# Characterization of relevant bovine dander allergen components

**Running title:** Occupational bovine dander allergy

Airaksinen L[1], Suojalehto H[1], Lindström I[1], Sund J[1], Pallasaho P[2], Puustinen A[3]

[1]Finnish Institute of Occupational Health

[2]City of Espoo

[3]Helsinki University

**Correspondence**:

Liisa Airaksinen

Finnish Institute of Occupational Health, PO Box 40, FI-00251 Helsinki, Finland

E-mail: liisa.airaksinen@ttl.fi



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**Abstract**

**Background:** The diagnostics of allergic occupational diseases is highly dependent on the quality of the allergen extracts and specific IgE tests available. To enhance the diagnostics of bovine-related occupational rhinitis, asthma and urticaria, we produced an in-house cow dander extract, assessed its allergen profile and performance in clinical tests, and compared it to commercial bovine dander extracts.

**Methods**: One hundred patients with a suspected bovine-related occupational disease underwent skin prick tests (SPTs) with in-house and one to two commercial bovine dander extracts. Nasal allergen provocation tests were performed on 31 patients with suspected occupational rhinitis. We used Western blot to study the specific IgE-protein reactions from the serums of the patients with positive provocation tests, and identified allergens from immunoblot bands using tandem mass spectrometry.

**Results**: Odorant-binding protein Bos d OBP, bovine serum albumin Bos d 6, and lipocalin Bos d 2 were identified as the major allergens. We found altogether 24 bovine dander allergens, of which several were formerly unknown. The in-house extract sensitivity and specificity in SPTs were 100% and 94%, in 87 patients respectively and SPTs appeared negative in 20 healthy controls. Nasal allergen provocation tests with inhouse extract detected occupational rhinitis with 100% sensitivity in 21 patients. Five healthy controls remained negative in the provocation tests.

**Conclusions:** Three major and several minor allergens were found from bovine dander as a cause of occupational rhinitis. A sufficient concentration and variety of tested allergens were essential in the diagnostics of bovine-related occupational diseases.

**Key words:** Animal worker. Rhinitis. Asthma. Cow. Allergy. Occupational disease. Farming. Sensitization. Skin prick test.



**Resumen**



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**Antecedentes:** El diagnóstico de las enfermedades profesionales alérgicas depende en gran medida de la calidad de los extractos alergénicos y de las pruebas de IgE específicas disponibles. Para mejorar el diagnóstico de la rinitis ocupacional, el asma y la urticaria relacionados con los bovinos, se hizo un extracto de caspa de vaca propio, se evaluó su perfil de alérgenos y su rendimiento en pruebas clínicas y se comparó con extractos de caspa bovina comerciales.

**Métodos:** Cien pacientes con sospecha de enfermedad ocupacional relacionada con bovinos se sometieron a pruebas de punción cutánea (SPT) con extractos de caspa bovina propios y uno o dos comerciales. Se realizaron pruebas de provocación nasal con alérgenos en 31 pacientes con sospecha de rinitis ocupacional. Se realizó un Western blot para estudiar las uniones específicas de las proteínas a la IgE de los sueros de los pacientes con pruebas de provocación positivas, se identificaron los alérgenos de las bandas de inmunotransferencia usando espectrometría de masas en tándem.

**Resultados:** La proteína fijadora de olores (OBP) Bos d OBP, la albúmina sérica bovina Bos d 6 y la lipocalina Bos d 2 se identificaron como los principales alérgenos. Se encontraron un total de 24 alérgenos de caspa bovina, de los cuales varios eran desconocidos anteriormente. La sensibilidad y la especificidad del extracto interno en las SPT fueron del 100 % y del 94 %, en 87 pacientes respectivamente, y las SPT resultaron negativas en 20 controles sanos. Las pruebas nasales de provocación con alérgenos con extracto interno detectaron rinitis ocupacional con una sensibilidad del 100 % en 21 pacientes. Cinco controles sanos resultaron negativos en las pruebas de provocación nasal.

**Conclusiones:** Se encontraron tres alérgenos mayores y varios menores de la caspa bovina como causa de la rinitis ocupacional. Una concentración suficiente y una variedad de alérgenos probados fueron esenciales en el diagnóstico de enfermedades profesionales relacionadas con los bovinos.

**Palabras clave:** Granjero. Rinitis. Asma. Vaca. Alergia. Enfermedad profesional. Agricultura. Sensibilización. Prueba de punción cutánea.





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**Introduction**

Bovine (*Bos domestic*, *Bos d*) epithelia allergens are a frequent cause of allergy and a significant occupational problem among agricultural cow farmers who provide meat and especially dairy products. Bovine dander is causative in most cases of animal-induced occupational rhinitis (OR), occupational asthma (OA) and occupational contact urticaria in Finland [1, 2, 3]. The German Cattle Allergy Study found that 9.1% of 5627 farmers had occupational airway diseases due to cattle allergies [4]. Cattle allergy may have significant economic consequences and cause occupational disability in affected farmers and workers [4]. In Europe, cattle farmers are known to be at an increased risk of developing allergic reactions to cattle: up to 20% of cattle farmers are sensitized to cattle allergens (dander, hair, meat and milk), which are found throughout cattle stables and farmers' homes [5].

Population-based studies have found the highest median concentrations of bovine hair allergens to be in the milking parlour (7154 µg/g), followed in decreasing order by concentrations in the computer room in the cowshed (2165 µg/g), the changing room (380 µg/g), the living room (109 µg/g), and finally the bedroom (63 µg/g) [6]. In cowsheds, the concentration of bovine hair allergens from conventional milking systems have been reported as being higher than those from automatic milking systems [7]. In cold climate zones generally, exposure increases during the winter when cattle are kept indoors.

Several different sized bovine dander antigens have been determined, a major one of which is the 22 kD allergen, called lipocalin Bos d 2. This is the best characterized bovine dander major allergen and has three variants. It has also been found in cattle urine [8]. Bos d 3, the 11 kD Ca-binding protein S100 homologue, is a minor allergen in bovine dander [9]. Alpha lactalbumins Bos d 4 (14 kD), Bos d 5 beta-lactoglobulin (18 kD), and 20-30 kD caseins are also bovine dander minor allergens, and are best known as milk allergens causing food allergy [10]. Further allergens identified in bovine dander are 67 kD bovine serum albumin (Bos d 6), and 160 kD immunoglobulin (Bos d 7) [11].

Allergen standardization in occupational allergic disease diagnostics is inconsistent: a missing or too low protein and allergen contents have been noted in the skin prick test (SPT) extracts of occupational allergens, and this hampers the diagnosis of OR and OA [12,13]. Recently, quantification of individual allergens has been the main focus of allergen standardization, because the allergenicity of most allergen extracts is known to be dependent on the content of a small number of allergen molecules. The concentration of individual major allergens is reported to correlate with the biological potency and IgE reactivity of allergen extracts [14].





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In this study, we produced an in-house bovine dander extract and investigated whether it 1) increased the prompt diagnostics of occupational allergic diseases and 2) assessed clinically relevant new bovine allergens.

## Participants and Methods

### Patients

Altogether 102 consecutive Finnish patients (48 men, 54 women) who were exposed at work to bovine dander and had a suspected bovine-related occupational disease between 2009–2012 were routinely tested by SPTs. Two patients were excluded due to wheals from the negative control. The mean age of the patients was 44 (range 22–61). A subset, 87 of the 100 subjects, was tested for bovine epithelia specific IgE (sIgE), (Phadia UniCAP, Uppsala, Sweden). A placebo-controlled nasal allergen provocation test (NPT), a golden standard test to diagnose allergic rhinitis, was performed on 31 of the patients with a history of rhinitis in relation to bovine contact and a detected (sIgE or SPT) sensitization to cows, to confirm possible OR (Figure 1). Due to practical reasons, only 21 of these patients were tested in NTP with both commercial and in-house allergen extracts and ten with only in-house allergen.

Twenty healthy adult volunteers participated as controls for the in-house bovine dander extract SPT and five as NPT controls. The participants gave their informed consent. The study and the use of patient databases was approved by the study institute's administration and the coordinating ethical committee of Helsinki University Central Hospital (130/13/03/00/2011).

### Preparation and testing allergen extract

Bovine dander was brushed from the necks of several breeds of healthy animals. Allergens were extracted by mixing 1 g of the dander in 20 ml of $NaHCO_3$ for 16 hours at +6–7 °C [16]. The mixture was filtered through a 0.07 mm nylon net to remove debris and centrifuged for 20 minutes with 5000x g. Supernatant was concentrated with ultrafiltration (3000 Da cut-off), after which it was sterile filtrated using 0.45 µm and 0.2 µm filters (Millipore Corporation, Bedford, MA, USA). The manufacturing was done aseptically. We measured the protein concentration using modified Lowry (BioRad, Hercules, CA, US), and the concentrations of endotoxins were defined at the Kuopio unit of the Finnish Institute of Occupational Health (FIOH), which is accredited as a testing laboratory T013 (EN ISO/IEC 17025) by the Finnish Accreditation Service. Measurements were made using a standard (SFS-EN 1 4031:2003) kinetic chromogenic method based on the Limulus amoebocyte lysate. We confirmed the similarity of the different extract batches by comparing the protein composition in SDS-PAGE and IgE reactivity intensity in the immunoblots of the new and previous preparations. Unspecific SPT and NPT reactivity was tested on 20 healthy volunteers: SPTs with an



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extract protein concentration of 22 mg/ml in 20 volunteers, and NPT with 2 mg/ml in five volunteers. The Finnish Medicines Agency permitted the preparation, storage and use of animal dander allergens for SPTs and NPTs at FIOH.

**Skin prick tests and bovine epithelia-specific IgE**

SPTs with common environmental allergens were performed using standardized allergen extracts of birch, alder, grass and mugwort pollens, cat, dog, horse, *Dermatophagoides pteronyssinus, Dermatophagoides farinae, Alternaria alternata, Cladosporium herbarum* (ALK-Abello, Hørsholm, Denmark), and diluent and histamine hydrochloride (10 mg/ml) as controls. We defined a positive SPT reaction, classified as sensitization, as a wheal diameter of at least 3 mm in the absence of a reaction to the diluent and in the presence of a reaction to histamine hydrochloride [17]. All patients were tested with the commercial bovine-epithelia SPT extracts (ALK 1:100 w/v, and from 22.9.11 onwards, 27 patients were also with Allergopharma (ALP) 317 10000 BU/ml) together with the in-house extract. To find the suitable concentration to test the patients, we initially used 5 mg/ml to test ten patients, then raised the concentration to 20-22 mg/ml to test 60 patients. After large 10–13 mm SPT wheals appeared on four patients, the routine test concentration was finally adapted to 2 mg/ml and tested on 30 patients. Bovine epithelia sIgE values of ≥0.35 kU/l were classified as sensitized. OA was diagnosed in sensitized patients with work-related asthma symptoms by placebo-controlled positive inhalation challenge to bovine extract (ALP) [15]. A sustained fall of ≥15% from the pre-challenge value of $FEV_1$ was considered positive, provided that $FEV_1$ fluctuations were <10% on a control day. In addition, positive workplace PEF monitoring combined with detected sensitization to bovine was considered diagnostic for OA.

**Nasal provocation tests**

To confirm OR and to compare the clinical relevance of the different allergen extracts, NPTs with both in-house extract and the available bovine dander extract (ALP 317) were performed on 21 patients with a history of rhinitis in bovine contacts and verified bovine dander sensitization based on SPTs or sIgE. Ten patients' NPTs were carried out using only the in-house extract, due to practical reasons (Figure 1). NPTs were performed using the in-house extract at a dilution of 2 mg/ml. The commercial allergen extract was concentrated to eight times the recommended strength, i.e., to 40000 BU/ml. We followed the published NPT recommendations [18, 19].

In the NPTs, we scored the bilateral nasal secretion from dry mucous membrane (0) to mucous dropping out of the nose (3) and blockage from no swelling (0) to completely obstructed nose (3) observed in anterior rhinoscopy before and after the provocation [18]. Score changes of ≥ 4 points



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were considered positive if the placebo provocation tests with the allergen dilution were negative or if the allergen reactions were many times stronger than the placebo test and we could identify no temporary reason for nasal hyperreactivity. In addition to scoring, we measured the nasal secretion amounts in 35 minutes and nasal volume change ($cm^3$) at 2–5 cm from the nostril (acoustic rhinometer A1, GM instruments UK) and nasal resistance changes (Pa/cc/s %) with active anterior rhinomanometry (GM instruments rhinomanometer NR6-2, UK). We followed the recommendations for the Standardization Committee on Objective Assessment of the Nasal Airway [19]. Nasal secretion was measured in accordance with Pirilä, but bilaterally [20]. Positive NPT reactions in sensitized patients, with a history of rhinitis in contact with cows, were diagnosed with OR.

**Identification and IgE-immunoblotting of allergens from bovine dander extracts**

Proteins from the in-house and commercial extracts (ALK 556 and ALP 317) were separated under reducing 12% SDS-PAGE conditions. One gel was silver stained, and the other was immunoblotted with pool sera from ten previously collected bovine dander allergic rhinitis patients and with 34 participants' individual sera (24 NTP positive and 10 SPT and IgE negative controls, Fig 1). Immunoblotting was performed as described previously, [22] with a few modifications. Briefly: We transferred proteins by electrophoresis (MiniTransBlot, BioRad, Hercules, CA, US) from the SDS-PAGE gels onto the PVDF membranes (Immobilon-P, Millipore Corporation, Bedford, MA, USA). The non-specific binding of antibodies was blocked by a non-ionic Tween®-20 detergent (0.1%), which does not interfere with protein identification from the immunoblot by LC-MS/MS. We then incubated the membranes with diluted (1/5) patients' sera overnight at +4°C. Biotinylated goat anti-human IgE (1:1000, Vector Laboratories Inc., Burlingame, CA, US) was followed by 1:12000 diluted streptavidin-conjugated alkaline phosphatase (Invitrogen, Waltham, MA, US) and the colour development solution (BioRad, Hercules, CA, US). Pooled positive patient sera was added to each patient blot to compare the intensities of the positive bands between patient blots.

To identify the allergens, we performed both in-gel and in-membrane trypsin digestions on the chosen bands, and the resulting peptides were extracted as previously described [23]. The peptide extracts were dried in a vacuum centrifuge. Each peptide mixture was analysed by automated nanoflow capillary LC–MS/MS, using an EASY nanoLC 1000 (Proxeon, Thermo Fisher Scientific Inc., Waltham, MA, US) coupled with an electrospray ionization quadrupole orbitrap mass spectrometer (QExactive, Thermo Fisher Scientific Inc., Waltham, MA, US). We reverse-phase separated the peptides using a 75 μm × 15 cm Acclaim PepMap100 C18 column (Dionex, Thermo Fisher Scientific Inc., Waltham, MA, US) at a flow rate of 300 nl/min. Peptides were eluted from the column at a linear gradient of 5–35% solvent B (0.1 % formic acid in 95% acetonitrile) in 80 minutes. Solvent A was 0.1% formic acid in 5% acetonitrile. The obtained mass fragment spectra were searched in Uniprot





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database (www.uniprot.org) against human entries using Proteome Discoverer 1.4 (Thermo Fisher Scientific Inc., USA).

**Statistics**

Differences between binomial variables were tested using the McNemar test for dependent variables, and the Chi-Squared test for independent variables. We tested the differences between the continuous variables using the Wilcoxon signed rank test for dependent variables, and the Mann–Whitney U-test for independent variables. Statistics were calculated using SPSS Statistics 22 (IBM, Armonk, NY, US). Two-by two tables were used to calculate the sensitivity and specificity of in-house extract SPT and NPT.

**Results**

**Sensitization**

We analysed the SPTs with in-house extract and 1-2 commercial bovine dander extracts of 100 patients. In-house extract gave 44 positive SPT reactions and ALK extract 34 positive reactions in 100 patients. ALP extract was positive in 3 out of 27 tested subjects. Sixty-three of the patients had sensitization to ubiquitous allergens in SPT. Figure 1 summarizes the suspected work-related diagnoses and test results done to these patients.

The in-house extract yielded 100% sensitivity and 94% specificity in SPTs compared to the commercial sensitization tests (Table S1). The mean wheal size was larger (2.7 mm) with the in-house extract than with the commercial extracts (1.6 and 0.4 mm, respectively, data not shown). No generalized reactions appeared in the tests. SPTs with the in-house extract were negative in all 20 healthy volunteers. Altogether 11 patients were diagnosed with OA to cows: nine with workplace PEF monitoring, one with a positive inhalation challenge test, and one case with ceasing of asthma after giving up of the cattle.

**Nasal provocation tests to the bovine dander extracts**

Table 1 presents the characteristics of the 31 patients who underwent NPTs. Five patients had negative and 26 positive reactions together with negative control challenges, confirming the suspected OR. A total of 55% of the patients had asthma, and 48% were sensitized to the tested common environmental allergens. A further 83% of the patients had elevated sIgE to bovine dander.

Of the 21 patients who underwent NPTs to both in-house and concentrated commercial extract, 13 (62%) reacted to only the in-house extract, whereas five (24%) had positive reactions to both extracts





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($p<0.001$) (Table 2). In-house extract sensitivity was 100%, but specificity 18%. In the NPTs with the in-house extract, we detected larger secretion amounts ($p=0.001$) and greater nasal congestion in acoustic rhinometry ($p=0.001$) and rhinomanometry ($p=0.032$) than in the NPTs with commercial extract. No generalized reactions occurred. All five healthy volunteers had negative NPTs to the in-house extract.

**Serum IgE reactivities to allergens**

Figure 2A presents an immunoblot of the bovine dander extracts (two in-house batches and two commercial batches) together with the patient sera. We found no differences between the protein composition or serum IgE reactivity of the separate in-house batches used in this study. The three major bovine epithelial allergens identified in the in-house extract were odorant-binding proteins (Bos d OBP), bovine serum albumin (Bos d 6), and lipocalins (Bos d 2), followed by several minor allergens such as alpha-2 macroglobulin, ceruloplasmin, lactotransferrin, and fibronectin (Figure 2 B, Table 3). Identification scores (Mascot) were thousands for the main allergens, and around 100 for others. Lipocalin Bos d 2 variants were identified in both commercial extracts. Cow epithelia from ALP also contained Bos d 6 albumin.

**Discussion**

To our knowledge, this is the first report on the use of an in-house bovine dander extract in both SPTs and NPTs for diagnosing OR. Altogether 24 allergens were detected in the NPT positive rhinitis patients. Among these, the major allergens were Bos d OBP, bovine serum albumin Bos d 6 and lipocalin Bos d 2. Several of the identified allergens had not previously been reported.

Our results confirm earlier reports that sensitivity to occupational SPT extracts vary between different producers [12,13]. In both the SPT and NPT tests, the in-house extract induced more positive allergic reactions than the commercial products. This finding is well in line with the majority of earlier findings. Heutelbeck et al. suggested [24] that if bovine dander allergy test results with commercial extracts are contradictory to the clinical symptoms, they should be supplemented by using extracts of the hair of the farmers' own cattle. In accordance with these reports, two studies have compared commercial bovine dander SPT epithelial allergens and concluded that their sensitivity is low; they have also found solutions with higher protein and antigen content to have higher sensitivities and test efficiencies [11,12]. They called for the standardization of occupational extracts for SPT. Our results indicate that the need for standardization also extends to provocation test extracts. The commercial SPT solutions contained glyserol, that prevented direct protein concentration comparison. After a buffer exchange the measured commercial concentrations were low, in line with the earlier findings.





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The concentration of bovine dander extract in our SPT varied during this study hampering the exact evaluation of sensitivity and specificity. Our initial concentration of 20-22 mg/ml was too high for a standard allergy testing for the sensitized patients, shown in the large SPT reaction among four patients. Our in-house extract was well concordant with sIgE results and ALK SPT results, but ALP detected sensitization poorly, (figure 1). Our healthy 20 controls group, however, were all negative with it, indicating good specificity of the test. Our final protein concentration of 2 mg/ml was is suitable for both SPT and NPT.

The allergenic potency in the crude in-house extract seemed to be superior to that of the commercial extracts, due to sufficient protein concentration to clinical diagnostics and wider allergen variety. One to two of the three main allergens in the in-house extract, *Bos d 6*, bovine serum albumin and *Bos d OBP*, odorant-binding protein, seemed to be missing from the commercial extracts altogether. The commercial allergens contained mainly *Bos d 2* lipocalin 18-20 kD variants. Both OR and OA diagnostics were hampered by the insufficient potency of the commercial extract.

Numerous major bovine dander allergens have been reported earlier; for example, in a study using German cattle [24]. Our immunoblot result corresponds to these earlier findings, which reported relevant bovine dander allergens with several molecular weights in more than 50% of farmers with cattle-related symptoms [24]. Our in-house bovine dander extract also contained a few allergens from bovine saliva and milk in very low amounts.

Only solitary reports of occupational respiratory allergy to *Bos d 6*, bovine serum albumin (BSA) have been published [25,26]. Although 40% of the control patients were sensitized to Bos d 6, the sensitization rate was over twice this amount, 86%, among the NPT test positive patients. Albumins are ubiquitous animal allergens, usually considered minor allergens, [27,28] but their relevance as respiratory allergens have not been fully explored [29]. Patients with persistent milk allergy and specific immunoglobulin E to BSA are reported to be at a greater risk of rhinoconjunctivitis and asthma from animal dander [30]. The first contact with BSA usually comes orally through cow's milk, and milk allergic patients develop sensitization to BSA without direct contact with animals. We were not particularly looking for oral allergies, but pasteurized milk is typical in the Nordic diet, and according to medical history, occupationally sensitized patients normally tolerate ingested milk. It is noteworthy that sensitization to bovine serum albumin has been identified as a cause of some generalized allergy reactions to pharmaceutical excipients in artificial insemination, surgery tissue adhesives and vaccines [31, 32,33]. However, an early study concluded that bovine serum albumin





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was of marginal significance for respiratory cow allergy diagnostics [34]. SIgE to several mammalian albumins are nowadays commercially available [34].

Most mammalian-derived respiratory allergens belong to the lipocalin superfamily of proteins. Previously, *Bos d 2* lipocalin variants have been the only major occupational bovine allergens identified [35]. Our data indicated another bovine lipocalin family allergen, an odorant-binding protein, as the third major bovine dander occupational allergen. Bos d OBP has previously been reported as a putative allergen in bovine colostrum [36] and one Bos d OBP has previously been identified as cross-reactive to the dog dander *Can f 4* allergen, which also belongs to odorant-binding proteins [37].

In addition to these three major cow dander allergens, lipocalins, OBP and albumins, the study identified several other proteins that orchestrated the allergic inflammation [38]. A known bovine allergen *Bos d 3*, is an antimicrobial calcium-binding protein S100A, a principal effector molecule of epithelial mucosal immunity and a regulator of keratinocytes function. It is found in amniotic fluid. Its homology in humans is called psoriasin (or S100A7) [39].
Caseins are the principal protein of milk. They have previously been identified as allergens in asthmatic farmers, and milk-derived pure or modified casein has been described as an occupational allergen in a few case-reports of people handling products containing it [40, 41]. Cathelicidins in turn are proteolytically activated antimicrobial and antiviral peptides, and human cathelicidin LL37 has been found to act in other roles as an eosinophil and neutrophil chemoattractant [42]. Fibrinogens in turn are essential cofactors in initiating exogenous proteinase-derived allergic reactions and eosinophil degranulation [43].
Lactoferrins belong to the lipocalin protein family and are also known milk allergens. A case of lactoferrin-induced occupational asthma has previously been described in workers handling a powdered milk product [44]. One of the most abundant proteins secreted to the airway surface liquid by epithelial and nonepithelial cells is Gelsolin. Its concentration has been found to be higher in patients with asthma [45]. Ceruloplasmin is a glycoprotein which functions in, for example, antioxidant defence and homeostasis. It has been suggested that its oxidase activity is an indicator of inflammation of allergy, especially among asthma patients [46].

**Conclusions**

Bovine dander contains several potential allergens that can cause IgE-mediated occupational diseases. The characterization of relevant bovine dander allergen components using the in-house extract in this study may promote the development of component standardized diagnostic products in the future.





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Currently, the use of in-house extracts is necessary in the diagnostics of immediate occupational allergies to bovine dander.

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**Conflicts of interest**

The writers declare no conflicts of interests.

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**Table 1**. Characteristics and sensitization of 31 patients who underwent nasal provocation tests using in-house extract. Data presented as means and ranges unless otherwise stated.

| | NPT with in-house extract positive n=26 | NPT with in-house extract negative n=5 | P-value |
|---|---|---|---|
| Age | 41 (23–56) | 53 (41–61) | 0.007 |
| Males n (%) | 17 (65) | 3 (60) | 0.146 |
| Bovine dander exposure, years | 15 (3–31) | 24 (8–40) | 0.115 |
| Current smoking n (%) | 11 (42) | 4 (80) | 0.019 |
| Rhinitis symptom, years | 5 (1–13) | 5 (1–10) | 1.00 |
| Asthma n (%) | 13 (50) | 4 (80) | 0.217 |
| Occupational skin disease to bovine dander n (%) | 8 (31) | 2 (40) | 0.686 |
| Sensitization in SPT n (%) common environmental allergens | 13 (50) | 2 (40) | 0.682 |
| Total IgE kU/l | 189 (17–1555) | 239 (5–496) | 0.448 |
| Bovine dander sIgE ≥0.35 n (%) kU/l | 20 (83) * 5.10 (0.01–34.1) | 4 (80) 1.93 (0.11–3.86) | 0.858 |
| SPT with bovine dander (in-house) positive n (%) size mm | 25 (96) 6.8 (0–13) | 4 (80) 5.0 (0–8) | 0.178 |
| SPT with bovine dander (ALK) positive n (%) size mm | 20 (77) 3.5 (0–6) | 3 (60) 2.6 (0–5) | 0.428 |

* n=24





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**Table 2.** The results of nasal provocation tests of 5 healthy controls and those 21 patients, to whom the tests were performed with both commercial and in-house bovine dander extracts.

| | Healthy controls n=5 | Patients with suspected occupational rhinitis n=21 | | | |
|---|---|---|---|---|---|
| | In-house extract | Saline control | Commercial ALP[§] bovine dander extract* | In-house bovine dander extract* | *P-value between test results with commercial and in-house extracts |
| Positive reaction[#] n (%) | 0 (0) | 0 (0) | 5 (24) | 18 (86) | <0.001 |
| Secretion amount (g) mean (range) | 0 (0) | 0 (0) | 0.2 (0–1.6) | 3.8 (0–11.3) | 0.001 |
| Nasal volume decrease cm$^{3}$[§] ≥30% n (%) | 0 (0) | 1 (5) | 1 (5) | 9 (43) | 0.001 |
| mean % (range) | -1 (-18–27) | 3 (-43–32) | 4 (-12–30) | 27 (-22–81) | |
| Nasal resistance increase Pa/cc/s >100% n (%) | 1(20) | 1 (5) | 1 (5) | 10 (48) | 0.032 |
| mean % (range) | 37 (-17–111) | 11 (-68–111) | 22 (-65–134) | 175 (-84–1534) | |

[§] nasal cavity volume at distance of 2–5cm from the nostril. [#] rhinorrhoea and mucosal swelling ≥4.
[§]Allergopharma



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**Table 3.** Allergen identifications from immunoblot of in-house cow dander extract with patient sera. Proteins of same size are listed in order of identification scores (Mascot).

| WB band | Identification (Mascot score) | Molecular weight (kD) | Allergen name | Homology with | NPT positive patients[§] reactivity % (n=24) | Controls[*] reactivity % (n=10) |
|---|---|---|---|---|---|---|
| 1 <15 kD | Protein S100-A7 (2 967) | 11.5 | Bos d 3 | | 25% | 0% |
| | Similar to Allergen Fel d I-B chain (2190) | 12.2 | | | | |
| | Beta-defensins (283) | 4-7 | | plant defensins | | |
| 2 15 kD | **Allergen Bos d 2** (891) | 19.5 | **Bos d 2** | | 25% | 0% |
| | Profilin-1 (140) | 15.0 | | Hom s Profilin | | |
| 3 18 kD | **Allergen Bos d 2** (1 745) | 19.5 | **Bos d 2** | | 29% | 0% |
| | **Odorant-binding protein** (981) | 18.5 | **Bos d OBP** | | | |
| | Cathelicidin-1 (137) | 17.6 | | | | |
| 4 20 kD | **Allergen Bos d 2** (48 051) | 19.5 | **Bos d 2** | | 21% | 0% |
| | Cathelicidins (2272) | 17-22 | | | | |
| | Kappa-casein (238) | 21.3 | Bos d 12 | | | |
| | Beta-lactoglobulin (84) | 19.9 | Bos d 5 | | | |
| 5 25 kD | **Allergen Bos d 2** (17 521) | | **Bos d2** | | 50% | 30% |
| | Short palate, lung and nasal epithelium carcinoma-associated proteins 2 (574) | 26.3 | | | | |
| | Alpha-caseins (124) | 24-26 | Bos d 8, Bos d 9, Bos d 10 | | | |
| 6 35 kD | **Odorant-binding protein** (3707) | | **Bos d OBP** | | 48% | 10% |
| | Carbonic anhydrase 4 (722) | 35.1 | | | | |
| 7 40-45 kD | **Odorant-binding protein** (3707) | | **Bos d OBP** | | 71% | 10% |
| | Serpin A3 (351) | 46.3 | | Der f 27, Hor v 33, Tri a 33 | | |
| | Alpha-1-antiproteinase (302) | 46.2 | | | | |
| 8 65 kD | **Serum albumin** (22 180) | 69.2 | **Bos d 6** | | 87% | 40% |
| | Fibrinogen alpha chain (322) | 67 | Bos d Fibrin | | | |
| | Kininogen-1 and -2 (115) | 68-69 | | | | |
| 9 75-80 kD | Lactotransferrin (6991) | 78 | Bos d LF | | 41% | 10% |
| | Gelsolin (3 481) | 80 | | Gelsolin-like allergen Der f 16 | | |
| | Serotransferrin (3 278) | 78 | | | | |
| 10 | Ceruloplasmin (1 538) | 122 | | | 45% | 20% |



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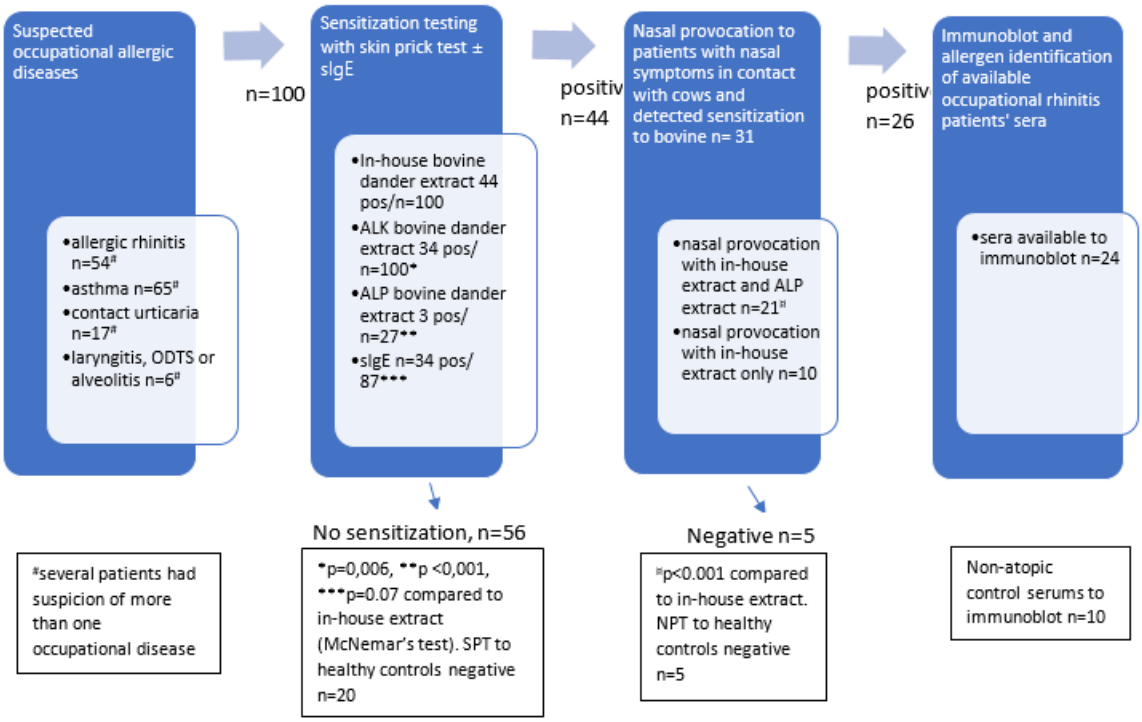
| | | | | | | |
|---|---|---|---|---|---|---|
| 120 kD | | | | | | |
| 11 >150 kD | Alpha-2-macroglobulin (4 154) | 187 | | | 42% | 20% |
| | Fibronectin (3 737) | 272 | | | | |

§ patients and * controls are identified in Figure 1.

**Figure 1.** Flow chart of the study.




Abbreviations: SPT: skin prick test, ODTS: organic dust toxic syndrome, ALK: ALK-Abelló, ALP: Allergopharma, NPT: nasal provocation test. #Several patients had suspicion of more than one occupational disease.

**Figure 2. A.** Immunoblot of two in-house bovine dander extract batches and two commercial extracts. Molecular weight marker (MWM) on the right. **B.** An example of different IgE-reactive allergens in pool serum (first lane after MWM) and the first ten available occupational rhinitis patient sera of the



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sample. The molecular weight marker (Precision Plus Protein standard, BioRad) is on the left. Table 3 presents mass spectrometry-based, identified bovine dander allergens for one of the patient lanes 1-11.