Original Article

Cohort study of subclinical sensitization against galactose-α-1,3-galactose in Japan: Prevalence and regional variations

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Running title: Prevalence of α-Gal sensitization in Japan

Abstract

Sensitization to galactose-α-1,3-galactose (α-Gal) leads to the development of α-Gal syndrome, which includes red meat allergy and cetuximab-induced anaphylaxis. Since tick bites represent the main cause of α-Gal sensitization, it was speculated that sensitization to α-Gal occurs throughout Japan. However, few cohort studies have investigated α-Gal sensitization in Japan. Therefore, we aimed to elucidate the subclinical sensitization rate to α-Gal in Japan. Sera were obtained from 300 participants without food or cetuximab allergy at Shimane University Hospital (Shimane prefecture), Tokyo Medical and Dental University Hospital (Tokyo metropolis), and Tohoku University Hospital (Miyagi prefecture). ImmunoCAP-bovine thyroglobulin (BTG), ImmunoCAP-beef, and IgE immunoblotting with cetuximab were performed to detect α-Gal-specific IgE. Clinical information was collected from participants using a questionnaire. The overall positivity rate of ImmunoCAP-BTG was 4.0% without significant inter-institute differences, whereas that for ImmunoCAP-beef was 9.7% with a significant inter-institute difference. Tokyo Medical and Dental University Hospital (19.0%) had the highest positivity rate. The positivity rate based on cetuximab IgE immunoblotting was 2.7%, without any significant inter-institute differences. The overall positivity rate for both ImmunoCAP-BTG and cetuximab immunoblotting was 2.0%, with a significant inter-institute difference; 5.0% of Shimane University Hospital was the highest. Two cases showed sensitization against the non-α-Gal epitope of cetuximab. The overall positivity rate for both ImmunoCAP-beef and cetuximab immunoblotting was 1.3%, without significant inter-institute differences. Male sex was associated with positive beef-specific IgE. The prevalence of subclinical sensitization to α-Gal was estimated at 2.0–4.0% in Japan and may be higher in rural areas, supporting an association between tick bites and α-Gal sensitization. In contrast, the

prevalence of subclinical sensitization to beef is 9.7% in Japan and is highest in Tokyo Metropolis, suggesting the presence of another IgE-binding epitope apart from α-Gal and another sensitization route in the sensitization to beef IgE.

Keywords: beef, cetuximab, galactose-α-1,3-galactose, red meat allergy, tick bites

Introduction

Sensitization to tick salivary proteins via tick bites causes several IgE-mediated allergic reactions, such as red meat allergy, anaphylactic reactions to cetuximab, and hypersensitivity to tick bites. Galactose-α-1,3-galactose (α-Gal) bound to tick salivary proteins is known to serve as the IgE-binding epitope in these allergic reactions, which are referred to α-Gal syndrome.[1-5]

Cetuximab is a chimeric mouse–human IgG1 monoclonal antibody that is specifically expressed against epidermal growth factor receptor (EGFR) and has been approved for use in patients with EGFR-positive unresectable progressive/recurrent colorectal cancer and squamous cell carcinoma of the head and neck.[6,7] Cetuximab contains glycosylation sites, including the α-Gal site in the Fab region.[8] Chung et al. showed that most of the severe hypersensitivity reactions to cetuximab are associated with IgE antibodies against α-Gal, and the prevalence of IgE against cetuximab varies among different regions in the USA, with a high incidence in Tennessee and low incidence in California and Massachusetts.[8] In 2009, Commins et al. reported that the late-onset urticaria and anaphylaxis that occurred 3–6 h after meat consumption are mediated by IgE specific to the α-Gal carbohydrate structure, similar to that in cetuximab allergy.[1] Moreover, the distribution of anaphylactic reactions to cetuximab overlaps the area of the high prevalence of Rocky Mountain spotted fever, which is spread by *Amblyomma americanum* and *Dermacentor variabilis*.[9] Subsequently, other studies in Australia, France, and Spain showed that tick bites are involved in the development of red-meat allergy via sensitization to α-Gal.[10-12] Hamsten et al. reported the existence of α-Gal in the gastrointestinal tract of *Ixodes ricinus*, which suggests the exposure of the host to α-Gal during a tick bite.[13] Moreover, they identified 39 patients with mammalian meat allergy in Sweden who had a history of repeated tick bites and serum IgE antibodies against *I. ricinus*.[14]

We previously demonstrated that the salivary gland of *Haemaphysalis longicornis* contains α-Gal-bearing proteins, and most of the patients with red meat allergy in Shimane (located in western Japan) have serum IgE antibodies against the salivary gland proteins of *H. longicornis*.[15] Similar to *A. americanum* that spreads Rocky Mountain spotted fever, *H. longicornis* is a dominant vector of Japanese spotted fever (JSF), which is endemic to the central and western regions of Japan.[16] Hashizume et al. reported a close association between the production of α-Gal-specific IgE antibodies and repeated bites by *Amblyomma testudinarium* in rural areas of Shizuoka, Japan.[17]

Three dominant tick species, *Amblyomma, Haemaphysalis,* and *Ixodes*, exist in Japan.[18] Few cohort studies have evaluated cetuximab and/or red meat allergy in Japan. It is speculated that sensitization to α-Gal occurs throughout Japan owing to the wide distribution of these ticks: *Amblyomma* from the central to western regions, *Ixodes* from the central to northern regions, and *Haemaphysalis* throughout Japan.[18] Since we have identified certain cases of cetuximab allergy even without a previous history of red meat allergy,[18] it is of clinical interest to determine the prevalence of sensitization to α-Gal for predicting the risk of allergic reactions before cetuximab administration. Therefore, this study was conducted to investigate subclinical sensitization rates to α-Gal in three areas of Japan: Shimane prefecture (western Japan), Tokyo metropolis (central Japan), and Miyagi prefecture (northeastern Japan).

Methods

Participants

We randomly recruited 100 participants each from Shimane University Hospital (Shimane prefecture), Tokyo Medical and Dental University Hospital (Tokyo metropolis), and

Tohoku University Hospital (Miyagi prefecture), for a total of 300 participants. The inclusion criterion was a chief complaint of anything other than food or cetuximab allergy. This study was conducted from February 2015 to March 2021 and was approved by the Ethics Committee of the Shimane University Faculty of Medicine (approval nos. 1788 and 4278). The purpose and procedures of the study were explained to eligible participants and written informed consent was obtained from each participant.

Structured questionnaire

The questionnaire assessed the following items: age, sex, blood type, history of urticaria, food allergy, cetuximab treatment, tick bites, history of JSF, and pet keeping.

Serum allergen-specific IgE values

Sera obtained from the participants were stored at -20 °C until use. Since bovine thyroglobulin (BTG) is a typical α-Gal-carrying glycoprotein,[19] and most patients with red meat allergy have anti-BTG IgE and anti-beef IgE,[20] serum allergen-specific IgE values were measured for both BTG and beef IgE antibodies using a CAP-fluorescent enzyme immunoassay system (ImmunoCAP®; Thermo Fisher Scientific, Uppsala, Sweden), and the results are expressed as units of allergen per milliliter (U_A/mL). Allergen-specific IgE values ≥0.35 U_A/mL indicated a positive result.

IgE immunoblotting analysis

Cetuximab (Erbitux®, Merck, Darmstadt, Germany) was used to detect specific IgE antibodies against α-Gal,[21] and was electrophoresed at 1 μg/lane via sodium dodecyl sulfate-

polyacrylamide gel electrophoresis using a 7.5% polyacrylamide gel. The electrophoresed proteins were transferred to polyvinylidene difluoride membranes (PVDFs; Immobilon-P; Millipore, Billerica, MA, USA). After blocking the PVDF membrane with 0.6% skim milk in Tris-buffered saline containing 0.1% Tween-20 (TBS-T) for 1 h, the membrane was incubated with 1:20 diluted serum for 20 h at room temperature (15-25 °C), washed three times with TBS-T, and incubated with horseradish peroxidase-conjugated mouse monoclonal anti-human IgE Fc (ab99806; Abcam, Cambridge, United Kingdom) for 1 h at room temperature. After washing with TBS-T, cetuximab-binding IgE antibodies were visualized on Super RX (FUJIFILM Co., Tokyo, Japan) using an Amersham ECL-Prime kit (GE Healthcare UK Ltd., Buckinghamshire, United Kingdom). Serum from a healthy participant with negative results for BTG-specific IgE (<0.35 U_A/mL, ImmunoCAP®) and serum from a patient with red meat allergy with positive BTG-specific IgE (21.8 U_A/mL, ImmunoCAP®) were used as negative and positive controls, respectively, in all experiments.

Statistical analyses

One-way analysis of variance and the post hoc Tukey multiple comparison or Games–Howell test were used to compare the positive rates among hospitals. The chi-square test was used to investigate the association of clinical factors with positivity for α-Gal-specific IgE and/or beef-specific IgE. Pearson's correlation analysis was used to confirm the correlation between the two parameters. Statistical analysis was performed using SPSS software version 25 (SPSS Inc., Chicago, IL, USA). $P = 0.05$ was considered significant.

Results

Clinical features of the participants

The clinical features of the 300 participants are presented in Table 1. Among the 300 participants with a median age of 56 years (range, 16–94 years), 173 were female and 127 were male. There were no significant differences in the clinical features of the participants among the three institutes.

Positivity rates of allergen-specific IgE and cetuximab IgE immunoblotting

The overall positivity rate of BTG-specific IgE was 4.0% among the 300 participants (Table 2). The positivity rates of BTG-specific IgE were 7.0% at Shimane University Hospital, 4.0% at Tokyo Medical and Dental University Hospital, and 1.0% at Tohoku University Hospital, with no significant inter-institute difference ($P = 0.059$, Table 2). The overall positivity rate of beef-specific IgE was 9.7%, and the positivity rates at Shimane University Hospital, Tokyo Medical and Dental University Hospital, and Tohoku University Hospital were 4.0%, 19.0%, and 6.0%, respectively, with significant inter-institute differences ($P = 0.003$, Table 2).

IgE immunoblotting with cetuximab showed IgE binding at an approximately 50 kDa band corresponding to cetuximab in eight participants (Figure 1), and the overall positivity rate was 2.7% for this study population (Table 2). Positive IgE binding was detected in 5.0%, 2.0%, and 1.0% of participants at Shimane University Hospital, Tokyo Medical and Dental University Hospital, and Tohoku University Hospital, respectively, without significant inter-institute differences ($P = 0.251$, Table 2). In the participants of Shimane University Hospital, the positive cetuximab-specific IgE antibodies were observed in five participants with ImmunoCAP-BTG values >1.2 U_A/mL (S7, S17, S19, S40, and S58) out of the seven participants with positive BTG-specific IgE values (Table 3). In the participants of Tokyo Medical and Dental University

Hospital, the positive cetuximab-specific IgE antibodies were observed in two participants containing ImmunoCAP-BTG values <0.1 U_A/mL (K19) and 8.25 U_A/mL (K51) (Table 3). Among the participants of Tohoku University Hospital, one participant (H27) had positive cetuximab-specific IgE antibodies and an ImmunoCAP-BTG value of <0.1 U_A/mL (Table 3). The overall rates for both positive BTG-specific IgE tests and positive cetuximab immunoblotting were 2.0%, with a significant difference among the three institutes with the highest rate of 5.0% at Shimane University Hospital (P = 0.028, Table 2). Two participants (K19 and H27) with negative BTG-specific IgE tests and negative beef-specific IgE test results showed positive immunoblotting with cetuximab, suggesting that these participants had IgE against the non-α-Gal epitope of cetuximab (Table 3). The overall rates for both positive beef-specific IgE tests and positive cetuximab immunoblotting were 1.3%, without significant differences among the three institutes (P = 0.171, Table 2).

Association between the BTG-specific IgE value and the beef-specific IgE value

On studying the association between the BTG-specific IgE and beef-specific IgE values, a significant correlation was observed between the positive results in the two tests (Table 4). Three of seven participants at Shimane University Hospital with positive BTG-specific IgE levels had positive beef-specific IgE antibodies. Four participants at the Tokyo Medical and Dental Hospital with positive BTG-specific IgE had positive beef-specific IgE. One participant at Tohoku University Hospital with positive BTG-specific IgE had positive beef-specific IgE. However, positive beef-specific IgE was detected in one of the 93 participants who had negative BTG-specific IgE tests at Shimane University Hospital, 15 of the 96 participants who had negative BTG-specific IgE tests at Tokyo Medical and Dental University Hospital, and five of

192 the 99 participants who had negative BTG-specific IgE tests at Tohoku University Hospital
193 (Table 4). Among the 33 participants showing either positive BTG-specific IgE or beef-specific
194 IgE, no significant association was observed between BTG-specific IgE values and beef-specific
195 IgE values (Figure 2).
196
197 **Association between the BTG- or the beef-specific IgE value with clinical characteristics**
198 Table 5 shows the associations of clinical characteristics of participants with positive
199 BTG-specific IgE. Positive BTG-specific IgE was detected only in the participants with non-B
200 blood type. Table 6 shows the association of clinical characteristics of participants with positive
201 beef-specific IgE. Positive beef-specific IgE was associated only with the male sex.
202
203 **Discussion**
204 In the present study, we performed three different tests to investigate subclinical
205 sensitization to α-Gal: BTG-specific IgE test, beef-specific IgE test, and cetuximab IgE
206 immunoblotting. We demonstrated that the prevalence of sensitization to α-Gal was 4.0% in
207 Japan using a BTG-specific IgE test in the investigation of three institutes covering Shimane
208 Prefecture (western part), Tokyo Metropolis (central part), and Miyagi Prefecture (northern part)
209 of Japan. True sensitization to α-Gal seemed to be lower than 4.0%, since the positivity rate
210 based on cetuximab IgE immunoblotting was 2.7% and the rate for both positive BTG-specific
211 IgE and positive cetuximab-immunoblotting was 2.0% (Table 2). The low positivity rate of
212 cetuximab IgE immunoblotting (2.7%) may be attributed to its higher detection limit (1.2
213 U_A/mL) than that of the BTG-specific IgE-test (≥0.35 U_A/mL was considered positive). Notably,
214 cetuximab immunoblotting showed a positive reaction in two participants who had negative

BTG- and beef-specific IgE tests, suggesting that these participants had IgE against the non-α-Gal epitope of cetuximab (Figure 1 and Table 3). The characteristics of IgE antibodies are yet to be investigated. Taken together, the overall sensitization rate to α-Gal was estimated to be 2.0–4.0% in Japan. This overall positivity rate is comparable to the α-Gal sensitization rates in previous reports, which are 1.8% in Denmark and 2.2% in Spain.[22] These results indicate that α-Gal sensitization occurs worldwide, although the rates vary among different regions.

The positive results in this study may indicate subclinical sensitization because the results were not related to clinical symptoms such as red meat allergy or cetuximab allergy. Nevertheless, these participants with subclinical α-Gal sensitization may develop anaphylaxis if they receive intravenous administration of cetuximab, since we previously found eight cases of cetuximab-induced anaphylactic shock that developed without a history of red meat allergy.[18]

The rate of α-Gal sensitization differed based on region. This difference became obvious when sensitization was evaluated using ImmunoCAP-BTG combined with cetuximab immunoblotting (Table 2). Among the three regions, the sensitization rate was highest in Shimane Prefecture (5.0%). The regional difference in the α-Gal sensitization found in this study is compatible to that in previous studies reporting that the prevalence of positive specific IgE to α-Gal varies among regions.[8,9,23,24] Commins et al. showed that the prevalence of IgE antibodies against cetuximab is 20% in the southeast region of the USA, whereas it is only 2% in northern California.[9] Furthermore, they reported that the prevalence of IgE antibodies against α-Gal is <1% in northern Sweden, 76% in Kabati (rural area) in Kenya, and 29% in Thika (a moderately sized industrial town) in Kenya.[9] The prevalence of α-Gal-specific IgE (≥0.01 U_A/mL) is 24.7% in rural villages and 1.2% in urban areas of the Friuli Venezia Giulia region in Italy.[24]

237 Sensitization to α-Gal is thought to be caused mainly by tick bites and, thus, the
238 relatively higher sensitization rate may be due to the higher chance of tick bites.[7] *A.*
239 *americanum*, *D. variabilis, Ixodes holocyclus*, *I. ricinus, H. longicornis,* and *A. testudinarium*
240 have been reported to be involved in α-Gal sensitization.[9-15, 17, 18] Thus, α-Gal sensitization
241 induced by tick bites can occur anywhere in Japan. However, in this study, we found a
242 difference in α-Gal sensitization rates depending on the region. The α-Gal syndrome is
243 specifically related to activities or occupations, such as hiking, hunting, or forest work, based on
244 frequent exposure to ticks.[14, 25, 26] Therefore, the rural population is likely to have a higher chance
245 of acquiring tick bites than that of the urban population. Taking these factors into consideration,
246 the participants examined at Shimane University Hospital possibly had a higher incidence of
247 tick bites because the forest area accounts for more than 80% of Shimane Prefecture; however,
248 we failed to collect information on the occupations of the participants. Furthermore, Shimane is
249 an endemic area for JSF which is spread by tick bites.[27] In contrast, the participants examined at
250 Tokyo Medical and Dental University Hospital had relatively fewer opportunities of acquiring
251 tick bites because the Tokyo Metropolis is an urban area with few forests and is located in the
252 Kanto Plain in central Japan. Similarly, the participants examined at Tohoku University Hospital
253 had a relatively lower risk of tick bites because Tohoku University Hospital is located in Sendai
254 city, which is a medium-sized city in northeastern Japan where thick clothing is preferred
255 because of the prevailing low temperatures.
256 In contrast, we found that the prevalence of sensitization to beef was 19% in the
257 participants examined at Tokyo Medical and Dental University Hospital and was significantly
258 higher than that in those examined at Shimane University Hospital (4%) and Tohoku University
259 Hospital (6%) (Table 2). Although red meat allergy is usually associated with α-Gal-specific IgE

antibodies,[1] 79% (15 out of 19), 83% (5 out of 6), and 25% (1 out of 4) of the participants in Tokyo Medical and Dental University Hospital, Tohoku University Hospital, and Shimane University Hospital, respectively, who had positive beef-specific IgE tested negative for BTG-specific IgE (Table 4). In addition, no correlation was found between BTG-specific IgE levels and beef-specific IgE levels in these participants (Figure 2). The discrepancy in these results between the two tests may be attributed to different sensitization routes and causative allergens; tick bites may represent the main cause of sensitization in the Shimane area, whereas gastrointestinal absorption of beef allergens may represent the predominant route for beef-sensitization in the Tokyo and Miyagi areas.

A key aspect of our study was the association between positive beef-specific IgE and male sex ($p < 0.001$, n = 300; Table 6), although no association was found between positive BTG-specific IgE test and sex ($p = 0.082$, n = 300; Table 5). Currently, there is no evidence of an association of α-Gal syndrome with difference in sex;[22, 24, 28,29] however, Orhan et al. reported 12 patients with beef allergy and a 3:9 sex ratio of female to male.[30] These cases may differ from those of α-Gal-related beef allergy, which is characterized by delayed-onset of allergic reactions (>3 h from beef ingestion)[1], because these 12 patients had the onset of beef allergy at a relatively younger age and short symptom-onset time of <2 h. Therefore, a primary beef allergy, and not α-Gal syndrome, can occur more frequently in males, whereas tick bites do not contribute to sex differences. A limitation of this study is the small sample size of 300 participants from only three institutes.

In conclusion, the prevalence of subclinical sensitization to α-Gal is likely to be 2.0-4.0% in Japan and high in Shimane prefecture. The risk of hypersensitivity reactions to

cetuximab and/or red meat should be carefully evaluated, even in subjects without a history of red meat allergy, especially in rural areas, such as Shimane prefecture.

Acknowledgements

The authors thank Mrs. Kiyoe Ueda for technical assistance with this research. This research was funded by the Japan Agency for Medical Research and Development, AMED grant number. 17ek0410020h0003, and JSPS KAKENHI grant number 16K10157.

Conflicts of interest

The authors declare that they do not have any potential conflict of interest to disclose.

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376

377 **Figure legends**

378 **Figure 1.** IgE immunoblot analysis using cetuximab.

379 Cetuximab (1 μg/lane) was separated via sodium dodecyl sulfate-polyacrylamide gel
380 electrophoresis and immunoblotted with the sera of the participants. The protein band at
381 approximately 50 kDa corresponded to the α-Gal-bound heavy chain of cetuximab in Coomassie
382 brilliant blue staining. Nos. S1–S100, K1–K100, and H1–H100 represent the participants at
383 Shimane University Hospital, Tokyo Medical and Dental University Hospital, and Tohoku
384 University Hospital, respectively. The results of 12 participants with positive ImmunoCAP-BTG
385 (S7, S17, S19, S33, S40, S58, S84, K31, K51, K52, K61, and H37) and two participants with
386 positive immunoblotting (K19 and H27) are shown. P: patient with beef allergy as positive
387 control; N, healthy control used as negative controls. α-Gal, galactose-α-1,3-galactose.

388

389 **Figure 2.** Association of BTG- specific IgE values and beef-specific IgE values.

390 No correlation was observed between BTG-specific IgE values and beef-specific IgE values in
391 33 participants with either positive BTG-specific IgE or beef-specific IgE. BTG, bovine
392 thyroglobulin.

393 **Table 1**. Clinical features of the participants

Institutes†	Shimane	Tokyo	Tohoku	*P*-value*
Number of participants	100	100	100	NA
Age, years, median (range)	57.8 (19–92)	45.0 (16–84)	52.4 (18–94)	0.342
Female/male	59/41	52/48	62/38	0.082
Blood type				
A	48.5% (48/99)	44.9% (44/98)	39.4% (39/99)	0.225
B	19.2% (19/99)	23.5% (23/98)	21.2% (21/99)	
O	27.3% (27/99)	23.5% (23/98)	26.3% (26/99)	
AB	5.1% (5/99)	8.2% (8/98)	13.1% (13/99)	
History of				
Urticaria	53.0% (53/100)	67.0% (67/100)	52.0% (52/100)	0.052
Red meat allergy	3.0% (3/100)	2.0% (2/100)	1.0% (1/100)	0.603
Cetuximab treatment	0.0% (0/100)	1.0% (1/96)	0.0% (0/100)	0.354
Tick bites	5.0% (5/99)	7.1% (7/99)	4.0% (4/100)	0.632
JSF	1.0% (1/100)	0.0% (0/100)	0.0% (0/100)	0.369
Keeping pets	71.0% (71/100)	61.0% (61/100)	67.0% (67/100)	0.329

394 †Shimane, Shimane University Hospital; Tokyo, Tokyo Medical and Dental University Hospital; Tohoku, Tohoku University Hospital.

395 NA, not applicable; JSF, Japanese spotted fever. **P*-values were obtained with Tukey's and Games–Howell tests as appropriate.

396

Table 2. Positive rates of serum allergen-specific IgE and cetuximab immunoblotting

Institutes†	Shimane	Tokyo	Tohoku	*P*-value*	Total
BTG-specific IgE	7.0% (7/100)	4.0% (4/100)	1.0% (1/100)	0.059	4.0% (12/300)
Cetuximab immunoblotting	5.0% (5/100)	2.0% (2/99)	1.0% (1/100)	0.251	2.7% (8/299)
Both BTG-specific IgE and cetuximab immunoblotting	5.0% (5/100)	1.0% (1/99)	0.0% (0/100)	0.028	2.0% (6/299)
Beef-specific IgE	4.0% (4/100)	19.0% (19/100)	6.0% (6/100)	0.003	9.7% (29/300)
Both beef-specific IgE and cetuximab immunoblotting	3.0% (3/100)	1.0% (1/99)	0.0% (0/100)	0.171	1.3% (4/299)

†Shimane, Shimane University Hospital; Tokyo, Tokyo Medical and Dental University Hospital; Tohoku, Tohoku University Hospital. BTG, bovine thyroglobulin. **P*-values were obtained with Tukey's and Games–Howell tests as appropriate.

401 **Table 3.** Characteristics of the participants with positive allergen-specific IgE and/or immunoblot analysis

No.†	Age	Sex	Blood type	Urticaria	Red-meat allergy	Cetuximab treatment	Tick bites	JSF	Keeping pets	ImmunoCAP (U_A/mL)		IgE Immunoblotting with cetuximab
										BTG	Beef	
S7	84	F	O	-	-	-	-	-	-	21.8	9.49	+
S17	84	M	ND	-	-	-	-	-	-	3.06	1.11	+
S19	61	F	O	-	-	-	-	-	-	1.31	0.161	+
S33	66	F	O	+	-	-	+	-	+	0.755	0.177	-
S40	54	M	O	-	-	-	-	-	+	1.20	0.347	+
S58	74	F	A	+	-	-	-	-	+	14.3	0.679	+
S84	82	M	A	-	-	-	-	-	-	0.675	<0.1	-
K19	38	F	O	-	-	-	-	-	-	<0.1	0.166	+
K31	26	M	O	-	-	-	-	-	+	0.396	0.624	-
K51	44	M	A	+	-	-	-	-	+	8.25	6.06	+
K52	56	M	A	+	-	-	-	-	+	0.765	0.626	-
K61	76	M	O	+	-	-	+	-	+	0.566	1.02	-
H27	47	M	A	+	-	-	-	-	+	<0.1	<0.1	+
H37	41	M	O	+	-	-	-	-	+	0.514	0.716	-

402 †Nos. S1–S100, K1–K100, and H1–H100 represent participants at Shimane University Hospital, Tokyo Medical and Dental University
403 Hospital, and Tohoku University Hospital, respectively. M, male; F, female; JSF, Japanese spotted fever; BTG, bovine thyroglobulin; IgE,
404 immunoglobulin E; ND, No data.

405 **Table 4.** Concordance ratio between BTG-specific IgE and beef-specific IgE

Institutes†		Shimane (n = 100)			Tokyo (n = 100)			Tohoku (n = 100)			Total (n = 300)		
BTG (U_A/mL) \ Beef (U_A/mL)		<0.35	≥0.35	*P*-value*	<0.35	≥0.35	*P*-value	<0.35	≥0.35	*P*-value	<0.35	≥0.35	*P*-value
<0.35		92	1	<0.001	81	15	<0.001	94	5	<0.001	267	21	<0.001
≥0.35		4	3		0	4		0	1		4	8	

406 †Shimane, Shimane University Hospital; Tokyo, Tokyo Medical and Dental University Hospital; Tohoku, Tohoku University Hospital. **P*-

407 values were obtained using chi-square tests. BTG, bovine thyroglobulin.

408

409 **Table 5**. Demographic characteristics of participants testing positive for BTG-specific IgE

Institutes†	Shimane			Tokyo			Tohoku			Total		
ImmunoCAP-BTG (U_A/mL)	<0.35	≥0.35	*P*-value*	<0.35	≥0.35	*P*-value	<0.35	≥0.35	*P*-value	<0.35	≥0.35	*P*-value
Sex												
Male (n = 127)	38	3	0.917	44	4	0.034	37	1	0.199	119	8	0.082
Female (n = 173)	55	4		52	0		62	0		169	4	
Blood type												
A (n = 128)	46	2	0.140	42	2	0.459	39	0	0.418	127	4	0.018
B (n = 63)	19	0		23	0		21	0		63	0	
O (n = 76)	23	4		21	2		25	1		69	7	
AB (n = 26)	5	0		8	0		13	0		26	0	
History												
Urticaria (n = 172)	51	2	0.179	64	3	0.728	48	0	0.334	166	6	0.600
Red-meat allergy (n = 6)	3	0	0.629	2	0	0.771	1	0	0.920	6	0	0.614
Cetuximab treatment (n = 1)	0	0	NA§	1	0	0.834	0	0	NA	1	0	0.837
Tick bites (n = 16)	4	1	0.242	6	1	0.150	4	0	0.837	14	2	0.750
JSF (n = 1)	1	0	0.783	0	0	NA	0	0	NA	1	0	0.838
Keeping pets (n = 199)	68	3	0.089	57	4	0.103	66	1	0.481	191	8	0.980

410 †Shimane, Shimane University Hospital; Tokyo, Tokyo Medical and Dental University Hospital; Tohoku, Tohoku University Hospital. Data

411 are presented as the number of participants. * *P*-values were obtained using chi-square tests. BTG, bovine thyroglobulin; NA, not applicable;

412 JSF, Japanese spotted fever

413

Table 6. Demographic characteristics of participants testing positive for beef-specific IgE

Institutes†	Shimane			Tokyo			Tohoku			Total		
ImmunoCAP-beef (U_A/mL)	<0.35	≥0.35	*P*-value*	<0.35	≥0.35	*P*-value	<0.35	≥0.35	*P*-value	<0.35	≥0.35	*P*-value
Sex												
Male (n = 127)	39	2	0.709	31	17	<0.001	33	5	0.018	103	24	<0.001
Female (n = 173)	57	2		50	2		61	1		168	5	
Blood type												
A (n = 128)	46	2	0.801	35	9	0.111	38	1	0.507	119	12	0.304
B (n = 63)	19	0		20	3		20	1		59	4	
O (n = 76)	26	1		20	3		23	3		69	7	
AB (n = 26)	5	0		4	4		12	1		21	5	
History												
Urticaria (n = 172)	52	1	0.252	56	11	0.348	50	2	0.345	158	14	0.299
Red-meat allergy (n = 6)	3	0	0.720	1	1	0.259	1	0	0.800	5	1	0.558
Cetuximab treatment (n = 1)	0	0	NA	1	0	0.619	0	0	NA	1	0	0.741
Tick bites (n = 16)	5	0	0.640	5	2	0.503	4	0	0.606	14	2	0.693
JSF (n = 1)	1	0	0.837	0	0	NA	0	0	NA	1	0	0.743
Keeping pets (n = 199)	69	2	0.345	48	13	0.461	61	6	0.760	178	21	0.466

†Shimane, Shimane University Hospital; Tokyo, Tokyo Medical and Dental University Hospital; Tohoku, Tohoku University Hospital.

Data are presented as the number of participants. **P*-values were obtained using chi-square tests. NA, not applicable; JSF, Japanese spotted fever

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