INFLUENZA D VIRUS IN CATTLE IN IRELAND

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CONCLUSIONS

- Influenza D virus is an emerging pathogen which has been detected in cattle in Ireland.
- Epidemiological analysis of infected herds did not detect any significant differences in herd level characteristics compared to herds reporting respiratory disease where influenza D was not found.
- Serological testing revealed widespread exposure in Irish cattle, with 94.6% of cattle sampled at slaughter testing positive for antibodies
- Serological exposure in pigs and sheep was much lower, at 5.8% and 4.5% respectively.
- Phylogenetic analysis of influenza D virus isolates from Irish cattle revealed two distinct lineages, one clustering with French influenza D isolates and one clustering with Italian influenza D isolates.
- This suggests that influenza D virus has been circulating widely in Irish cattle since at least 2014, and that at least two separate introductions are likely to have occurred.
- Further research is needed to determine the impact of this novel respiratory virus in Irish bovine production systems.

INTRODUCTION AND OBJECTIVES

Influenza D is a newly characterised virus associated with bovine respiratory disease. The objective of this study was to determine whether this virus was present in cattle in Ireland, to investigate the characteristics of any infected herds, and to estimate the prevalence of exposure. A secondary objective was to determine the degree of exposure in sheep and pigs in Ireland.

MATERIALS & METHODS

The materials and methods are fully described in Flynn et al (2018) and O'Donovan et al (2019).

320 archived bovine nasal swabs submitted by private veterinary practitioners for routine respiratory virus PCR testing from 2014-2016 to Virology Division, Central Veterinary Research Laboratory, Ireland, were tested for influenza D using a real-time PCR assay described by Hause et al (2013). RNA was extracted using an automated extraction robot. Samples with a Ct value of ≤25 were selected for further molecular characterisation using 3 primer sets targeting the Haemagglutinin-esterase (HE) gene (667F & 1350R) and Matrix (M) (8F & 670R; 602F & 1212R). cDNA synthesis was performed using Quantabio qScript cDNA SuperMix according to manufacturer's instructions. PCR amplification was performed using Quantabio AccuStart II PCR ToughMix with the following thermocycling conditions - initial denaturation at 94°C for 3 min (1 cycle), 40 cycles of 94°C for 30 sec, 65°C for 30 sec and 72°C for 3 min. Samples were resolved on a 1.5% agarose gel stained with gel red. PCR products were treated according to manufacturer's instructions (Illustra ExoProStar 1-Step) prior to being sent for sanger sequencing. Sequenced data was analysed using DNASTAR Lasergene 12 SeqMan Pro and sequence alignment carried out using Clustal W in MEGA 5.01. Phylogenetic trees were constructed using Maximum Likelihood in MEGA 5.01.

Herd level information for the year 2015 was available for 84 herds of origin of these nasal swabs (10 positive and 74 negative for influenza D). The data was obtained from the Animal Identification and Movement System database of the Department of Agriculture, Food and the Marine. Univariate statistical analysis was carried out using Stata/SE14.1(StataCorp LP, College Station, Texas, USA). The factors investigated for a possible association with influenza D status were herd size, numbers of stillbirths, dairy cows in herd, beef cows in herd, inward movements from marts, inward movements to farm, and carcases moved to knackeries.

The serology aspect of this study used 1,219 bovine serum samples taken at slaughter from healthy beef cattle aged 30-36 months which had passed ante-mortem veterinary inspection. These samples were taken in January 2017 from a range of slaughter plants across Ireland to ensure a representative geographical spread. In addition, 1,183 serum samples from cattle were included which had been taken during 2016 and early 2017 for diagnostic purposes to screen for antibodies to bovine respiratory disease (BRD) pathogens were used. A smaller number of swine and ovine sera, 377 and 288 respectively, were also included in the study. The swine and ovine sera had been submitted for routine general diagnostic testing. Each sample was tested for antibodies to influenza D virus. Haemagglutination Inhibition (HAI) assay was performed. Briefly, sera were inactivated with receptor-destroying enzyme (RDE), 50uL of sera to 200uL of RDE, and incubated overnight at 37°C. 200uL of 1.5% sodium citrate was added to each sample and heat-inactivated at 56°C for 30 minutes. Finally, sera were treated with 50uL of 50% Turkey red blood cells to give a final dilution of 1 in 10. HAI assay was performed using 0.75% Turkey red bloods cells in vee-bottomed plates. Samples with titres of ≥ 40 were considered positive.

RESULTS

18 samples tested positive for influenza D by PCR. 13 of these 18 positives also tested positive by PCR for one or two of several other viral pathogens (BHV1, PI3, BoCoV, BRSV, BVD). 9 of these influenza D samples had a Ct value ≤25 which were selected for sequencing . Sequences were obtained for 5 samples. Phylogenetic analysis showed Irish influenza D viruses clustered in two distinct lineages, one with French viruses and one with Italian viruses (fig 1).

Herd level information was available for 10 of the positive herds and for 74 comparison herds for which the nasal swabs were negative for influenza D. No associations between herd characteristics and influenza D status were detected (table 1).

Of the 1,219 samples collected randomly from healthy beef cattle at routine slaughter, 1153 were positive for antibodies to IDV, resulting in a seroprevalence of 94.6% (95% confidence interval 95.87%, 93.33%). A lower seroprevalance of 64.9% was observed in the samples taken from cattle for diagnostic testing for BRD; 768 positive samples from a total of 1183 tested. Swine and ovine serum had much lower prevalence; 5.8% for swine and 4.5% for ovine samples. The results are shown in table 2.

RESULTS

Table 1: Herd level characteristics for influenza D virus positive and negative herds

Herd level factor (2015 data)	Mean value for influenza D positive herds (with 95% CI)	Mean value for influenza D negative herds (with 95% CI)
Herd size	204.6 (140.6-268.6)	250.8 (182.7-318.9)
Inward moves from mart	69.7 (10.8-128.6)	110 (0-288.2)
Moves onto farm	13 (0-26.3)	27 (15.7-38.4)
Stillbirths	1.1 (0-3.3)	2.1 (1-3.2)
Moves to knackery	10.1 (5.6-14.6)	12.4 (8.5-16.3)
Dairy cows in herd	76.1 (13.6-138.6)	121.8 (86.2-157.4)
Beef (suckler) cows in herd	48.8 (19.1-78.5)	78.3 (60.9-95.5)

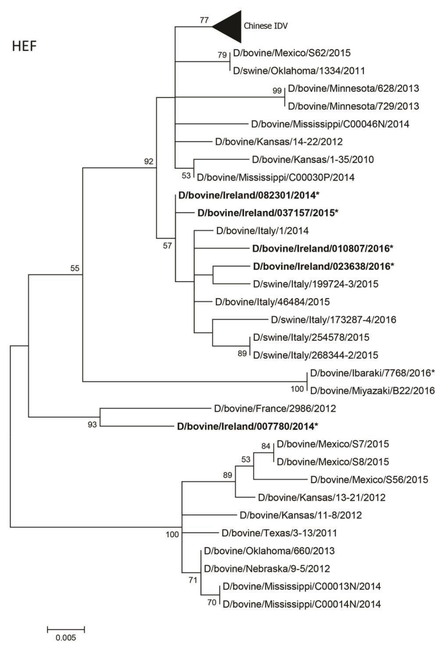

Figure 1: Phylogenetic tree for hemagglutinin esterase fusion (HFP) segment of influenza D viruses obtained from cattle in Ireland and comparison with sequences from GenBank. Other genomic segments (PB1. PB2, P3, NP, MP, NS) reported in Flynn et al (2018).

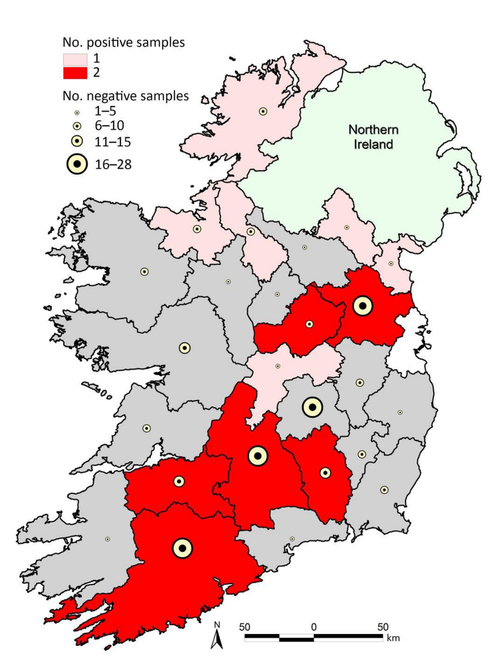

Figure 2: Geographic distribution of positive and negative samples for influenza D virus, by county, in Ireland. Pink indicates 1 positive detected; red indicates 2 positive samples detected; gray indicates no positive samples detected. Circles indicate number of negative samples per county.

Table 2: Prevalence of antibodies to IDV in sampled farm animal groups

Sampled group	Antibody prevalence
Cattle at routine slaughter aged 30-36 months	94.6% (1153/1219)
Cattle sampled for diagnostic reasons	64.9% (768/1183)
Pigs sampled for diagnostic reasons	5.8% (22/377)
Sheep sampled for diagnostic reasons	4.5% (13/288)

DISCUSSION

We present evidence that influenza D virus is circulating widely in Irish cattle, and is present but less common Irish sheep and pigs.

Its presence in swabs submitted from cases of bovine respiratory disease supports the hypothesis that it plays a role in the bovine respiratory disease complex, although further evidence is needed to determine the impact of any pathogenic effect in an Irish context.

This highlights the ongoing need for effective surveillance for new and emerging pathogens in Irish livestock systems.

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