Seroprevalence of influenza D virus in selected sample groups of Irish cattle, sheep and pigs

Tom O'Donovan1*, Leah Donohoe1, Mariette F. Ducatez2, Gilles Meyer2 and Eoin Ryan3

Abstract

Influenza D virus (IDV) is a new member of the Orthomyxoviridae family. It was first reported in swine in 2011 and isolated from bovine samples received for routine respiratory disease diagnosis in Ireland during 2014–2016. The goal of this study was to determine the seroprevalence in selected populations of IDV in cattle, pigs and sheep. Results showed a high prevalence of IDV in cattle sampled at slaughter (94.6%) or for diagnostic reasons (64.9%), whereas prevalence in samples taken for diagnostic reasons from sheep (4.5%) and pigs (5.8%) was much lower. This study suggests that IDV is widespread in Irish cattle.

Keywords: Influenza, Cattle, Virus, Respiratory, Surveillance

Introduction

Influenza viruses are enveloped RNA viruses that are members of the Orthomyxoviridae family [1]. The natural reservoir of influenza A virus (IAV) is waterfowl and as well as causing outbreaks in poultry, it is responsible for both seasonal and pandemic influenza in humans [2]. The main reservoir of influenza B virus is humans and it causes seasonal influenza [3]. Influenza C virus causes milder disease, primarily lower-respiratory-tract infections in children [4]. In 2011, a novel influenza virus was isolated from swine in America that was designated influenza D virus (IDV). It was shown to share approximately 50% sequence similarity with influenza C virus [5]. Although this was first detected in swine, surveillance data suggest the natural reservoir for this virus is cattle [6]. IDV has subsequently been detected in several European countries including Italy and France [7, 8]. IDV was detected in Irish cattle submitted for routine diagnosis during 2014–2016 [9] and on this basis a seroprevalence study was carried out to determine the prevalence of IDV in Irish cattle. A smaller number of swine and ovine sera were also tested for the presence of IDV antibodies.

Materials and methods

This study used 1219 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, 1183 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. The number of samples selected was based on availability rather than design prevalence; the samples taken at slaughter were originally selected for surveillance for another disease, while samples submitted for diagnostic purposes from cattle, sheep and pigs were used as convenience samples rather than random samples.

Each sample was tested for antibodies to influenza D virus. Haemagglutination Inhibition (HAI) assay was performed as described in standard protocols [10]. 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 min. 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 V-well plates. The HAI assay was then 
conducted using the stock virus D/Bovine/France/5920/ 
2014. A homologous positive control serum was also 
included in the assay. A 1 in 40 dilution of the stock virus 
was required to produce a working dilution of 4HAU.

Samples with titres of ≥40 were considered positive as 
per previous studies [6]. Serological cross reactivity 
against influenza C virus was not considered as it has 
been previously demonstrated that no cross reactivity 
between these two viruses is present [5].

Results

Of the 1219 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 sero-

prevalence of 64.9% was observed in the samples taken 
cattle for diagnostic testing for BRD; 768 positive 
samples from a total of 1183 tested. A breakdown of the 
titres observed in positive bovine samples is as follows; a 
1/40 titre in 7% of samples, a 1/80 titre in 15% of samples, 
a 1/160 titre in 23% of samples, a 1/320 titre in 19% of 
samples, a 1/640 titre in 11% of samples, a 1/1280 in 3% 
of samples, 1/2560 in 1% of samples and 1/5120 in 0.2% of 
samples. Finally, 0.2% of samples had a titre ≥1/10240.

Swine and ovine serum had much lower prevalence; 5.8% 
for swine and 4.5% for ovine samples. Confidence intervals 
were not calculated for the diagnostic samples since they 
were non-random submissions.

Discussion

The results reported in this study are important as they 
establish that IDV infection in the Irish cattle population 
is far more widespread than previously thought, and that 
cattle are a more important host for this virus, rather 
than pigs or sheep. It is notable that broadly similar 
findings were reported from Luxembourg, where 80.2% 
seroprevalence was reported in cattle [11].

It has previously been reported that IDV may have a 
role to play in the BRD complex [12]. Therefore, it 
might be expected that a higher prevalence of IDV anti-
bodies would be observed in cattle samples taken for 
diagnostic testing for BRD than in samples taken from 
healthy cattle at slaughter. However, the data show the 
reverse to be the case. This finding may be the result of 
an age difference in the two groups. Although one of the 
limitations of the study is that animal-level age data was 
not available for diagnostic submissions, the beef cattle 
which were sampled at slaughter were all aged 30 to 36 
months; in contrast, samples submitted for routine BRD 
diagnostic testing are most frequently younger animals, 
including calves and weanlings. Age may therefore be 
acting as a confounder in comparing the two groups; as 
cattles get older, they may have more opportunities to be 
come exposed and infected with IDV. However, the 
 multifactorial aetiology and epidemiology of BRD means 
that it is important to take a nuanced approach to inter-

preting these results; it may be that IDV plays a role as a 
co-factor or potentiating agent in BRD in Irish cattle, 
and that it also spreads through the clinically normal 
population in such a way as to result in the remarkably 
high prevalence reported here. Further research is neces-

sary to understand the epidemiology and any clinical im-

pact of IDV in cattle.

The prevalence rates for pigs and sheep were much 
lower and the pig data were in line with those reported 
in other European countries. Italy recorded a prevalence 
in pigs of 11.7% in 2015 [13], Luxembourg of 5.9% in 
2014–2015 [11]. 

The zoonotic potential of IDV is not yet clear but as it 
is closely related to ICV, the possibility is worth consid-
erring. Given the high prevalence observed in Irish cattle, 
further investigation in people with increased exposure 
to cattle may be warranted. This study has demonstrated that infection with IDV 
is widespread in two populations of Irish cattle studied 
(beef cattle aged 30–36 months and cattle sampled for 
diagnostic reasons), but has a relatively low prevalence 
in populations of Irish sheep and pigs which have been 
sampled for diagnostic reasons. Further research is 
needed to address the role IDV may play in bovine 
respiratory disease and to explore the potential for any 
zoonotic risk.

Abbreviations

BRD: Bovine respiratory disease; HAI: Haemagglutination inhibition; 
IAV: Influenza A virus; IBV: Influenza B virus; ICV: Influenza C virus; 
IDV: Influenza D virus; RDE: Receptor destroying enzyme

Acknowledgements

None.

Authors’ contributions

TOD and LD carried out the lab work; MFD and GM provided technical 
guidance; ER analysed the data. All authors contributed to writing the 
manuscript. All authors read and approved the final manuscript.

Funding

This work was funded by the Department of Agriculture, Food and the 
Marine, Ireland.

Availability of data and materials

The datasets used and/or analysed during the current study are available 
from the corresponding author on reasonable request.

Ethics approval and consent to participate

Samples used were archived samples originally taken for diagnostic purposes 
and therefore no ethical approval was required.

Consent for publication

No human data used, not applicable.
Competing interests
The authors declare that they have no competing interests.

Author details
1 Central Veterinary Research Laboratory, Celbridge, Co. Kildare, Ireland. 2 INRA UMR 1225 IHAP-ENVT, 31076 Toulouse, France. 3 Ruminant Animal Health Division, Department of Agriculture, Food and the Marine, Celbridge, Ireland.

Received: 11 June 2019 Accepted: 16 October 2019
Published online: 29 October 2019

References
1. Paules C, Subbarao K. Influenza. Lancet. 2017;390(10095):697–708.
2. Olsen B, Munster VJ, Wallensten A, Waldenstrom J, Osterhaus ADME, Fouchier RAM. Global patterns of influenza a virus in wild birds. Science. 2006;312(5772):384–8.
3. Huang SSH, Banner D, Paquette SG, Leon AJ, Kelvin AA, Kelvin DJ. Pathogenic influenza B virus in the ferret model establishes lower respiratory tract infection. J Gen Virol. 2014;95(Pt_10):2127–39.
4. Matsuzaki Y, Katsushima N, Nagai Y, Shojo T, Itagaki T, Sakamoto M, et al. Clinical features of influenza C virus infection in children. J Infect Dis. 2006;193(9):1229–35.
5. Hause BM, Ducatez M, Collin EA, Ran Z, Liu R, Sheng Z, et al. Isolation of a novel swine influenza virus from Oklahoma in 2011 which is distantly related to human influenza C viruses. PLoS Pathog. 2013;9(2):e1003176.
6. Ferguson L, Eckard L, Epperson WB, Long L-P, Smith D, Huston C, et al. Influenza D virus infection in Mississippi beef cattle. Virol. 2015;486:28–34.
7. Chiapponi C, Faccini S, Mattia AD, Baioni L, Barbieri I, Rosignoli C, et al. Detection of influenza D Virus among swine and cattle, Italy. Emerg Infect Dis. 2016;22(3):524–4.
8. Ducatez MF, Pelletier C, Meyer G. Influenza D virus in cattle, France, 2011–2014. Emerg Infect Dis. 2015;21(2):568–71.
9. Flynn O, Gallagher C, Mooney J, Irvine C, Ducatez M, Hause B, et al. Influenza D virus in cattle, Ireland. Emerg Infect Dis. 2018;24(2):389–91.
10. OIE. Manual of diagnostic tests and vaccines for terrestrial animals part 2, section 2.8, chapter 2.8.7, (2018).
11. Snoeck CJ, Oliva J, Pauly M, Losch S, Wildschutz F, Muller CP, Hübschen JM, Ducatez MF. Influenza D virus circulation in cattle and swine, Luxembourg, 2012-2016. Emerg Infect Dis. 2018;24(7):1388–9. https://doi.org/10.3201/eid2407.171937.
12. Ng TFF, Kondov NO, Deng X, Eenennaam AV, Neibergs HL, Delwart E. A metagenomics and case-control study to identify viruses associated with bovine respiratory disease. J Virol. 2015;89(10):5340–9.
13. Foni E, Chiapponi C, Baioni L, Zanni I, Merenda M, Rosignoli C, et al. Influenza D in Italy: towards a better understanding of an emerging viral infection in swine. Sci Rep. 2017;7(1):11660.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.