BACKGROUND: Agriculture-related dust exposures to antimicrobial-resistant pathogens can have significant health implications for animal agricultural industry workers. In the U.S., occupational exposure is thought to be involved in 30% of asthma and 15% of chronic obstructive pulmonary disease cases, and associated medical costs are estimated in the billions[1 2]. Moreover, as the Western U.S. becomes more arid due to climate change, dust exposure is increasing and contributing to poorer health outcomes in areas such as inland California. This particularly affects poorer socioeconomic communities and is a leading contributor to health disparities[3-4]. While dust particles and dust-borne pathogens primarily enter the body by respiratory exposure, mucociliary clearance and swallowing into the gastrointestinal tract causes intestinal infection[5]. Analysis of the microbial composition of air from a pig farm found that swine farm dust is highly abundant (>98%) in gram-positive and negative bacteria, and lipopolysaccharides (LPS; endotoxins) [6,7]. LPS has the potential to weaken the gut barrier and alter gut microbial composition. Therefore, we seek to identify how ***agricultural dust LPS*** triggers gastrointestinal inflammation and barrier dysfunction.

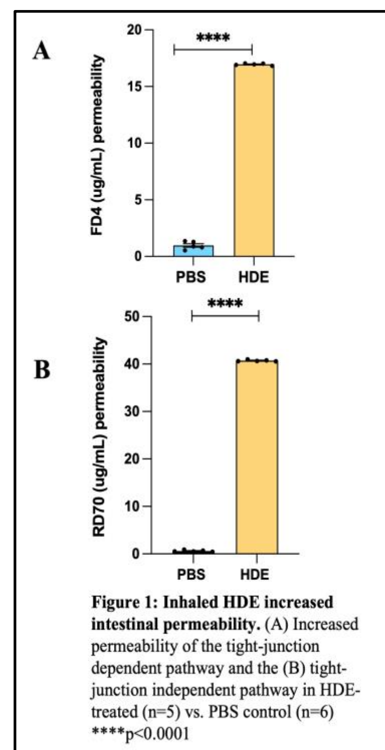

Figure 1: Inhaled HDE increased intestinal permeability. (A) Increased permeability of the tight-junction dependent pathway and the (B) tight-junction independent pathway in HDE-treated (n=5) vs. PBS control (n=6) ****p<0.0001

PRINCIPLE AIM: The goal of this study is to characterize and identify mechanisms by which ***agricultural dust can disrupt interspecies interactions between resident gut microbes that leads to host inflammation***. <u>**Preliminary data:**</u> We identified that intranasal exposure of 8-week-old male or female C57BL/6 mice to 12.5% hog dust extract (HDE; containing 22.1-91.1 endotoxin units [EU]/mL) (n=6) for 15 days caused chronic airway inflammation marked by significantly elevated bronchoalveolar lavage total cell (p=0.0061) and neutrophil levels (p=0.0027; not shown) and increased intestinal permeability (Fig. 1A-B) vs. saline controls. We also reported that HDE-treated mice have ***elevated serum LPS*** (Fig. 2) indicating endotoxemia. Characterization of the gut microbiome found reduced abundance of the beneficial species, *Akkermansia muciniphila* (*A. muciniphila*) in fecal samples of HDE-exposed mice (Fig.3). These data indicate that agricultural dust exposure induces endotoxemia, promotes intestinal inflammation and alters gut microbial communities causing depletion of a beneficial bacterium.

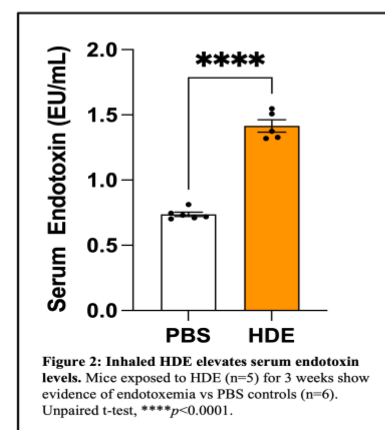

Figure 2: Inhaled HDE elevates serum endotoxin levels. Mice exposed to HDE (n=5) for 3 weeks show evidence of endotoxemia vs PBS controls (n=6). Unpaired t-test, ****p<0.0001.

SPECIFIC AIMS: <u>***Aim 1: Determine the contribution of a gut-lung axis in tissue-specific responses to dust exposure.***</u> An unresolved question with respect to inhalation of pollutants and their effects on intestinal inflammation is whether pollutants initiate inflammation in the lung that then migrates to the gut to compromise the intestinal barrier, or whether inhaled pollutants directly access the intestinal lumen and initiate inflammation independent from lung exposure. We propose to answer this question by comparing intestinal responses to inhaled vs. direct intestinal administration of HDE.

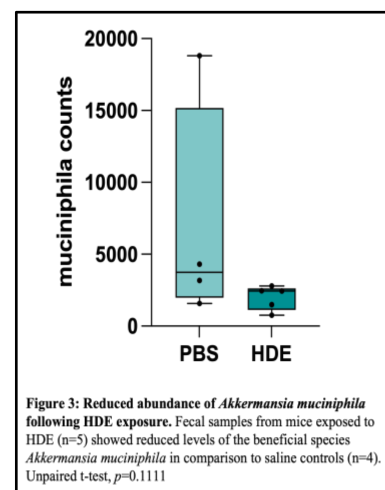

Figure 3: Reduced abundance of *Akkermansia muciniphila* following HDE exposure. Fecal samples from mice exposed to HDE (n=5) showed reduced levels of the beneficial species *Akkermansia muciniphila* in comparison to saline controls (n=4). Unpaired t-test, p=0.1111

Aim 1.1: To distinguish the site of origin of increased serum endotoxin and intestinal permeability of mice intranasally exposed to HDE (Fig. 1 & 2), we will directly administer HDE (12.5%) into the stomach of mice and compare intestinal inflammatory responses induced by respiratory (inhaled) vs. intestinal routes of HDE administration. We will measure: 1) serum LPS; 2) FD4 vs. RD70 permeability; 3) histology (small intestine and large intestine); 4) cytokine levels (i.e., *Tnf*α); 5) flow cytometric analysis of gut mucosal immune populations[7,8].

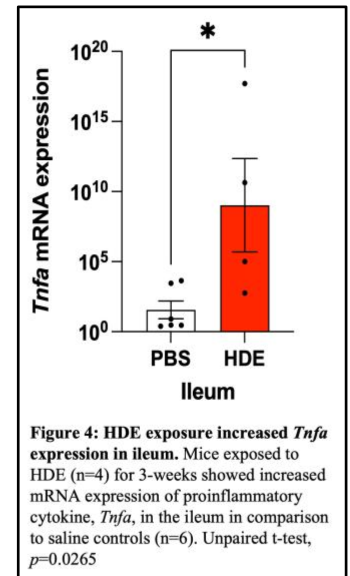

Figure 4: HDE exposure increased *Tnfa* expression in ileum. Mice exposed to HDE (n=4) for 3-weeks showed increased mRNA expression of proinflammatory cytokine, *Tnfa*, in the ileum in comparison to saline controls (n=6). Unpaired t-test, p=0.0265

Aim 1.2: To examine gut microbial alterations following oral gavage of HDE, fecal samples will be collected, and microbial communities will be examined by Illumina MiSeq at the UCSD Microbiome Core and analyzed using QIIME with the help of the UCR Statistical Consulting Collaboratory (collaboratory.ucr.edu).

Aim 1.3: We propose that acute exposure to bacterial LPS found in 12.5% HDE[9] is the predominant mediator of HDE-induced intestinal inflammation and barrier dysfunction. To verify this, mice will be exposed (intranasally vs. oral gavage) to one dose of fluorescently labeled LPS (22.1-91.1 EU/mL; concentration of LPS in 12.5% HDE) for 24 hours. LPS transit from the lungs-to-gut vs. gut-to-lungs will be monitored over time in a fluorescence in vivo imaging system[10]. This will distinguish if inhaled vs. intestinal LPS exposure elevates serum LPS and triggers gut inflammation.

Aim 1.4 To address a key translational question as to whether previous dust exposures could worsen disease outcomes, we will identify if mice exposed to HDE (12.5%; 15 days) have more severe responses to either LPS administration (oral gavage; 24 hrs.), or in a mouse model of human colitis (3-5% dextran sulfate sodium given in drinking water; 5 days)[11].

Aim 2: Identify the effects of A. muciniphila treatment during 12.5% HDE exposure. We demonstrated that exposure to hog dust extract reduced levels of the beneficial species *A. muciniphila* in fecal samples (Fig. 3). We hypothesize that reduction in *A. muciniphila* may contribute to barrier dysfunction during HDE exposure. **Therefore, we propose that loss of *A. muciniphila* contributes to HDE-induced intestinal inflammation, and that administration of *A. muciniphila* can rescue normal barrier function in HDE-exposed mice.**

Aim 2.1 In collaboration with Dr. Ansel Hsiao's germ-free mouse facility at UCR, we will perform fecal-microbial transplants of stool from control vs. HDE-treated mice into germ-free mice to identify if the intestinal inflammatory response to HDE can be transmitted by bacterial transfer.

Aim 2.2 *A. muciniphila* MucT (ATTC® BAA-835; ATCC, Manassas, VA) will be cultured anaerobically as previously described[9]. Saline controls and 12.5% HDE exposed mice will be concurrently treated with *A. muciniphila* (oral gavage; 4.0×10^{8} CFU in PBS[12]) in a "protection" protocol; or treated with *A. muciniphila* for 15 days after HDE treatment concludes to determine if *A. muciniphila* accelerates "rescue" of barrier function.

Subsequent ***metabolomic analysis*** of *A. muciniphila* treated vs. untreated mice will serve as a "discovery" approach to drive the next phase of this project and identify biomarkers of disease and recovery from dust-induced intestinal inflammation that I will utilize to establish my own research program.

SIGNIFICANCE: This study has direct relevance to understanding how air pollutants drive chronic disease by modifying gut physiology. I will not only identify mechanisms by which agricultural dust causes inflammation, but I will also identify if restoration of a beneficial commensal bacterium can rescue gut barrier function. This project is significant because it lies at the intersection of climate change-induced environmental pollution, the rapid increase in respiratory driven diseases in inland California, and addresses causes of health disparities in poorer rural areas of the U.S. that are dependent on agriculture.

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