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SHORT COMMUNICATION

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Influenza D virus exposure among US cattle workers: A call for surveillance

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Abstract

Although cattle are a reservoir for influenza D virus (IDV), little is known about human exposure to IDV. We assessed IDV exposure and associated health effects among United States dairy workers, a population at heightened risk of cattle zoonoses. In prospective, cross-shift sampling of 31 workers employed at five large-herd dairy operations in two states, we found evidence of IDV in the nasal washes of 67% of participants at least once during the 5-day study period. IDV exposure was not associated with respiratory symptoms in these workers. These findings suggest that IDV is present in dairy cattle environments and can result in worker exposure.

KEYWORDS
cattle, influenza virus, occupational health, pandemic, zoonoses

1 | INTRODUCTION

Cross-species exchange of influenza viruses between animals and humans may accelerate viral genetic changes that result in enhanced viral morbidity. (Taubenberger & Morens, 2010) Although influenza A viruses (IAVs) have been responsible for global influenza pandemics to date, research considering the pandemic potential of other influenza viruses is more limited. (Reperant et al., 2012) Influenza D virus (IDV) is a newly recognized zoonotic influenza virus species of the *Deltainfluenzavirus* genus in the *Orthomyxoviridae* family. It was first identified in 2011 and cattle are a leading animal reservoir. (Collin et al., 2015; Hause et al., 2013) Although IDV circulates widely in cattle, little is known about IDV infections among humans. (Liu et al., 2020) To date, IDV infections among the general population appear limited, but our pilot study of cattle workers in Florida, USA, identified a very high IDV seroprevalence. (White et al., 2016) A

more comprehensive assessment of cattle and cattle workers could clarify worker exposure and consider human health risks posed by this emerging pathogen.

We prospectively assessed United States dairy workers for up to five consecutive workdays, collecting daily pre- and post-shift nasal wash specimens, personal bioaerosol samples, and respiratory symptoms self-reports. We assessed the prevalence of IAV, IDV, influenza C virus (ICV) and pan-coronaviruses (CoV) in personal bioaerosol and nasal wash specimens, and we evaluated respiratory symptoms associated with IDV nasal carriage to evaluate health concerns.

Impacts

- Influenza D virus (IDV) is an emerging genus of zoonotic influenza virus identified commonly in dairy cattle, although little is known about human exposure.
- More than 2/3 of dairy workers in a longitudinal, cross-shift study (n = 31) had an IDV-positive nasal wash at least once during the study period.
- IDV was not associated with respiratory symptoms in this study, and the 'silent' nature of carriage reinforces the need to actively monitor spillover of this pathogen to humans.

2 | MATERIALS AND METHODS

2.1 | Study design

Participants were employees of five large-herd dairy operations in the Western and Southwestern US and were recruited on-site from May 2019 to January 2020. This research was nested within a prospective study assessing the effect of saline nasal washes on respiratory irritation among US dairy workers. Participants were monitored daily for one to five consecutive workdays. Nasal washes were administered using 10 ml sterile saline before and immediately after their workshift as previously described. (Burch et al., 2010) Participants were queried on current experiences of 10 respiratory symptoms (eye irritation, blurred vision, nasal congestion, excessive mucus, shortness of breath, headache, wheezing, sore throat, cough or fever) and rated worsening severity on a 0–4 scale. During each workshift, participants wore individual SKC Button samplers (SKC Inc.) for continuous personal sampling of inhalable bioaerosols (0–100 μm in aerodynamic diameter). These samplers, containing PVC filters with a 5 μm pore size, were connected to individual pumps calibrated to 4 L/min.(Martenies et al., 2020) All human subjects protocols were approved by Colorado State University's IRB.

2.2 | Laboratory analysis

Bioaerosol filters and wash specimens were examined for molecular evidence of IAV, ICV, IDV and CoV using previously described pan-species, real-time, and conventional reverse transcription polymerase chain reaction (qRT-PCR and RT-PCR) (Gray et al., 2021). Viral load was established through cycle threshold (C_t) values. Samples with positive RT-PCR results were submitted to Eton Bioscience Inc. for Sanger sequencing. Recovered sequences were compared with the NCBI sequence database using BLAST 2.11.0. Matches with greater than 95% identity were recorded.

2.3 | Statistical analysis

Questionnaire and laboratory data were studied with descriptive and bivariate statistics (chi-square or Fisher's exact tests and t-tests). We hypothesized that any respiratory symptoms associated with an IDV infection would manifest two or more days after an IDV-positive wash. To evaluate this hypothesis, we used t-tests to compare the mean symptom severity score two or more days after a positive IDV wash to mean scores from other study days.

3 | RESULTS

3.1 | Study participants

Thirty-one (n = 31) dairy workers participated in this study, including 23 men and eight women, who worked at five different dairies representative of large-herd operations in the U.S. (>1000 lactating cows). Median age was 32 years (range: 23–55). Participants provided 123 full sets of samples, defined as pre- and post-shift nasal washes, pre- and post-shift symptom diaries, and a workday personal aerosol sample on a single workday. Median duration of participation was 3 days (range:1–5 days).

3.1.1 | Influenza D—bioaerosol samples

Seven workday bioaerosol specimens had qRT-PCR evidence of IDV (5.7%) (Table 1). These specimens were from seven participants (prevalence: 22.6%). Mean cycle threshold (C_t) value was 29.25 (range: 26.11–31.46). Due to limitations in RNA quantity from aerosol samples, we were not able to amplify IDV RNA for sequence typing. *Influenza D—nasal wash*: Twenty-one workers had at least one IDV-positive nasal wash (prevalence: 67.7%); (Table 1). Of 123 specimens, 36 had molecular evidence of IDV (29.3%). Post-shift prevalence was higher than pre-shift prevalence (21.1% vs. 12.5%), but this difference was not statistically significant. Four workers (19.0%) had both pre- and post-shift IDV positivity on the same workday. For the remaining 17 participants, the nasal carriage was transient during the workday and the study period. We observed low concordance between aerosol and washed IDV positivity, with one participant having IDV-positive aerosol and washing specimen on the same workday. All

TABLE 1 Influenza D-positive nasal wash among dairy cattle workers ($n = 31$), by sampling day[a]

Worker ID	Dairy of employment (A-E)[b]	Age in years; sex (M/F)	Day 1	Day 2	Day 3	Day 4	Day 5
1	A	43; M	post+	post+	neg		
2	A	36; M	pre+	post+	neg		
3	A	38; F	neg	post+	post+	post+	post+
4	B	26; M	neg	neg	neg		
5	B	30; M	post+	post+	neg	neg	
6	B	26; F	post+	post+	neg	post+	post+
7	B	26; F	neg	neg	neg	neg	
8	B	34; F	neg	neg	neg	neg	neg
9	B	39; M	neg	neg	neg		
10	B	50; M	neg	neg	neg	neg	neg
11	B	41; M	neg	pre+	neg		
12	B	50; M	neg	neg	neg	neg	neg
13	B	27; F	neg	neg	pre+		
14	B	42; M	neg	neg	neg		
15	C	31; M	neg	neg	pre+		
16	C	32; M	pre+	neg	pre+	neg	
17	C	55; M	neg	neg	pre+		
18	C	39; F	neg	neg			
19	D	27; M	pre+	neg	pre+	neg	
20	D	30; M	neg	neg	neg		
21	D	39; F	neg	neg	neg	neg	
22	E	30; M	pre+/post+	neg	post+	neg	neg
23	E	29; M	neg	post+	neg	neg	
24	E	31; F	neg	pre+	neg	neg	neg
25	E	35; M	pre+/post+	neg	neg	neg	neg
26	E	25; M	neg	post+	neg	neg	neg
27	E	34; M	pre+/post+	post+	neg	neg	neg
28	E	23; M	post+	post+	neg	neg	neg
29	E	35; M	post+	neg	post+	neg	neg
30	E	23; M	pre+/post+	neg	neg	neg	
31	E	26; M	neg	post+	neg	neg	neg

[a]Pre + indicates pre-shift positive IDV nasal wash (red). Post+ indicates post-shift positive IDV wash (blue). Pre+/post+ indicates an IDV positive was at both pre- and post-shift sampling on that day (green). Neg indicates an IDV-negative nasal wash (yellow). Hatching indicates an IDV-positive aerosol sample from the workshift on the specified day. Blank boxes indicate that the participant did not participate on that day.

[b]Workers participating in each dairy (A-E) as follows: A = 3 workers; B = 11 workers; C = 4 workers; D = 3 workers; E = 10 workers.

five dairies had participants with IDV carriage (positivity ranged from 18.2% to 72.7% of participants by facility). Of the four workers with concurrent pre- and post-shift IDV positivity on the same day, three worked at the same facility and were positive on the same day.

3.1.2 | Other viruses—bioaerosol samples

IAV was detected in five aerosol specimens (4.1%) collected from five individuals (prevalence: 16.1%). Two of the five IAV-positive samples were successfully sequenced using specific primers for the hemagglutinin (HA) and neuraminidase (NA) genes. BLAST results showed that one sample had 100% identity with avian (duck) influenza A H6N2 and H4N6 (HA segment: A/Peking duck/Mexico/CPA-5009/2007(H6N2), NA segment: (A/mallard duck/Alberta/136/00[H4N6]). The other sample matched avian (chicken) influenza A H9N2 with 100% identity (HA segment: A/chicken/XinjiangBaicheng/1/2014(H9N2), NA segment: (A/chicken/Japan/AQ-HE30-35C2/2018(H9N2). All bioaerosol samples were negative for ICV and CoV.

3.1.3 | Other viruses—nasal wash: Influenza A

Six nasal wash specimens (4.9%) had molecular evidence of IAV from six distinct individuals (prevalence: 19.4%). C_t values were high (range: 37.2 to 39.7). Post-shift prevalence was higher than pre-shift prevalence (16.1% vs. 3.2%). No participants were IAV-positive at pre- and post-shift on the same day or on multiple days. All samples were negative using the HA/NA RT-PCR assay due to low nucleic acid. *Influenza C*: Three specimens (2.4%) from different individuals were qRT-PCR positive for ICV (prevalence: 9.7%) No participants were ICV positive at both pre- and post-shift sampling on the same day. Due to assay technical difficulties, RT-PCR assay work for sequencing was not successfully performed. We identified six workers with molecular evidence of more than one influenza virus genus (IAV, ICV or IDV) during the study period (19.4%). Two workers had IDV-, ICV- and IAV-positive wash specimens during a 5-day period. *Coronaviruses (CoV)*: Five specimens (4.1%) from distinct individuals had evidence of coronavirus (prevalence: 16.1%) in nasal wash specimens. We obtained sequencing results from one sample, and it had 100% identity with human coronavirus 229 E, a human pathogen associated with respiratory symptoms and not observed in cattle. (Forni et al., 2022).

3.2 | Symptoms assessments

Nine of 21 participants with a positive IDV nasal wash reported respiratory symptoms two or more days after the positive specimen (42.9%), although this did not differ significantly from reports of respiratory symptoms among participants with an IDV-negative wash (51.7%). Participants reported lower mean severity scores two or more days after a positive IDV wash than they did at other times (mean severity score 1.8 vs. 2.3), but these values did not differ significantly. IAV-, IAC- and CoV-positive washes were not associated with respiratory symptoms.

4 | DISCUSSION

More than two-thirds of dairy workers enrolled this prospective, a cross-shift study had molecular evidence of IDV exposure, and this exposure appears transient. IDV-positive nasal washes were obtained from workers at all dairies sampled, suggesting widespread occupational exposure in this industry. Overall, these findings suggest that dairy workers experience chronic IDV exposure, but that the pathogen is a transient constituent of the nasal microbiome. IDV exposure was not associated with respiratory symptoms, and to date, there is no evidence that IDV causes clinical disease in humans.

In our study, we identified concurrent positive dairy worker nasal washes with IAV, ICV and IDV, each of which has been recognized to infect cattle. (Sreenivasan et al., 2019; Zhang et al., 2018) The relevance of pathogen-pathogen interactions within the respiratory microbiome of livestock workers is an important area for future study.

We recovered avian IAV from aerosol monitoring. This finding likely reflects inter-species interaction within open dairy operations, where wild birds are known to frequent. Cross-species transmission of avian influenza viruses poses risks for IAV pandemic emergence, and our observations highlight the ongoing need for influenza surveillance in these facilities. The role of livestock facilities as 'mixing bowls' for multiple influenza viruses remains an important consideration. Collecting information on contact between avians and domesticated animals—and potential human exposures to multiple species at work—is a key priority in future research to elaborate on the role of industrial livestock facilities in the generation of novel viruses.

This study is limited by its small size, which reduced our ability to infer occupational risk factors. Sequencing IDV was challenging due to the lack of established and optimized assays for amplifying and subtyping IDV. It remains possible that IDV carriage identified here is a function of community, and not occupational exposure, despite an understanding of IDV's animal reservoir. Data on IDV carriage within the dairy herds would clearly have strengthened our understanding of the role of occupation in these exposures but was beyond the scope of this small study. Information on animal contact at home or otherwise outside of primary work would have strengthened our understanding of pre-shift IDV positives, but in the context of a small sample, this information was difficult to interpret. Likewise, serology on these workers would have also advanced our understanding of IDV exposure in this population. These issues remain important areas for follow-up study. Our work is strengthened by cross-shift and repeated sampling using paired environmental and respiratory samples, granting a robust understanding of exposure within large-herd dairy operations.

Our findings suggest that IDV is present in dairy cattle environments and can result in worker exposure. As IDVs have been detected across geographical areas, it is prudent to conduct surveillance for emergent, and possibly more virulent, IDVs that may cause human disease.

AUTHOR CONTRIBUTIONS

JHL, JWS and GCG conceptualized the study. JWS is a co-PI of the parent study and provided specimens for viral analyses. JS provided data and specimen support and management. SJR is PI of the CDC/NIOSH High Plains Intermountain Center for Agricultural Health and Safety, co-PI of the parent study and supported recruitment of dairies. GCG supervised laboratory analyses and interpretation of laboratory data. AA conducted laboratory analyses. RFW and WEJ provided guidance and support towards study design and analysis. JHL conducted data analysis and wrote the paper with contributions from all authors.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to report.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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