One Health 11 (2020) 100175

Contents lists available at ScienceDirect

One Health

journal homepage: www.elsevier.com/locate/onehlt

Farm animal contact is associated with progression to Hemolytic uremic syndrome in patients with Shiga toxin-producing *Escherichia coli* — Indiana, 2012–2018

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ARTICLE INFO

Keywords:
Shiga toxin-producing *E. coli*
Hemolytic uremic syndrome
Farm animal
stx2
Mediation

ABSTRACT

Background: Hemolytic uremic syndrome (HUS) is a life-threatening complication of Shiga toxin-producing *Escherichia coli* (STEC) infection. The relationship between STEC exposure and severity of clinical outcomes is not well documented. We examined whether direct contact with farm animals increased the likelihood of HUS among Indiana residents diagnosed with STEC.

Methods: Exposure data for laboratory-confirmed STEC cases among Indiana residents during 2012–2018 were retrieved. Logistic regression and mediation analysis were performed to determine the extent to which a history of direct contact with farm animals was associated with post-diarrheal HUS independent of age and mediated by *stx2* gene presence.

Results: A total of 784 STEC cases were retrieved. Of these, 46 (6%) developed HUS. Complete exposure data were available for 600 (77%) cases. A total of 24 (52%) HUS patients reported direct contact with farm animals, while 114 (21%) STEC patients who did not develop HUS reported this exposure. Among all STEC cases, HUS was associated with direct farm animal contact after adjusting for age (OR = 3.40, 95% CI: 1.81, 6.40). Detection of *stx2* genes mediated 12% of the association between farm animal contact and HUS.

Conclusions: Direct farm animal contact was a risk factor for development of HUS among laboratory-confirmed STEC cases, independent of *stx2* presence. Direct farm animal contact should be considered a potential predictor of progression to HUS when patients present for care and the mechanism for its effect on virulence investigated.

1. Introduction

Hemolytic uremic syndrome (HUS) is a serious condition characterized by hemolytic anemia, thrombocytopenia, and acute renal dysfunction. [1] HUS can be precipitated by infection with several different bacterial enteric pathogens; the leading cause of post-diarrheal HUS in young children is Shiga toxin-producing *Escherichia coli* (STEC). [2] *E. coli* O157:H7 is the STEC serotype most commonly associated with HUS. Approximately 15% of children younger than five years of age and 6% of people in all age groups who are diagnosed with *E. coli* O157:H7 progress to HUS. Dialysis is required in over 50% of children diagnosed with HUS and 3–5% of cases result in death. [3] Although infection with most non-O157 serotypes of STEC is less likely to result in severe clinical consequences, HUS has been observed in these cases. [2]

STEC bacteria are transmitted fecal-orally. [4] Healthy ruminant animals, including cattle, goats, sheep, and deer, are natural reservoirs for STEC. Because ruminants do not have specific cell receptors that allow Stx to enter endothelial cells, they carry STEC without experiencing illness. [5] STEC can be transmitted to humans in water, food, soil, or surfaces that have been contaminated with animal feces. [6] Incidence of both O157 and non-O157 STEC have seasonal variability, with disease incidence peaking in the summer months. [7]

Shiga toxin (Stx) is the principal virulence factor associated with the severe sequelae of STEC infections and is encoded by *stx1* and *stx2* genes. [8] Both Stx types have the same mode of action but are antigenically distinct. [9] Presence of *stx2* variants has been more commonly associated with HUS onset. [10,11] The high risk of HUS

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https://doi.org/10.1016/j.onehlt.2020.100175
Received 17 July 2020; Received in revised form 23 September 2020; Accepted 23 September 2020
Available online 29 September 2020

associated with Stx2 production is likely due to the toxin's greater ability to pass through epithelial cells of the intestine and enter the bloodstream, where it has toxic effects on the renal endothelium and blood cells. [12]

The severity of clinical outcomes as a result of STEC infections has not previously been attributed to specific sources of exposure. An association between exposure source and virulence may allow clinicians to better predict the likelihood of progression to HUS. This information would also enable public health efforts to target exposure sources with the greatest impact on severe disease. This may be particularly relevant in states with greater animal agriculture operations. As of 2017, the state of Indiana was home to 56,649 farming operations. There were 17,014 farms with 844,187 cattle and calves. There were 11,753 farms with beef cows and 2049 farms with dairy cows. Approximately 90% of farms with cattle had less than 100 animals per farm and 0.4% had 1000 or more animals per farm. [13] STEC prevalence has been observed to be higher in beef cows than in dairy cows, and smaller farms have been associated with fecal shedding of Shiga toxin-encoding bacteria when compared to larger herds, likely due to variation in biosecurity practices. [14,15]

We conducted a population-based study in Indiana to examine the effects of direct contact with farm animals and livestock on HUS onset, overall and independent of potential mediating effects by *stx2* detection.

2. Methods

Laboratory-confirmed STEC cases reported to the Indiana State Health Department (ISDH) from 2012 to 2018 were retrieved for analysis. Indiana laboratories are required to forward positive STEC isolates to the ISDH laboratory for serotyping immediately upon identification, and hospitals are required to report confirmed cases of HUS to the ISDH immediately upon diagnosis. [16] Confirmed STEC cases were determined based on the national surveillance case definition applicable to the year of disease notification. Confirmatory laboratory evidence included either isolation of *E. coli* O157:H7 or isolation of other non-O157 strains supplemented by *stx* detection or evidence of Stx production. [17–19] Among the STEC cases, HUS cases were classified according to the national surveillance case definition, which requires acute illness diagnosed as HUS or thrombotic thrombocytopenic purpura (TTP) accompanied by anemia and renal injury. [1] Medical records were reviewed to verify clinical diagnoses of HUS for all HUS cases reported to ISDH.

All STEC patients were interviewed at the time of case reporting to determine potential sources of exposure. Patients were asked if they had direct contact with farm animals and livestock in the two weeks prior to illness onset, and if so, what type of animal.

We summarized cases by serotype and *stx2* detection and compared the seasonality of farm animal contact between patients who did and did not develop HUS. Using logistic regression, we estimated the effect of direct contact with farm animals and livestock on HUS onset, adjusted for age as a continuous variable. Regression coefficients were exponentiated to obtain odds ratios (ORs), and exact 95% confidence intervals (CIs) were calculated.

We used mediation analysis to measure the average direct effect of farm animal contact on HUS independent of potential mediation by *stx2* (Fig. 1). [20] The direct effect was calculated as the difference in the potential HUS outcome between those with and without animal exposure for a given *stx2* status, assuming no interaction between the exposure and mediator. The indirect, or mediated, effect was calculated as the difference in the potential HUS outcome between those with and without *stx2* for a given animal exposure status. The average direct and indirect effects were calculated by averaging across the direct and indirect effects for both levels of *stx2* and animal exposure status, respectively. We also calculated the average causal mediated effect and percent of the total effect mediated. CIs were estimated using 10,000 bootstrap replicates. Data were analyzed using the STATA/IC™ Software Suite version 16.0.

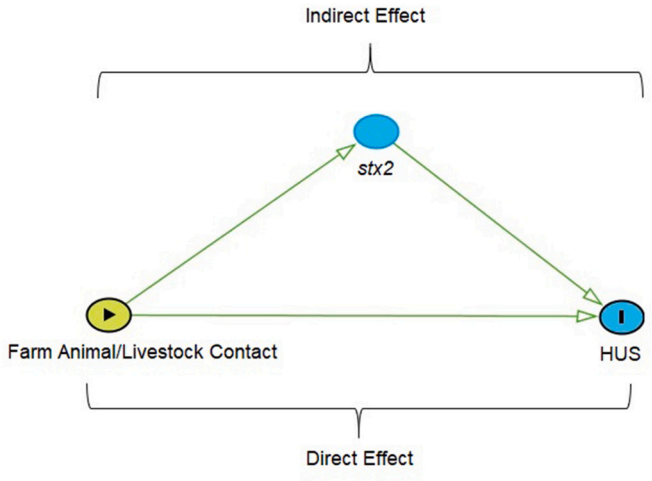

Fig. 1. Directed acyclic graph of hypothesized relationship between farm animal contact, age, stx2 genes, and HUS. The variable in yellow with the "►" symbol inside the oval is the exposure variable. The variable in blue with the letter "I" inside the oval is the outcome variable. Variables in blue are antecedents of the outcome variable. Abbreviations: HUS, Hemolytic Uremic Syndrome; stx2, Shiga toxin 2. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3. Results

A total of 784 confirmed STEC cases were reported during the study period. Of these, 46 (6%) developed HUS. There were 176 STEC patients aged ≤5 years. Of these, 26 (15%) developed HUS (Table 1).

The most commonly reported serotype was *E. coli* O157 (Table 2). Among all patients with confirmed *E. coli* O157 only, 41 (10%) developed HUS; among *E. coli* O157 patients who were children ≤5 years, 26 (23%) developed HUS. All HUS cases in patients ≤5 years were attributable to *E. coli* O157. By contrast, 1% of *E. coli* non-O157 patients developed HUS, and none were ≤ 5 years old. Of *E. coli* O157 strains, 92.6% expressed *stx2* (Table 2).

Among all STEC cases, information regarding exposure to farm animals or livestock was available for 600 (77%). The distributions of

Table 1
Demographic Distribution of Shiga Toxin-Producing *Escherichia coli* (STEC) cases, by Post-diarrheal Hemolytic Uremic Syndrome (HUS) Status — Indiana, 2012–2018.

	STEC Patients			
Characteristic	NO HUS		HUS	
	n	(%)	n	(%)
Age group				
≤5 years	150	(20.3)	26	(56.5)
6–10 years	71	(9.6)	10	(21.7)
11–18 years	112	(15.2)	6	(13.0)
19–59 years	315	(42.7)	2	(4.3)
≥60 years	90	(12.2)	2	(4.3)
Female sex	422	(57.2)	25	(54.3)
Race				
White	477	(64.6)	39	(84.8)
Black or African-American	28	(3.8)	–	
Asian	15	(2.0)	1	(2.2)
Native American or Alaska Native	1	(0.14)	–	
Other	9	(1.2)	–	
Unknown	208	(28.2)	6	(13.0)

Abbreviations: STEC, Shiga toxin-producing *E. coli*; HUS, Hemolytic Uremic Syndrome.

Table 2
STEC Serotype and Detection of *stx2* genes among STEC cases, by HUS status — Indiana, 2012–2018.

	STEC Patients							
	No HUS (n = 738)				HUS (n = 46)			
	Serotype n (%)		*stx2* detected n (%)		Serotype n (%)		*stx2* detected n (%)	
O157	363	(49.2)	336	(92.6)	41	(89.1)	38	(92.7)
O103	112	(15.2)	11	(9.8)	0	(−-)	–	–
O26	92	(12.5)	8	(8.7)	1	(2.2)	–	–
O111	80	(10.8)	15	(18.8)	1	(2.2)	0	(0.0)
O45	32	(4.3)	5	(15.6)	0	(−-)	–	–
O145	25	(3.4)	16	(64.0)	1	(2.2)	0	(0.0)
O121	18	(2.4)	15	(83.3)	2	(4.3)	2	(100)
O113	1	(0.1)	1	(100.0)	0	(0.0)	–	–
O28	1	(0.1)	1	(100.0)	0	(0.0)	–	–
O50	1	(0.1)	0	(0.0)	0	(0.0)	–	–
O69	1	(0.1)	0	(0.0)	0	(0.0)	–	–
O76	1	(0.1)	0	(0.0)	0	(0.0)	–	–
O8	1	(0.1)	1	(100.0)	0	(0.0)	–	–
O5	1	(0.1)	0	(0.0)	0	(0.0)	–	–
O103 & O111	2	(0.3)	1	(50.0)	0	(0.0)	–	–
O103 & O26	2	(0.3)	0	(0.0)	0	(0.0)	–	–
O103 & O121	1	(0.1)	0	(0.0)	0	(0.0)	–	–
O103 & O45	1	(0.1)	0	(0.0)	0	(0.0)	–	–
O45 & O26	1	(0.1)	1	(100.0)	0	(0.0)	–	–
O91 & O39	1	(0.1)	0	(0.0)	0	(0.0)	–	–
O118 & O111	1	(0.1)	1	(100.0)	0	(0.0)	–	–

Abbreviations: STEC, Shiga Toxin-Producing *Escherichia coli*; HUS, Hemolytic Uremic Syndrome; *stx2,* Shiga toxin 2 genes.

Table 3
Association Between Farm Animal Contact and HUS adjusted for age (Total Effect) and independent of the mediating effects of *stx2* (Direct Effect)— Indiana, 2012–2018.

HUS	Total Effect		Direct Effect	
	OR	*95% CI*	*OR*	*95% CI*
Farm Animal Contact	3.40	(1.81, 6.40)	3.13	(1.64, 5.98)
stx2	–		5.00	(1.91, 13.11)
Age	0.94	(0.92, 0.97)	0.94	(0.92, 0.97)

Abbreviations: OR, Odds Ratio; CI, confidence interval; stx2, Shiga toxin 2.

age, sex, race, serotype, and *stx2* detection were approximately equal in subjects with and without available animal exposure information (See Supplementary Table 1). Of those with available exposure information, 138 (23%) reported animal exposure, of whom 24 (17%) progressed to HUS. Of the 462 cases without direct animal contact, 22 (5%) developed HUS. Among children under the age of 5, 40% of those with reported farm animal contact developed HUS. Among children aged 6–10, 28% of those with reported farm animal contact developed HUS (Fig. 2).

Incidence of STEC increased in summer months with a peak in July. STEC cases with reported farm animal contact also peaked in July. However, HUS was proportionally most common among STEC cases with farm animal contact in the fall months; at its maximum in September, 30% of STEC cases with farm animal contact progressed to HUS (Fig. 3). The only months when the incidence of HUS among STEC cases without farm animal contact exceeded that among cases with farm animal contact were January-March, when very few cases reported animal contact.

Overall, farm animal contact was significantly associated with HUS onset after adjusting for age (OR 3.40; 95% CI 1.81, 6.40) (See Table 3). Both direct (independent of *stx2*) and indirect (mediated by *stx2*) effects were > 0 (See Supplementary Table 2). For the direct effect, we estimated the odds of HUS were 3.13 (95% CI 1.64, 5.98) times higher for

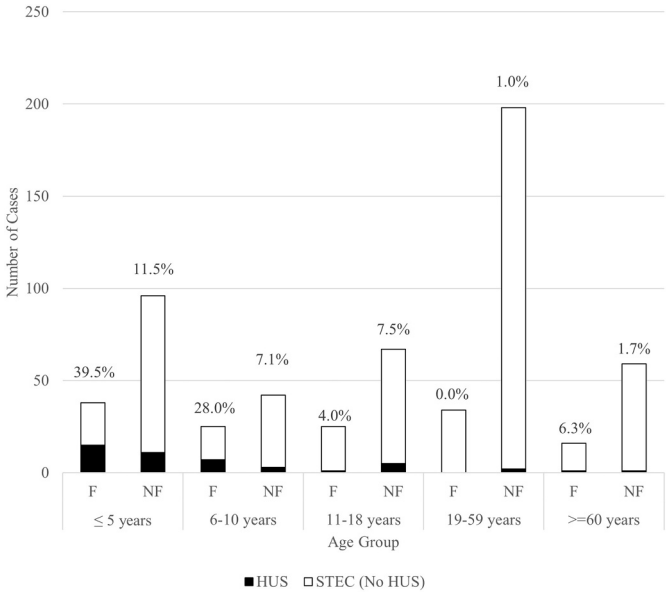

Fig. 2. Age Distribution of STEC and HUS by Known Farm Animal Contact and Percent HUS by Exposure Status. Patients with known farm animal exposure information were used to calculate the percent of patients who developed HUS by age by exposure status (n = 600). The labeled percentages represent the percent of patients that developed HUS corresponding with the bar underneath. The majority of patients aged 5 years under and aged 6–10 years who developed HUS reported direct farm animal contact. Abbreviations: HUS, Hemolytic Uremic Syndrome; STEC, Shiga toxin-producing *E. coli*; F, Farm Animal Contact; NF, No Farm Animal Contact.

cases with farm animal contact. In that model, the odds of HUS were 5.00 (95% CI 1.91, 13.11) times higher for cases in whom *stx2* was detected. The proportion of the total effect mediated by *stx2* was 12.2% (95% CI 7.03%, 24.2%) (See Supplementary Table 2).

4. Discussion

The odds of HUS were over three times higher among STEC cases with farm animal contact than those without (OR 3.40; 95% CI 1.81, 6.40). We found that only 12% of this association was mediated by *stx2*, yielding a direct effect of OR 3.13 among Indiana residents diagnosed with STEC. A greater proportion of younger children with farm animal contact developed HUS. HUS was proportionally most common among STEC cases with animal contact in the fall, but absolute numbers were highest in the summer months.

Farm animal contact is a well-known risk factor for STEC infection. [6,21,22] Ours is the first study of which we are aware that demonstrates a higher risk for developing HUS after direct exposure to farm animals and livestock. Clinical measures such as hemoglobin, leukocyte count and creatinine have been used to predict HUS development and subsequent outcomes. [23,24] Given the results of our study, measures of exposure such as farm animal contact and seasonality may also be considered when determining the prognosis for STEC cases.

Additionally, this information could be provided to public health agencies when healthcare providers report HUS, expediting the process of identifying potential sources of exposure. Rapid notification of enteric disease outbreaks is essential for effective response, identification of the source of contamination, and prevention of further morbidities. [25] Between 2009 and 2017, there were 57 animal-associated outbreaks of STEC reported to the National Outbreak Reporting System, resulting in 563 illnesses, 113 hospitalizations, and 3 deaths, demonstrating the need for providers to promptly recognize and report cases of STEC and HUS suspected to be associated with animal contact. [26] Additional research is needed to compare clinical outcomes of animal-

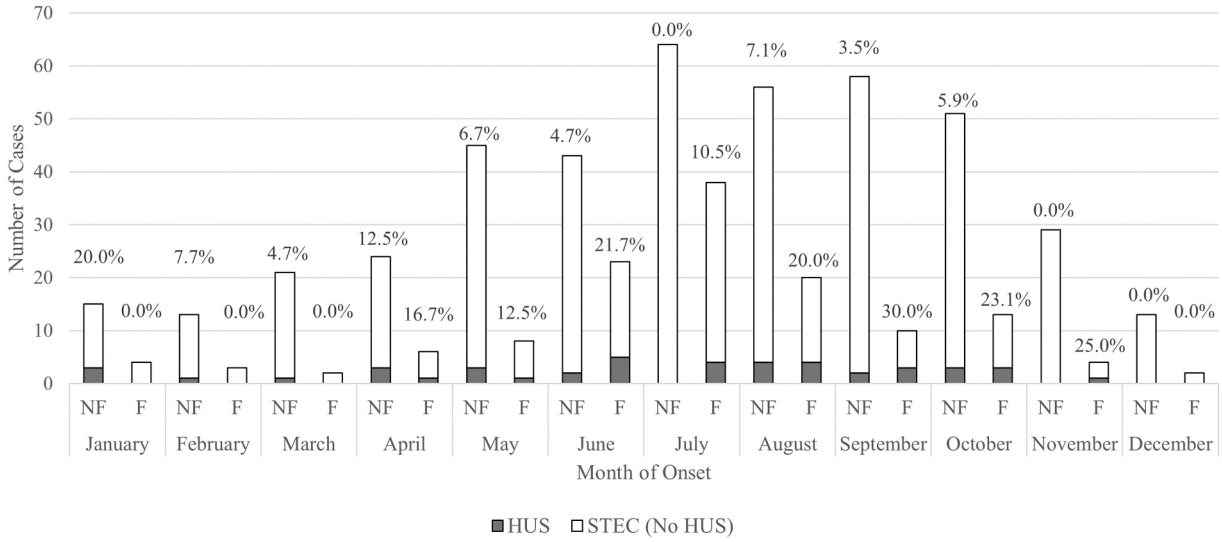

Fig. 3. Seasonal Distribution of STEC and HUS by Known Farm Animal Contact and Percent HUS by Exposure Status. Patients with known farm animal exposure information were used to calculate the percent of patients who developed HUS by month by exposure status (n = 600). The labeled percentages represent the percent of patients that developed HUS corresponding with the bar underneath. STEC incidence peaks in July independent of reported farm animal exposure. There is a greater proportion of HUS cases with farm animal contact between April and October, with the highest percentage of HUS cases with farm animal contact reported in September. Abbreviations: F, Farm Animal Contact; NF, No Farm Animal Contact.

associated STEC outbreaks in the United States to outbreaks of STEC associated with other sources.

Our mediation analysis demonstrated that only a small portion of the association between animal contact and HUS is due to *stx2,* implying the importance of other mechanisms. One potential mechanism is the dose received via direct animal contact. The pathogenicity of STEC is determined by both host and virulence factors, and STEC has a relatively low infectious dose of 10–100 CFU. [27,28] In cattle, *E. coli* O157 typically adhere to and colonize the recto-anal junction of the gastrointestinal tract, which leads to pathogen shedding and subsequent contamination of the surrounding environment. [29] The term "super-shedder" is defined as shedding $\geq 10^4$ CFU/g of feces; cattle that are super-shedders are capable of spreading significantly more pathogens when compared to other similar hosts. [30,31] Moreover, increased stress on animals exhibited in public settings, which may include transportation, confinement to limited physical space, overcrowding, over-handling, or comingling, can increase shedding and increase the risk of spreading harmful pathogens to people. [32] The association between animal contact in these settings and HUS should be studied. Several studies have been conducted to categorize STEC serotypes and virulence factors in ruminant animals, although few have compared the distribution of virulence factors in animals in public contact settings with free-range animals. [33–35] Analysis of feces collected from ruminant animals at agricultural events such as fairs, festivals, and petting zoos could help characterize both the pathogen load being shed into the surrounding environment and the distribution of known virulence factors in these settings, such as detection of stx_{2c} variants and activation of *eae* genes, that may inform pathogenicity of STEC strains. [36–38]

The proportion of HUS patients in our Indiana dataset diagnosed with *E. coli* O157 (10%) was on the higher limit of estimates reported in the literature (5–10%). [39] We also observed a higher proportion of HUS cases among patients ≤5 years with confirmed *E. coli* O157 (23%) when compared with previous studies (14–15%). [3,40] This may be explained by our use of the CSTE case definition, which has been shown to overestimate the burden of post-diarrheal HUS. [41]

In this study, animal exposure may have been misclassified. "Direct contact" may have been interpreted literally by respondents, even though contact with animal feces can occur without coming into physical contact with an animal. From a review of the comments sections in the surveillance data, some case investigators noted that patients who did not report direct animal contact did report consuming home-raised and home-butchered ground beef, visiting petting zoos, farms, or animal barns at state or local fairs, and residing near farms. This indicates that animal exposure may be underreported and/or underrecognized. Additionally, location of animal exposure was not documented in all case investigations during the study period precluding analysis of this characteristic. Location information would allow us to compare clinical outcomes among patients with exposures at agritourism events to patients who had contact with free range animals. Future studies may examine this.

5. Conclusions

Our results suggest that, among STEC cases, direct exposure to farm animals and livestock was a risk factor for HUS. Only a small portion of this association is mediated by *stx2*, suggesting the existence of other important mechanisms. This relationship between exposure source and severity of clinical outcomes has not been previously documented. Exposure variables, such as farm animal contact and seasonality, should be considered by clinicians when establishing the prognosis for STEC patients. Operators of venues allowing direct contact between farm animals and members of the public should take precautions to prevent transmission of STEC.

6. Potential conflicts of interest

None of the authors have any commercial or other association that might pose a conflict of interest.

Acknowledgements

The authors would like to thank Jamie Yeadon, Timothy Hey, Eric Hawkins, MS, Harold Gil, MSPH, Nicole Stone, MPH, Hailey Vest, MPH, and Peter Krombach, MPH in collaboration with the Indiana State Department of Health for their assistance with laboratory testing, data retrieval and contributions during the development of this project.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author Statement.

Madhura S. Vachon: Conceptualization, Software, Formal Analysis, Data Curation, Writing-Original Draft. **Myda Khalid**: Investigation, Resources, Writing-Review & Editing. **Gillian A.M. Tarr**: Conceptualization, Methodology, Software, Visualization, Writing-Review & Editing. **Craig Hedberg:** Conceptualization, Methodology, Writing-Review & Editing. **Jennifer A. Brown:** Conceptualization, Methodology, Writing-Review & Editing, Supervision.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.onehlt.2020.100175.

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