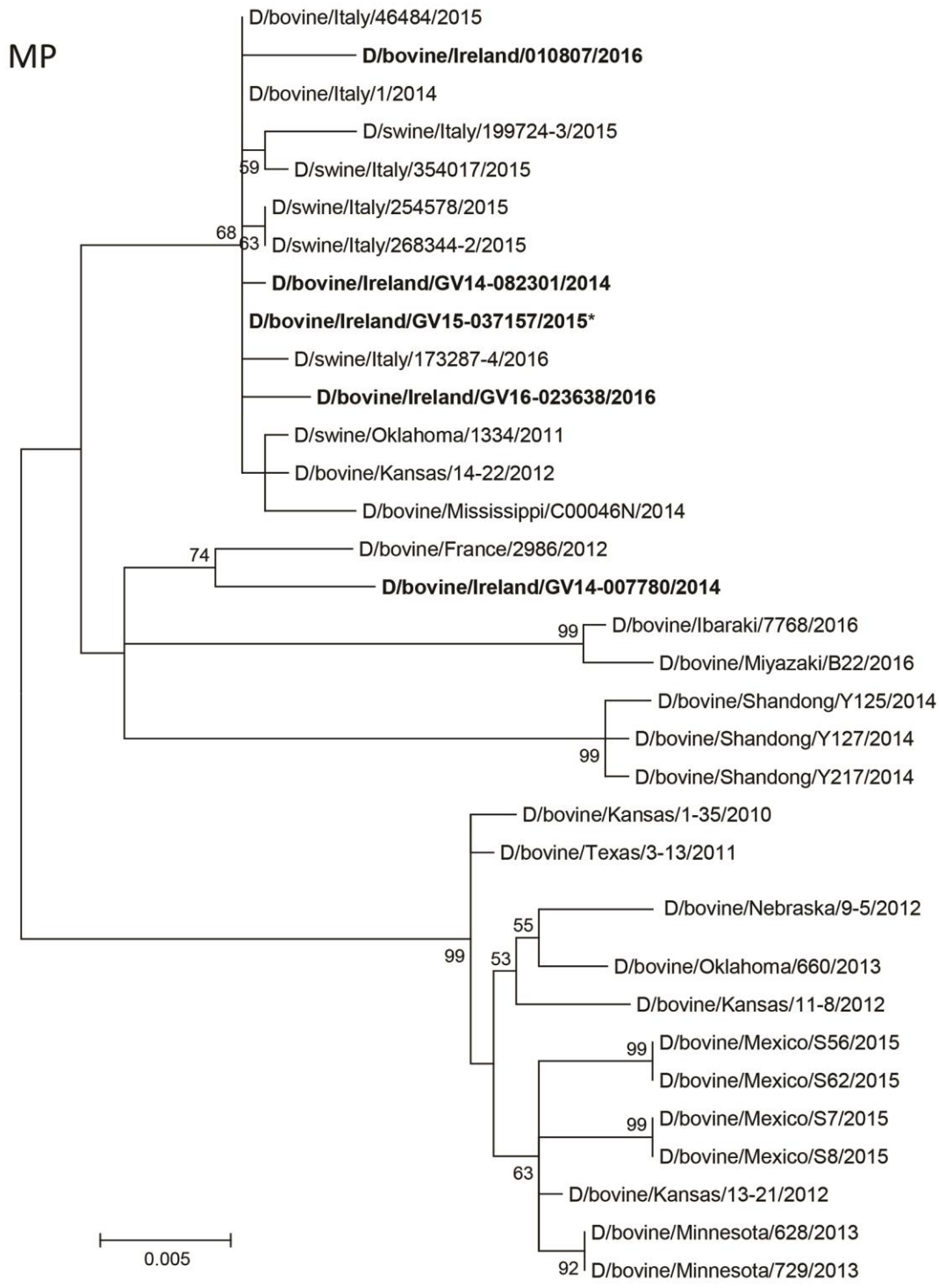
In March 2015, a 24-year-old man (patient 1) was admitted to the Infectious Diseases Unit of Befelatanana Hospital (Antananarivo) seeking treatment for fever, headache, and unilateral sixth nerve palsy. One year later, in March 2016, a 60-year-old woman (patient 2) was admitted to the same unit with meningitis and sudden hearing loss. Patient 1 worked in a slaughterhouse and patient 2 as a cook; both were frequently exposed to pork meat. Both patients were febrile (temperature $\geq 39.9^{\circ}\text{C}$) and confused and had a score of ≤ 13 out of 15 on the Glasgow Coma Scale. Laboratory results for lumbar puncture showed turbid cerebrospinal fluid (CSF) with increased cell numbers (446 cells/ μL , 56% neutrophils for patient 1; 1,180 cells/ μL , 86% neutrophils for patient 2 [reference range $<10/\mu\text{L}$]); high protein levels (1.4 g/L for patient 1, 2.34 g/L for patient 2 [reference range 0.15–0.45 g/L]); and low glucose levels (0.28 mmol/L for patient 1; 0.73 mmol/L for patient 2 [reference range 2.7–4.2 mmol/L]). CSF Gram stain results showed gram-positive diplococci resembling *Streptococcus pneumoniae*. CSF samples inoculated onto chocolate agar showed microbial growth that was further found to contain optochin-resistant α -hemolytic streptococci. In-house real-time PCR on CSF and colonies, targeting the specific gene for *S. pneumoniae*, were negative. The colonies were finally identified as *S. suis* by using ApiStrep20 (bioMérieux, Marcy l'Étoile, France) and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry with a log (score) value of spectra >2.3 (cutoff ≥ 2.0). Both patients achieved overall recovery after a complete course of intravenous ampicillin (patient 1) or ceftriaxone (patient 2), but a mild hearing deficit acquired during the infection remained for both patients.

We retrieved only strain Ss1223, isolated from patient 2, for further investigation. By using slide agglutination with type-specific hyperimmune serum and specific multiplex PCR (4), we identified the Ss1223 strain as *S. suis* serotype 2. We performed whole-genome sequencing by

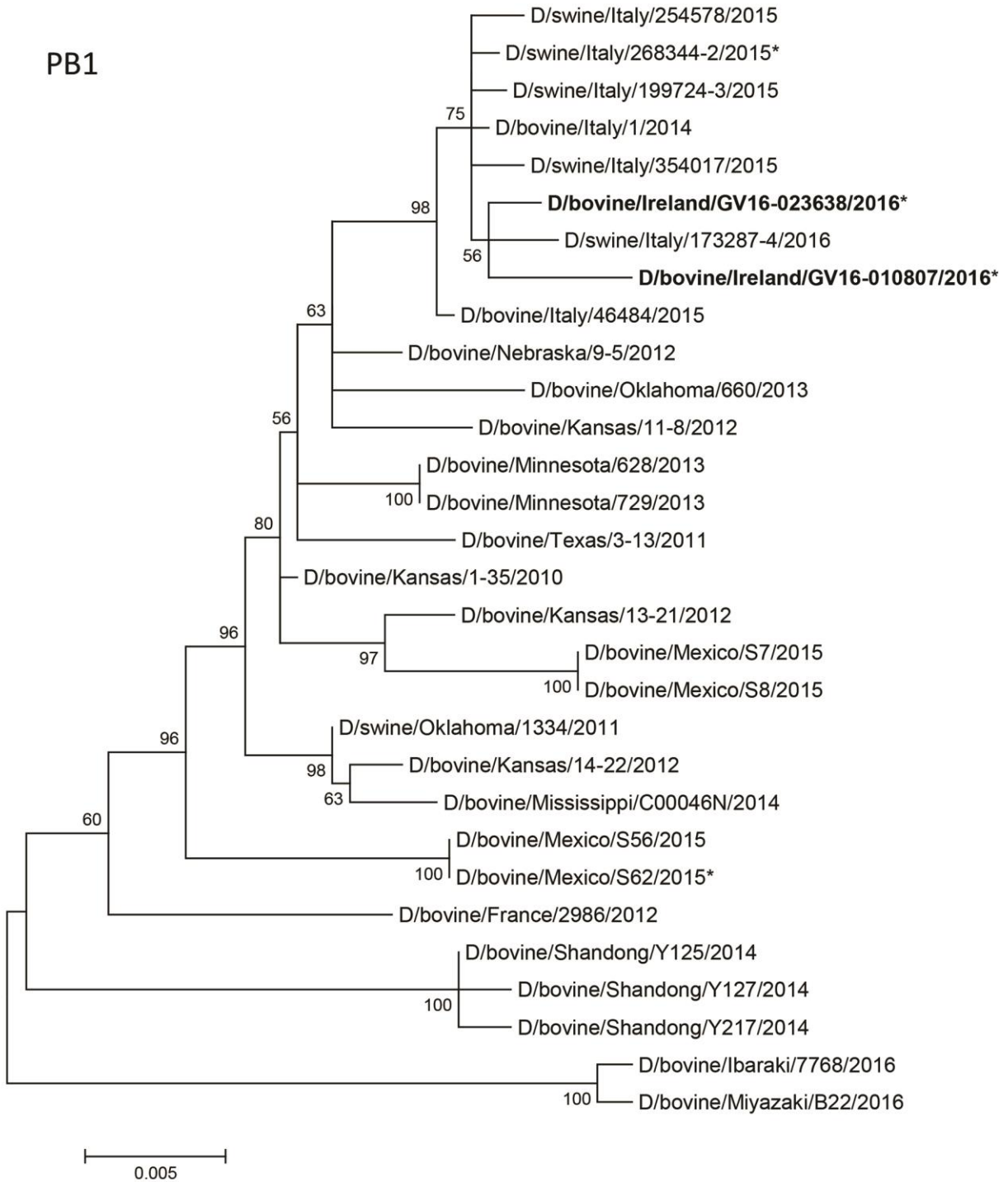
Influenza D Virus in Cattle, Ireland

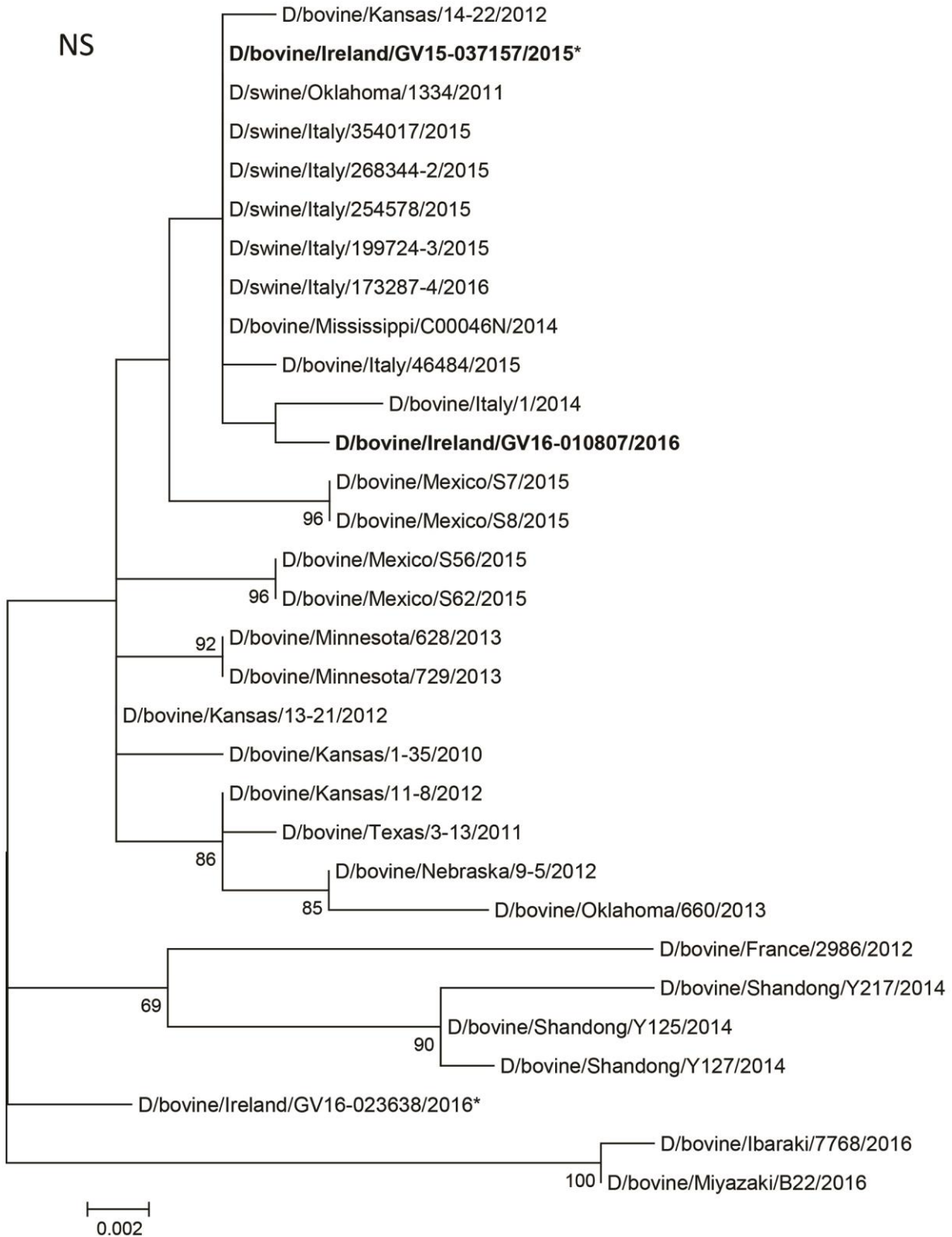
Technical Appendix

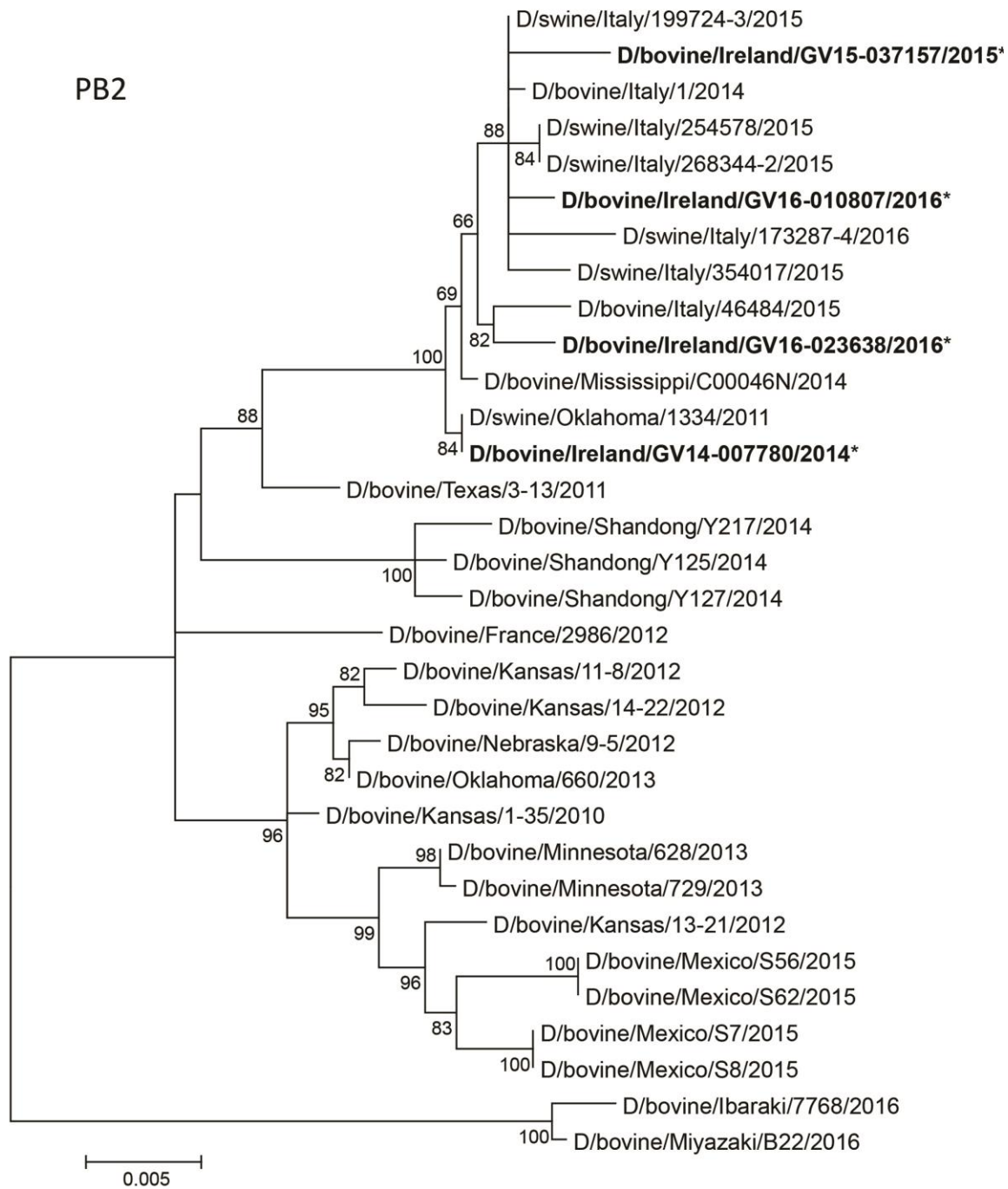




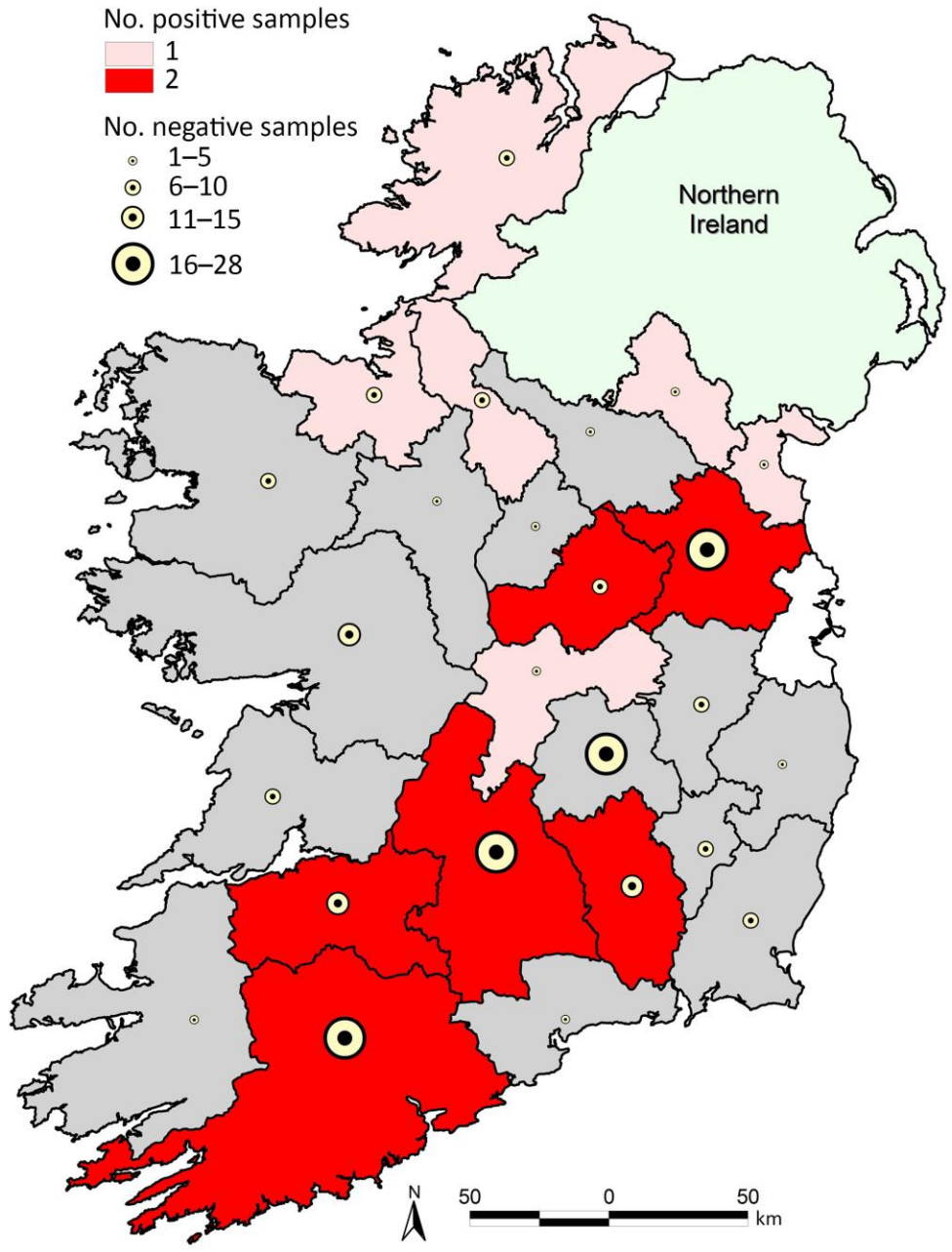
PB1







Technical Appendix Figure 1. Phylogenetic trees for 7 genomic segments of influenza D viruses obtained from cattle in Ireland and comparison with virus sequences obtained from GenBank. *Indicates incomplete sequences (only bootstraps >50 are shown). Bold indicates strains isolated in this study. Scale bars indicate nucleotide substitutions per site. PB1, polymerase basic 1; PB2, polymerase basic 2; P3, polymerase 3; HEF, hemagglutinin esterase fusion; NP, nucleoprotein; MP, matrix protein; NS, nonstructural.



Technical Appendix Figure 2. Geographic distribution of positive and negative samples for influenza D virus, by county, in Ireland. Pink indicates 1 positive detected; red indicates 2 positive samples detected; gray indicates no positive samples detected. Circles indicate number of negative samples per county.