

Mammalian milk allergy: clinical suspicion, cross-reactivities and diagnosis

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Purpose of review

Cow's milk allergy affects 2–3% of young children, the economic impact of which necessitates search for simple diagnostic tools and affordable milk substitutes.

This review examines recent studies on the diagnosis of cow's milk allergy as well as on the allergenicity of milk from other mammalian species.

Recent findings

Resolution of symptoms during strict milk avoidance and their re-appearance during the double-blind, placebo-controlled milk challenge remains the gold standard for the diagnosis of cow's milk allergy. Allergic eosinophilic esophagitis/gastroenteritis requires confirmatory endoscopic biopsy. There are increasing data in various populations on cut-off points based on positive predictive values for skin prick test and milk-specific IgE measurements to aid in the diagnosis of cow's milk allergy and to decrease the number of unnecessary food challenges. For non-IgE-mediated manifestations, noninvasive diagnostic tests are still largely lacking. The significant homology between milk from cow, sheep and goat results in clinical cross-reactivity. However, mare's or donkey's milk may be tolerated by some individuals.

Summary

Data have been accumulating on the utility of diagnostic tools for mostly IgE-mediated milk allergy and allergenicity of milk from other mammalian species, although further studies are sought.

Keywords

allergenicity, bovine, cow's milk, cross-reactivity, diagnosis

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Introduction

Cow's milk allergy (CMA) affects 2–3% of young children and presents with a wide range of IgE-mediated and non-IgE-mediated clinical syndromes. Diagnosis is based on a physician-supervised oral food challenge, but measurement of cow's milk-specific IgE can aid in the diagnosis of IgE-mediated CMA and occasionally eliminate the need for oral food challenges. However, diagnostic tests for non-IgE-mediated manifestations are lacking. This review examines the basic literature and most recent studies within the past 18 months on the clinical suspicion and diagnosis of CMA as well as the cross-reactivity between milk from bovine and other mammalian species.

Clinical suspicion

Clinical symptoms of CMA often appear during the first months of life, usually within days or weeks after the commencement of feeding with cow's milk-based formula, or occasionally already earlier during breastfeeding. Patients with CMA present with a wide range of IgE-

mediated and non-IgE-mediated clinical syndromes (Table 1) [1^{••},2–5]. IgE-mediated reactions generally tend to occur immediately or within 1–2 h of ingestion, whereas non-IgE-mediated reactions occur later [6].

Cutaneous manifestations

In addition to causing immediate reactions such as hives and angioedema, CMA plays a pathogenic role in a subset of patients, primarily infants and children, with atopic dermatitis. Approximately 40% of infants and young children with moderate-to-severe atopic dermatitis have food allergy, hen's eggs, cow's milk, soy and wheat accounting for about 90% of allergenic foods [7,8]. In another study [9], hen's egg showed the highest percentage of positive reactions (70%) followed by cow's milk (51%).

Gastrointestinal manifestations

Gastrointestinal manifestations of CMA may be nonspecific, and similar symptoms can be due to other causes. IgE-mediated manifestations include mouth and lip pruritus, abdominal pain, vomiting and diarrhea shortly after ingestion of cow's milk. In non-IgE-mediated

Table 1 Presentation of cow's milk allergy

IgE-mediated	
Cutaneous	Urticaria Angioedema
Gastrointestinal	Oral itching and abdominal pain Nausea and vomiting Diarrhea
Respiratory	Rhinoconjunctivitis Wheeze, asthma Laryngeal edema
Systemic	Anaphylaxis
Mixed IgE and non-IgE mediated	
Cutaneous	Atopic dermatitis
Gastrointestinal	Eosinophilic gastroenteropathies
Non-IgE mediated	
Gastrointestinal	Gastroesophageal reflux Protein-losing enteropathy Dietary protein enterocolitis Dietary protein proctitis/proctocolitis Colic Constipation
Respiratory	Pulmonary hemosiderosis (Heiner syndrome)

Adapted from [1••] and [5].

manifestations, the causal relationship to foods is more difficult to detect.

Symptoms in gastroesophageal reflux (GER) associated with CMA are the same as those observed in primary gastroesophageal reflux disease (GERD). Immunologic tests and esophageal pH monitoring may be helpful [4]. A study by Nielsen *et al.* [10] examined the role of CMA, verified by elimination and food challenge, in infants with severe GERD. A positive cow's milk challenge was not associated with a significant increase in the reflux index.

Eosinophilic esophagitis is a disorder identified in patients with symptoms suggestive of GERD but unresponsive to conventional reflux therapies and characterized by eosinophilic inflammation of the esophagus. Foods play a role in allergic eosinophilic esophagitis (AEE) in a subset of patients. In addition, pain, nausea, vomiting, diarrhea, poor appetite, food aversion, failure to thrive, growth failure, dysphagia and food impaction can also be seen in allergic eosinophilic gastroenteritis (AEG), eosinophilic gastroenteropathy of the stomach and intestines [11•].

Food protein-induced enterocolitis syndrome (FPIES) typically presents with profuse vomiting and diarrhea within 2–3 h after ingestion of the offending allergen, causing profound dehydration and lethargy in a formula-fed infant [11•]. Diarrhea may have occult blood, and fecal smears reveal leukocytes and eosinophils. Chronic exposure to the offending allergen results in a clinical presentation consistent with failure to thrive and hypoalbuminemia [11•,12]. Milk, soy and grains are the major offending allergens [1••].

Allergic proctitis usually presents by 6 months of life in breastfed (60%) or occasionally formula-fed (40%) infants

with bloody streaked, mucousy, loose stools and occasionally diarrhea in an otherwise well appearing infant [11•]. Cow's milk and soy are the major causative foods [11•]. CMA in infants with proctitis may be less common than previously thought, and other causes such as viruses may cause a similar presentation [13].

The role of diet in infantile colic is still controversial [14]. Studies [15–17] have shown an improvement in colic symptoms after cow's milk elimination or change of formula. Worsening of the symptoms was confirmed by a cow's milk challenge. A recent randomized trial [18] also found that maternal exclusion of allergenic foods was associated with a reduction in distressed behavior among breastfed infants with colic, although challenges were not performed to demonstrate relapse of symptoms after reintroduction of food proteins into the maternal diet.

CMA/intolerance has been suggested as a cause of constipation in infants and children [19–21]. Studies using double-blind [21] or open food challenges [22] demonstrated that in one-third to one-half of children with refractory chronic constipation, it was related to cow's milk. The incidence may vary according to selection criteria and age of patients. Findings include proctitis with eosinophil infiltrate of the rectal mucosa and a reduced thickness of the rectal mucus layer [21] as well as lymphonodular hyperplasia in the terminal ileum and colon [22].

CMA can present already in the neonatal period with bilious vomiting, massive bloody stools [23,24] and difficulty of spontaneous defecation mimicking Hirschsprung's disease [25]. Radiologic findings show irregular bowel wall or marked narrowing of intestinal lumen indicating massive mucosal edema. Peripheral eosinophilia, eosinophilic infiltration in the lamina propria and positive serum cow's milk-specific IgE support the diagnosis of CMA [23,24].

Respiratory manifestations

Food allergy may present with a variety of respiratory tract symptoms that generally involve IgE-mediated responses, including rhinorrhea and wheezing. However, chronic or isolated asthma or rhinitis induced by food is unusual [25]. Furthermore, food allergy is a risk factor for severe asthma [26].

Heiner syndrome is a food hypersensitivity pulmonary disease that affects primarily infants and is mostly caused by cow's milk. The symptoms include cough, wheezing, hemoptysis, nasal congestion, dyspnea, recurrent otitis media, recurrent fever, anorexia, vomiting, colic or diarrhea, hematochezia and failure to thrive [27]. Radiologic evidence of pulmonary infiltrates and high serum titers of precipitating antibodies (IgG) to cow's milk proteins are seen. Milk-specific IgE may be detected.

Cow's milk allergens

Cow's milk contains caseins and whey proteins, accounting for approximately 80 and 20% of total protein, respectively [28]. Caseins (α_{s1} -caseins, α_{s2} -caseins, β -caseins and κ -caseins, Bos d 8) as well as the whey proteins, α -lactalbumin (ALA, Bos d 4) and β -lactoglobulin (BLG, Bos d 5) are the most important allergens [28–30]. Minor milk allergens include BSA (Bos d 6), lactoferrin and immunoglobulins (Bos d 7) [30]. Recently, high intensity and frequent recognition (41%) of lactoferrin was reported from young patients suffering from CMA [31*].

Cooking diminishes the antigenicity of ALA and BLG [32,33] and may explain why cooked milk is better tolerated by many patients [34**]. High temperature reduces allergenicity of many food proteins, presumably by altering the conformation of heat-labile proteins that results in loss of conformational epitopes [35].

Allergy to other mammalian milk

The protein composition between different mammals has been reported (Table 2) [28,35,36]. Mammals that are phylogenetically related, such as sheep and goat, cow and water buffalo and horse and donkey (i.e. ass), have quite similar milk protein expression [36]. Although high degree of cross-reactivity between milk proteins from different mammals has been described based on the very homologous proteins (Table 3), there are studies that emphasize heterogeneity between proteins from various mammalian milks.

Most of the patients allergic to cow's milk cannot tolerate goat's (i.e. caprine) milk or sheep's (i.e. ewe's or ovine) milk and vice-versa. A study [37] in 26 children with proven IgE-mediated CMA, using skin tests, specific IgE and double-blind, placebo-controlled food challenge (DBPCFC), CAP inhibition and immunoblotting inhibition assays revealed strong cross-reactivities between cow's milk and goat's milk. However, selective allergy to goat's milk or sheep's milk but not to bovine milk has also been reported in 28 older children with severe allergic reactions, including anaphylaxis, after consumption of

Table 3 Comparison of amino acid sequences of major proteins from different mammalian species

	Sequence homology (%)					
	Cow versus goat	Cow versus ewe	Cow versus human ^a	Cow versus mare ^a	Cow versus donkey ^a	Cow versus camel ^a
ALA	96	96	81	69	69	66
BLG	95	94	^b	69	^b	^b
Casein						
α_{s1}	87	89	40	51	^b	^b
α_{s2}	88	89	^b	^b	64	^b
β	90	90	63	62	^b	45
κ	85	84	58	61	43	64

ALA, α -lactalbumin; BLG, β -lactoglobulin. Adapted from [28].

^a Homology assessed using Vector NTI (Invitrogen, Carlsbad, California, USA).

^b NA (not available).

goat's milk or sheep's milk products, but tolerance to cow's milk products. IgE antibodies recognized α_{s1} -caseins, α_{s2} -caseins and β -caseins from goat's milk or sheep's milk, but cow's milk caseins were not at all or poorly recognized [38]. Increasing concentrations of cow's milk caseins failed to inhibit the binding of patient's IgE to goat's milk or sheep's milk caseins. In contrast, a case report in an adult with goat's milk allergy without CMA found specific IgE to goat's milk ALA [39].

Recently, Katz *et al.* [40] suggested allergy to artiodactyls (even-toed ungulates, i.e. 'pawed animals') and ruminants, such as cow, sheep and goat, to be due to the 'kosher epitope'. All 24 patients allergic to CMA also tested positive by skin prick test (SPT) to deer, ibex (wild mountain goat) and buffalo milk, but only one-fifth tested positive to pig's milk and 25% to camel's milk. Interestingly, although reindeer is also considered a ruminant, Suutari *et al.* [41] had shown only partial cross-reactivity of human antbovine IgE with reindeer BLG.

Previous studies [42,43] on allergenicity of equine milk (mare's and donkey's milk) have suggested it to be less allergenic. More recently, a randomized, crossover study showed that donkey's milk was better tolerated and more effective than goat's milk in reducing symptoms of atopic dermatitis in children with CMA. In food challenges in 26 children, 23 had a positive reaction with goat's milk and one with donkey's milk [44*]. A prospective study [45] of 46 selected children with CMA confirmed that 80% of children tolerated donkey's milk. The degree of cross-reactivity of IgE with donkey's milk proteins was very weak and nonspecific. However, the study utilized heated donkey's milk and fresh and skimmed cow's milk, both for the food challenges and the in-vivo and in-vitro testing, which may have important implications for the tolerability. Furthermore, 80% of children with prior anaphylaxis reacted to donkey's milk as well [46].

Table 2 Protein composition of milk from different mammalian species

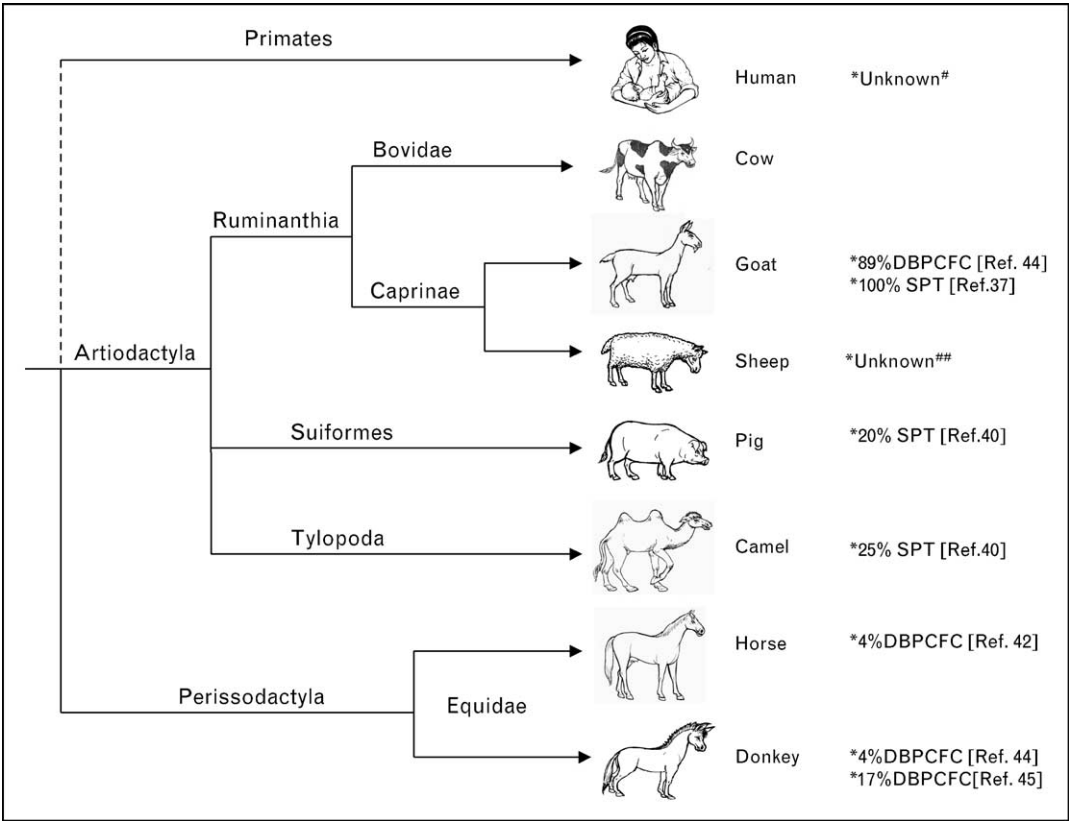
	Cow ^a	Goat ^a	Ewe ^a	Mare ^b	Donkey ^b	Human ^b
Proteins (g/l)						
Casein	28–30	25–30	50–60	9.4–12.0 ^b	6.4–10.3 ^b	3.2–4.2 ^b
α -casein	14	2–6	25	2.5 ^c		0.8 ^c
β -casein	11	18	25	11.0 ^c		4.0 ^c
κ -casein	4	4	10	0.25 ^c		1.0 ^c
Whey	6	4	9	7.4–9.1 ^b	4.9–8.0 ^b	6.8–8.3 ^b

^a Adapted from [28].

^b Adapted from [35].

^c Adapted from [36].

Fig. 1 Clinical cross-reactivity between milk from cow and other mammalian species that are phylogenetically either close or far-related



DBPCFC, double-blind, placebo-controlled food challenge; SPT, skin prick test. *Cross-reactivity with cow's milk; #clinical cross-reactivity suggested [50,51]; ##no clinical studies available.

The protein expression of human milk differs remarkably from cow, being more similar to those of horse and donkey, which are relatively poor in casein [36]. Human milk also differs from other mammalian milks in that BLG is absent. Despite this, a significant sequence homology between other bovine and human milk proteins has led to the suggestion that cross-reactivity between conserved regions of bovine and human casein may exist [47,48]. Indeed, the first case of IgE-mediated allergy to endogenous human milk protein described a young father who showed contact urticaria upon exposure to breast milk, but not cow's milk, had specific IgE antibodies to human but not bovine ALA [49]. Schulmeister *et al.* [50] found IgE antibodies to human milk in more than 80% of the tested cow's milk-allergic patients. Both cross-reactive IgE-reactive human antigens such as ALA and noncross-reactive human milk antigens were identified, and immediate-type skin reactions could be elicited with human milk. We utilized peptides representing the known IgE-binding regions of bovine ALA and β -casein and the corresponding, highly similar peptides on human ALA and β -casein [51]. Recognition of both bovine and the corresponding human milk peptides was seen in infants with CMA who continued

having symptoms even when their mothers were no longer ingesting cow's milk-containing products and only show resolution of symptoms when breastfeeding is discontinued. However, low-level IgE binding to human milk peptides was also infrequently seen in those infants who responded to maternal milk elimination [51]. Interestingly, IgE binding to a human peptide in the presence of no binding to the corresponding bovine peptide was infrequently seen, suggesting sensitization primarily to endogenous human milk. Furthermore, human ALA was capable of cross-linking IgE on effector cells in an assay utilizing rat basophil leukemia cells expressing a human IgE receptor α chain that are passively sensitized with IgE antibodies from cow's milk-allergic infants (Jarvinen *et al.*, unpublished data). Clinical cross-reactivity between milk from various mammalian sources is summarized in Fig. 1.

Cross-reactivity to other protein sources

Apart from being an important allergen in milk, serum albumin is found in meat and is considered to be involved in the cosensitization to milk and beef, reported in

13–20% of cow's milk-allergic children [52]. With regard to beef allergy, monoclonal antibodies specific for BSA cross-reacted only with sheep serum albumin, whereas the recognition of other mammalian serum albumins depended directly on the closeness of the phylogenetic relationship between animal species [43].

Given the potential for cross-reactivity based on serum albumin present in milk, meat and epithelia of different mammals, Vicente-Serrano *et al.* [53] examined whether patients with persistent CMA showed sensitization to serum albumin in animal dander. Seven of eight patients sensitized to milk, BSA and animal dander recognized serum albumin in different meats (beef, lamb, deer and pork), epithelia (dog, cat and cow) and cow's milk, although all the patients were tolerant to heated meats, and their sera showed no recognition of heated meat extracts. The authors suggested that patients with allergy to both BSA and cow's milk must avoid raw meats and furry pets.

Adverse reactions in cow's milk-allergic individuals fed soy milk have been associated with a 30-kDa, glycinin-like protein from soybean that cross-reacts with cow's milk casein [54]. In the study by Katz *et al.* [40], cross-sensitization without clinical reactivity to soy milk was noted in 17%. Klemola *et al.* [55] reported 10% incidence of soy allergy, mostly non-IgE mediated, in cow's milk-allergic infants fed soy formula.

Diagnosis

The diagnostic workup of CMA includes the patient's history, SPT, milk-specific IgE antibodies and the atopy patch test (APT). The DBPCFC is the gold standard for the diagnosis of food allergy and part of the diagnostic procedure including elimination of the suspected food, challenge and re-elimination. In breastfed infants, this includes maternal exclusion diet avoiding cow's milk protein, and in formula-fed infants, use of either extensively hydrolyzed or amino acid-based infant formulae [56[•]]. If there is no improvement on a milk avoidance diet, the food in question either is not responsible for

symptoms or the diet has not been restricted enough, and additional foods may be considered suspicious. Although numerous efforts have been made to standardize the DBPCFC, there is a need for improvement. The current status of the indication and performance of controlled oral food challenges in children with suspected food-related symptoms has been recently reviewed [57[•]].

DBPCFC is time-consuming, expensive and can induce severe and potentially life-threatening allergic reactions. Therefore, a number of studies [58–60,61[•],62,63[•]] have investigated the relationship between the specific IgE levels and SPT wheal size to cow's milk and the outcome of milk DBPCFC to identify cut-off values above which the high likelihood of a positive oral food challenge would eliminate the need for a challenge (Table 4). A critical analysis comparing these studies was recently written by Miceli Sopo *et al.* [64], which emphasized that all the studies have been performed on highly selected pediatric populations and positive predictive values (PPVs), which are influenced by the prevalence of the disease in the studied population, are all different between the studies. The predictive values for clinical reactivity associated with food-specific IgE levels determined by ImmunoCAP (Phadia, Uppsala, Sweden) should not be applied to results from other assays [65[•]].

In the recent years, the APT has been suggested as an addition to the work-up for children with atopic dermatitis and suspected CMA, although results are conflicting [66–72]. The most recent study [73] found no additional value of the APT when compared with immediate skin testing in the diagnosis of CMA, although the occlusion was only 24 h, and CMA was only confirmed by an open food challenge. However, APT was found to be useful for evaluating children with CMA manifesting with gastrointestinal symptoms, mainly vomiting and chronic diarrhea [74]. The PPV using fresh cow's milk was 95% and the negative predictive value (NPV) 67% when both immediate and delayed reactions were considered together, although APT performed better in the delayed type reactions.

Table 4 Cut-off values based on 95% positive predicted values for cow's milk-specific IgE and skin prick test in children with cow's milk allergy

Study	Age (years)	95% PPV	Methods
CM-specific IgE			
Sampson [59]		15 kU/l	CAP System FEIA ^b
Garcia-Ara <i>et al.</i> [60]	<1	5 kU/l	CAP System FEIA ^b
van der Gughten <i>et al.</i> [61 [•]]	<2.5	7.5 kU/l	CAP System FEIA ^b
SPT			
Verstege <i>et al.</i> [62]		12.5 mm wheal or 2.7 SI ^a	Fresh CM
Calvani <i>et al.</i> [63 [•]]		15 mm wheal	Fresh CM

CM, cow's milk; PPV, positive predictive value; SI, stimulation index; SPT, skin prick test.

^aSI, that is, the ratio of allergen-induced wheal diameter to histamine-induced wheal diameter.

^bPhadia, Uppsala, Sweden.

Most recently, protein microarrays using purified natural cow's milk-allergen components have been introduced to the diagnostic armamentarium of milk allergy [75]. They showed performance characteristics comparable with the current diagnostic tests with an advantage of using small blood volumes especially lucrative for use in small children. Peptide microarray immunoassay is a novel method that can be used to analyze IgE and IgG4 binding to sequential epitopes on individual milk allergens and has been shown to differentiate between tolerant and milk-reactive patients [76]. Further studies are needed to validate its utility in clinical practice.

The diagnosis of AEE/AEG is based on clinical presentation and biopsy with at least 15 eosinophils/high power field after aggressive therapy with anti-GERD medications and the disappearance of eosinophils following an appropriate dietary elimination diet [77*]. The combination of SPT and APT correctly identified a suitable diet in about 70% of the population with resolution of the symptoms and biopsies, with the remaining 30% requiring an elemental diet [78]. However, the NPV for milk APT was unacceptably low [78]. Unfortunately, there are no other reports to confirm these findings.

In FPIES, traditional allergy testing is commonly negative, and the diagnosis is based on a typical presentation. A small cohort of 19 infants with FPIES most commonly to cow's milk and soy found APT to have a PPV of 75% and a NPV of 100% [79]. A study [80] on gastric juice analysis with more than 10 leukocytes/high power field suggested that it could provide a confirmatory diagnosis for cow's milk protein-induced enterocolitis when vomiting or lethargy after an oral food challenge is not apparent or difficult to interpret.

Conclusion

Data have been accumulating on the utility of cut-off values for specific IgE and SPT to decrease the need for cow's milk challenges. For non-IgE-mediated manifestations, diagnostic tests are sought, and resolution of symptoms and their reappearance during the DBPCMC remains the gold standard for the diagnosis. Significant amino acid sequence homology and resulting high rate of clinical cross-reactivity between milk from ruminants renders sheep's or goat's milk an inappropriate feeding alternative for a cow's milk-allergic individual. Mare's or donkey's milk might be better tolerated, although further studies are needed on the allergenicity of alternative milk protein sources.

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Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 000–000).

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