ORIGINAL ARTICLE

Characterization of Relevant Bovine Dander Allergen Components

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Abstract

Background: Diagnostic tests in occupational allergic diseases are highly dependent on the quality of available allergen extracts and specific IgE tests. To enhance diagnostic testing in cattle-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 with commercial bovine dander extracts.
Methods: One hundred patients with a suspected cattle-related occupational disease underwent skin prick tests (SPTs) with in-house and 1 or 2 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 sera of the patients with positive provocation test results and identified allergens from immunoblot bands using tandem mass spectrometry.
Results: The odorant-binding protein Bos d OBP, bovine serum albumin (Bos d 6), and the lipocalin (Bos d 2) were identified as the major allergens. We found a total of 24 bovine dander allergens, of which several were formerly unknown. The sensitivity and specificity of the in-house extract in SPTs were 100% and 94%, respectively, in 87 patients. The SPT results were negative in 20 healthy controls. Nasal allergen provocation tests with in-house extract detected occupational rhinitis with 100% sensitivity in 21 patients. The provocation results remained negative in 5 healthy controls.
Conclusions: Three major and several minor allergens in bovine dander caused occupational rhinitis. Diagnosis of bovine allergen–related occupational diseases requires a sufficient concentration and variety of tested allergens.

Key words: Cattle farmer. Rhinitis. Asthma. Cow. Allergy. Occupational disease. Cattle farming. Sensitization. Skin prick test.

Resumen

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 y 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: Ganadero. Rinitis. Asma. Vaca. Alergia. Enfermedad profesional. Ganadería. Sensibilización. Prueba de punción cutánea.

Summary box

- **What do we know about this topic?**
  Cattle allergens from *Bos domesticus* (Bos d) are a frequent cause of occupational allergy and a significant occupational problem among farmers. Inconsistent sensitivity to commercial diagnostic allergen extracts has hampered diagnosis of occupational diseases.
- **How does this study impact our current understanding and/or clinical management of this topic?**
  This study found that in-house bovine dander extract improved clinical tests. The article characterized a total of 24 relevant bovine allergen components, several of which were previously unknown. The major cow allergens assessed were Bos d OBP, Bos d 6, and Bos d 2.

Introduction

Allergens from the epithelia of *Bos domesticus* (Bos d) are a frequent cause of allergy and a significant occupational problem among cattle farmers who provide meat and, especially, dairy products. Bovine dander is responsible for most cases of animal-induced occupational rhinitis (OR), occupational asthma (OA), and occupational contact urticaria in Finland [1-3]. The German Cattle Allergy Study found that 9.1% of 5627 farmers had occupational airway diseases due to cattle allergy [4]. Cattle allergy can 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 have been detected in stables and farmers' homes [5].

Population-based studies have found the highest median concentrations of bovine hair allergens to be in the milking parlor (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 has been reported to be higher than that recorded in automatic milking systems [7]. In cold climate zones, exposure generally increases during winter, when cattle are kept indoors.

Several bovine dander antigens of different sizes have been identified. One of the major allergens is the 22-kD lipocalin Bos d 2, which is also the best characterized major bovine dander allergen and has 3 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]. The α lactalbumin Bos d 4 (14 kDa), ß-lactoglobulin Bos d 5 (18 kDa), and 20- to 30-kDa caseins are also minor bovine dander allergens and are best known as milk allergens causing food allergy [10]. Further allergens identified in bovine dander include a 67-kDa bovine serum albumin (Bos d 6) and a 160-kDa immunoglobulin (Bos d 7) [11].

Allergen standardization in the diagnosis of occupational allergic disease is inconsistent: a missing or excessively low protein and allergen content in the skin prick test (SPT) extracts of occupational allergens hampers the diagnosis of OR and OA [12,13]. Quantification of individual allergens was recently the 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].

In this study, we produced an in-house bovine dander extract, investigated whether this enabled earlier diagnosis of occupational allergic diseases, and assessed clinically relevant new bovine allergens.

Participants and Methods

Patients

We performed routine SPTs on 102 consecutive Finnish patients (48 men, 54 women) who were exposed to bovine dander at work and had a suspected cattle-related occupational disease between 2009 and 2012. Two patients were excluded because of wheals induced by the negative control. The mean age of the patients was 44 years (range, 22-61). A subset comprising 87 of the remaining 100 patients was tested for bovine epithelia–specific IgE (sIgE) (Phadia UniCAP). A placebo-controlled nasal allergen provocation test (NPT), the gold standard test for diagnosis of allergic rhinitis, was performed on 31 of the patients with a history of rhinitis in relation to bovine contact and confirmed (sIgE or SPT) sensitization to cows to confirm possible OR (Figure 1). For practical reasons, only 21 of these patients underwent NPT with both commercial and in-house allergen extracts, and 10 were tested with in-house allergen only.

Twenty healthy adult volunteers participated as controls for the in-house bovine dander extract SPT and 5 as NPT controls. The participants gave their informed consent. The study and the use of patient databases was approved by the administration of the institution where the study was performed and the Coordinating Ethics Committee of Helsinki University Central Hospital (130/13/03/00/2011).

Preparation and Testing of 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 [15]. The mixture was filtered through a 0.07-mm nylon

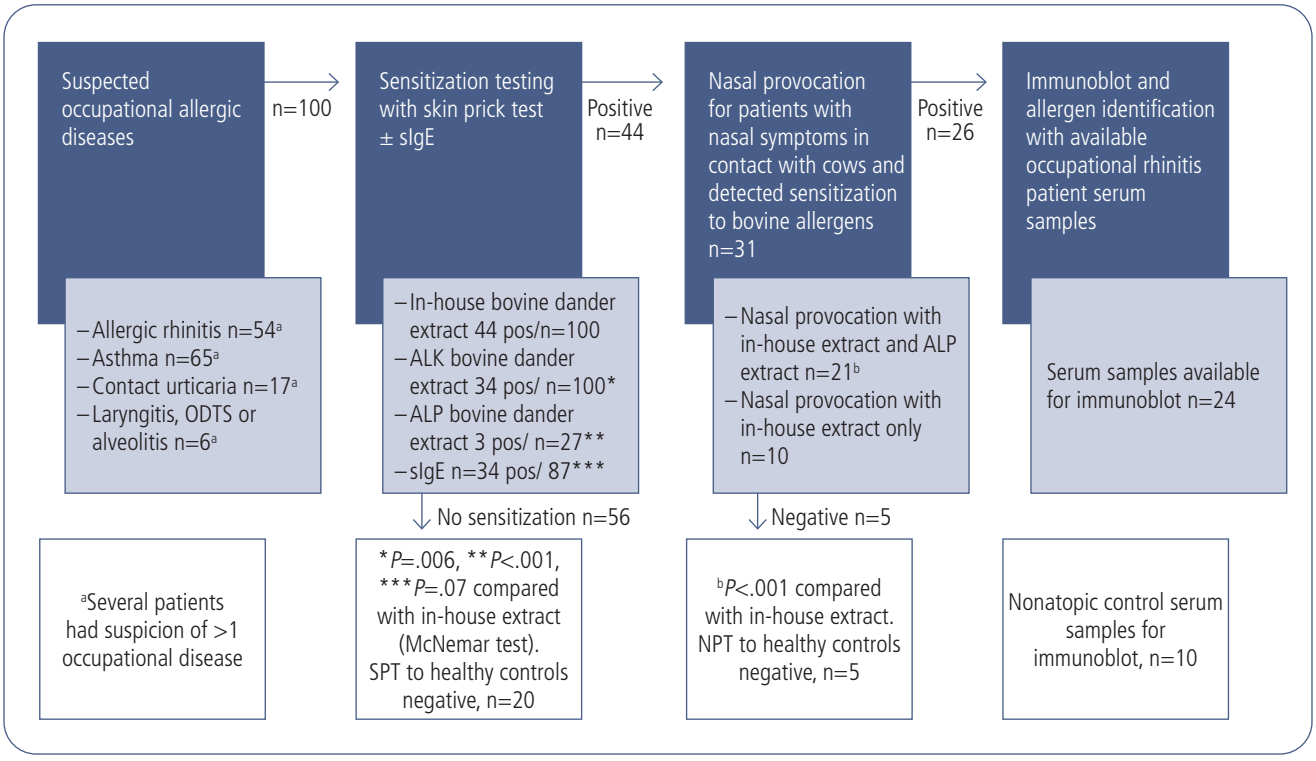

Figure 1. Study flow chart. SPT indicates skin prick test; ODTS, organic dust toxic syndrome; ALK, ALK-Abelló; ALP, Allergopharma; NPT, nasal provocation test.

net to remove debris and centrifuged for 20 minutes at 5000*g*. Supernatant was concentrated with ultrafiltration (3000-Da cut-off), after which it was sterile filtered using 0.45-µm and 0.2-µm filters (Millipore Corporation). The manufacturing process was aseptic. We measured the protein concentration using a modified Lowry approach (Bio-Rad), 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 by the Finnish Accreditation Service (T013 [EN ISO/IEC 17025]). Measurements were made using a standard kinetic chromogenic method based on Limulus amebocyte lysate (SFS-EN 1 4031:2003). We confirmed the similarity of the different extract batches by comparing the protein composition using SDS-AGE and the intensity of IgE reactivity in the immunoblots of the new and previous preparations. Nonspecific SPT and NPT reactivity was tested on 20 healthy volunteers, SPTs with an extract protein concentration of 22 mg/mL in 20 volunteers and NPT with 2 mg/mL in 5 volunteers. The Finnish Medicines Agency gave permission for 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 pollen allergen extracts (birch, alder, grass, and mugwort), cat, dog, horse, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Alternaria alternata*, and *Cladosporium herbarum* (ALK-Abello), with both 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 [16]. All patients were tested with the commercial bovine epithelia SPT extracts (ALK, 1:100 wt/vol, and from September 22, 2011 onward, 27 patients were also tested with Allergopharma 317 [10 000 BU/mL]), together with the in-house extract. To find a suitable concentration for testing, we initially used 5 mg/mL to test 10 patients, then raised the concentration to 20-22 mg/mL to test 60 patients. After large SPT wheals (10- to 13-mm) appeared on 4 patients, the routine test concentration was finally adapted to 2 mg/mL and tested on 30 patients. Sensitization was defined as bovine epithelia sIgE values of ≥0.35 kU/L. OA was diagnosed in sensitized patients with work-related asthma symptoms by placebo-controlled positive inhalation challenge with bovine extract (Allergopharma) [17]. A sustained fall of ≥15% from the prechallenge value of FEV_1 was considered positive, provided that FEV_1 fluctuations were <10% in a control challenge performed a day earlier. In addition, a positive result in workplace monitoring of peak expiratory flow combined with detected sensitization to bovine epithelia 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 bovine dander extract (Allergopharma 317) were performed on 21 patients with a history of rhinitis after contact with cattle and verified bovine dander sensitization based on SPTs or sIgE. Ten patients' NPTs were carried out using only the

in-house extract for 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 8 times the recommended strength, ie, to 40 000 BU/mL (according to the manufacturer's recommended concentration, ie, 5000 BU/mL). We followed published NPT recommendations [18,19].

In the NPTs, we scored bilateral nasal secretion from 0 (dry mucous membrane) to 3 (mucus dripping out of the nose) and blockage from 0 (no swelling) to 3 (completely obstructed nose), according to anterior rhinoscopy before and after the provocation [18]. Score changes of ≥4 points 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 nasal secretion in 35 minutes and change in nasal volume (cm^3) at 2-5 cm from the nostril using acoustic rhinometry (A1, GM Instruments) and nasal resistance (Pa/cc/s %) using active anterior rhinomanometry (NR6-2, GM Instruments). We followed the recommendations for the Standardization Committee on Objective Assessment of the Nasal Airway [20]. Nasal secretion was measured in accordance with Pirilä and Nuutinen [21], although bilaterally. Positive NPT reactions in sensitized patients with a history of rhinitis caused by 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 Allergopharma 317) were separated under reducing 12% SDS-PAGE conditions. One gel was silver-stained, and the other was immunoblotted with pooled serum samples from 10 patients diagnosed with allergic rhinitis induced by bovine dander and the individual serum samples from 34 participants (24 NTP-positive and 10 SPT- and IgE-negative controls, Figure 1). Immunoblotting was performed as previously described [22], with a few modifications. Briefly, we transferred proteins by electrophoresis (MiniTransBlot, Bio-Rad) from the SDS-PAGE gels onto the PVDF membranes (Immobilon-P, Millipore Corporation). The nonspecific binding of antibodies was blocked by a nonionic Tween-20 detergent (0.1%), which does not interfere with protein identification from the immunoblot based on liquid chromatography with tandem mass spectrometry (LC-MS/MS). We then incubated the membranes with diluted patients' sera (1/5) overnight at +4°C. The reaction with biotinylated goat antihuman IgE (1:1000, Vector Laboratories Inc.) was then followed by addition of 1:12 000 diluted streptavidin-conjugated alkaline phosphatase (Invitrogen) and the color development solution (Bio-Rad). Pooled positive patient sera were added to each patient blot to compare the intensities of the positive bands between 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 analyzed using automated nanoflow capillary LC–MS/MS, with an EASY nanoLC 1000 device (Proxeon, Thermo Fisher Scientific Inc.) coupled to an electrospray ionization quadrupole orbitrap mass spectrometer (QExactive, Thermo Fisher Scientific Inc.). We reverse-phase separated the peptides using a 75-µm × 15-cm Acclaim PepMap100 C18 column (Dionex, Thermo Fisher Scientific Inc.) 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 mass fragment spectra obtained were analyzed in the Uniprot database (www.uniprot.org) against human entries using Proteome Discoverer 1.4 (Thermo Fisher Scientific Inc.).

Statistical Analysis

Differences between binomial variables were tested using the McNemar test for dependent variables, and the χ^2 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 test for independent variables. The statistical analysis was performed using SPSS Statistics for Windows, Version 22.0 (IBM Corp.). Two-by two tables were used to calculate the sensitivity and specificity of SPTs and NPTs based on the in-house extract.

Results

Sensitization

We analyzed the SPT results of 100 patients with in-house extract and 1 or 2 commercial bovine dander extracts. The in-house extract yielded 44 positive SPT reactions, and the ALK extract 34 positive reactions in 100 patients. The Allergopharma extract was positive in 3 out of 27 patients tested. Sensitization to ubiquitous allergens was detected in SPT for 63 patients. Figure 1 summarizes the suspected work-related diagnoses and test results for these patients.

The in-house extract yielded 100% sensitivity and 94% specificity in SPTs compared with 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 the 20 healthy volunteers. Altogether, 11 patients were diagnosed with OA to cow: 9 based on workplace peak expiratory flow monitoring, 1 based on a positive inhalation challenge test, and 1 based on resolution of asthma after discontinuing work with cattle.

Nasal Provocation Tests With Bovine Dander Extracts

Table 1 presents the characteristics of the 31 patients who underwent NPTs. Five patients had negative and 26 positive reactions, and control challenges were negative, confirming the suspected OR. Slightly more than half of the patients (55%) had asthma, and 48% were sensitized to the common environmental allergens tested. 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 only to the in-house extract, whereas 5 (24%) had positive reactions

to both extracts (*P*<.001) (Table 2). In-house extract sensitivity was 100%, and specificity was 18%. In the NPTs with the in-house extract, we detected larger volumes of secretion (*P*=.001) and greater nasal congestion in acoustic rhinometry (*P*=.001) and rhinomanometry (*P*=.032) than in the NPTs with commercial extract. No generalized reactions occurred. All 5 healthy volunteers had negative NPT results with the in-house extract.

Table 1. Characteristics and Sensitization of 31 Patients Who Underwent Nasal Provocation Tests Using the In-House Extract.[a]

	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)	.007
Males, No. (%)	17 (65)	3 (60)	.146
Bovine dander exposure, y	15 (3-31)	24 (8-40)	.115
Current smoking, No. (%)	11 (42)	4 (80)	.019
Rhinitis symptoms, y	5 (1-13)	5 (1-10)	1.00
Asthma, No. (%)	13 (50)	4 (80)	.217
Occupational skin disease caused by bovine dander, No. (%)	8 (31)	2 (40)	.686
Sensitization to common environmental allergens in SPT, No. (%)	13 (50)	2 (40)	.682
Total IgE, kU/L	189 (17-1555)	239 (5-496)	.448
Bovine dander sIgE ≥0.35 kU/L, No. (%) 5.10 (0.01-34.1)	20 (83)[b]	4 (80) 1.93 (0.11-3.86)	.858
SPT with bovine dander (in-house)			
Positive, No. (%)	25 (96)	4 (80)	.178
Size, mm	6.8 (0-13)	5.0 (0-8)	
SPT with bovine dander (ALK)			
Positive, No. (%)	20 (77)	3 (60)	.428
Size, mm	3.5 (0-6)	2.6 (0-5)	

Abbreviation: ALK, ALK-Abelló; SPT, skin prick test.
[a]Data presented as means and ranges unless otherwise stated.
[b]n=24.

Table 2. Results of Nasal Provocation Tests for 5 Healthy Controls and the 21 Patients on 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 Allergopharma bovine dander extract	In-house bovine dander extract	*P* Value between test results with commercial and in-house extracts
Positive reaction[a] No. (%)	0 (0)	0 (0)	5 (24)	18 (86)	<.001
Mean (range) secretion amount, g	0 (0)	0 (0)	0.2 (0–1.6)	3.8 (0–11.3)	.001
Mean decrease in nasal volume, cm^3[b]					
≥30%, No. (%)	0 (0)	1 (5)	1 (5)	9 (43)	.001
Mean % (range)	–1 (–18 to 27)	3 (–43 to 32)	4 (–12 to 30)	27 (–22 to 81)	
Nasal resistance increase, Pa/cc/s					
>100%, No. (%)	1 (20)	1 (5)	1 (5)	10 (48)	.032
Mean % (range)	37 (–17 to 111)	11 (–68 to 111)	22 (–65 to 134)	175 (–84 to 1534)	

[a]Rhinorrhea and mucosal swelling ≥4.
[b]Nasal cavity volume at distance of 2-5 cm from the nostril.

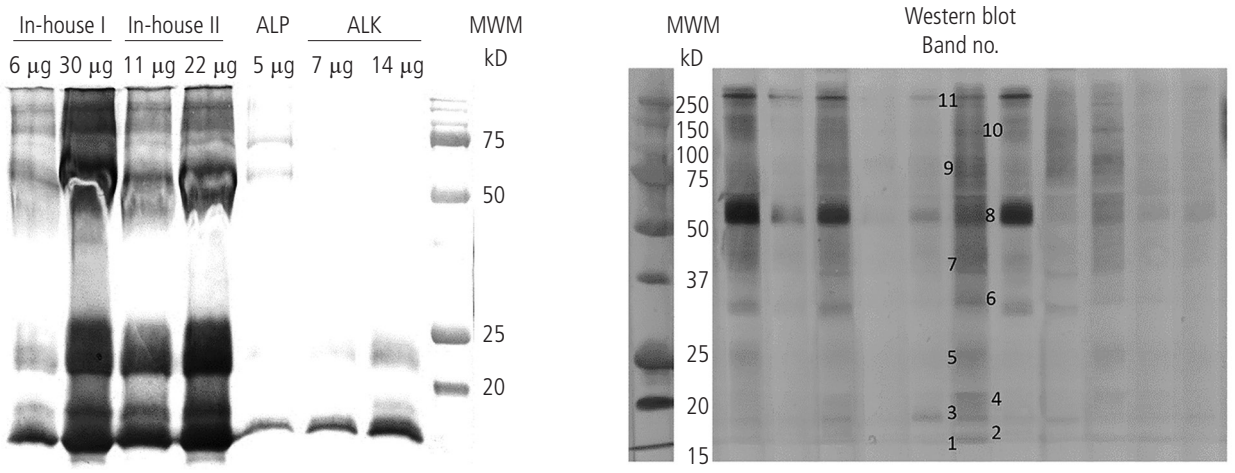

Figure 2. A, Immunoblot of 2 in-house bovine dander extract batches and 2 commercial extracts. Molecular weight marker (MWM) on the right. B, An example of different IgE-reactive allergens in pooled serum (first lane after MWM) and the first 10 available serum samples from patients with occupational rhinitis. The molecular weight marker (Precision Plus Protein standard, Bio-Rad) is on the left. Table 3 presents mass spectrometry-based, identified bovine dander allergens for each of the patient lanes 1-11. ALK indicates ALK-Abelló; ALP, Allergopharma.

Serum IgE Reactivity to Allergens

Figure 2A presents an immunoblot of the bovine dander extracts (2 in-house batches and 2 commercial batches), together with 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 3 major bovine epithelial allergens identified in the in-house extract were an odorant-binding protein (Bos d OBP), bovine serum albumin (Bos d 6), and a lipocalin (Bos d 2), followed by several minor allergens, such as α-2 macroglobulin, ceruloplasmin, lactotransferrin, and fibronectin (Figure 2B, Table 3). Identification scores (Mascot) were in the thousands for the main allergens and around 100 for the others. Lipocalin Bos d 2 variants were identified in both commercial extracts. Cow epithelia from Allergopharma 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. The major allergens were Bos d OBP, Bos d 6, and Bos d 2. Several of the identified allergens had not previously been reported.

Our results confirm earlier reports that sensitivity to occupational SPT extracts varies between producers [12,13]. In both the SPTs and the NPTs, the in-house extract induced more positive allergic reactions than the commercial products. This finding is well in line with most earlier findings. Heutelbeck et al [24] suggested that if bovine dander allergy test results with commercial extracts are contradictory to clinical symptoms, they should be supplemented with extracts from the hair of the farmers' own cattle. In accordance with these reports, 2 studies compared commercial bovine dander epithelial allergens used in SPT and concluded that their sensitivity was low; they also found solutions with higher protein and antigen content to ensure higher sensitivities and test efficiencies [11,12]. The authors 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 glycerol, which prevented direct comparison of protein concentrations. After a change in the buffer used, the measured commercial concentrations were low, in line with earlier findings.

The concentration of bovine dander extract in our SPT varied, thus hampering the exact evaluation of sensitivity and specificity. Our initial concentration of 20-22 mg/mL was too high for standard allergy testing for the sensitized patients, as seen in the large SPT reaction in 4 patients. Our in-house extract was highly concordant with sIgE results and the results of SPT with the ALK extract, although detection of sensitization was poor with the Allergopharma extract (Figure 1). However, results with in-house extract were negative in our group of 20 healthy controls, indicating the good specificity of the test. Our final protein concentration of 2 mg/mL was 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 owing to the sufficient protein concentration for clinical diagnostic tests and wider allergen variety. One to two of the 3 main allergens in the in-house extract, Bos d 6 (bovine serum albumin) and/or Bos d OBP (odorant-binding protein), seemed to be missing from the commercial extracts altogether. The commercial allergens contained mainly Bos d 2 variants (lipocalin, 18-20 kDa). Diagnostic testing in both OR and OA was hampered by the insufficient potency of the commercial extract.

Numerous major bovine dander allergens have previously been reported, for example, in a study based on German cattle [24]. Our immunoblot result is consistent with 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 isolated reports of occupational respiratory allergy to Bos d 6 (bovine serum albumin [BSA]) have been

Table 3. Allergen Identification From Immunoblot of In-house Cow Dander Extract With Patient Sera.[a]

WB band	Identification (Mascot score)	Molecular weight, kD[a]	Allergen name	Homology with	Reactivity, NPT-positive patients, % (n=24)[b]	Reactivity, controls, % (n=10)[c]
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				
	ß-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 (1745)	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				
	κ-Casein (238)	21.3	Bos d 12			
	ß-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				
	κ-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		
	α-1-Antiproteinase (302)	46.2				
8 65 kD	Serum albumin (22 180)	69.2	Bos d 6		87%	40%
	Fibrinogen α 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				
10 120 kD	Ceruloplasmin (1 538)	122			45%	20%
11 >150 kD	α-2-Macroglobulin (4 154)	187			42%	20%
	Fibronectin (3 737)	272				

Abbreviation: NPT, nasal provocation test.
[a]Proteins of the same size are listed in order of identification scores (Mascot).
[b]Patients (Figure 1).
[c]Controls (Figure 1).

published [25,26]. Although 40% of the control patients were sensitized to Bos d 6, the sensitization rate was over twice this amount, ie, 86% among the NPT-positive patients. Albumins are ubiquitous animal allergens, usually considered minor allergens [27,28], although their relevance as respiratory allergens has not been fully explored [29]. Patients with persistent milk allergy and specific IgE 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. While we were not specifically searching for oral allergies, pasteurized milk is a typical component of the Nordic diet, and, according to the medical history, occupationally sensitized patients normally tolerate ingested milk. It is noteworthy that sensitization to BSA has been identified as a cause of some generalized allergy reactions to pharmaceutical excipients in artificial insemination, surgical tissue adhesives, and vaccines [31-33]. However, an early study concluded that BSA was of marginal significance in the diagnosis of respiratory cow allergy [34]. sIgE to several mammalian albumins are commercially available [34].

Most mammalian-derived respiratory allergens belong to the lipocalin superfamily of proteins. Previously, Bos d 2 lipocalin variants were 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 been reported to be an allergen in bovine colostrum [36] and shown to cross-react to the dog dander allergen Can f 4, which is also an odorant-binding protein [37].

In addition to these 3 major cow dander allergens, namely, lipocalins, OBP, and albumins, the study identified several other proteins involved in allergic inflammation [38]. A known bovine allergen, Bos d 3, is an antimicrobial calcium-binding S100A protein, a principal effector molecule of epithelial mucosal immunity and a regulator of keratinocyte function. It is found in amniotic fluid. Its homologue in humans is called psoriasin (or S100A7) [39].

Caseins are the principal milk proteins. They have previously been identified as allergens in asthmatic farmers, and milk-derived pure or modified casein has been reported to be an occupational allergen in people handling products containing it [40,41]. Cathelicidins are proteolytically activated antimicrobial and antiviral peptides, and human cathelicidin LL37 has been found to act as an eosinophil- and neutrophil-specific chemoattractant [42]. Fibrinogens are essential cofactors in initiating exogenous proteinase-induced 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 in the airway surface liquid by epithelial and nonepithelial cells is gelsolin, which is found at higher concentrations in asthma patients [45]. Ceruloplasmin is a glycoprotein which functions in, for example, antioxidant defence and homeostasis. Its oxidase activity is thought to be an indicator of allergic inflammation, 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-based standardized diagnostic products in the future. In-house extracts play a key role in diagnostic testing to detect immediate occupational allergy to bovine dander.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

Previous Presentations

Some of the results reported in this work were presented in poster format at the European Respiratory Society meeting held on September 3-7, 2016 (Lindström I, Airaksinen L, Suojalehto H, Puustinen A: In-house and commercial bovine dander extracts in the diagnostics of allergic occupational diseases).

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