Animal Allergens, Endotoxin, and β-(1,3)-Glucan in Small Animal Practices: Exposure Levels at Work and in Homes of Veterinary Staff

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Abstract

Objectives: In veterinary settings, high exposures to animal allergens and microbial agents can be expected. However, occupational exposure levels are largely unknown. The objective of this study was to estimate the allergen, endotoxin, and β-(1,3)-glucan concentrations in small animal practices and in the homes of practice employees.

Methods: Dust samples were collected using electrostatic dust fall collectors in diverse rooms of 36 small animal practices, as well as in employees’ homes. Major animal allergens (Fel d 1, Can f 1, Ory c 3, Cav p 1, Equ c 1, Bos d 2), domestic mite (DM) allergens, and β-(1,3)-glucan levels were measured using enzyme immunoassays. Endotoxin was determined using the Limulus amoebocyte lysate assay. Influences on exposure levels were analyzed using multilevel models.

Results: The levels of Can f 1, Fel d 1, Ory c 3, and Cav p 1 were up to 30 times higher in practices compared with homes without animals, but significantly lower compared with the homes with the...
respective pet. Although horses were not treated in the practices, Equ c 1 was found in 87.5% of samples, with the highest concentrations measured in changing rooms. DM levels were significantly lower in practices than in all private homes, and endotoxin levels were similar to those in homes with pets. In the practice itself, exposure levels were significantly influenced by animal presence, type of the room, and area per employee; whereas, room volume and diverse cleaning measures had mostly no effect.

Conclusions: Exposure to animal allergens is high in veterinary practices, but it does not reach levels of households with pets. Domestic mite allergen and endotoxin exposure seem to be low for workers in veterinary practices. The high Equ c 1 detection rate strongly indicates dispersal of allergens, most likely through clothing and hair.

Keywords: animal allergens; cat; dog; endotoxin; β-(1,3)-glucan; guinea pig; horse; occupational exposure; rabbit; veterinary practice

Introduction

Exposure to animal allergens is a relevant risk factor in the development of sensitization, and allergic diseases (Konradsen et al., 2015). Animal allergies tend to affect the general population mainly due to the large number of pet owners. Contact with animals also represents an occupational health hazard. Both the prevalence of allergic diseases and allergen (Jones, 2015) levels have been well investigated for laboratory animal workers handling mice and rats, and cattle farmers (Heutelbeck et al., 2007; Zahradnik et al., 2011; Schlünssen et al., 2015). Interestingly, although veterinarians are among those most exposed to animal allergens, only a few studies have investigated allergic symptoms among veterinary staff. For example, 40% of California veterinarians reported animal-related respiratory and/or skin symptoms. The most commonly reported causes of symptoms were cats (26%), dogs (19%), horses (7%), and cattle (7%) (Susitaival et al., 2003). In a Canadian survey, 39% of the study participants developed allergies during their veterinary career, and the most commonly reported allergy triggers were hair and dander from companion animals (Epp and Waldner, 2012). In Germany, the ‘Berufsgenossenschaft für Gesundheitsdienst und Wohlfahrtspflege’ (BGW), a part of the German Social Accident Insurance, provides a mandatory service for all veterinary practices in order to identify the most important health risks to veterinarians and their staff. According to their database, allergic reactions to animals (respiratory symptoms or allergic contact dermatitis) accounted for 23.8% of all verified occupational diseases in this group (Nienhaus et al., 2005).

The exposure levels to animal allergens in veterinary practices are largely unknown. Thus far, only one study has been published examining allergen exposure in a companion animal hospital (Samadi et al., 2010). In contrast, there are numerous publications on the quantitative measurements of diverse animal allergens [dog (Can f 1), cat (Fel d 1), mouse (Mus m 1), horse (Equ c 1)] in homes, schools, and other public places (Zahradnik and Raulf, 2014). In general, exposure to animal allergens occurs in every type of indoor environment, even in locations where no animals reside. In addition, there is strong evidence that human clothing and hair are the primary means by which allergens are transferred (Lucca et al., 2000; Krop et al., 2006).

Allergen concentrations vary considerably among different environments and are dependent on numerous factors. For example, in addition to animal presence, differences in allergen concentrations are associated with the number of pet owners and building-related factors, such as size and type of room, type of flooring and furnishing, cleaning frequency, and ventilation system (Zahradnik and Raulf, 2014).
Exposure to microbial components, such as endotoxins (part of the outer membrane of Gram-negative bacteria) and β-(1,3)-glucans (part of fungal cell walls) are considered potential health hazards in the field of veterinary medicine. Exposure of animal farmers to β-glucan has been identified in several studies (Hilger et al., 2011; Swiontek et al., 2021). Exposure to rabbit allergens (Ory c 1 and Ory c 3) has been investigated in small animal veterinary practices. In addition, these biological agents were also measured in the homes of practice employees to compare home and work environment.

Methods

Study design

The study was conducted in 36 small animal practices across North Rhine-Westphalia, Germany, and in 101 of their employees’ homes from October 2017 until February 2019. Dust sampling and sampling documentation (i.e. duration, position and height of sampling equipment, room size, room ventilation) were carried out in the practices by a professional field worker, who tried to include all available rooms during sampling. The rooms (n = 304) were grouped into 11 categories: reception/waiting room, examination room, X-ray/ultrasound room, operating room, surgery preparation room, inpatient ward, pharmacy/laboratory, storage/utility room, break room, office, changing room. Short questionnaires were used to collect information about practice characteristics (location, size, number of rooms, number of employees, opening hours), type and percentage of treated animals, and type and frequency of cleaning measures. Dust samples were collected at home by the study participants, who received detailed instructions on how to use electrostatic dust fall collectors (EDCs) in rooms they occupied the most. They were then required to fill a questionnaire addressing sampling characteristics, as well as information on the presence of pets (cat, dog, rabbit, guinea pig, hamster) at home, and any direct contact to animals during leisure activities.

This study was part of the project AllergoMed which was approved by the Ethics Committee of the Ruhr University Bochum in Germany (registration number: 17-6022). Participation in the project was voluntarily and all participants signed an informed consent before taking part in the study.

Dust sampling

Dust sampling was performed using EDCs consisting of a polypropylene folder with two dust-binding cloths (Techmed-Textil-Service-GmbH, Dipperz, Germany), each with a surface exposure area of 0.0209 m². The cloths were made pyrogen-free by heating for 4 h at 200°C. In most cases, the EDCs were left in a horizontal position at a median height of 1.9 m (range: 1–2.6 m) above the floor for the recommended time of 14 days (range: 7–36 days) to collect settling airborne dust. After the sampling period, the EDCs were closed, individually placed into Ziploc bags and sent to the laboratory by regular mail in a pre-addressed envelope. Once received, one cloth from each EDC was removed from the folder and placed in a 150 ml beaker and stored at 4°C until extraction of endotoxin. The second cloth was frozen in the polypropylene folder, placed in a Ziploc bag overnight at −20°C to eliminate mite proliferation on the cloth, and then stored at room temperature until extraction of allergens and β-(1,3)-glucan.

Extraction

Allergens and β-glucans were sequentially extracted from the cloths in 15 ml phosphate-buffered saline (PBS), pH 7.4 with 0.05% Tween 20 (PBST) by rotation for 1 h at room temperature. After removing the cloths, the extracts were centrifuged at 3000 g for 15 min. The supernatants were stored in aliquots at −80°C until allergen analysis. For the β-(1,3)-glucan analysis, the pellets were re-suspended with 2 ml of the supernatant and autoclaved at 121°C and 1 bar for 20 min. These autoclaved resuspensions were centrifuged at 3000 g for 15 min and stored in aliquots at −80°C.

Endotoxin was extracted from the second EDC cloth in 20 ml pyrogen-free water (Aqua ad inyectabilia, DeltaSelect, Reutlingen, Germany) by shaking (160
shakes min⁻¹) for 1 h at room temperature. The extracts were transferred into pyrogen-free tubes and centrifuged at 1000 g for 10 min. The supernatants were stored in aliquots at −80°C until analysis.

Quantification of allergens, endotoxin, and β-(1,3)-glucan
Allergen levels of Fel d 1, Can f 1, and Bos d 2 were determined using monoclonal antibodies and calibration standards purchased from Indoor Biotechnologies Inc. (Charlottesville, VA, USA) according to protocols described previously for cat and dog (Sander and Lotz et al., 2016) and for cattle (Zahradnik et al., 2015). Equ c 1 concentrations were quantified using an immunoassay based on polyclonal antibodies and naturally-purified Equ c 1 as a standard (Zahradnik et al., 2018). To improve assay sensitivity, the chromogenic substrate ABST [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)] was exchanged with the fluorogenic substrate QuantaBlu (ThermoScientific, Rockford, IL, USA). A sensitive immunoassay based on polyclonal antibodies to Dermatophagoides farinae extract was used to estimate domestic mite levels. Due to strong cross-reactivity, this assay detects allergens from several house dust and storage mite species (Sander et al., 2012). β-(1,3)-Glucan measurements were performed as previously described by (Sander et al., 2008). Endotoxin was determined using a kinetic chromogenic limulus amoebocyte lysate assay (Charles River, Sulzfeld, Germany) according to the manufacturer's instructions.

For the detection of Ory c 3 and Cav p 1, newly developed enzyme immunoassays were applied. The production of recombinant proteins and polyclonal antibodies (pAb) is described in the supplementary material provided online. Briefly, 384 well microtiter plates (Nunc MaxiSorp, ThermoFisher Scientific, Waltham, Massachusetts) were coated with purified anti-Ory c 3 or anti-Cav p 1 pAb at 0.5 µg ml⁻¹ in PBS (50 µl well⁻¹) overnight at 4°C, followed by a blocking step with 3% bovine serum albumin in PBST (100 µl well⁻¹). Dust samples were added in duplicate to the microplates (undiluted and diluted ½ in PBS). Standard curves were established using a native Ory c 3 purified from rabbit hair or a mixture of rCav p 1 isoallergens (0.001 to 50 ng ml⁻¹) at 50 µl well⁻¹. Bound allergens were quantified using biotinylated anti-Ory c 3 or anti-Cav p 1 pAb diluted 1/2000 in blocking buffer (50 µl well⁻¹) followed by incubation with horse-radish peroxidase labeled streptavidin (Fitzgerald, Concord, MA, USA) diluted 1/20 000 in blocking buffer. All incubations were carried out for at least 1 h at room temperature whilst shaking, followed by three washes with PBST between successive steps. The assays were developed using QuantaBlu.

Values below the lower limit of detection (LOD) were replaced by 2/3 LOD. All values above the LOD were divided by the number of sampling days and multiplied by 14 to adjust the values to the recommended dust sampling duration of two weeks. This was done because allergen levels increase proportionally with deployment time over a period of 4 weeks (Sander and Lotz et al., 2016). All values were then calculated as ng m⁻² or EU m⁻². The LODs for EDC samples were 5.7 ng m⁻² for Cav p 1, 7.2 ng m⁻² for Fel d 1, Can f 1, and Equ c 1, 14.4 ng m⁻² for Bos d 2, 16.5 ng m⁻² for Ory c 3, 35.9 ng m⁻² for DM, and 95.7 ng m⁻² for β-(1,3)-glucan.

Statistical analysis
The concentrations of all analytes were log-transformed and analyzed using multilevel-level models with sample as level-one unit and practice as level-two unit to determine (i) differences between home and practice environments, (ii) differences between rooms with and without animals, and (iii) influencing factors on exposure levels within the practices. For the latter, independent variables in the models were: room type, room ventilation (by window), frequency of cleaning (wiping, sweeping, vacuuming), opening hours, room volume, and area per employee. Additionally, for each model the conditional intraclass correlation coefficient (ICC) was calculated, which is a measure of the degree of within-group homogeneity or between-group heterogeneity after controlling for contextual variables (Wang et al., 2011). The ICC approaches one when the between-practice variation is very large relative to the within-room variation, indicating that samples collected from one practice are similar. Conversely, ICC approaches zero when the grouping of samples by practice conveys no additional information. Each analyte was analyzed in a separate model.

Statistical analyses were conducted using SAS, version 9.4 (SAS Institute, Inc. Cary, NC). If some of the exposure levels were below the limit of detection, the multilevel-models with a censored dependent variable were estimated as previously described (Vaida and Liu, 2009), and calculated using software R (R Core Team, 2015). The descriptive statistics and graphs were made with GraphPad Prism version 8.4.3 (GraphPad Software, Inc., La Jolla, CA).

Results
Exposure levels in practices and homes
Characteristics of the veterinary practices and homes of their employees are given in Table 1. In practices, Can f 1 was detected in all, Fel d 1 in 99.7%, Ory c 3 in 81.6%,
Cav p 1 in 82.9%, and DM allergens in 64.8% of the samples. Although horses and cattle were not treated in practices, Equ c 1 was found in 87.5% and Bos d 2 in 22% of the samples. In homes, 92.1% of samples contained Can f 1, 79.2% Fel d 1, 18.8% Ory c 3, 50.5% Cav p 1, and 88.1% DM allergens. Moreover, Equ c 1 was found in 82.2% and Bos d 2 in 20.8% of the samples. All samples were above the limit of detection for endotoxin and β-(1,3)-glucan.

In order to classify the levels of allergen and endotoxin exposure in the practices, concentrations were compared with those in employees’ homes (Table 2), which were grouped according to the presence of the specific animal, or the presence of pets for DM and endotoxin. In the case of Equ c 1 and Bos d 2, homes were classified according to whether employees had contact with horses or cattle during their leisure time. In general, analytes’ concentrations varied widely (up to three orders of magnitude). Allergens levels of the animals treated at practices (Can f 1, Fel d 1, Ory c 3, Cav p 1) were significantly higher (up to 30 times) in the practices compared with homes where the animals were not present, but significantly lower (except Ory c 3) compared with the homes with the respective animals. Although not significant, Ory c 3 levels were higher in homes with rabbits compared with levels measured at practices. A similar trend was observed for Equ c 1 where compared with the practices, 11-fold higher Equ c 1 levels were found at the homes of employees who were in contact with horses outside of work. Conversely, employees with no contact to horses outside of work exhibited 6-fold lower median Equ c 1 levels compared with those in the practices. DM levels in the practices were significantly lower in households with and without pets. Furthermore, endotoxin levels did not differ between practices and homes with pets, and were twice as high in practices compared with homes without pets, albeit not significant.

Exposure levels in practices classified by room type
Concentrations of all allergens, endotoxin, and β-(1,3)-glucan were sorted according to room type (Table 3), and classified as rooms with or without animals (Fig. 1). A detailed overview of the Bos d 2 results is not provided due to the large percentage of samples below the LOD and very low concentrations compared with the other allergens (maximum 128 ng m⁻²). The levels of all analytes, except β-(1,3)-glucan were significantly higher in rooms with animals compared with those without, but these differences were small. For example, Can f 1, Fel d 1, and Cav p 1 concentrations only differed by about 2.5-fold. Of all allergens, the highest levels were found for Can f 1 followed by Fel d 1, and the lowest for DM allergens. Can f 1 values were on average twice as high as Fel d 1 and 6-fold higher than Cav p 1. Differences were also found among the individual room types following a similar pattern for all determinants, except Ory c 3 and Equ c 1. In rooms occupied by animals, the highest allergen concentrations were found in the examination room and the lowest in the operating room. In contrast, the highest concentrations of Ory c 3 and Equ c 1 were measured in the inpatient ward and reception/waiting area, respectively. Changing rooms had the highest allergen, endotoxin and β-(1,3)-glucan levels among those where no animals were present. Interestingly, Equ c 1 levels were much higher in changing rooms than in rooms with animals (Table 3).

Influences on exposure levels in practices
Analyte concentrations in the practices were significantly influenced by the room type in the multilevel models (Table 4), confirming the results of the descriptive analysis. In general, the examination room had significantly higher concentrations than the majority of other room types. Equ c 1 was the only allergen where the concentration in the examination room was significantly lower compared with the changing room. The second factor that strongly and significantly influenced exposure levels was the area per employee. The more space available per employee, the lower the allergen and β-(1,3)-glucan exposure. However, this effect was not significant for endotoxin and Cav p 1. Less frequent ventilation led to reduced analyte concentration, which was only significant for Fel d 1, endotoxin, and β-(1,3)-glucan. Cleaning measures, opening hours and room volume had no influence on concentrations of analytes with the exception of DM allergens. In particular, increasing room volume was associated with reduced allergen levels of DM. The highest conditional ICC value was achieved for Equ c 1 (0.76) indicating that about 76% of the total variance in the outcome measure was due to variations between practices. However, no specific practice had an influence on DM allergen concentrations (ICC = 0.001).

Discussion
This is the first study to compare the levels of exposure to allergens from furred animals and selected microbial agents between the workplace and homes of German veterinary practice employees. Although active airborne dust sampling using pumps is the gold standard to assess occupational exposure, we selected EDC for dust sampling for several reasons. Loud noises from the pumps may increase nervousness in animals, thus elevating the risk of injury to
Table 1. Characteristics of practices and homes of practice employees.

| Characteristic                        | Practices (n = 36) | Homes (n = 101) |
|---------------------------------------|--------------------|-----------------|
|                                      | Median       | IQR            | Range         | Median       | IQR            | Range         |
| Size of the practice                 | 145 m²       | 112.5–204.5 m² | 80–420 m²     | 22 m²       | 18–30 m²       | 9–60 m²       |
| Number of rooms                      | 9            | 7–12.5         | 3–18          | 4           | 0–2           | 1–6           |
| Size of the rooms                    | 15 m²        | 12–20 m²       | 4–30 m²       | 1           | 0             | 0–1           |
| Height of the rooms                  | 2.5 m        | 2.5–2.9 m      | 2–4 m         | 1           | 0             | 0–1           |
| Number of employees                  | 5            | 4–8            | 1–14          | 2           | 2–3           | 1–5           |
| Opening hours                        | 29 h week⁻¹  | 24–37 h week⁻¹ | 18–53 h week⁻¹| 12           | 0–2           | 0–1           |
| Treated animal species               |              |                |               |              |                |               |
| Dogs                                  | 40%          | 40–45%         | 35–60%        | 1           | 0             | 0–1           |
| Cats                                  | 40%          | 35–45%         | 24–50%        | 1           | 0             | 0–1           |
| Rabbits                               | 8%           | 5–10%          | 0–20%         | 1           | 0             | 0–1           |
| Guinea pigs                           | 5%           | 3–9.75%        | 0–10%         | 1           | 0             | 0–1           |
| Hamster                               | 1%           | 0–2%           | 0–5%          | 1           | 0             | 0–1           |
| Other animals*                        | 0%           | 0–1%           | 0–5%          | 1           | 0             | 0–1           |
| Cleaning measures                     |              |                |               |              |                |               |
| Wiping every day                      | 24 (66)      |                |               | 12           | 0             | 0–1           |
| Wiping less than every day           | 12 (33)      |                |               | 9            | 0             | 0–1           |
| Vacuuming every day                   | 27 (75)      |                |               | 16           | 0             | 0–1           |
| Vacuuming less than every day        | 9 (25)       |                |               | 20           | 0             | 0–1           |
| Sweeping every day                    | 16 (44)      |                |               | 20           | 0             | 0–1           |
| Sweeping less than every day         | 20 (56)      |                |               | 20           | 0             | 0–1           |
| Pets in the home                      |              |                |               |              |                |               |
| None                                  | 20           |                |               | 20           | 2             | 0–5           |
| Dogs                                  | 51           |                |               | 51           | 1             | 0–5           |
| Cats                                  | 38           |                |               | 38           | 1             | 0–5           |
| Rabbits                               | 4            |                |               | 4            | 1             | 0–5           |
| Guinea pigs                           | 4            |                |               | 4            | 1             | 0–5           |
| Hamster                               | 1            |                |               | 1            | 0             | 0–1           |
| Other animals$                        | 8            |                |               | 8            | 0             | 0–1           |

Contact to animals (outside the home during leisure time) (0%)

| Contact to animals (outside the home during leisure time) (0%) | None | Dogs | Cats | Horses | Cattle |
|---------------------------------------------------------------|------|------|------|--------|--------|
| None                                                          | 10   | 76   | 51   | 24     | 3      |
### Table 1. Allergen, endotoxin, and β-(1,3)-glucan levels in small animal practices and homes of practice employees.

|                   | N  | ND | Median | IQR   | Range   | P-value* |
|-------------------|----|----|--------|-------|---------|----------|
| Can f 1 (ng m⁻²)  |    |    |        |       |         |          |
| Practices         | 304| -  | 793    | 300–1606 | 9-10967 | 0.0193   |
| Homes with dogs   | 52 | -  | 1617   | 563–2163 | 16-17084 | <0.0001  |
| Homes without dogs| 49 | 8  | 34     | 11–73  | <LOD-874 | <0.0001  |
| Fel d 1 (ng m⁻²)  |    |    |        |       |         |          |
| Practices         | 304| 1  | 440    | 180–1058 | <LOD-41254 | <0.0001  |
| Homes with cats   | 38 | -  | 1617   | 362–9009 | 59-166238 | <0.0001  |
| Homes without cats| 63 | 21 | 15     | <LOD-52 | <LOD-324 | <0.0001  |
| Ory c 3 (ng m⁻²)  |    |    |        |       |         |          |
| Practices         | 304| 56 | 282    | 50–81  | <LOD-13880 | <0.0001  |
| Homes with rabbits| 4 | -  | 973    | 230–4342 | 32–5417  | 0.2558   |
| Homes without rabbits| 97 | 82 | <LOD   | <LOD-52 | <LOD-324 | <0.0001  |
| Cav p 1 (ng m⁻²)  |    |    |        |       |         |          |
| Practices         | 304| 52 | 93     | 23–257 | <LOD-4427 | <0.0001  |
| Homes with guinea pigs | 4 | -  | 3972   | 900–6236 | 134–6732 | 0.0011   |
| Homes without guinea pigs | 97 | 50 | <LOD   | <LOD-32 | <LOD-496 | <0.0001  |
| Equ c 1 (ng m⁻²)  |    |    |        |       |         |          |
| Practices         | 304| 38 | 77     | 17–325 | <LOD-8781 | <0.0001  |
| Homes of employees with contact to horses | 24 | -  | 877   | 136–2058 | 20-62009 | <0.0001  |
| Homes of employees without contact to horses | 77 | 18 | 12    | 7–28   | <LOD-1258 | <0.0001  |
| Bos d 2 (ng m⁻²)  |    |    |        |       |         |          |
| Practices         | 304| 237| <LOD   | <LOD- <LOD | <LOD-128 | n.a.     |
| Homes of employees with contact to cattle | 3 | 1  | 21    | <LOD-456 | <LOD-131 | n.a.     |
| Homes of employees without contact to cattle | 98 | 79 | <LOD   | <LOD-456 | <LOD-131 | n.a.     |
| DM (ng m⁻²)       |    |    |        |       |         |          |
| Practices         | 304| 107| 60     | <LOD-163 | <LOD-2597 | n.a.     |
| Homes with pets   | 80 | 8  | 157    | 61–408  | <LOD-84292 | <0.0001  |
| Homes without pets| 21 | 4  | 127    | 40–261  | <LOD-7614 | 0.0495   |
| Endotoxin (EU m⁻²)|    |    |        |       |         |          |
| Practices         | 304| -  | 140    | 72–281  | 23-3362 | 0.0897   |
| Homes with pets   | 79 | -  | 164    | 67–434  | 20-6512 | 0.0839   |
| Homes without pets| 21 | -  | 64     | 39–185  | 16-1380 | 0.0495   |

*other rodents, birds, reptiles.

†gerbil, squirrel, mice, frogs, fish, geckos, axolotl, corn snake, aquatic turtles.

* birds, mice, goats, sheep.

Including mixed use (living room/bedroom, kitchen-living room, living room/office).

### Table 2. Allergen, endotoxin, and β-(1,3)-glucan levels in small animal practices and homes of practice employees.

|                      | N  | ND | Median | IQR   | Range   | P-value* |
|----------------------|----|----|--------|-------|---------|----------|
| Rabbits             |    |    | 14     |       |         |          |
| Guinea pigs         |    |    | 7      |       |         |          |
| Hamster             |    |    | 4      |       |         |          |
| Other animals*      |    |    | 4      |       |         |          |

*other rodents, birds, reptiles.

†gerbil, squirrel, mice, frogs, fish, geckos, axolotl, corn snake, aquatic turtles.

* birds, mice, goats, sheep.

Including mixed use (living room/bedroom, kitchen-living room, living room/office).

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N: number of measurements; ND: number of not detectable samples; IQR: interquartile range; LOD: limit of detection; n.a.: not applicable.

#P-value in comparison to practices (determined in two-level-model), P-values <0.05 are printed in bold

*β-(1,3)-Glucan was determined only in EDC-samples from practices.
Table 3. Allergen, endotoxin, and β-(1,3)-glucan levels in different room types of small animal practices.

| Room category               | Can f 1 (ng m⁻²) | Fel d 1 (ng m⁻²) | Ory c 3 (ng m⁻²) | Cav p 1 (ng m⁻²) | Equ c 1 (ng m⁻²) | Domestic mite (ng m⁻²) | Endotoxin (EU m⁻²) | β-Glucan (ng m⁻²) |
|-----------------------------|------------------|------------------|------------------|------------------|------------------|------------------------|---------------------|------------------|
|                             | N M Range        | M Range          | M Range          | M Range          | M Range          | M Range                | M Range            | M Range          |
| Rooms with animals          |                  |                  |                  |                  |                  |                        |                     |                  |
| Examination room            | 61 2184 21-10967 | 1377 14-6374     | 723 <LOD-5099    | 400 <LOD-4427    | 137 <LOD-4128    | 161 <LOD-1126          | 247 28–1069        | 59 3181 1506–23572|
| Reception/waiting room      | 58 1193 142–10743| 438 9-7939       | 286 <LOD-4770    | 107 <LOD-1463    | 180 <LOD-8137     | 93 <LOD-1481           | 200 36–1453        | 56 3248 329–22564 |
| Operating room              | 33 399 18-6918   | 210 8-7587       | 324 <LOD-2548    | 41 <LOD-1874     | 42 <LOD-565       | 28 <LOD-665            | 84 28–952          | 33 1628 216–3721  |
| X-ray/ultrasound room       | 31 733 60–2973   | 405 19–1478      | 206 <LOD-1306    | 57 <LOD-860      | 133 <LOD-1112     | 52 <LOD-217            | 99 23–856          | 31 2046 714–5801  |
| Inpatient ward              | 16 476 102–2288  | 859 32–2719      | 1198 <LOD-13880  | 173 <LOD-1041    | 35 <LOD-629       | 45 <LOD-599            | 117 42–726         | 16 2102 590–5476  |
| Surgery preparation         | 13 830 166–1303  | 671 163–10031    | 913 <LOD-2465    | 137 <LOD-500     | 79 <LOD-1020      | 58 <LOD-359            | 120 46–309         | 13 2158 395–6085  |
| Rooms without animals       |                  |                  |                  |                  |                  |                        |                     |                  |
| Pharmacy/laboratory         | 30 307 38–2290   | 173 9-1252       | 71 <LOD-2078     | 39 <LOD-1927     | 41 <LOD-1181     | 137 <LOD-2597          | 73 24–600          | 30 2165 432–7420  |
| Break room                  | 28 855 60–5000   | 373 32–2835      | 120 <LOD-1843    | 97 <LOD-637      | 129 <LOD-8781    | 47 <LOD-428            | 161 36–3852        | 28 2457 575–8274  |
| Storage/utility room        | 17 212 9-3276    | 125 <LOD-624     | 52 <LOD-969      | 19 <LOD-123      | 14 <LOD-1069     | 137 <LOD-558           | 53 29–566          | 17 1468 200–7141  |
| Office                      | 9 184 15-1909    | 160 50–661       | <LOD-680        | 32 <LOD-211      | 11 <LOD-254      | 137 <LOD-745           | 81 55–574          | 9 1863 841–2223  |
| Changing room               | 8 901 46–1531    | 566 41-1254      | 356 <LOD-728     | 176 <LOD-492     | 589 <LOD-6764    | 83 <LOD-376            | 259 67–699         | 8 3551 769–13260  |

N: number of measurements, M: median, LOD: limit of detection.
Approximately 66% of all reported accidents in veterinary practices are due to scratches, bites, or kicks from animals (Nienhaus et al., 2005). Moreover, using pumps is expensive, needs trained staff for recharging and calibration, and due to short sampling time (2–8 h) increases the probability of obtaining samples with allergen concentrations below the LOD. In addition, passive dust collection with EDC has proven to be a suitable and practical method in other studies (Krop et al., 2014; Schlünssen et al., 2015; Sander et al., 2018), especially if comparative exposure measurements are conducted in homes, which is dependent on study participants who are not trained in exposure measurement techniques.

**Exposure levels in practices and homes**

**Dogs and cats**

More than 80% of the animals treated at the veterinary practices were cats and dogs. Accordingly, Can f 1 and Fel d 1 were found in all but one sample from all practices. Samples from employees’ homes with dogs or cats were also positive for Can f 1 and Fel d 1, respectively. The percentage of dog (52%) and cat (38%) ownership in this study was much higher than the average ownership (dogs 19%; cats 23%) in Germany (Zentralverband Zoologischer Fachbetriebe e.V.). In addition, there was also a very high percentage of positive samples in homes without dogs (84%) or cats (66%). In comparison, the positive rate in homes of children and day-care center staff was only 39% (Can f 1—homes without dog) and 27% (Fel d 1—homes without cat) (Sander et al., 2018). Allergen levels were found to be approximately 40-fold (Can f 1) and 100-fold (Fel d 1) higher in homes with pets than in homes without, which agrees with earlier studies examining cat and dog allergens in reservoir dust from floors or mattresses (Custovic et al., 1999; Arbes et al., 2004; Heinrich et al., 2006; Stemeseder et al., 2017). Can f 1 and Fel d 1 values were also elevated in the homes of practice employees with and without cats/
|Room type| Examination Room| Reception/waiting room| Operating room| X-ray-ultrasound room| Inpatient ward| Surgery preparation| Pharmacy/laboratory| Break room| Storage/utility room| Office| Changing room|
|---|---|---|---|---|---|---|---|---|---|---|---|
|**N**| 61| 58| 33| 31| 16| 13| 30| 28| 17| 9| 8|
|**exp(β)**| 1.000| 0.632 0.0066| 0.189 <.0001| 0.341 <.0001| 0.232 <.0001| 0.360 0.0002| 0.173 <.0001| 0.445 <.0001| 0.109 <.0001| 0.136 <.0001| 0.399 0.0090|
|**P-value**| <.0001| <.0001| <.0001| <.0001| <.0001| <.0001| <.0001| <.0001| <.0001| <.0001| <.0001|

|Ventilation| daily| occasionally| never|
|---|---|---|---|
|**N**| 169| 76| 59|
|**exp(β)**| 1.000| 0.809 0.1346| 0.790 0.1675|
|**P-value**| <.0001| <.0001| <.0001|

|Wiping| every day| less than every day|
|---|---|---|
|**N**| 202| 102|
|**exp(β)**| 1.000| 1.444 0.2021|
|**P-value**| <.0001| <.0001|

|Vacuuming| every day| less than every day|
|---|---|---|
|**N**| 218| 86|
|**exp(β)**| 1.000| 0.795 0.4473|
|**P-value**| <.0001| <.0001|

|Sweeping| every day| less than every day|
|---|---|---|
|**N**| 148| 156|
|**exp(β)**| 1.000| 0.688 0.1404|
|**P-value**| <.0001| <.0001|

|Opening hours (per 1 h increase)| 304| 0.101 0.4752|
|**exp(β)**| 0.999 0.7050|
|**P-value**| <.0001| <.0001|

|Room volume (per 1 m³ increase)| 304| 0.999 0.7050|
|**exp(β)**| 0.999 0.7050|
|**P-value**| <.0001| <.0001|

|Area per employee (per 1 m² increase)| 304| 0.974 0.0064|
|**exp(β)**| 0.974 0.0064|
|**P-value**| <.0001| <.0001|

|Conditional ICC| 0.36| 0.25|
|**P-value**| 0.63| 0.63|

### Table 4. Influences on allergen, endotoxin, and β-(1,3)-glucan levels in small animal practices determined by multilevel models.

|Can f I| Fel d 1| Ory c 3| Can p 1| Equ c 1| Domestic mite| Endotoxin| β-(1,3)-Glucan|
|---|---|---|---|---|---|---|---|
|**N**| 1.000| 1.000| 1.000| 1.000| 1.000| 1.000| 1.000|
|**exp(β)**| <.0001| 1.000| <.0001| 1.000| <.0001| 1.000| <.0001|
|**P-value**| <.0001| <.0001| <.0001| <.0001| <.0001| <.0001| <.0001|

N: number of measurements; β: regression coefficient; ICC: intraclass correlation coefficient; P-values <.05 are printed in bold.

#: The conditional ICC is a measure of the degree of within-group homogeneity or between-group heterogeneity after controlling for contextual variables.
dogs compared with the homes of those not occupationally exposed to animals (Krop et al., 2014; Sander et al., 2018). The higher levels in our study may be due to (i) the transfer of allergens from the practice to homes via clothing and hair, (ii) higher number of animals (up to six cats or dogs) at home, and (iii) more frequent and intensive contact between study participants and other pet owners or pets outside their home.

In practices, Can f 1 levels were approximately twice as high as Fel d 1 values (809 versus 440 ng m⁻²), although similar percentages of cats and dogs were examined (mean: 40% for cats and 44% for dogs). One reason is that dogs may release more allergens due to their higher average body mass. Similar Can f 1 (720 ng m⁻²), but lower Fel d 1 (56 ng m⁻²) levels were previously reported in a companion animal hospital using EDCs; however, cats represented only 15% of animals treated in this study (Samadi et al., 2010).

Although concentrations of 1 µg g⁻¹ for Fel d 1 and 2 µg g⁻¹ for Can f 1 have been associated with allergic sensitization and 8 µg g⁻¹ for Fel d 1 and 10 µg g⁻¹ with asthma symptoms in sensitized individuals (Salo et al., 2009), these thresholds have not been established so far. Anyway, no such risk levels have been suggested for airborne samples. Nevertheless, it has been demonstrated that respiratory symptoms were induced in sensitized individuals with a brief exposure to airborne levels of Feld 1 that were found in many homes with cats and occasionally in homes without cats (BOLLINGER et al., 1996). For EDC samples, Sander et al. defined exposure levels that predicted the presence of dogs (≥75 ng m⁻² for Can f 1) and cats (≥46 ng m⁻² for Fel d 1) in dwellings (Sander et al., 2018). In our study, 280 out of 304 practice rooms had concentrations that exceeded both values, indicating probable health risk for veterinary staff.

**Rabbits and guinea pigs**

This is the first study measuring allergens from rabbits (Ory c 3) and guinea pigs (Cav p 1) using newly developed immunoassays. Both were examined in 35 or 34 of 36 investigated practices and represented 9 and 6% of all treated species, respectively. Accordingly, the percentage of positive samples in practices, and the median allergen levels for both species were lower compared with cats and dogs. The median Cav p 1 level (93 ng m⁻²) was three-times lower than the Ory c 3 level (282 ng m⁻²), which may be related to the smaller surface of guinea pigs compared with rabbits. According to a survey (7000 households) by the Central Association of Zoological Specialized Companies, approx. 5% of households in Germany have a small animal (except cats and dogs) (Zentralverband Zoologischer Fachbetriebe e.V.). Among the practice employees, the percentage of small animal ownership (rabbit, guinea pig, and hamster) was almost twice as high (mean 9%). In all homes with rabbits or guinea pigs, allergens of the respective species could be detected; whereas, the median allergen level was below the LOD in households without. Similar to our study, another allergen Ory c 1 was found in dust from all households with rabbits and was non-detectable in control dwellings (Willerton and Mason, 2018).

**Horse**

The very high percentage of Equ c 1 positive samples in practices and homes (over 70%) was surprising. The possibility that our Equ c 1 assay cross-reacts with the structurally related cat and dog allergens, Fel d 4 and Can f 6 (Hilger et al., 2012; Nilsson et al., 2012; Yamamoto et al., 2019) was excluded by testing recombinant Feld 4 and Can f 6, as well as cat and dog allergen preparations (data not shown). The specific recognition of Equ c 1 was also supported by showing that employees’ homes with horse contact had considerably higher Equ c 1 values (70-fold) compared with those without. This strongly indicates that allergens can be easily spread to non-animal environments due to passive transfer via clothing or hair. Compared with the allergen levels in homes with pets (1416 ng m⁻² for Can f 1; 1617 ng m⁻² for Fel d 1), the relatively high Equ c 1 value (877 ng m⁻²) found in homes of employees in contact with horses indicates that animal size influences amount of allergen transferred.

**Domestic mite**

DM allergen levels were 2–3 times lower in practices than in homes, with or without pets. One reason may be increased cleaning frequency in practices compared with homes, and the absence of upholstered furniture, beds, and carpets—the most important indoor mite habitats (Colloff, 2009; Solarz and Pajak, 2019)—and thus a reservoir for mite allergens (Sander and Neumann et al., 2016). The DM allergen level in practices (median 60 ng m⁻²/2 weeks) was also much lower than the levels measured in Dutch schools (geometric mean 133.5 ng m⁻²/week (Krop et al., 2014)) or German day care centers (median 364 ng m⁻²/2 weeks (Sander et al., 2018)) using the same sampling and quantification methods. No significant difference was seen in DM levels between homes with and without pets (P = 0.255), which is consistent with other studies showing that pet presence at homes has no significant effect on DM allergen levels on EDC (Krop et al., 2014;
Sander and Neumann et al., 2016; Sander et al., 2018). In contrast, rooms occupied by animals in practices had significantly higher DM allergen levels than those without, probably due to increased use and therefore enhanced air disturbance in these rooms. In undisturbed conditions, airborne mite allergen concentrations are mostly undetectable (Paufler et al., 2001).

Endotoxin and β-(1,3)-glucan exposure
In general, direct comparison of endotoxin and β-(1,3)-glucan levels among different studies is often hampered by methodological differences. Measuring both microbial agents can be performed using various quantitative assays (from different manufacturers or laboratories) that produce different nominal values that do not always correlate (Brooks et al., 2013; Liebers et al., 2020). Furthermore, extraction procedure and subsequent storage can influence the results. For example, endotoxin activity in frozen samples was significantly lower than in fresh samples, and adding the detergent Tween 20 to the extraction medium generated significantly higher endotoxin values (Liebers et al., 2007; Spaan et al., 2008). Therefore, comparisons of endotoxin levels are only appropriate within a study. In our study, endotoxin levels were slightly lower in practices than in homes with pets, and twice as high as in homes without pets. Significant difference was obtained between houses with and without pets (P = 0.005). This is consistent with other studies reporting that pets (dog or cat) significantly contribute to elevated endotoxin values (Liebers et al., 2007; Spaan et al., 2008). This is also seen in the practices, where rooms occupied by animals had significantly higher endotoxin levels than rooms without animals (161 versus 100 EU m⁻²). In the companion animal hospital, highest endotoxin exposure levels were measured in areas with close contact to animals (Samadi et al., 2010).

Influences on exposure levels within the practices
Room type significantly influenced exposure levels in practices. Most of the investigated analytes were similarly distributed throughout the practices, with the highest concentrations in examination rooms and the lowest in operating rooms. Examination rooms tend to be used more frequently and for longer periods compared with other rooms with animals. In addition, stress of examination may result in more active animals. Conversely, in operating rooms, animals are usually sedated, and these areas are cleaned and disinfected regularly. Consistent with our results, a Dutch study also found the lowest Can f 1 and Fel d 1 levels in the operating room (Samadi et al., 2010). However, Can f 1 levels were lower in the examination room than the waiting or ultrasound room. In rooms without animals, changing rooms had the highest allergen and endotoxin levels, supporting the hypothesis that clothing is an important allergen carrier. For Equ c 1, the main route of allergen transfer into practices may be via clothing worn by employees who had contact with horses. Pet owners who ride horses may also bring allergens into the practice, explaining the higher levels of Equ c 1 in the waiting room compared with the examination room. Significantly high levels of cow hair allergens were also discovered in cattle farmers’ changing rooms due to allergen transfer from stables (Zahradnik et al., 2011). Apart from room type, exposure was strongly influenced by the area per employee. Allergen and β-(1,3)-glucan levels decreased with lower occupancy, which may be due to lower air and dust turbulence. This is supported by higher allergen levels measured in break rooms (used by all employees) compared with individual offices (mainly used by only one person). More frequent ventilation (per window) did not reduce exposure levels. In fact, slightly lower exposure was observed in rooms that were never ventilated, although the difference was significant only for Fel d 1, endotoxin, and β-(1,3)-glucan. This may be also explained by lower dust turbulence in non-ventilated rooms. Finally, more frequent cleaning measures had no influence on exposure levels, but the differences between ‘cleaning every day’ and ‘less than every day’—which we used for grouping—might be rather small.

Conclusions
Our data demonstrated that high exposures to animal allergens occur in certain areas of veterinary practices, while the mite allergen levels were low. Although the animal allergen concentrations in veterinary practices were lower than in households with the corresponding animals, it cannot be excluded that such levels are sufficient to cause symptoms in allergic patients. A practical option to reduce the allergen levels could be the use of air filtration systems or portable air cleaners with HEPA filters. To prevent the transfer of allergens from the workplace to the home and vice versa, it would be important to avoid contamination of clothing as much as possible.

Supplementary Data
Supplementary data are available at Annals of Work Exposures and Health online.
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Conflict of interest
The authors declare no conflicts of interest.

Data availability
The data underlying this article are available in the article and in its online supplementary material.

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