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Research Note

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Research Note

Dispersal and risk factors for airborne *E. coli* in the proximity to beef cattle feedlots

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ABSTRACT

California Leafy Green Products Handler Marketing Agreement (LGMA) established food safety metrics with guidance recommendations of 366 m (1200 feet) and 1609 m (1-mile) distances between production fields of leafy greens and a concentrated animal feeding operation (CAFO) containing >1000 and >80,000 head of cattle, respectively. This study evaluated the effect of these distance metrics and environmental factors on the occurrence of airborne *E. coli* in proximity to seven commercial beef cattle feedlots located in Imperial Valley, California. A total of 168 air samples were collected from seven beef cattle feedlots during March and April, 2020, which were the month's implicated in the 2018 Yuma Arizona lettuce outbreak of *E. coli* O157:H7. The distance between air sampling sites and the edge of the feedlot ranged from ~0 to ~ 2200 m (~1.3 mile), with each sample comprised of 1000 liters of processed air taken at a 1.2 m elevation over a 10-minute duration. *E. coli* colonies were enumerated on CHROMagar ECC selective agar and confirmed with conventional PCR. Meteorological data (air temperature, wind speed, wind direction, relative humidity) was collected *in situ*. The prevalence and mean concentration of *E. coli* was 6.55% (11/168) and 0.09 CFU per 1000 L of air, with positive samples limited to within 37 m (120 ft) of the feedlot. Based on logistic regression, the odds of airborne *E. coli* detection were associated with little to no wind and close proximity to a feedlot. This pilot study found limited dispersal of airborne *E. coli* in proximity to commercial feedlots in Imperial Valley, with light to no wind and proximity within 37 m of a feedlot significant factors associated airborne *E. coli* in this produce growing region of California.

HIGHLIGHTS

- *E. coli* was only detected in air samples within 37 m (120ft) from the feedlot.
- There is a negative association between wind speed and the odds detecting *E. coli*.

From 2016 to 2022, there have been one or more outbreaks of foodborne *E. coli* O157:H7 associated with leafy greens per year in the United States (Center for Disease Control and Prevention, 2022). For example, during the 2018 Yuma outbreak there were 210 reported cases, with 96 hospitalizations and 27 cases of hemolytic uremic syndrome (HUS) (Center for Disease Control and Prevention, 2018). The biological or environmental source of the *E. coli* O157:H7 contamination was not determined or only speculated for most of these foodborne outbreaks associated with consumption of leafy greens (U.S. Food & Drug Administration, 2020). For example, regarding the *E. coli* O157:H7 outbreaks linked to contamination of romaine lettuce in 2019, FDA speculated that the biological source of bacterial contamination might have been nearby cattle, but definitive confirmation was lacking as to the true source of in-field contamination of these leafy greens (U.S. Food & Drug Administration, 2020). In an effort to implement good agricultural practices that minimize the risk of leafy green contamination from microbial pathogens such as *E. coli* O157:H7, the California Leafy Greens Products Handler Marketing Agreement (LGMA) recommends minimal distances of 1200 ft from concentrated animal feeding operations (CAFOs) with >1000 cattle and 1 mile from CAFOs with >80,000 cattle (California Leafy Green Products Handler Marketing Agreement, 2019). The LGMA recognizes that these numbers are interim recommendations and are subject to change as new science becomes available, which was a motivation for conducting this pilot study.

Prior research has been conducted on the relationship between distances from a CAFO (mainly beef cattle feedlots) and the occurrence of microorganisms (e.g., *E. coli* O157) in matrices such as air, soil, or leafy green samples. With respect to air samples, prior research has generally processed 100 to 1000 liters (L) of air per sample and used culture-based methods to detect the presence/absence of indicator bacteria or to enumerate microorganisms/L (Berry et al., 2015; Glaize et al., 2021; Riccardi et al., 2021; Sanz et al., 2015; Wilson et al., 2002a). Glaize et al (Glaize et al., 2021) detected airborne *E. coli* in 6.7% (8/119) of air samples in proximity to a CAFO. These studies (Berry et al., 2015; Glaize et al., 2021; Riccardi et al., 2021; Sanz et al., 2015) characterized the effect of specific distances between a CAFO (e.g., dairy farm or cattle feedlot) and the occurrence of airborne *E. coli*, with an observed mean concentration of *E. coli*/1000L of air being 68 CFU at 0 m, 4 CFU at 60 m, 2 CFU at 120 m, and 1 CFU at 180 m from the CAFO (Berry et al., 2015).

Building on this prior research, our objective for this pilot study was to characterize the concentration of airborne *E. coli* at varying distances from multiple commercial feedlots located in the Imperial Valley during March and April, which were the months associated with the 2018 Yuma leafy green outbreak of *E. coli* O157:H7 (Center for Disease Control and Prevention, 2018). In addition, meteorological data was collected in order to characterize the association between air temperature, wind speed, wind direction and relative humidity and the occurrence of airborne *E. coli* in proximity to commercial feedlots in order to identify environmental conditions at higher risk of airborne *E. coli*.

MATERIAL AND METHODS

Sites selection. We conducted in-person meetings in 2019 with the feeder cattle industry in Imperial Valley, California, to solicit voluntary and confidential participation in this study. Seven beef cattle feedlots from different locations were recruited for this study. Due to confidentiality, the number of head of cattle for each feedlot was not provided by the owner, and instead we estimated the physical area of each feedlot using global positioning system (GPS) coordinates of each feedlot's visible boundary. The area surrounding the seven feedlots was generally arid agricultural land characterized as blocks of fallow fields, cultivated row crops, or flood-irrigated alfalfa, with sporadic residential housing or commercial buildings located at varying distances from the feedlots. Feedlots were generally a dry-lot design, bunk feeding, pen floors comprised of dry packed manure, limited shade, with 50 to 150 head of cattle per pen. Cattle are typically Holstein or Holstein x beef crossbred cattle fed high-energy rations for 300-360 days based on the management system. Manure was scraped and removed from the pens 1-2 times per year, typically as cattle are moved out of the pens and off the feedlot or during periods of high mud buildup. All seven feedlots were visited in March and April, 2020.

During each sampling event, the location for 12 different sampling sites at each feedlot were determined based on the predicted next-day prevailing wind direction using the cell phone application, Weather Mate 6.4.2. Three of the 12 sites were located upwind of the feedlot to function as controls (i.e., air prior to passing over or through the feedlot). The remaining nine sampling sites were divided into three groups, with each group at a different distance downwind of the feedlot edge: group A was located several feet downwind from the edge of the closest feedlot pen, group B located about 366 m (1200 ft) downwind from the feedlot, and group C located about 1609 m (1 mile) downwind from the feedlot. The 12 sites (3 upwind controls, 9 downwind exposures) formed an arc shape to accommodate potential shifts in wind direction, as shown in Figure 1. Due to unforeseen accessibility issues or limited access to adjoining private property, the exact distance of some sampling sites was adjusted to the nearest accessible sampling location which either increased or decreased the intended distance from the feedlot edge.

Sample collection. A total of 168 air samples were collected from seven feedlots during March and April, 2020 (7 feedlots/month × 12 1000L air samples/feedlot × 2 months = 168 1000L air samples). Sampling time occurred between ~8:30 a.m. to ~4:30 p.m. Air samples were collected using MAS-100 Eco microbial air samplers (Merck KGaA, Darmstadt, Germany), which had been factory calibrated prior to the onset of the study. For each air sample, 1000 L of air were processed at a flow rate of 100 L/min over 10 minutes, with the air sampler attached to a portable tripod at a height of 1.2 m above the ground level. Samplers were disinfected using 70% ethanol before and after air sampling each site. In parallel, *in situ* meteorological data (air temperature, wind speed, wind direction, relative humidity) were collected at 30-second intervals using a tripod-mounted Kestrel 5500, whose internal compass was calibrated daily. Bacteria in the air were impinged onto plates of CHROMagar ECC (CHROMagar Microbiology, Pairs, France), which were then incubated at 37 °C for 24 hours to allow for enumeration of colonies of *E. coli*. All isolates were confirmed as generic *E. coli* based on conventional PCR, using the forward primer (5' CCG ATA CGC TGC CAA TCA GT 3') and reverse primer (5' ACG CAG ACC GTA GGC CAG AT 3') targeting the *uspA* gene, with positive *E. coli* and negative controls (Anastasi et al., 2010; Chen & Griffiths, 1998).

Two categorization schemes for the position of the air sampler relative to the feedlot and wind direction were utilized. For scheme A, with the prevailing wind direction set at 0°, *downwind* from a feedlot was a 180° partition or 270° to 90°, and *upwind* from a feedlot was a 180° partition or 90° to 270° (Figure 2A). For scheme B, with the prevailing wind direction set at 0°, *downwind* from a feedlot was a 90° partition or 135° to 225° when windspeed was ≥ 1.8 m/s (Figure 2B); *other* category included 90° partitions for upwind (315° to 45°) and both lateral side winds (45° to 135° and 225° to 315°) when wind speed was ≥ 1.8 m/s; *light wind* was when wind speed was <1.8 m/s regardless of wind direction. The cutoff for designating wind as light (<4 mph or <1.8ms) was based on a definition used by the U.S. National Weather Service (www.weather.gov/mediaas/pqr/wind/wind.pdf). Rainfall data was obtained from CIMIS (https://cimis.water.ca.gov) for the seven days preceding the sampling date.

Statistical analysis. Linear distances between each sampling site and the nearest edge of a feedlot cattle pen were calculated based on GPS coordinates using SAS University software. SAS University and R Studio were used to compile, summarize the data, and generate descriptive statistics for the percentage of positive samples of airborne *E. coli* at different distances between air sampling sites and the edge of the feedlot, and different positions of sampling sites relative to feedlot and wind direction (e.g., upwind, downwind of feedlot). Logistic regression was used to characterize the association between feedlot distance, environmental parameters, meteorological factors, and the occurrence of airborne *E. coli*/1000L of air, using a forward stepping algorithm for inclusion of variables in the order of their significance from a univariate analysis. A p-value ≤0.05, based on a likelihood ratio statistic, was used for retention in the final model. Two-way interactions were also examined for significance during the forward-stepping model building process.

RESULTS

Detection of indicators. The metric for distances between air samplers and the edge of the feedlot are based on the unit foot since California LGMA uses this distance metric for their guidelines (California Leafy Green Products Handler Marketing Agreement, 2019). For the distance partitions in Table 1, we used 120, 420 ft, etc. as the cut-points instead of 100-foot partitions due to the inability of obtaining precise 100-ft distance measurements in the field due to limited physical access, private property borders, and other limitations; in other words, a 20-foot buffer was added to capture distances up to and slightly exceeding 100, 400-ft, etc. distances.

Indicator *E. coli* was detected and confirmed in 6.55% (11/168) of all air samples, with an overall mean and range of concentrations of 0.09 CFU/1000L and 0 to 2 CFU/1000L air, respectively. Using scheme B in Figure 2 to classify the location of the air sampler relative to the feedlot and wind direction, the mean concentration of airborne *E. coli* for *downwind* samples was 0.07, *light-wind* samples was 0.25, and *other* (upwind or side-wind controls) was 0.06 CFU/1000L. The prevalence of airborne *E. coli* for these same three locations was 5.71% for *downwind*, 20.00% for *light-wind*, and 3.85% for *other* (Table 1). The prevalence of airborne *E. coli* was 9.52% (8/84) in March and 3.57% (3/84) in April. Lastly, detection of *E. coli* was limited to within 120 feet (37 m) of the feedlot; distances beyond 120 feet (37 m) all tested negative for *E. coli* for a 1000L volume of ambient air.

Assessment of meteorological and environmental risk factors. The air temperature, wind speed, and relative humidity in Imperial Valley, CA, during the sampling months of March and April, 2020, ranged from 17.0 to 32.0 °C (mean: 23.0 °C), 0.6 to 7.5 m/s (mean: 3.3 m/s), and 16% to 65% (mean: 37.2%), respectively. There was no rainfall during the sampling days in March and

April. However, in the seven days prior to sampling, the total cumulative precipitation was 2.4 mm and 18.6 mm for the March and April sampling event, respectively. Based on univariate logistic regression analyses, there were no significant associations between the odds of airborne *E. coli* detection and relative humidity (p=0.92), air temperature (p=0.97), position of air sampler relative to feedlot and wind direction (180° partition) (p = 0.95), month when sampling occurred (p = 0.13), feedlot area (p=0.51), and hour the sample was taken (1-24 hr) (p = 0.71). In contrast, the odds of airborne *E. coli* detection were significantly associated with little to no wind conditions (<4 mph or <1.8 m/s) (p=0.02 and 0.03) and close proximity to a feedlot (p=0.01) (Table 2). Specifically, the odds of detecting airborne *E. coli* decreased 0.52-times ($e^{-0.65\times1} = 0.52$) for each additional meter of wind speed occurring during air sampling, indicating that non-windy conditions were at higher risk for airborne *E. coli*. Similarly, the odds of detecting airborne *E. coli* during light to no wind (<1.8 m/s) conditions were almost 6.0-times higher ($e^{1.77} = 5.88$) compared to air samples taken during windy conditions located either downwind, upwind or side-wind of the feedlot (Table 2). Lastly, for each additional 100 feet of distance between the feedlot edge and the air sampler, the odds of detecting airborne *E. coli* decreased 0.05-times ($e^{-0.03\times100} = 0.05$). In terms of developing a multivariate logistic regression model, if distance from feedlot was included in the model, then no other variables or interactions were significantly associated with detecting airborne *E. coli*. Considering this, only the variable for distance from the feedlot was included in the final logistic regression model.

DISCUSSION

Key findings from this pilot study conducted in the arid region of Imperial Valley, CA, include an updated estimate of 6.55% (11/168) for the apparent prevalence of *E. coli* in 1000L of ambient air in proximity to beef cattle CAFOs. In addition, the mean and range of *E. coli* concentration was 0.09 CFU/1000L and 0 to 2 CFU/1000L air, respectively. All air samples that were positive for *E. coli* were within 120 feet (37 m) distance from a beef cattle feedlot. Prior research on airborne *E. coli* in proximity to cattle or poultry CAFOs have generally found similar results of low bacterial CFU per 100L or 1000L of air and/or low prevalence for detectable *E. coli* (≥1 CFU) in similar air volumes. For example, Glaize et al. (2021) observed a similar prevalence of detectable *E. coli* of 6.7% (8/119) at 10, 61, and 122 m distance from dairy and poultry farms (Glaize et al., 2021), while Berry et al. (2015) documented a range of concentration of *E. coli* from 0 to 68 CFU/1000L at distances of up to 180 m (Berry et al., 2015). The higher *E. coli* levels in this latter study conducted in Nebraska could be due to a variety of local factors that differ between our study conducted in the arid desert conditions of Imperial Valley, California, and that of Nebraska, such as their closer proximity to the feedlot pens (180 m maximum) compared to our maximum distance of a 2000 m, different densities of cattle per pen, month when sampling occurred, variable winds, and/or different hours of the day when air samples were taken (Berry et al., 2015). In our study, air samples were collected between 8:30 a.m. to 4:30 p.m. which may not be the most active time of cattle according to previous research (Berry et al., 2015; Wilson et al., 2002b; Zhao et al., 2014). Periods of high activity for cattle are often in the morning (5:30-8:00 a.m.) and evening (5:00-11:00 p.m.) when air temperature is cooler (Berry et al., 2015; Wilson et al., 2002b), which could elevate airborne concentrations of *E. coli*.

A strong negative association was found between *E. coli* and wind speed, as demonstrated by (1) the higher prevalence of airborne *E. coli* during light-to-no wind conditions shown in Table 1, (2) the negative coefficient (β = -0.65) for wind speed (m/s) characterizing the reduction in ln(odds) of detecting *E. coli* per additional meter of wind velocity shown in Table 2, and (3) the positive coefficient (β = 1.77) for the light-to-no wind category shown in Table 2. This key finding is somewhat counterintuitive given that high wind velocity can function to suspend CAFO surface aggregates and particulate matter that can carry attached fecal *E. coli* into the air (Zhong et al., 2019). In contravention to this process, higher wind velocity can also decrease the concentration of suspended fecal bacteria in the air by atmospheric dilution (Sabariego et al., 2000). Previous studies have found a variety conclusions regarding meteorological and environmental risk factors for airborne bacteria: higher concentration of bacteria downwind compared to upwind of a CAFO (Berry et al., 2015; Dungan et al., 2010), positive association between the concentration of *E. coli* and air temperature and wind speed (Berry et al., 2015; Sanz et al., 2015), a negative association between the concentration of airborne bacteria and relative humidity (Dungan et al., 2011), and no significant association between concentration of heterotrophic bacteria and ambient weather conditions (Dungan et al., 2010). Other studies detected 0% *E. coli* from the air around CAFOs or crop farmland, making it impossible to analyze the association between *E. coli* and meteorological risk factors (Atwill et al., 2015; Glaize et al., 2021).

The interaction of dynamic wind with CAFO manured surfaces under a variety of local management practices that vary depending on time of day and prevailing environmental conditions likely combine to create a complex system that makes it difficult to generate consistent predictions regarding airborne *E. coli* around CAFOs. In addition, *E. coli* is often ubiquitous in an agricultural environment and its source not limited to CAFOs, which then complicates any effort to document the impact of proximity to CAFOs on the occurrence of airborne bacteria such as *E. coli*. This pilot study, in combination with prior research, suggest that airborne *E. coli* is generally low in concentration downwind of a CAFO, but we recommend that larger volumes of air per sampling event to be used in future research in order to generate a more robust measure of airborne bacterial concentrations under the influence of CAFOs, and to focus on conditions that either generate or mitigate fugitive dust (i.e., scraping pens, feeding cattle, before and after dust abatement procedures) to better understand the acute conditions that can create or mitigate fluxes of airborne bacteria from anthropogenic surfaces such as CAFOs. The cattle can be most active during early morning (5:30-8:00 a.m.) and evening (5:00-11:00 p.m.). Sampling during these active times, or during conditions when cattle are physically active or agitated would be helpful to obtain a more complete understanding of the influence of cattle and other activity on airborne bacteria loads, and in addition to measure the potentially beneficial effect of dust mitigation practices (e.g, watering roads) on reducing airborne bacterial loads in proximity to CAFOs

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TABLE 1. *Occurrence of airborne E. coli in 1000 L air taken at 1.2 m elevation above ground level, stratified by position of air sampler relative to feedlot and wind direction from commercial feedlots in Imperial Valley, California, Spring (March & April), 2020.*

Distance from the edge of feedlot	*E. coli*					
	All samples	Position of air sampler relative to feedlot and wind direction (90° partitions)			Position of air sampler relative to feedlot and wind direction (180° partitions)	
		Downwind	Light wind	Other	Downwind	Upwind
	Positive % (Positive number/N)					
0-50 ft	37.50% (9/24)	80.00% (4/5)	50.00% (3/6)	15.38% (2/13)	54.55% (6/11)	23.08% (3/13)
51-120 ft	9.52% (2/21)	0 (0/5)	33.33% (1/3)	7.69% (1/13)	15.38% (2/13)	0 (0/8)
121-420 ft	0 (0/22)	0 (0/6)	0 (0/2)	0 (0/14)	0 (0/9)	0 (0/13)
500-820 ft	0 (0/7)	0 (0/3)	0 (0/1)	0 (0/3)	0 (0/3)	0 (0/4)
821-1220 ft	0 (0/12)	0 (0/6)	0 (0/1)	0 (0/5)	0 (0/10)	0 (0/2)

1221-2020 ft	0 (0/33)	0 (0/12)	0 (0/5)	0 (0/13)	0 (0/25)	0 (0/5)
2021-6500 ft	0 (0/49)	0 (0/33)	0 (0/2)	0 (0/17)	0 (0/47)	0 (0/5)
Total	6.55% (11/168)	5.71% (4/70)	20.00% (4/20)	3.85% (3/78)	6.78% (8/118)	6.00% (3/50)

* Means there were no air samples located between 421-499 ft. The range of distance from the edge of feedlot started from 500 ft instead of 421 ft. Light wind is wind speed < 1.8 m/s; other category includes 90° partitions for upwind and both lateral sidewinds with wind speed ≥ 1.8 m/s.

TABLE 2. *Univariate logistic regression analyses for the association between airborne E. coli and various environmental and meteorological variables in proximity to seven commercial feedlots in Imperial Valley, California, March & April 2020.*

Model variable	**Level of categorical variable**	**Coef.**	***p*-value**	**OR (95% CI)**
Relative humidity (%)	-	0.003	0.92	1.00 (0.93,1.07)
Air temperature (°C)	-	0.004	0.97	1.00 (0.82,1.23)
Wind speed (m/s)	-	-0.65	0.03*	0.52 (0.29,0.95)
Distance between feedlot edge and air sampler (ft)[a]	-	-0.03	0.01*	0.97 (0.94,0.99)
Position of air sampler relative to feedlot and wind direction (180° partitions)	Downwind	0.04	0.95	1.04 (0.28,3.84)
	Upwind[b]	0	-	Referent
Position of air sampler relative to feedlot and wind direction (90° partitions)[c]	Downwind	0.38	0.61	1.46 (0.34,6.19)
	Light wind[c]	1.77	0.02*	5.88 (1.29,26.84)
	Other[b]	0	-	Referent
Month when sampling occurred	March	1.04	0.13	2.84 (0.73,11.11)
	April[b]	0	-	Referent
Hour sample was taken (1-24hr)	-	0.06	0.71	1.06 (0.77,1.47)

Coef. = beta coefficient from logistic regression model, OR = odds ratio, CI = confidence interval,

* Represents $p<0.05$.

[a] Remained variable after performing model selection.

[b] Referent category for calculating the odds ratio (OR).

[c] Light wind is wind speed less than 1.8 m/s; other category includes 90° partitions for upwind and both lateral sidewinds with wind speed ≥ 1.8 m/s.

There is no competing interest.

FIGURE 1

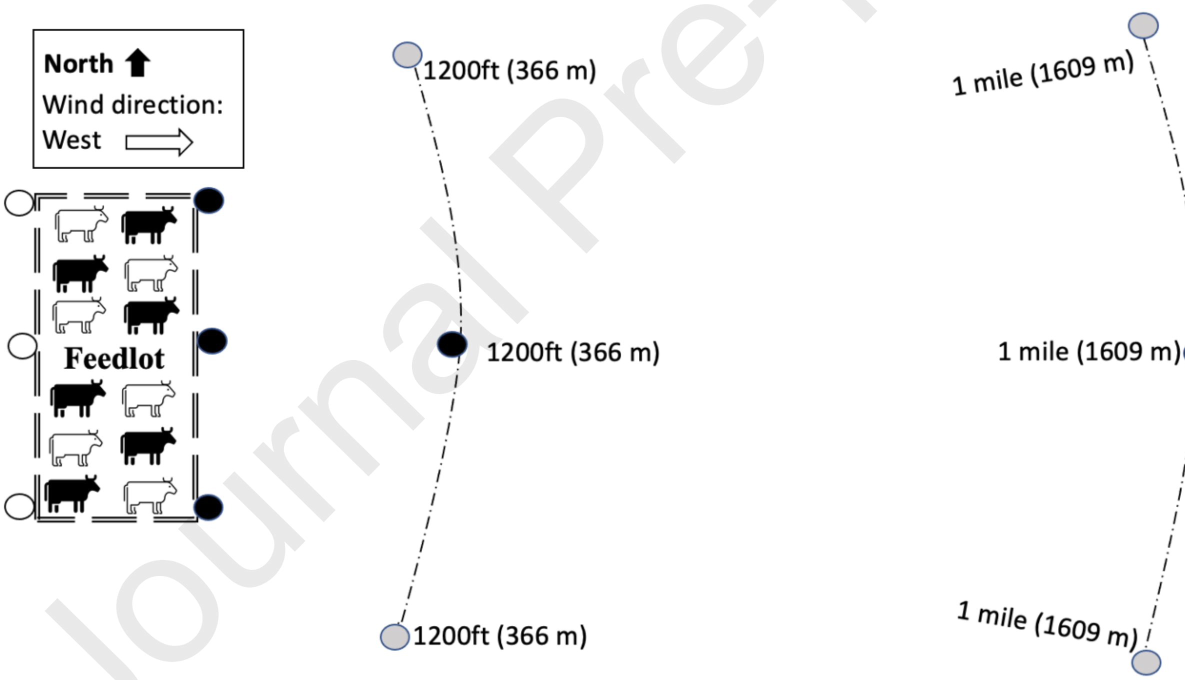

FIGURE 1. *An example distribution of sampling sites with proximity of each feedlot when the wind is from the West in the ideal situation (unlimited access). White circle = upwind sites; black circle = downwind sites; grey circle = sidewind sites.*

FIGURE 2

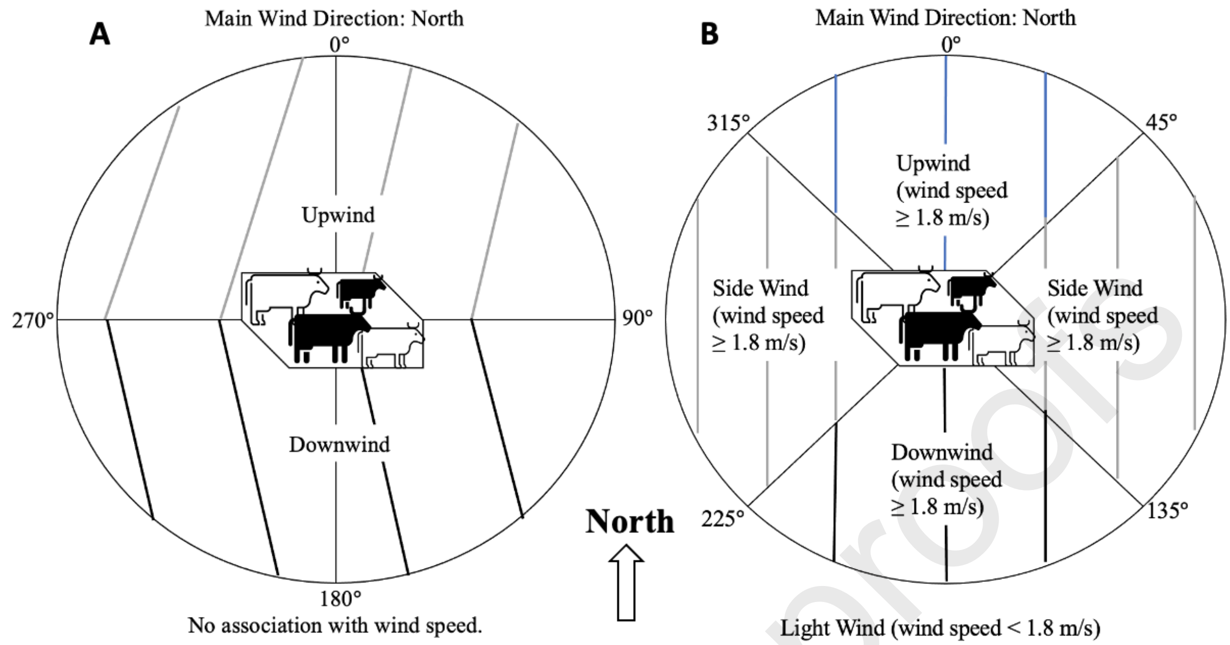

FIGURE 2. *Two different categorization schemes (A and B) for indicating position of the air sampler relative to feedlot location and wind direction, with an example when wind is from the North.*