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An Analysis of the Association between Animal Exposures and the Development of Type 1 Diabetes in the TEDDY Cohort

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An Analysis of the Association between Animal Exposures and the Development of Type 1 Diabetes in the TEDDY Cohort

by

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Dedication

To my parents, for the extraordinary example of love and hard work they have been and continue to be every day of my life.

To Brian, for being courageous enough to stand by my side as I tackled this final educational feat and for being crazy enough to ask me to marry him in the process.

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Abstract

Research on exposure to animals and risk of type 1 diabetes (T1D) has had conflicting results with some researchers finding that animal exposure reduces the risk of T1D and others finding no association between animal exposure and T1D. Previously conducted studies on the association between animal exposure and T1D are case-control studies that have been limited by recall bias. The purpose of this study is to investigate the association between early life animal exposure and the risk of persistent, confirmed islet autoantibodies (IA) and T1D diagnosis among an eligible cohort of genetically high T1D risk participants enrolled in the international prospective cohort study, The Environmental Determinants of Diabetes in the Young (TEDDY). It is hypothesized that children who are exposed to animals in early life will have a lower risk of developing IA and T1D than children who are not exposed to animals in early life.

A total of 7,432 TEDDY participants were included in the study. The associations between early life animal exposure and the outcomes of interest were explored using Cox proportional hazards models. In order to control for confounding, a propensity score analysis was applied by three different methods: adjustment for the propensity score variable in the Cox proportional hazards model, stratification on propensity score groups, and propensity score pair matching.

Early life animal exposure was not associated with diabetes autoimmunity or T1D onset in this genetically high T1D risk population. These findings were consistent across all three propensity score analysis methods and when directly adjusting for HLA type. The hypothesis that children who are exposed to animals in early life will have a lower

risk of developing IA and T1D than children who are not exposed to animals in early life is not supported by this study.

The results of this study suggest that there is no association between early life animal exposure and development of T1D. Performing this analysis again after longer follow-up has been completed for the study population is recommended as it may elucidate the effect of animal exposure on T1D and IA risk. Further studies are also needed on animal exposure and T1D in different types of environments (e.g., high residential density) and the effect of different types of animal exposures (e.g., species, duration) on T1D and IA risk. Additionally, studies on differences in perceptions of pets across countries could also aid the interpretation of studies on animal exposure and health outcomes.

Introduction

Background

Type 1 diabetes (T1D) is a chronic disease characterized by the autoimmune destruction of pancreatic beta-cells leading to absolute insulin deficiency (1). Insulin is a hormone produced by the pancreatic beta-cells to lower the glucose level in the body and is essential for survival. Uncontrolled glucose levels can result in complications such as diabetic ketoacidosis, retinopathy, neuropathy, and nephropathy (1). There is currently no standard practice available to prevent or cure T1D. The standard treatment for T1D is multiple daily injections of insulin through a pump or needle and patient self-monitoring of blood glucose with the challenging goal of obtaining and maintaining glycemic control. The onset of T1D is known to occur early in life with three-quarters of all individuals with T1D diagnosed under the age of 18 years (1).

The incidence of T1D has increased over time. A study assessing T1D incidence trends among Colorado youth enrolled in the Colorado IDDM Registry and SEARCH for Diabetes in Youth Study reported an annual increase in T1D incidence of 2.3% (95% CI: 1.6-3.1) from 1978 to 2004 (2). The EURODIAB study, an international collaborative effort consisting of 20 population-based registries across 17 countries, reported a 3.2% (95% CI: 2.7-3.7) annual increase in T1D incidence from 1989 to 1998 and a 3.9% (95% CI: 3.6-4.2) annual increase from 1989 to 2003 (3, 4). The DIAMOND project, an international collaborative effort across 57 countries, reported an annual increase of 2.4% (95% CI=1.3-3.4) from 1990 to 1994 and an annual increase of 3.4% (95% CI: 2.7-4.3) from 1995 to 1999 (5). T1D incidence is predicted to continue to increase, with the

incidence in children under 5 years of age predicted to double between 2005 and 2020 (4, 5).

Understanding why the incidence of T1D is increasing and what factors trigger the chain of reactions that cause the body to attack its own pancreatic beta-cells are among the many unanswered questions about T1D. Studies on the relationship between genetic disposition and T1D have reported associations between several genes and T1D risk, most notably human leukocyte antigens (HLA); however, less than 10% of genetically susceptible individuals actually develop T1D (6, 7, 8). Additionally, the pairwise concordance rate of monozygotic twins has been reported at 39% to 50% (9). These findings imply that genetics alone is not responsible for T1D risk and development. Reports of large T1D incidence variation across countries (5, 8), incidence increases too rapid to be explained by shifts in genetic susceptibility (5, 10), and reduced frequency of high risk HLA genotypes (11, 12) have led researchers to suspect that environmental exposures play a significant role in disease incidence. Thus, the question has been raised as to which environmental exposures play a role in triggering the autoimmune response leading to the destruction of beta-cells in the pancreas and development of T1D.

Interest in environmental exposures and autoimmune disease development is rooted in the hygiene hypothesis, also known as the old friends hypothesis. The hygiene hypothesis is the assumption that individuals who are not exposed to organisms early in life that help to develop appropriate immunoregulation are more susceptible to autoimmune diseases as the immunoregulation deficit is believed to potentially cause an overreaction to later environmental exposures and attack important cells, such as the beta-cells critical to insulin production (13). Environmental exposures assessed in studies of the hygiene hypothesis and autoimmune disease development include elements that could lead to earlier and more antigenic exposure in life such as the

number of individuals in the household, number of siblings, room sharing, social contact (e.g., daycare, class size), geographical location (e.g., rural, urban), and animal contact.

Researcher Graham A. W. Rook has identified several critical organisms that have an important contribution to immunoregulation and are relevant to chronic autoimmune disorders. Specifically, Rook has proposed *Helicobacter pylori, Salmonella,* and many species of helminths as protective against allergies, Hepatitis A virus and *Necator americanus* as protective against asthma, and coxsackievirus B and rotavirus as protective against T1D (13). These particular organisms were selected because they were each abundant during mammalian evolution, are virtually absent from the present environment, and have had therapeutic effects in animal models or human clinical trials on chronic inflammatory disorders (13).

The hygiene hypothesis has been repeatedly supported in studies of autoimmune diseases such as asthma and allergies (14, 15, 16). Strachan noted in his 10-year review of epidemiological literature on the hygiene hypothesis that the scientific community has consistently found a higher prevalence of hay fever, eczema, skin prick positivity, and allergen specific IgE among individuals raised in smaller, more affluent families (15). Another example of evidence for the hygiene hypothesis is the case-control study conducted by Boneberger et al. to test the validity of the hygiene hypothesis in regard to childhood asthma, which showed that daycare attendance (OR: 0.31, 95% CI: 0.10-0.94) and regular farm animal contact (OR: 0.38, 95% CI: 0.17-0.85) in the first year of life had a statistically significant protective effect on childhood asthma (16).

There is also evidence supporting a protective association between several of the environmental exposures related to the hygiene hypothesis and risk of T1D, including crowded living conditions, sharing a room, having older siblings, and social contact with other children (17, 18); however, minimal research has been conducted on

the relationship between animal contact and T1D risk specifically. The few studies that have assessed the relationship between animal contact and T1D risk have had conflicting results and notable design flaws. Marshall et al. conducted a matched case-control study in Lancashire and Cumbria, United Kingdom to identify environmental risk factors for T1D in children up to age 16 years (17). Marshall et al. reported a statistically significant protective association (OR: 0.552, 95% CI: 0.309-0.987) between regular contact with pets or animals and the risk of developing T1D (17). Radon et al. conducted a case-control study in children age 6 to 16 years living in rural areas of Germany to explore the relationship between exposure to farming environments and T1D (19). Radon et al. reported no statistically significant associations related to regular contact with farm animals, defined as contact at least once per week, in the first year of life (OR: 1.2, 95% CI: 0.5-2.7) nor during the second to sixth year of life (OR: 0.81, 95% CI: 0.41-1.6) (19).

The major limitation of the previously conducted studies on animal contact and T1D is that exposure data was collected after T1D diagnosis, resulting in possible recall bias. Recall bias could result in overestimation of the association away from the null due to a systemic difference in the way that cases and controls recall the exposures of interest. For example, individuals diagnosed with T1D may be more cognizant of all environmental exposures and more likely to recall animal exposures than individuals who have not been diagnosed with T1D. An additional limitation of these studies is a possible lack of statistical power to detect weak effects due to sample size, as specifically noted by Radon et al. (19). Another general challenge of case-control studies is difficulty in selecting appropriate controls, which can result in selection bias and distortion of the reported measure of association.

The Environmental Determinants of Diabetes in the Young (TEDDY) study is a multinational prospective cohort study with the primary goal of identifying environmental

exposures that contribute to increased risk of the development of beta-cell autoimmunity and T1D in genetically susceptible children from birth to 15 years of age (20). Among the vast amount of data collected as a part of the study is the report of pet and animal exposures by the primary caregivers of children followed in the study. The TEDDY study offers an ideal body of data for studying the relationship between animal contact and T1D risk as its study design allows for the collection of data on environmental exposures prior to T1D diagnosis, thereby limiting recall bias. Exploring the potential association between animal exposures and T1D without recall bias would greatly contribute to existing scientific knowledge on the hygiene hypothesis and T1D development.

Another advantage of the cohort study design as opposed to the case-control study design is that multiple outcomes can be evaluated in cohort studies. This will allow for evaluation of both T1D diagnosis and pancreatic islet autoantibodies (IA), which are highly predictive of T1D risk and can be assessed prior to T1D diagnosis (21). An additional advantage of the TEDDY dataset is that it has a diverse international study population, which will allow possible effect modifiers such as country of residence to be evaluated and described. Data on possible confounders were also collected throughout the study; therefore, possible confounders can be controlled for when investigating the association between early life animal exposures and the risk of IA and T1D.

Study Objectives

In response to the minimal existing body of literature on the role of animal exposures in T1D development, the study design limitations of the existing literature, and the new possibilities and advantages offered by the TEDDY dataset, this study aims to investigate the association between reported early life animal exposures and T1D development, considering both persistent, confirmed IA and T1D diagnosis, among the 8,677 individuals enrolled in the prospective international TEDDY cohort between

September 2004 and February 2010. Specifically, this study aims to answer the following questions:

1. Does the risk of developing IA differ between children who are exposed to animals in early life and children who are not exposed to animals in early life?
2. Does the risk of developing T1D differ between children who are exposed to animals in early life and children who are not exposed to animals in early life?

Study Hypotheses

It is hypothesized that children who are exposed to animals in early life will have a lower risk of developing IA than children who are not exposed to animals in early life. It is also hypothesized that children who are exposed to animals in early life will have a lower risk of developing T1D than children who are not exposed to animals in early life. These hypotheses are based on the assumption that animals increase a child's exposure to organisms that help to develop appropriate immunoregulation and that children not exposed to organisms that help to develop appropriate immunoregulation are more susceptible to experience an autoimmune overreaction triggering the development of islet autoimmunity and T1D, as suggested by the hygiene hypothesis.

Methods

Methods Overview

The Environmental Determinants of Diabetes in the Young (TEDDY) Study is an international cohort study consisting of 8,677 participants at genetically high risk for type 1 diabetes (T1D) who were enrolled as newborns between September 2004 and February 2010 and followed through the time of T1D diagnosis or age 15 years, depending on the time point encountered first for each participant. An abundance of data on environmental exposures that may contribute to increased risk of the development of IA and T1D are collected for each participant throughout study follow-up. Early life data and outcomes data on the TEDDY cohort will be used for the proposed study.

The proposed observational study aims to investigate the association between early life animal exposure and development of IA and T1D using an eligible cohort of participants enrolled in the TEDDY study. Early life is considered to be prior to 9 months of age for the purposes of this analysis. This particular time period was selected, because the 9-month study visit is the first study visit at which animal exposure data is collected from TEDDY participants. Data on potential confounding variables will also be limited to early life exposure, before age 9 months. Propensity scores were incorporated into the analysis methodology to compare outcome risk between participants with similar animal exposure propensity scores and differing actual animal exposure status in order to control for confounding in the evaluation of the association between early life animal exposure and risk of IA and T1D. The propensity score calculated for each participant represents the predicted probability of early life animal exposure for that participant

based on other individual characteristics. Details on the study population, variables of interest, data preparation, and analysis methodology follow.

TEDDY Study Overview

TEDDY participants are recruited by six U.S. and European clinical centers located in Washington, Colorado, Georgia/Florida, Finland, Sweden, and Germany. The TEDDY data coordinating center is located at the University of South Florida in Tampa, Florida. The TEDDY study population to date includes children up to age 8 years who were enrolled in the TEDDY study during the screening period from September 2004 to February 2010. Newborns younger than 4.5 months of age who have high-risk HLA alleles in the general population (GP) or who have a first degree relative (FDR) with T1D were eligible for enrollment in the TEDDY cohort. The HLA alleles listed in Table 1 were considered high-risk alleles for the purposes of study screening. Newborns with an illness or birth defect that would prevent long term follow-up or would involve use of a treatment that may alter the natural history of diabetes were not eligible for enrollment.

Table 1: Eligible HLA Haplotypes for TEDDY Study Enrollment

HLA Haplotypes	HLA Haplotype Category
DR4*030X/0302*DR3*0501/0201	DQ8/2
DR4*030X/0302*DR4*030X/0302	DQ8/8
DR4*030X/0302*DR4*030X/020X	DQ8/8
DR4*030X/0302*DR8*0401/0402	DQ8/4
DR4*030X/0302*DR1*0101/0501	FDR
DR4*030X/0302*DR13*0102/0604	FDR
DR4*030X/0302*DR4*030X/0304	DQ8/8
DR4*030X/0302*DR9*030X/0303	FDR
DR3*0501/0201*DR3*0501/0201	DQ2/2
DR3*0501/0201*DR9*030X/0303	FDR

A total of 424,788 newborns were screened for the TEDDY study. Study screening involved HLA testing for HLA class II genes DRB1, DQA1, and DQB1. Parent

or primary caretaker consent was obtained from each potential participant prior to screening. Among the 418,367 GP newborns screened, 20,152 (4.8%) were eligible for enrollment. Among the 6,421 FDR newborns screened, 1,437 (22.4%) were eligible for enrollment. A separate consent was obtained from the parents or primary caretakers of eligible newborns prior to study enrollment. A total of 8,677 participants were enrolled in TEDDY as of February 2010.

Newborns enrolled in the TEDDY study are followed until 15 years of age or T1D diagnosis, depending on which is encountered first. Follow-up study visits for exposure data collection occur quarterly (every three months) until age 4 years. The follow-up study visit schedule then continues biannually for participants who are negative for IA and quarterly for participants who are persistently positive for IA until age 15 years.

Scope of Proposed Analysis: Animal Exposures, T1D, and Autoimmunity

The purpose of this analysis is to explore the relationship between reported early life animal exposure reported at the 9-month study visit and the outcomes persistent, confirmed islet autoimmunity and T1D diagnosis among those eligible enrolled in TEDDY cohort (N=8,677) during the screening period. The exposure of interest is any exposure to animals prior to the 9-month study visit. Outcomes of interest include persistent, confirmed IA and T1D diagnosis. Data cleaning and statistical analyses were conducted using SAS version 9.2.

Inclusion Criteria and Exclusion Criteria for Analysis

Inclusion Criteria:

1. Participant was enrolled in the TEDDY study between September 2004 and February 2010.
2. Participant has a 9-month study visit record.

Exclusion Criteria:

1. HLA results are pending or HLA results indicate that the participant does not have a high risk allele.
2. Participant was diagnosed with T1D prior to the 9-month study visit.
3. Participant had persistent, confirmed IA prior to the 9-month study visit.

Of the 8,677 participants in the TEDDY cohort, 7,604 participants had a 9-month study visit record. Differences between participants who withdrew from the TEDDY study within one year of enrollment and those who remained in the TEDDY study have been previously described by Johnson et al. (22). Significant predictors of early withdrawal identified by Johnson et al. include country of residence, young maternal age, no father participation, and female gender. Exclusions were performed on the 7,604 participants who met the defined inclusion criteria in the order listed above. As outlined in Figure 1, 135 participants were excluded due to pending or ineligible HLA results and 37 participants were excluded due to having persistent, confirmed IA prior to the 9-month visit. The final study population for the analysis included 7,432 participants.

Animal Exposure Classification

History of animal exposures was collected from the primary caregiver at the 9-month follow-up visit using a questionnaire. Questions 15 and 16 on the questionnaire focused on animal exposures. Question 15 asked if there were any animals or pets in the TEDDY child's house with the option to select "Yes" or "No" in response. If the caretaker completing the questionnaire indicated that there were animals or pets in the TEDDY child's house, the caretaker was also prompted to indicate the type of pet in the TEDDY child's house and the number of pets in the TEDDY child's house. Question 16 asked if the TEDDY child lives on a farm with animals or if there were animals that lived

outside of the child's house with the option to select "Yes" or "No" in response. Additionally, the caretaker was prompted to indicate the type of animals that live outside of the TEDDY child's house. The full 9-month questionnaire which includes the described questions on animal exposures is provided in Appendix 2.

The animal exposure data collected on the 9-month questionnaire were used to create a variable indicating whether or not each participant had any exposure to animals, regardless of whether the animal lived inside or outside the house, prior to the 9-month visit. This variable was created as a binary variable with "1" indicating that the participant was exposed to at least one animal as of the 9-month visit and "0" indicating that the participant was not exposed to any animals as of the 9-month visit. A participant was coded as "1" for this variable if the caretaker answered "Yes" to question 15, answered "Yes" to question 16, or selected a type of indoor or outdoor animal. Otherwise, the participant was coded as "0".

Outcome Classification

The study analysis was performed for two separate outcomes: persistent, confirmed IA and T1D. Since the maximum age of the study population is only 8 years, it is expected that the number of participants diagnosed with T1D may not be large enough to make appropriate association conclusions. Islet autoimmunity is highly predictive of T1D and usually occurs prior to T1D diagnosis (21); therefore, it is expected that analyzing the relationship between animal exposures and persistent, confirmed IA will allow for more confident conclusions at this stage.

A power analysis was conducted using the program Power and Sample Size (PASS) 12 by the Lakatos method. For the outcome IA, it is estimated that early life animal exposure will need to be associated with IA at a hazard ratio (HR) of 1.4 or greater in order for the study to have 80% power (Table 2), which would allow the study

to have an 80% probability of rejecting the null hypothesis when it is false. For the outcome T1D, it is estimated that early life animal exposure will need to be associated with T1D at a HR of 1.65 in order for the study to have 80% power (Table 3).

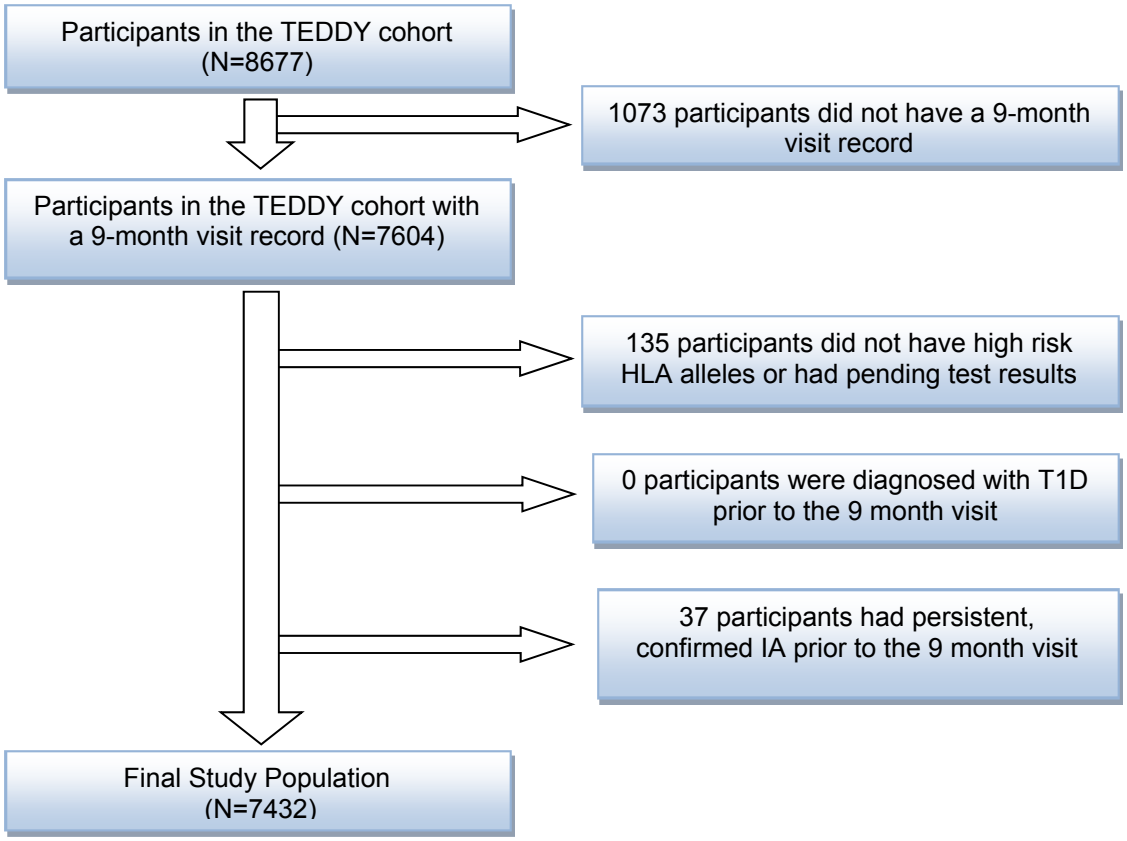

Figure 1: Eligibility Flowchart

Table 2: Power for IA Outcome, Alpha=0.05, N=8,677, 50% Exposed, 4 Years Accrual, Follow-up to Age 8 Years, 14% Loss, 5% Conversion

HR	Power
1.30	0.62
1.35	0.75
1.40	0.85
1.45	0.91
1.50	0.96

Table 3: Power for T1D Outcome, Alpha=0.05, N=8,677, 50% Exposed, 4 Years Accrual, Follow-up to Age 8 Years, 14% Loss, 2% Conversion

HR	Power
1.60	0.78
1.65	0.83
1.70	0.88
1.75	0.91
1.80	0.94

Persistent, Confirmed Islet Autoantibodies (IA) Outcome

The persistent, confirmed IA outcome is defined as the presence of IA at two consecutive visits confirmed by two separate laboratories. TEDDY participants are screened for glutamic acid decarboxylase autoantibodies (GAD65), insulinoma-associated antigen-2 autoantibodies (IA-2), and insulin autoantibodies (IAA) every 3 months, starting at age 3 months. Samples are initially sent to one of two TEDDY Central Autoantibody Laboratories for testing depending on geographical residence: Barbara Davis Center (Aurora, Colorado) or University of Bristol (Bristol, UK). If the test result is positive for GAD65, IA-2, or IAA, the sample is sent to the second laboratory for confirmation. Participants who receive positive results from both laboratories are considered to have confirmed IA. The participant will also be tested at his/her next study visit 3 months later. If both laboratories again indicate positive results, then the participant is considered to have persistent, confirmed IA. Children who had a confirmed antibody-positive result and were diagnosed with T1D prior to or at their next study visit were also deemed to have persistent, confirmed IA. Only participants identified as having persistent, confirmed IA are considered to have developed the autoimmunity outcome for the purposes of this analysis.

Two variables related to the persistent, confirmed IA outcome were created for this analysis. A binary variable was created to indicate whether the participant had developed persistent, confirmed IA throughout the course of study follow-up with “1”

indicating the participant had developed persistent, confirmed IA and “0” indicating that the participant had not developed persistent, confirmed IA. A continuous variable was also created to indicate the time until the participant developed persistent, confirmed IA or was censored. For participants with the event of interest (development of persistent, confirmed IA), this time variable represents the number of days from participant birth to the date of the blood draw associated with the persistent, confirmed IA determination (event date). For participants without the event of interest (no persistent, confirmed IA), this time variable represents the number of days from participant birth to the date of the participant’s last antibody-negative specimen sample date (censor date).

Type 1 Diabetes (T1D) Outcome

The TEDDY Study recognizes T1D diagnosis if at least one of the following American Diabetes Association (ADA) criteria is met on two occasions (unless criterion 4 is present, in which case a single occasion is sufficient for diagnosis):

1. Casual (any time of day without regard to time since last meal) plasma glucose ≥ 200 mg/dL, if accompanied by unequivocal symptoms (i.e. polyuria, polydipsia, polyphagia, and/or weight loss)
2. Fasting (no food or drinks except water for at least 8 hours) plasma glucose ≥ 126 mg/dL
3. 2-hour plasma glucose ≥ 200 mg/dL in oral glucose tolerance test
4. Unequivocal hyperglycemia with acute metabolic decompensation (diabetic ketoacidosis)

These criteria are defined on the TEDDY T1D diagnosis data collection form (Appendix 2). Once a T1D diagnosis is established for a participant using this criteria, data is collected on the diagnosis (i.e., date of diagnosis, symptoms, lab results) and further

study follow-up is discontinued. The TEDDY definition based on ADA criteria was also utilized for the purposes of this analysis.

Two variables related to T1D diagnosis outcome were created for this study. A binary variable was created to indicate whether the participant had been diagnosed with T1D with "1" indicating the participant had been diagnosed with T1D and "0" indicating that the participant had not been diagnosed with T1D. A continuous variable was also created to indicate the time until the participant was diagnosed with T1D or was censored. For participants with the event of interest (T1D diagnosis), this time variable represents the number of days from participant birth to the T1D diagnosis date (event date). For participants without the event of interest (no T1D diagnosis), this time variable represents the number of days from participant birth to the date of the participant's last visit date (censor date).

Potential Confounders

Potential confounders for the proposed analysis include: country of residence, age, sex, maternal education, HLA type, first degree relative with T1D status, smoking during pregnancy, drinking during pregnancy, illnesses/conditions during pregnancy, maternal age at birth, birth weight, exclusive breastfeeding, formula exposure, cow milk exposure, vaccination, social group exposure, and crowding (residence density).

Potential Confounder Definitions

Demographic variables including participant sex and maternal education level were collected during infant screening for the TEDDY study. Sex was classified as either male or female. Maternal education was collected at the 3-month study visit and classified as primary school through some trade school, graduated from trade school or some college/university education, or graduated from a college/university. Country

classification was based on where the clinical center which enrolled the participant was located (United States, Finland, Germany, or Sweden). Participant age was calculated as the number of days from the participant's date of birth to the participant's last study visit date.

T1D risk characteristics including HLA testing results and first degree relative (FDR) status were collected during TEDDY study screening. Each participant was assigned to a HLA category based on the high risk allele identified during screening as outlined in Table 1. A separate variable for FDR status was coded to indicate whether or not the participant had a mother, father, and/or sibling who had been diagnosed with T1D, with "1" assigned to participants who have a FDR with T1D and "0" assigned to participants who do not have a FDR with T1D.

Prenatal exposures were collected from the TEDDY child's mother at the 3-month study visit. Whether or not the mother smoked during each individual pregnancy trimester was reported during the visit. This information was then recoded into one variable that indicated whether or not the mother had smoked at all during pregnancy with "1" assigned to participants whose mother had smoked at least once during pregnancy and "0" assigned to participants whose mother had not smoked at all during pregnancy. Drinking during pregnancy was coded into one variable for the analysis structured in the same manner as described for the smoking during pregnancy variable. Illnesses and conditions during pregnancy were reported by the mother at the 3-month visit as well. A binary variable was created from these data to indicate whether the participant's mother reported at least one illness or condition during pregnancy, assigned as "1", or whether the mother did not report any illnesses or conditions during pregnancy, assigned as "0". Maternal age at birth was calculated from the mother's date of birth and participant's date of birth and utilized as a continuous variable. Birth weight

was collected during the 3-month study visit in pounds and ounces. The measurement was converted to grams and utilized as a continuous variable.

Breastfeeding, formula, and cow milk exposures were collected every 3 months beginning at the 3-month study visit. Exclusive breastfeeding data on exposure and duration were recoded into one variable to summarize the length of time each participant was exposed to exclusive breastfeeding with the following categories: no exclusive breastfeeding (includes participants who breastfed once in the hospital only), <3 months of exclusive breastfeeding, and ≥3 months of exclusive breastfeeding. Exclusive breastfeeding duration is defined as the period of time for which breast milk was the only source of nutrition for the TEDDY child with no other food or formula introduced during this period. Data indicating whether or not the participant had been introduced to any infant formula and the age at introduction was combined into one summary variable with the following categories: not introduced in the first 9 months of life, introduced at age <3 months, introduced between age 3 months to <7.5 months, and introduced at age 7.5 months to <9 months. Data on cow milk exposure, consumed as a drink or mixed into another food product, was summarized in the same manner as described for formula.

Vaccination history was also collected every 3 months beginning at the 3-month visit. This data was compiled into one binary variable indicating whether or not the participant had ever received any type of vaccination. Participants for whom at least one vaccination was received in the first 9 months of life were coded as “1” and participants for whom no vaccinations were received in the first 9 months of life were coded as “0”.

At the 9-month study visit, the primary caretaker was asked to report the number of children under the age of 18 years living in the TEDDY child’s household, the number of adults age 18 years and older living in the TEDDY child’s household, and the number of rooms in the TEDDY child’s home not including bathrooms, porches, halls, or balconies. A household crowding score representing residence density was calculated

for each participant from this information by dividing the total number of people in the household, adults plus children, by the number of rooms in the house. Additionally, at the same visit the primary caretaker was asked to identify which of the following residence types best describes where the TEDDY child lives: rural area, small city/village, suburb, or big city.

Social group exposure data was collected from the TEDDY child's primary caretaker every 3 months beginning at the 3-month study visit. Social group exposure was defined as regular (once a week or more) day care or other social gathering that included at least one other child, who is not a sibling, in addition to the TEDDY child. A binary variable was created to indicate whether the TEDDY child had been exposed to a social group in the first 9 months of life. Children for whom an early life (in the first 9 months) social group experience had been reported were coded as "1" and children for whom an early life social group experience had not been reported were coded as "0".

Data Preparation for Statistical Analysis

The dataset was reviewed for data entry errors to prepare the dataset for analysis. Crowding space (residence density) values were coded as missing if any of the variables included in the calculation (number of children, number of adults, or number of rooms in home) were reported as an inappropriate value (e.g., -3 rooms, -2 adults). Crowding space was coded as missing for a total of nine participants due to inappropriate values. No other data entry errors were identified.

Skewness was calculated for each variable. The skewness measure indicates the degree and direction of asymmetry of the data distribution for a given variable. Crowding space was the only continuous variable with a skewness value greater than three standard deviations. To normalize the skewed distribution, participants were categorized as having a crowding score of less than or equal to 1 or greater than 1.

Missingness was assessed for each variable by reviewing the frequencies of values for each variable. All variables were missing less than 3% of values. Missing trends were further assessed by tabulating the number of missing variables of interest for each participant in order to determine if a few participants were missing the majority of the variables and to consider whether participants should be removed from the analysis due to a high number of missing variables. A total of 174 participants were missing one variable only, 65 participants were missing two variables, and two participants were missing three variables. Since participants were only missing three variables at most and very few participants were missing more than two variables, no participants were removed due to frequency of missing variables.

Statistical Analysis

Analysis Overview

The association between early life animal exposure and persistent, confirmed IA and the association between early life animal exposure and T1D were explored using Cox proportional hazards (PH) models. A propensity score analysis was applied to control for confounding when assessing the relationship between the exposure and outcomes of interest. Propensity scores, calculated by logistic regression, represent the probability of a participant being exposed to animals based on the other data collected on that participant. The propensity score analysis was applied in three different ways: by adjustment for the propensity score variable in the Cox proportional hazards model, by stratification on propensity score groups, and by propensity score pair matching. Details on the methods addressed in this brief overview follow.

Univariate Analysis

Frequencies were tabulated for each variable in order to assess missingness and distribution by exposure status. Chi-square tests were performed on each categorical variable to determine whether the proportions for each variable were equal for the exposed and unexposed study groups. Chi-square test p-values of less than 0.05 indicate a statistically significant difference in proportions by exposure status. T-tests were utilized to determine if the means of continuous variables were equal across the exposed and unexposed groups. T-test p-values of less than 0.05 indicate a statistically significant difference in the means of the tested variable by exposure status. Degree of correlation between variables and early life animal exposure status was also explored for the purpose of assessing multicollinearity.

The association between early life animal exposure and each outcome of interest was first examined by Kaplan-Meier survival curves to determine if there was any difference in outcome risk by exposure status. The log rank test was performed to provide a quantitative value indicating whether there was a statistically significant difference in risk by exposure status. Log rank test p-values of less than 0.05 indicate a statistically significant difference between assessed survival curves.

Logistic Regression for the Calculation of Propensity Scores

Propensity scores allow for the investigation of the association between early life animal exposure and risk of IA and T1D by simulating a randomized controlled trial from the dataset. The propensity score is the probability of a participant receiving “treatment” based on that participant’s characteristics. In this case, the treatment of interest is early life animal exposure.

An early life animal exposure propensity score was calculated for each study participant by stepwise logistic regression. The propensity score calculated for each

participant represents the predicted probability of early life animal exposure for that participant based on other characteristics of the participant. Early life animal exposure is considered the outcome and other covariates (e.g., sex, country of residence) are considered predictors when calculating the propensity scores. Logistic regression is appropriate for the analysis due to the binary structure of the outcome variable, early life animal exposure (1=animal exposure in first 9 months of life, 0=no animal exposure in first 9 months of life). Stepwise logistic regression was utilized so that all potential variables that may affect the probability of a participant having early life animal exposure can be considered and variables that do not affect the probability of a participant having early life animal exposure could be excluded with the overall goal being to determine the most effective set of variables for predicting the outcome.

Environmental and demographic characteristics that may affect the probability of one having early life exposure to animals were of interest for the logistic regression model. All covariates were included in the initial stepwise logistic regression model, with the exception of HLA and participant age at last visit. HLA is an indicator of genetic risk for T1D and was included in the survival analysis. Age at last visit is represented by the time to event (outcome or censoring) in the survival analysis. The regression model was set to require a significance level of 0.1 for entry into the model and a significance level of 0.1 to stay in the model. Propensity scores predicting early life animal exposure were calculated utilizing the final logistic regression model.

The propensity score calculated for each participant was added to the dataset as a single continuous variable. Additionally, each study participant was assigned a quintile based on propensity score, ranging from quintile 1 with the lowest propensity scores (least likely to have early life animal exposure) to quintile 5 with the highest propensity scores (most likely to have early life animal exposure). Interactions between early life animal exposure and predictors in the final logistic regression model were assessed by

calculating the measure of association for early life animal exposure and each outcome of interest by predictor variable category. For example, for the early life vaccination variable the measure of association between early life animal exposure and IA was calculated among participants who received early life vaccinations and then separately among participants who did not receive early life vaccinations for comparison.

Cox Proportional Hazards Analysis

The risk of persistent, confirmed IA and T1D by early life animal exposure status was examined by Cox proportional hazards modeling. Cox proportional hazards modeling is a type of survival analysis. In a conventional sense, survival would be interpreted as the probability of death and time until death. In health outcomes research, survival can be applied to the probability of a health outcome (e.g., T1D diagnosis) and the time until the health outcome (e.g., the time from birth to diagnosis of T1D). In other words, we can use survival analysis techniques to calculate the instantaneous risk for an outcome. Advantages of using survival analysis methods include the ability to account for the differing periods of time each participant contributes to the study and the ability to analyze the effect of predictors on time to outcome in addition to whether or not the outcome occurred. The Cox proportional hazards model is considered a semiparametric method, because the distribution of the underlying hazard does not need to be known or assumed.

The primary assumptions of the Cox proportional hazards model are that the hazards are proportional and independent of time and that censoring is independent of event occurrence. The proportionality assumption was tested by randomly simulating 1000 empirical score processes, based on martingale residuals, that meet the proportional hazards assumption for the variable early life animal exposure in regard to each outcome of interest and then calculating a p-value representing the percent of

simulated paths which had extreme points exceeding the most extreme point on the observed path for the variable of interest. A resulting p-value less than 0.05 would indicate evidence against the proportional hazards assumption.

The binary early life animal exposure variable indicating actual animal exposure status was included in each Cox proportional hazards model as the independent variable. The risk of T1D and persistent, confirmed IA for the exposed group compared to the unexposed group (which serves as the reference group) is reported as a hazard ratio (HR) and corresponding 95% confidence interval (CI) for each model. HRs equal to 1 indicate that there is no difference in risk between the exposed and unexposed groups. The 95% CIs that include 1 indicate that the calculated HR is not statistically significant.

An unadjusted Cox proportional hazards model was run on the entire study population for each outcome, including only early life animal exposure as the independent variable and the outcome (T1D or IA) as the dependent variable, to determine the risk of the outcomes by exposure status prior to adjusting for any confounders. The risks of T1D and IA by early life animal exposure were then further assessed by incorporating the propensity scores into the Cox proportional hazards models in three ways, as described in detail in the three following sections.

Propensity Score Application 1: Adjusting for Propensity Score within the Cox Proportional Hazards Model

In order to measure the risk of persistent, confirmed IA by early life animal exposure status while controlling for confounding, a Cox proportional hazards model was created that included the binary early life animal exposure variable and the continuous propensity score variable as independent variables and the binary IA outcome variable as the dependent variable. The model was run on the entire study dataset and the resulting HR and 95% CI were reported. The same procedure was repeated for the T1D

outcome. A second adjusted model that included early life animal exposure, propensity score, and HLA was also created for each outcome and then run on the entire study dataset in order to control for HLA as a potential confounder in addition to propensity score. DQ8/8 was defined as the reference group for HLA. The HR indicating the risk of the exposed group compared to the unexposed group (the reference group) was reported along with the 95% CI for each model.

Propensity Score Application 2: Stratification on Propensity Score Quintiles

The effect of early life animal exposure on the risk of each endpoint (T1D and persistent, confirmed IA) was examined within propensity score quintiles in order to further explore the relationship between early life animal exposure and the endpoints of interest. This method provided the opportunity to examine the effect of early life animal exposure on T1D and IA risk in groups of participants with similar propensity for early life animal exposure. This allows one to control for differences within the study population by studying the association in homogenous groups of people (participants with similar propensity scores and therefore similarities in the characteristics included in the final logistic regression model used to calculate the propensity score).

Two Cox proportional hazards models were run in each propensity score quintile to determine the risk of the outcome (persistent, confirmed IA or T1D diagnosis) among those actually exposed to animals within specified quintile compared to those not actually exposed to animals within specified quintile:

1. An unadjusted Cox proportional hazards model for each outcome that included only early life animal exposure in the model.
2. An adjusted Cox proportional hazards model for each outcome that included both early life animal exposure and HLA in the model. DQ8/8 was defined as the reference group for HLA.

Two sets of these models were created, one for the outcome T1D and one for the outcome persistent, confirmed IA. Therefore, a total of four different Cox proportional hazards models were run in each quintile. For each endpoint, Kaplan-Meier survival curves were plotted by propensity quintile and homogeneity of risk across propensity quintiles was assessed by log rank test.

Propensity Score Application 3: Propensity Score Pair Matching

Exposed and unexposed participants were pair-matched by propensity score in order to ensure that compared participants were as similar as possible in an effort to thoroughly tease out any potential association between early life animal exposure and the endpoints IA and T1D. Participants were matched using the greedy match macro available from Mayo Clinic (23) as described by Faries et al. (24). A difference in propensity score of up to 0.1 was permitted. Two Cox proportional hazards models were run, stratified by matched pair, to determine the risk of the outcome (persistent, confirmed IA or T1D diagnosis) among those actually exposed to animals in early life compared to those not actually exposed to animals in early life:

1. An unadjusted Cox proportional hazards model for each outcome that included only early life animal exposure in the model, stratified by matched pair.
2. An adjusted Cox proportional hazards model for each outcome that included both early life animal exposure and HLA in the model, stratified by matched pair. DQ8/8 was defined as the reference group for HLA.

Two sets of these models were created, one for the outcome T1D and one for the outcome persistent, confirmed IA. Therefore, a total of four different Cox proportional hazards models were run on the matched dataset.

Results

Descriptive Statistics

A total of 7,432 TEDDY participants met the study eligibility criteria and were included in the final study population. Of this study population, 3,987 (54%) participants had been exposed to animals prior to 9 months of age and 3,445 (46%) participants had not been exposed to animals prior to 9 months of age. The study population overall, as well as broken down by exposure status, is described in Table 4.

The proportions of sex, FDR, pregnancy conditions, maternal age, early life formula introduction, and early life vaccination categories were consistent between the exposed and unexposed study populations ($p \geq 0.05$); however, there were significant differences across exposure groups for other variables. Compared to the unexposed population, the exposed population included fewer mothers who had graduated college ($p < 0.0001$), more mothers who had smoked during pregnancy ($p = 0.0005$), more mothers who drank alcohol during pregnancy ($p = 0.003$), more participants without an exclusive breastfeeding experience ($p = 0.0002$), and more participants who were introduced to cow milk prior to 3 months of age ($p = 0.0003$). Additionally, participants in the exposed group had a lower mean birth weight ($p = 0.008$) and were slightly younger as of the last study visit ($p = 0.04$) than participants in the unexposed group.

Characteristics of the environment in which the participants lived, both in terms of crowding (residence density) and residence type, differed significantly between the exposed and unexposed groups ($p = 0.0003$ and $p < 0.0001$, respectively). The exposed group had a much higher percentage of participants who lived in a rural environment

(20%) than the unexposed group (8%). The exposed group also had fewer participants in a crowded environment (9%) than the unexposed group (11%).

Additionally, country distribution was vastly different between the exposed and unexposed populations ($p<0.0001$). The most striking difference is the distribution of U.S. participants. The exposed group included 2,091 (52%) U.S. participants; whereas, the unexposed group included only 966 (28%) U.S. participants. Furthermore, the exposed group included fewer participants from Finland (15%), Germany (6%), and Sweden (27%) than the unexposed group which included 30%, 7%, and 35%, respectively. Distribution of HLA allele type also varied greatly between the exposed and unexposed populations ($p<0.0001$). Notably, there were more DQ8/4 allele types in the unexposed population (19%) than in the exposed population (15%), which is likely linked to higher prevalence of Finnish participants in the unexposed population, since the HLA DQ8/4 genotype is more prevalent in this TEDDY country-specific population (25).

A total of 417 (6%) participants in the study population developed persistent, confirmed IA. Of these participants, 221 (53%) were exposed to animals early in life (prior to 9 months of age) and 196 (47%) were not exposed to animals early in life (Table 5). The Kaplan-Meier survival curves detailing cumulative incidence of IA by early life animal exposure are shown in Figure 2. There is not a statistically significant difference between the survival curves for IA by early life animal exposure ($p=0.92$).

A total of 113 (2%) participants in the study population were diagnosed with T1D. Of these participants, 59 (52%) were exposed to animals early in life and 54 (48%) were not exposed to animals early in life (Table 6). The Kaplan-Meier survival curves detailing cumulative incidence of T1D by early life animal exposure are shown in Figure 3. There is not a statistically significant difference between the survival curves for T1D by early life animal exposure ($p=0.89$).

Table 4: Descriptive Statistics of the Study Population for the Analysis of Early Life Animal Exposures and Risk of IA and T1D

Variable	All N=7432 N (%)	Exposed to Animals in Early Life N=3987 N (%)	Not Exposed to Animals in Early Life N=3445 N (%)	P-value
Sex				
Female	3646 (49.06)	1970 (49.41)	1676 (48.65)	0.5132
Male	3786 (50.94)	2017 (50.59)	1769 (51.35)	
Age at Last Visit, years				
(mean, SD)	4.23 (1.83)	4.19 (1.81)	4.28 (1.86)	0.0422
HLA Allele				
DQ2/2	1541 (20.73)	844 (21.17)	697 (20.23)	<0.0001
DQ8/2	2894 (38.94)	1631 (40.91)	1263 (36.66)	
DQ8/8	1476 (19.86)	766 (19.21)	710 (20.61)	
DQ8/4	1283 (17.26)	613 (15.37)	670 (19.45)	
Other	238 (3.2)	133 (3.34)	105 (3.05)	
First Degree Relative with T1D				
Yes	816 (10.98)	449 (11.26)	367 (10.65)	0.4028
No	6616 (89.02)	3538 (88.74)	3078 (89.35)	
Country of Residence				
United States	3057 (41.13)	2091 (52.45)	966 (28.04)	<0.0001
Finland	1617 (21.76)	584 (14.65)	1033 (29.99)	
Germany	497 (6.69)	252 (6.32)	245 (7.11)	
Sweden	2261 (30.42)	1060 (26.59)	1201 (34.86)	
Residence Type				
Rural Area	1064 (14.35)	785 (19.74)	279 (8.11)	<0.0001
Small City/Village	2378 (32.06)	1127 (28.34)	1251 (36.37)	
Suburb	2859 (38.55)	1566 (39.38)	1293 (37.59)	
Big City	1116 (15.05)	499 (12.55)	617 (17.94)	
Missing	15			
Crowding Space (Residence Density)				
>1 (Crowded)	727 (9.85)	344 (8.68)	383 (11.20)	0.0003
≤ 1 (Not Crowded)	6654 (90.15)	3617 (91.32)	3037 (88.80)	
Missing	51			
Mother's Education Level				
Basic Primary Education	1299 (17.74)	737 (18.77)	562 (16.55)	<0.0001
Graduated Trade School	1853 (25.31)	1070 (27.25)	783 (23.06)	
Graduated College	4170 (56.95)	2120 (53.99)	2050 (60.38)	
Missing	110			
Mother Smoked During Pregnancy				
Yes	895 (12.15)	529 (13.38)	366 (10.73)	0.0005
No	6469 (87.85)	3424 (86.62)	3045 (89.27)	
Missing	68			
Mother Drank Alcohol During Pregnancy				
Yes	2546 (34.56)	1426 (36.08)	1120 (32.81)	0.0032
No	4820 (65.44)	2526 (63.92)	2294 (67.19)	
Missing	66			

Mother Experienced a Condition or Illness During Pregnancy				
Yes	6767 (91.05)	3634 (91.15)	3133 (90.94)	0.7600
No	665 (8.95)	353 (8.85)	312 (9.06)	
Mother's Age at Birth of Participant, years				
mean (SD)	31.13 (5.16)	31.09 (5.28)	31.18 (5.03)	0.4768
Participant Birth Weight, grams				
mean (SD)	3503.6 (543.80)	3487.7 (543.4)	3521.8 (543.7)	0.0077
Missing	189			
Exclusive Breastfeeding Duration				
No exclusive breastfeeding	2764 (37.19)	1551 (38.90)	1213 (35.21)	0.0002
<3 months	2838 (38.19)	1523 (38.20)	1315 (38.17)	
≥ 3 months	1830 (24.62)	913 (22.90)	917 (26.62)	
Early Life Formula Introduction Age				
Not introduced	1094 (15.11)	554 (14.36)	540 (15.97)	0.0574
Introduced at <3 months	5196 (71.78)	2821 (73.12)	2375 (70.25)	
Introduced at 3-<7.5 months	827 (11.42)	419 (10.86)	408 (12.07)	
Introduced at 7.5-<9 months	122 (1.69)	64 (1.66)	58 (1.72)	
Missing	193			
Early Life Cow Milk Introduction Age				
Not introduced	25 (0.36)	13 (0.35)	12 (0.37)	0.0003
Introduced <3 months	5058 (73.01)	2769 (75.14)	2289 (70.58)	
Introduced 3-<7.5 months	1618 (23.35)	786 (21.33)	832 (25.66)	
Introduced 7.5-<9 months	227 (3.28)	117 (3.18)	110 (3.39)	
Missing	504			
Early Life Vaccination				
Yes	7077 (95.22)	3783 (94.88)	3294 (95.62)	0.1393
No	355 (4.78)	204 (5.12)	151 (4.38)	
Early Life Social Group				
Yes	4800 (64.59)	2624 (65.81)	2176 (63.16)	0.0172
No	2632 (35.41)	1363 (34.19)	1269 (36.84)	

Table 5: Cross-tabulation of Early Life Animal Exposure and Persistent, Confirmed IA

	IA N (%)	No IA N (%)	Total N (%)
Early Life Animal Exposure	221 (52.99)	3766 (53.68)	3987 (53.65)
No Early Life Animal Exposure	196 (47.00)	3249 (46.32)	3445 (46.35)
Total	417 (5.61)	7015 (94.39)	7432 (100)

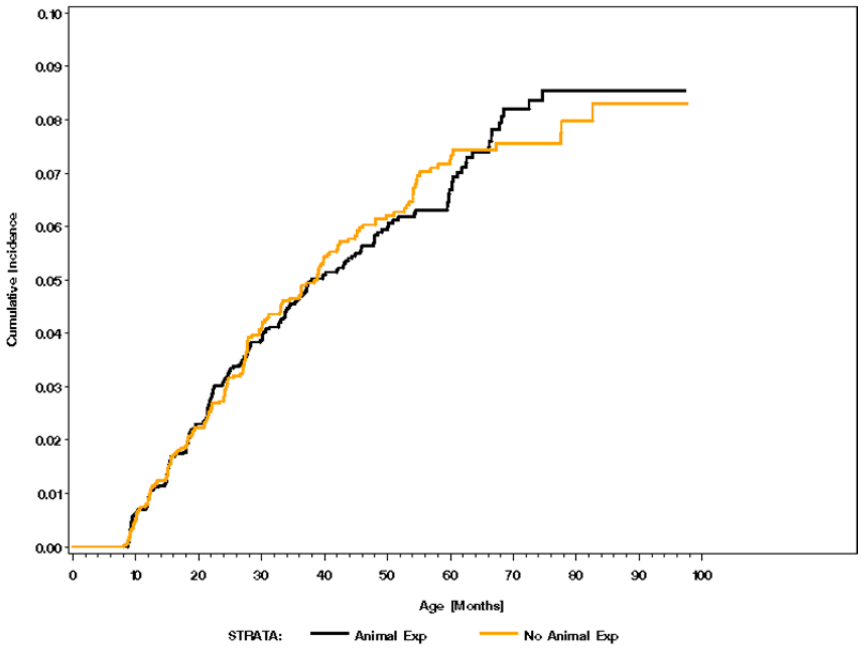

Figure 2: Kaplan-Meier Survival Curve, Cumulative Incidence of Persistent, Confirmed IA by Early Life Animal Exposure, Log Rank P=0.92

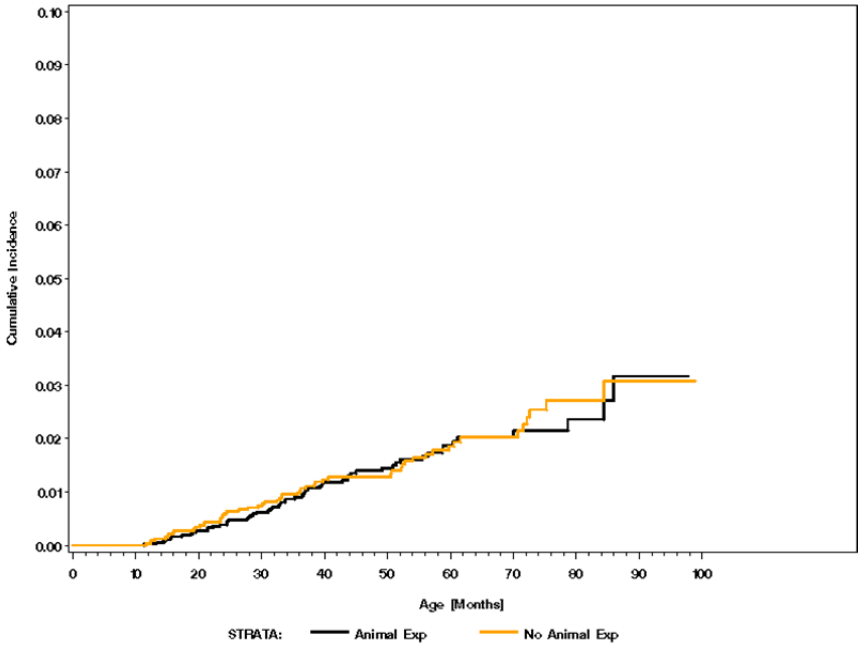

Figure 3: Kaplan-Meier Survival Curve, Cumulative Incidence of T1D by Early Life Animal Exposure, Log Rank P=0.89

Table 6: Cross-tabulation of Early Life Exposure to Animals and T1D

	T1D N (%)	No T1D N (%)	Total N (%)
Early Life Animal Exposure	59 (52.21)	3928 (53.67)	3987 (53.65)
No Early Life Animal Exposure	54 (47.79)	3391 (46.33)	3445 (46.35)
Total	113 (1.52)	7319 (98.48)	7432 (100)

Logistic Regression

All variables of interest (listed in Table 4) that were thought to potentially impact the probability that one would be exposed to an animal/pet were included in the initial logistic regression model and then assessed by stepwise logistic regression. This resulted in all variables of interest being included in the initial model with the exception of HLA allele type and participant age at last visit, which would be accounted for in the survival analysis instead. Stepwise logistic regression, based on a significance level of 0.1 for entry and to stay in the model, resulted in a final model including eight variables: country of residence, residence type, smoking by the mother during pregnancy, crowding space (residence density), early life vaccination, maternal education, early life social group exposure, and drinking alcohol by the mother during pregnancy.

The final logistic regression model was used to create the propensity score for each participant, representing the probability of early life animal exposure based on the eight variables included in the final model (country of residence, residence type, smoking by the mother during pregnancy, crowding space, early life vaccination, maternal education, early life social group exposure, and drinking alcohol by the mother during pregnancy) as predictors. Only those participants with complete data (N=6,532) for the variables assessed in the stepwise logistic regression were included in the logistic regression analysis. The odds ratios (OR) and corresponding 95% confidence intervals

(CI) as well as the p-values associated with each of the variables included in the final model are listed in Table 7.

Table 7: Final Logistic Regression Model for Early Life Animal Exposure Propensity Score Calculation, Stepwise Logistic Regression, Significance Level of 0.1 for Model Entry and to Stay in Model, N=6,532

Variable	OR (95% CI)	P-value
Country of Residence (Ref = United States)		
Finland	0.20 (0.17-0.23)	<0.0001
Germany	0.39 (0.31-0.48)	
Sweden	0.31 (0.27-0.36)	
Residence Type (Ref = Big City)		
Rural	5.87 (4.75-7.25)	<0.0001
Small city/village	1.81 (1.52-2.14)	
Suburb	1.50 (1.28-1.76)	
Smoking During Pregnancy (Ref= None)	1.35 (1.14-1.59)	<0.0001
Crowding Space (Ref = Crowding Score ≤1)	0.74 (0.61-0.89)	0.0010
Early Life Vaccination (Ref = None)	1.50 (1.15-1.94)	0.0036
Maternal Education (Ref = Primary Education)		
Graduated Trade School/Some College	0.96 (0.81-1.13)	0.0027
Graduated College	0.78 (0.67-0.91)	
Early Life Social Group Exposure (Ref = None)	1.17 (1.04-1.31)	0.0045
Drinking During Pregnancy (Ref = None)	1.11 (0.99-1.25)	0.0601

These results indicate that the odds of being exposed to animals early in life among participants in Finland, Germany, or Sweden are lower than the odds of being exposed to animals early in life among participants in the U.S. The odds of being exposed to animals early in life among those living in a rural environment are 5.9 times (OR: 5.87, 95% CI: 4.75-7.25) the odds of being exposed to animals early in life among those living in a big city. Also in regard to environment, those who live in a crowded environment have lower odds (OR: 0.74, 95% CI: 0.61-0.89) of early life animal exposure than those who do not live in a crowded environment. Those participants whose mothers smoked during pregnancy have higher odds (OR: 1.35, 95% CI: 1.14-1.59) of early life animal exposure than those whose mothers did not and those whose mothers drank alcohol during pregnancy have higher odds (OR: 1.11, 95% CI: 0.99-1.25) of early life animal exposure than those whose mothers did not. Additionally, those

who received early life vaccinations have higher odds (OR: 1.50, 95% CI: 1.15-1.94) of early life animal exposure than those who did not and those who had early life social group experiences have higher odds (OR: 1.17, 95% CI: 1.04-1.31) of early life animal exposure than those who did not.

Propensity Score Application 1

The association between early life animal exposure and each outcome was first explored by examining the study population (N=7,432) as a whole. Propensity scores could not be calculated for 241 participants who were missing at least one of the eight variables included in the final logistic regression model used to calculate the propensity scores. The Cox proportional hazards models utilized to assess the association between early life animal exposure and each outcome were run on the 7,191 participants for whom propensity scores could be calculated.

Outcome: Persistent, Confirmed IA

Before controlling for any other variables, the hazard ratio (HR) for the risk of persistent, confirmed IA among those with early life animal exposure compared to those without early life animal exposure was 1.02 with a 95% CI of 0.84-1.24. Since the 95% CI for this basic unadjusted association includes 1, the finding is not statistically significant. Therefore, no difference in risk of persistent, confirmed IA was found between those participants with early life animal exposure and those without early life animal exposure.

The propensity score variable was added to the original unadjusted model in order to control for the variables that were predictive of early life animal exposure and determine if the HR was altered by the adjusted model (Table 8, Adjusted Model 1). When adjusted for early life animal exposure propensity, the resulting HR was 1.11

(0.90-1.37). This measure of association indicates that there is no significant difference in IA risk between those with early life animal exposure and those without early life animal exposure.

Adjusting for HLA type in addition to propensity score (Table 8, Adjusted Model 2) had little additional impact on the HR (HR 1.09, 95% CI: 0.89-1.35). This finding was not statistically significant and indicates no significant difference in risk between the exposed and unexposed. Ultimately, no statistically significant associations were identified between early life animal exposure and persistent, confirmed IA by the models run on the entire study population. The findings of these models are summarized in Table 8.

Table 8: HR & 95% CI for Early Life Animal Exposure and Persistent, Confirmed IA by Cox PH Models Fit to the Entire Study Population

	Risk of IA for Participants with Early Life Animal Exposure[1]		
Variable	Unadjusted Model N=7191 HR (95% CI)	Adjusted Model 1 N=7191 HR (95% CI)	Adjusted Model 2 N=7191 HR (95% CI)
Early Life Animal Exposure	1.02 (0.84-1.24)	1.11 (0.90-1.37)	1.09 (0.89-1.35)

[1] The unadjusted model includes early life animal exposure only (reference group unexposed). Adjusted model 1 is adjusted for early life animal exposure propensity score. Adjusted model 2 is adjusted for propensity score and HLA (reference group DQ8/8).

Outcome: T1D

Before controlling for any other variables, the HR for the risk of T1D among those with early life animal exposure compared to those without early life animal exposure was 0.98 with a 95% CI of 0.67-1.42. Since the 95% CI for this basic unadjusted association includes 1, the finding is not statistically significant and indicates no difference in risk between the exposed and unexposed groups. As was done for analysis of IA risk, propensity score was added to the analysis for T1D risk in order to control for the

variables that were predictive of early life animal exposure and determine if the HR was altered by the adjusted model. When adjusted for early life animal exposure propensity (Table 9, Adjusted Model 1), the resulting HR for T1D risk was 1.04 (0.69-1.54). This measure of association indicates that there is no significant difference in T1D risk between those with early life animal exposure and those without early life animal exposure.

Adjusting for HLA type in addition to propensity score (Table 9, Adjusted Model 2) also resulted in no significant difference in the risk of T1D between the exposed and unexposed groups (HR: 1.01, 95% CI: 0.67-1.50). In summary, none of the measured associations between early life animal exposure and T1D, examined by running models on the entire study population, indicated a statistically significant difference in risk between the exposed and unexposed. The findings of these models are summarized in Table 9.

Table 9: HR & 95% CI for Early Life Animal Exposure and T1D by Cox PH Models Fit to the Entire Study Population

	Risk of T1D for Participants with Early Life Animal Exposure[1]		
Variable	Unadjusted Model N=7191 HR (95% CI)	Adjusted Model 1 N=7191 HR (95% CI)	Adjusted Model 2 N=7191 HR (95% CI)
Early Life Animal Exposure	0.98 (0.67-1.42)	1.04 (0.69-1.54)	1.01 (0.67-1.50)

[1] The unadjusted model includes early life animal exposure only (reference group unexposed). Adjusted model 1 is adjusted for early life animal exposure propensity score. Adjusted model 2 is adjusted for propensity score and HLA (reference group DQ8/8).

Propensity Score Application 2

Quintiles Overview

The total number of participants in each propensity score quintile ranges from 1396 to 1481 with each quintile representing 19% to 20% of the total study population.

The propensity score range of each quintile, as well as the distribution of exposure and outcomes across quintiles, is detailed in Table 10. Quintile 1 includes those participants with the lowest propensity scores indicating that they have a low probability of early life animal exposure. Conversely, quintile 5 includes those participants with the highest propensity scores indicating that these participants have a high probability of early life animal exposure. As expected, quintile 1 (the quintile with the lowest propensity scores) had the lowest percent of exposed participants. The percent exposed then increased with each quintile through quintile 5 (the quintile with the highest propensity scores) which had the highest percent of exposed participants. This indicates that the propensity scores were appropriately predicting early life animal exposure in the study population. Propensity scores could not be calculated for 241 participants who were missing at least one of the eight variables included in the final logistic regression model used to calculate the propensity scores. As shown in Table 10, the group of participants for whom propensity score could not be calculated had a high proportion of early life animal exposure (54%) and a low proportion of IA (4%) and T1D (<1%) which may attenuate the HRs calculated for these outcomes. The characteristics of study participants by propensity score quintile are further detailed in Table 11.

The Kaplan-Meier survival curves for persistent, confirmed IA by early life animal exposure propensity quintile are shown in Figure 4 and the Kaplan-Meier curves for T1D by early life animal exposure propensity quintile are shown in Figure 5. There is not a statistically significant difference between the survival curves for IA by early life animal exposure propensity quintile ($p=0.57$) or between the survival curves for T1D by early life animal exposure propensity quintile ($p=0.76$).

Table 10: Early Life Animal Exposure Propensity Score Quintiles: Range, Total, Number Exposed, Number with Persistent Confirmed IA, and Number with T1D

Propensity Score Quintile	Propensity Score Range	Total N (%)	Exposed N (%)	IA Outcome N (%)	T1D Outcome N (%)
1	0.140-<0.355	1444 (19.43)	409 (28.32)	99 (6.86)	29 (2.01)
2	0.355-<0.470	1431 (19.25)	554 (38.71)	83 (5.80)	23 (1.61)
3	0.470-<0.619	1439 (19.36)	857 (59.56)	80 (5.56)	22 (1.53)
4	0.619-<0.689	1481 (19.93)	1017 (68.67)	77 (5.20)	22 (1.49)
5	0.689-<0.938	1396 (18.78)	1019 (72.99)	68 (4.87)	15 (1.07)
Missing		241 (3.24)	131 (54.36)	10 (4.15)	2 (0.83)

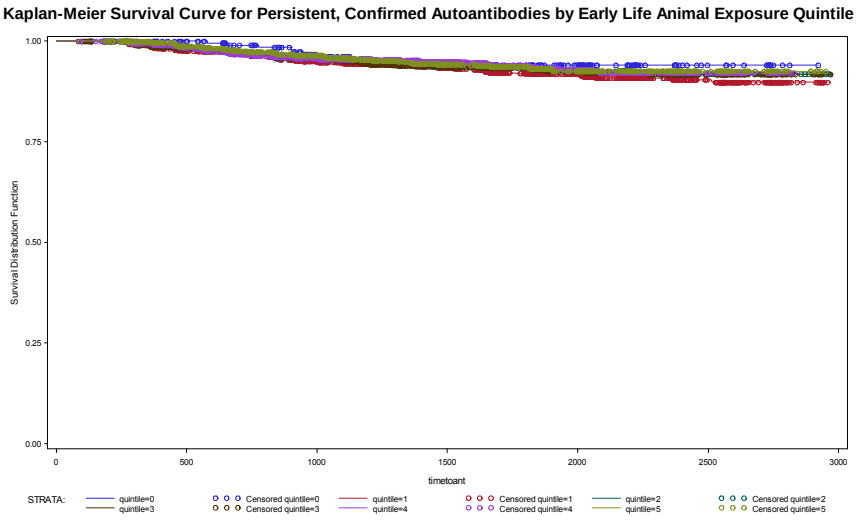

Figure 4: Survival Curves for Persistent, Confirmed IA by Early Life Animal Exposure Propensity Score Quintile (Survival by Time to Event), Log Rank P=0.57

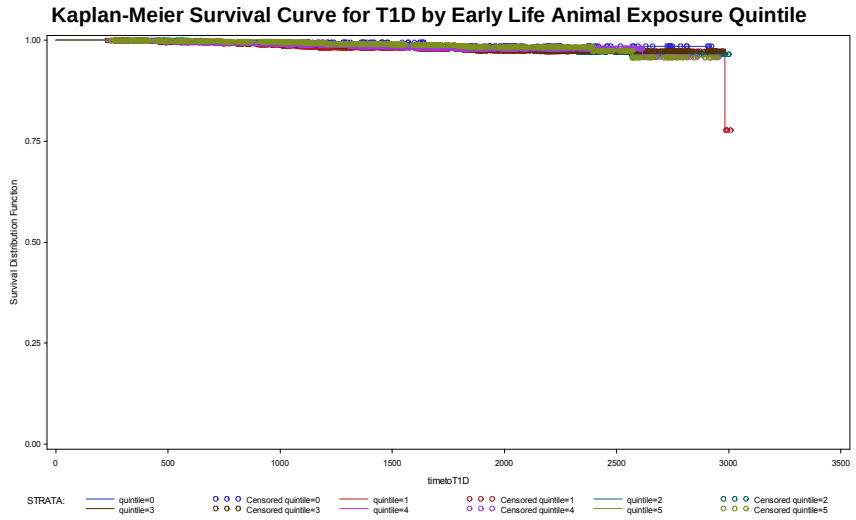

Figure 5: Survival Curve for T1D by Early Life Animal Exposure Propensity Score Quintile (Survival by Time to Event), Log Rank P=0.76

Table 11: Descriptive Statistics of the Study Population by Early Life Animal Exposure Propensity Quintile

Variable	Early Life Animal Exposure Propensity Quintiles (Lowest Propensity, Q1, to Highest Propensity, Q5, for Animal Exposure)				
	Quintile 1 N=1444 N (%)	**Quintile 2 N=1431 N (%)**	**Quintile 3 N=1439 N (%)**	**Quintile 4 N=1481 N (%)**	**Quintile 5 N=1396 N (%)**
Early Life Animal Exposure					
Yes	409 (23.32)	554 (38.71)	857 (59.56)	1017 (68.67)	1019 (72.99)
No	1035 (71.68)	877 (61.29)	582 (40.44)	464 (31.33)	377 (27.01)
Sex					
Female	723 (50.07)	692 (48.36)	708 (49.20)	727 (49.09)	671 (48.07)
Male	721 (49.93)	739 (51.64)	731 (50.80)	754 (50.91)	725 (51.93)
Age at Last Visit, years					
(mean, SD)	4.50 (1.84)	4.45 (1.93)	4.14 (1.87)	4.12 (1.69)	3.95 (1.75)
HLA Allele					
2/2	224 (15.51)	302 (21.10)	317 (22.03)	330 (22.28)	319 (22.85)
8/2	510 (35.32)	564 (39.41)	543 (37.73)	620 (41.86)	565 (40.47)
8/8	260 (18.01)	307 (21.45)	305 (21.2)	275 (18.57)	284 (20.34)
8/4	399 (27.63)	221 (15.44)	210 (14.59)	218 (14.72)	185 (13.25)
Other	51 (3.53)	37 (2.59)	64 (4.45)	38 (2.57)	43 (3.08)
First Degree Relative with T1D					
Yes	134 (9.28)	136 (9.50)	179 (12.44)	174 (11.75)	170 (12.18)
No	1310 (90.72)	1295 (90.50)	1260 (87.56)	1307 (88.25)	1226 (87.82)
Country of Residence					
United States	0 (0)	10 (0.70)	586 (40.72)	1333 (90.01)	1007 (72.13)
Finland	1055 (73.06)	192 (13.42)	193 (13.41)	85 (5.74)	27 (1.93)
Germany	73 (5.06)	155 (10.83)	181 (12.58)	19 (1.28)	67 (4.80)
Sweden	316 (21.88)	1074 (75.05)	479 (33.29)	44 (2.97)	295 (21.13)
Residence Type					
Rural Area	0 (0)	4 (0.28)	194 (13.48)	133 (8.98)	702 (50.29)
Small City/Village	489 (33.86)	808 (56.46)	597 (41.49)	102 (6.89)	316 (22.64)
Suburb	525 (36.36)	513 (35.85)	191 (13.27)	1172 (79.14)	369 (26.43)
Big City	430 (29.78)	106 (7.41)	457 (31.76)	74 (5.00)	9 (0.64)
Crowding Space (Residence Density)					
>1 (Crowded)	243 (16.83)	112 (7.83)	148 (10.28)	100 (6.75)	91 (6.52)
≤ 1 (Not Crowded)	1201 (83.17)	1319 (92.17)	1291 (89.72)	1381 (93.25)	1305 (93.48)

Mother's Education Level					
Basic Primary Education	91 (6.30)	286 (19.99)	413 (28.70)	108 (7.29)	365 (26.15)
Graduated Trade School	239 (16.55)	328 (22.92)	382 (26.55)	253 (17.08)	611 (43.77)
Graduated College	1114 (77.15)	817 (57.09)	644 (44.75)	1120 (75.62)	420 (30.09)
Mother Smoked During Pregnancy					
Yes	73 (5.06)	187 (13.07)	213 (14.80)	75 (5.06)	310 (22.21)
No	1371 (94.94)	1244 (86.93)	1226 (85.20)	1406 (94.94)	1086 (77.79)
Mother Drank Alcohol During Pregnancy					
Yes	438 (30.33)	440 (30.75)	529 (36.76)	543 (36.66)	530 (37.97)
No	1006 (69.97)	991 (69.25)	910 (63.24)	938 (63.34)	866 (62.03)
Mother Experienced a Condition or Illness During Pregnancy					
Yes	1324 (91.69)	1322 (92.38)	1304 (90.62)	1357 (91.63)	1297 (92.91)
No	120 (8.31)	109 (7.62)	135 (9.38)	124 (8.37)	99 (7.09)
Mother's Age at Birth of Participant, years					
mean (SD)	31.24 (4.67)	31.35 (4.82)	31.36 (5.15)	31.78 (5.14)	30.01 (5.70)
Participant Birth Weight, grams					
mean (SD)	3554.25 (541.01)	3550.55 (532.34)	3510.41 (547.57)	3422.53 (524.29)	3472.89 (556.80)
Exclusive Breastfeeding Duration					
No exclusive breastfeeding	442 (30.61)	425 (29.70)	501 (34.82)	723 (48.82)	589 (42.19)
<3 months	571 (39.54)	573 (40.04)	588 (40.86)	449 (30.32)	540 (38.68)
≥ 3 months	431 (29.85)	433 (30.26)	350 (24.32)	309 (20.86)	267 (19.13)
Early Life Formula Introduction Age					
Not introduced	232 (16.08)	271 (19.02)	201 (14.36)	187 (13.24)	181 (13.67)
Introduced at <3 months	970 (67.22)	974 (68.35)	997 (71.21)	1069 (75.71)	998 (75.38)
Introduced at 3-<7.5 months	204 (14.14)	165 (11.58)	177 (12.64)	128 (9.07)	132 (9.97)
Introduced at 7.5-<9 months	37 (2.56)	15 (1.05)	25 (1.79)	28 (1.98)	13 (0.98)
Early Life Cow Milk Introduction Age					
Not introduced	7 (0.52)	3 (0.21)	6 (0.45)	9 (0.67)	0 (0)
Introduced <3 months	912 (67.16)	968 (68.75)	988 (74.29)	1028 (77.06)	978 (76.89)
Introduced 3-<7.5 months	387 (28.50)	418 (29.69)	285 (21.43)	229 (17.17)	264 (20.75)
Introduced 7.5-<9 months	52 (3.83)	19 (1.35)	51 (3.83)	68 (5.10)	30 (2.36)
Early Life Vaccination					
Yes	1407 (97.44)	1392 (97.27)	1299 (90.27)	1420 (95.88)	1346 (96.42)
No	37 (2.56)	39 (2.73)	140 (9.73)	61 (4.12)	50 (3.58)
Early Life Social Group					
Yes	789 (54.64)	929 (64.92)	1000 (69.49)	975 (68.83)	968 (69.34)
No	655 (45.36)	502 (35.08)	439 (30.51)	506 (34.17)	428 (30.66)

Notably, the quintiles are largely characterized by country of residence (Table 11). For example, 73% of quintile 1 is comprised of Finland participants and there are no U.S. participants in this quintile. Quintile 2, on the other hand, includes mostly participants from Sweden (75%). The distribution of quintile 3 is less extreme with 33% of participants from Sweden, 13% from Germany, 13% from Finland, and 41% from the U.S.; however, quintile 4 is almost entirely made up of U.S. participants (90%). Quintile 5 is then basically the opposite of quintile 1 with 72% U.S. participants and very few participants from Finland (2%). The DQ8/4 allele also has higher prevalence in quintile 1 (the quintile comprised mostly of Finland participants) than the other quintiles. The quintiles are also largely characterized by residence type, specifically rural residence. No participants in quintile 1 (the group with the lowest predicted probably of animal exposure) lived in rural areas. Conversely, 50% of participants in quintile 5 (the group with the highest predicted probably of animal exposure) lived in rural areas.

Outcome: Persistent, Confirmed IA

No statistically significant associations between early life animal exposure and persistent, confirmed IA were found for quintiles 2, 3, 4, or 5. This was true even after directly adjusting for HLA type. Quintile 1, however, did produce a statistically significant finding. The results indicate that those participants in quintile 1 who were exposed to animals in early life have a 54% higher risk of developing persistent, confirmed IA than those participants who were not exposed to animals in early life (HR: 1.54, 95% CI: 1.02-2.31). Adjusting for HLA type had little impact on the resulting HR (HR: 1.53, 95% CI: 1.02-2.30). These findings are detailed in Table 12. Despite the statistically significant association identified in the quintile 1 population, a statistically significant difference in risk of persistent, confirmed IA across the quintiles was not found (p=0.57), indicating no overall association between early life animal exposure and persistent, confirmed IA .

Outcome: T1D

No statistically significant associations between early life animal exposure and T1D were found for any of the propensity score quintiles (Table 13). This was true for all quintiles even after adjusting for HLA type. Additionally, a statistically significant difference in risk of T1D across the quintiles was not found (p=0.76).

Table 12: HR & 95% CI for Cox PH Models for Outcome Persistent, Confirmed IA run within Early Life Animal Exposure Propensity Score Quintiles

		Risk of IA by Propensity Score Quintile[1]	
Propensity Score Quintile	Exposure Group	Unadjusted Model HR (95% CI)	Adjusted Model 1 HR (95% CI)
1	Exposed	1.54 (1.02-2.31)	1.53 (1.02-2.30)
	Unexposed	1 (Ref)	1 (Ref)
2	Exposed	0.79 (0.50-1.24)	0.76 (0.48-1.21)
	Unexposed	1 (Ref)	1 (Ref)
3	Exposed	0.86 (0.56-1.34)	0.86 (0.55-1.34)
	Unexposed	1 (Ref)	1 (Ref)
4	Exposed	1.35 (0.80-2.26)	1.30 (0.78-2.19)
	Unexposed	1 (Ref)	1 (Ref)
5	Exposed	1.12 (0.64-1.97)	1.10 (0.63-1.94)
	Unexposed	1 (Ref)	1 (Ref)

[1] The unadjusted model includes early life animal exposure only (reference group unexposed). Adjusted model 1 is adjusted for HLA (reference group DQ8/8).

Table 13: HR & 95% CI for Cox PH Models for Outcome T1D run within Early Life Animal Exposure Propensity Score Quintiles

		Risk of T1D by Propensity Score Quintile[1]	
Propensity Score Quintile	Exposure Group	Unadjusted Model HR (95% CI)	Adjusted Model 1 HR (95% CI)
1	Exposed	1.52 (0.72-3.23)	1.46 (0.69-3.10)
	Unexposed	1 (Ref)	1 (Ref)
2	Exposed	0.35 (0.12-1.04)	0.33 (0.11-0.97)
	Unexposed	1 (Ref)	1 (Ref)
3	Exposed	0.97 (0.42-2.27)	0.92 (0.39-2.15)
	Unexposed	1 (Ref)	1 (Ref)
4	Exposed	1.48 (0.55-4.02)	1.45 (0.53-3.92)
	Unexposed	1 (Ref)	1 (Ref)
5	Exposed	2.10 (0.47-9.31)	2.07 (0.46-9.20)
	Unexposed	1 (Ref)	1 (Ref)

[1] The unadjusted model includes early life animal exposure only (reference group unexposed). Adjusted model 1 is adjusted for HLA (reference group DQ8/8).

Propensity Score Application 3

Exposed and unexposed participants with propensity scores within 0.1 of one another were matched as pairs. This resulted in a total of 2,510 matched pairs (5,020 participants). The characteristics of the matched study population for the variables used to calculate propensity score are detailed in Table 14.

Table 14: Descriptive Statistics of the Matched Study Population for Variables Used in the Calculation of Propensity Score, N=5,020

Variable	All N=5,020 N (%)	Exposed to Animals in Early Life N=2,510 N (%)	Not Exposed to Animals in Early Life N=2,510 N (%)	P-value
Country of Residence				
United States	1886 (37.57)	956 (38.09)	930 (37.05)	<0.0001
Finland	1030 (20.52)	511 (20.36)	519 (20.68)	
Germany	402 (8.01)	208 (8.29)	194 (7.73)	
Sweden	1702 (33.90)	835 (33.27)	867 (34.54)	
Residence Type				
Rural Area	574 (11.43)	302 (12.03)	272 (10.84)	<0.0001
Small City/Village	1850 (36.85)	940 (37.45)	910 (36.25)	
Suburb	1820 (36.25)	883 (35.18)	937 (37.33)	
Big City	776 (15.46)	385 (15.34)	391 (15.58)	
Crowding Space (Residence Density)				
>1 (Crowded)	500 (9.96)	246 (9.80)	254 (10.12)	0.0829
≤ 1 (Not Crowded)	4520 (90.04)	2264 (90.20)	2256 (89.88)	
Mother's Education Level				
Basic Primary Education	1008 (20.08)	529 (21.08)	479 (19.08)	<0.0001
Graduated Trade School	1301 (25.92)	655 (26.10)	646 (25.74)	
Graduated College	2711 (54.00)	1326 (52.83)	1385 (55.18)	
Mother Smoked During Pregnancy				
Yes	601 (11.97)	310 (12.35)	291 (11.59)	0.3209
No	4419 (88.03)	2200 (87.65)	2219 (88.41)	
Mother Drank Alcohol During Pregnancy				
Yes	1670 (33.27)	824 (32.83)	846 (33.71)	0.3287
No	3350 (66.73)	1686 (67.17)	1664 (66.29)	
Early Life Vaccination				
Yes	4781 (95.24)	2390 (95.22)	2391 (95.26)	0.4629
No	239 (4.76)	120 (4.78)	119 (4.74)	
Early Life Social Group				
Yes	3218 (64.10)	1592 (63.43)	1626 (64.78)	0.2966
No	1802 (35.90)	918 (36.57)	884 (35.22)	

Outcome: Persistent, Confirmed IA

No statistically significant associations between early life animal exposure and persistent, confirmed IA were found when stratifying by matched pair. This was true even when directly adjusting for HLA type. These findings are detailed in Table 15.

Outcome: T1D

No statistically significant associations between early life animal exposure and TID were found when stratifying by matched pair. This was true even when directly adjusting for HLA type. These findings are detailed in Table 16.

Table 15: HR & 95% CI for Persistent, Confirmed IA, Cox PH Models on Matched Study Population, N=5,020

	Risk of IA for Participants with Early Life Animal Exposure[1]	
Variable	Unadjusted Model HR (95% CI)	Adjusted Model 1 HR (95% CI)
Early Life Animal Exposure	1.02 (0.78-1.32)	0.99 (0.75-1.29)

[1] The unadjusted model includes early life animal exposure only (reference group unexposed). Adjusted model 1 is adjusted for HLA (reference group DQ8/8).

Table 16: HR & 95% CI for T1D, Cox PH Models on Matched Study Population, N=5,020

	Risk of T1D for Participants with Early Life Animal Exposure[1]	
Variable	Unadjusted Model HR (95% CI)	Adjusted Model 1 HR (95% CI)
Early Life Animal Exposure	0.77 (0.45-1.32)	0.77 (0.44-1.36)

[1] The unadjusted model includes early life animal exposure only (reference group unexposed). Adjusted model 1 is adjusted for HLA (reference group DQ8/8).

Additional Analyses

Testing the Proportional Hazards Assumptions

The assumptions of the Cox proportional hazards model were explored and confirmed. Details of this analysis are provided in Appendix 3.

Testing for Interactions

The unadjusted Cox proportional hazards models for each outcome (persistent, confirmed IA and T1D) were run within categories of variables of interest to determine if there were interactions between the early life animal exposure variable and other covariates. Variables of interest are those variables included in the final logistic regression model, as well as HLA which was included in adjusted models. The HR and corresponding 95% CI for each outcome by variable category are summarized in Table 17. No statistically significant interactions were identified.

Table 17: HR & 95% CI for Unadjusted Cox PH Models run within Variable Categories to Check for Potential Interactions

Variable	Category	IA Outcome HR (95% CI)	T1D Outcome HR (95% CI)
Country of Residence	US	0.992 (0.687-1.432)	1.037 (0.498-2.162)
	Finland	1.393 (0.955-2.030)	1.311 (0.670-2.562)
	Germany	1.032 (0.497-2.141)	1.136 (0.425-3.039)
	Sweden	0.931 (0.670-1.294)	0.763 (0.354-1.644)
Residence Type	Rural	1.363 (0.756-2.456)	3.243 (0.753-13.973)
	Small City/Village	0.775 (0.551-1.088)	0.797 (0.427-1.485)
	Suburb	1.058 (0.772-1.452)	0.780 (0.412-1.476)
	Big City	1.005 (0.594-1.703)	0.939 (0.326-2.707)
Maternal Education	High	1.075 (0.839-1.377)	0.964 (0.586-1.586)
	Low	0.925 (0.679-1.260)	0.998 (0.571-1.745)
Crowding - Binary	Score >1	1.954 (0.978-3.902)	2.070 (0.606-7.075)
	Score ≤ 1	0.925 (0.757-1.132)	0.879 (0.595-1.299)
Crowding - Quintiles	Quintile 1	1.169 (0.738-1.852)	0.778 (0.365-1.662)
	Quintile 2	0.983 (0.635-1.524)	0.944 (0.372-2.395)
	Quintile 3	0.877 (0.566-1.360)	0.863 (0.378-1.969)
	Quintile 4	0.866 (0.515-1.456)	0.637 (0.227-1.791)
	Quintile 5	1.095 (0.75-1.600)	1.518 (0.722-3.190)
Smoking During Pregnancy	Yes	1.221 (0.615-2.423)	1.419 (0.260-7.749)
	No	0.992 (0.811-1.214)	0.965 (0.661-1.411)
Early Life Vaccination	Yes	1.00 (0.823-1.215)	0.978 (0.669-1.429)
	No	0.662 (0.177-2.478)	0.848 (0.170-4.236)
Early Life Social Group	Yes	1.020 (0.804-1.294)	1.189 (0.732-1.932)
	No	0.939 (0.676-1.303)	0.737 (0.411-1.320)
Drinking During Pregnancy	Yes	0.856 (0.614-1.194)	0.863 (0.461-1.618)
	No	1.077 (0.850-1.364)	1.034 (0.655-1.631)
HLA	2/2	0.779 (0.455-1.335)	0.680 (0.208-2.230)
	8/2	1.021 (0.779-1.339)	0.851 (0.515-1.409)
	8/8	1.045 (0.656-1.665)	0.990 (0.347-2.825)
	8/4	1.037 (0.635-1.694)	1.865 (0.677-5.133)
	FDR	0.714 (0.275-1.850)	0.971 (0.296-3.181)

Discussion

The purpose of this study was to assess the association between early life animal exposure and development of T1D, considering two separate endpoints: persistent, confirmed IA and T1D diagnosis. The relationship between early life animal exposure and each of these endpoints was assessed utilizing three different propensity score analysis techniques: model adjustment for propensity score, stratification on propensity score, and propensity score pair matching. Overall, this study does not support an association between early life animal exposure and persistent, confirmed IA or between early life animal exposure and T1D diagnosis. The findings of this study indicate no significant difference in T1D risk or IA risk among those with early life animal exposure compared to those without early life animal exposure. These findings were consistent even when directly adjusted for HLA type.

This study is one of only a few studies that have previously been conducted on animal exposure and T1D in children and the first we are aware of that examines early life animal exposure and persistent, confirmed IA. The results of this study are consistent with the results reported by Radon et al. (19). Radon et al. specifically focused on regular (at least once per week) contact to stables and T1D diagnosis and examined the association for those exposed in the first year of life and those exposed in the second to sixth year of life. Radon et al. did not find a statistically significant association, defined as $p<0.05$, between stables (farm animals) exposure and T1D, regardless of age at exposure. Radon et al. also did not find a statistically significant association between pets and T1D. The study by Radon et al. differs from the study currently being reported in that they examined farm animal exposure specifically, included only participants from

6 to 16 years of age who lived in rural areas of Germany with less than 100,000 residents, and utilized only T1D diagnosis as an endpoint. Additionally, the Radon et al. study is a case-control study including 242 cases and 224 controls. In comparison, the study currently being reported is a prospective cohort study including 7,432 participants enrolled in four different countries without limitation in scope to a particular residence type (e.g., rural) or particular animal type (e.g., farm animal) and with examination of both T1D diagnosis and persistent, confirmed IA as endpoints.

The results of this study differ from the results reported by Marshall et al. (17). Marshall et al. reported a statistically significant protective association between regular contact with pets/animals and T1D ($p=0.045$). Their finding was not reproduced in the study currently being reported. Marshall et al. performed a matched case-control study including 196 cases and 381 controls consisting of children under the age of 16 years living in the United Kingdom. It is not clear whether the data used by Marshall et al. to study the association between animal exposure and T1D was limited to exposure during a certain period of life, or even if it was limited to exposure prior to T1D diagnosis.

Studying the association between early life animal exposure and development of T1D in a multinational cohort is an advance for the field of study since previous studies on animal exposure and T1D have only focused on effects within national populations. However, studying a population that includes individuals from several countries also produces challenges. In this study, country appears to be a major factor in animal exposure propensity. It is possible that attitudes toward pet ownership and animal contact may differ by country. Koivusilta and Ojanlatva conducted a study on pet ownership in the Finnish population and found that pet ownership is associated with poor perceived health among the population (26). This perception could result in pet avoidance by a parent due to the knowledge of their child being at genetically high risk for T1D to a greater extent in Finland than in other countries. Finland also differs from

the other studied countries in that it has the highest incidence of T1D worldwide (5). Of the countries participating in TEDDY, the country of next highest incidence is Sweden, followed by the U.S. and then Germany.

The study questionnaires had to be translated to multiple languages in order to accommodate the multiple countries in the TEDDY study. It is possible that exposure misclassification could have occurred due to slight differences in these translations that affect a participant's understanding of the questions on animal exposure and whether or not exposure is recorded accurately for how the question was intended. For example, one of the questions pertaining to animal exposure on the questionnaire is whether the TEDDY child lives on a farm with animals or if there are animals that live outside of the child's house. It is possible, depending how this question translates, that individuals in some countries may report any animals they encounter that live outside of the child's house (regardless of who owns the animal); whereas, individuals in other countries may only report animals living outside the home that they own. These differences in interpretation could result in exposure misclassification that may attenuate the HR and cause concern for the internal validity of the study.

While exposure misclassification cannot be ruled out, outcome misclassification is unlikely. T1D diagnosis was defined by uniform criteria across sites and was documented in detail by each of the clinical centers. IA cases were limited to persistent, confirmed cases only for the purposes of this analysis. This means that an individual must test positive for IA at two separate consecutive study visits and that these findings were confirmed by a second laboratory. Considering only persistent, confirmed IA cases as opposed to all cases of positive IA results limits the likelihood of outcome misclassification for the IA endpoint.

External validity is limited in this study due to selection bias in regard to a systematic difference in the characteristics of the individuals selected for the TEDDY

study compared to the individuals not selected for the TEDDY study. The TEDDY study is specifically designed to enroll participants who are genetically at high risk for T1D, which does not accurately represent the general population. A population aware of its high genetic risk for T1D may have different exposure patterns than a general population that is not at high risk for T1D or is unaware of its genetic susceptibility to T1D. Therefore, findings in this study population may not apply to a general population.

An additional disadvantage of the cohort study design is the length of follow-up needed to obtain a large enough population of those with the outcomes of interest for analysis. In the present study, there were 113 (2%) participants with T1D and 417 (6%) participants with persistent, confirmed IA. However, the mean study participant age is only 4.23 (1.83) years. Study follow-up is intended to continue until age 15 years or T1D diagnosis, depending on the event that occurs first for each participant. At this time, power is limited in the ability to detect differences in risk for T1D. Performing this analysis again in the future after longer follow-up has been completed may elucidate on how cumulative animal exposure affects risk of T1D. An additional disadvantage associated with the length of follow-up is the risk for loss to follow-up. To minimize the effect of loss to follow-up on study analyses, the TEDDY study group has studied predictors for loss to follow-up in the study population to target individuals at high risk for loss to follow-up (22). Identifying the individuals at high risk for loss to follow-up allows clinical centers to take extra care in maintaining contact with these individuals and encouraging continued participation throughout the course of the study.

Despite the noted limitations, there are several important strengths of the present study. One strength of the study is the prospective cohort study design. This study design allows for temporality between the exposure and outcome to be established. In the TEDDY study, participants are enrolled prior to T1D diagnosis which allows for the collection of exposure data before diagnosis and ensures temporal sequence. In

comparison, the other studies on this topic are case-control studies for which temporality can be questionable since exposure data is collected after diagnosis.

Another advantage of the cohort study design is that it allows for the study of multiple outcomes. In this case, both persistent, confirmed IA and T1D diagnosis were studied as outcomes. Furthermore, the prospective cohort study design minimizes recall bias, which is a major limitation of the other referenced case-control studies on animal exposures and T1D. Recall bias is a type of measurement bias that is characterized by a systemic difference in the way cases and controls recall exposures. For example, those diagnosed with T1D may be more cognizant of exposures they had than those not diagnosed with T1D, which may cause a difference in the accuracy of the reported exposures by case status. To minimize this potential bias, the present study limited its population to individuals diagnosed with persistent, confirmed IA and T1D after 9 months of age to ensure that exposure data was collected prior to determination of either outcome. Additionally, the recall of animal exposure in this study was only for a period of 9 months; whereas, the other referenced case-control studies required recall for a period of many years.

This study is further strengthened by its use of propensity scores and multiple propensity score techniques. By comparing exposed and unexposed participants with similar propensity scores, one is simulating a random allocation of treatment. The propensity score is intended to capture all the background characteristics of the participant; therefore, if an exposed participant and unexposed participant have the same propensity score, it is expected that the only difference between these participants in regard to risk for the endpoint would be the exposure of interest, early life animal exposure. This simulation of random allocation helps to minimize any selection bias in regard to differences in the characteristics of the treatment (exposed and unexposed) groups (27, 28). Initially, propensity score analysis was applied by adjusting for

propensity score in the Cox proportional hazards models. While this method allows one to explore the association between early life animal exposure and the endpoints of interest while controlling for confounding, it does not allow one to explore how confounding and distribution of variables play a role across the propensity score range. Therefore, quintiles on propensity score were created in order to further explore the association in this regard. The third propensity score application, propensity score pair matching, was pursued in order to define highly matched participants on propensity score to tease out any effect between early life animal exposure and the outcomes of interest.

Stratification on propensity score brought to light a few variables that appear to drive propensity for early life animal exposure, including crowding (residence density), residence type (e.g., rural), and country of residence. For instance, the group with the lowest predicted probability of early life animal exposure (quintile 1) included a higher proportion (17%) of participants living in a crowded environment than the other quintiles. Furthermore, the group with the lowest propensity for early life animal exposure (quintile 1) included no participants who lived in rural areas; whereas, 50% of the participants in the group with the highest propensity for early life animal exposure (quintile 5) lived in rural areas. The most striking difference between quintiles was in regard to the distribution of country of residence, which largely characterized each quintile. Quintile 1 included mostly participants from Finland (73%), quintile 2 included mostly participants from Sweden (75%), and quintiles 4 and 5 included mostly participants from the U.S. (90% and 72% respectively). Quintile 3 was a more heterogeneous population in terms of country of residence than the other quintiles; however, it still was largely characterized by participants residing in the U.S. (41%) and Sweden (33%) compared to Germany (13%) and Finland (13%). These findings emphasized the need for consideration of potential interactions between early life animal exposure and these variables; however,

no significant interactions were found. Further research on the effect of crowding, residence type, and country of residence on animal exposure, IA, and T1D is warranted.

It was hypothesized that this study would find that children exposed to animals in early life would have a lower risk of developing IA and T1D than children not exposed to animals in early life. This hypothesis was based on the idea, rooted in the hygiene hypothesis, that animals would increase a child's exposure to organisms that help to develop appropriate immunoregulation; thus, minimizing the risk of a later immune system disregulation triggering the development of IA and T1D. The results of this study do not support the originally stated hypothesis.

While an overall difference in risk of T1D and persistent, confirmed IA by early life animal exposure status was not found in this study, additional research is needed to definitively rule out any potential role of animal exposure in the development of T1D. Suggested research questions include the effect of exposure to different types of animals, animal exposure duration, primary pet residence (e.g., exposure to family pets who live inside the home versus exposure to family pets who live outside the home), and exposure to animals outside the child's residence (e.g., animals at the zoo, animals the child visits at another residence) on T1D and IA risk. Studies on animal exposure in crowded environments are also recommended in order to discern whether the overload hypothesis, which suggests that individuals who experience an overload of the islet cells in early life due to characteristics such as physical and psychological stress may result in accelerated islet autoimmunity and cell death (18, 29), applies to the relationship between early life animal exposure and T1D development. Additionally, the collection of data on differences in perceptions of pets across countries could aid the interpretation of studies on animal exposure and health outcomes.

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Appendices

Appendix 1

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Appendix 2

The TEDDY study forms utilized to collect data on early life animal exposures and T1D diagnosis are provided on the following pages.

47649

Local Use Only

SubjectID

9 Month Primary Caretaker Interview

Office Use Only

Local Code:

Clinical Center:

Subject ID:

Visit Location Code:

Interview Date: / /
(DD/MMM/YYYY - *Example 01/JAN/2004*)

Interviewer: _____

TEDDY Staff Code of Interviewer:

47649 *Local Use Only*

SubjectID

We would like some information about the TEDDY child's parents and family. Please remember that all answers are confidential.

1. What is your relationship to the TEDDY child?

○ Mother ○ Father ○ Other Primary Caretaker ○ Other, specify _____

Code (office use only)

2. Who does the TEDDY child live with in this household? (Mark all that apply)

○ Mother
○ Step-mother
○ Father
○ Step-father
○ Brothers or sisters
○ Step-brothers or step-sisters
○ Grandparents
○ Other, specify _____

Code (office use only)

3. How many children (under the age of 18 years) live in your household?

Please include the TEDDY child in this total:

4. How many adults (18 and older) currently live in your household?

5. How many rooms are in your home? (Do not count bathrooms, porches, halls or balconies)

6. Which of the following best describes where you live? (Interviewer: Read all of the choices to the person being interviewed)

○ Rural area ○ Small city/village ○ Suburb ○ Big city

7. What is the marital status of the TEDDY child's parents? (Interviewer: Read all of the choices to the person being interviewed)

- ○ Married
- ○ Unmarried but living together
- ○ Separated
- ○ Divorced
- ○ Unmarried and living apart
- ○ Widowed

8. If the TEDDY child's parents are living apart, think about the parent the child sees less often. How often does this parent see the child? ○ Parents live together

☐☐ times per:
- ○ day
- ○ week
- ○ month
- ○ year

9. These next few questions are about the child's mother OR the primary female caretaker living in the household that this form pertains to. (Interviewer: The following questions relate to the mother or primary female caretaker that the child lives with in this household. If the child does not live with the mother or a female caretaker in this household please indicate this and go to question 10).

○ Does not live with mother or female caretaker

a. What is your (her) first language? _____ Code (office use only) ☐☐☐☐☐

b. What is your (her) country of birth? _____ Code (office use only) ☐☐☐☐☐

c. Is this your (her) first child? ○ No ○ Yes

d. What is your (her) highest grade or level of schooling completed?

- ○ Grades 1-9
- ○ Grades 10-12
- ○ Graduated High School or awarded a GED
- ○ Some trade school
- ○ Graduated from trade school
- ○ Some college or university
- ○ Graduated with a bachelor's degree (for example BA, AB or BS degrees)
- ○ Some graduate or professional school
- ○ Graduated with a master's degree (for example MA, MS, MBA, MEng, MEd, MSW)
- ○ Graduated with a doctoral degree (for example MD, DDS, JD, Ph.D., Ed.D degree)

e. **Do you (she) work outside the home now?** ○ No ○ Yes

If yes:
How many hours per week do you (she) work? ☐☐☐ hours per week

10. **Interviewer: If the child lives with the father or a partner in this household please get the following information.** (If child does not live with father (partner) in this household please indicate this and go to question 11)

○ Does not live with father or partner

a. **What is his (partner's) first language?** _____ Code (office use only) ☐☐☐☐☐

b. **What is his (partner's) country of birth?** _____ Code (office use only) ☐☐☐☐☐

c. **Is this his (partner's) first child?** ○ No ○ Yes

d. **What is his (partner's) highest grade or level of schooling completed?**

○ Grades 1-9
○ Grades 10-12
○ Graduated High School or awarded a GED
○ Some trade school
○ Graduated from trade school
○ Some college or university
○ Graduated with a bachelor's degree (for example BA, AB or BS degrees)
○ Some graduate or professional school
○ Graduated with a master's degree (for example MA, MS, MBA, MEng, MEd, MSW)
○ Graduated with a doctoral degree (for example MD, DDS, JD, Ph.D., Ed.D degree)

e. **Does he (partner) work outside the home now?** ○ No ○ Yes

If yes:

How many hours per week does he (partner)work? ☐☐☐ hours per week

11. **What is the biological father's height?** ☐ feet ☐☐ inches

47649 *Local Use Only* **SubjectID**

Smoke can affect the results of one of our laboratory tests. It will help us to know if the TEDDY child is exposed to smoke of any kind including cigarettes, cigars, or pipes. (Interviewer: Questions 12 and 13 refer to the primary caretakers (asked about in questions 9 and 10) that the child lives with in this household).

12. Do you (mother, female primary caretaker living in this household) currently smoke?"

○ No ○ Yes ○ Not applicable

If yes,

a. Do you (mother, female primary caretaker living in this household) smoke in the home?

○ No ○ Yes

b. Do you (mother, female primary caretaker living in this household) smoke in the car?

○ No ○ Yes

13. Does the child's father (or other partner living in this household) currently smoke?

If yes, ○ No ○ Yes ○ Not applicable

a. Does he (child's father or other partner living in this household) smoke in the home? ○ No ○ Yes

b. Does he (child's father or other partner living in this household) smoke in the car? ○ No ○ Yes

14. Does the child regularly spend time with anyone else who smokes? ○ No ○ Yes

15. Are there any animals or pets in the TEDDY child's house (the household that this form pertains to)? If yes, please tell us what kind of pet and how many: ○ No ○ Yes

☐☐	Cat	☐☐	Snake	☐☐	Other, what? _____
☐☐	Dog	☐☐	Rabbit		
☐☐	Bird	☐☐	Fish		
☐☐	Guinea Pig	☐☐	Turtle		
☐☐	Hamster	☐☐	Rat		
☐☐	Mouse	☐☐	Lizard		

Code (office use only) ☐☐☐☐☐

16. Does the TEDDY child live on a farm with animals, or are there animals that live outside the house (the household that this form pertains to)

○ No ○ Yes

○ Cat	○ Goat
○ Dog	○ Chicken
○ Cow	○ Horse
○ Pig	○ Goose
○ Duck	○ Other, what? _____
○ Sheep	

Code (office use only) ☐☐☐☐☐

Diagnosis of Diabetes Form

Office Use Only

Local Code: **Clinical Center:**

Subject ID: **Visit Location Code:**

Date form Completed: / / (DD/MMM/YYYY - *Example 01/JAN/2004*)

Person Completing Form: _____

TEDDY Staff Code of person completing form:

23218

Subject ID

Diagnosis of Diabetes

The following ADA criteria must met on two occasions (unless criterion 4 is present): At least one plasma, serum or whole blood glucose should be measured in a local laboratory. Hyperglycemia must not be attributable to other causes (e.g. acute stress, exogenous glucocorticoid use).

1. Casual (or: Random) (any time of day without regard to time since last meal) plasma glucose >= 200 mg/dL (11.1mmol/L), if accompanied by unequivocal symptoms (i.e. polyuria, polydipsia, polyphagia, and/or weight loss)

Or

2. Fasting (no food or drinks except water for at least 8 hours) plasma glucose >= 126 mg/dL (7 mmol/L)

Or

3. 2-hour plasma glucose >=200 mg/dL (11.1 mmol/L) in oral glucose tolerance test (OGTT)

Or

4. Unequivocal hyperglycemia with acute metabolic decompensation (diabetic ketoacidosis).

Unless criterion 4 is present or the fasting glucose is >=250 mg/dL (13.8 mmol/L) at the bedside or in the local laboratory on the day of testing, it is preferred that at least one of the two testing occasions involve an oral glucose tolerance test (OGTT). If the first criterion met is #3, i.e. by the 2-hour OGTT value, the OGTT should be repeated within 60 days.It is essential that every effort be made to obtain the necessary tests to establish the diagnosis of diabetes. Subjects will be instructed to eat a balanced diet and not to do any excessive exercises in the days leading up to the OGTT.

Date of Diagnosis of Diabetes by ADA criteria ☐☐ / ☐☐☐ / ☐☐☐☐
(DD/MMM/YYYY - Example 01/JAN/2004)

Diabetes Diagnosis made

○ By TEDDY Study Staff Member TEDDY Staff Code ☐☐☐☐

○ Elsewhere Location ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

Do we have signed medical release? ○ Yes ○ No ○ Don't Know

Current Weight ☐☐☐.☐☐ kg. Date of Measurement: ☐☐/☐☐☐/☐☐☐☐

Height ☐☐☐.☐ cm.

Signs and/or Symptoms:

Was the child symptomatic? ○ Yes ○ No ○ Unknown

If yes, complete the following:

Polyuria	○ Yes	○ No	○ Unknown	If yes, Date of Onset:	☐☐/☐☐☐/☐☐☐☐
Polydipsia	○ Yes	○ No	○ Unknown	If yes, Date of Onset:	☐☐/☐☐☐/☐☐☐☐
Polyphagia	○ Yes	○ No	○ Unknown	If yes, Date of Onset:	☐☐/☐☐☐/☐☐☐☐
Weight Loss	○ Yes	○ No	○ Unknown	If yes, amount	☐☐☐.☐☐ kg.

Subject ID

Glucose Values: Report the glucose values at the two occasions required to establish the diagnosis of diabetes. Meter readings must be supported by at least one diagnostic laboratory sample, drawn at a different time than the meter read sample.

For each glucose value, report the following:

Result mg/dL ☐☐☐ **Result mmol/L** ☐☐☐.☐☐ **Date** ☐☐/☐☐☐/☐☐☐☐

Hours since last meal ☐☐

Type	**Test**	**Draw Site:**
○ Meter	○ Random Glucose	○ Venous Plasma
○ TEDDY Lab	○ Fasting Glucose	○ Venous Blood
○ Other Lab	○ Postprandial Glucose	○ Capillary Blood
	○ OGTT (2 hr value)	

Result mg/dL ☐☐☐ **Result mmol/L** ☐☐☐.☐☐ **Date** ☐☐/☐☐☐/☐☐☐☐

Hours since last meal ☐☐

Type	**Test**	**Draw Site:**
○ Meter	○ Random Glucose	○ Venous Plasma
○ TEDDY Lab	○ Fasting Glucose	○ Venous Blood
○ Other Lab	○ Postprandial Glucose	○ Capillary Blood
	○ OGTT (2 hr value)	

Glycosylated hemoglobin

Hemoglobin A1c: Result ☐☐.☐ % Date ☐☐/☐☐☐/☐☐☐☐

☐☐☐ mmol/mol

Normal Range ☐.☐ - ☐.☐

○ Not done ○ Subject's medical chart not available to TEDDY staff

23218

Subject ID

Laboratory Values - Report as many of the following as available

Initial Values at time of Diabetes Diagnosis

Date ☐☐ / ☐☐☐ / ☐☐☐☐

	Result	Time (Please record time in Universal Time - for example 2 pm would be recorded as 14:00)
pH		
○ Venous ○ Arterial ○ Capillary	☐☐☐.☐☐	☐☐:☐☐ hr : min
○ Not done ○ Subject's medical chart not available to TEDDY staff		
Bicarbonate/ Total Co2	☐☐☐.☐☐ mEq/L ☐☐☐.☐☐ mmol/L	☐☐:☐☐ hr : min
○ Not done ○ Subject's medical chart not available to TEDDY staff		
pCO2	☐☐☐.☐☐ torr ☐☐☐.☐☐ kPa ☐☐☐.☐☐ mmHg	☐☐:☐☐ hr : min
○ Not done ○ Subject's medical chart not available to TEDDY staff		
Base Excess (BE) (value can be positive or negative. If reported as base deficit record as excess using opposite sign)	☐☐☐.☐☐ mEq/L ☐☐☐.☐☐ mmol/L ○ Positive ○ Negative	☐☐:☐☐ hr : min
○ Not done ○ Subject's medical chart not available to TEDDY staff		
Potassium	☐☐☐.☐☐ mmol/L	☐☐:☐☐ hr : min
○ Not done ○ Subject's medical chart not available to TEDDY staff		
Sodium	☐☐☐.☐☐ mmol/L	☐☐:☐☐ hr : min
○ Not done ○ Subject's medical chart not available to TEDDY staff		

Initial Values at time of Diabetes Diagnosis

	Result	Time (Please record time in Universal Time - for example 2 pm would be recorded as 14:00)
Chloride	☐☐☐.☐☐ **mmol/L**	☐☐:☐☐ **hr : min**
	○ Not done ○ Subject's medical chart not available to TEDDY staff	
BUN	☐☐☐.☐☐ **mg/dL** ☐☐☐.☐☐ **mmol/L**	☐☐:☐☐ **hr : min**
	○ Not done ○ Subject's medical chart not available to TEDDY staff	
Plasma Creatinine	☐☐☐ **micromol/L** ☐.☐☐ **mg/dL**	☐☐:☐☐ **hr : min**
	○ Not done ○ Subject's medical chart not available to TEDDY staff	
Beta OHB(blood ketone levels) ○ Blood ○ Serum ○ Plasma	☐☐☐.☐☐ **mg/dL** ☐☐☐.☐☐ **mmol/L**	☐☐:☐☐ **hr : min**
	○ Not done ○ Subject's medical chart not available to TEDDY staff	
Urine Ketones	○ Negative ○ Trace ○ Small ○ Moderate ○ Large	☐☐:☐☐ **hr : min**
	○ Not done ○ Subject's medical chart not available to TEDDY staff	

If laboratory evaluations were not obtained to evaluate for ketoacidosis, please comment on the subject's clinical situation at diagnosis

pH and Bicarbonate Values at time of nadir

pH

○ Venous
○ Arterial
○ Capillary

[ ][ ][ ] . [ ][ ]

Date [ ][ ] / [ ][ ][ ] / [ ][ ][ ][ ]

Time [ ][ ] : [ ][ ] **hr : min**

○ Not done ○ Subject's medical chart not available to TEDDY staff

Bicarbonate

[ ][ ][ ] . [ ][ ] **mEq/L**

[ ][ ][ ] . [ ][ ] **mmol/L**

Date [ ][ ] / [ ][ ][ ] / [ ][ ][ ][ ]

Time [ ][ ] : [ ][ ] **hr : min**

○ Not done ○ Subject's medical chart not available to TEDDY staff

Treatment

Was the child hospitalized? ○ Yes ○ No ○ Unknown

If yes, name and address of the hospital

[ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ]

[ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ]

[ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ]

If child was hospitalized, list below any additional diagnoses from hospital discharge summary

ICD-10 Code [ ][ ][ ] . [ ] _____

ICD-10 Code [ ][ ][ ] . [ ] _____

ICD-10 Code [ ][ ][ ] . [ ] _____

Date of admission [ ][ ] / [ ][ ][ ] / [ ][ ][ ][ ]

Date of discharge [ ][ ] / [ ][ ][ ] / [ ][ ][ ][ ]

Was this child treated in emergency room only? ○ Yes ○ No ○ Unknown

If yes, name and address of the emergency room

Date of admission / /

Date of discharge / /

Was this child treated in outpatient clinic only? ○ Yes ○ No ○ Unknown

If yes, name and address of clinic

Date of initial visit / /

Insulin (including insulin drip and/or s.c. insulin):

Has insulin been started? ○ Yes ○ No ○ Unknown

Date of starting insulin therapy / /

Family has given permission to be contacted again ○ Yes ○ No ○ Not asked

Appendix 3

The assumptions of the Cox proportional hazards model were explored by randomly simulating 1000 empirical score processes, based on martingale residuals, that meet the proportional hazards assumption for the variable early life animal exposure in regard to each outcome of interest (IA and T1D) and then calculating a p-value representing the percent of simulated paths that had extreme points that exceeded the most extreme point of the observed path for the variable of interest. A p-value less than 0.05 would be evidence against the proportional hazards assumption. The resulting p-values for each predictor variable are summarized in Table A1 for the outcome persistent, confirmed IA and in Table A2 for the outcome T1D.

All p-values produced by the models for the association between early life animal exposure and persistent, confirmed IA were greater than the significance level of 0.05; therefore, the proportional hazards assumption is supported. All p-values produced by the models for the association between early life animal exposure and T1D were greater than the significance level of 0.05; therefore, the proportional hazards assumption is supported.

Table A1: Test for Proportional Hazards Assumption, P-values for each variable included in model, H0: Hazards Proportional; alpha = 0.05, IA Outcome

	Model[1]		
Variable	Unadjusted Model P-value	Adjusted Model 1 P-value	Adjusted Model 2 P-value
Animal Exposure	0.6570	0.6410	0.6470
Propensity Score		0.2250	0.1920
HLA DQ2/2			0.3900
HLA DQ8/2			0.6830
HLA DQ8/4			0.5870
Other HLA			0.7190

[1] The unadjusted model includes early life animal exposure only (reference group unexposed). Adjusted model 1 is adjusted for early life animal exposure propensity score. Adjusted model 2 is adjusted for propensity score and HLA (reference group DQ8/8).

Table A2: Test for Proportional Hazards Assumption, P-values for each variable included in model, H0: Hazards Proportional; alpha = 0.05, T1D Outcome

	Model[1]		
Variable	Unadjusted Model P-value	Adjusted Model 1 P-value	Adjusted Model 2 P-value
Animal Exposure	0.6690	0.5230	0.5380
Propensity Score		0.0800	0.0930
HLA DQ2/2			0.5480
HLA DQ8/2			0.5870
HLA DQ8/4			0.2410
Other HLA			0.6760

[1] The unadjusted model includes early life animal exposure only (reference group unexposed). Adjusted model 1 is adjusted for early life animal exposure propensity score. Adjusted model 2 is adjusted for propensity score and HLA (reference group DQ8/8).

Additionally, the first 20 simulated paths and the actual path for early life animal exposure were plotted for outcome persistent, confirmed IA (Figure A1) and T1D (Figure A2). The actual paths do not vary drastically from the simulated paths. This is evidence in support of the proportional hazards assumption.

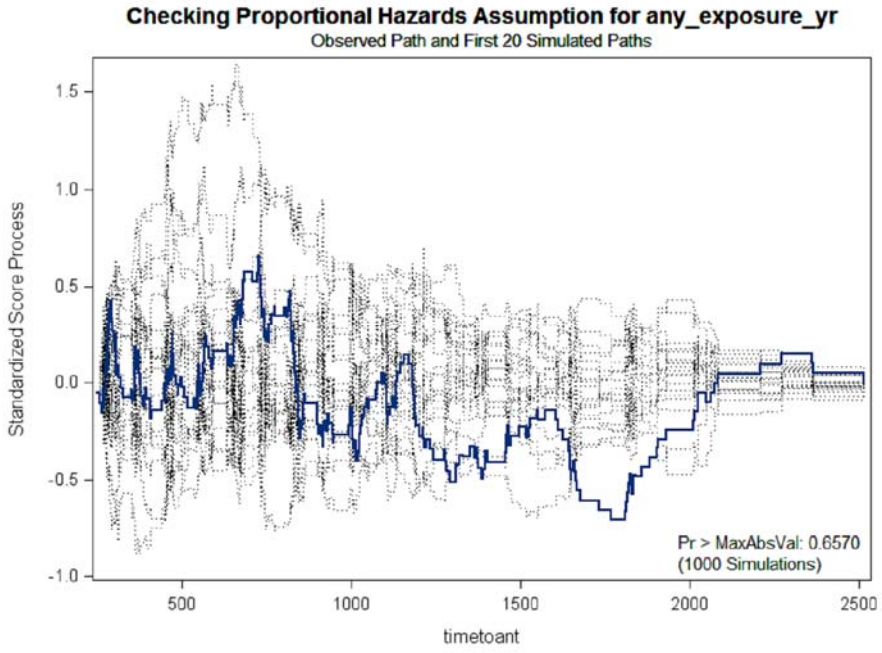

Figure A1: Checking Proportional Hazards Assumption for Early Life Animal Exposure Variable, Outcome Persistent, Confirmed IA, Unadjusted Model

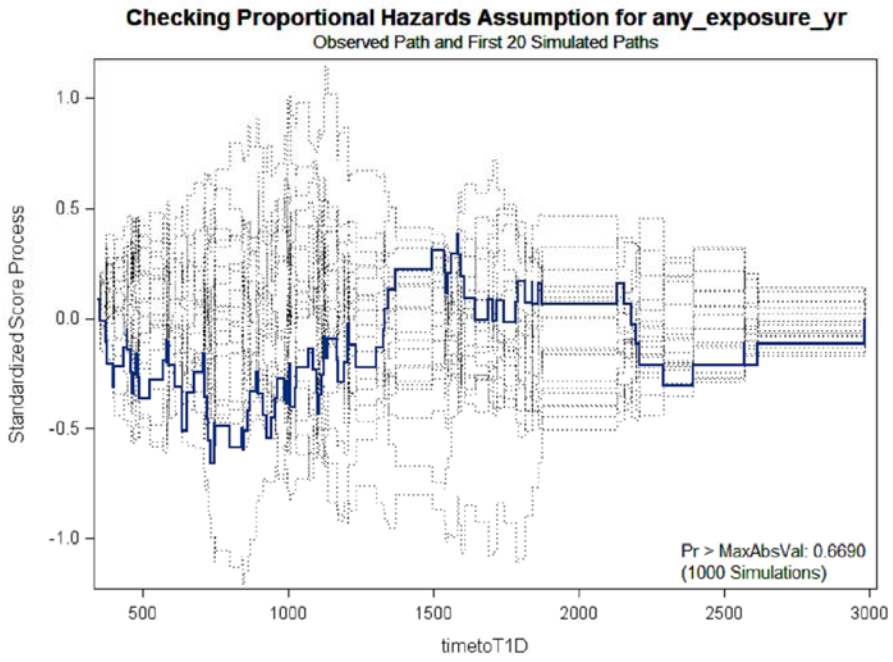

Figure A2: Checking Proportional Hazards Assumption for Early Life Animal Exposure Variable, Outcome T1D, Unadjusted Model

Appendix 4

The Environmental Determinants of Diabetes in the Young (TEDDY) study is approved by the University of South Florida Institutional Review Board (IRB). The IRB approval for Callyn Hall, author of this thesis, to conduct analyses on the TEDDY study data is provided on the following page.

USF
UNIVERSITY OF
SOUTH FLORIDA

DIVISION OF RESEARCH INTEGRITY AND COMPLIANCE
Institutional Review Boards, FWA No. 00001669
12901 Bruce B. Downs Blvd., MDC035 • Tampa, FL 33612-4799
(813) 974-5638 • FAX (813) 974-5618

December 17, 2012

Jeffrey Krischer, PhD
Pediatrics
3650 Spectrum Blvd., Suite 100
Tampa, FL 33612

RE: **Approved** Amendment Request
IRB#: MS1_100638
Title: The Consortium for Identification of Environmental Triggers of Type I Diabetes – Data Coordinating Center for the Environmental Determinants of Diabetes in the Young (TEDDY) Study

Dear Dr. Krischer:

On 12/17/2012 the Institutional Review Board (IRB) reviewed and approved your Amendment by expedited review procedures.

The submitted request has been for the following:

Change in study staff: Add Martha Butterworth, Callyn Hall, Kristian Lynch and Roy Tamura.

We appreciate your dedication to the ethical conduct of human subject research at the University of South Florida and your continued commitment to human research protections. If you have any questions regarding this matter, please call 813-974-5638.

Sincerely,

V Jorgensen MD

E. Verena Jorgensen, MD, Chairperson
USF Institutional Review Board