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Persistence of elevated concentrations of PM, affiliated pharmaceuticals, and tetracycline resistance genes downwind of feedyards☆

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ABSTRACT

Beef cattle feedyards have been identified as sources of large amounts of particulate matter (PM) which may transport affiliated chemicals including steroids, beta agonists, and antibiotics from feedyards into the environment. This study is the first to examine persistence of PM-affiliated pharmaceuticals downwind of feedyards using multiple downwind samples collected at increasing distances from feedyard boundaries (n = 5). Concentrations of antibiotics and ractopamine per gram of PM remained consistent at all downwind locations (out to 4.8 km) whereas concentrations per m^3 air decreased significantly at distances between 0.1 and 0.7 km downwind, corresponding to significant decreases in mass of PM. Monensin was present in the highest concentrations of any measured pharmaceutical, with concentrations of 37 μg/g PM (376 ng/m^3) air in samples collected within 0.1 km downwind of feedyards. Total copy count of tetracycline resistance genes (tetW, tetQ, tetO, tetM, tetL, and tetB) were also significantly increased in samples collected within 0.1 km downwind of feedyards (10^6 copies) as compared to samples collected upwind (10^3 copies) and farther downwind (10^4 copies) of feedyard boundaries. These results suggest that transport of pharmaceutical-laden PM into the terrestrial environment is occurring primarily via PM deposition within 0.7 km of the feedyard, while aerial transport persists over longer distances (>4.8 km).

1. Introduction

Beef cattle feedyards emit large quantities of particulate matter (PM). Airborne PM from feedyards is primarily composed of manure, with smaller contributions from feed particles and road dust (Huang et al., 2013). The manure component of PM results from disruption of pen surface material via cattle movements; factors such as pen material depth and humidity can impact PM formation and aerosolization (Razote et al., 2006). Emission models estimate that up to 127 kg per 1000 head day^{-1} total suspended particulates (reviewed in Bonifacio et al., 2014), are emitted from feedyards daily, but there is little information available on where deposition of PM from feedyards occurs and how far downwind elevated concentrations of PM in air persist. Hiranuma et al. (2011) compared PM concentrations and mean particle sizes at the edge of and 3.5 km downwind of a feedyard. Larger particles settled out of the air at 3.5 km, but fine particles (~PM_2) were present downwind at >99% of their concentration at the feedyard boundary. Increased $PM_{2.5}$ was measured in a west Texas city with a high number of feedyards (>35) nearby as compared to a city in the same region with only one nearby feedyard (Purdy et al., 2010), suggesting that feedyards are important sources of airborne PM for communities in agriculture-intensive regions.

Transport of PM is of interest due to potential health effects of PM itself, and because of the possibility that chemicals excreted in manure or applied in the feedyard setting are also transported offsite via PM. Recent studies have quantified veterinary pharmaceuticals (Blackwell et al., 2015; McEachran et al., 2015; Wooten et al., 2018) in PM emitted from feedyards in west Texas. A variety of veterinary pharmaceuticals are administered to cattle for growth promotion or disease prophylaxis including steroids, beta adrenergic agonists, and antibiotics. Transport of these pharmaceuticals into regions surrounding feedyards may lead to chemical exposures among non-target species. Environmental exposure to estrogenic and androgenic steroids can result in deleterious effects

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on reproduction (i.e. Ankley et al., 2003; Nash et al., 2004), population sex ratios (Li et al., 2015), and behavior (i.e. Dugard et al., 2001). Ractopamine, the only beta agonist currently approved for use in beef cattle production in the United States, has affinity for both β-adrenergic receptors in muscle (β2 receptors) and cardiac tissue (β1 receptors). Following exposure to ractopamine, a range of effects including altered gene expression (Sun et al., 2016) and locomotor behavior (Zhuang et al., 2014), and acute myocardial toxicity (Yaeger et al., 2012) have been observed. Antibiotics in the environment are of concern due to development and dissemination of antibiotic resistance, a phenomenon well documented in situations where chronic, sublethal concentrations of antibiotics occur (reviewed in Andersson and Hughes, 2014).

Quantitation of veterinary pharmaceuticals in feedyard PM has, to date, only been accomplished using PM samples collected within the boundaries of or adjacent to feedyards. Samples collected within or at feedyard boundaries represent worst-case exposure scenarios; understanding the distribution of PM and affiliated pharmaceuticals in areas around feedyards is therefore important for determining the areas with the greatest deposition/exposure potential. The goal of the current study was to examine downwind persistence of PM concentrations and affiliated pharmaceuticals emitted from feedyards. Pharmaceuticals, including antibiotics, ractopamine, and steroids were quantified. Additionally, tetracycline resistance genes were quantified as a potential indicator of environmental dissemination of antibiotic resistance via aerialized PM. Feedyard-emitted PM is predominantly comprised of large particles (~80% larger than PM_{10}, Blackwell et al., 2015), and we hypothesized that there would be a rapid decrease in PM and pharmaceutical concentrations downwind as larger particles settle out of air, and then a more gradual decrease as smaller particles slowly settle out.

2. Materials and methods

2.1. PM sampling

Particulate matter samples were collected around five feedyards in west Texas during the spring and summer of 2015. Feedyards were selected based on vehicular accessibility to roads at multiple distances upwind and downwind of feedyard perimeters based on wind direction at the time of sampling. Sampling was conducted from dirt/caliche roads that typically experienced minimal vehicular traffic (approximately two vehicles encountered per 30 min sampling event). Thus, road dust was not considered to be a significant contributing source to particulate matter samples. Due to the high density of feedyards in the region, it was impossible to select sites without potential influence of other upwind feedyards, though efforts were made to select sites with maximum distance to the next feedyard in combination with vehicle accessibility. Particulate samples collected upwind of feedyards were included to quantify potential contributions from other upwind sources, as well as to further characterize low concentrations of pharmaceuticals that have previously been observed in PM collected immediately upwind of feedyards (McEachran et al., 2015; Wooten et al., 2015). Feedyards included in this study were estimated to have capacities of 20,000–50,000 animals; exact number of head on feed during sampling and pharmaceutical administration (number of cattle administered/dose per animal) was not available for any feedyard.

Samples were collected from 1–2 upwind and 2–3 downwind locations at each feedyard. The predominant wind direction is variable in the region (Table S2), and upwind and downwind locations were determined the day of sampling to accurately account for wind conditions. Total suspended particulates were collected on 4" glass fiber filters (CF-902, Hi-Q Environmental Products, San Diego, CA) for 30 min at each location; generally, samples were collected beginning with the farthest upwind site and ending with the farthest downwind site. References to PM throughout the remainder of the methods and results refer to total suspended particulates. Sample collection was intended to capture downwind samples during the well documented evening dust period (i.e. Purdy et al., 2007), when the confluence of weather conditions and cattle activity produce the largest quantities of PM on feedyards in west Texas. A 30 min sample collection period was employed at each feedyard because it permitted enough time for collection of all samples at a single feedyard in one evening, but was also sufficient for collection of PM with quantifiable concentrations of pharmaceuticals as confirmed in previous studies (McEachran et al., 2015; Wooten et al., 2015). At each upwind or downwind location, 3 PM samples were collected concurrently, and were later designated for steroid, antibiotic, and ractopamine analysis. Between each collection, the filter holding apparatus was cleaned with 70% ethanol. After sampling, filters were stored in protective storage tins on ice for transport to the laboratory where they were weighed to determine mass of PM collected and then stored at −80 °C until analysis. Field blanks, where filters were removed from storage tins, put into the filter holding apparatus to sit while the remaining filters for that site were set up, and then treated in the same manner as PM-laden filters, were collected at random intervals; 2–3 field blanks were included with PM-laden filters in the extractions for each class of pharmaceutical.

2.2. Quantitation of steroids in PM

One filter from each location was analyzed for steroids via the methods of Blackwell et al. (2013). Briefly, filters were shaken with methanol, and supernatant was centrifuged and filtered through 0.2 μm regenerated cellulose filters to remove particulates. Samples underwent solvent exchange to 95:5 hexane:dichloromethane prior to a Florosil (Supelco Supelclean; 1 g, 6 cc) SPE cleanup; samples were eluted in methanol and divided in half prior to evaporation under nitrogen. One sample aliquot was reconstituted in 25 μL of ethanol and stored at −20 °C for possible later usage in *in vitro* assays. The second sample aliquot had internal standards added (d_3-β trenbolone and d_5-β estradiol) prior to evaporation and was reconstituted in 60:40 water:methanol for LC-MS/MS analysis on a Thermo TSQ Quantum (Thermo Fisher Scientific, San Jose, CA). Instrumental methodology was the same as that in Blackwell et al. (2013), with the use of a Gemini-NX C18 column (150 × 2.0 mm; Phenomenex, Torrance, CA) to allow for the separation of the α and β isomers of trenbolone and estradiol, a methanol:water gradient elution, and atmospheric pressure chemical ionization (positive for trenbolone and melengestrol, negative for estrogen). Quality control/quality assurance measures included with all pharmaceutical analyses included extraction of blank and spiked manure or filters with all batches of PM-laden filters (approx. 10 filters per batch) and solvent blanks and check standards within all instrument runs at least every 12 samples.

2.3. Quantitation of antibiotics and tetracycline resistance genes in PM

One eighth of each filter designated for antibiotic analysis was removed for qPCR analysis of 6 tetracycline resistance genes previously quantified in PM emitted from beef cattle feedyards in west Texas (McEachran et al., 2015). These genes were selected to represent multiple mechanisms of tetracycline resistance: ribosomal protection proteins (tetW, tetM, tetO, tetQ) and efflux pumps (tetB and tetL). Quantitation of tet genes was performed at RTLGenomics (Lubbock, TX); details of methodology are included in

Supporting Information.

The remaining 7/8 of each filter underwent analysis to quantify antibiotics previously detected in PM emitted from feedyards in west Texas (McEachran et al., 2015) – monensin, tylosin, tetracycline, oxytetracycline, and chlortetracycline. Antibiotics included in analysis represent multiple classes, and an existing multiclass antibiotic extraction method for manure (Hou et al., 2015) was optimized for use with PM samples and for addition of monensin to target analytes. Filters were extracted via shaking (30 min at 275 rpm) with a series of solvents: 25 mL of McIlvaine's buffer with EDTA (pH ~4); 25 mL of 2:2:1 acetonitrile:methanol:acetone; and 15 mL of 1 mM ammonium hydroxide in water (pH ~10). Between each extraction, samples were centrifuged to remove large particulates, and supernatants were combined. 100 mL of milliQ water was added to the final supernatant mixture prior to SPE cleanup. Oasis HLB cartridges (Waters; 500 mg, 6 cc) were conditioned with methanol and water, samples were passed through under vacuum, and cartridges were washed with water and allowed to dry under vacuum for 30 min. Samples were eluted in methanol, spiked with simetone (internal standard for quantitation), evaporated under nitrogen, and reconstituted in 10:90 methanol:water. Antibiotics were quantified via LC-MS/MS using the instrumental methods of McEachran et al. (2015), which include chromatographic separation with an acetonitrile:water + 0.1% formic acid gradient elution and a Kinetix PFP column (100 × 2.1 mm, Phenomenex), and positive electrospray ionization. Extraction efficiencies were determined, prior to PM sample analysis, in dried manure (the primary component of feedyard PM, Huang et al., 2013; tetracyclines) or in potting soil (monensin and tylosin; no manure without these was available), and were 40 ± 4, 54 ± 1, 65 ± 6, 43 ± 2, and 37 ± 2 for monensin, tylosin, chlortetracycline, oxytetracycline, and tetracycline, respectively (n = 3). Concentrations are reported without correction for extraction efficiency.

2.4. Quantitation of ractopamine in PM

Quantitation of ractopamine was done using the methods of Wooten et al. (2018). Filters were sonicated twice with 0.1 M hydrochloric acid; between each extraction samples were centrifuged to remove large particulates, and supernatants were filtered (0.2 μm nylon syringe filter) and combined. Samples underwent SPE cleanup via HyperSep SCX cartridges (strong cation exchange; ThermoFisher; 500 mg, 3 cc), ractopamine was eluted with methanol + 5% ammonium hydroxide, evaporated under nitrogen, and reconstituted in 20:80 acetonitrile:water for instrumental analysis. The internal standard d_6-ractopamine was added to each sample for quantitation. Ractopamine was quantified via LC-MS/MS using a Gemini C18 column (150 × 2.0 mm; Phenomenex), an acetonitrile:water + 0.1% formic acid gradient, and positive electrospray ionization.

2.5. Data analysis

Reporting limits for each analyte were conservatively set at the highest observed concentration in the field blanks (Table S5). Chemical and qPCR samples below reporting limits were assigned a value of "0" for statistical analyses. As sampling locations were not available at the same distance from all feedyards, sample locations were divided into 2 upwind and 4 downwind distance categories (Table 1). Differences in concentration of PM, antibiotics, ractopamine, and tet genes were determined using ANOVA, followed by a Tukey's HSD post-hoc test when ANOVA results indicated significant differences ($p < 0.05$). All statistical analyses were performed in R Statistical Software (version 3.3.1; R Core Development Team, 2010).

3. Results and discussion

3.1. PM concentrations

High concentrations of total suspended particulates were observed in samples collected <0.1 km downwind of feedyards (Fig. 1). Within 1 km downwind, PM had returned to concentrations observed in upwind samples. PM concentrations upwind and downwind were similar to those previously collected in this region under similar weather conditions (Table S2); Wooten et al. (2015) observed mean upwind concentrations of 180 μg/m^3 and downwind concentrations at sampling locations adjacent to feedyards of 10,000–35,000 μg/m^3.

3.2. Pharmaceutical concentrations

Individual steroids were detected infrequently and at low concentrations (ng/g PM, Table 2). Detection frequency of steroids, especially estrogens, in downwind samples was lower than that reported in previous studies of PM emitted from feedyards in West Texas (39.3% androgens/98.5% estrogens, Blackwell et al., 2015; 95% androgens/100% estrogens, Wooten et al., 2015). Lower detection frequencies are not surprising given that masses of PM collected in samples in the current study, outside of those in D1, were much smaller than those collected in previous studies. When steroids were detected, however, concentrations were similar to those previously reported on either a per g PM (Blackwell et al., 2015) or per m^3 air (Wooten et al., 2015) basis.

All filters collected downwind of feedyards had at least one antibiotic or ractopamine present above reporting limits. Concentrations were significantly higher <0.1 km downwind of feedyards (D1) than at other downwind locations per m^3 air, but not per g PM (Fig. 2, Table S3). The sharp decrease in air concentrations was expected, as it reflects the significant decrease in airborne PM at this same distance. Monensin was the antibiotic present at the highest concentrations, followed by tylosin and chlortetracycline, which was similar to the pattern observed in samples previously collected in the same region (McEachran et al., 2015).

It is expected that the relative abundance of different PM size classes changes at differing distances downwind, with smaller particles persisting farther downwind than larger particles. The similarity of concentrations of antibiotics and ractopamine in PM at all distances suggests that they are present at similar concentrations in all size classes of PM. This has been previously documented for steroids (total suspended particulates vs. PM_{10} vs. $PM_{2.5}$, Blackwell et al., 2015), but has not been investigated for antibiotics or ractopamine. This also suggests that feedyard-emitted PM is the predominant source of total PM collected at downwind sites. The introduction of PM from other sources would be expected to decrease concentrations of pharmaceuticals on a per g PM basis. It is possible that due to the timing of sampling (in the evening to capture the maximal feedyard PM emission), the relatively large amounts of feedyard PM overwhelmed all other sources. Future sampling should include collection during various times of day and weather conditions to determine if dilution with PM from other sources has an impact on observed concentrations of pharmaceuticals.

Monensin, chlortetracycline, and tetracycline were detected in filters collected upwind of feedyards. Monensin was only detected in the closest upwind site (U1), while tetracycline and chlortetracycline were present in far upwind samples from three and one feedyard(s), respectively. Due to the high number of cattle facilities in the region, it is possible that these detections indicate PM from another cattle facility in the sample. The nearest upwind cattle facilities were located between 6 and 50 km of the sampled

Table 1
Definition of abbreviations of sampling location distance groupings used to categorize airborne PM samples collected upwind and downwind of beef cattle feedyards.

Abbreviation	Description	km from feedyard boundary	n
U2	farther upwind	0.9–2.5	5
U1	upwind - closest access	<0.5	4
D1	downwind – collected on road adjacent to feedyard	<0.1	3
D2		0.7–1.0	3
D3	progressively farther downwind	1.6–1.8	3
D4		2.0–4.8	3

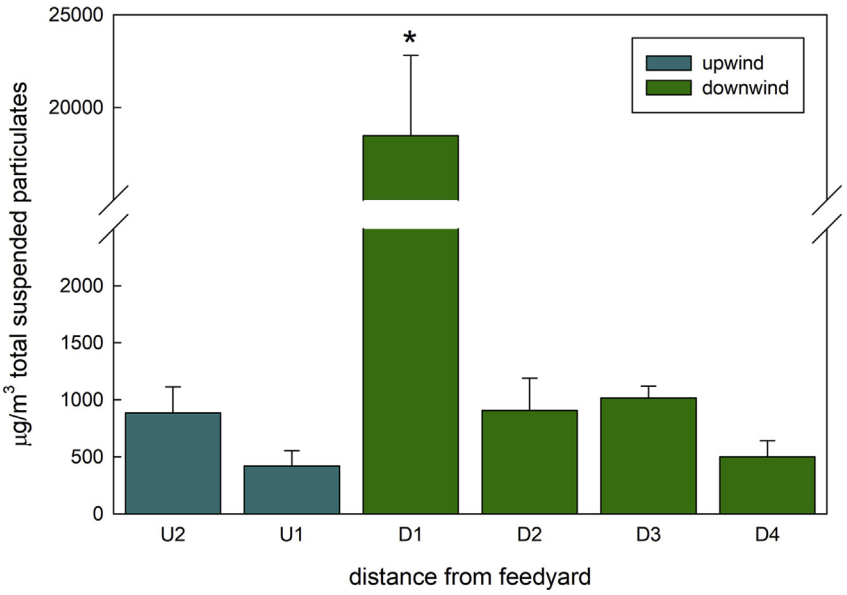

Fig. 1. Concentration of particulate matter in air samples collected upwind and downwind of beef cattle feedyards. n = 15 for U2, 12 for U1, and 9 for all downwind distance groups; the asterisk represents a significant difference in PM concentration as compared to other distances (ANOVA and Tukey's HSD, $p < 0.05$).

Table 2
Quantitation of steroids in PM samples collected at various distances up and downwind of beef cattle feedyards; distance group definitions are explained in Table 1.

Steroid class[a]	Detection frequency by distance group						Concentration range[b]	
	U2	U1	D1	D2	D3	D4	ng/g PM	pg/m^3 air
androgens	2/5	2/4	1/3	1/3	1/3	0/3	2–107	3–68
estrogens	0/5	2/4	1/3	1/3	2/3	0/3	4–51	19–66
progestin	0/5	0/4	0/3	1/3	1/3	0/3	11–31	17–23

[a] Androgens includes α and β trenbolone and trendione, estrogens includes α and β estradiol and estrone, and progestin is melengestrol acetate.
[b] Range in samples above reporting limits.

feedyards, based on wind direction at the time of sampling. If other facilities were the source, it would be expected that additional pharmaceuticals would be present in the sample, not just tetracyclines. Tetracyclines may be coming from sites of land application of feedyard manure; an additional possibility is deposition and reemission, similar to the mechanism recently identified for feedyard-emitted ammonia (McGinn et al., 2016), of tetracyclines in PM from upwind cattle facilities. Pharmaceutical degradation rates vary greatly depending on environmental conditions; tetracyclines, however, are considered more persistent in manure than other classes of antibiotics ($t_{1/2}$ up to 100 days, reviewed in Masse et al., 2014), and may be more likely to be present in quantifiable concentrations in reemitted PM than pharmaceuticals that degrade rapidly.

3.3. Tetracycline resistance genes

Copy counts of tet W, Q, O, and M, as well as the total number of copies of tetracycline resistance genes (Fig. 3; Table S4) were significantly higher in samples from D1, closest to feedyard boundaries, than in PM collected at any other distance. Samples from all distances contained predominantly tetW, tet Q, and tet M (Fig. 4). Both the predominant genes and the fold increase between samples collected upwind and downwind (U1 and D1) are similar to those observed by McEachran et al. (2015). Tetracycline resistance genes are frequently detected in a variety of environmental matrices (i.e. Nesme et al., 2014), so their presence, at relatively low copy numbers, in upwind samples and filter blanks is not surprising. Increases in tet gene prevalence have been observed in response to non-tetracycline stimuli (i.e. metals, Ji et al., 2012), so increases in tet genes downwind may be reflective of multiple contributing factors within the feedyard, not just tetracycline administration.

3.4. Implications for risk assessment

This study was intended as an initial investigation into the persistence of pharmaceutical-laden PM downwind of feedyards. As such, sample sizes limit broad interpretation of the data. Nonetheless this study focussed on feedyards of similar size, within the same region, and during similar weather conditions to assess variability in downwind PM, pharmaceutical and resistance gene concentrations between feedyards while limiting variability due to

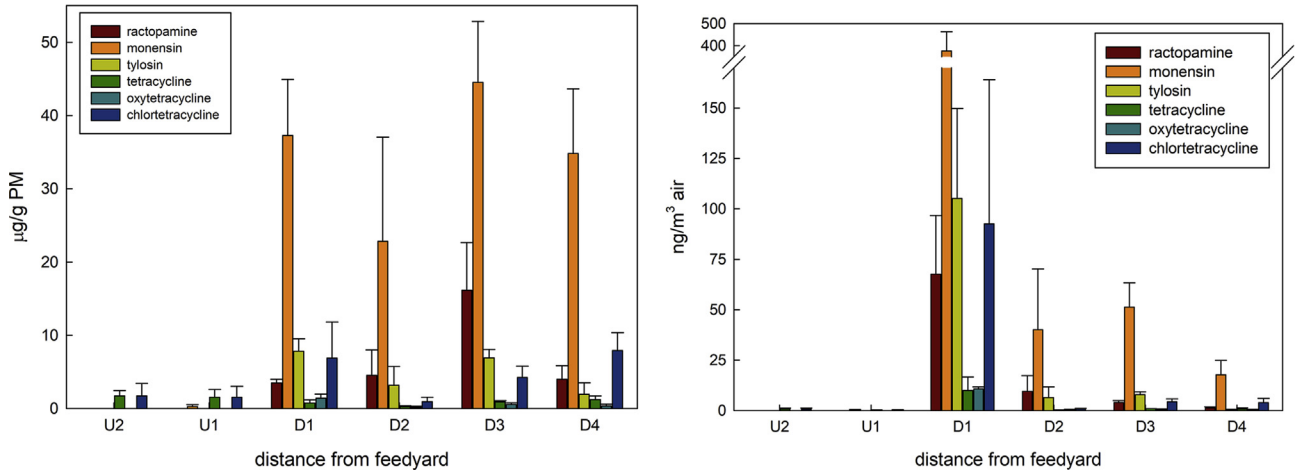

Fig. 2. Concentration (mean ± SE) of five antibiotics and the beta-adrenergic agonist ractopamine in PM collected at varying distances up and downwind of beef cattle feedyards. Concentrations are included on a per unit PM (left) and per unit air (right) basis. Distance abbreviations and n values are defined in Table 1; significant differences are indicated in Table S3.

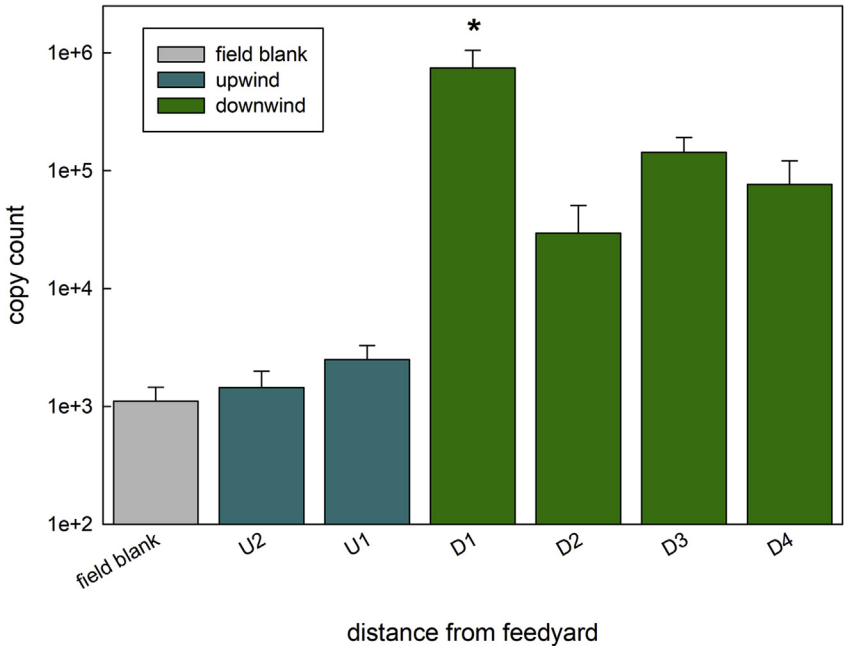

Fig. 3. Copy count (mean ± SE) for the sum of six tetracycline resistance genes (tetW/Q/O/M/L/B) in PM samples collected at various distances from beef cattle feedyards. Distance abbreviations and n for each bar are defined in Table 1. Field blanks were included (n = 3) to account for tet genes coming from blank filters, storage conditions, and collection procedures and not from the PM sample. The asterisk indicates a significant difference from all other groups (ANOVA + Tukey's $p < 0.05$).

ancillary factors such as precipitation, feedyard size, etc. Sample collection occurred during conditions expected to result in high concentrations of emitted PM (Purdy et al., 2007), so results may be interpreted as worst-case scenario. Additional study is warranted to determine the impact of weather conditions, time of day, feedyard size, and other factors on concentrations of emitted PM and pharmaceuticals. Results of this study provide a foundation for future studies which may consider sampling within or adjacent to feedyards, and further downwind.

To determine total area impacted by feedyard emitted PM, it is necessary to trace PM contributions outside of the limits of obvious visual impairment due to increased PM concentration. Rogge et al. (2006) previously identified organic compounds that were distinct to PM emitted from open air feedyards and dairies. These included an increase in C18:C16 fatty acid ratio and the presence of stanols (plant sterols following metabolism in the rumen). Based on their persistence downwind in this study, veterinary pharmaceuticals like monensin and ractopamine may also be useful as organic markers of feedyard PM. Tylosin and the tetracyclines quantified in this study transitioned from being available over the counter to available by veterinary feed directive only on Jan 1, 2017 (FDA, 2017). Without knowing how this change will impact the amount of tylosin and tetracyclines administered on feedyards, it is unclear if they will remain prevalent enough in feedyard-emitted PM to be useful as chemical tracers.

Results from this study, in conjunction with the particle size data from Hiranuma et al. (2011), indirectly provide information on particle deposition around feedyards. Deposition of the majority of

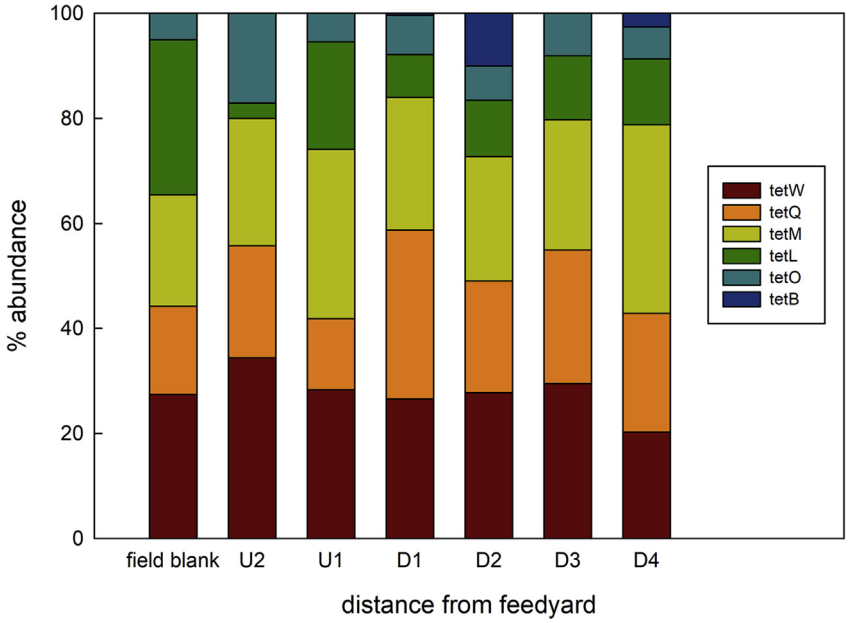

Fig. 4. Relative contributions of individual tetracycline resistance genes to total observed gene copy number in PM collected at varying distances up and downwind of feedyards. Distance categories are explained in Table 1.

the mass of emitted PM, presumably as larger particles, occurs within 1 km of feedyard boundaries. Living receptors outside of this area would have a lesser risk of coming into contact with contaminated water or sediments following PM deposition into playas, or contaminated food sources following PM deposition onto plants and soils. Potential inhalation of pharmaceuticals, however, is not limited to areas close to feedyard boundaries, as pharmaceutical-laden particles persist for at least 4.8 km downwind of feedyards. Recent studies in west Texas have quantified veterinary pharmaceuticals on wildflowers (<1 km downwind of feedyards; Peterson et al., 2017) and in playa water and sediments (<15 km downwind of feedyards; Sandoz et al., 2017), confirming that potential exposure to feedyard pharmaceuticals in this region is not limited to areas within feedyard boundaries. Additional sampling of environmental matrices at varying distances from feedyards is warranted to further elucidate the transport and deposition of PM-affiliated pharmaceuticals.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2018.12.047.

References

Andersson, D.I., Hughes, D., 2014. Microbiological effects of sublethal levels of antibiotics. Nat. Rev. Microbiol. 12, 465–478.
Ankley, G.T., Jensen, K.M., Makynen, E.A., Kahl, M.D., Korte, J.J., Hornung, M.W., Henry, T.R., Denny, J.S., Leino, R.L., Wilson, V.S., Cardon, M.C., Hartig, P.C., Gray, L.E., 2003. Effects of the androgenic growth promoter 17-β-trenbolone on fecundity and reproductive endocrinology of the fathead minnow. Environ. Toxicol. Chem. 22, 1350–1360.
Blackwell, B.R., Buser, M.D., Johnson, B.J., Baker, M., Cobb, G.P., Smith, P.N., 2013. Analysis of veterinary growth promoters in airborne particulate matter by liquid chromatography-tandem mass spectrometry. In: Evaluating Veterinary Pharmaceutical Behavior in the Environment. American Chemical Society, pp. 137–148.
Blackwell, B.R., Wooten, K.J., Buser, M.D., Johnson, B.J., Cobb, G.P., Smith, P.N., 2015. Occurrence and characterization of steroid growth promoters associated with particulate matter originating from beef cattle feedyards. Environ. Sci. Technol. 49, 8796–8803.
Bonifacio, H.F., Maghirang, R.G., Trabue, S.L., McConnell, L.L., Prueger, J.H., Razote, E.B., 2014. Particulate emissions from a beef cattle feedlot using the flux-gradient technique. J. Environ. Qual. 43 (4), 1131–1142.
Dugard, M.L., Tremblay-Leveau, H., Mellier, D., Caston, J., 2001. Prenatal exposure to ethinylestradiol elicits behavioral abnormalities in the rat. Dev. Brain Res. 129, 189–199.
FDA (US Food and Drug Administration), 2017. Drugs transitioning from over-the-counter (OTC) to veterinary feed directive (VFD) status. In: https://www.fda.gov/AnimalVeterinary/DevelopmentApprovalProcess/ucm482107.htm.
Hiranuma, N., Brooks, S.D., Gramann, J., Auvermann, B.W., 2011. High concentrations of coarse particles emitted from a cattle feeding operation. Atmos. Chem. Phys. 11, 8809–8823.
Hou, J., Wan, W., Mao, D., Wang, C., Mu, Q., Qin, S., Luo, Y., 2015. Occurrence and distribution of sulfonamides, tetracyclines, quinolones, macrolides, and nitrofurans in livestock manure and amended soils of Northern China. Environ. Sci. Pollut. Res. 22, 4545–4554.
Huang, Q., McConnell, L.L., Razote, E., Schmidt, W.F., Vinyard, B.T., Torrents, A., Hapeman, C.J., Maghirang, R., Trabue, S.L., Prueger, J., Ro, K.S., 2013. Utilizing single particle Raman microscopy as a non-destructive method to identify sources of PM 10 from cattle feedlot operations. Atmos. Environ. 66, 17–24.
Ji, X., Shen, Q., Liu, F., Ma, J., Xu, G., Wang, Y., Wu, M., 2012. Antibiotic resistance gene abundances associated with antibiotics and heavy metals in animal manures and agricultural soils adjacent to feedlots in Shanghai; China. J. Hazard Mater. 235, 178–185.
Li, Y.Y., Xu, W., Chen, X.R., Lou, Q.Q., Wei, W.J., Qin, Z.F., 2015. Low concentrations of 17β-trenbolone induce female-to-male reversal and mortality in the frog Pelophylax nigromaculatus. Aquat. Toxicol. 158, 230–237.
Massé, D.I., Saady, N.M.C., Gilbert, Y., 2014. Potential of biological processes to eliminate antibiotics in livestock manure: an overview. Animals 4, 146–163.
McEachran, A.D., Blackwell, B.R., Hanson, J.D., Wooten, K.J., Mayer, G.D., Cox, S.B., Smith, P.N., 2015. Antibiotics, bacteria, and antibiotic resistance genes: aerial transport from cattle feed yards via particulate matter. Environ. Health Perspect. 123, 337.
McGinn, S.M., Janzen, H.H., Coates, T.W., Beauchemin, K.A., Flesch, T.K., 2016. Ammonia emission from a beef cattle feedlot and its local dry deposition and re-emission. J. Environ. Qual. 45, 1178–1185.
Nash, J.P., Kime, D.E., Van der Ven, L.T., Wester, P.W., Brion, F., Maack, G., Stahlschmidt-Allner, P., Tyler, C.R., 2004. Long-term exposure to environmental

concentrations of the pharmaceutical ethynylestradiol causes reproductive failure in fish. Environ. Health Perspect. 112, 1725–1733.
Nesme, J., Cécillon, S., Delmont, T.O., Monier, J.M., Vogel, T.M., Simonet, P., 2014. Large-scale metagenomic-based study of antibiotic resistance in the environment. Curr. Biol. 24 (10), 1096–1100.
Peterson, E.M., Wooten, K.J., Subbiah, S., Anderson, T.A., Longing, S., Smith, P.N., 2017. Agrochemical mixtures detected on wildflowers near cattle feed yards. Environ. Sci. Technol. Lett. 4 (6), 216–220.
Purdy, C.W., Clark, R.N., Straus, D.C., 2007. Analysis of aerosolized particulates of feedyards located in the Southern High Plains of Texas. Aerosol Sci. Technol. 41, 497–509.
Purdy, C.W., Rice, W.C., Clark, R.N., Straus, D.C., 2010. Influence of feedyards on bioaerosols of two small towns on the southern high plains. Tex. J. Sci. 62, 83–110.
R Development Core Team, 2010. R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0. http://www.R-project.org.
Razote, E.B., Maghirang, R.G., Predicala, B.Z., Murphy, J.P., Auvermann, B.W., Hamer III, J.P., Hargrove, W.L., 2006. Laboratory evaluation of the dust-emission potential of cattle feedlot surfaces. Trans. ASABE (Am. Soc. Agric. Biol. Eng.) 49, 1117–1124.
Rogge, W.F., Medeiros, P.M., Simoneit, B.R., 2006. Organic marker compounds for surface soil and fugitive dust from open lot dairies and cattle feedlots. Atmos. Environ. 40, 27–49.
Sandoz, M.A., Wooten, K.J., Clendening, S.L., Hensley, L.L., Smith, L.R., Smith, P.N., 2017. Transport mechanisms for veterinary pharmaceuticals from beef cattle feedyards to wetlands: is aerial deposition a contributing source? Agric. Ecosyst. Environ. https://doi.org/10.1016/j.agee.2017.09.016.
Sun, L., Wang, S., Lin, X., Tan, H., Fu, Z., 2016. Early life exposure to ractopamine causes endocrine-disrupting effects in Japanese medaka (Oryzias latipes). Bull. Environ. Contam. Toxicol. 96 (2), 150–155.
Wooten, K.J., Sandoz, M.A., Smith, P.N., 2018. Ractopamine in particulate matter emitted from beef cattle feedyards and playa wetlands in the Central Plains. Environ. Toxicol. Chem. 37, 970–974.
Wooten, K.J., Blackwell, B.R., McEachran, A.D., Mayer, G.D., Smith, P.N., 2015. Airborne particulate matter collected near beef cattle feedyards induces androgenic and estrogenic activity in vitro. Agric. Ecosyst. Environ. 203, 29–35.
Yaeger, M.J., Mullin, K., Ensley, S.M., Ware, W.A., Slavin, R.E., 2012. Myocardial toxicity in a group of greyhounds administered ractopamine. Vet. Pathol. 49, 569–573.
Zhuang, Z., Zhao, Y., Wu, Q., Li, M., Liu, H., Sun, L., Gao, W., Wang, D., 2014. Adverse effects from clenbuterol and ractopamine on nematode Caenorhabditis elegans and the underlying mechanism. PLoS One 9 (1), e85482.