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Skin Testing With Water Buffalo's Milk in Children With Cow's Milk Allergy

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Abstract

Background—Cow's milk allergy is the most common food allergy in young children. In areas outside the United States, milk from other mammals has been studied as a possible and desirable alternative for children with cow's milk allergy.

Objectives—We chose to further investigate water buffalo's milk as an alternative for cow's milk allergic children in the United States.

Methods—Children with cow's milk allergy were skin prick tested with water buffalo's milk. Additionally, subjects were followed clinically for 1 year after the test to determine how many of the subjects had persistent cow's milk allergy.

Results—In total, 30 children, age 8 months to 8 years, were skin prick tested to water buffalo's milk with 73% (22/30) having a positive test. All children with a negative water buffalo's milk skin test also had a negative cow's milk skin test. In follow-up, most (7 of 8) of the children with a negative skin prick test (SPT) to water buffalo's milk were found to have outgrown their cow's milk allergy. In comparison, all of the subjects with a positive skin test to water buffalo's milk had persistent cow's milk allergy. After adjusting for this, we determined that 96% (22/23) of the children with persistent cow's milk allergy were positive on skin testing to water buffalo's milk.

Conclusions—In this population, the vast majority of children with persistent cow's milk allergy were positive on skin prick testing to water buffalo's milk. These results indicate that water buffalo's milk is unlikely to be a successful alternative for children with cow's milk allergy.

Introduction

It is estimated that up to 6% of children in westernized countries have a food allergy.¹ In particular, cow's milk allergy is the most common food allergy affecting toddlers with a rate of 2%–3% in the first 2 years of life.^{1–4} While most children eventually develop tolerance to cow's milk,⁵ parents must utilize other milk sources until that time. In the United States, apart from breastfeeding, alternatives to cow's milk include hydrolyzed cow's milk, elemental cow's milk, soy milk, and rice milk. Additionally, other mammalian sources of milk have been studied outside of the United States. Sheep's milk and goat's milk are generally not recommended as an alternative in cow's milk allergic patients due to cross-reactivity between sheep's and goat's milk with cow's milk,^{6–8} whereas horse's milk showed a much greater degree of success.⁹

Milk from water buffalo (*Bubalus bubalis*) is a common drinking milk in certain countries, and is the main component of buffalo mozzarella eaten throughout the world.¹⁰ Water buffalo's milk as a substitute for children with cow's milk allergy has never been formally studied in a controlled intervention trial. *In vitro* studies suggest antibody cross-reactivity and demonstrate a similar proteomic evaluation between cow and water buffalo's milk proteins.^{11,12} Similarly, one human study from Israel showed a high degree of skin test positivity to water buffalo's milk in patients with cow's milk allergy.¹³ In contrast, a recent case report discussed a child with cow's milk allergy who was able to tolerate water buffalo's milk.¹⁴ However, the use of water buffalo's milk in these patients has never been further studied or reported. We chose to further investigate the possibility of water buffalo's milk as an alternative for cow's milk in American children. For this study, we performed skin prick testing with water buffalo's milk in children with cow's milk allergy.

Materials and Methods

Subjects were recruited from cow's milk allergic children who were being evaluated in our allergy and immunology program. Subjects were approached for inclusion into the study on the day of their allergy clinic visit. Inclusion criteria consisted of all of the following: (1) physician diagnosis of cow's milk allergy, (2) a history of clinical reaction after the ingestion of cow's milk, and (3) currently a cow's milk elimination diet. After informed consent was obtained, subjects were skin prick tested to fresh pasteurized water buffalo's milk (Woodstock Water Buffalo Company, Woodstock, VT). The use of the pasteurized water buffalo's milk for skin testing was approved and monitored as an Investigational New Drug by the United States Food and Drug Administration (IND 13459).

Skin prick testing was performed on the back or volar surface of the forearm with the Multitest applicator (Lincoln Diagnostics, Inc., Decatur, IL) using standard methods.¹⁵ Positive histamine and negative saline controls (Hollister-Stier, Spokane, WA) were used. A wheal 3 mm greater than the negative control at 15 min was considered positive. Subjects were excluded if they had recently taken any medications that might interfere with skin testing (short-acting antihistamines within 3 days, long-acting antihistamines within 10 days, and cetirizine or levocetirizine within 14 days). All subjects had a positive histamine control.

When available, additional information regarding cow's milk allergy was obtained on each subject. This included diagnostic evaluations performed in our program such as skin prick testing to cow's milk extract (Greer Laboratories, Lenoir, NC) and allergen-specific IgE levels (ImmunoCAP 250; Phadia AB, Portage, MI) to cow's milk. The decision to perform these supplementary tests was determined solely by the primary allergy physician as clinically indicated, and therefore, these tests were not performed in all subjects. The research team did not have any influence or knowledge of supplementary tests performed until after the water buffalo's milk skin prick test (SPT) results were finalized. The study was approved by the Institutional Review Board of Children's Hospital Boston. Informed consent was obtained from the parents of all subjects prior to inclusion in the study.

Statistical analysis

The subjects with a water buffalo's positive SPT were compared to those with a negative test. Continuous variables, such as age and cow's milk-specific IgE levels, were compared between the 2 groups by using the Wilcoxon rank-sum test (Mann-Whitney *U*). Binomial variables, such as gender and cow's milk SPT results, were compared using the Fisher's exact test.

Results

Thirty children with a diagnosis of cow's milk allergy were included in our study and skin prick tested to water buffalo's milk. The subjects' ages ranged from 8 months to 105 months with 60% male. The clinical history of all subjects is presented in Table 1. All subjects had a clinical diagnosis of cow's milk allergy at the time of their inclusion into the study. All children had a history of a reaction associated with the ingestion of milk. Additionally, 93% (28/30) of the subjects had a history of either a positive SPT or a positive cow's milk-specific IgE (>0.35).

Of the 30 subjects with cow's milk allergy, 73% (22/30) were found to have a positive SPT to water buffalo's milk. The median wheal diameter for these subjects was 15 mm, with 86% (19/22) having a wheal greater than 10 mm. None of the subjects had any systemic reaction or cutaneous reaction outside of the skin testing area. Table 2 provides a comparison of the subjects with a positive water buffalo's milk SPT to those with a negative water buffalo's milk SPT. There were no differences noted in the gender or age of these 2 groups. However, there were significant differences noted in their testing for cow's milk allergy. The group with a positive water buffalo's milk SPT had a higher median cow's milk-specific IgE level (5.28 kU/L versus 0.76 kU/L; $P < 0.001$). Similarly, subjects with a positive water buffalo's milk SPT were highly likely to also have a positive cow's milk SPT on the same day (94% versus 0%; $P < 0.001$).

Water buffalo's milk and cow's milk skin prick testing usually yielded the same result when tested simultaneously. Of the subjects who were skin prick tested to both water buffalo's and cow's milk on the same day, 96% (25/26) had corresponding results on both tests. Seventeen subjects were positive on both tests and 8 subjects were negative on both tests. The one subject with differing results was an infant male with a negative SPT to cow's milk, but a positive SPT to water buffalo's milk (11 mm wheal). This subject had a history of clinical reaction to cow's milk, a positive cow's milk-specific IgE level, and had been improving on a strict cow's milk elimination diet. Of note, there were no subjects who had a positive SPT to cow's milk and a negative SPT to water buffalo's milk.

Subjects were followed clinically for 8 to 12 months after the water buffalo's milk SPT was performed to determine if they were outgrowing their cow's milk allergy. Of the subjects with a positive water buffalo's milk SPT, all of them were considered to have persistent cow's milk allergy and continued on a cow's milk elimination diet. In contrast, of the subjects with a negative water buffalo's milk SPT, only one subject was considered to have persistent cow's milk allergy. The other 7 subjects were considered, by their primary allergy provider, to be likely outgrowing their cow's milk allergy based on the results of the evaluation performed on that day. In fact, all 7 of these subjects had passed or were scheduled for an open challenge to cow's milk within 1 year of inclusion into the study (Table 3). After removing these 7 subjects from our analysis, 96% (22/23) of the subjects with persistent cow's milk allergy demonstrated a positive SPT to water buffalo's milk.

Discussion

This study adds to the growing amount of literature regarding alternative mammalian sources of milk for use in children with cow's milk allergy. In our group, we found 96% of the children with persistent cow's milk allergy were found to have a positive SPT to water buffalo's milk with a majority of these subjects having wheal sizes >10 mm. Additionally, we found that the SPT results for cow's milk and water buffalo's were in concordance 96% of the time when these tests were performed on the same day. We did not have any subjects with a positive cow's milk SPT and a negative water buffalo's milk SPT on the same day.

Although challenge data are not available, these data indicate that water buffalo's milk is unlikely to be a suitable alternative for children with cow's milk allergy.

A limitation of the study is the lack of an oral food challenge to water buffalo's milk. We recognize that this is the best method for definitive confirmation of true clinic allergy. However, due to patient safety concerns, we did not perform food challenges. Given the strongly positive skin tests, we did not proceed with food challenges for concern of a severe reaction. A second limitation of our study is that testing was performed in children who may be outgrowing their milk allergy, due to the evolving natural history of food allergies. Originally, we found that 73% of our children with cow's milk allergy had a positive SPT to water buffalo's milk. It is important to note that, of the subjects with a negative water buffalo's milk SPT, all of them also had a negative SPT to cow's milk performed on the same day. Furthermore, most of the negative water buffalo's milk SPT subjects were considered to be likely outgrowing their cow's milk allergy at the time of the testing (Table 3). We therefore removed these subjects from our final analysis and found that nearly all (96%) of the children with persistent cow's milk allergy demonstrated a positive water buffalo's milk SPT.

Our data agree with previous studies that show a high degree of cross-reactivity for certain mammalian milk products. A number of *in vitro* studies have shown a high degree of cross-reactivity for cow's milk when compared to sheep's, goat's, and water buffalo's milk.^{8,12,16} More recently, Katz et al. in Israel showed a high degree of cross-sensitization when cow's milk allergic children were skin prick tested to milk from deer, ibex, and water buffalo.¹³ Goat's milk has undergone the most thorough clinical trials. Bellioni-Businco et al. in Italy reported that 92% (24 of 26) of children with cow's milk allergy were found to have positive double-blind, placebo-controlled, food challenges to goat's milk.⁶ Similar food challenge studies have not been performed for sheep's milk. Our study suggests that a majority of patients with cow's milk allergy would also fail an oral food challenge of water buffalo's milk. In contrast to our results, other mammalian milk products have been shown to have a much lower degree of cross-reactivity. *In vitro* studies from Italy have shown a weak cross-reactivity between cow's milk proteins and milk proteins from camels, donkeys, and horses.^{12,16} A small *in vivo* study demonstrated that only 25% (2 of 8) of patients with cow's milk allergy reacted to camel's milk on skin prick testing.¹³ Iacono et al. demonstrated that donkey's milk was tolerated well by children with cow's milk allergy.¹⁷ The most complete study was performed using horse's milk in Italy. Businco et al. demonstrated that 96% of children with cow's milk allergy tolerated horse's milk in double-blind, placebo-controlled, food challenges.⁹ It is reasonable that future studies of alternative mammalian milk products would focus on these animals. However, these products are much less commercially available, particularly in the United States, when compared to products made from goat, sheep, and water buffalo's milk.

In conclusion, our study found the majority of cow's milk allergic children had strongly positive skin tests to water buffalo's milk. This study agrees with previous *in vitro* findings. Our study, performed in the United States, agreed with a similar study in Israel. Based on our data, we expect that water buffalo's milk is likely not a safe option for most children with cow's milk allergy; although, future studies with food challenges to water buffalo's milk may be considered for confirmation.

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Table 1

Clinical Characteristics and Results of Children With Cow's Milk Allergy who Were Skin Prick Tested to Water Buffalo's Milk

Patient	Gender	Age (months)	Reaction to cow's milk	CM-specific IgE (kU/L)	CM SPT (wheal in mm)	WBM SPT (wheal in mm)
1	F	50	Rash	5.28	ND	18
2	M	21	GI, Resp	0.76	0	0
3	M	57	Rash, Resp	>100.00	20	15
4	F	11	Rash	49.40	ND	15
5	F	46	GI	9.04	25	20
6	F	19	Rash	1.70	0	0
7	F	105	Rash, GI	<0.35	0	0
8	M	8	Rash, Resp	6.11	6	12
9	M	34	Rash, Resp	5.34	13	25
10	M	11	Rash	10.40	ND	10
11	M	100	Rash	7.78	20	30
12	M	49	Rash	ND	10	12
13	F	52	Rash	0.88	13	9
14	F	11	Rash	<0.35	11	10
15	M	50	Rash	<0.35	18	20
16	M	27	Rash	2.00	20	25
17	M	49	GI	0.63	20	32
18	M	12	Rash, GI	ND	11	14
19	M	52	Rash	1.37	0	0
20	M	25	Rash	7.47	10	33
21	F	52	Rash	0.44	0	0
22	M	12	Rash, GI	0.92	0	11
23	M	87	Rash	98.20	ND	22
24	M	24	GI, ENT	6.83	0	0
25	M	18	Rash, GI	ND	0	0
26	F	74	Rash, GI	1.77	18	17
27	F	30	GI	0.38	4	3
28	F	38	Rash	<0.35	0	0
29	M	16	Rash, GI	ND	5	8

Patient	Gender	Age (months)	Reaction to cow's milk	CM SPT (wheal in mm)	WBM SPT (wheal in mm)
30	F	50	Rash, GI	20	11

All reported CM-specific IgE levels and CM SPT results were recent (performed on the same day or within 6 months prior to the WMB SPT).

Abbreviation: ND, not done.

Clinical reaction to cow's milk:

Rash: Any cutaneous manifestation.

GI: Any vomiting, diarrhea, abdominal pain.

Resp: Any breathing difficulties, cough, or airway compromise.

ENT: Any rhinorrhea, facial swelling, oral swelling, or eye changes.

Table 2

Comparison of Children Based on Water Buffalo's Milk Skin Prick Test Result

	Positive WBM SPT	Negative WBM SPT	P value
Water buffalo's milk SPT: Number	22	8	
Wheal diameter: Median (range)	15 mm (3–33 mm)	All negative	
Gender: % Male	64% (14/22)	50% (4/8)	0.678
Age: Median (range)	40 months (8–100 months)	31 months (18–105 months)	0.707
CM-specific IgE (recent): Median (range) (# Tested)	5.28 kU/L (0.35–100 kU/L) (19)	0.76 kU/L (0.35–6.83 kU/L) (7)	<0.001
CM SPT (same day): % Positive	94% (17/18)	0% (0/8)	<0.001
Wheal diameter: Median (range)	13 mm (0–25 mm)	All negative	

All cow's milk (CM)-specific IgE levels used for comparison were recent (performed within 6 months).

All cow's milk SPT (Skin Prick Test) studies were performed on the same day as the WBM SPT.

Table 3

Children With Negative Water Buffalo SPT: Clinical Outcomes

Gender	Age (months)	Reaction history (cow's milk)	Cow's milk RAST (most recent)	Cow's milk SPT (most recent)	Clinical outcome
M	21	GI, Resp	0.76 kU/L	Negative	Presumed to have outgrown milk allergy. Passed open cow's milk challenge
F	19	Skin	1.70 kU/L	Negative	Presumed to have outgrown milk allergy. Passed open cow's milk challenge
F	105	Skin, GI, Resp	0.35 kU/L	Negative	Presumed to have outgrown milk allergy. Passed open cow's milk challenge
M	52	Skin	1.37 kU/L	Negative	Presumed to have outgrown milk allergy. Passed open cow's milk challenge
F	52	Skin	0.44 kU/L	Negative	Presumed to have outgrown milk allergy. Passed open cow's milk challenge
M	24	GI, ENT	6.83 kU/L	Negative	Presumed to have persistent milk allergy. Continues to avoid cow's milk
M	18	Skin, GI	Not obtained	Negative	Presumed to have outgrown milk allergy. Scheduled for open cow's milk challenge
F	38	Skin	0.35 kU/L	Negative	Presumed to have outgrown milk allergy. Scheduled for open cow's milk challenge

All cow's milk RAST levels were drawn within 6 months prior to water buffalo's milk SPT.

All cow's milk SPT studies were performed on the same day as the water buffalo's milk SPT.