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RED MEAT ALLERGY: INVESTIGATING THE INFLUENCE OF COOKING AND LIPID CLASSES ON ALPHA-GAL CONTENT AND UNDERSTANDING THE DISEASE PREVALANCE IN KANSAS RURAL COMMUNITIES

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I. INTRODUCTION

Alpha-gal syndrome (AGS), also known as red meat allergy, is an acquired allergy to galactose-alpha-1,3-galactose (α-Gal), which can be conjugated to both glycoproteins and glycolipids. α-Gal is ubiquitous in the tissues of mammals, but humans and other Old-World primates lack the enzyme necessary to synthesize the carbohydrate. As a result, humans can be sensitized to the α-Gal antigen. In North America, the major vector is the Lone Star tick (*Amblyomma americanum)*; after a bite, individuals may then experience an allergic reaction upon the consumption of food products containing mammalian tissues [1]. The reaction severity varies greatly but can range from hives and gastrointestinal distress to potentially fatal anaphylaxis [2]. While little is known about the true number of effected individuals, the species range and population of the Lone Star tick is increasing as aided by climate change and increasing populations of primary hosts like white-tail deer. Today, states like Kansas are experiencing historically unprecedented incidence rates of AGS. While prior work in our lab has evaluated the contribution of glycoproteins to α-Gal content [3], limited work has been done to evaluate glycolipids and their relevance to AGS. Thus, our objectives for this study were to 1) to understand the impact of cooking and lipid species on the α-Gal content of beef striploins and 2) gain a better understanding of AGS awareness, prevalence, and disease management throughout rural Kansas communities.

II. MATERIALS AND METHODS

To determine the influence of cooking and lipid species on the α-Gal content of beef glycolipids, strip steaks (*longissimus lumborum*) were collected from 10 beef carcasses. One steak from each carcass was left raw, while the other was cooked on a clamshell grill to 77°C. Total lipid samples were then extracted from each sample and solid-phase extraction was utilized to separate the extracted lipid into two classes: neutral and polar lipids. Lipid extracts were adjusted to a standard concentration of 0.5 mg/mL using a spotting buffer ($CHCl_3$:MeOH:50 mM HCl [250:500:200 v/v]). A lipid blot was performed by dotting 10 µg of sample on a nitrocellulose membrane along with 0.01 µg of α-Gal conjugated phosphatidylethanolamine (PE) standard (CAT NGL0334; Dextra, United Kingdom) with a known α-Gal content. Also included on each membrane were 10 µg of total chicken lipid as a negative control and 10 µl of spotting buffer as a blank. Membranes were blocked and blotted against an anti-α-Gal IgG1 primary antibody overnight before incubation with a horseradish peroxidase-conjugated anti-IgG secondary antibody. α-Gal content was calculated by standardizing each sample to the standard (810 pmol/µg lipid) and dividing by 10 to express α-Gal content on a per µg of lipid sample basis. To accomplish our second objective, three listening sessions were conducted across the state (Parson, KS, Salina, KS, and online via Zoom) to establish relationships with a network of K-State extension agents for survey distribution. Incentivized paper and electronic (Qualtrics) were distributed through Kansas County Extension Agents to local communities across Kansas (IRB-12256). Surveys were validated using CAPTCHA scores, geographical data, and the provided home addresses to ensure submissions were from individuals residing in the state of Kansas. The survey consisted of 23 questions aimed at understanding respondents' awareness and knowledge of AGS, their familiarity with other affected individuals, key demographic and lifestyle factors, and their interest in learning more about AGS.

III. RESULTS AND DISCUSSION

A representative image of a lipid blot is provided in Figure 2. There was no significant interaction between cooking and lipid species ($P>0.05$). While cooking did not significantly impact the α-Gal content ($P>0.05$), there was a main effect on lipid species. Polar lipids had a significantly greater α-Gal content as compared to the neutral lipids with 21.7 and 3.7 pmol of α-Gal/µg lipid, respectively ($P<0.05$; Figure 1). Previous work done in our lab has shown the heat stability of the α-Gal content of glycoproteins of beef strip steaks [3]. For the second objective, only 165 surveys were validated from the 579 surveys returned (28.5%), with the highest response rate coming from the northeastern (35.2%) and south central (32.1%) regions. The lowest response rate came from the southwestern (1.21%) and

northwestern (1.21%) regions. 95.8% of respondents had heard of AGS and 86.1% responded that they were familiar with the symptoms of the allergy. Though more than half of all respondents knew that AGS was associated with abdominal pain, diarrhea, hives, nausea, and swelling, few knew AGS patients may experience respiratory symptoms like cough, wheezing, and shortness of breath. When assessing prevalence, 80.6% of respondents said they themselves or someone they knew have/had AGS. Of the 553 reported AGS cases, Crawford County, followed by Montgomery County, both located in the southeastern region, had the highest number of reported cases with 78 and 51, respectively, while only 1.4% of the reported cases were from the western region of Kansas (Figure 3). Despite the large number of reported cases, only 1.8% of respondents believed there was sufficient information about AGS available, and 75.2% were interested in receiving more information regarding AGS.

IV. CONCLUSION

This work represents one of the first attempts to quantify α-Gal within lipid. Insights were gained into the heat stability of the α-Gal glycolipid epitope and the high concentration of α-Gal within the polar lipid fraction. However, this work is preliminary, and further investigation is necessary to reduce antibody cross-reactivity and explore the role of lipid-bound α-Gal in eliciting AGS reactions. Furthermore, our survey results revealed that many Kansans suffer from AGS or know someone in their community who does. Most of the AGS cases seem to be concentrated in the eastern portion of the state, particularly in the southeastern corner. However, as climate change drives the northern and westward migration of the Lone Star tick, the incidence rate of AGS in northern and western Kansas may continue to rise. To expand upon this work, our group is conducting interviews with diagnosed AGS patients to better understand the challenges they face. Ultimately, we hope to establish a targeted approach to help alleviate the biological, social, and economic impacts of AGS in Kansas. A complimentary approach of both basic, biochemical research, coupled with patient-centered research is paramount to building understanding and awareness, and improving quality of life for patients with AGS around the world.

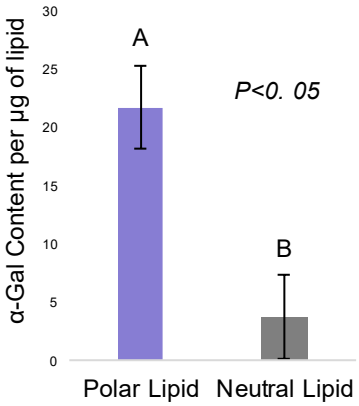


Figure 1. Lipid species influence on *α*-Gal content.

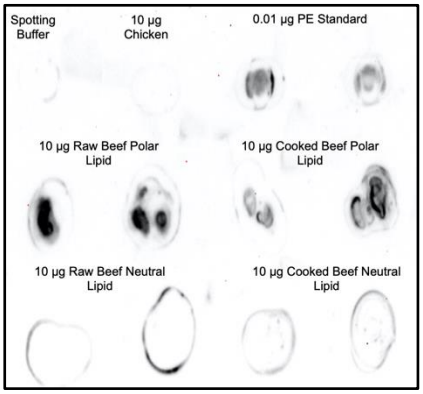


Figure 2. Representative lipid blot row 1) spotting buffer, chicken lipid, PE standard 2) raw polar lipid (duplicate), cooked polar lipid (duplicate), 3) raw neutral lipid, cooked neutral lipid.

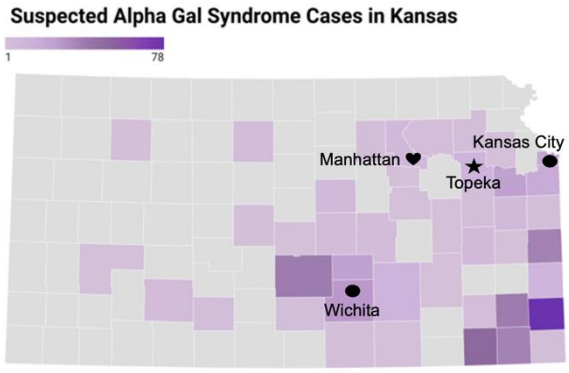


Figure 3. Map of Kansas counties and the suspected number of AGS cases by density.

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