2024 Research Project Abstracts

Histological and imaging analysis of the diarrheal parasite, *Cryptosporidium parvum*

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The protozoan parasite *Cryptosporidium* is a leading cause of diarrhea-associated morbidity and mortality in young children globally. Besides being a pediatric pathogen, *C. parvum* is an important zoonotic veterinary parasite that can be transmitted from infected animals to humans and is a common cause of neonatal diarrheal disease in ruminants. Currently, there is no effective treatment available that can fully treat cryptosporidiosis or prevent infection in animals and humans. Our knowledge on the fundamental biology of *Cryptosporidium* and the molecular mechanism governing host-parasite interactions is limited. Identification of essential genes and understanding their biological functions is necessary to advance drug discovery. However, the complex lifecycle of *Cryptosporidium*, that includes multiple asexual and sexual stages, and the small size of the parasites makes it challenging to visualize these stages by standard histological analysis. Ultrastructure expansion microscopy (U-ExM) is a technique that physically expands a biological sample making it easier to visualize intracellular parasites at high resolution. In this study, we will perform histological analysis of intestinal tissue from interferon-gamma knockout (IFN-g KO) mice infected with *Cryptosporidium* transgenic strains to measure changes in intestinal architecture. Additionally, we aim to develop a tissue expansion microscopy method to image all *Cryptosporidium* lifecycle stages in the infected intestinal tissue and compare it to histological analysis. Development of this new method will provide a high resolution of intracellular *Cryptosporidium* stages *in vivo* and allow us to gain new insights into cell biology of this important animal and human pathogen.

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What did the dog bring in?! Do dogs increase their owner's exposure to ticks and mosquitoes?

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Tick and mosquito borne illnesses continue to threaten the health of people and their pets in Illinois. The purpose of this study is to determine risk factors associated with exposure to ticks, mosquitoes, and vector-borne disease in adults, with a focus on dog owners. We hypothesize that there is a relationship between human exposure to ticks and dog ownership, and a relationship between dog exposure to ticks and time spent outdoors, activity level, and home environment type. We created a survey to collect information about tick and mosquito exposure, time spent outside, dog ownership, and vector-borne disease prevention use. Survey participants were recruited from online social media posts, emails, and in-person outreach events. Outreach events included festivals, markets, and educational events at nature centers throughout different regions of Illinois. Data analysis and visualization was conducted in RStudio to identify risk factors associated with exposure to ticks or mosquitoes. The incidence of vector-borne diseases has been increasing rapidly in Illinois, and this study will help to provide a systematic and scientifically sound assessment of the risks as well as potentially effective prevention recommendations and communication methods.

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Epigenetic remodeling of somatic cancer cells through knockdown of KDM6A&B to modulate chemotherapy resistance

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The platinum derivative cis-diamminedicholoplatinum(II) (cisplatin) is a commonly used chemotherapy for patients suffering from solid tumors such as testicular, ovarian, colorectal, lung, cerebral, craniovertebral, and bladder cancers. Cisplatin, in combination with other chemotherapies, can eliminate aggressive metastatic cancers, however, this is uncommon in many patients as cisplatin cell resistance often occurs. Studies are lacking which report cisplatin's mechanism of action and how cell resistance is acquired. Further, within the last decade literature has identified resistance to cisplatin corresponding with polycomb repressive complex 2 (PRC2) function and histone H3 on lysine K27 methylation (H3K27me) in testicular germ cell tumors (TGCT). Restoring H3 K27 methylation using JSK4 (H3K27 demethylase inhibitor) reverse cisplatin resistance in TGCT. This study aims to conduct genetic intervention through knockdown of KDM6A and KDM6B for testicular germ cells and solid tumor cell lines to further confirm and investigate the involvement of histone H3 on lysine K27 methylation. Knock down of KDM6A and KDM6B defends previous pharmaceutical findings for testicular germ cell tumors, and furthermore, establishes the association of histone methylation with decreased cisplatin resistance. However, these findings do not hold true for all solid tumor cell lines investigated in this study.

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Combining coagulopathy diagnosis and SIRS scores to enhance survival prediction in horses experiencing colic

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Acute abdominal pain (colic) is a serious health concern in horses that may disrupt coagulation homeostasis. This study aimed to determine if coagulation abnormalities identified by viscoelastic testing (VCM Vet™) could predict outcomes in horses with colic and if combining coagulopathy diagnosis and systemic inflammatory response syndrome (SIRS) scores improved prognostication. Horses with VCM Vet™ tests at admission were included. Data included diagnosis, PCV, SIRS score, and survival. The coagulopathy cutoff was determined by receiver operator curve analysis. Backward stepwise logistic regression identified parameters differentiating survivors from non-survivors. Forty-four horses were included with 23 survivors and 21 non-survivors. Coagulopathy diagnosis cutoff was ≥1 abnormal coagulation parameter (sensitivity 65.2%, specificity 42.9%). The final model included SIRS score, coagulopathy and clinical diagnosis (area under curve 0.781, sensitivity 52.2%, specificity 100%). Only SIRS scores were significantly associated with survival ($p = 0.005$) in the model but retaining the other two variables improved model specificity. This predictive model includes SIRS score, coagulopathy, and clinical diagnosis. It predicted survival with high specificity and moderate sensitivity, identifying nonsurvivors better than survivors. Although a model excluding coagulopathy had better sensitivity (72.7%) and specificity (85.7%), the final model is more clinically relevant as it correctly identifies non-survivors. These findings suggest that patients with abnormal coagulopathy are less likely to survive, highlighting the importance of viscoelastic testing in colic patients to improve prognostication by identification of coagulopathy.

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Characterizing the epidemiology of a CT-derived BCI calculation in free-ranging Blanding's turtles in Illinois

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Body condition index (BCI) is a standard measurement used to assess the health and fitness of free-ranging Blanding's turtle (*Emydoidea blandingii*) populations. The BCI formula validated for this species integrates mass and straight carapace length to calculate an individual turtle's fat percentage (FP). The objective of this study was to create reference intervals for the expected BCI of turtles based on factors, including location, year, age class, and sex. The specific hypothesis we tested was that the BCI of adult female turtles would be lower when gravid. The weight, shell measurements, age class, sex, and gravidity were measured from 2701 free-ranging Blanding's turtles from Lake, Kane, Cook, and DuPage counties in Illinois between 2016 and 2023. Results indicated that year, county, and age class were significant predictors of BCI, but not gravidity. Overall, the turtles caught in 2016 had a greater BCI than turtles in 2020. The turtles sampled in DuPage County had a statistically lower BCI compared to the turtles caught in the other counties. Lastly, adults were found to have a lower BCI than subadults and juveniles, with no significant differences between females and males. Understanding the differences and trends in the BCI of Blanding's turtle populations across the state of Illinois will improve health surveys and conservation efforts to better manage these endangered species by supporting habitat areas with greatest turtle body condition.

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Investigating prostate-specific membrane antigen as a rapidly detectable marker for canine hemangiosarcoma

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Canine hemangiosarcoma (cHSA) is an often aggressive neoplasm of mesenchymal origin believed to arise from endothelial cells of blood vessels. The gold standard diagnostic test for cHSA uses histologic evaluation with immunohistochemistry (IHC) to detect the expression of tumor cell biomarkers. However, IHC can be time consuming, thus delaying definitive diagnosis and leaving patients and owners uncertain of disease prognosis. A quick cytologic diagnostic test using a cell surface enzyme alkaline phosphatase (ALP) was created for osteosarcoma, which eliminates IHC and instead relies on the enzyme-substrate relationship. Our goal was to identify a specific cell membrane target and validate reagents that can be used to develop a test for cHSA that parallels the ALP test for osteosarcoma. We investigated prostate-specific membrane antigen (PSMA) that is located on the cell surface of prostate carcinoma and cHSA cells but not healthy endothelial cells. An optical imaging ligand of PSMA, DUPA-FITC, was used for fluorescent staining and validation experiments. Four different control prostate carcinoma cell lines were cultured: human PSMA-positive LNCaP and PSMA-negative PC3, and canine PSMA-positive CPA and PSMA-negative ACE-1. Cells were stained with 100 nM DUPA-FITC for 1 hour. Then fluorescent staining was analyzed with confocal microscopy. Four cHSA cell lines were also cultured and stained. We found fluorescent staining on the cell surface of the positive and cHSA cell lines, while no staining was present on the negative cell lines. Future validation experiments will include flow cytometry and cytospin cell preparation using a biotinylated DUPA molecule with streptavidin horseradish peroxidase and substrate chromogen.

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Investigating MGMT expression as a resistance mechanism to temozolomide in canine hemangiosarcoma cell lines

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Hemangiosarcoma (HSA) is the most common splenic cancer diagnosed in dogs. Despite treatment with surgery and adjuvant doxorubicin (DOX) chemotherapy, long term prognosis remains poor. In an attempt to improve outcomes, several clinical studies have assessed combination chemotherapy protocols; however, none appear vastly superior and are more toxic compared to single-agent DOX. Temozolomide (TMZ), an oral chemotherapy drug, has safety data available when combined with DOX. TMZ works by methylating nucleotide bases. Overexpression of O-6-methylguanine-DNA methyltransferase (MGMT), a DNA repair protein, is a major determinant of TMZ resistance. This study aimed to investigate canine HSA cell line sensitivity to TMZ and the correlation with MGMT expression. If such correlation exists, MGMT expression may be explored as a biomarker to stratify patients that may benefit from the combination of DOX and TMZ. MGMT expressions were investigated in five canine HSA and one canine endothelial cell lines by qPCR and western blot. Cytotoxicity assays (+/- an MGMT inhibitor) were performed to assess the sensitivity of canine HSA cell lines to TMZ. MGMT was variably expressed by the canine HSA cell lines and expression patterns were comparable between gene and protein assessments. Overall, canine HSA cell lines are considered resistant to TMZ, and their resistance did not correlate with MGMT expression levels. This in vitro investigation demonstrates that MGMT status cannot predict sensitivity to TMZ and therefore suggests canine HSA cells have an MGMT-independent resistance mechanism. These in vitro data do not support the clinical exploration of TMZ in combination with DOX for treating dogs with splenic hemangiosarcoma.

Research Grant: Veterinary Student Scholar
Student Support: Morris Animal Foundation

Quantifying kisspeptin & GnRH expression in 10 day old piglets exposed to atrazine perinatally

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Atrazine is a widely used herbicide that is especially prevalent in vast agricultural areas of the United States, such as the Midwest. Known as an endocrine-disrupting chemical on the adult hypothalamic-pituitary-gonadal axis, atrazine can have negative reproductive effects. In the hypothalamus, two neuron populations are critical in reproductive regulation: kisspeptin (Kiss) and gonadotropin-releasing hormone (GnRH). Rodent models have confirmed that acute atrazine toxicity can reduce GnRH neuronal activation, lower *Kiss1* mRNA expression, or reduce reproductive hormone secretion. However, how developmental atrazine exposure impacts GnRH and Kiss protein expression in the brain remains relatively unknown. Our goal was to determine how atrazine exposure impacts Kiss and GnRH expression in the piglet model. Pregnant sows were exposed to atrazine (20µg/liter water) or untreated water from gestation day 28 to postnatal day 10. We used immunohistochemistry to stain for Kiss and GnRH on 10-day old female piglet brains. We found no significant difference between atrazine-exposed individuals (n=5) and controls (n=5) for cell counts, fiber density, or percent area stained for Kiss or GnRH. Although, when we analyzed variables with Cohen's D, we found a large effect size between atrazine and control piglets. Our data suggests that perinatal exposure to atrazine minimally affects Kiss and GnRH neuron expression in 10-day old, pre-pubertal piglets. Future work will include male piglet brains and data will be analyzed across groups and sex. It is critical to identify how atrazine exposure impacts the health of animals in agriculture, specifically in food animal production, as reproductive viability is necessary for its success.

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Characteristics of meconium impaction/retention in newborn foals: 2006 to 2024.

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Meconium impaction/retention is a significant cause of colic in foals; about 75% of foals treated medically survive but case population has not recently been described. Our objective was to describe signalment, presentation, treatment, outcome, and comorbidities in newborn foals 0-3 days of age with meconium impaction. Medical records from 2006-2024 were searched and foals with a history of straining to defecate, abdominal distention, colic, and/or tail flagging were included. Forty-three foals met the inclusion criteria. Foals were 0.8±0.9 (range 0-3) days of age at presentation. Male foals dominated (30/43; 70%). Breeds reflected hospital population: Standardbred (17/43; 40%), Thoroughbred (9/43; 21%) and Quarter Horse (7/43; 16%). All foals were managed with enemas; Fleet™ (11/43; 30%), water with soap/lube (25/43; 58%), retention (1; 2%), multiple types (8; 19%); no type specified in 9/43 (21%). Forty foals (93%) survived to discharge, 3 (7%) were humanely euthanized. Of surviving foals, 36/40 (90%) responded fully to medical treatment. Surgical treatment was undertaken in 4 foals (9%); 3/4 (75%) survived. An additional 3 foals underwent surgery unrelated to meconium impaction: umbilical resection, ruptured bladder, and periosteal stripping. Comorbidities were common including sepsis (10/43; 23%), pneumonia (10/43; 23%), and failure of passive transfer (6/43; 14%), Hypoxic-Ischemic Encephalopathy (5/43; 12%). Non-survivors (3/43; 7%) were euthanized due to sepsis, limb malformation, and pneumonia. Medical management of meconium impaction/retention is successful in most cases. Prognosis depends on comorbidities present.

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Research Grant: None

Prevalence and MRI characterization of subclinical lumbosacral disc disease in dogs

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Degenerative lumbosacral stenosis (DLSS) is a common cause of lower back pain in dogs. The condition is considered multifactorial due to narrowing of the intervertebral canal or intervertebral foramina causing compression of the cauda equina nerve roots. Magnetic resonance imaging (MRI) is often used for DLSS diagnosis due to its soft tissue contrast. However, the diagnosis remains challenging as degenerative changes to the lumbosacral (LS) joint are often present in older animals. Thus, some patients may erroneously be diagnosed with DLSS based on non-clinical changes. The purpose of this study was to determine the prevalence of subclinical LS disc protrusions in dogs and compare the MRI findings to those diagnosed with DLSS. MR images of the LS spine in dogs presenting to the University of Illinois Veterinary Teaching Hospital (VTH) were examined. A search via the VTH's PACs system was performed to identify dogs who underwent LS MRI between 01/01/17 and 05/31/24. Dogs were excluded from the study if they were diagnosed with or exhibited neurologic signs of LS disc disease at the time of their MRI. A total of 234 dogs had LS protrusions on MRI; of those, 93 met inclusion criteria. 168 dogs had evidence of degenerative changes to the LS junction on MRI and, of those, 92 were asymptomatic. Two measurements on MRI will be compared between dogs with subclinical and clinical LS compression to determine associations with subclinical protrusions at the LS junction: LS angle and compression ratio. By finding associations between MR characteristics and subclinical protrusions, this research could potentially lead to an improved imaging diagnosis of DLSS, thus allowing improvement in the guidance of potential treatment options.

Research Grant: None
Student Support: University of Illinois College of Veterinary Medicine

Acute phthalate exposure during adulthood and its late-life effects on ovarian aging markers in mice

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Phthalates, such as di(2-ethylhexyl) phthalate (DEHP) and di-isononyl phthalate (DiNP), are low-cost manufactured plasticizers identified as reproductive toxicants. In females, their endocrine disrupting properties may affect sexual development, maturity, and fertility. We hypothesized that phthalate-induced ovarian aging is contributing to these health issues. This study examined DEHP and DiNP exposure and their effects on ovarian aging through investigating inflammation, fibrosis, and telomere associated genes—markers of ovarian aging in females. Adult female CD-1 mice were orally dosed daily for 10-days. Vehicle controls (n=8) were dosed with corn oil. Treated groups (n=7-10) concentrations consisted of DEHP 20 µg, 200 µg, 20 mg, 200 mg and DiNP 20 µg, 100 µg, 20 mg, 200 mg per kg/BW. At fifteen months post-exposure, ovaries were harvested and frozen for gene expression and inflammation marker evaluation. Cytokine arrays were used to compare the effects of DEHP and DiNP on inflammation markers (IMs). qPCR was used to analyze gene expression of collagen, antioxidant, inflammatory (InF), and telomere associated genes (TAG). DEHP caused more changes in IMs than DiNP. DEHP (20 mg, 200 mg) and DiNP (20 µg) both increased IMs, whereas DEHP (20 µg) and DiNP (20mg, 200 mg) decreased IMs. DiNP decreased (20 µg) and increased (100 µg, 20 mg) a TAG, known as *Terc,* compared to control. DiNP (20 mg) and DEHP (20 µg) both increased the InF gene, *IL-13,* when compared to control. DiNP (20 mg) also decreased the InF gene, *Nlrp3,* compared to control. In conclusion, acute exposure to phthalates may cause changes in inflammatory markers in the ovary, with DEHP causing more changes than DiNP.

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The role of plasma-derived extracellular vesicles in exercise-induced adult hippocampal neurogenesis.

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Many studies have established that exercise can improve physical and mental health. At least some of the mental health benefits have been attributed to increased adult hippocampal neurogenesis which occurs with exercise in mice. However, we currently do not know how exercise increases neurogenesis. One hypothesis is that extracellular vesicles (EVs) released from muscles or other organs travel in the blood of exercising mice and carry molecular signals that interact with the brain. The goal of this study was to determine whether EVs extracted from the blood of exercising mice are sufficient to increase neurogenesis when injected into sedentary mice. EVs were isolated from both sedentary mice (SedV) and mice with access to a running wheel (ExerV) and then injected interperitoneally into a new group of sedentary mice twice a week for 4 weeks. An additional cohort of sedentary mice were injected with PBS to serve as the control. BrdU (bromodeoxyuridine) injections were given the first 10 days to label dividing cells. Preliminary results show sedentary mice receiving ExerV injections had 1.2-fold increased numbers of BrdU-positive cells compared to sedentary mice receiving SedV injections or PBS injections. Approximately 88% of the BrdU+ cells were co-labeled with the mature neuronal marker, NeuN, and 5% were co-labeled with the mature astrocyte marker, S100. This result suggests ExerVs increased both the number of new astrocytes and new neurons in the hippocampus. The experiment is currently being repeated to determine the reliability of the results. If ExerVs can increase hippocampal neurogenesis they may provide a useful novel therapeutic for reversing hippocampal atrophy associated with multiple common mental illnesses.

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Whole genome sequencing and identification of genes related to domestication in tame and aggressive red foxes

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The recent domestication of red foxes (*Vulpes vulpes*) provides a unique opportunity to evaluate the role of genetics in animal domestication. The success of fox farming at Prince Edwards Island in the 1900's allowed breeding stock derived from Atlantic Canada to expand throughout North America and Eurasia and due to its recent and widespread domestication, allows for a global comparison between farmed and wild fox population to identify the impact of domestication on the genome. Our aim was to whole genome sequence wild and farm fox populations with varying degrees of tameness to identify genomic signals of domestication. Prior genome comparison identified two regions on chromosome 4 linked to adaptation in captivity. We plan a detailed analysis of these regions to identify positional candidate genes and potentially impactful sequence variants. The recently produced chromosome-level assembly of the fox genome will also allow us to perform fine scale analysis of whole genome sequences to compare and identify previously overlooked regions of interest linked to tame behavior. 56 wild (Great Britain, Ontario, Quebec, Norway, Newfoundland, Wisconsin, and Maryland) and 112 farm-bred (Iowa, Nebraska, Newfoundland, Wisconsin, Poland, Russia (Moscow & Novosibirsk)) foxes were sequenced. Reads were trimmed and aligned to the new reference genome to call single nucleotide variants. Then fixation index analysis will help identify the most divergent regions between wild and farm bred populations with different behavioral phenotypes. This study's findings will benefit our genetic knowledge of animal behavior considering its role in fox domestication and will provide insight into species with more complex domestication histories.

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Panthera uncia papillomavirus 1 association with sublingual plaques and squamous cell carcinoma in snow leopards (*Panthera uncia*)

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Snow leopards (*Panthera uncia*) (SLs) managed through the Association of Zoos and Aquariums Species Survival Plan are commonly diagnosed with sublingual mucosal plaques. A papillomavirus, *Panthera unica* papillomavirus 1 (PuPV1), was isolated from a single sublingual plaque; however, prevalence and potential association with hyperplastic sublingual lesions throughout the SL population are unknown. In addition to plaques, the SL population under human care also has a high prevalence of oral and sublingual squamous cell carcinoma (SCC). In other species, including domestic cats, there is an association between papillomavirus-induced hyperplastic lesions and the transformation to Bowenoid *in situ* carcinoma and invasive SCC. This research aims to determine if PuPV1 is associated with sublingual plaques and oral squamous cell carcinomas in SLs. Formalin-fixed paraffin-embedded tissue samples (n=63) from SLs including 7 SCCs, 36 sublingual plaques, and 20 negative controls were evaluated. Polymerase chain reaction (PCR) and RNAscope™ in situ hybridization (ISH) were performed to test for the presence of PuPV1 DNA and RNA expression. In SLs with sublingual plaques, 97% were PuPV1 positive as evidenced by both positive PCR and ISH. Microscopically viral cytopathic effects (hypergranulosis, koilocytosis, clumped tonofilaments) and hyperkeratosis were strongly associated with PuPV1 infection ($p<0.05$). SCC was not associated with PuPV1 infection as samples were negative for PuPV1 using PCR and ISH. SLs are considered vulnerable to extinction; this information can aid conservation efforts by guiding veterinary medical care and management decisions for the population under human care.

Student Support: Office of the Director, NIH, T35 OD011145

Malassezia microbiology: characterization of yeast isolates from canine otitis externa cases

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Otitis externa (OE) is a common disease of dogs, characterized by inflammation of the ear canal, and frequently complicated by secondary bacterial and fungal infections. The yeast *Malassezia pachydermatis* is traditionally presumed to be associated with OE. Literature reports, as well as clinical experience in the dermatology/otology service at the University of Illinois Veterinary Teaching Hospital, suggest an increase in the incidence of persistent or recurrent OE cases associated with the presence of yeast cells. Traditional miconazole treatment does not produce the clinical resolution previously observed in most cases. Hypotheses to explain this observation include i). emerging miconazole resistance in *M. pachydermatis* isolates and/or ii). involvement of *Malassezia* species other than *M. pachydermatis* that cause OE in dogs and have intrinsically lower miconazole susceptibility. Work presented here involved adaptation of assays to evaluate miconazole susceptibility for a collection of yeast isolates sampled from OE ears of dogs enrolled in our ongoing clinical study. Although the cellular morphology of these strains was consistent with *Malassezia* species, two of these isolates could not be identified as *M. pachydermatis* using MALDI-TOF mass spectrometry. We also worked toward development of a method for removing the *Malassezia* cell wall to create spheroplasts that can be readily lysed to release high-molecular-weight DNA. Long-read whole genome sequencing will reveal the diversity within the isolate collection and indicate whether some isolates represent other *Malassezia* species. Results from this work will aid diagnostic identification of OE yeast isolates as well as inform treatment protocols to resolve disease.

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