**Title:** Iodine and Canine Hypothyroidism

**Principal Investigators:** Brian K. Petroff, PDI and Co-I John Buchweitz, VDL

**Estimated Project Dates:** June, 2023 - May, 2024

**Total Amount Requested:** $49,828

**Introduction and Problem Statement**: Canine hypothyroidism is the most common veterinary endocrinopathy and among the most common disorders encountered in veterinary practice. Treatment requires lifelong thyroid hormone replacement [1,2]. The Veterinary Diagnostic Laboratory at MSU CVM (MSUVDL) pioneered the diagnosis of canine hypothyroidism and is considered the premier testing facility for the diagnosis of this disorder [3,4]. In this proposal, we leverage recent studies about hypothyroidism in humans and laboratory animals to explore iodine as a modifiable risk factor for autoimmune thyroiditis and hypothyroidism. In a second aim we will use the enormous database of past testing results at MSUVDL to gain insight into recent trends in demographics and geographical distribution of hypothyroidism in dogs across North America. This work builds upon unique strengths of MSUCVM in the veterinary community to discover novel strategies to decrease canine hypothyroidism

**Specific Aims:** Hypothyroidism is the most common endocrine disorder in dogs and humans [5-7]. Iodine is critical for the production of thyroid hormones (T3 and T4) [**5**]. Human and rodent studies have documented profound effects of dietary iodine on thyroid function – both in excess and deficiency [8-13]. This work investigates the relationship between iodine and thyroid disease in dogs through the following aims: **Aim 1: Determine serum iodine concentrations in normal dogs and dogs with autoimmune thyroiditis and hypothyroidism**. Both deficient and excess dietary iodine can compromise thyroid function through decreased thyroid hormone synthesis [5] and increased autoimmune thyroid disease [8] (the major cause of canine hypothyroidism [7,14]). We will document iodine concentrations in sera from dogs with normal thyroid function vs. dogs with autoimmune thyroid disease and/or hypothyroidism. Our hypothesis is that serum iodine will be higher in dogs with thyroid disease as has been shown in human populations and laboratory animals [8,15]. We also plan to identify dogs with high and low iodine results to allow future study of dietary and geographicrisk factors for the disease. **Aim 2: Characterize historical trends in canine hypothyroidism using retrospective testing data**. The MSUVDL developed the now commonly used canine diagnostic thyroid panel and receives plentiful submissions for thyroid testing in dogs of nearly all breeds and ages from across the United States and Canada [3,4]. A census of testing over the past 10 years indicates a database of over 500,000 diagnostic thyroid profiles in dogs during that period. We would like to use this resource to update and expand past work correlating incidence of canine hypothyroidism with breed, age, sex, reproductive status and geographic location. A long term goal is to identify clusters of canine hypothyroidism and use these to investigate modifiable risk factors such as iodine status.

**Significance and Impact:** This work will provide critical information concerning modifiable risk factors for hypothyroidism in dogs. This will underpin new clinical tests and trials to better diagnose and prevent canine hypothyroidism. This work offers immediate and tangible improvements in canine health by identifying previously unknown causes of hypothyroidism in dogs.

**Method:**

**Aim 1: Determine serum iodine concentrations in normal dogs and dogs with autoimmune thyroiditis and hypothyroidism**.

*Overview:* Synthesis of thyroid hormones is the main use of iodine in the body [5]. Low iodine intake results in a failure of thyroid hormone production and accumulation of precursors of thyroid hormone in the thyroid follicles leading to goiter. Paradoxically, more recent data indicate that increased iodine intake can lead to hypothyroidism as well - through increased autoimmune disease [8-13]. Iodination of thyroid proteins is thought to increase their autoantigenicity predisposing to autoimmune thyroiditis [16-21] – the main cause of hypothyroidism in dogs [3] and humans [16,22]. Our own preliminary data support this link between increased iodine and hypothyroidism (**Table 1**). In this aim, we will measure iodine in dogs with normal thyroid function and dogs with autoimmune thyroiditis and hypothyroidism. This will result in a diagnostic reference interval for serum iodine and will test iodine as a modifiable risk factor for canine hypothyroidism.

*Study population, experimental design and statistics:* Submissions from euthyroid (n=100) and dogs with autoimmune thyroiditis (AIT) and hypothyroidism (n=100) [21]. will be identified through thyroid panel submission to MSU VDL. We routinely receive serum samples from healthy dogs for prebreeding testing (Orthopedic Foundation for Animals, OFA) in additional to diagnostic samples from dogs with clinical signs of hypothyroidism (**Table 2**). Total T4 and free T4 will be measured using radioimmunoassay kits (50, 200 and 100 µl serum; MP Biomed, Santa Ana, CA) [22,23]. TSH will be assayed via automated chemiluminescent reagents for canine TSH on the Immulite 2000 platform (300 µl serum, Siemens, Malvern, PA). These assays are validated and used routinely for canine diagnostic samples [4,24]. Euthyroid serum samples are defined as having total T3 and T4, free T4, TSH and TgAA concentrations within their reference intervals. Hypothyroidism is defined as decreased free T4 and/or total T4 and elevated TSH [28]. Elevated TgAA (>35% Tg binding in comparison to control) will be used as evidence of autoimmune thyroiditis [3,4].

**TABLE 1. Preliminary data: serum iodine concentrations in euthyroid and hypothyroid dogs.**

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Mean** | **Range** | **2.5 - 97.5% pepercenti**  **le** |
| ***Normal (n=144)*** |  |  |  |
| Total iodine (ng/ml)  (ng/ml) | 123 | 24-1543 | 54-394 |
| Inorganic iodine (ng/ml)  (ng/ml) | 82 | 13-1361 | 27-318 |
| **Hypothyroid (n=26)\***  **(n((n=26)** |  |  |  |
| Total iodine (ng/ml)  (ng/ml) | 687 | 19-2384 | 32-2256 |
| Inorganic iodine (ng/ml)  (ng/ml) | 626 | 12-2240 | 25-2107 |

\*17 of 26 HT dogs (65%) had total iodine above 97th percentile for normal dogs.

*Iodine analysis.* Iodine will be assessed in the Nutrition and Toxicology section of the Veterinary Diagnostic Laboratory, MSU. Total and inorganic iodine will be measured in 100 µl of serum using an Agilent 7900 Inductively Coupled Plasma – Mass Spectrometer (ICP-MS) at MSU as described previously ([25] based on the technique of Benkhedda et al, 2009 [26], preliminary data). Briefly, samples are predigested with a perchloric and nitric acid mixture prior to ICP-MS monitoring m/z ratios of 115 and 127. Total iodine is a measure of all species of iodine, bound and free, in the serum. Inorganic represents circulating iodine not bound to protein. Once inorganic and organic iodine tests have been completed, we will run two sample t-tests to check for differences between the euthyroid and hypothyroid groups (power analysis in budget).

*Preliminary Data and Expected Results:* Previous studies in humans and laboratory rodents have observed an increased prevalence and progression of autoimmune thyroiditis and hypothyroidism with increased iodine intake [16-18]. Our preliminary data from historical comparison in a small cohort of hypothyroid dogs support this trend (**Table 1**). We hypothesize that iodine concentrations will be higher during hypothyroidism (with or without autoimmune thyroiditis) than in serum from dogs with normal thyroid profiles reflecting a role of increased iodine in the promotion of canine hypothyroidism. These findings would provide a rationale for better managing iodine intake as a practical strategy to decrease the incidence of autoimmune thyroiditis and hypothyroidism in dogs.

*Potential Problems and Future Studies:* Our preliminary data in a small cohort suggest that hypothyroidism is associated with increased serum iodine. This appears to affect nearly two-thirds of the hypothyroid dogs in a preliminary cohort (**Table 1**). However, should the full study fail to confirm this relation these data will establish a diagnostic reference interval for serum iodine in dogs. Preliminary data contain few (2) dogs with active autoimmune thyroiditis. However, AIT is a transient condition reflecting active inflammatory disease in the thyroid glands and most hypothyroid dogs exhibit normal autoantibodies [3,4]. A critical secondary benefit of this work will be the identification of dogs with low and high iodine status. In a future study, we plan to characterize the diets of these animals in an effort to identify problem diets for thyroid function. Additionally, obvious work to follow would include characterization of the pathophysiology of increased hypothyroidism and a clinical prevention trial.

**Aim 2: Characterize historical trends in canine hypothyroidism using retrospective testing data**.

*Overview:* MSUVDL is a leader in the diagnosis of canine hypothyroidism and has an immense record of past testing that is an important resource for the veterinary community. Previous historical surveys of MSUVDL testing data have helped to define our understanding of canine hypothyroidism, most recently in 2008 [3,4]. In this aim, we would like to use more recent testing data to characterize modern canine hypothyroidism in collaboration with professional statistical expertise at MSU CSTAT.

**MSUVDL Diagnostic Thyroid Submissions, 2003-2022**

|  |  |  |
| --- | --- | --- |
| **Region** | **Total Cases** | **Hypothyroid** |
| Canada | 1622 | 139 |
| Northeast | 150769 | 12833 |
| Midwest | 257276 | 23792 |
| West | 58212 | 4731 |
| Southwest | 14918 | 1295 |
| Southeast | 111888 | 8679 |
| US Territory | 662 | 45 |
| Grand Total | 595347 | 51514 |

**Table 2.** MSU VDL submissions for canine diagnostic thyroid profiles. Hypothyroid defined strictly here as low total T4 and elevated TSH.

*Study Population and Statistics:* The MSU VDL holds approximately 500,000 records on historical thyroid testing over the last 10 years obtained from throughout the United States (**Table 2**). With the help of MSU Center for Statistical Training and Consulting (CSTAT), we will investigate risk factors of canine hypothyroidism including age, sex, geographic location, and breed. Thyroid hormones, TSH and thyroid autoantibodies were measured as described in Aim 1. When submitting samples, owners fill out information on each animal’s date of birth, sex, reproductive status (spayed or neutered), breed, and the address of the clinic where blood samples were taken. This information will allow us to look at the incidence of hypothyroidism among each group and assess risk factors for the disease. Incidences of hypothyroidism (defined as low thyroid hormone(s) and increased TSH) and autoimmune thyroiditis (TgAA positive status) can be parsed by these factors to identify and/or update risk factors for canine hypothyroidism.

In our initial consultation with CSTAT, a discussion of this data and possible statistical analysis lead us to plan for regression analysis to determine which, if any, factors had an impact on the incidence of hypothyroidism. Regression analysis is an array of statistical methods designed to estimate the relationship between a dependent variable and one or more independent variables. Once data can be pulled from the VDL system, it will be sent to CSTAT where it will be cleaned and checked for the assumptions of regression. If the data conforms to the underlying assumptions, the analysis will be run with model diagnostics to be examined for goodness of fit. Additional analyses may be performed, under the direction of CSTAT.

*Preliminary Data and Expected Results:* Historical studies, including work from MSUCVM, established significant effects of age and breed on the incidence of canine hypothyroidism. Roughly 20 breeds were defined as high risk led by English Setter dogs with a lifetime incidence of nearly 1 in 3 for that breed [6,27]. As a result, breed associations have been attempting to decrease propagation of animals at elevated risk for hypothyroidism through the Orthopedic Foundation for Animals thyroid database. Our preliminary data (**Table 2**) should also allow insight into correlations between location and canine hypothyroidism – a novel insight that may intersect with the iodine data in Aim 1.

*Potential Problems and Alternative Approaches:* There are weaknesses to the data obtained during routine diagnostic testing. Clinical history, including treatment information, often is limited and not captured as categorical data amenable to statistical analysis. However, the vast majority of submissions for canine diagnostic thyroid testing have similar clinical signs and are not receiving treatment with significant effect on thyroid function (monitoring of dogs being treated for hypothyroidism is typically performed using an abbreviated thyroid profile not included in these data. We anticipate that by using a very large dataset, effects of uncommon variable factors will be minimized. Smaller datasets can be sampled in detail if needed. This big data work is intended as hypothesis generation allowing more definitive studies of individual risk factors of canine hypothyroidism. A similar approach has been used successfully by corporate practices in the United Kingdom [29]

**Timeline:**

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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **AIM 1** | | Jun | | Jul | | Aug | | Sep | | Oct | | Nov | | Dec | | Jan | | Feb | | Mar | | Ap | | May |
| Sample selection | | X | | X | | X | | X | | X | | X | |  | |  | |  | |  | |  | |  |
| Iodine analysis | |  | |  | |  | |  | |  | |  | | X | | X | |  | |  | |  | |  |
| Statistics and manuscript preparation | |  | |  | |  | |  | |  | |  | |  | |  | | X | | X | | X | | X |
| **AIM 2** | |  | |  | |  | |  | |  | |  | |  | |  | |  | |  | |  | |  |
| Database preparation | | X | | X | |  | |  | |  | |  | |  | |  | |  | |  | |  | |  |
| Data cleaning and analysis | |  | |  | | X | | X | | X | | X | | X | | X | |  | |  | |  | |  |
| Statistics and manuscript preparation | |  | |  | |  | |  | |  | |  | |  | |  | | X | | X | | X | | X |
| Statistics and manuscript preparation |  | |  | |  | |  | |  | |  | |  | |  | | X | | X | | X | | X | |

**Budget:**

|  |  |  |
| --- | --- | --- |
| **Category** | **Cost per sample**  **(if applicable)** | **Cost** |
| Iodine analysis | $45/sample x 200 samples | $9,000 |
| Project Coordinator | 50% effort x 1 year | $34,372 |
| Statistical analysis | CSTAT Consult | $6,456 |
| **Total Costs** |  | **$49,828** |

We have budgeted for 200 samples for our iodine testing. Estimating a moderate effect size, 200 ng/mL, between the euthyroid and hypothyroid group gives us a conservative sample size of 100 animals per group to measure a difference at 80% power and 0.05 type I error rate (code below) for total iodine. While our preliminary data showed a larger effect size, the small sample size in the hypothyroid group and the large range of values seen in both groups justify using a more conservative estimate of the true effect size between the two populations. Using the same power parameters for inorganic iodine, 89 animals are needed per group (code also below). As we would like to power both tests sufficiently, we will use the larger estimate of 100 animals per group. Iodine testing will be $45/sample for both inorganic and total iodine, resulting in a budget of $9,000 for iodine testing.

Two-sample t test power calculation with unequal variances – Total Iodine

##

## n = 99.66435, 99.66435

## delta = 200

## sd = 173, 685

## sig.level = 0.05

## power = 0.8

## alternative = two.sided

Two-sample t test power calculation with unequal variances – Inorganic Iodine

##

## n = 88.46762, 88.46762

## delta = 200

## sd = 153, 650

## sig.level = 0.05

## power = 0.8

## alternative = two.sided

##

## NOTE: n is number in \*each\* group

CSTAT currently employs graduate level research assistants, overseen by PhD level statisticians to provide professional statistical services to internal and external clients of MSU. We will utilize the help of a research assistant to perform statistical work including data cleaning and regression analysis. CSTAT personnel will also help to write and prepare the manuscript and create any figures or heat maps needed for data visualization.

A research assistant, Ms. Sichao Wang, is budgeted at 5% FTE. With an annual salary of $92,820, a 5% FTE amounts to $4,641 in salary and $1,665 in fringe costs ($92,820\*.3588 = $1,665) and software costs over the course of a year are budgeted at $150. Total costs for CSTAT services are $6,456.

Petroff, Brian K. (PI; tenure track; no salary billed): Dr. Petroff will provide oversightfor the study, assist with sample collection and enrollment and lead data analysis and manuscript preparation.

Buchweitz, John (Co-I; tenure track; no salary billed) Dr. Buchweitz will provide oversight for the iodine sample testing as well as analysis and interpretation of iodine testing.

Egbert, Rebecca M. (Research assistant, 50% effort): Ms. Egbert will serve as study coordinator providing sample selection and preparation as well as data collection. Ms. Egbert will work on data analysis with the oversight of the MSU Center for Statistical Training and Consulting (CSTAT). The budget includes .5FTE for Ms. Egbert’s effort, $22,309, with fringe costs for the position at 54.07%, or $12,063. The total for Ms. Egbert’s position is $34,372.

**Team:** The Endocrinology Section at MSU VDL includes 6 full time veterinarians with specialized training and experience relevant to veterinary endocrinology and 10 laboratory staff who perform approximately 24 laboratory tests for endocrine disease in animals. The section is the busiest specialty laboratory of its kind and performs approximately one million diagnostic tests for 250,000 patients per year. Clients include individual practices, researchers and corporate laboratories from the United States, Canada and around the world. Endocrinologists provide written interpretations of test results and telephone consultations to assist in the diagnosis and management of veterinary patients with endocrine disease. Brian Petroff D.V.M. Ph.D. is Section Chief of Endocrinology at the Veterinary Diagnostic Laboratory. Dr. Petroff has a research background in comparative endocrinology and endocrine disruption and has been Section Chief at MSU VDL for 7 years. He has received external support from the Canine Health Foundation for research into thyroid disease in dogs

Toxicology and Nutrition at MSUVDL: The toxicology, nutrition and mineral sections are supervised by Dr. John Buchweitz, sections and include 1 resident/PhD student, 1 APHL fellow, 1 analytical chemist, 1 laboratory supervisor, 1 project manager, 1 research associate, 3 laboratory technicians, 2.5 preparatory technicians, and 5 undergraduate students. This group has been critical in updating the technical capability of the VDL during characterization of toxicities in animals.

The toxicology and endocrinology sections have worked closely together in the past and are on a trajectory of increased collaboration in the future. Several recent widespread thyroid and vitamin D toxicity outbreaks in dogs and cats were detected by the Endocrinology section (vitamin D is measured by immunoassay in the Endocrinology section), verified by advanced methodology in the Toxicology section who then communicated with the Food and Drug Administration resulting in withdrawal of the problematic diets. The sections have worked together to develop new testing methods for vitamin D and are developing a new methodology for the measurement of parathyroid hormone related protein (PTHrP), a widely used marker for hypercalcemia of malignancy that now can be measured by laser capture-mass spectroscopy (LC-MS/MS). We anticipate that a number of our current immunoassays used in the Endocrinology section will be transitioned to LC-MS/MS methodology which is more specific.

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| BIOGRAPHICAL SKETCH Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2. Follow this format for each person. **DO NOT EXCEED FOUR PAGES.** | | | |
|  | | | |
| NAME  **PETROFF, Brian K.** | POSITION TITLE  Professor | | |
| eRA COMMONS USER NAME  BPETROFF |
| EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)* | | | |
| INSTITUTION AND LOCATION | DEGREE  *(if applicable)* | YEAR(s) | FIELD OF STUDY |
| Ohio State University, Columbus, OH | B.S. | 1991 | Animal Sciences |
| Ohio State University, Columbus, OH | Ph.D. | 1996 | Physiology |
| Ohio State University, Columbus, OH | D.V.M. | 1998 | Veterinary Medicine |
| KU Medical Center, Kansas City, KS | Postdoc. | 2000 | Endocrinology |
| Marine Biological Laboratory, Woods Hole, MA |  | 2001 | Frontiers in Reproduction |

1. **Personal Statement:**

In 2016 I began as section chief of endocrinology at the Veterinary Diagnostic Laboratory (VDL), Michigan State University after twelve years as an NIH and Komen Foundation funded PI at the University of Kansas Medical Center. I was previously co-leader of the NCI funded Cancer Prevention Program focused on hormone-dependent cancers. Since moving to MSU my focus has been on refining diagnostic testing in the Endocrinology section, particularly concerning the study of ovarian and thyroid autoimmune disease and their impact on animal health and fertility.

**B. Positions and Honors**

Positions and Employment:

1988 – 1992 Veterinary Assistant, Dr. David Ockenga, Wakeman, OH

1992 – 1998 Graduate Fellow in Reproductive Physiology, College of Veterinary Medicine, Dept. of Animal Sciences, Ohio State University, Columbus, OH (Advisors: Pate and Ottobre)

1998 Veterinary Intern, Village Veterinary Care, Pataskala, OH

1998 – 2000 NIH Postdoctoral Fellow in Reproductive Sciences, Center for Reproductive Sciences, University of Kansas Medical Center, Kansas City, KS (Advisor: Terranova)

2000 – 2004 Research Assistant Professor, Department of Molecular and Integrative Physiology , University of Kansas Medical Center, Kansas City, KS.

2004 – 2014 Assistant and Associate Professor, Department of Internal Medicine, University of Kansas Medical Center, Kansas City, KS.

2015-2021 Associate Professor, Pathobiology and Diagnostic Investigation, Michigan State University CVM, Lansing, MI.

2022-present Professor, Pathobiology and Diagnostic Investigation, Michigan State University CVM, Lansing, MI.

2016-present Section Chief, Endocrinology; Veterinary Diagnostic Laboratory, Michigan State University, Lansing, MI

Honors:

Ohio State University Veterinary Reproductive Medicine Award, 1998; NCI Breast SPORE Career Development Award, 2004; Susan Love Research Foundation Pilot Award, 2005; Komen Promise Grantee, 2011; Chancellor’s Teaching Award, 2011.

Professional and Scientific Societies:

AACR, American Veterinary Medical Association**,** International Society for Fertility Preservation, Ohio Veterinary Medical License # 7635**,** Michigan Veterinary Medical License #6901011061, Society for the Study of Reproduction**,** Society for Comparative Endocrinology.

Reviewer:

Biology of Reproduction, Biomarkers, Biomarkers in Medicine, BMC Cancer, Breast Cancer: Basic and Translational Research, Cancer Research, Carcinogenesis, Comparative Biochemistry and Physiology, Congenital Diseases and Environment, Current Medicinal Chemistry, Endocrine, Endocrinology, Fertility and Sterility, Journal of Assisted Reproduction and Genetics, Journal of Endocrinology, Molecular and Cellular Probes, Reproduction, Reproductive Biology, Reproductive Biology and Endocrinology, Reproduction Nutrition and Development, Reproductive Toxicology, Toxicology, Toxicology and Applied Pharmacology, Toxicology Letters, Zoological Sciences. NA Managing Editor: Reprod Biol.

Grant reviewer: NICHD, NCI, Komen for the Cure, DOD, EPA

1. **Contributions to Science**
2. The original focus of both my postdoctoral and independent research was environmental reproductive toxicology, specifically the impact of ligands of the aryl hydrocarbon receptor (AhR) pathway on female reproductive function. Beginning with EPA-funded work in the laboratory of Dr. Paul Terranova and continuing with funding from my own NIEHS RO1 and R21, we characterized the pathways of endocrine disruption, ovulatory blockade and premature reproductive senescence from aberrant AhR activation, predominantly using dioxins as model pollutants. These publications documented both acute neuroendocrine and chronic anti-steroidogenic effects of AhR pollutants, effects that were most profound when exposure was early in life leading to lingering dysfunction. This work was incorporated into the EPA risk assessment documentation for reproductive effects of dioxins.
   1. **Petroff BK**, Croutch CR, Hunter DM, Weirman M, and Gao X. 2003. 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) disrupts gonadotropin secretion through an estradiol and pentobarbital-sensitive mechanism but does not alter GnRH secretion by immortalized GnRH neurons in vitro. Biol Reprod 68:2100-2106.
   2. Franczak A, Nynca A, Valdez KE, Mizinga KM, **Petroff BK.** 2005. Effects of acute and chronic exposure to the aryl hydrocarbon receptor agonist 2,3,7,8-tetrachlorodibenzo-p-dioxin on the transition to reproductive senescence in female Sprague Dawley rats. Biol Reprod, 74:125-130.
   3. Hutt K, Shi Z, Albertini DF, **Petroff BK**. 2008. The environmental toxicant 2,3,7,8-tetrachloro dibenzo-p-dioxin disrupts morphogenesis of the rat embryo. BMC Dev Biol. 8:1-10. PMC2254588.
   4. Valdez KE, Shi Z, Ting A and **Petroff BK**. 2009. Effect of chronic exposure to the aryl hydrocarbon receptor agonist 2,3,7,8-tetrachlorodibenzo-p-dioxin in female rats on ovarian gene expression. Reprod Toxicol. 28:32-37. PMC2691863.
3. My background is in animal agriculture and my undergraduate research, Ph.D. study and early veterinary practice were focused on improving fertility in farm animals, particularly through better understanding ovarian and uterine physiology in large animals. This has remained a thread in the laboratory to this day, particularly through collaboration with European colleagues. Resulting publications have contributed to our understanding of luteal regression, ovulation and endocrine disruption in horses, pigs and cattle.

* 1. **Petroff BK**, Dabrowski K, Ciereszko RE, Ottobre JS. 1997. Total ascorbate and dehydroascorbate concentrations in porcine ovarian stroma, follicles and corpora lutea throughout the estrous cycle and pregnancy. Theriogenology 47:1265-1273.
  2. **Petroff BK**, Ciereszko RE, Dabrowski K, Ottobre AC, Pope WF, and Ottobre JS. 1998. Depletion of vitamin C from pig corpora lutea by prostaglandin F2a-induced secretion of the vitamin. J Reprod. Fert. 112:243-247.
  3. **Petroff BK**, Wegner KM. 2005. Preovulatory tranquilization with xylazine does not alter the timing or hormonal profiles of ovulation in the mare. J Equine Sci, 16:67-72.
  4. Franczak AG, Kotwica G, Kurowicka A, Oponowicz I, Woclowek-Potocka G, **Petroff BK**. 2006. Effect of oxytocin on prostaglandin F2a and E2 synthesis and secretion by myometrium during luteolysis and early pregnancy. Theriogenology, 66:1049-1056.

1. A shared research topic in the lab has been the role of the immune system in controlling and disrupting ovarian function. This work has been in collaboration with Dr. Margaret Petroff and initially with Dr. Joy Pate, both leading reproductive immunologists. Most recently this work has focused on the role of the Autoimmune Regulator (*Aire*) gene, a coordinator of central tolerance to peripherally restricted tissue antigens. Proper Aire function is essential to avoid autoimmune disease and my role has been to characterize the ovarian phenotype in rodents lacking functional Aire. Publications here have helped develop a new model and understanding of premature ovarian failure from autoimmune disease. This work was featured in the keynote address of the 2015 Society for Reproductive Investigation by Dr. Margaret Petroff (Coinvestigator here).

* 1. Petroff MG, **Petroff BK**, Pate JL. 2001. Mechanisms of cytokine-induced cell death of cultured bovine luteal cells. Reproduction 121:753-760.
  2. Jasti S, Warren BD, McGinnis LK, Kinsey WH, **Petroff BK**/MG (shared senior authorship). 2012. The auto-immune regulator (Aire) prevents premature reproductive senescence in female mice. Biol Reprod. 86:110. PMC3338656. *Editorial: Cushman, R.A. doi:10.1095/biolreprod.112.099242.*
  3. Warren BD, Kinsey WK, McGinnis LK, Christenson LK, Jasti S, Stevens AM, **Petroff BK**, Petroff MG. 2014. [Ovarian autoimmune disease: clinical concepts and animal models.](http://www-ncbi-nlm-gov.proxy1.cl.msu.edu/pubmed/25327908) Cell Mol Immunol 11(6):510-21.

1. While I have had an independent research laboratory since 2000, I simultaneously began work as scientific director of a biomarker and translational laboratory in support of one of the leading breast cancer prevention and survivorship programs with Dr. Carol Fabian at the University of Kansas Medical Center. I was recruited into this leadership position as an early adopter of molecular biomarkers in my endocrine disruption work. In this role, we developed novel animal models of breast and ovarian cancer, arguing for a combined approach to prevention of these diseases, and conducted many biomarker-based human trials of chemoprevention agents and novel biomarkers for breast cancers in high risk women. This work culminated in our selection as Komen Promise Scholars in 2011.
   1. Ting AY, Kimler BF, Fabian CJ, **Petroff** **BK**. 2006 [Characterization of a preclinical model of simultaneous breast and ovarian cancer progression.](http://www.ncbi.nlm.nih.gov.proxy.kumc.edu:2048/pubmed/16891317) Carcinogenesis. 28:130-5. PMID: 16891317.
   2. Fabian CJ, Kimler BF, Zalles CM, Khan QJ, Mayo MS, Phillips TA, Simonsen M, Metheny T, **Petroff** **BK**. 2007. [Reduction in proliferation with six months of letrozole in women on hormone replacement therapy.](http://www.ncbi.nlm.nih.gov.proxy.kumc.edu:2048/pubmed/17221152) Breast Cancer Res Treat. 106:75-84. PMID: 17221152. (PMC# not required; not NIH funded).
   3. **Petroff BK**. 2012. A case for combined ovarian and breast cancer prevention. Med Hypoth. 79:549-551. PMID: 22867867. (PMC # not required; not NIH funded).
   4. Delman D, Geiser J, Fabian CJ, Kimler BF, **Petroff BK**. 2015 Effects of flaxseed lignan secoisolariciresinol diglucoside on preneoplastic biomarkers of cancer progression in a model of simultaneous breast and ovarian cancer development. Nutr Canc, 67:857-861. PMID: 26010915. (PMC # not required; not NIH funded)
2. While analyzing ovarian samples from a cancer chemoprevention study using the SERM tamoxifen (TAM), we noticed that, while the ovarian carcinogen used to induce ovarian cancer in treated rats depleted follicular reserves, there were many ovarian follicles remaining in the rats receiving carcinogen + TAM. Based on this incidental finding, we designed and conducted multiple experiments in vivo and in vitro testing the hypothesis that TAM could protect the ovary from toxic exposures including chemotherapy agents cyclophosphamide and doxorubicin. These publications demonstrated a protective local ovarian action of TAM that was previously unknown. Other laboratories have joined in this work, demonstrating a similar protective ovarian action of TAM for toxic doses of therapeutic ionizing radiation (Gleicher et al., 2013).
   1. Ting AY, Kimler BF, Fabian CJ, **Petroff** **BK**. 2008 [Tamoxifen prevents premalignant changes of breast, but not ovarian, cancer in rats at high risk for both diseases.](http://www.ncbi.nlm.nih.gov.proxy.kumc.edu:2048/pubmed/19139004) Cancer Prev Res 1:546-53. PMC2748942.
   2. Ting AY, **Petroff** **BK**. 2010 [Tamoxifen decreases ovarian follicular loss from experimental toxicant DMBA and chemotherapy agents cyclophosphamide and doxorubicin in the rat.](http://www.ncbi.nlm.nih.gov.proxy.kumc.edu:2048/pubmed/20711751) J Assist Reprod Genet. 11:591-7. PMC2995431.
   3. Ting A, **Petroff BK**. 2015. Challenges and potential for fertility preservation with SERMs. Biol Reprod 92:133-138. PMID: 25810474.
   4. Piasecka-Srader J, Blanco FF, Delman DH, Dixon DA, Geiser JM, Ciereszko RE, **Petroff BK**. 2015. Tamoxifen prevents apoptosis and follicle loss from cyclophosphamide in cultured rat ovaries. Biol Reprod 92:132-139. PMID: 25833159.

**D. Research Support**

Current Research Support

(Petroff, BK) 1June, 2019 – 31 May, 2023

*02659: Breed Specific Reference Ranges for Canine Thyroid Testing, Role: PI*

(Petroff MG) 05/01/2020-04/30/2025

NICHD

*Endocrine regulation of maternal immunity in pregnancy* Role: Co-I

Completed Research Support (recent):

(Petroff MG) 05/18/2018-04/30/2019

NIH 1R21HD083058 *Shared Placenta/Tumor Antigens and Immunity, Role: Co-I*

(Petroff BK) 01/01/2018-12/31/2019

Glassen Foundation

*Canine autoimmune thyroiditis and risk of autoimmune ovarian disease, Role: PI*

(Petroff BK and Refsal K,) 09/01/2016-12/31/2017

Canine Health Foundation/Orthopedic Foundation for Animals

*Thyroglobulin autoantibody and progression of canine autoimmune thyroiditis*, *Role:PI*

*Past ERF Funding:*

(Petroff BK) 06/01/2015-05/31/2016

*Mechanisms of canine autoimmune hypothyroidism, Role: PI*

Data from this study were used for successful grant application to the Canine Health Foundation (above). Presented, European College of Veterinary Internal Medicine.

OMB No. 0925-0001 and 0925-0002 (Rev. 10/2021 Approved Through 09/30/2024)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.  
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Buchweitz, John Philip

eRA COMMONS USER NAME (credential, e.g., agency login): JOHN.BUCHWEITZ

POSITION TITLE: Associate Professor Health Professions & Toxicology/Nutrition Section Chief

EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)

| INSTITUTION AND LOCATION | DEGREE  (if applicable) | Completion Date  MM/YYYY | FIELD OF STUDY |
| --- | --- | --- | --- |
| Michigan State University | BS | 09/1992 | Biochemistry |
| Michigan State University | MS | 09/2001 | Animal Science |
| Michigan State University | Dual PhD | 09/2007 | Pharmacology and Toxicology & Environmental Toxicology |
| Van Andel Research Institute | Postdoctoral | 03/2009 | Protein Biochemistry |
| University of Florida | Grad Cert. | 05/2015 | Clinical Toxicology |

**A. Personal Statement**As Nutrition and Toxicology Section Chief for the MSU Veterinary Diagnostic Laboratory I am responsible for overseeing the multitude of analytical tests performed, the results attained and reported from those tests, and providing their accompanying interpretations within the context of animal health and disease. Analysis of unknown contaminants and intentional poisoning incidents in companion animal and wildlife populations are routine within my laboratory. Additionally, my laboratory helps veterinary scientists and regulatory professionals quantitate various minerals, drugs and pesticides in a variety of matrices through the development or use of well-characterized and stringently validated diagnostic tests. As a PI- or co-Investigator on several university- and FDA-funded grants, my laboratory continues to build the capacity to respond to chemical adulterants within the nation’s food/feed supply. Accordingly, I am responsible for seeking internal and external support for equipment purchases, technical staff, and support of undergraduate, graduate and professional students across the university and within the college. In summary, I have the expertise, leadership, supporting personnel and state-of-the-art equipment necessary to successfully carry out proposed research projects.

**Ongoing and recently completed projects that have received funding are highlighted here:**

**MSU CVM Endowed Research Funds**

**Buchweitz (PI)**

07/01/22-07/01/24

Seed Grant: Evaluation of Food Fraud within the Commercial Pet Food Industry

**Food and Drug Administration F0052U**

Dodd (PI), Role: **Buchweitz (co-PI)**

06/01/22-05/31/27

Michigan State University VDL VetLRN 2022

**Michigan Dept. of Agriculture M1254M**

Tsoi (PI), Role: **Buchweitz (co-investigator)**

05/01/22-06/30/25

Pilot study to develop a model of hypocalcemia in laying hens.

**B. Positions, Scientific Appointments, and Honors**

**Positions**

2018 - 2023 Nutrition Section Chief Veterinary Diagnostic Laboratory Michigan State University, East Lansing, MI

2012 - 2023 Toxicology Section Chief Veterinary Diagnostic Laboratory Michigan State University, East Lansing, MI

2019 - 2023 Associate Professor, Health Professional Veterinary Diagnostic Laboratory Michigan State University, East Lansing, MI

2012 - 2019 Assistant Professor Health Professional, Veterinary Diagnostic Laboratory Michigan State University, East Lansing, MI

2009 - 2012 Toxicologist, Michigan Department of Agriculture and Rural Development, Lansing, MI

**Scientific Appointments**

2022 - 2026 Executive Board, American Board of Toxicology (ABT)

2013 - 2023 Member, American Association of Veterinary Laboratory Diagnosticians (AAVLD)

2004 - 2017 Member, Society of Toxicology (SOT)

2004 - 2017 Member, Michigan Regional Chapter of the Society of Toxicology (MI-SOT)

2014 - 2016 Representative, MISOT Regional Chapter K-12 Education Committee

2014 - 2015 Executive Board, Association of Analytical Communities Central Chapter (AOAC)

2012 - 2015 Member, Association of Analytical Communities (AOAC)

**Honors**

2020 FDA Group Recognition Award. Method Collaboration Team for Aflatoxin B1 in Dog Food.

2018 FDA Group Recognition (Agency Crosscutting) Award. Pentobarbital in Pet Food Investigation and Recall Group.

2017 MSU Center for Service Learning and Civic Engagement. Flint companion animal blood lead testing.

2015 FDA Group Recognition Award. Lascadoil Animal Feed Contamination Response Group.

2013 FDA Commissioner’s Special Citation (Group). Salmonella Contaminated Pet Food Investigation Team.

2013 FDA Group Recognition Award. Development of the RRT Best Practices Manual.

2010 Michigan Department of Agriculture Team Excellence Award.

**C. Contributions to Science**

1. **My primary responsibility as a Section Chief is to provide a diagnostic service to clients that makes use of standard and novel approaches to analyze a sundry of mineral and organic compounds in matrices of veterinary importance. Publications derived from this service highlight method improvements that may add value for other diagnostic laboratories within the veterinary network. I generally provide initial input to the conduct of these studies and serve as a senior author to manuscripts.**
2. Lehner, A.F. and **Buchweitz, J.P.,** 2022. Benefits and Malefits of Solvent Vent Mode in Combination with Tandem Mass Spectrometry for Static Headspace Analysis of Organic Solvents by Gas Chromatography. *Chromatographia*, *85*(4), pp.315-331.
3. Lehner, A., Johnson, M., Zimmerman, A., Zyskowski, J. and **Buchweitz, J.,** 2021. Vitamin D analyses in veterinary feeds by gas chromatography-tandem mass spectrometry. *European Journal of Mass Spectrometry*, *27*(1), pp.48-62.
4. Lehner, A.F., Johnson, M. and **Buchweitz, J.,** 2018. Veterinary utility of dried blood spots for analysis of toxic chlorinated hydrocarbons. *Toxicology Mechanisms and Methods*, *28*(1), pp.29-37.
5. **Additionally, from a clinical perspective, publications arise that detail unique client-based case reports detailing a more complete clinical picture. These reports are often published in collaboration with the submitting clinician, or pathologist. For these publications, my target audience is generally the clinical forensic toxicologist and I typically serve as the primary author.**
6. **Buchweitz, J.P.,** Johnson, M., Wixson, M. and Puschner, B., 2022. Quantitation of Methamphetamine and Amphetamine in Postmortem Canine Tissues and Fluids. *Journal of Analytical Toxicology*, *46*(2), pp.e92-e96.
7. **Buchweitz, J.P.,** Zyskowski, J. and Lehner, A.F., 2022. Heroin Fatality in a Feline: A Case Report with Postmortem Liver Concentrations. *Journal of Analytical Toxicology*, *46*(1), pp.e36-e41.
8. **Buchweitz, J.P.,** Mader, D. and Lehner, A.F., 2019. Bifenthrin fatality in a canine: a case report with postmortem concentrations. *Journal of Analytical Toxicology*, *43*(1), pp.72-78.
9. Moreover, the diagnostic work conducted in my lab is frequently tied to food safety concerns and accompanying regulatory measures. It is important to disseminate the diagnostic approaches taken and provide awareness to the agriculture industry and the appropriate regulatory authorities of the necessity to monitor for these types of adulterants. Depending on the audience type selected, I serve as either first or senior author.
10. Lehner, A.F., Zyskowski, J., Fulton, R.M. and **Buchweitz, J.P.,** 2020. Haloxyfop determination by gas chromatography/tandem mass spectrometry in eggs. *Rapid Communications in Mass Spectrometry*, *34*(19), p.e8895.
11. **Buchweitz, J.P.,** Johnson, M., Jones, J.L. and Lehner, A.F., 2018. Development of a Quantitative Gas Chromatography–Tandem Mass Spectrometry Method for the Determination of Pentobarbital in Dog Food. *Journal of agricultural and food chemistry*, *66*(42), pp.11166-11169.
12. **Buchweitz, J.,** McClure-Brinton, K., Zyskowski, J., Stensen, L. and Lehner, A., 2015. Lead isotope profiling in dairy calves. *Regulatory Toxicology and Pharmacology*, *71*(2), pp.174-177.
13. **As a collaborator with researchers in academia and public and private sectors, I work closely with my colleagues to ensure that methodologies are well executed and written to their needs. Since I am providing little input to the direction or actual conduct of the study, my role in providing method write-ups and manuscript review leads to variable inclusion and position placement on manuscripts.**
14. Viner, T.C., Kagan, R.A., Lehner, A. and **Buchweitz, J.P.,** 2022. Anticoagulant exposure in golden eagle (aquila chrysaetos) power line electrocution and wind turbine mortalities. *The Journal of Wildlife Diseases*, *58*(2), pp.348-355.
15. Johnson, S.D., **Buchweitz, J.P.** and Lehner, A.F., 2022. Single oral or intravenous administration of voriconazole achieved recommended therapeutic minimum inhibitory concentrations against Aspergillus in the common raven (Corvus corax). *American Journal of Veterinary Research*, *83*(10).
16. Czaran, V., Edwards, J., **Buchweitz, J.,** Finney, C., Chikweto, A., Butler, B.P. and Marancik, D., 2022. Occurrence of Histamine Toxicity and Metal and Mineral Contaminants in Invasive Lionfish (Pterois volitans) in Grenada, West Indies. *Caribbean Journal of Science*, *52*(1), pp.114-125.
17. **Lastly, as a mentor, or committee member to residents, undergraduate, and graduate students, the work generated by, and in collaboration with, my students/mentees are instrumental to their professional development and scholarship.**
18. Slabe, V.A., Anderson, J.T., Millsap, B.A., Cooper, J.L., Harmata, A.R., Restani, M., Crandall, R.H., Bodenstein, B., Bloom, P.H., Booms, T. and **Buchweitz, J.,** 2022. Demographic implications of lead poisoning for eagles across North America. *Science*, *375*(6582), pp.779-782.
19. Slabe, V.A., Anderson, J.T., Cooper, J., Miller, T.A., Brown, B., Wrona, A., Ortiz, P., **Buchweitz, J.,** McRuer, D., Dominguez‐Villegas, E. and Behmke, S., 2020. Feeding ecology drives lead exposure of facultative and obligate avian scavengers in the eastern United States. *Environmental toxicology and chemistry*, *39*(4), pp.882-892.
20. Strickland, J.M., Herdt, T.H., Sledge, D.G. and **Buchweitz, J.P.,** 2019. Survey of hepatic copper concentrations in Midwest dairy cows. *Journal of dairy science*, *102*(5), pp.4209-4214.

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In: Canine and feline endocrinology, 4th ed. USA: Elsevier; 2015: 77-135.

2. Kemppainen RJ, Clark TP. Etiopathogenesis of canine hypothyroidism. Vet Clin North Am Small Anim Pract 1994; 24:467-476.

3. Graham PA, Nachreiner RF, Refsal KR, Provencher-Bolliger AL. Lymphocytic thyroiditis. Vet Clin North Am Small Anim Pract 2001; 31:915-933, vi-vii.

4. Graham PA, Refsal KR, Nachreiner RF. Etiopathologic findings of canine hypothyroidism. Vet Clin North Am Small Anim Pract 2007; 37:617-631.

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6. Bellumori TP, Famula TR, Bannasch DL, Belanger JM, Oberbauer AM. Prevalence of inherited disorders among mixed-breed and purebred dogs: 27,254 cases (1995-2010). J Am Vet Med Assoc 2013; 242:1549-1555.

7. Mooney CT. Canine hypothyroidism: a review of aetiology and diagnosis. N Z Vet J 2011; 59:105-114.

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9. Teti C, Panciroli M, Nazzari E, Pesce G, Mariotti S, Olivieri A, Bagnasco M. Iodoprophylaxis and thyroid autoimmunity: an update. Immunol Res 2021 69:129-138.

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22. Caturegli P, De Remigis A, Chuang K, Dembele M, Iwama A, Iwama S.

Hashimoto's thyroiditis: celebrating the centennial through the lens of the Johns Hopkins hospital surgical pathology records. Thyroid 2013; 23:142-150.

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