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RESEARCH LETTER

Allergy to all mammalian Bovidae proteins but cow's milk in a child

To the Editor,

Cross reactivity between mammalian proteins (cow, goat, ewe, buffalo) has been previously described in both in vitro and in vivo studies. The highest homologies are observed between the milk proteins from cows and other Bovidae, being on average 96.1% for buffalo, 91.1% for ewe, and 87.6% for goat.¹

Lower sequence similarities are associated with proteins contained in milks from Suidae (pigs and boars, 64.2%), Equidae (horse 62.4% and donkey 62.8%), Camelidae (dromedary 60.0%) and humans (58.4%). Despite the high degree of homology with cow's milk, allergy to buffalo's milk is poorly investigated and so far only one case of severe allergy to buffalo's milk has been shown in a 70-year-old German patient who indeed tolerated cow's milk and had negative goat milk skin tests.²

We report the case of a six-year-old boy with a past medical history of atopic dermatitis. At the age of two years, he developed asthma. The skin tests with common inhalants (Alk-Abelló extracts)³ resulted positive to grass, dust mite, cypressus, cat and mugwort. The child was eating regular cow's milk proteins from the first year of life. At the age of three years he had vomit immediately after the intake of ewe cheese. Prick-to-prick with ewe's and goat's milks resulted positive as well as skin prick tests with goat's milk and ewe's milk caseins (Alk-Abello, Milan, Italy). Cow's milk prick-to-prick and skin prick test (SPT) with purified casein, α -lactalbumin and β -lactoglobulin (Alk-Abello, Milan, Italy) from cow's milk were negative. He was advised to eat only cow's milk and cow's milk products. At the age of four years after the intake of cheese made with cow's and goat's milk (the last ingredient was not declared) he developed asthma plus vomit. The in vitro diagnostic test confirmed the presence of specific IgE to goat and ewe milk: >100 KUA/L and 78.9 KuA/L respectively, and the absence of specific IgE to cow's milk, bovine casein and whey proteins α -lactalbumin and β -lactoglobulin (ImmunoCAP system, Phadia Diagnostics, Milan Italy). The total IgE level was 100 KUA/L.⁴

At the age of five years he had an anaphylactic reaction (asthma, vomit, generalised urticaria)⁵ immediately after the intake of buffalo's mozzarella cheese. At the time of

anaphylaxis the child could eat cow's milk proteins without adverse reactions. Wheal and flare (W/F) reactions by means of prick-to-prick tests with buffalo's milk, buffalo's mozzarella and histamine at 10 mg/ml were 10/20, 10/20 and 4/8, respectively.

Prick-to-prick with buffalo's mozzarella was performed in three non-allergic children as controls.

Under double-blinded, placebo-controlled oral provocation with buffalo's fresh untreated milk,⁶ the patient developed urticaria and rhinitis (1 mL = single provoking dose). The reaction was treated with oral corticosteroids and anti-histamines.

This study was performed with the approval of the ethics committee, and both parents gave written consent.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis with different mammalian milks and with purified proteins from cow's milk (α - and β -casein, α -lactalbumin, β -lactoglobulin and bovine serum albumin) was performed. As shown in Fig. 1, different electrophoretic patterns were obtained according to the mammalian species considered. Cow's milk contains four different bands from top to bottom: α - and β -casein, β -lactoglobulin and α -lactalbumin.

In the goat's milk sample (both fresh and commercial), the α -casein amount is lower than that in cow's milk according to previous evidence.¹ Ewe's milk electrophoretic profile is slightly different in terms of electrophoretic mobilities of casein and whey proteins. Buffalo's milk shows an electrophoretic profile overlapping that of cow's milk.

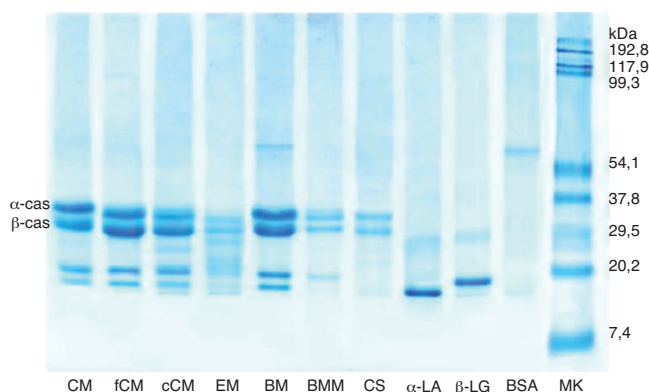


Figure 1 SDS-PAGE of different mammalian milk samples and purified bovine proteins.

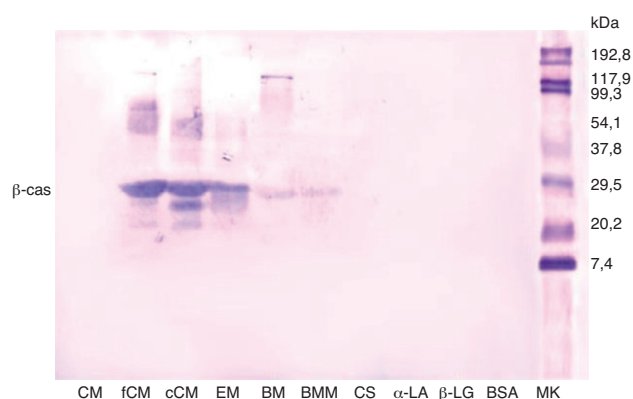


Figure 2 Immunoblotting of samples illustrated in Fig. 1 with the serum of the allergic subject.

In the buffalo's mozzarella both α -casein and β -casein are easily identified, whereas whey proteins are less clearly visible (only a weak band corresponding to β -lactoglobulin).

After transfer of proteins from gel to PVDF membrane, incubation with the serum of the allergic patient was performed. The immunoblotting showed specific IgE reactivity against β -casein in goat's milk and ewe's milk; a lower but still significant reaction was observed versus buffalo's milk and mozzarella. A marked IgE-mediated reaction with another protein having an electrophoretic run higher than β -casein, can be observed in commercial goat's milk. This is probably due to the presence of γ -caseins (a fraction of β -casein) coming from a partial proteolysis. The same band is also present in fresh goat's milk but in traces; in fact γ -caseins are fragments due to proteolysis whose abundance increases progressively from fresh milk to cheese. No reactivity with cow's milk proteins can be detected (Fig. 2).

Cross reactivity between goat or ewe's milk and cow's milk has been frequently described.⁷

Moreover, allergy to milk of goat and ewe, two phylogenetically closely related mammals, without allergy to cow's milk has also been reported.⁸ Again, despite the close phylogenetic relationship between buffalo and cow, an isolated buffalo's milk allergy has been described.²

In this case,² specific IgE against two bands were demonstrated and in immunoblot experiments, specific IgE reactivity could be reproduced against non-glycosylated and glycosylated bufaline K-caseins, corresponding to the 17-kDa band.

In our study, we demonstrated a sensitisation to β -casein from buffalo's, ewe's and goat's milk without any reactivity to cow's milk proteins.

So far very few clinical studies have been published on the relationship between buffalo and cow's milk allergy, probably because of a general awareness of the high homology between these two animal species and the usual avoidance of buffalo's milk derivatives (such as mozzarella) by cow's milk allergic subjects. Buffalo's milk allergy may be isolated, without any other Bovidae mammalian allergy,² but this case report suggests that there is the possibility of an associated goat-ewe-buffalo's milk allergy without cow's

milk allergy, and it helps in order to give the right advice on milk avoidance.

Ethical disclosures

Protection of human and animal subjects. We declare that no experiments were performed on humans or animals for this investigation.

Confidentiality of data. We declare that we have followed the protocols of our work centre on the publication of patient data and that the patient included in the study has received sufficient information and has given his informed consent in writing to participate in that study.

Right to privacy and informed consent. We have obtained the informed consent of the patient's relatives in the study. We for correspondence are in possession of this document.

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