Original Research Article

Ambient Air Pollution-Related Mortality in Dairy Cattle Corroborates Human Findings

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Ambient Air Pollution and Dairy Cattle Mortality

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The authors declare no conflicts of interest.

ABSTRACT

Background: Although epidemiologic studies in animals may provide additional insights, the short-term effects of air pollution on mortality have never been studied in animal populations. We investigated the association between ambient air pollution and the risk of mortality in dairy cows and we assessed potential effect modification by season.

Methods: We collected ozone (O_3), particulate matter (PM_{10}), and nitrogen dioxide (NO_2) concentrations at municipality level for 87,108 dairy cow deaths in Belgium from 2006 to 2009. We combined a case-crossover design with time-varying distributed lag non-linear models.

Results: We found acute and delayed effects of air pollution on dairy cattle mortality during the warm season. The increase in mortality for a 10 $\mu g/m^3$ increase in 2-day (lag 0–1) O_3 was 1.2% (95% confidence interval: 0.3% to 2.1%), and the corresponding estimates for a 10 $\mu g/m^3$ increase in same-day (lag 0) PM_{10} and NO_2 were 1.6% (0.0% to 3.1%) and 9.2% (6.3% to 12.2%), respectively. Compared to the acute effects, the cumulative 26-day (lag 0–25) estimates were considerably larger for O_3 (3.0%; 0.2% to 6.0%) and PM_{10} (3.2%; -0.6% to 7.2%), but not for NO_2 (1.4%; -4.9% to 8.2%). In the cold season, we only observed increased mortality risks associated with same-day (lag 0) exposure to NO_2 (1.4%; -0.1% to 3.1%) and with 26-day (lag 0–25) exposure to O_3 (4.6%; 2.2% to 7.0%).

Conclusions: Our study adds to the epidemiologic findings in humans and reinforces the evidence on the plausibility of causal effects. Furthermore, our results indicate that air pollution effects go beyond short-term mortality displacement.

Introduction

Although experimental studies on laboratory animals have been widely used to study mechanisms of air pollution-related health effects,[1-3] only few studies have investigated the effects of air pollution on animal health in an epidemiological context. Pet animals have been used to study cancer, lung disease, and brain abnormalities in relation to urban air pollution,[4,5] or indoor exposures.[4,6] Other studies have investigated effects of toxic gases, dusts and endotoxins inside farm facilities on livestock health.[7] We are not aware of any study on the association between ambient air pollution and animal mortality, except for some reports of pet and farm animal deaths during historical air pollution episodes.[7-10] In the 1870s, death of cattle during a livestock show in England was associated with a dense industrial fog.[8] Also in Belgium cattle died in the fog of 1911,[9] and during the Meuse valley air pollution episode in 1930.[10]

Even at current concentration levels, the relation between air pollution and excess morbidity and mortality remains, as shown by numerous epidemiologic studies on human data.[11] However, still some debate exists about the lag period associated with these exposures, which may include both harvesting and delayed effects, contributing differently to the net cumulative effects. The harvesting hypothesis states that short-term increases in air pollution simply shorten the life span of frail individuals, implying a short-term positive correlation between exposure and daily deaths, followed by a deficit in mortality at longer lags.

Studies on the association between air pollution and mortality in animal populations can corroborate or inform epidemiologic studies in humans. The advantages of using animals as comparative models of human disease accrue in part from their relative freedom from concurrent exposures, bias due to confounding, and, to some extent, exposure misclassification.[4] Dairy cows have a relatively long life span, limited population variability

in lifestyle and dietary habits, limited geographical mobility, and (partial) outdoor housing. Moreover, in many countries farm animals are subject to a stringent mandatory registration procedure from birth till death, which is at the individual level for ruminants.

We investigated whether the short-term association between air pollution and mortality in humans can be corroborated in an animal population in which confounding and exposure misclassification are limited, to further elaborate on the causality of this association. We used distributed lag non-linear models (DLNM)[12] to investigate the effect of ozone (O_3), particulate matter with aerodynamic diameter less than 10 μm (PM_{10}), and nitrogen dioxide (NO_2) on mortality over a 25-day period among dairy cows in Belgium. A DLNM has the advantage of providing cumulative effects of air pollution by flexibly estimating contributions at different lag times, thus accounting for delayed effects and short-term harvesting. In addition, we assessed potential effect modification by season by using time-varying DLNM models.[13]

Methods

Data

Data on cattle mortality were extracted from Sanitrace (Sanitel), a national-level computerized database for the registration and traceability of farm animals, managed by the Federal Agency for the Safety of the Food Chain.[14] For all adult dairy cows (≥ 2 years) that died (different from culling in slaughterhouse) in Belgium during the period 2006-2009, we obtained information on date of birth and death, farm identification and postal code. The majority of dairy cows in Belgium are of the Holstein Friesian breed.

Daily maximum 8-hour average O_3 concentrations and daily average PM_{10} and NO_2 concentrations were obtained from the Belgian Interregional Environment Agency. For its assessment, a dense network of automatic monitoring sites, as implemented by the three

Belgian regions, collected real-time data on a half-hourly basis. The average distance between the nearest measuring stations is about 25 km. Data from monitoring stations are combined with land cover data obtained from satellite images in a spatial-temporal (Kriging) interpolation model, described by Janssen et al.[15] This provides estimates for O_3, PM_{10}, and NO_2 on a 4 x 4 km^2 grid, which are then used to calculate area-weighted average concentrations per municipality (average size of 43.9 km^2). Air pollution levels are linked to cattle mortality data through the postal code of the farm. For O_3, the interpolation model explained more than 90% of the temporal variability (R^2) and 60% of the spatial variability in Belgium.[16] For PM_{10} the temporal variability explained was more than 70%, and the spatial variability explained was 50%. The corresponding numbers for NO_2 were more than 75% and more than 80% respectively. Previous studies suggest that PM_{10} estimates correlate well with individual exposure, as assessed by carbon load in human macrophages.[17]

Because climate is a known confounder of the association between air pollution and health,[18,19] data on mean air temperature and average relative humidity were provided by the Belgian Royal Meteorological Institute (KMI). We used data from one central and representative station in Uccle (Brussels), because Belgium is very uniform for temperature, as a result of extremely small altitudinal and latitudinal gradients: elevations range from 0 to 694 m above sea level, and the distance between the northernmost and southernmost part is only 224 km.

Statistical Analysis

The case-crossover design is widely used for analyzing short-term exposures with acute outcomes.[20] It is a variant of the matched case-control study, where each subject serves as its own control so that known and unknown time-invariant confounders are inherently adjusted for by study design.[21] This design samples only cases (deaths in this study) and compares

each subject's exposure in a time period just before a case event (the hazard period) with that subject's exposure at other times (the control periods). Selection bias was avoided by applying a bidirectional time-stratified design.[22] Control days are taken from the same calendar month and year as the case day (*i.e.* day of death), both before and after the case, thus controlling for long-term trends and season by design. Cases and controls were additionally matched by day of the week to control for any weekly patterns in deaths or pollution. A case on 1 September, for example, has four control days (8, 15, 22, and 29 September), whereas a case on 21 September has three control days (7, 14, and 28 September).

To account for potential harvesting and delayed effects of air pollution on dairy cow mortality, we combined the case-crossover design with distributed lag non-linear models (DLNM), using a separate model for each of the three air pollutants. This study applies recent extensions of the DLNM methodology beyond aggregated time series data,[23] specifically implementing them in a conditional logistic regression model with individual-level exposure measures. The DLNM is defined through a "cross-basis" function, which allows the simultaneous estimation of a non-linear exposure-response association and non-linear effects across lags, the latter termed lag-response association. The maximum lag was set to 25 days, meaning that the hazard period contains up to 24 days before the case day and each of the control periods contains up to 24 days before the control day. We assumed a linear air pollutant–mortality association and the lag structure was modelled with a natural cubic splines with 6 degrees of freedom (df). The knots in the lag space were set at equally spaced values in the log scale of lags to allow more flexible lag effects at shorter delays.[24]

We also included a cross-basis for mean temperature in the model to capture the (potentially delayed) effects of heat and cold on mortality. The maximum lag was set to 25 days. We used a natural cubic spline with 5 df for the temperature–mortality function and a natural cubic spline with 6 df (with knots at equally spaced values in the log scale) for the lag structure.

Spline knots for temperature were placed at equally spaced values of the actual temperature range to allow enough flexibility in the two ends of the temperature distribution. Models were additionally adjusted for the moving average of humidity on the current day and the two previous days (lag 0–2), using a natural cubic spline with 3 df.

Potential seasonal heterogeneity in air pollution effects was investigated because: 1) human studies have reported larger effect estimates in summer than in winter,[18,19] and 2) free-ranging cows are, apart from the daily milking moments, the majority of their time on pasture from March-May until October, whereas they are continuously in the stable during the other months of the year. Seasonal effect modification was addressed with time-varying DLNM models, expressed through an interaction between the cross-basis for the air pollutant and an indicator variable for season.[13] Season was defined as the warm (April-September) and the cold (October-March) period of the year. Seasonal variation was formally tested by comparing models with and without the interaction term (likelihood ratio test on 6 df).

In sensitivity analyses we used an unconstrained distributed lag model to define the lag structure, that is, a model in which each lag is entered as a separate variable.[25,26] Because of the correlation between air pollution concentrations on days close together, the unconstrained distributed lag model will result in unstable estimates for the individual lags, but it is known as more flexible and less prone to bias for the estimate of the overall effect.[25] We also investigated the potential influence of Bluetongue disease on the observed results. There were two outbreaks of Bluetongue Virus Serotype 8 in Belgium within the study period: from August to December 2006 and from July to December 2007.[27] Because the spread of Bluetongue in Belgium has been found to be associated with weather conditions[28] and because of the correlation between meteorology and air pollution, we examined whether our results were robust to the exclusion of these epidemics from the analyses by using both constrained (as in the main analyses) and unconstrained distributed lag models. In a last

sensitivity analysis, we checked the robustness of results with respect to the specification of the temperature cross-basis, varying the df for the exposure-response and for the lag-response functions from 3 to 7. Model fit was assessed based on the Akaike Information Criterion (AIC).

We calculated relative risks (RR) of mortality for a 10 $\mu g/m^3$ increase in air pollutant concentrations. Reported estimates, computed as the overall cumulative risk accounting for the 0–25 lag period, are presented as percent change in mortality with corresponding 95% confidence interval (CI). All analyses were performed with the statistical software R (R Foundation for Statistical Computing, Vienna, Austria) using the "dlnm" package.[24]

Results

Data Description

There were 87,108 cow deaths in Belgium from 2006 to 2009. Table 1 shows descriptive statistics for daily mortality, air pollutants and weather variables. In the warm season there were on average 55 cases per day and in the cold season 65 cases. The average concentrations of O_3, PM_{10} and NO_2 in the warm season were 81.2 $\mu g/m^3$, 24.9 $\mu g/m^3$ and 14.6 $\mu g/m^3$ respectively, whereas the corresponding concentrations in the cold season were 48.3 $\mu g/m^3$, 27.3 $\mu g/m^3$ and 21.2 $\mu g/m^3$ respectively. To highlight sufficient variation around a non-zero mean value as suggested in case-crossover studies,[29] table 1 also presents the "relevant exposure term" which is the absolute difference between each pollutant's levels on the case day and its average concentrations over the control days.

Spearman correlation coefficients (r) between air pollutants and meteorological variables are presented in Table 2. Correlations were highest between PM_{10} and NO_2 ($r > 0.7$ in both seasons) and between O_3 and NO_2 (only in the cold season, $r = -0.66$). The correlation

between PM_{10} and O_3 was strongest in the cold season (r = -0.53, compared with r = 0.34 in the warm season). O_3 was positively correlated with temperature in both seasons (r = 0.30), whereas PM_{10} and NO_2 were negatively correlated with temperature in the cold season (r = -0.30 and r = -0.41 respectively).

DLNM Analyses

Results from the time-varying DLNM models indicated seasonal heterogeneity in the association between air pollution and dairy cattle mortality, so the interaction term between season and the cross-basis for the air pollutant (p-value = 0.024 for O_3, p-value = 0.342 for PM_{10}, p-value < 0.001 for NO_2) was kept in the final models.

During the warm season, highest relative risks of mortality were observed on the day of exposure (PM_{10} and NO_2) or the day after (O_3), immediately followed by a 2- to 3-day deficit in mortality (Figures 1A, 1C, and 1E). For O_3 and PM_{10}, the deficit in mortality was followed by a significantly increased mortality risk lasting for one (O_3) to two (PM_{10}) weeks. Different from results for the warm season, the lag-specific curves for O_3 (Figure 1B) and PM_{10} (Figure 1D) in the cold season did not show acute effects (at lag 0 or lag 1), but mortality was significantly increased 4 to 11 days after O_3 exposure (Figure 1B). The lag structure for NO_2 in the cold season was similar to the temporal pattern in the warm season, i.e. an increase in mortality at lag 0 followed by a deficit lasting for few days, but the effect in the warm season was much smaller than the effect in the cold season (Figure 1F).

Cumulative effects of air pollutants on dairy cow mortality are presented in Table 3. The increase in the risk of mortality for a 10 $\mu g/m^3$ increase in air pollutant concentration in the warm season was 1.2% (95% CI = 0.3%, 2.1%) for O_3 (lag 0–1), 1.6% (95% CI = 0.0%, 3.1%) for PM_{10} (lag 0), and 9.2% (95% CI = 6.3%, 12.2%) for NO_2 (lag 0). The overall 26-

day estimates, incorporating the harvesting and delayed effects, were considerably larger than the acute effects for O_3 (3.0%; 95% CI = 0.2%, 6.0%) and PM_{10} (3.2%; 95% CI = -0.6%, 7.2%), but not for NO_2 (1.4%; 95% CI = -4.9%, 8.2%). In the cold season, we only observed increased mortality risks associated with same-day (lag 0) exposure to NO_2 (1.4%; 95% CI = -0.1%, 3.1%) and with 26-day (lag 0–25) exposure to O_3 (4.6%; 95% CI = 2.2%, 7.0%).

Sensitivity analyses gave similar results. The use of unconstrained distributed lag models and especially the exclusion of the Bluetongue epidemics resulted in higher estimates, except for O_3 in the cold season (eTable 1). Overall (26-day) warm season estimates from the unconstrained models without Bluetongue epidemics were 3.3% (95% CI = 0.1%, 6.6%) for O_3, 4.2% (-0.2%, 8.8%) for PM_{10}, and 3.9% (-3.3%, 11.6%) for NO_2 respectively. Corresponding estimates for the cold season were 3.6% (95% CI =0.9%, 6.3%), 3.2% (95% CI = 0.0%, 6.5%), and 0.0% (95% CI = -5.0%, 5.3%) respectively. Effect estimates were quite robust to changes in the df for the exposure-response and lag-response functions of the temperature cross-basis (eTable 2).

Discussion

To the best of our knowledge, this is the first epidemiological study that uses an animal population to investigate short-term variations in mortality in association with recent exposure to air pollution. We found significant increases in the risk of dairy cattle mortality associated with air pollution, both particulate (PM_{10}) and gaseous (O_3 and NO_2), during the warm period of the year. Exposure to NO_2 was associated with a same-day increase in mortality, but this was largely compensated by a subsequent 2-day deficit in mortality. For O_3 and PM_{10}, however, the overall (lag 0–25) effects were substantially larger than the acute effects (up to lag 0–1), indicating that the adverse response to air pollution persists up to weeks after the exposure. In the cold season, we only observed acute effects for NO_2 and delayed effects for O_3. Overall, our study in cattle corroborates findings in humans and provides further evidence that air pollution-related mortality goes beyond short-term harvesting. In broader perspective, a quantification of dairy cattle mortality related to environmental exposure is important for animal welfare and health,[30] as well as for economic reasons.[31]

Despite the recognition that animals could be useful role models for human health risks,[32] the full potential of linking animal and human health data in environmental research has not been realized.[33] Possible reasons for this include the professional segregation of human and animal health communities, the separation of human and animal surveillance data, and evidence gaps in the linkages between human and animal responses to environmental health hazards.[33] Our study shows that epidemiological observations in animal populations can add to findings from human studies. Evidence for causality is strengthened because dairy cattle are expected to be relatively free from confounding factors such as the use of air conditioning, housing construction, occupational exposures, and lifestyle factors.[4] The restricted daily mobility and low frequency of migration in cattle populations contribute to the likelihood that exposure assessment can be conducted relatively accurately. Moreover, the majority of adult dairy cows

are on pasture during summer, making outdoor exposure a good proxy for actual individual exposure, at least in summer. In this study the case-crossover design represents an attractive alternative to Poisson models to investigate acute health effects, because of the possibility to use individual-level information on exposures. The use of DLNM models enabled the investigation of the net effect of air pollution on cattle mortality, accounting for harvesting as well as delayed effects.

Whereas it has been argued that risk assessments using relatively short timescales might have overestimated the public health impact of air pollution because of harvesting, our analysis indicates that such studies might have underestimated the total effects. This was also suggested by some human studies that have examined the temporal pattern of the association using longer follow-up periods.[26,34-37] As in our study, they found extended effects of O_3[37] and particulates,[26,34-36] with larger estimates for the overall cumulative effect than for the acute effects. As pointed out by Zanobetti et al,[26] numerous epidemiologic studies have shown that air pollution is associated with exacerbation of illness, which might result in an increased recruitment into the pool of individuals at risk of dying. This may occur at different lags, depending on the mechanism and individual, and at a slower pace than death out of the risk pool, which might result in delayed increases in mortality persisting for several days or weeks. Extended air pollution effects on mortality are supported by evidence from the historical London smog episode in 1952, which was followed by elevated human mortality rates up to 3 months after the exposure.[38]

Results of our study provide evidence for seasonal variation in the association between ambient air pollution and dairy cattle mortality, indicating that the triggering effect of air pollution is not equally harmful under different weather conditions, even after strong adjustment for immediate and delayed effects of outdoor temperature. This finding is consistent with results from a study on human mortality in the northern part of Belgium

(Flanders), reporting much larger effects of particulates during the warmer period of the year.[19] Stronger associations between air pollution and daily mortality in the warm season have also been found in other human studies.[18,39] We can only speculate about the mechanisms underlying the effect modification by season. It is unlikely that the difference is due to differences in outdoor concentration levels, because concentrations of both PM_{10} and NO_2 were higher and more variable in the cold season (Table 1). However, seasonal differences in air pollution mixture or composition of PM may exist, as suggested by studies reporting a higher inflammatory activity of PM during warm periods.[40,41] Human studies have shown that outdoor exposure contributes more to personal human exposure during warm periods than during cold periods,[42] which is likely to be related to differences in ventilation and time spent outdoors between the two periods.

It is expected that $PM_{2.5}$ (particles with an aerodynamic diameter of ≤ 2.5 μm) imposes stronger health risks than the coarse part of PM_{10}. We were not able to use $PM_{2.5}$ data in the current study, because the number of measuring stations for $PM_{2.5}$ in Belgium before 2008 was less than 15, with no data in the Southern part of the country.[43] For PM_{10} on the other hand, the number of measuring stations was above 45 in 2006 and above 60 in 2009.[43] The (Spearman) correlation between daily PM_{10} and $PM_{2.5}$ concentrations in 2009 was 0.9. Another limitation of our study is that we used interpolated air pollution estimates at the level of the municipality as a proxy for individual exposure since farm addresses were not available. Although larger farms might extend across municipalities, this phenomenon should be rather exceptional because farms in Belgium are much smaller than in the US for example. Milk specialized farms in Belgium have on average 56 cows and 47 hectares of forage area.[44]

Cattle are known to be susceptible for respiratory disease because of their small physiological gaseous exchange capacity, greater basal ventilatory activity, and greater anatomical compartmentalization of the lung as compared with other mammals.[45] This makes bovine

lungs highly susceptible to bacterial infection and lung damage. Despite physiological and anatomical differences between cattle and human, biochemical and physiological changes in response to air pollution exposure are expected to be similar for both species. Compared with large-scale meta-analyses and multi-city studies on human populations, which typically have examined the risk of mortality up to only a few days after exposure, our estimates for the immediate effects of air pollutants during the warm season are considerably larger. Assuming that 10-ppb O_3 equals 20 $\mu g/m^3$ and converting the 8-hour maximum concentration to the daily average,[46] our (lag 0–1) estimate corresponds to a 4.7% increase in mortality for a 10-ppb increase in daily average O_3. Estimates for humans range from 0.2% to 1.4% per 10-ppb in daily average O_3.[46-55] Meta-analytic and multi-city estimates for a 10 $\mu g/m^3$ increase in PM_{10} range from 0.2% to 0.6%,[48-50,54,56,57] whereas corresponding estimates for NO_2 range from 0.1% to 1.2%.[48,49,54,58]

Only a limited number of studies on the association between air pollution and human mortality have considered lag periods longer than a few days.[26,34-37] A study combining data from 21 European cities found a 21-day increase in respiratory deaths of 3.3% (95% CI = 1.9%, 4.8%) for each 10 $\mu g/m^3$ increase in O_3, whereas effects on total and cardiovascular mortality were only found in summer and were counterbalanced by negative effects thereafter.[37] Based on data from 10 European cities, Zanobetti et al[35] investigated the effect of PM_{10} on deaths up to 40 days after the exposure and found a 4.2% (95% CI = 1.1%, 7.4%) increase in respiratory deaths and a 2.0% (95% CI = 1.4%, 2.5%) in cardiovascular deaths for each 10 $\mu g/m^3$ increase in PM_{10}. Similarly, the 41-day increase in mortality associated with a 10 $\mu g/m^3$ increase in black smoke in Dublin was 3.6% (95% CI = 3.0%, 4.3%) for respiratory mortality, but only 1.1% (95% CI = 0.8%, 1.3%) for total mortality.[36] The 41-day increased risk of total mortality associated with a 10 $\mu g/m^3$ increase in PM_{10} estimated for 10 European cities was 1.6% (95% CI = 1.0%, 2.2%).[34]

Our study replicates epidemiological findings for humans, but in a more controlled and stable context, as socio-demographic confounding and exposure misclassification are limited in dairy cattle. This reinforces the evidence on the plausibility of causal effects in humans, and suggests that there are common pathophysiological patterns. In addition, our findings provide further evidence that acute exposures have long lasting effects.

References

1. Kodavanti UP, Costa DL. 10 - Animal Models to Study for Pollutant Effects. In: Holgate ST, Samet JM, Koren HS, Maynard RL, eds. *Air Pollution and Health*. New York: Academic Press, 1999;165-197.
2. Godleski JJ, Verrier RL, Koutrakis P, et al. Mechanisms of morbidity and mortality from exposure to ambient air particles. *Res Rep Health Eff Inst*. 2000:5-88; discussion 89-103.
3. Watkinson WP, Campen MJ, Nolan JP, Costa DL. Cardiovascular and systemic responses to inhaled pollutants in rodents: effects of ozone and particulate matter. *Environ Health Perspect*. 2001;109 Suppl 4:539-46.
4. Reif JS. Animal sentinels for environmental and public health. *Public Health Rep*. 2011;126(suppl 1):50-7.
5. Calderon-Garciduenas L, Mora-Tiscareno A, Ontiveros E, et al. Air pollution, cognitive deficits and brain abnormalities: a pilot study with children and dogs. *Brain Cogn*. 2008;68:117-27.
6. Bukowski JA, Wartenberg D. An alternative approach for investigating the carcinogenicity of indoor air pollution: pets as sentinels of environmental cancer risk. *Environ Health Perspect*. 1997;105:1312-9.
7. Van den Hoven R. Air Pollution and Domestic Animals. In: Moldoveanu A, ed. *Air Pollution New Developments*. Rijeka: Intech, 2011;179-202.
8. Veterinarian. The effects of the recent fog on the Smithfield Show and the London dairies. *Veterinarian*. 1874;47:32-33.
9. Bertyn F. Le brouillard et le bétail. *Ann Gembloux*. 1913;25:153-173.
10. Nemery B, Hoet PH, Nemmar A. The Meuse Valley fog of 1930: an air pollution disaster. *Lancet*. 2001;357:704-8.

11. Brunekreef B, Holgate ST. Air pollution and health. *Lancet*. 2002;360:1233-42.
12. Gasparrini A, Armstrong B, Kenward MG. Distributed lag non-linear models. *Stat Med*. 2010;29:2224-34.
13. Gasparrini A, Guo Y, Hashizume M, et al. Temporal variation in heat-mortality associations: a multicountry study. [published online ahead of print May 1, 2015]. *Environ Health Perspect*.
14. Federal Agency for the Safety of the Food Chain. Sanitel [in Dutch]. http://www.favv-afsca.be/dierlijkeproductie/dieren/sanitel/. Accessed February 26, 2015.
15. Janssen S, Dumont G, Fierens F, Mensink C. Spatial interpolation of air pollution measurements using CORINE land cover data. *Atmos Environ*. 2008;42:4884-4903.
16. Maiheu B, Veldeman N, Viaene P, et al. Identifying the best available large-scale concentration maps for air quality in Belgium, study commissioned by the Flemish Environment Agency (VMM), MIRA, MIRA/2013/01. Flemish Institute for Technological Research (VITO), 2012.
17. Jacobs L, Emmerechts J, Mathieu C, et al. Air pollution related prothrombotic changes in persons with diabetes. *Environ Health Perspect*. 2010;118:191-6.
18. Katsouyanni K, Touloumi G, Spix C, et al. Short-term effects of ambient sulphur dioxide and particulate matter on mortality in 12 European cities: results from time series data from the APHEA project. Air Pollution and Health: a European Approach. *BMJ*. 1997;314:1658-63.
19. Nawrot TS, Torfs R, Fierens F, et al. Stronger associations between daily mortality and fine particulate air pollution in summer than in winter: evidence from a heavily polluted region in western Europe. *J Epidemiol Community Health*. 2007;61:146-9.

20. Nawrot TS, Perez L, Kunzli N, Munters E, Nemery B. Public health importance of triggers of myocardial infarction: a comparative risk assessment. *Lancet*. 2011;377:732-40.
21. Maclure M. The case-crossover design: a method for studying transient effects on the risk of acute events. *Am J Epidemiol*. 1991;133:144-53.
22. Levy D, Lumley T, Sheppard L, Kaufman J, Checkoway H. Referent selection in case-crossover analyses of acute health effects of air pollution. *Epidemiology*. 2001;12:186-92.
23. Gasparrini A. Modeling exposure-lag-response associations with distributed lag non-linear models. *Stat Med*. 2014;33:881-99.
24. Gasparrini A. Distributed lag linear and non-linear models in R: the package dlnm. *J Stat Softw*. 2011;43:1-20.
25. Schwartz J. The distributed lag between air pollution and daily deaths. *Epidemiology*. 2000;11:320-6.
26. Zanobetti A, Wand MP, Schwartz J, Ryan LM. Generalized additive distributed lag models: quantifying mortality displacement. *Biostatistics*. 2000;1:279-92.
27. Meroc E, Herr C, Verheyden B, et al. Bluetongue in Belgium: episode II. *Transbound Emerg Dis*. 2009;56:39-48.
28. Ensoy C, Aerts M, Welby S, Van der Stede Y, Faes C. A dynamic spatio-temporal model to investigate the effect of cattle movements on the spread of bluetongue BTV-8 in Belgium. *PLoS One*. 2013;8:e78591.
29. Kunzli N, Schindler C. A call for reporting the relevant exposure term in air pollution case-crossover studies. *J Epidemiol Community Health*. 2005;59:527-30.
30. Silanikove N. Effects of heat stress on the welfare of extensively managed domestic ruminants. *Livest Prod Sci*. 2000;67:1-18.

31. St-Pierre NR, Cobanov B, Schnitkey G. Economic losses from heat stress by US livestock industries. *J Dairy Sci*. 2003;86:E52-E77.
32. van der Schalie WH, Gardner HS, Jr., Bantle JA, et al. Animals as sentinels of human health hazards of environmental chemicals. *Environ Health Perspect*. 1999;107:309-15.
33. Rabinowitz P, Conti L. Links among human health, animal health, and ecosystem health. *Annu Rev Public Health*. 2013;34:189-204.
34. Zanobetti A, Schwartz J, Samoli E, et al. The temporal pattern of mortality responses to air pollution: a multicity assessment of mortality displacement. *Epidemiology*. 2002;13:87-93.
35. Zanobetti A, Schwartz J, Samoli E, et al. The temporal pattern of respiratory and heart disease mortality in response to air pollution. *Environ Health Perspect*. 2003;111:1188-93.
36. Goodman PG, Dockery DW, Clancy L. Cause-specific mortality and the extended effects of particulate pollution and temperature exposure. *Environ Health Perspect*. 2004;112:179-85.
37. Samoli E, Zanobetti A, Schwartz J, et al. The temporal pattern of mortality responses to ambient ozone in the APHEA project. *J Epidemiol Community Health*. 2009;63:960-6.
38. Bell ML, Davis DL. Reassessment of the lethal London fog of 1952: novel indicators of acute and chronic consequences of acute exposure to air pollution. *Environ Health Perspect*. 2001;109 Suppl 3:389-94.
39. Peng RD, Dominici F, Pastor-Barriuso R, Zeger SL, Samet JM. Seasonal analyses of air pollution and mortality in 100 US cities. *Am J Epidemiol*. 2005;161:585-94.

40. Hetland RB, Cassee FR, Lag M, Refsnes M, Dybing E, Schwarze PE. Cytokine release from alveolar macrophages exposed to ambient particulate matter: heterogeneity in relation to size, city and season. *Part Fibre Toxicol*. 2005;2:4.
41. Happo MS, Hirvonen MR, Halinen AI, et al. Seasonal variation in chemical composition of size-segregated urban air particles and the inflammatory activity in the mouse lung. *Inhal Toxicol*. 2010;22:17-32.
42. Sorensen M, Loft S, Andersen HV, et al. Personal exposure to PM2.5, black smoke and NO2 in Copenhagen: relationship to bedroom and outdoor concentrations covering seasonal variation. *J Expo Anal Environ Epidemiol*. 2005;15:413-22.
43. Fierens F, Vanpoucke C, Adriaenssens S, et al. Air Quality in Belgium 2011. Annual Report. Belgian Interregional Environment Agency (IRCEL-CELINE), 2011. http://www.irceline.be/en/documentation/publications/annual-reports/annual-report-2011/view. Accessed November 18, 2015.
44. European Commission. EU dairy farms report 2013 based on FADN data. European Commission - EU FADN, 2014. http://ec.europa.eu/agriculture/rica/pdf/Dairy_Farms_report_2013_WEB.pdf. Accessed November 18, 2015.
45. Veit HP, Farrell RL. The anatomy and physiology of the bovine respiratory system relating to pulmonary disease. *Cornell Vet*. 1978;68:555-581.
46. Bell ML, Dominici F, Samet JM. A meta-analysis of time-series studies of ozone and mortality with comparison to the national morbidity, mortality, and air pollution study. *Epidemiology*. 2005;16:436-45.
47. Thurston GD, Ito K. Epidemiological studies of acute ozone exposures and mortality. *J Expo Anal Environ Epidemiol*. 2001;11:286-94.

48. Stieb DM, Judek S, Burnett RT. Meta-analysis of time-series studies of air pollution and mortality: update in relation to the use of generalized additive models. *J Air Waste Manag Assoc*. 2003;53:258-61.
49. Dominici F, McDermott A, Daniels M, Zeger S, Samet J. Mortality among residents of 90 cities. *Revised analyses of time-series studies of air pollution and health*. Special report. Boston, MA: Health Effects Institute, 2003;9-24.
50. Anderson HR, Atkinson RW, Peacock JL, Marston L, Konstantinou K. Meta-analysis of time-series studies and panel studies of Particulate Matter (PM) and Ozone (O3). Report of a WHO task group. WHO Regional Office for Europe, 2004.
51. Bell ML, McDermott A, Zeger SL, Samet JM, Dominici F. Ozone and short-term mortality in 95 US urban communities, 1987-2000. *JAMA*. 2004;292:2372-8.
52. Ito K, De Leon SF, Lippmann M. Associations between ozone and daily mortality: analysis and meta-analysis. *Epidemiology*. 2005;16:446-57.
53. Levy JI, Chemerynski SM, Sarnat JA. Ozone exposure and mortality: an empiric bayes metaregression analysis. *Epidemiology*. 2005;16:458-68.
54. Wong CM, Vichit-Vadakan N, Kan H, Qian Z. Public Health and Air Pollution in Asia (PAPA): a multicity study of short-term effects of air pollution on mortality. *Environ Health Perspect*. 2008;116:1195-202.
55. Peng RD, Samoli E, Pham L, et al. Acute effects of ambient ozone on mortality in Europe and North America: results from the APHENA study. *Air Qual Atmos Health*. 2013;6:445-453.
56. Katsouyanni K, Touloumi G, Samoli E, et al. Confounding and effect modification in the short-term effects of ambient particles on total mortality: results from 29 European cities within the APHEA2 project. *Epidemiology*. 2001;12:521-31.

57. Samoli E, Peng R, Ramsay T, et al. Acute effects of ambient particulate matter on mortality in Europe and North America: results from the APHENA study. *Environ Health Perspect*. 2008;116:1480-6.
58. Samoli E, Aga E, Touloumi G, et al. Short-term effects of nitrogen dioxide on mortality: an analysis within the APHEA project. *Eur Respir J*. 2006;27:1129-38.

Figure 1. Lag-specific relative risks (RR, with 95% confidence interval) for dairy cow mortality associated with a 10 µg/m^3 increase in air pollutant concentrations during the warm season (left panel: A, C, E for O_3, PM_{10}, NO_2 respectively) and the cold season (right panel: B, D, F for O_3, PM_{10}, NO_2 respectively), Belgium 2006-2009.

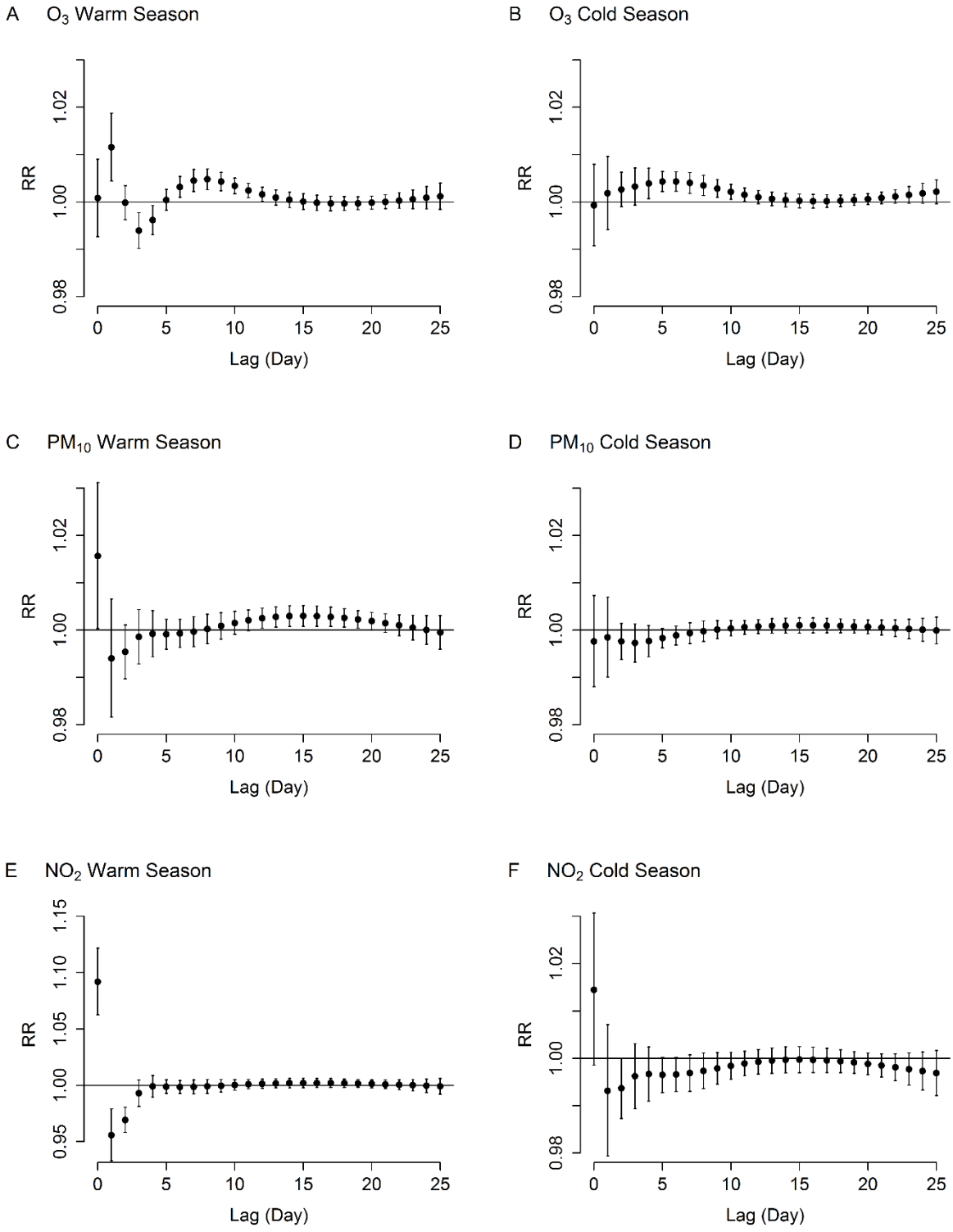

TABLE 1. Summary Statistics for Daily Cattle Mortality, Weather Conditions and Air Pollution Levels and for the Absolute Differences between the Daily Levels of Each Pollutant (Case Days) and the Average Concentrations over the Control Days, Belgium 2006-2009.

	Warm Season (N=39,979)				Cold Season (N=47,129)			
Variable	**Mean**	**SD**	**Min**	**Max**	**Mean**	**SD**	**Min**	**Max**
Daily Number of Cow Deaths								
	54.6	22.2	12	145	64.6	25.9	0	162
Exposure on Case Days								
O_3 (µg/m^3)	81.2	26.0	16.2	222.0	48.3	20.4	2.4	115.5
PM_{10} (µg/m^3)	24.9	11.8	2.6	95.0	27.3	16.6	1.3	142.2
NO_2 (µg/m^3)	14.6	7.8	1.0	59.6	21.2	11.4	1.0	93.4
Temperature (°C)	15.6	4.2	4.1	28.6	6.2	4.4	-8.2	18.7
Humidity (%)	69.9	11.0	32.1	94.5	81.0	9.0	40.0	99.8
Exposure Difference between Case Days and Average over Control Days [a]								
O_3 (µg/m^3)	18.1	15.8	0.0	126.7	14.6	10.7	0.0	59.8
PM_{10} (µg/m^3)	8.5	7.8	0.0	69.1	13.0	12.2	0.0	117.2
NO_2 (µg/m^3)	4.7	3.9	0.0	31.7	8.4	7.1	0.0	55.9

[a] The relevant exposure term in a case-crossover design.[29]

SD, Standard Deviation; Min, minimum; Max, maximum.

TABLE 2. Matrix of Spearman Correlation Coefficients* between Air Pollutants and Weather Variables, Belgium 2006-2009.

	O_3	PM_{10}	NO_2	Temperature	Humidity
Warm Season					
O_3	1.00				
PM_{10}	0.34	1.00			
NO_2	0.09	0.70	1.00		
Temperature	0.30	0.13	-0.06	1.00	
Humidity	-0.64	-0.32	-0.22	-0.29	1.00
Cold Season					
O_3	1.00				
PM_{10}	-0.53	1.00			
NO_2	-0.66	0.75	1.00		
Temperature	0.30	-0.30	-0.41	1.00	
Humidity	-0.42	-0.04	0.10	-0.16	1.00

*All $P < 0.01$

TABLE 3. Cumulative Effects of Air Pollution on Dairy Cow Mortality along the Lag Days, Belgium 2006-2009. Estimates Represent the Percent Change in Dairy Cow Mortality for a 10 $\mu g/m^3$ Increase in Air Pollutant Concentration.

	Lag	**Percent Change (95% CI)**		
Season	**(Day)**	**O_3**	**PM_{10}**	**NO_2**
Warm	0	0.1 (-0.7, 0.9)	1.6 (0.0, 3.1)	9.2 (6.3, 12.2)
	0–1	1.2 (0.3, 2.1)	1.0 (-0.5, 2.4)	4.3 (1.5, 7.3)
	0–25	3.0 (0.2, 6.0)	3.2 (-0.6, 7.2)	1.4 (-4.9, 8.2)
Cold	0	-0.1 (-0.9, 0.8)	-0.2 (-1.2, 0.7)	1.4 (-0.1, 3.1)
	0–1	0.1 (-0.8, 1.0)	-0.4 (-1.3, 0.5)	0.7 (-0.8, 2.3)
	0–25	4.6 (2.2, 7.0)	-0.5 (-3.1, 2.2)	-4.0 (-8.4, 0.6)

Supplemental Digital Content

Ambient Air Pollution-Related Mortality in Dairy Cattle Corroborates Human Findings

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eTABLE 1. Cumulative (Lag 0–25) Effects of Air Pollution on Dairy Cow Mortality, Belgium 2006-2009, for the Total Study Period (Main Analysis) and Excluding the Bluetongue Epidemics (August-December 2006 and July-December 2007), and Using a Natural Cubic Spline with 6 Degrees of Freedom (Main Analysis) and an Unconstrained Distributed Lag to Model the Lag Structure. Estimates Represent the Percent Change in Dairy Cow Mortality for a 10 $\mu g/m^3$ Increase in Air Pollutant Concentration.

		Percent Change (95% CI)			
		Total Study Period		**Excluding Bluetongue Epidemics**	
Season	**Exposure**	**Natural Spline**	**Unconstrained**	**Natural Spline**	**Unconstrained**
Warm	O_3	3.0 (0.2, 6.0)	3.1 (0.2, 6.1)	3.1 (-0.1, 6.3)	3.3 (0.1, 6.6)
	PM_{10}	3.2 (-0.6, 7.2)	3.7 (-0.2, 7.9)	4.0 (-0.3, 8.3)	4.2 (-0.2, 8.8)
	NO_2	1.4 (-4.9, 8.2)	1.8 (-4.8, 8.8)	3.6 (-3.4, 11.0)	3.9 (-3.3, 11.6)
Cold	O_3	4.6 (2.2, 7.0)	4.0 (1.7, 6.5)	4.4 (1.8, 7.1)	3.6 (0.9, 6.3)
	PM_{10}	-0.5 (-3.1, 2.2)	0.6 (-2.1, 3.4)	1.5 (-1.6, 4.7)	3.2 (0.0, 6.5)
	NO_2	-4.0 (-8.4, 0.6)	-2.7 (-7.2, 2.1)	-1.8 (-6.6, 3.3)	0.0 (-5.0, 5.3)

eTABLE 2. Cumulative (Lag 0–25) Effects of Air Pollution on Dairy Cow Mortality, Belgium 2006-2009, Using Different Specifications for the Temperature Cross-basis. Estimates Represent the Percent Change in Dairy Cow Mortality for a 10 µg/m^3 Increase in Air Pollutant Concentration. The Models Used in the Main Analysis Are Shown in Bold.

	Temperature			**Percent Change (95% CI)**	
Exposure	**Var Df [a]**	**Lag Df [b]**	**AIC**	**Warm Season**	**Cold Season**
O_3	3	6	257123.0	3.1 (0.4, 5.9)	5.0 (2.8, 7.2)
	5	**6**	**257127.5**	**3.0 (0.2, 6.0)**	**4.6 (2.2, 7.0)**
	7	6	257121.7	3.6 (0.6, 6.7)	4.9 (2.4, 7.5)
	5	3	257143.4	3.7 (0.8, 6.6)	4.9 (2.5, 7.3)
	5	5	257128.1	3.0 (0.1, 5.9)	4.5 (2.2, 6.9)
	5	7	257112.1	3.1 (0.2, 6.0)	4.6 (2.2, 7.0)
PM_{10}	3	6	257153.5	3.7 (-0.1, 7.7)	-1.2 (-3.7, 1.4)
	5	**6**	**257154.2**	**3.2 (-0.6, 7.2)**	**-0.5 (-3.1, 2.2)**
	7	6	257149.7	3.8 (-0.2, 7.9)	-0.4 (-3.1, 2.4)
	5	3	257175.8	2.6 (-1.2, 6.5)	-0.3 (-2.9, 2.4)
	5	5	257160.4	3.0 (-0.8, 7.0)	-0.4 (-3.0, 2.3)
	5	7	257137.3	3.2 (-0.7, 7.1)	-0.5 (-3.1, 2.2)
NO_2	3	6	257111.9	1.7 (-4.6, 8.4)	-5.3 (-9.5, -0.8)
	5	**6**	**257115.5**	**1.4 (-4.9, 8.2)**	**-4.0 (-8.4, 0.6)**
	7	6	257108.1	2.4 (-4.3, 9.6)	-3.3 (-7.9, 1.4)
	5	3	257142.9	0.6 (-5.6, 7.3)	-4.7 (-9.0, -0.1)
	5	5	257128.3	1.2 (-5.1, 8.0)	-4.0 (-8.4, 0.6)
	5	7	257098.7	1.6 (-4.8, 8.3)	-3.9 (-8.3, 0.7)

[a] Degrees of Freedom for the Temperature-Response Function

[b] Degrees of Freedom for the Lag-Response Function