

Clinical Characteristics and Sensitization Profile of Patients Allergic to Cow Epithelium

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Keywords

Allergy · Cow epithelium · Major allergen · Occupational allergy · Sensitization

Abstract

Introduction: Cow epithelium allergy (CEA) has been described in workers highly exposed to cattle, such as farmers and veterinarians, being a health problem in this population since it is their main livelihood. This study aimed to characterize the main clinical manifestations and define the sensitization profile of the cow epithelium-allergic population treated in our health area. **Methods:** This is a retrospective study including a total of 34 patients with a clinical diagnosis of CEA, confirmed by skin tests, bovine epithelium-specific IgE levels and allergen-specific conjunctival challenge test in some cases. They were distributed by age, sex, profession, clinical symptoms, specific IgE levels to other mammalian epithelia, pollens, mites, and foods. Immunoblotting was performed with extracts from cow dander, cow body fluids (urine and saliva), bull urine, and 17 sera from immunotherapy-untreated CEA patients. **Results:** The mean age of the patients was 44 years, with a higher incidence in cattle farmers. Rhinoconjunctivitis occurred in 100% of cases, with 35% having monosensitization to cow epithelium. Sera from

most patients detected a 20-kDa IgE-binding band in cow dander, cow saliva, cow urine, and bull urine, corresponding to the major allergen Bos d 2 (bovine lipocalin). In 70% of the patients, a 25-kDa band was detected in cow and bull urine extracts, whose identification by mass spectrometry and investigation with protein databases led to the identification of a *Bos taurus* lipocalin (UniProt protein ID: A0A3Q1LGU7_BOVIN). **Conclusion:** CEA should be considered in patients exposed to cattle and as a cause of occupational disease. The IgE immunodetection revealed sensitization to a protein present in cow and bull urine (odorant-binding protein) not previously described.

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Introduction

The importance of occupational allergies which manifest in the form of respiratory and/or skin disorders in people exposed to fluids or substances from different bovine species has long been recognized. Our health area occupies the leading position in the total number of cattle heads in Galicia, with almost half a million animals. What

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Table 1. Summary of table 2

Patients, N (total)	Age, years mean	Sex, N/%		Profession, N/%						Symptoms, N/%				slgE CD (kU _A /L) mean
		F	M	CF-FW	V	OWT	S	P	RC	As	CU	CMPA		
34	44	15 44%	19 56%	22 64%	5 15%	2 6%	2 6%	1 3%	2 6%	34 100%	13 38%	10 29%	1 3%	11.47

CF, cattle farmer; FW, farm worker; V, veterinarian; OW, office worker; T, teacher; S, student; P, pensioner; Rc, rhinoconjunctivitis; As, asthma; Cu, contact urticaria; Cmpa, cow milk protein allergy; CD, cow dander.

could be considered medium-sized cattle farms (11–99 animals) predominate, and cattle farming represents the basis of the economic activity in our region. Thus, bovine exposure in our rural population is high.

Different bovine allergens have been described, including Bos d 2, which has a molecular weight of 20 kDa. This allergen belongs to the lipocalin family and is considered a major allergen. It is found in the sweat glands, dander, and body fluids. Bos d 1 allergen has been detected in dander and urine as well as Bos d 3 which has a molecular weight of 11 kDa [1].

Cow epithelium allergy (CEA) is a generic name used to define the clinical symptoms caused by exposure of susceptible patients to proteins from cattle epithelium (dander) but also from various cattle body fluids in their occupational environment. Major allergens can be found in saliva, urine, and dander. The latter is the common source of the presence of these, and it is for this reason that we adopt the term CEA.

More recently, our group has reported the presence of the galactose-alpha-1,3 epitope in bovine amniotic fluid [2] as a cause of occupational allergy. The objective of the present study was to analyze the clinical characteristics and sensitization profile of patients allergic to cow epithelium in our population given its medical and legal implications.

Material and Methods

Patients

We conducted a retrospective study spanning 2000 to the beginning of 2020 including a total of 34 patients with a clinical diagnosis of CEA which included: rhinoconjunctivitis, asthma, and contact urticaria confirmed by skin tests, determination of specific IgE, and specific allergen conjunctival provocation tests in specific patients. Age distribution, sex, profession, clinical symptoms, specific IgE levels against cow epithelia, other mammalian epithelia, dust mites, pollens, and foods were recorded and are shown in Table 1.

In vivo Tests

Skin prick tests (SPTs) were performed following EAACI methodology [3] with a standard battery of commercial extracts (*Dermatophagoides pteronyssinus*, *Lepidoglyphus destructor*, *Tyrophagus putrescentiae*, *Glycyphagus domesticus*, *Chortoglyphus arcuatus*, *Alternaria alternata*, *Cladosporium herbarum*, *Aspergillus fumigatus*, cat epithelium, dog epithelium, cow epithelium, rabbit epithelium, horse epithelium, latex and pollens from *Lolium perenne*, *Phleum pratense*, *Plantago lanceolata*, *Parietaria judaica*, *Betula verrucosa*, *Olea europaea*, and *Cupressus arizonica* (from Roxall Laboratories, Bizkaia, Spain) and positive and negative control solutions (histamine hydrochloride 10 mg/mL and diluent, respectively) on the volar forearms. Tests were considered positive when the wheal was larger than 3 mm after 15–20 min of application.

Conjunctival provocation tests (CPTs) were performed with cow dander extract in 9 patients who showed sensitization to other airborne allergens to verify and confirm the clinical relevance of the allergy to cow epithelium. Tests were conducted following guidelines for daily practice [4] and EAACI norms and were considered positive following the criteria of Abelson [5].

In vitro Tests

Specific IgE against cow epithelium was determined using the ImmunoCAP system (Phadia, Uppsala, Sweden), and a cut-off point of 0.35 kU_A/L was used. SDS-PAGE immunoblotting was carried out as described by Laemmli [6] under standard conditions (with 2-mercaptoethanol) with extracts from cow dander, cow milk, cow saliva, cow urine, and bull urine and 17 sera (out of the 34 subjects) who had not previously received immunotherapy for bovine epithelium in order to avoid a modification of their basal allergen sensitization profile.

A 25-kDa protein band was identified by mass spectrometry. Protein identification was performed by searching the nonredundant protein sequence database (NCBI) using the Mascot program (<http://www.matrixscience.com>).

Results

The mean age of the patients was 44 years, and there was a slight predominance of males (56%). Rhinoconjunctivitis was the most frequent manifestation, affecting 100% of patients, followed by asthma (38%) and contact urticaria (29%) summarized and shown in Table 1.

Table 2. Demographic clinical characteristics and sensitization profile

Patient No.	Age, years	Sex	Profession	Symptoms*	Total IgE, kU/L	slgE Cow dander, kU _A /L	slgE mites, kU _A /L	SlgE pollen, kU _A /L	slgE Other epithelia, kU _A /L	slgE Food, kU _A /L
1	65	F	Cattle farmer	Rc, As	82	1.74				
2	47	M	Teacher	Rc	21	8.32			1.51 (HE)	
3	36	M	Office worker	Rc		0.99				
4	62	F	Cattle farmer	Rc, Cu	167	52				
5	46	M	Cattle farmer	Rc	127	14.5				
6	74	F	Pensioner	Rc, As		10.9				
7	49	M	Cattle farmer	Rc, As, Cu		15.4		4.27 (BV)		
8	39	F	Veterinarian	Rc, As		1.47		1.34 (PP)	6.82 (CE) 3.74 (HE)	1.12 (SS)
9	55	M	Cattle farmer	Rc, As		8.32	5.78 (DP)			
10	33	M	Office worker	Rc	317	2.05	25 (DP) 1.15 (LD)	6.23 (LP)		
11	29	F	Veterinarian	Rc, As, Cu		12.1			25 (DE) 0.66 (CE)	
12	59	F	Farm worker	Rc, As		25				
13	37	M	Cattle farmer	Rc	516	1.07	65 (DP)			
14	36	F	Cattle farmer	Rc	17	1.19				
15	39	M	Cattle farmer	Rc	136	4.7				
16	30	F	Farm worker	Rc		9				
17	30	F	Veterinarian	Rc, Cu	38	0.51	8 (DP) 2.5 (LD)	2.48 (PP)	0.73 (DE) 2.46 (CE)	
18	46	M	Cattle farmer	Rc, As, Cu	295	15	10.8 (DP) 61 (LD)	1.02 (LP)		
19	29	M	Cattle farmer	Rc, As, Cu	1,670	33	75 (DP) >100 (LD) 75 (TP)			
20	11	F	Student	Rc, Cmpa	68	9.24				30 (M)
21	50	F	Farm worker	Rc, Cu	81	8.44	8.5 (DP)	12 (LP)		
22	34	M	Cattle farmer	Rc	140	0.98	13 (DP) 7 (LD)			
23	44	M	Cattle farmer	Rc, As, Cu	453	15	39 (DP) 42 (LD)			
24	24	M	Veterinarian	Rc	32	6.6			42 (HE)	
25	40	M	Cattle farmer	Rc, As	122	1.5			1.06 (DE)	
26	40	F	Cattle farmer	Rc	36	4.24				
27	50	F	Farm worker	Rc	128	10.5				
28	36	M	Cattle farmer	Rc, As, Cu	258	34.4	15 (DP)	28 (PP)		
29	60	M	Veterinarian	Rc	62	1.25	10 (DP) 1.32 (LD)			
30	52	F	Farm worker	Rc, Cu	145	12.7	35 (DP)	6.18 (PP)		
31	45	M	Cattle farmer	Rc, As	568	36	81.2 (DP)		18 (CE)	
32	58	F	Farm worker	Rc	48	0.89	22 (DP)			
33	50	M	Teacher	Rc	156	10				
34	66	M	Pensioner	Rc	224	21	95 (DP)			

CE, cat epithelium; DE, dog epithelium; HE, horse epithelium; BV, *Betula verrucosa*; PP, *Phleum pratense*; LP, *Lolium perenne*; SS, sunflower seed; DP, *Dermatophagoides pteronyssinus*; LD, *Lepidoglyphus destructor*; TP, *Tyrophagus putrescentiae*; M, cow milk. *Rc, rhinoconjunctivitis; As, asthma; Cu, contact urticaria; Cmpa, cow milk protein allergy.

The highest incidence of the disorder was observed in cattle farmers/farm workers (64%), veterinarians (15%) and to a lesser extent in teachers, office workers, and pensioners (6%) (shown in Table 1). Analysis of the sensitization

profile of our population showed six possible combinations: 35% of patients were only sensitized to cow epithelium and the most frequent profile was polysensitized to cow epithelium and dust mites and/or pollens (41%).

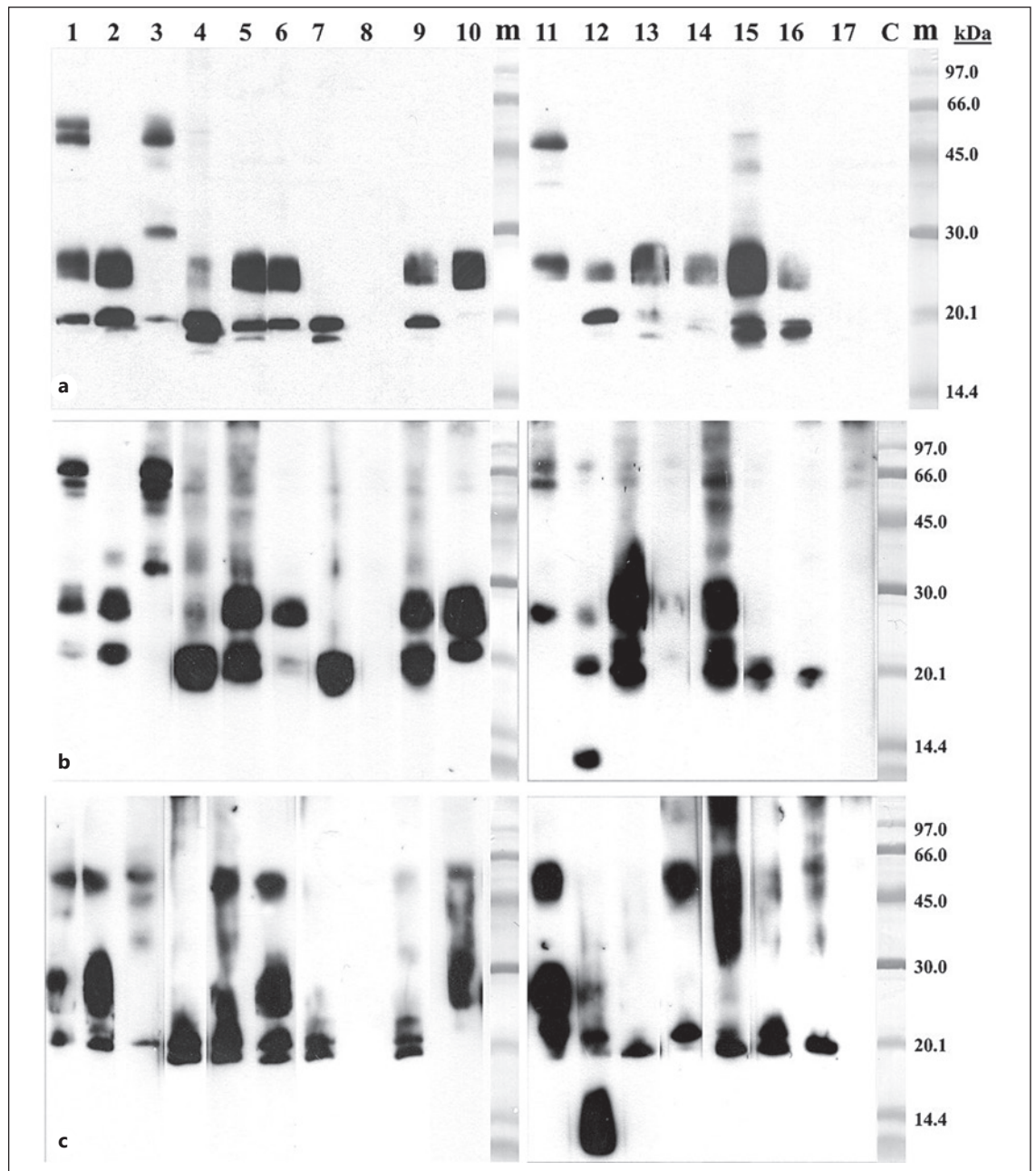


Fig. 1. SDS-PAGE immunoblotting. Cow urine extract (a), bull urine extract (b), cow dander extract (c). Lane 1–17: patient sera, lane C: control serum (pool of sera from non-atopic subjects), lane M: molecular mass standard.

Sensitization to other epithelia was found in 11% of the cases, and the remaining combinations did not exceed 6%, as shown in Table 2.

Immunoblotting results showed a highly prevalent IgE-binding band of approximately 20 kDa detected in cow urine extract with 12 sera and in bull urine extract

with 12 sera (shown in Fig. 1a, b), in cow dander extract with 12 sera (shown in Fig. 1c), and cow saliva extract with 10 sera. An IgE-reactive band of approximately 25 kDa was also detected in cow and bull urine extracts with 12 out of 17 sera (shown in Fig. 1a, b). Surely the 20-kDa band is the major allergen Bos d 2 (bovine lipocalin).

No IgE-reactive band was detected in cow milk extract. The 25-kDa band was identified as a lipocalin from *Bos taurus* (UniProt protein ID: A0A3Q1LGU7_BOVIN).

Discussions

The importance of cattle farming in our health area explains the high number of CEA patients that we see daily in our practices. We sought to further analyze this allergy in order to provide our patients with a more complete and personalized treatment. We found that rhinoconjunctivitis due to cow epithelium was the most frequent clinical manifestation in the patients, followed by bronchial asthma, which is in line with findings from previous studies conducted in Finland [7].

The exposure to cow epithelium in our population began at birth, given the rural and farm origin of our population. Some patients who currently live in urban areas maintain the exposure to cow epithelium due to their profession or for social and family reason. We believe this must be the explanation to find sensitization to cow epithelium in professions with no apparent exposure to this animal. The early, prolonged, and sustained exposure to proteins from bovine dander and urine could be a relevant factor in the development of CEA.

In the immunoblotting assay, we detected the sensitization to a 20-kDa protein, probably Bos d 2, present in the saliva, dander, and urine from cows and bulls, which is consistent with the findings of the group of Rautiainen et al. [1] in Finland. According to these findings, Bos d 2 (lipocalin) would be the major allergen in this group of patients.

We found that 12 of the 17 patients (70%) showed sensitization to a 25-kDa protein from bull and cow urine. To identify the protein, we used proteomics technology and detected various peptide sequences corresponding to an odorant-binding protein (OBP) from *Bos taurus*.

The detection of chemical molecules through the olfactory system is a vital process for animal life as some of these molecules modify important animal behaviors (reproduction, feeding, and flight from predators). OBPs, a subclass of soluble proteins called outlier lipocalins (within the calycin superfamily), have been reported as a major shuttle for odor perception, olfactory stimulus, and chemical communication, especially in insects and mammals, and have been shown to be essential for the olfactory system to function normally [8]. The bovine OBP here described binds a range of hydrophobic odiferous chemical molecules, transporting them to the organ responsible for their reception in the nasal mucosa [9]. This

protein presents a molecular mass of 18.5013 kDa according to its protein sequence, although it showed an apparent molecular mass of 25 kDa in electrophoresis with SDS. This protein could be the 23-kDa bovine protein described by Mattsson et al. [10] as a cross-reactive protein with lipocalin Can f 4.

Mammal OBPs have been detected in nasal mucus (bovine and buffalo OBP) and in body fluids such as urine (mouse major urinary protein I), vaginal secretion (aphrodisin of hamster), and saliva (boar lipocalin, buffalo OBP, and panda). Pheromone-binding OBPs in body fluids and odor-binding OBP in nasal mucus are identical in structure, although they may have different chemical and physiological properties. They can be found in the epithelium but at low concentrations [11].

Ylönen et al. [12] in studies with cow dander and urine identified proteins with masses of 20, 22, and 24 kDa and found that the allergenic activity in urine is lower than in dander based on IgE inhibition assays and crossed immunoelectrophoresis. They consider that the 20-kDa protein is the major allergen in substances derived from cows and that it corresponds to the Bos d 2 lipocalin.

In our study, we identified a 25-kDa band mainly in urine, with a low presence in dander and nondetected in saliva, which after proteomic characterization corresponded to OBP, a finding that has not been reported in previous studies as a cow allergen. Furthermore, we found that the epithelium-sIgE levels did not correlate with the odorant-binding protein sensitization: the sera from some patients with low sIgE levels detected the OBP-band, whereas others with high levels did not.

The clinical implications of OBP sensitization require further studies, but we can guess its location (mainly in urine) and its physicochemical properties due to its biological function (air carrier of molecules) would facilitate the aerosolization of this protein when the urine impacts against the ground surface, enabling the OBP-aero-sensitization and its contribution to the respiratory symptoms. Anyhow it is not easy to analyze the correlation between clinical manifestations and OBP sensitization as most of the patients were also sensitized to Bos d 2. We observed that some patients sensitized to Bos d 2 and not sensitized to OBP showed contact urticaria in addition to respiratory symptoms, surely due to the presence of Bos d 2 in dander and sweat glands. For this reason, in future research, we plan to perform nasal and/or conjunctival challenge test with purified OBP to assess its role in the patient symptomatology.

In line with immunoregulatory effects of lipocalins, recent studies have linked apo-Bos d 5 status (beta-lactoglobulin without binding iron-siderophore) to a diversion

toward a Th2 immune response [13]. Although it was not the objective of this study, serum iron levels were measured in our patients and were found to be normal in 100% of cases.

Given the difficulty of avoiding exposure to the cow allergens in this population, there is a clear need to further investigate specific immunotherapy treatments with standardized extracts which present the major allergens. In our opinion, such research should take into account the presence of OBP as it behaved as a major allergen in this group of patients.

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Statement of Ethics

The study protocol was approved by the Santiago-Lugo Research Ethics Committee (Register code: CSAE-HULA 2022/061). Written informed consent was obtained from all participants and from parents/legal guardians of all participants under 18 years of age.

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Conflict of Interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Author Contributions

L.A.G.G., M.B.P., R.N.O, and F.C.G. designed, performed, and interpreted the research and also provided serum samples; B.B. performed immunoblot analysis and provided critical feedback; and C.P.-V. contributed to protein characterization. L.A.G.G. and F.C.G. also analyzed data and wrote manuscript. All authors approved this submission.

Data Availability Statement

The data that support the findings of this study are not publicly available due to their containing information that could compromise the privacy of research participants. The data are available from the corresponding author upon reasonable request.