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Sensitization to bovine serum albumin as a possible cause of allergic reactions to vaccines

Rajiva de Silva [a,*], W.M.D.K. Dasanayake [a], G.D. Wickramasinhe [a], Chandima Karunatilake [a], Nayani Weerasinghe [a], Peshala Gunasekera [b], Gathsaurie Neelika Malavige [c]

[a] Department of Immunology, Medical Research Institute, Colombo 08, Sri Lanka
[b] Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo, Sri Lanka
[c] Department of Microbiology, Faculty of Medical Sciences, University of Sri Jayewardenapura, Sri Lanka

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ABSTRACT

Background: Immediate type hypersensitivity to vaccines containing bovine/porcine excipients, such as the measles, mumps and rubella (MMR) vaccine is probably due to sensitization to bovine/porcine gelatin. Most patients with such reactions in Sri Lanka have cow's milk (CM) or beef allergy.
Objectives: We investigated whether those who had beef and CM allergy had a higher incidence of hypersensitivity reactions to vaccines and the possible trigger of such reactions.
Material and methods: Twenty patients with immediate type hypersensitivity reactions to vaccines containing bovine/porcine excipients, controls with allergy to beef/pork (n = 11) or CM (n = 11), and 8 non atopic controls were recruited. Total serum IgE, specific IgE to beef, CM, casein, beta lactoglobulin, gelatin and bovine serum albumin (BSA) by Phadia ImmunoCap and IgE to porcine gelatin by Western blot were evaluated.
Results: 11/20, 5/20, 2/20, 2/20, 1/20 and 1/20 patients reported allergic reactions to measles containing, JE, rabies primary chick embryo, pentavalent, diphtheria and tetanus, and adult diphtheria and tetanus vaccines, respectively. Only one patient with allergy to vaccines had gelatin specific IgE, whereas IgE to BSA was seen in 73.3%, 90%, 66.6% and 0 of vaccine, beef or CM allergic and non-atopic controls, respectively. The mean IgE to BSA was higher in patients with allergy to vaccines, although not significant. Specific IgE to BSA was present in 54.7% of children with allergy to CM, of whom 11.8% had high levels (>17.5 kUA/L). In contrast, 66.6% of these children did not have specific IgE to β-lactoglobulin, which is one of the major components of whey protein.
Conclusion and clinical relevance: Gelatin does not appear to play a major role in Sri Lankan children with allergy to vaccines. In contrast, due to the higher levels of BSA specific IgE, sensitization to BSA is possibly playing a role.

1. Introduction

Anaphylaxis following immunization with the measles, mumps or rubella containing vaccines [1–5] (the measles, mumps and rubella (MMR), measles and rubella (MR) and measles vaccines), varicella [6] and live Japanese encephalitis vaccines [7] has been reported. Anaphylaxis in these instances was thought to occur due to an IgE mediated reaction to bovine or porcine gelatin or polygeline (a polymer of bovine gelatin) used as a stabilizer in these vaccines [1–3]. Some of those who developed anaphylaxis following vaccination were shown to have prior or subsequent allergy to gelatin containing foods [1–3]. High rates of allergic reactions to the MMR vaccine have been reported in Sri Lanka. For instance, in 2015, 119 cases were reported in the second quarter of the year, which gives a rate of 0.685% [8]. In 2008, two girls aged 13 years, died of anaphylaxis following immunization with the rubella vaccine in Sri Lanka (unpublished data). One of them was known to be allergic to cow's milk (CM) and the other allergic to beef and pork (confirmed by the presence of specific IgE to CM or beef and pork by Phadia ImmunoCap carried out with the assistance of the World Health Organization (WHO)). The gelatin used in the MMR is of porcine or bovine origin [9].

Patients with known allergy to beef or pork are at risk of developing immediate hypersensitivity reactions to gelatin containing

* Corresponding author at: Department of Immunology, Medical Research Institute, Danister de Silva Mawatha, Colombo 08, Sri Lanka.
E-mail address: nilhandesilva@gmail.com (R. de Silva).

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vaccines [10]. Allergy to casein has also been implicated in patients with severe cow's milk allergy (CMA) who developed anaphylaxis following immunization with the diphtheria, tetanus and pertussis vaccine [11]. Although beef allergy is rare in the West, two to three percent of children are believed to have CMA [12,13]. In contrast, many adults and children with beef allergy have been referred to our clinical at the Medical Research Institute (MRI), Colombo and have been confirmed by detection of specific IgE. Therefore, the prevalence of beef allergy is likely to be higher in Sri Lanka when compared to many other countries. Although, beef consumption is less in Sri Lanka as the majority of individuals are either Buddhists or Hindus, it is possible that those with CMA become sensitized to the bovine serum albumin (BSA) component more frequently, thus reacting to BSA in vaccines.

Studies have shown that 73% [14] and 93% [15] of children allergic to beef also had CMA and 10% of patients with CMA were also allergic to beef [16]. The sensitivity to bovine serum albumin (BSA), a major allergen in beef was the main predictive marker of CMA in children with beef allergy [15]. The vaccines used in Sri Lanka have BSA and gelatin. The MMR contains trace amounts of BSA and porcine gelatin 2.5 mg/dose, live JE vaccine has BSA <50 ng/dose and gelatin and the rabies chick embryo vaccine (PCEC) contains polygeline. The pentavalent vaccine (against diphtheria, tetanus, whole cell pertussis, H influenza type b, and hepatitis B), manufactured by the Serum Institute, India has components produced in bovine protein containing media, including casein.

Most patients attending the Allergy Clinic at MRI, following anaphylactic reactions to vaccines gave a history of allergy to CM or beef. As bovine and porcine products are present in the MMR, MR, measles, live JE, pentavalent, adult diphtheria and tetanus (AdT) and PCEC vaccines, we sought to investigate if patients with CMA and concurrent beef allergy are at a higher risk of allergic reactions when immunized with such vaccines and the possible trigger of such reactions.

2. Material and methods

Patients who developed immediate hypersensitivity reactions, including anaphylaxis following immunization from 2014 to May 2016 were recruited following informed written consent. Ethical approval for the study was obtained from the Ethics Review Committee of the MRI (Research Project No 41/2014). Informed written consent was obtained from the parent if the patient was below 18 years, of age.

Anaphylaxis was diagnosed according to ICON guidelines [17]. A detailed clinical history was obtained, and clinical records were scrutinized. In addition, patients with CMA or allergy to beef/pork and who did not have reactions to vaccinations, as well as age matched controls who had no atopy related diseases who were attending the Thalassemia Clinic, at the Department of Haematology, MRI were recruited as controls.

The total serum IgE was assessed by ELISA (DRG instruments, GmbH, Germany) and specific IgE to CM, beef, β lactoglobulin, BSA, casein and gelatin was performed using the Phadia ImmunoCap system in all patients. A positive IgE titre was defined as specific IgE levels of >0.35 kUA/L. In order to determine the relationship between the presence of high titres of IgE to cow's milk and cow's milk components, the patients were grouped as previously described [18]. Accordingly, those with IgE levels of <3.5 kUA/L considered to have low levels, specific IgE levels of 3.5 to 17.5 kUA/L as moderate levels and levels >17.5 kUA/L were considered to be high.

The presence of IgE to gelatin was also evaluated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and by Western blot [19] in patients who developed allergic reactions to the vaccine. BSA was used as a positive control, using serum from a patient who had specific IgE to BSA. SDS-PAGE was performed using a Mini Protein-R apparatus (Bio-Rad). Ten μl containing 10 μg of porcine gelatin (Bloom number 180-Sigma Aldrich) was added to 5 μl of 3X Lammeni sample buffer (80 mM Tris-HCl buffer (pH 6.8), containing 2% SDS and 10% glycerol, 0.1% Bromophenol Blue), incubated at 95 °C for 10 min and subjected to electrophoresis through a 15% polyacrylamide gel at 70 V at 4 °C for 3 h. Gels were stained with Coomassie stain for 1 h and de-stained using de-staining solution containing glacial acetic acid, methanol and deionized water. The process was repeated with the BSA standard (Sigma Aldrich).

For western blotting, the proteins separated by SDS-PAGE were first electro transferred from the gel to a nitrocellulose blotting membrane using a mini protein tetra system (Bio-Rad) for 2 h at 70 V constant voltage. The membrane was then washed with phosphate buffered saline (PBS) containing 0.05% Tween 20 (PBST) and blocked with blocking solution (5% non-fat milk in PBST) at 4 °C for 1 h. After washing with PBST for 5 min (x3), the membrane was reacted with patient's serum (diluted 1:40) in antibody diluting buffer (5% non-fat milk in PBST) at 4 °C for overnight. The membrane was washed with PBST for 5 min (x3) and reacted at 4 °C for 2 h with the peroxidase-labelled goat anti-human IgE antibody diluted in 1:1000 (Sigma Aldrich). After re-washing with PBST, antigen antibody binding was visualized using 4CN substrate.

2.1. Statistical analysis

PRISM version 6 was used in the statistical analysis. As the data were not normally distributed, differences in means were compared using the Mann-Whitney *U* test (two tailed). The degree of association between the development of anaphylaxis and the presence of high (>17.5 kUA/L) casein specific IgE was expressed as the odds ratio (OR), which was obtained from standard contingency table analysis by Haldane's modification of Woolf's method. Chi Square tests, Chi square for trend or the Fisher's exact test was used to determine the p value.

3. Results

Of the 20 patients 08 had immediate hypersensitivity reactions to the MMR, 02 for the MR, 01 for measles, 05 for live JE vaccine, 02 for rabies PCEC, 02 for the pentavalent vaccine, 01 for the diphtheria and tetanus (DT) and 01 for the AdT vaccine (Table 1 and 2). Two patients reacted to 2 vaccines. Fourteen patients developed anaphylaxis, of whom 8 developed anaphylaxis to measles containing vaccines (measles, MR, MMR), and 4 developed anaphylaxis following administration of the live JE vaccine, with one patient developing anaphylaxis to both MMR and live JE vaccines. Two patients developed anaphylaxis to the pentavalent vaccine, while 1 patient developed anaphylaxis to the PCEC. One patient who had anaphylaxis following the MMR vaccine previously had an anaphylactic reaction to the DT vaccine. Six patients developed immediate hypersensitivity reactions not considered to be anaphylaxis. Of the 6, two patients developed a dry cough immediately after the MMR vaccine, while 1 patient each developed cough and shortness of breath/wheezing after the MR and live JE vaccines. One patient each developed urticaria following the AdT and PCEC vaccines.

16/20 (80%) patients were known to have either CM and/or beef allergy. These included 7, 6 and 3 patients with CM, beef, or beef and CM allergy. One patient, who was 2 months at the time of the allergic reaction to the vaccine (pentavalent), had not been exposed to CM or beef. A total of 8 patients (40%) had never ingested beef.

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Table 1
Summary of clinical features in those who developed allergies to vaccines.

Clinical reaction	Number (%) N = 20
Urticaria	14 (70)
Wheezing/ shortness of breath/rhonchi	09 (45)
Vomiting	02 (10)
Abdominal pain	01 (5)
Loss of consciousness	02 (10)
Lowering of blood pressure	03 (15)
Anaphylaxis	14 (70%)[a]
Type of allergic reaction	
MMR/MR/M	
Anaphylaxis	08 (40)[b,c]
Respiratory allergy	03 (15)
Dry Cough	02 (10)
Cough and wheezing	01 (05)
Total	11 (55)
Live JE	
Anaphylaxis	04 (20)[b]
Respiratory allergy	01 (05)
Cough and wheezing	01 (05)
Total	05 (25)
Penta	
Anaphylaxis	02 (10)
Total	02 (10)
AdT	
Urticaria	01 (5)
Total	01 (05)
Rabies PCEC	02 (10)
Anaphylaxis	01 (5)
Urticaria	01 (05)
Total	02 (10)
DT	
Anaphylaxis	01 (05)[c]
Total	01 (05)
Other allergies	16 (80)
CMA alone (Beef not ingested = 06)	07 (anaphylaxis = 05, respiratory = 02 to vaccine))
Beef/ pork/ bovine products allergy	06 (anaphylaxis = 03, respiratory = 01, skin = 02 to vaccine)
CMA plus beef/ pork/ bovine product allergy	03 (anaphylaxis = 03 to vaccine)
No allergies (Beef not ingested = 01, beef and CM not ingested = 01)	04 (anaphylaxis = 03, respiratory = 01 to vaccine)

[a] 01 patient reacted to both MMR and live JE, 01 patient reacted to both MMR and DT.
[b] 01 patient reacted to MMR and live JE.
[c] 01 patient reacted to MMR and DT.

IgE to gelatin by Phadia ImmunoCap was seen (0.44 kUA/L) in 1/15 patients (6.6%) who developed immediate hypersensitivity to the vaccines, 4/10 (40%) who had allergy to beef and in none of 9 patients who had CMA. Those who had detectable levels of gelatin specific IgE (>0.35 kUA/L) had low levels (<1.0 IgE to gelatin kUA/L). The absence of sensitization to gelatin was confirmed by immunoblotting (data not shown). In contrast, IgE to BSA was present in 8/14 (57.1%) patients who developed immediate hypersensitivity to vaccines (one other patient was not tested for IgE to BSA, as the IgE to beef was <0.1 kUA/L), 9/10 (90%) in children allergic to beef and 6/9 (66.6%) patients with CMA. Two patients with CMA who were not tested for IgE to BSA or gelatin had IgE to beef <0.15 kUA/L. Although the mean BSA specific IgE levels were higher in those who developed immediate hypersensitivity to vaccines, this was not statistically significant (Fig 1).

3.1. Analysis of specific IgE to different components of CM

Individuals who have CMA have been shown to have varied sensitizations to different components of CM, which is shown to associate with severity and subsequent response to immunotherapy [18]. As an initial step to determine the association of specific IgE to cow's milk allergy and the risk of development of vaccine associated anaphylaxis, we determined general sensitization patterns to CM components in our cohort of patients (n = 26) who reported to have CM allergy. All these patients had detectable levels (>0.35 kUA/L) of CM specific IgE and casein specific IgE. However, 66.6% of these children did not have specific IgE to β-lactoglobulin, which is one of the major components of whey protein, 16.6% had low IgE levels to β-lactoglobulin, and none had high IgE levels to β-lactoglobulin. In contrast, only 23.5% of patients did not have specific IgE to BSA and 11.8% had high IgE levels to BSA. Furthermore, the children with CM allergy in our study had higher mean IgE levels to BSA, whereas their mean IgE levels to β-lactoglobulin was lower (Fig 2).

3.2. Relationship between IgE levels for CM and CM components and the risk of anaphylaxis

Total CM specific IgE levels (p = 0.03) were significantly higher in patients with CMA who developed vaccine allergy (mean 49.07, SD ± 37.8 kUA/L), when compared to those who did not (mean 17.88, SD ± 24.8 kUA/L) (Fig 3) The mean β-lactoglobulin specific IgE and BSA specific IgE levels were also higher in patients who developed allergy to vaccines, although again this was not significant. Patients with CMA who developed allergy to vaccines, were significantly more likely (p = 0.03) to have higher levels (>17.5 kUA/L) of casein specific IgE than patients who did not develop reactions to vaccines (odds ration = 8 95% CI = 1.24 to 52.27). However, high casein specific IgE levels (>17.5 kUA/L) had a poor sensitivity (66.7%) and specificity (80%) for the development of vaccine associated anaphylaxis. The ROC curves gave an area under the curve value of 0.72, with a p value of 0.07, which was not satisfactory for use of high casein specific IgE levels alone as a predictive factor of vaccine associated anaphylaxis.

4. Discussion

Sixteen of the 20 (80%) patients who had allergic reactions to the vaccines had CMA or allergy to beef/pork. Of 4/20 patients who did not have CMA or allergy to beef/pork, one was not exposed to beef or pork. None of the patients, who developed anaphylaxis to the measles containing vaccines in the study from the US [5], or from Finland [4], were allergic to gelatin containing foods. Seven of 26 (26.9%) Japanese patients [3] who developed allergic reactions to monovalent mumps, measles or rubella vaccine (19 with anaphylaxis and 7 with urticaria) and the first patient with anaphylaxis to the MMR reported from the US gave a history of allergy to foods containing gelatin [1].

All vaccines implicated in allergic reactions in Sri Lanka had BSA and gelatin (MR, MMR, live JE), polygeline (PCEC) or were grown on media containing bovine protein. Only one of our patients who developed allergic reactions (including 14 with anaphylaxis) to vaccines had IgE to gelatin, which is in contrast to studies in Japan. IgE to gelatin was seen in 24/26 allergic patients (19 with anaphylaxis) [3], and also 25 of 27 (93%) with anaphylaxis, 27 of 48 (56%) with urticaria, and 8 of 90 (9%) with a generalized eruption [20] following immunization in Japan. In the west the presence of IgE to gelatin in patients with allergy to MMR was less than in Japan; in the US [5] where 6/22, and in Finland [4] where 5/36 (by ImmunoCap) and 10/36 (by Immunospot) had IgE to gelatin. HLA DR 9 was significantly associated with IgE to gelatin, compared to controls in Japan [21] where most of the gelatin allergic patients were detected. This allele is rarely identified in Sri Lanka [22].

In contract, the majority of our patients (73.3%) had IgE to BSA. BSA is a minor allergen in CMA [23], but it is a major allergen in

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Table 2
Clinical features .

No	History	History of CM allergy	History of beef / pork/product allergy	Vaccines (measles containing, live JE)	IgE to milk kUA/L	IgE to beef kUA/L	IgE to lactoglobulin kUA/L	IgE to casein kUA/L	IgE to BSA kUA/L	IgE to gelatin kUA/L
1	5 year male u, c, sob 40 min after **MR II**	No	Jujubes - u	No reaction with **M I** Live **JE** not given	10.3	47.3	<0.1	0.28	1.0	0.12
2	3 year male c 5 min after **MMR** II	CM - rh, sn 5 min later. Refusal of milk Cheese – r, sn, u	Not ingested	No reaction after **M I** at 9 months	>100	59.7	18.9	>100	77.7	<0.1
3	4 year male At 11 months of age u, sob, w, f after **M I**	CM – red eyes, c, u, w Goat milk – within minutes- u, c, sob, w, r SpO_2 82%	Not ingested	Reaction with **M I** at 11 months	55.4	0.12	0.68	75.8	<0.1	<0.1
4	5 years female At 3 years of age u, c, h, loc 30 min after **MMR II** At 4 years u, a, p, h (70/40 later 90/60) immediately after **JE**	CM - u	Not ingested	No reaction to **M I** at 9 months	7.36	22.8	0.03	0.2	1.12	0.15
5	3 year male p, sn, sob, v immediately after **MMR II**	Cheese at 6 months a, u, sn, sob immediately	No allergy to mutton, pork	No reaction to **MMR 1, JE**	38.2	<0. 1	4.08	42.2	0.62	<0.1
6	3 year female u, sn, c, w, loc, cyanosis, cardiac arrest immediately after **MMR II** Cardio pulmonary resuscitation given	Triposha[a] – immediate u, a, sn, c, w, rhonchi spO_2 94, 70, 50 Contact with CM– u, w Cake, biscuit,- u, sob, w	Not ingested	No reaction with M I Sn,c, w, rhonch 10 min after **DT** at 5 years	>100	<0.1	20.5	>100	0.11	<0.1
7	3 year female C 10 min after **MMR 2**	Cheese, chocolate, triposha[a], tipi tip[a] u, rh, sn, c, sob	Not ingested	No reaction to **M I**	78.8	0.42	<0.1	71.1	<0.1	<0.1
8	3 year female u, c, rhonchi 30 min after **MMR 2**	No	Not ingested	No reaction to **MMR 1**	2.17	6.07	0.01	0.08	0.35	0.44
9	3 years female pallor, sweating, floppy, pulse absent, rhonchi within minutes of **MME II**	No	No	No reaction to **MR 1**	<0.1	<0.1	–	–	–	–
10	5 year female 3 years – c, w 45 min after **MR II**	No	W, c, pr after jelly	No reaction to **M 1**, live **JE**	4.69	7.62	0.02	0.33	2.19	0.17
11	3 years female u, pr, c 30 min after **MMR II**	Chocolate, ice cream - u, c	Pork - u, ab pain, v within 15 min Jelly – u	No reaction to **MR 1,** live **JE**	16.1	56.6	<0.1	0.52	2.59	0.23
12	7 year male u,, fl, c, ab 15 min after live **JE**	CM – ab u, c after 20 min	Beef, pork, porcupine meat - u, f, ab after 15 min	No reaction to **MR II**	10.3	36.3	0.07	0.64	0.78	<0.1
13	8 year female c, sob 20 min after live **JE**	No	No	No reaction to **MR II**	ND	4.97	ND		ND	ND
14	9 year female p, u, a, w, h (systolic BP 40 mm), v, ab 1 **½ hours after JE**	CM, yoghurt - u	Beef – u	No reaction to **MR II**	4.88	9.65	ND	ND	ND	ND
15	4 year female 1 h c, rh, u 1 h after live **JE**	No	Beef - u, a, rh, c after 30 min	No reaction to **MMR II, PCEC**	ND	3.39	ND	ND	ND	ND
16	8 year female 5 years – u immediately after **AdT**	Does not drink milk. Can eat cake	Ab pain, u 30 min after beef X 1/pork x 2 at 2 years	No reaction to **MR II**	1.06	2.79	0.04	0.1	0.36	<0.1
17	10 months female, fl, u, sob, w, low volume pulse, SpO_2 80 mm 1 h after Pentavalent vaccine	Cheese, contact with yoghurt - u	Not ingested	No reaction to **MMR I**	46.7	10.7	12.5	19.9	17.7	<0.1
18	2 months male u, respiratory arrest after pentavalent, 1st dose	Not ingested	Not ingested	Not given	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
19	24 year male u, p at injection sites immediately after **PCE rabies** (4 sites, intradermal regime)	No	Pork, beef - U		ND	12.1	ND	ND	ND	ND
20	18 years female v, f, unrecordable BP, low volume pulse, rhonchi 5 min after **PCEC rabies** vaccine (2nd dose) 1st dose – u at site	No	Pork, beef – u, a, hoarseness (X 3 times with beef)		ND	3.37	ND	ND	ND	ND
	Controls beef									
1	5 year male anaphylaxis	No	Beef and pork – a, u, ab, v	No reaction to **MR, M**	4.77	13.9	0.34	0.36	0.6	0.4
2	3 year male	Cheese – u, a Can eat cake, biscuits	Beef, pork, jelly – u, a	No reaction to **MMR**	12.9	39.3	<0.1	0.6	3.68	0.37
3	10 year male anaphylaxis	No	Beef – u, p, stridor, sob, w, h after few minutes	No reaction to **M, MR**	3.64	12.4	0.32	0.55	1.54	0.35

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Table 2 (*continued*)

No	History	History of CM allergy	History of beef / pork/product allergy	Vaccines (measles containing, live JE)	IgE to milk kUA/L	IgE to beef kUA/L	IgE to lactoglobulin kUA/L	IgE to casein kUA/L	IgE to BSA kUA/L	IgE to gelatin kUA/L
4	15 year female anaphylaxis	No	Beef, pork, jelly Immediate u, v, sob, h	No reaction to **M**	4.22	9.7	<0.1	0.21	0.98	0.1
5	8 years female anaphylaxis	No	Beef – p, u, a, c, w, sob,r, spO_2 86%, dizziness, v after 30 min, on 2 occasions	No reaction to **MMR II**	7.05	8.05	0.12	0.5	2.32	0.34
6	10 year male anaphylaxis	CM, ice cream, yoghurt, chocolate - U, sn, c, ab, v	Mutton - u, c v, ab	No reaction to **M**	25.6	57.9	0.11	1.3	7.02	0.92
7	3 year female	CM, yoghurt, curd – c, w after 30 min	Jujubes, jelly - c, w after 30 min Beef/pork not given	No reaction to **M, MMR**	2.14	4.99	<0.1	<0.1	0.74	<0.1
8	7 year male	CM, curd – u	Pork – u after 2 h	No reaction to **M, MMR**, live **JE**	19.2	57.7	<0.1	0.49	2.34	0.25
9	5 year female anaphylaxis	No	Pork 1 h later u, sob, v, ab, loc, h (70/33)	No reaction to **MMR**	20.2	13.0	<0.1	0.54	2.68	0.27
10	10 year female	No	Beef, venison - u after 30 min	No reaction to **MMR**, live **JE**	–	1.59			0.17	<0.1
11	7 year male	CM, cheese, butter - u	Beef, pork, venison - u	No reaction to **M, MMR**	10.3	36.2				
	Controls CM									
1	10 month female	Yoghurt, cerelac[a], CM ice cream - u immediately	Not ingested	Not given	10.6	0.3	1.07	10.6	0.82	<0. 1
2	10 month female	Yoghurt, cerelac[a], CM, ice cream- u	Not ingested	No reaction to **MMR 1**	30.9	3.66	13.8	27.6	6.18	<0.1
3	1 year female	CM, cheese -p, u, sn with CM, cheese, yoghurt	Not ingested	No reaction to **MMR 1**	1.29	0.25	0.3	0.4	0.44	<0. 1
4	11 months female	CM, cheese – U after 20 min Can eat yoghurt, biscuits	Not ingested	Not given	0.45	<0.1	<0.1	0.14	<0.1	<0.1
5	7 month female	CM – u, p, v immediately	Not ingested	Not ingested	2.35	<0.1	<0.1	<0.1	<0.1	<0.1
6	1 year female	CM, cake - fl, a, v, immediately	Not ingested	No reaction to **MMR I**	42.1	6.25	1.17	24.1	9.08	<0.1
7	1 year 10 female anaphylaxis	+	Not ingested		7.31	1.39	5	5.76	1.27	<0.1
8	7 month female	CM – urticaria after 10 min Biscuit – v, u	Not ingested	Not ingested	2.01	<0.1	0.28	1.4	<0.1	<0.1
9	3 year female	Cerelac[a], yoghurt, sunquick (fruit drink – CM present) 10 m fl, a, sneezing, v	Not ingested	No reaction to **M, MMR**	9.26	0.14	<0.1	5.14	–	–
10	8 year male	Triposha[a], biscuit, sunquick, chocolate – u, fl, sob, v	Not ingested	No reaction to **M, MMR**, live **JE**	100	23	2.23	>100	33.3	<0.1
11	9 month male	Cheese, yoghurt – fl, p,,sn, rh, immediately	Not ingested	No reaction to **MMR 1**	9.64	<0.1	6.83	3.79	–	–
	Controls Non atopic				CM	Beef				
1					<0.1	0.13				
2					<0.1	<0.1				
3					<0.1	<0.1				
4					<0.1	<0.1				
5					<0.1	<0.1				
6					<0.1	0.12				
7					<0.1	<0.1				
8					<0.1	<0.1				

a = angioedema., u = urticaria, p = pruritus, fl = flushing, sn = sneezing, rh = rhinorrhoea, c = cough, sob = shortness of breath, w = wheezing, f = faintness, h = hypotension, loc = loss of consciousness.
ND – not done.
Not done as IgE to CM/beef negative.
[a] CM containing weaning food.

beef allergy [13], and is the major allergen responsible for cross reactivity between CM and beef [16]. BSA as a cause of allergy to vaccines have not been described, even though anaphylaxis has been described following artificial insemination [24] and contact with BSA [25], and asthma with inhalation in a laboratory setting [26]. A study from Turkey found 3 patients with anaphylaxis to

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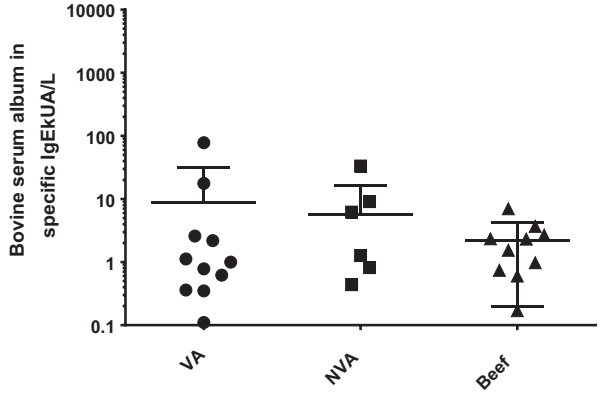

Fig. 1. Specific IgE to BSA in patients who developed hypersensitivity reactions to vaccines. The BSA IgE levels indicate those with VA (n = 14), those with CMA who did not develop reactions (NVA, n = 9) and those with beef allergy (n = 10). The bars indicate the mean and the standard deviation. VA – Vaccine allergy, NVA – Non vaccine allergy.

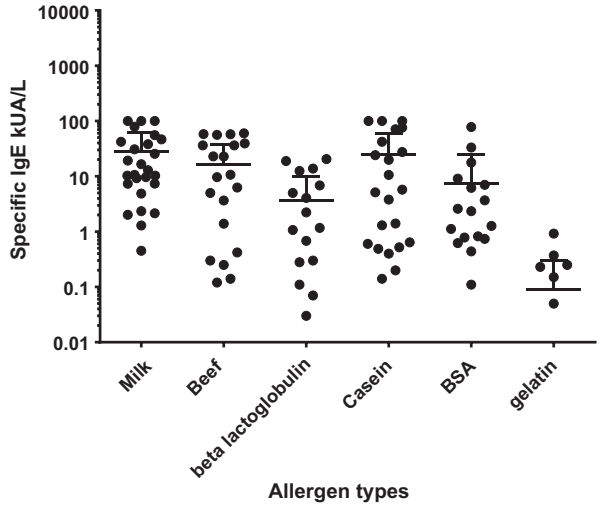

Fig. 2. Patterns of sensitization to cow's milk components in those who had cow's milk allergy (n = 20). The serum specific IgE levels for milk, beef, beta lactoglobulin, casein, BSA and gelatin are shown. The lines represent the means and the bars the standard deviations.

MMR, who had IgE to CM, but not to gelatin, but IgE to BSA was not evaluated [27]. However, those who did not develop hypersensitivity reactions to vaccines with allergy to beef and cow's milk had IgE to BSA. Therefore, the presence of IgE to BSA alone does not appear to be a pre requisite for developing reactions after vaccination.

Of the 4 patients who developed anaphylaxis to the pentavalent, Adt, or DT vaccines, 2 had IgE to casein, which is present in all 3 vaccines. Two patients had very high levels (>100 kUA/L). One of them did not have IgE to BSA. Casein has been implicated as the allergen responsible for anaphylaxis following immunization with the diphtheria, tetanus and pertussis vaccines in 8 patients in the US, 6 of whom were allergic to CM [28]. However, IgE to casein was not evaluated, unlike in our study. It is possible that casein is the allergen responsible for the anaphylactic reaction in at least two patients in our study.

Many of our patients who develop allergy to vaccines had CMA or were allergic to beef/pork. All of our patients were Sinhalese and had not been exposed to beef or pork as beef consumption among the mainly Buddhist Sinhalese and Hindu Tamil population in Sri Lanka is limited. Nine out of 12 patients who consumed beef or

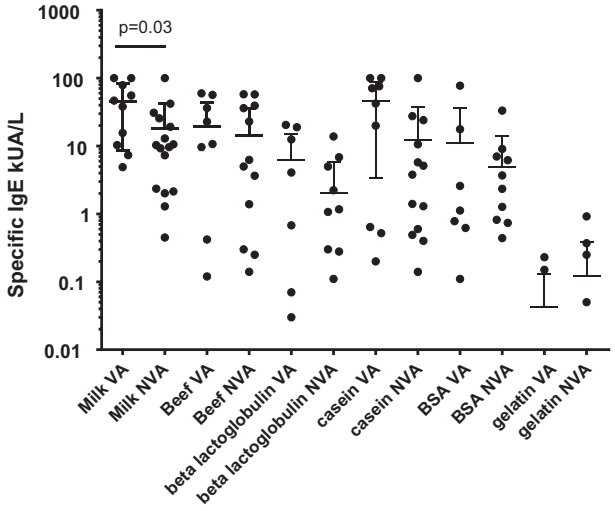

Fig. 3. Specific IgE levels in children with cow's milk allergy who developed vaccine associated allergy (VA) and those who did not (NVA). The specific IgE in serum for milk, beef, beta lactoglobulin, casein BSA and gelatin is shown in those who developed VA (n = 9) and those who did not (n = 16). The lines indicate mean values and the bars the standard deviation.

pork had allergic symptoms after ingestion. As the majority of our children had CMA, it is likely that they were sensitized to BSA, and thus reacting to the BSA component in these vaccines, whether or not the patient had ingested beef/pork. Although other studies have shown that the majority of the children with CMA were sensitized to β lactoglobulin, in our cohort, β lactoglobulin specific IgE was not detected in 66.6% of the children. In contrast, 76.5% of children in our cohort were sensitized to BSA. However, the children who had CMA in our study, and who did not develop anaphylaxis to vaccines also had specific IgE to BSA without concomitant allergy to vaccines. Therefore, the mere presence of IgE to BSA alone does not appear to be sufficient for clinical symptoms. It is possible that the epitopes that are recognized by vaccine allergic patients may be different from epitopes recognized by controls. All except one patient who developed allergic reactions to the measles containing vaccines, and live JE vaccine had been immunized with the measles, MR or MMR vaccines previously. It is also possible that the initial vaccination may have been the priming event for the allergic reaction. However, as our sample size was relatively small (n = 20) as allergy to vaccines is fortunately a rare event, this smaller sample size could have an impact on the statistical analysis.

In summary, our results show that the majority who developed anaphylaxis to vaccines in Sri Lanka had CMA. Although sensitization to BSA was not frequent among children with CMA in other countries, 76.5% of our children had BSA specific IgE. Therefore, sensitization to BSA in those with CMA probably resulted in reaction to the BSA component in the vaccines. Two patients with CMA and also possible allergy to beef, who were immunized with the rubella vaccine in a school or field center in Sri Lanka developed anaphylaxis and subsequently died. This resulted in the rubella immunization programme being suspended for a few years resulting in an increase in congenital rubella syndrome. It is therefore essential that especially in less developed countries, potential vaccinees who have CMA or allergy to beef should be immunized in centers where resuscitation measures are available.

Conflict of interest

The authors declare no conflicts of interest.

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