



HAL
open science

Buffalo's milk allergy: Role of sensitization to caprine β -casein

Anays Piotin, Stéphane Hazebrouck, Hervé Bernard, Frédéric De Blay, Carine Metz-favre

► To cite this version:

Anays Piotin, Stéphane Hazebrouck, Hervé Bernard, Frédéric De Blay, Carine Metz-favre. Buffalo's milk allergy: Role of sensitization to caprine β -casein. *Pediatric Allergy and Immunology*, 2023, 34 (6), 10.1111/pai.13971 . hal-04151181

HAL Id: hal-04151181

<https://hal.inrae.fr/hal-04151181>

Submitted on 4 Jul 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 Buffalo's milk allergy: role of sensitization to caprine β -casein

2
3 A. Piotin MD ¹, S. Hazebrouck PhD ², H. Bernard PhD ², F. de Blay MD PhD ^{1,3}, C. Metz-Favre
4 MD ¹

5
6 1. Allergology Unit, Chest Disease Department, Strasbourg University Hospital, Strasbourg,
7 France

8 2. Paris Saclay University, CEA, INRAE, Département Médicaments Et Technologies Pour La
9 Santé (DMTS), SPI, Gif-Sur-Yvette, France

10 3. Federation of Translational Medicine, University of Strasbourg, BP 426, 67091, Strasbourg,
11 France

12
13 *This is the pre-peer reviewed version of the following article: "Buffalo's milk allergy: role of*
14 *sensitization to caprine β -casein", which has been published in final form at*
15 *<https://doi.org/10.1111/pai.13971>. This article may be used for non-commercial purposes in*
16 *accordance with Wiley Terms and Conditions for Use of Self-Archived Versions.*

17
18 Preprint version:

19 **Publication History**

20 **Issue Online:**

21 **31 May 2023**

22 **Version of Record online:**

23 **31 May 2023**

24 **Manuscript accepted:**

25 **21 May 2023**

26 **Manuscript revised:**

27 **10 May 2023**

28 **Manuscript received:**

29 **07 April 2023**

30 **© 2023 European Academy of Allergy and Clinical Immunology and John Wiley & Sons Ltd.**

31
32
33
34

35 **Corresponding author:**
36 Dr Anays Piotin, MD
37 Service de Pneumologie
38 Nouvel Hôpital Civil
39 1 place de l'hôpital
40 BP 426
41 67091, Strasbourg Cedex
42 France
43 anays.piotin@chru-strasbourg.fr
44 **Word count of the manuscript: 1109**

45

46 **Table: 1**

47

48 **Figure: 1**

49

50 **Conflicts of interest**

51 All authors except Frédéric de Blay have no conflict of interest.

52 Frédéric de Blay perceived financial support: Clinical Grants (Aimmune, Stallergènes-Greer, ALK,
53 Novartis, AstraZeneca, DBV, Sanofi, GSK), board membership (Aimmune, Stallergènes-Greer, ALK,
54 Novartis, AstraZeneca, DBV, Sanofi).

55

56 **Funding Source**

57 None.

58

59 **Key words**

60 buffalo's milk allergy, caprine β -casein, cross-reactivity, mozzarella allergy, primary sensitization

61

62 **Abbreviations**

63 specific Immunoglobulin E (sIgE)

64 skin prick test (SPT)

65

66

67

68 To the Editor,

69

70 Water buffalo milk is one of the non-cow's milks increasingly produced and consumed. Looking at the
71 global worldwide milk production, buffalo's milk proportion as nearly tripled over the last fifty years¹.

72 It currently accounts for about 15% of world milk production¹. Mostly produced in India, Pakistan,
73 and Nepal, it is also widely used to produced typical Italian cheese. Food allergy to buffalo's milk is
74 exceptionally report, although its consumption is nowadays widespread even among children.

75 Furthermore, its cross-reactivity with other milks needs to be further investigated. Herein, we report
76 a rare case of buffalo's milk allergy in a goat's and sheep's milk allergic patient tolerant to cow's milk.
77 We analyzed specific IgE sensitization to buffalo milk proteins and investigated the cross-reactivity
78 with cow's and goat's milk to identify the initial sensitizer.

79 The patient was a 4-year-old French girl, with a history of severe food allergy to goat's and sheep's
80 milks, allergic asthma and rhinoconjunctivitis to birch pollen with a pollen-food syndrome and atopic
81 dermatitis. The child used to eat cow's milk from her first year of life without any reaction. One hour
82 after eating buffalo's mozzarella, she developed abdominal pain, feeling weak and fainting leading to
83 intramuscular injection of epinephrine. She had never consumed buffalo's milk before. Allergy
84 workup to buffalo's milk was performed eight months later. Skin prick test (SPT) with buffalo's milk
85 (1:10 dilution) and buffalo's mozzarella were positive after 20 minutes: wheal diameter of 10 mm
86 with pseudopods and 5 mm respectively, both with a skin flare. SPT were positive for goat's milk
87 (wheal diameter 8 mm with pseudopods, at 1:1000 dilution) and sheep's milk (wheal diameter 6 mm,
88 at 1:1000 dilution). SPT to cow's milk was negative. Positive control with histamine at 10 mg/mL
89 revealed a wheal of 5 mm diameter and control with saline solution was negative. Specific IgE (sIgE)
90 assays by ImmunoCap[®] system (ThermoFisher Scientific, Uppsala, Sweden) provided the following
91 results: goat's milk (10.30 kU/L), sheep's milk (8.18 kU/L), bovine casein (0.59 kU/L), bovine β -
92 lactoglobulin (0.17 kU/L), bovine α -lactalbumin (<0.1 kU/L), bovine serum albumin (<0.1 kU/L).

93 Buffalo milk proteins were purified from raw milk. Caseins were isolated and characterized using a
94 combination of isoelectric precipitation at pH 4.6 and reverse phase-high performance liquid
95 chromatography as previously described². Using indirect ELISA (see Supplementary methods), with
96 purified caseins adsorbed on the solid phase, we confirmed that the patient was sensitized to all
97 caseins. The patient had higher levels of sIgE to caprine and buffalo's α S1- and β -caseins than to the
98 bovine homologs (Table 1). However, indirect ELISA does not differentiate low- from high- affinity IgE-
99 binding to the different caseins. We then used a second immunoassay based on the capture of serum
100 IgE antibodies by a monoclonal antibody immobilized on the solid phase and the binding of
101 biotinylated β -caseins³. The IgE cross-reactivity between β -caseins was analyzed by performing
102 competitive inhibitions of IgE-binding to caprine and buffalo's β -caseins, as previously described⁴. As
103 shown in Figure 1, competitive inhibitions revealed a strong IgE cross-reactivity between buffalo's and
104 caprine β -caseins. The IgE binding to caprine β -casein was partially inhibited by buffalo's β -casein
105 (Figure 1A), whereas the IgE binding to buffalo's β -casein was totally inhibited by caprine β -casein
106 (Figure 1B). Therefore, the IgE-reactivity of the buffalo's β -casein results from the primary
107 sensitization to caprine β -casein for our patient. Moreover, no inhibitory capacity of the bovine β -
108 casein was observed. This confirms that sIgE-binding to bovine caseins detected by ImmunoCap[®]
109 system and by indirect ELISA is of low avidity without clinical relevance.

110 The parents of the child gave their informed consent for the investigations and the publication of this
111 case.

112

113 Nowadays, there is a growing interest in non-cow's milks for their nutritional benefits as well as their
114 hypoallergenic potential, although their allergenicity needs to be further investigated. Compared to
115 cow's milk, buffalo milk has higher rate of proteins and fat^{1,5}. Buffalo milk proteins are mostly caseins,
116 with on average 32 to 40g/L of caseins^{1,5}.

117 So far, very few clinical cases of buffalo milk allergy have been published.

118 Broekaert et al. published the first clinical case of buffalo's milk allergy in a 70 years old man without
119 any medical history of allergy⁶. He presented a severe anaphylaxis after eating Italian buffalo's
120 mozzarella. No sensitization to cow's or goat's milk was revealed by SPT.

121 Specific IgE assay and molecular diagnosis of buffalo's milk allergy is not usually performed because
122 no commercial assay is currently available. Seven potential molecular allergens have been described
123 in the buffalo's milk allergen source⁷. However, none has been registered by the WHO/IUIS Allergen
124 Nomenclature Subcommittee yet.

125 The main buffalo's milk caseins, β -casein and α S1-casein, are highly similar to bovine caseins with 95
126 to 97 % sequence homology⁸. Thus, cross-reactivity with other mammalian milks raise a significant
127 issue.

128 Two cases of buffalo's milk allergy were previously reported in goat's and sheep's milk allergic patient
129 who tolerated cow's milk^{9,10}. Although, co-sensitization between cow's, buffalo's, sheep's, and goat's
130 milks has been described both in vitro and in vivo, the cross-reactive molecular allergen and the
131 initial sensitizer has never been demonstrated experimentally⁸. To the best of our knowledge, we
132 provide evidence for the first time that allergy to buffalo's milk was triggered by a primary
133 sensitization to goat's milk.

134 Currently, the use of buffalo's milk in cow's milk allergic patient is usually not recommended because
135 of the high similarity degree and the cross-reactivity between cow's and buffalo's milk proteins^{8,11}.

136 Furthermore, in vivo cross-sensitization to buffalo's milk has been also described in patients with IgE-
137 mediated cow's milk allergy. Indeed, in two cohort studies, all cow's milk allergic patients with
138 positive SPT to cow's milk had a positive SPT to buffalo's milk^{12,13}. However, the clinical relevance of
139 this skin sensitization has not been evaluated.

140 Conversely, a rare clinical case of a young boy allergic to cow's milk and clinically tolerant to buffalo's
141 milk was reported¹⁴.

142 Thus, further study on larger cohort should be interesting to evaluate the clinical relevance of
143 buffalo's milk allergens and their cross-reactivity other mammalian milks.

144

145 In conclusion, we investigated a rare case of buffalo's milk allergy in a young girl allergic to goat's and
146 sheep's milk, and tolerant to cow's milk. Our study showed that buffalo's milk allergy was due to
147 primary sensitization to goat's milk because of an IgE cross-sensitization to caprine β -casein. Of note,
148 the patient was not sensitized to buffalo's β -lactoglobulin (data not shown). Nevertheless, several
149 clinical phenotypes of buffalo's milk allergy may exist with or without concurrent cow's milk allergy,
150 suggesting that different allergenic sensitization pathways should be involved. Further investigations
151 on molecular allergen sensitization, IgE cross-reactivity with other ruminants' milks and allergen
152 epitope identification should improve our knowledge of buffalo's milk allergy.

153

154

155 **Acknowledgments**

156 We would like to thank the breeder who provided us with the fresh buffalo milk, which allowed us to
157 carry out this study.

158

159

160 **References**

161

- 162 1. Roy D, Ye A, Moughan PJ, Singh H. Composition, Structure, and Digestive Dynamics of Milk from
163 Different Species-A Review. *Front Nutr.* 2020;7:577759.
- 164 2. Ah-Leung S, Bernard H, Bidat E, Paty E, Rancé F, Scheinmann P, Wal JM. Allergy to goat and sheep
165 milk without allergy to cow's milk. *Allergy.* 2006;61(11):1358-65.
- 166 3. Bernard H, Drumare MF, Guillon B, Paty E, Scheinmann P, Wal JM. Immunochemical
167 characterisation of structure and allergenicity of peanut 2S albumins using different formats of
168 immunoassays. *Anal Bioanal Chem.* 2009;395(1):139-146.
- 169 4. Bernard H, Ah-Leung S, Tilleul S, Drumare MF, Paty E, Bidat E, Wal JM, Hazebrouck S. Specificity of
170 IgE antibodies from patients allergic to goat's milk and tolerant to cow's milk determined with
171 plasmin-derived peptides of bovine and caprine β -caseins. *Mol Nutr Food Res.* 2012;56(10):1532-40.
- 172 5. Ahmad S, Anjum FM, Huma N, Sameen A, Zahoor T. (2013). Composition and physico-chemical
173 characteristics of buffalo milk with particular emphasis on lipids, proteins, minerals, enzymes and
174 vitamins. *J Anim Plant Sci.* 2013;23 Suppl 1:62-74.
- 175 6. Broekaert SM, Darsow U, Ollert M, et al. Anaphylactic shock caused by buffalo's mozzarella cheese.
176 *Ann Allergy Asthma Immunol.* 2008;101(1):105-107.
- 177 7. Allergome. <http://www.allergome.org/>. Accessed April 7, 2023.
- 178 8. Restani P, Ballabio C, Di Lorenzo C, Tripodi S, Fiocchi A. Molecular aspects of milk allergens and
179 their role in clinical events. *Anal Bioanal Chem.* 2009;395(1):47-56.
- 180 9. Pétrus M, Rayssac M, Cossarizza G, Lasserre J, Dutau G. Allergy to buffalo milk mozzarella. Case
181 report concerning a 16-year-old adolescent. *Revue Française d'Allergologie.* 2011; 51(6): 553-555.
- 182 10. Mori F, Restani P, Pucci N, et al. Allergy to all mammalian Bovidae proteins but cow's milk in a
183 child. *Allergol Immunopathol (Madr).* 2013;41(5):349-350.
- 184 11. Restani P, Gaiaschi A, Plebani A, et al. Cross-reactivity between milk proteins from different animal
185 species. *Clin Exp Allergy.* 1999;29(7):997-1004.
- 186 12. Sheehan WJ, Gardynski A, Phipatanakul W. Skin Testing With Water Buffalo's Milk in Children With
187 Cow's Milk Allergy. *Pediatr Asthma Allergy Immunol.* 2009;22(3):121-125.
- 188 13. Katz Y, Goldberg MR, Zadik-Mnuhin G, Leshno M, Heyman E. Cross-sensitization between milk
189 proteins: reactivity to a "kosher" epitope?. *Isr Med Assoc J.* 2008;10(1):85-88.
- 190 14. Sheehan WJ, Phipatanakul W. Tolerance to water buffalo milk in a child with cow milk allergy. *Ann*
191 *Allergy Asthma Immunol.* 2009;102(4):349.

192

193 **Table**

194

195

196 Table 1. Specific IgE (sIgE) levels to cow's, buffalo's, and goat's milks caseins.

197

sIgE levels	Cow's milk	Buffalo's milk	Goat's milk
sIgE to α S1-casein (UI/mL)	0.35	1.30	1.8
sIgE to β -casein (UI/mL)	0.48	1.13	3.36

198

199

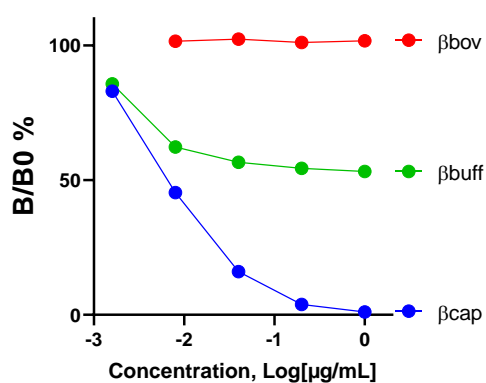
200

201 **Figure**

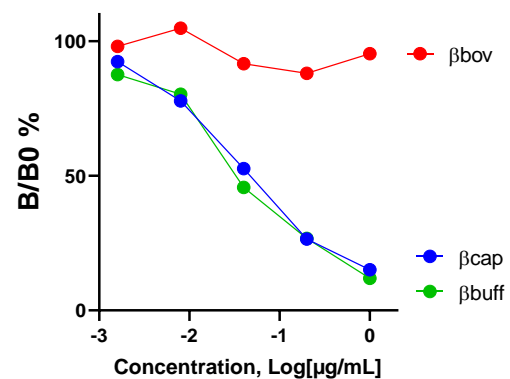
202

203 Figure 1. A: Competitive inhibition of the IgE binding to caprine β -casein by caprine β -casein (blue),
204 buffalo's β -casein (green), and bovine β -casein (red). B: Competitive inhibition of the IgE binding to
205 buffalo's β -casein by caprine β -casein (blue), buffalo's β -casein (green), and bovine β -casein (red).
206 Results were expressed as B/B₀, B₀ and B representing the amount of labeled β -casein bound to
207 immobilize IgE antibodies in the absence or presence of a known concentration of inhibitor,
208 respectively.

A Competitive inhibition of IgE-binding to caprine β -casein



B Competitive inhibition of IgE-binding to buffalo's β -casein



209

210

211

212

213

214 **Supplementary methods**

215

216 **Indirect ELISA**

217 Microtiter plates were coated with purified milk proteins (5 µg/mL) and then saturated with EIA
218 buffer (0.1 M phosphate buffer, 0.1% bovine serum albumin, 0.15 M NaCl, 0.01% sodium azide, pH
219 7.4). After ON incubation with sera diluted 1:5 and 1:20, plates were washed and IgE-binding was
220 revealed by addition of a mouse anti-human IgE mAb (clone BS17) labeled with acetylcholinesterase
221 (AChE, 2 Ellman Unit (EU)/mL). AChE activity was revealed after addition of Ellman's reagent and
222 absorbance was measured at 414 nm.

223 **IgE-capture ELISA**

224 sIgE levels were also evaluated by measuring the binding of biotinylated allergens to serum IgE
225 antibodies captured by a monoclonal antibody (mAb) immobilized on the solid phase.^{27,31} Briefly,
226 mouse anti-human IgE mAb (Clone LE27) was adsorbed on microtiter plates (2.5 µg/mL).³⁰ After ON
227 incubation with diluted sera (1:5), plates were washed and biotinylated β-casein was added (0.05
228 nmol/mL) for 4h at RT. After washing, AChE-labeled neutravidin was added before revelation with
229 Ellman's reagent and absorbance was measured at 414 nm and expressed in Absorbance Unit
230 (AU_{414nm}).

231 For competitive inhibition of IgE-binding, after ON incubation with diluted sera (1:5), 25 µL of inhibitors
232 (i.e. increasing concentration of unlabeled milk protein) were mixed with 25 µL of biotinylated β-casein
233 protein (0.05 µg/mL), and incubated at RT for 4h. IgE-binding was revealed as described above. Results
234 were expressed as B/B0, B0 and B representing the amount of labeled SFS protein bound to immobilize
235 IgE antibodies in the absence or presence of a known concentration of inhibitor, respectively.

236

237

238