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Short Communication

Is the association between pet ownership and indoor endotoxin levels confounded or modified by outdoor residential greenspace?

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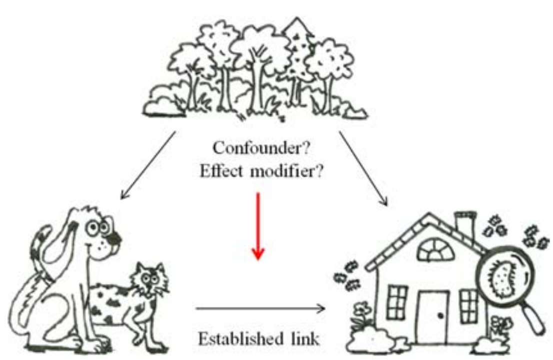
HIGHLIGHTS

- Pet ownership is consistently identified as a predictor of indoor endotoxin levels.
- Pets may be endotoxin sources, surrogates of greener areas, or assist in transmission.
- Confounding and/or effect modification by outdoor greenspace is plausible.
- However, we found no evidence that this is true in an urban German study area.
- Future studies are needed to determine how pets influence indoor endotoxin levels.

GRAPHICAL ABSTRACT



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ABSTRACT

Background: Pet ownership is consistently associated with higher indoor endotoxin concentrations, but may also be related to the amount of greenspace around the home. This study examined whether the association between pet ownership and higher indoor endotoxin concentrations is confounded or modified by residential greenspace.
Methods: Information on pet ownership was collected at the time of recruitment of the German LISA birth cohort. Endotoxin levels were measured in settled house dust sampled from mothers' mattresses ($N = 1197$) and living room floors ($N = 390$). Greenspace around the home was assessed as the mean Normalized Difference Vegetation Index in 100 m, 500 m and 1000 m buffers around the home, and as the distance to the nearest urban and natural green space. Linear regression models assessed cross-sectional associations between pet ownership and log-transformed endotoxin levels, adjusted for known predictors of endotoxin levels. Confounding by greenspace was assessed by additionally adjusting the models for each greenspace variable. Effect modification was assessed by including interaction terms between pet ownership and each greenspace variable, and by model stratification.
Results: Dog and cat ownership were associated with higher endotoxin levels in mothers' mattresses, whereas only dog ownership was associated with endotoxin levels in the floor samples. All associations were highly robust to further adjustment for greenspace, and there was little evidence to suggest any effect modification (interaction terms had p-values >0.05).

Abbreviations: LISA, Influence of Life-style related factors on the development of the Immune System and Allergies in East and West Germany; NDVI, Normalized Difference Vegetation Index.

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Conclusions: Residential greenspace did not confound or modify the association between pet ownership and indoor endotoxin levels. Studies should continue investigating whether pets influence the indoor environment only by their presence, or also by acting as transmission vectors of the outdoors.
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1. Introduction

Endotoxins are ubiquitous in the indoor environment and early-exposure to these compounds (and other microbials) is likely to play an important role in respiratory and allergic health development (von Mutius, 2016). Several studies have attempted to identify the sources of indoor endotoxin and have consistently identified pet ownership as an important predictor in European (Bischof et al., 2002; Casas et al., 2013; Gehring et al., 2004; Giovannangelo et al., 2007; Heinrich et al., 2001; Waser et al., 2004) and American (Gereda et al., 2001; Ownby et al., 2013; Park et al., 2001; Thorne et al., 2009) studies, in addition to other factors, such as number of occupants, presence of mold/dampness and season of measurement sampling.

Pets (and their food) may act as direct sources of endotoxin, similar to the way that humans are a predominant source of bacteria in (non-pet) homes (Täubel et al., 2009). Pets may also act as transmission factors for the outdoor (microbial) environment into the home, if they go outside. The level of this transmission may depend on the outdoor environment itself. Alternatively, pet ownership may be a proxy for living in more suburban or rural areas, which tend to be greener (Markevych et al., 2017a). In this latter situation, the residential outdoor environment could be confounding the consistently observed associations between pet ownership and higher indoor endotoxin concentrations.

Using available data from the German LISA (Influence of Life-style related factors on the development of the Immune System and Allergies in East and West Germany) - Munich cohort, for which associations between dog ownership and indoor endotoxin levels have already been reported (Casas et al., 2013), we explored the link between residential greenspace, pet ownership and indoor endotoxin levels. Specifically, we examined whether the association between pet ownership and higher indoor endotoxin concentrations is confounded or modified by residential greenspace.

2. Methods

The ongoing multicentre LISA birth cohort has been previously described (Heinrich et al., 2002; Zutavern et al., 2006). Ethical approval was obtained from local ethics committees and written informed consent was obtained from all participating families. We here considered only participants living in the Munich study center (47.3% of the original multicentre cohort of 3094 participants), for whom mattress and floor endotoxin levels, as well as pet and greenspace data were all available.

Endotoxin levels were collected in mattress dust from the mother's bed and from the living room floor at the three-month follow-up of cohort participants, using standard methods previously described (Casas et al., 2013; Heinrich et al., 2002). All samples were stored below −20 °C until endotoxin measurement analysis. The mattress samples were analyzed close to the time of sample collection and expressed as concentrations per gram of dust (EU/g) and loads per surface area (EU/m^2) (Heinrich et al., 2002). The floor samples were analyzed 10 years after storage, as part of the "Health effects of Indoor pollutants: integrating microbial, Toxicological and Epidemiological Approaches" project, and are expressed as concentrations per gram of dust only (EU/g) (Casas et al., 2013). The floor samples are available for only a random subsample of the LISA cohort, which had a higher response rate and contained more non-movers than the general cohort (further details of the subsample selection are provided elsewhere (Casas et al., 2013)).

Information on housing conditions and other potential determinants of microbial exposure was obtained using questionnaires administered at the time of cohort recruitment (i.e. birth) and during the dust sampling (age three months). Based on these data, the following pet ownership variables were defined: any pet (dogs, cats, bunnies, birds, hamsters, guinea pigs or "other pets" versus no pets), outdoor pet (dogs or cats versus no pets) and indoor pet (bunnies, birds, hamsters or guinea pigs versus no pets). Owning individual pets (e.g. dog, yes versus no) was also examined.

Residential greenspace (Taylor and Hochuli, 2017) was defined using two indicators of amount and proximity of greenspaces around a home. The first indicator was residential greenness, defined using the Normalized Difference Vegetation Index (NDVI; Tucker, 1979), which is a measure of vegetation level whose calculation is based on the difference of surface reflectance in visible (0.4–0.7 μm) and near infrared (0.7–1.1 μm) wavelengths. NDVI values range from negative one (water) through to zero (rock, sand, snow) to positive one (dense green vegetation; Weier and Herring, 2000). NDVI values were calculated at a resolution of 30 m by 30 m from cloud-free Landsat 5 Thematic Mapper satellite images obtained from the Global Visualisation viewer from the U.S. Geological Survey (http://earthexplorer.usgs.gov/). Three images (one each on 01/08/1998 (path 193, rows 26–27), 08/08/1998 (path 194, rows 26–27), and 10/10/1998 (Path 192, rows 26–27)) were merged and used, as a single image covering the entire study territory was not available for any given summer day. We focused on images taken in the summer to capture the greatest variation in vegetation and 1998 is the year closest in time to the endotoxin sampling. After excluding negative values of NDVI, as these normally correspond to water, mean NDVI values in 100 m, 500 m and 1000 m buffers around the home address were calculated.

The second greenspace indicator was the shortest Euclidean distance from a participant's home address to the edge of the nearest green space. In this study, we considered distance to the nearest urban green space (cemeteries, gardens, parks, plant nurseries) and natural green space (forests) separately. Data for these calculations were obtained from the local Bavarian land use dataset (vector with spatial resolution of <5 m; Markevych et al., 2014).

As the distribution of the endotoxin concentrations and loads were skewed to the right, they were naturally log-transformed. Geometric means with their 95% confidence intervals are thus presented. Correlations between the endotoxin variables and the greenspace variables were assessed using Spearman correlation coefficients.

Cross-sectional associations between the pet ownership variables and the log-transformed endotoxin variables were assessed using linear models, adjusted for covariates known to be associated with endotoxin levels in the LISA cohort (Casas et al., 2013; Gehring et al., 2004). These covariates included season of sampling, number of occupants, presence of mold/dampness, animal contact outside of the home, age of the home and age of the mother's mattress (latter covariate only for mattress samples). The resulting regression coefficients were back-transformed to their exponential [$\exp(\beta)$], resulting in the presentation of mean ratios with their corresponding 95% confidence intervals. Mean ratios can be interpreted as the percentage change in the mean of the outcome variable in one group compared to the reference group. Mean ratios are presented per 0.2 increase in mean greenness and per 500 m increase in distance to the nearest urban or natural green space.

Potential confounding by greenspace was assessed by individually adjusting the models for all greenspace variables treated as continuous

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