

REVIEW ARTICLE

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Ass's milk in allergy to Cow's milk protein: a review

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ABSTRACT

Several studies have recently focused attention on ass's milk (AM) due to its special composition and nutritional properties, which are very close to human milk. Whenever a mother cannot breastfeed, or chooses not to breastfeed, or the child is intolerant of cow's milk (CM), the use of a human and CM substitutes must provide the best option to meet the nutritional and health needs of the infant. The authors reviewed all the published studies about AM tolerance, safety and efficacy in the treatment of infants and children with a food allergy, i.e. CM protein allergy (CMPA). In all the reviewed studies, AM was well tolerated and acceptable, due to its palatability. It is a low-calorie food. Researchers enrolled children over 6 months of age, who did not have an exclusive milk diet, and/or had medium-chain triglycerides added. Overall data showed an adequate increase in auxological parameters measured after several months of AM consumption. Finally, potential cross-reactivity between AM protein and CM proteins must be considered. Some studies have reported severe reactions to AM in their study cohorts. However, taken together, all these results suggest that AM might be considered nutritionally adequate in infants and children with CMPA or multiple food allergies, included CMPA.

Key words: *Milk Proteins; Milk Allergy; Allergy and Immunology; Soy Milk*
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INTRODUCTION

Everyday a female ass or donkey produces between 0.2 to 0.3 liters of milk per day. Ass milk (AM) or donkey's milk has a long history; as a matter of fact, its healing and cosmetic virtues have been claimed since antiquity, when doctors recommended it to treat several afflictions. Hippocrates (460-370 BC), the father of medicine, prescribed it for numerous purposes, such as fevers, edema, wounds, poisonings, infectious diseases, nose bleeds and liver troubles (1). It is said that Cleopatra, Queen of Ancient Egypt (69-30 BC), took baths in AM to preserve the beauty and youth of her skin. Legend has it that no less than 700 asses were needed to provide the quantity of milk necessary for her daily bath. This baths were also performed by Poppaea Sabina (30-65

AD), Roman Emperor Nero's second wife (2). Pliny the Elder (23-79 AD), in his encyclopedic work, *Naturalis Historia*, dealing with remedies derived from animals, proposed using it to fight fever, fatigue, eye strain, weakened teeth, face wrinkles, poisonings, ulcerations, asthma and certain gynecological troubles (2). Similarly, Georges-Louis Leclerc, Comte de Buffon (1707-1788) mentions the benefits of AM in his *Histoire naturelle* (3) and Pauline Bonaparte (1780-1825), Napoleon's sister, is also reported to have used AM for her skin's health care. It was also used until the beginning of the twentieth century as a substitute for breast milk. Dr. Charles Porcher (1872-1933) of the Lyon National Veterinary Institution testimony, in 1928, showed that the

practice was still used, to a lesser extent, in the interwar years (4).

Today AM is used in the manufacture of soaps and moisturizers, but it has been recently re-evaluated for medical purposes, especially to treat infants and children with cow's milk (CM) protein allergy (CMPA).

We reviewed international literature, using PubMed, and the searching terms "ass's milk", "donkey's milk" and "cow's milk protein allergy", highlighting how AM may be a valid nutritional support for patients affected with CMPA, and it is able to ensure adequate increase in auxological parameters, filling nutritional deficiencies of these subjects.

BIOCHEMICAL PROPERTIES OF ASS'S MILK

AM has been the focus of several studies due to its special composition and nutritional properties, which are very close to human milk. Whenever a mother cannot breastfeed, or chooses not to breastfeed, or the child is intolerant to CM, the use of human and CM substitutes may provide the best option to meet the nutritional and health needs of the infant. AM has been widely used in the past to replace human milk, because its chemical composition and protein content are close to breast milk, and also because of its low allergic potential. AM digestibility is better than CM and similar to human milk, due to the high whey protein and the low casein content, so it may be used in infants and children with CMPA.

Guo et al. investigated the chemical composition, nitrogen fraction distribution, and amino acid (AA) profile of milk samples obtained during lactation from donkeys in Northwest China. Results showed that AM contained 9.53% total solids, 1.57% protein, 1.16% fat and 6.33% lactose, and 0.4% ash on average, which is more similar to human and mare milk than to other mammals (Table-1). All the samples collected throughout lactation showed constant

pH and density, whereas protein and ash content displayed an apparent negative trend; lactose content exhibited an increase during 120 days postpartum, followed by a decrease; fat content showed wide variability, and variations in content and percentage of casein, whey protein and AA were small. A casein to whey protein ratio of 52:37 was evidenced, between the lower human milk and the higher CM value. Sodium dodecyl sulfate-PolyAcrylamide Gel Electrophoresis (PAGE) results demonstrated that AM is rich in β -lactoglobulin and lysozyme. The percentage of 8 essential AA in AM protein was 38.2%, higher than those of cow and mare milk. AM also had higher levels of serine (6.2%), glutamic acid (22.8%), arginine (4.6%), and valine (6.5%) and a lower level of cystine (0.4%) (5-8).

Some authors, Bertino et al. and Cunsolo et al., conducted an extensive proteomic study and a detailed comparative analysis among the protein fractions (i.e. casein and whey proteins) of AM, CM and human milk. The detailed protein composition and structural features reported in these studies provided insight into the molecular reasons of AM hypo-allergenic quality. The already demonstrated low AM allergenic properties when compared with those of CM seems to be mainly related to the remarkable differences in the primary structure of their proteins, which determine deep divergence between the amino acid sequences of IgE-binding linear epitopes of CM allergens and the corresponding domains present in donkey's milk proteins (9-14).

In regard to lipid fraction, AM has been indicated as a nutraceutical food due to some bioactive compounds, i.e. lipids, which are able to directly or indirectly modify the intestinal environment and immunity, taking active part in the prevention and treatment of some pathologies. Chiofalo et al. examined triacylglycerol (TAG) composition and the positional isomers of AM samples. They identified 72 TAGs in analyzed samples, and examined similarities and differences among the ass and human milk TAGs fraction.

Allergy to Cow's milk protein

Table 1 - Composition of donkey's, mare's, human and cow's milk.

| Composition of donkey's, mare's, human and cow's milk (g/100 g) | | | | |
|---|-----------|-----------|-----------|-----------|
| Composition | Donkey | Mare | Human | Cow |
| pH | 7.0-7.2 | 7.18 | 7.0-7.5 | 6.6-6.8 |
| Total Solids g/100 g | 8.8-11.7 | 9.3-11.6 | 11.7-12.9 | 12.5-13.0 |
| Protein g/100g | 1.5-1.8 | 1.5-2.8 | 0.9-1.7 | 3.1-3.8 |
| Fat g/100g | 0.3-1.8 | 0.5-2.0 | 3.5-4.0 | 3.5-3.9 |
| Lactose g/100g | 5.8-7.4 | 5.8-7.0 | 6.3-7.0 | 4.4-4.9 |
| Casein Nitrogen g/100 g | 0.64-1.03 | 0.94-1.2 | 0.32-0.42 | 2.46-2.80 |
| Whey protein g/100 g | 0.49-0.80 | 0.74-0.91 | 0.68-0.83 | 0.55-0.70 |
| Non-protein nitrogen g/100 g | 0.18-0.41 | 0.17-0.35 | 0.26-0.32 | 0.1-0.19 |
| Casein Nitrogen % | 47.28 | 50 | 26.06 | 77.23 |
| Whey protein % | 36.96 | 38.79 | 53.52 | 17.54 |
| Non-protein nitrogen % | 15.76 | 11.21 | 20.42 | 5.23 |

From: Guo HY, Pang K, Zhang XY, Zhao L, Chen SW, Dong ML, Ren FZ. Composition, physicochemical properties, nitrogen fraction distribution, and amino acid profile of donkey milk. J Dairy Sci. 2007; 90: 1635-43 (modified).

AM presents TAGs with partition number values from 30 to 50. In human milk, short-chain fatty acids (FAs) are not well represented and the PN values range between 36 and 52. Other significant differences are among TAGs containing polyunsaturated fatty acids (PUFA). AM presents a larger number and amount of ω 3 and ω 6 FAs than human milk, which contains only significant amounts of ω 6 FA (linoleic). AM high PUFA n-3 content, and especially its low n-6/n-3 ratio, acquires particular interest in subjects with CMPA. In addition, both donkey and human milk present the saturated FA preferably on the sn-2 position. This fact, together with the relatively high content of medium-chain triglycerides, results in high bioavailability and digestibility of AM lipids, despite their low amount, and confirms the increasing interest toward AM as an alternative food for a hypoallergenic diet in humans (15-18).

In another study, 8 bioactive amines (histamine, tyramine, tryptamine, 2-phenylethylamine,

cadaverine, putrescine, spermidine and spermine) in 13 AM samples were found by high performance liquid chromatography atmospheric pressure chemical ionization mass spectrometry (HPLC-APCI-MS). Among the identified bioactive amines, putrescine, spermine and spermidine proved to be the most represented AM amines. Their concentration levels in the present study were lower than the values found in mature human, cow and sow milk (19).

ASS'S MILK AND FOOD HYPERSENSITIVITY

Food hypersensitivity is one of the most frequent causes of poor absorption and growth deficiency in unweaned infants in the first few months after birth. It has been reported that CMPA alone affects 2-7.5% of the general population, and the diagnostic incidence of this pathology is certainly increasing, as shown by the 1:200

diagnoses recorded in Stockholm in 1979 compared to the 1:7.500 recorded in 1948. However, it is well known that CM protein represents only some of the many possible allergens which can trigger food hypersensitivity reactions. In fact, there are several well-documented case reports of allergy to soy, rice, peanuts, hen's egg and other foods. Furthermore, these rarer food hypersensitivities are known to be more frequent in patients allergic to CM protein first; therefore, in these cases, there is multiple food hypersensitivity, which is a very difficult condition to treat. Although the remarkable progress gained in parenteral nutrition has led to substantial improvement in the prognosis of serious multiple food hypersensitivity, the need to reintroduce alimentation per os as soon as possible, to stimulate the functional recovery of the damaged intestine, is by now universally accepted. In this respect, breast-feeding is considered the safest realimentation method. However, it is obvious that human milk is not often readily available, and, therefore, other efficacious solutions have been sought. The use of formulas containing soy protein or hydrolysed protein formulas can, however, cause severe hypersensitivity reactions. Therefore, the use of AM might offer an important solution to treat infants affected with multiple food hypersensitivity, including CMPA. This food has the basic value of being extremely similar in composition to human milk so it could eventually represent a very valid alternative, especially considering its ability to integrate fat intake in CMPA patients. The high lactose content makes it pleasant for infant, and also qualitatively preferable to semi-elemental formulas containing protein hydrolysates or soy formulas, which contain carbohydrates other than lactose. In fact, it has been proved that lactose stimulates calcium intestinal absorption and this could lead to better bone mineralization in the first few months after birth. Therefore, it could be concluded that, in areas where it is readily available, AM is certainly preferable to a lactose-free artificial dietary milk. In addition, the renal solutes load, mainly

determined by the dietary amount of proteins and inorganic substances, is substantially very similar in both breast-fed infants and those fed with AM. For this reason it is not necessary to dilute AM before feeding the infants. This represents a considerable advantage, considering the lower fat, and obviously caloric, content of AM compared to CM (20,21).

Iacono et al., in 1992 (Table-2), reported the clinical data of 9 patients with multiple food hypersensitivity, including CMPA, treated over the last 2 years, and initially re-fed exclusively with AM. The patients presented severe symptoms of CMPA and successive attempts, using milk containing soy protein and/or a semi-elemental formula in their alimentation, did not improve their clinical condition, due to the onset of hypersensitivity to these allergens as well. After a short period of parenteral alimentation, the infants were re-fed per os with AM (250 mL/kg/day) plus medium chain triglycerides (40 mL/L milk). This food was well tolerated by all patients. No negative clinical reactions were recorded and during hospitalization average weight increase was 39.8 g/day. The follow-up of patients showed that AM was tolerated without any problem up to an age ranging from 15 to 20 months, when CM was reintroduced in some patients. Thus, the authors demonstrated that the use of this natural food, which, in their experience, has proved to be very useful in successfully treating the more complex cases of multiple food hypersensitivity, including CMPA, should be encouraged (22).

In another retrospective study, in 2000 (Table-2), the same authors evaluated the clinical characteristics of patients affected with CMPA and/or multiple food hypersensitivity, including CMPA, and hydrolysed protein (HP) intolerance and the long-term outcome of their treatment with AM, focusing their attention on its nutritional value. Intolerance to hydrolysed protein formulas has been considered, in the past, a very rare event, but later reports hypothesized that it might not be uncommon; however, very few data have

been published about the natural history of CMPA subjects intolerant to hydrolysed protein milk formulas too. They reported the clinical characteristics of 21 CM- and casein hydrolysate (CH) formula-intolerant infants, treated with an AM-based diet, and 70 CM-intolerant infants, treated with CH milk-based diet, as controls. All patients were followed-up for a median period of 4 years. Both casein hydrolysate formula-intolerance and intolerance to other foods were diagnosed according to the double-blind placebo-controlled procedure. Formal CM-challenges were conducted at yearly intervals until tolerance was demonstrated. The study demonstrated that the patients intolerant to extensively CH formula had a more severe clinical framework than the CMPA subjects successfully treated with CH formulas. Only 52% of the CM- and CH-intolerant patients included in this study achieved CM-tolerance at the end of the study, after a median follow-up period of 4 years, whereas 78% of CH-tolerant patients became CM-tolerant at the end of the study. Furthermore, the CH-intolerant patients achieved CM-tolerance at a median age significantly older than the CH-tolerant ones. Moreover, the higher hyperactivity of CH-intolerant subjects seems to be confirmed by the higher frequency (100%) of multiple food hypersensitivity they exhibit (i.e. to soy, goat's and sheep's milk, soy, oranges, tomatoes and fish) compared to CH-tolerant patients. We could hypothesize, in general, that the intolerance to extensively hydrolysed protein demonstrated in these patients represents the epiphenomenon of an elevated reactivity which is the basis of a more prolonged and severe food intolerance history. Two-thirds of the CH-intolerant patients had high serum IgE levels. Furthermore, in these patients the authors pointed out a higher frequency and more elevated levels of total serum IgE and specific IgE to CM antigens than in the CH-tolerant subjects. Whenever treated with an AM diet, the subjects gained satisfying weight and height and the more common hemo-chemical nutritional parameters returned to the normal range after 1

year of the CM-free diet. Besides, the authors did not observe any difference in growth parameters during the follow-up period between the AM and the CH-milk treated group. This last result is very significant, as in the CH-milk treated group only 14% patients showed multiple food intolerance, whereas all infants treated with AM had multiple food intolerance and this greatly limited food choice. The study demonstrated that AM is a safe solution even in infants in whom hydrolysed-milk formulas had failed. For the latter, L-aminoacid-based formulas have been recently used and achieved good results, providing satisfactory growth recovery. However, the authors observed similar excellent nutritional results using natural milk, AM, which is certainly more palatable than the elemental formulas and is similar, in its biochemical composition, to human milk. Finally, it must be considered that hydrolyzed formula products have an unpleasant taste and are quite expensive, so the use of AM might be encouraged in CMPA CH-tolerant subjects also (23).

Monti et al., in 2007 (Table-2), with a prospective study, investigated in vivo tolerance, palatability and nutritional adequacy of AM in a population of 46 infants and children with CMPA and other food allergies (mainly soy, wheat, egg and fish), for whom maternal milk was not available and no available CM substitute could be used. CMPA was diagnosed on the basis of a CMP elimination diet, followed by double-blind, placebo-controlled food challenge (DBPCFC). Before food challenge, CM proteins skin prick tests and RAST were also performed. Thirty-three children, CM protein SPT- and/or RAST-positive, were found to have an IgE-mediated CMPA; the remaining 13 were classified as non-IgE-mediated CMPA. AM challenge proved positive in 8 children (17.4%), whereas the remaining 38 (82.6%) both liked and tolerated AM at the challenge and throughout the follow-up period. Twenty-six of the 33 children (78.8%) with IgE-mediated CMPA and all 13 children with non-IgE-mediated CMPA tolerated AM. Catch-up growth

Allergy to Cow's milk protein

Table 2 - Ass's milk in the treatment of cow's milk protein allergy and others food allergies.

| Reference number in the text | Author | Year of Publication | Clinical presentation | Cases number | Age (month range) | Cow's milk hypersensitivity |
|------------------------------|---------------------|---------------------|---|--|-------------------|---|
| 22 | Iacono G. et al. | 1992 | Diarrhea, abdominal pain, vomiting and growth retardation | 9 unweaned infants with CMPA and other food allergies | 0 - 3 | 9/9 (100%) |
| 23 | Carroccio A. et al. | 2000 | Diarrhoea, abdominal pain, vomiting | Group A: 21 cow milk and hydrolyzed protein intolerant patients Group B: 70 cow milk intolerant patients Total: 91 | 2 | Group A: 21/21 (100%) Group B: 70/70 (100%) Total: 91/91 (100%) |
| 26 | Monti G. et al. | 2007 | Cutaneous symptoms, gastrointestinal symptoms and growth retardation | 46 CMPA patients | 1 - 146 | 46/46 (100%) |
| 27 | Vita D. et al. | 2007 | Atopic dermatitis | 28 CMPA patients | 6 - 36 | 28/28 (100%) |
| 28 | Tesse R. et al. | 2009 | Cutenous symptoms, gastrointestinal symptoms and respiratory symptoms | 25/25 CMPA patients | 6 - 11 | 25/25 (100%) |

(in terms of length/stature and weight and Z-scores for length/stature and weight increases) was observed in all subjects characterized by growth deficit during CM protein challenge. IgE cross-reactivity intensity *versus* AM proteins was very weak and aspecific. Therefore, AM was found to be a valid alternative, both in terms of palatability and weight-height gain, in IgE-mediated and non-IgE-mediated CMPA (24-26).

Vita et al., in 2007 (Table-2), relying on the frequent association between CMPA and atopic dermatitis, followed, in frequency, by urticaria/angioedema, gastrointestinal symptoms, wheezing

and asthma, carried out a crossover randomized-controlled trial to objectively compare the tolerance of AM, with goat's milk (GM), used as the control, in order to evaluate AM in the treatment of CMPA related atopic dermatitis. GM was chosen as the control since it is still widely used as a CM substitute in clinical practice. In several countries GM is available and recommended by some physicians, for infants and young children with CMPA. Twenty-eight children with CMPA and AD were enrolled in the study. The children were randomized to AM or GM diet for 6 months, then switched to the other milk for a further 3 months.

Allergy to Cow's milk protein

| Hydrolyzed formula hypersensitivity | Soy milk hypersensitivity | Goats' milk hypersensitivity | Ass' milk hypersensitivity | Auxological response after ass's milk treatment |
|---|-------------------------------------|------------------------------|--|--|
| 9/9 (100%) | 9/9 (100%) | Not determined | 0/9 (0%) | 9/9 (100%) |
| Group A: 21/21 (100%) Group B: 20/70 (29%) Total: 41/91 (45%) | Not determined | Not determined | Group A: 3/21 (14%) Group B: not determined | Group A: 18/21 (86%) Group B: not determined |
| 7/46 (15%) 39/46 refused hydrolyzed formula | 35/46 (76%) 11/46 not determined | Not determined | 8/46 (17%) | 33/46 (72%) 8/46 (17%): not determined due to AM hypersensitivity 5/46 (11%): not determined due to follow-up drop-out |
| Not determined | Not determined | 23/26 (88%) Drop-out: 2 | 1/26 (4%) Drop-out: 2 | Not determined, but improvement of atopic dermatitis |
| Not determined | Not determined | Not determined | 1/25 (4%) | 24/25 (96%) |

The SCORAD index (SI) and a visual analogue scale (VAS) were blindly evaluated. At the end of the study, food challenges with GM and AM were performed. An SDS-PAGE analysis of two different milks was performed. Two children from the GM group dropped out after randomization and 26 completed the study. AM invariably led to a significant improvement of SI and VAS symptoms ($p < 0.03$ vs. baseline and inter-group), whereas GM had no measurable clinical effect. At the end of the study 23 of 26 children had a positive food challenge with GM versus one of 26 with AM. In other words the study indicates that

AM is tolerated by 88% of children with CMPA and produces a significant improvement in AD. On the contrary, symptoms remained unchanged or even worsened, in all patients receiving GM. In particular, all children previously on an AM diet had a relapse of AD after switching to GM. Of note, at the end of the study, the majority of the children had a positive DBPCFC for GM too, although none of them had been previously fed with GM-containing foods. We speculate that this may be due to the GM protein profile which is quite similar to CM, as confirmed by SDS-PAGE analysis. This was not the first report documenting that Equidae

milk (milk derived from the taxonomic family of horses and related animals including donkeys), could be an appropriate alternative to CM, but is the first demonstration that AM is better tolerated than GM, which is still widely used. In conclusion, our revision results suggest that GM should not be used in children with CMA and AD, whereas AM, if available, may be an effective and safe alternative (27).

Tesse et al., in 2009 (Table-2), evaluated 30 children with suspected CMPA. They underwent skin prick tests, using fresh CM, AM, pear juice and other common food and aero-allergens, and double-blind, placebo-controlled food challenge to CM proteins. After confirming CMPA diagnosis, patients received fresh AM in open challenge. Specific serum CM and AM protein IgE, and blood biochemical parameters were also assessed. Auxological evaluations (standing height, weight and BMI) were evaluated in all subjects at entry and after 4-6 months of AM intake. Twenty-five children were considered suitable for the study, and 24 out of 25 subjects (96%) tolerated AM at the food challenge, 22 had IgE-mediated CMPA, and 2 had non-IgE-mediated disease. In cases of clinical tolerance, AM was included in the child's diet, which was appropriately balanced depending on age demand. Auxological data in all patients improved by the end of the study, while blood biochemical parameters did not vary during the follow-up. These data confirm a high rate of AM tolerance in children with moderate CMPA symptoms, and demonstrated that AM seems to be nutritionally adequate in subjects on a relatively free diet (28).

Pilla et al., in 2010, examined hygienic and health characteristics of 101 half-udder AM samples, determining somatic cell count (SCC), bacteriological analysis and total bacteria count (TBC). The major pathogens were tested for antimicrobial susceptibility, and *Staphylococcus aureus* isolates were further genotyped by nano-array analysis. Whey lysozyme and NAGs (NAG) activities were also assessed, they showed very

low TBC (<250 CFU/ml) and SCC (<50.000 cells/ml) values and a minor prevalence of pathogens: *Staphylococcus aureus* was only isolated from 5 milk samples (3 animals), *Streptococcus equi* from 2 samples and *Streptococcus equisimilis* from a single sample. All the isolates were sensitive to all antibiotic classes used in veterinary medicine. None of the *Staphylococcus aureus* isolates was found positive to harbor genes coding for any enterotoxin, toxic-shock syndrome toxin, or antibiotic resistance. Lysozyme levels were always very high (4.000-5.000 U/ml), while NAG values were mostly low (<50 U/ml), during the last part of lactation. The results of this study confirmed the low prevalence of intramammary infections in donkeys and the absence of food-borne pathogens, suggesting that AM could be a safe food, if the mammary gland is healthy and the animals are milked in proper hygienic conditions (29).

In contrast to the above mentioned study, Conte et al., in 2008, described isolation of two *Enterobacter sakazakii* (Es) strains from 50 samples of AM in Sicily. The antibiotic resistance profile of the isolates revealed a multiple resistance profile, including fluoroquinolones, commonly used to treat animal infections. In 2002, the International Commission for Microbiological Specifications for Foods (ICMFS) ranked *Enterobacter sakazakii* (Es) as a 'severe hazard for restricted populations, life threatening or substantial chronic sequelae of long duration'. Es ('yellow pigmented *Enterobacter cloacae*') has been found among the common food-born pathogens, such as *Listeria monocytogenes*, etc. The genus *Enterobacter* was associated with the phytic flora and it was supposed that the principal environmental sources of Es are water, soil and vegetables, and a secondary contamination media may be vectors such as flies and rodents; nevertheless the organism is considered ubiquitous. Es infection cases were reported in several countries. Neonatal infections have been reported to arise via birth canal Es contamination or through post-birth environmental sources.

Many neonatal meningitis cases may have some relationship with necrotizing enterocolitis, which is associated with several bacterial pathogens and is the most common newborn gastrointestinal disease. Neonatal pathologies also include bacteraemia, wound exudates, appendicitis, and conjunctivitis; in adults the organism usually causes bacteraemia. Intrinsic *Es* and *Salmonella* contamination of powdered infant formula can be a cause of infection and illness in infants, including severe disease which can lead to serious sequelae and death. No link has been established between illness and other microorganisms in powdered formula, although such a link was considered plausible for other *Enterobacteria*. Therefore, the authors pointed out survey importance because in Italy AM is used as one of the possible solutions for infants with hypersensitivity to some animal milk proteins. This is the first report of *Es* from AM and their recovery is noteworthy, especially because infants consume raw milk. The uncertainty about *Es* infectious dose and its antimicrobial susceptibility profile would be reasons for caution on this topic. A full risk assessment of the organism will require greater knowledge of its presence in food, especially those consumed by neonates and infants (30).

CONCLUSIONS

In all the above reviewed studies, the authors reported experiences of clinical AM extremely high tolerance at the food challenges conducted in Italian Regions, i.e. Apulia and Sicily, where AM is readily available and frequently used because of the presence of several ass farms. AM was tolerated by the patients either with the IgE- and non-IgE-mediated CMPA. The enrolled subjects generally found AM acceptable due to its palatability, and did not interrupt the studies. Therefore, AM might be considered an alternative to CM considering that its protein composition is similar to human milk. However, it is a low-calorie

food and thus in some studies the researchers enrolled children older than 6 months, who did not have an exclusive milk diet, and/or added medium-chain triglycerides to the diet. Overall data showed an adequate increase in auxological parameters (i.e. weight, length/stature and BMI), measured after several months of AM administration. It is possible to argue that the effect of AM on growth is related to its ability to fill some nutritional gaps present in the diet of treated subjects. However, all the studies suggest that a longer follow-up is needed in order to achieve reliable results. Moreover, during and after AM administration period, patients' biochemical and metabolic blood parameters did not vary. Finally, AM proteins potential cross-reactivity with CM proteins must be considered, suggested by the above mentioned studies that sometimes reported severe reactions to AM in their study cohorts. However, taken together, all these results suggest that AM might be considered nutritionally adequate in children with CMPA or multiple food allergies including CMPA.

To date, however, AM costs much more than CM and hydrolysed formulas, making this food, especially for poorer patient families, difficult to access. For this, further studies would be needed to confirm AM usefulness in filling the nutritional gap of CMPA patients and make it considered among medicaments approved by public health services. Another critical point which should be stressed, is lack of easy availability of AM even in countries where donkeys are very common; this could be explained both because of history of these animals, usually used for heavy works, especially in the fields, but also to the difficulty in finding the knowledge and resources to a farm for food supply purpose. To this should be added lack of knowledge among the population about AM nutritional characteristics and its low markets' demand, also related to subjective aspects of consumers, who consider as poor palatability the products derived from donkey die, although not having them ever tasted.

Abbreviations

AM - Ass's milk

CM - Cow's milk

CMPA - Cow's milk protein allergy

CONFLICT OF INTEREST

None declared.

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