Do dogs increase their owner's exposure to ticks and mosquitoes?

Holly E. Black, Rebecca L. Smith

Department of Pathobiology, College of Veterinary Medicine, University of Illinois, Urbana IL, Midwest Center of Excellence for Vector-Borne Disease, Madison WI

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 movement, activities, and home environment type. We created a survey to collect information about tick and mosquito exposure, time spent outside, dog ownership, and prevention use. Survey participants were recruited from online social media posts and in-person outreach events. Outreach events included festivals, markets, and educational events at nature centers throughout different regions of Illinois. Survey recruitment is ongoing, but preliminary analysis includes logistic regression to identify factors associated with exposure to ticks or mosquitoes or having been diagnosed with a tick-borne disease. Incidence of vector-borne diseases has been increasing rapidly in Illinois, and we believe that this study will help to provide a systematic and scientifically sound assessment of the risks and potentially effective prevention and communication methods.

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Effects of non-native plant invasions on tick-borne disease risk in Illinois

Jessica L. Brooks, Maria G. Muñoz, Brian F. Allan

College of Veterinary Medicine (Brooks) and School of Integrative Biology (Munoz, Allan), University of Illinois at Urbana-Champaign, Urbana, IL

Non-native plant species can greatly alter the ecological areas they invade by affecting a variety of both biotic and abiotic factors in an ecosystem. While this can have a negative impact on some species, recent research suggests that some non-native plants may provide a more conducive environment for ticks. This may occur by multiple ecological mechanisms, including altering habitat suitability for ticks or their wildlife hosts. This is a concern for both humans and animals as ticks are important vectors for multiple emerging pathogens. This study aimed to gauge the effects of multiple invasive plant species on tick populations compared to their native counterparts in natural areas located in Northern, Southern, and Central Illinois. Ticks were collected through "drag sampling" (i.e. dragging a 1m² white cloth over vegetation) in paired plots either dominated by non-native plant species or control plots dominated by native plant species. After collection, ticks were preserved in ethanol and identified to species and life stage. Preliminary results suggest that some species of non-native plants appear to be more conducive to ticks than others. Continued research is planned to quantify the effects of different non-native plant species on the microclimates experienced by ticks, resulting differences in tick survival, and the prevalence of tick-borne pathogens in invaded and uninvaded habitats.

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Prenatal phthalate exposure alters the development of sex-specific vocalization calls in neonatal mice

Joseph Caffarini, Jacob Maxon, Howard Gritton, Megan Mahoney

Department of Comparative Biology, College of Veterinary Medicine, University of Illinois at Urbana-Champaign

In many species, social interactions can be characterized by vocalizations, including calls for maternal care, courtship, and play, that often differ across sexes due to changes in hormone signaling during neural development. Estradiol notably organizes changes in the fetal brain to promote sexual differentiation. Some environmental chemicals, including phthalates, resemble estrogen and can alter hormone signaling resulting in unspecified effects on social-sexual development. Therefore, we tested the hypothesis that in utero phthalate exposure alters hormone signaling in the developing brain, leading to reduced male-specific social behaviors after birth. We dosed pregnant CD1 mice with oil (control) or phthalates from gestation day 10.5- birth (20 ug/kg/day. 200 ug/kg/day and 200 mg/kg/day). Pups (male and female) were isolated for 5 min from their litter briefly at postnatal day (PND) 3 (n=32), 6 (n=33), 9 (n=37), and 12 (n=35) to elicit calls for maternal care that were captured at each time point. We utilized machine learning tools to identify syllables that we sorted by shape, resulting in eight syllables: Short, Slope Up, Concave, Flat, Tilde, Complex, Slope Down, and Harmonic. Using statistical analysis, we compared syllable occurrences across dosage and age groups. We discovered that in control mice, sex specific vocalizations are divergent beginning at PND 12, with female animals showing more complex, uniformly distributed syllables. In contrast, male mice show a reduced vocabulary with highly repeated syllables. Our results suggest that exposed mice are born with feminized call repertoires, and that in utero phthalate treatment changes neural circuits important for producing sex-specific social communication.

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Host-derived PDCD6 mediates proliferation and egress of intracellular *Toxoplasma gondii* in rat macrophages.

Jordan Demanty, Shahbaz Khan, William H. Witola

Department of Pathobiology, College of Veterinary Medicine, University of Illinois Urbana-Champaign

Toxoplasma gondii is an important zoonotic parasite capable of causing profound disease in almost any mammalian host. Toxoplasmosis can manifest as miscarriages, encephalitis, and blindness in humans, and it is a main cause of abortions in small ruminants. It is well documented that this parasite utilizes a plethora of mechanisms to manipulate host machinery at the molecular level, but novel interactions continue to be elucidated. Several recent studies have demonstrated that Toxoplasma exploits the host endosomal sorting complex required for transport (ESCRT) machinery for scavenging nutrients and organelles from the host. In this study, we investigated the role of programmed cell death protein 6 (PDCD6), an adaptor ESCRT protein, in mediating T. gondii survival within rat macrophages. Using PDCD6-KO- and wildtype-NR8383 rat macrophage cell lines, we found that the genetic disruption of host PDCD6 restricts the *in vitro* growth and multiplication of the parasite within macrophages. Furthermore, we observed a "delayed-egress" phenotype in T. gondii tachyzoites growing within PDCD6deficient cells as compared to those infecting wild-type cells, indicating that PDCD6 is required for egress of the parasite from host cells. Overall, our results provide additional evidence for the idea that Toxoplasma utilizes components of the host ESCRT machinery to carry out important functions for its survival and proliferation. Future in-depth studies elucidating this novel relationship between *T. gondii* and host PDCD6 will be crucial in understanding this important host-parasite interaction.

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The effects of neonicotinoid pesticide imidacloprid on genes that regulate steroidogenesis in canine ovaries.

<u>Justin Huff</u>, Vasiliki Mourikes, and Jodi A. Flaws University of Illinois College of Veterinary Medicine, Department of Comparative Biosciences

Neonicotinoid insecticides are widely used in agriculture, horticulture, forestry and domestic settings to control pests such as fleas and ticks on pets. In insects, neonicotinoids work by binding to the same receptors in the nervous system as nicotine, causing overstimulation of the nervous system which leads to paralysis and death. Imidacloprid (IMI) is a neonicotinoid insecticide commonly used for its high affinity for insect nicotinic cholinergic receptors (nAChRs) and low affinity for mammalian nAChRs. Despite the common use of IMI, limited information exists on how IMI affects the ovary and its functions. Previous studies using mouse ovarian follicles have shown that IMI increased progesterone levels in vitro. Canines are exposed to IMI through flea and tick preventatives, but to our knowledge, the impact of IMI on the canine reproductive system is unknown. To bridge this knowledge gap, we investigated the effects of IMI on the canine ovary. Our hypothesis was that IMI exposure interferes with steroidogenic enzymes and genes that control follicle health in the canine ovarian follicle. To test this hypothesis, antral follicles were dissected from canine ovaries and cultured in media containing vehicle control (DMSO) or IMI (0.2µg/ml - 200µg/ml). After 96 hours, follicles were collected for gene expression analysis of genes that regulate steroidogenesis (Star, Cyp11a1, Cyp17a1, Cvp19a1, Hsd17b1, Hsd3b1) and follicle health (Bcl2 and Bax). We found that IMI did not significantly alter the selected genes. Future studies will investigate hormone production by antral follicles.

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Bold and blue: how lighting environments influence risk-taking behavior in bluefin killifish

Haley Kenyon, Ratna Karatgi, Jolene Blodgett, Becky Fuller

University of Illinois College of Veterinary Medicine, Urbana, IL (Kenyon) University of Illinois School of Integrative Biology, Urbana, IL (Karatgi, Fuller) University of Illinois College of Agricultural, Consumer, and Environmental Sciences, Urbana, IL (Blodgett)

Phenotypic plasticity is the phenomenon where a single genotype elicits multiple phenotypes in a population because of external factors such as environment, diet, and behavior. In bluefin killifish, (Lucania goodei), sire plasticity is responsible for male color polymorphism. Red and vellow phenotypes are largely genetic and controlled by a locus of large effect. Blue coloration, however, depends on the lighting conditions sires are exposed to. These color morphs exhibit various behavior patterns in different lighting environments: blue males are more dominant and less likely to be targeted by predators in dark waters than red and yellow males. However, it's unknown the extent to which extrinsic and intrinsic factors influence specific behaviors. Boldness is an important behavior to study as it affects how an animal interacts with its environment, which in turn shapes its life history. We evaluated how the extrinsic factor of lighting environment and the intrinsic factor of morph type alter risk-taking behavior in bluefin killifish. Two populations of bluefin killifish from differing light environments were studied. Individual fish from the same population were arranged into a triad that was placed into either a tea-stained or clear water tank. One blue male, one female, and one red or yellow male composed a triad. Each triad member's risk-taking behavior was recorded following the introduction of a predator. Afterwards, the lighting environments were switched to the opposite spectrum, and the triads were observed again. If the risk-taking behaviors of both killifish populations are comparable, this could indicate that the grade of boldness a morph exerts in varied lighting arose because of an evolutionary drive.

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Can Early Exercise Be Used to Prevent Fractures in Racehorses? A Look into Fetlock Angle Forces Over Time

<u>Colleen McDonnell</u>, Melany Opolz, Griffin Sipes, Mariana Kersh, and Annette McCoy University of Illinois College of Veterinary Medicine

Fractures in the equine distal limb are a common cause of morbidity and mortality in racehorses. There is a critical need to identify factors that can reduce the number of racing injuries and fatalities. Controlled exercise early in life could increase bone strength, potentially making bones more resistant to fracture, but the ideal exercise regimen is unknown. In silico musculoskeletal models can be used to help predict skeletal responses to exercise but require inputs from in vivo experiments. One such input is joint angles, which allow calculation of vectors of force exerted on the bones. The aim of this study was to determine how fetlock joint angles change over time and with exercise. Twelve foals were enrolled in the study at 8 weeks of age. Six were pasture raised under standard management conditions, while six underwent an 8-week exercise intervention consisting of 1600m trotting at 3.5m/s, 5 days a week. Motion capture videos were collected of age-matched exercised and non-exercised foals prior to the exercise protocol, at the mid-point, and at the end of the protocol. Videos were reviewed using DeepLabCut software and 21 anatomical markers were manually placed to train the software to recognize foal anatomy. Joint angles were measured within DeepLabCut after software training. Changes in joint angles over time will be compared between the exercised and non-exercised foals; foal joint angles will also be compared to adults. If joint angles change with age and exercise, then the forces exerted on bones are different between life stages and with exercise. This will allow us to conclude if it is necessary to input age- or activity-specific joint angles into an in silico musculoskeletal model.

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The effects of epigenetic manipulation on chemotherapy response in somatic versus germ cell tumors

Christine Powell, Ratnakar Singh, Michael J Spinella

College of Veterinary Medicine, University of Illinois, Urbana-Champaign, Urbana, IL, 618101 (Powell), Department of Comparative Biosciences, University of Illinois, Urbana-Champaign, Urbana, IL, 618101 (Singh and Spinella)

Relative to other tumor types, testicular germ cell tumors (TGCTs) are highly sensitive to the chemotherapy drug cisplatin, but the mechanisms behind this sensitivity are unclear. Cisplatin has been used to treat many types of cancer and acquired resistance remains a major challenge to therapy. Previous work in our lab has shown that cisplatin resistance in TGCTs can be overcome with combination therapy that includes agents manipulating the epigenome. This study tested whether a specific epigenetic mechanism influencing cisplatin resistance (i.e., histone tri-methylation at H3K27) in TGCTs can be applied to somatic cell cancers to alter cisplatin response. A total of five cancer cell lines, colon adenosarcoma, osteosarcoma, breast adenosarcoma, and control cisplatin-resistant and cisplatin-sensitive TGCTs, were utilized. First, dose-sensitivity assays were performed to establish appropriate cisplatin concentrations for each tumor type. Cells were treated with cisplatin concentrations ranging from 0 to 20 µM for 6 hours and cell viability was measured after 72 hours using a luminescent based cell viability assay. Next, each tumor type was pretreated with either GSK-126 (H3K27 methyltransferase inhibitor), GSK-J4 (H3K27 demethylase inhibitor), or vehicle control (DMSO). Cells were then treated with previously established doses of cisplatin and measured for cell viability as described above. RT-PCR and immunoblot analysis confirmed H3K27me3 alterations. The results of this head-to-head comparison of TGCTs and other cancers will help us further understand the mechanisms for the unique hypersensitivity of TGCTs to cisplatin.

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Understanding contact between humans and deer in Illinois to identify possible routes of cross-species COVID-19 spread

Ambrielle Pratt, Tara Prezioso, Nohra Mateus-Pinilla, Kimberly Pepin, Rebecca Smith

College of Veterinary Medicine (Pratt), Department of Pathobiology (Prezioso, Mateus-Pinilla, Smith), Department of Animal Sciences, Department of Natural Resources and Environmental Sciences (Mateus-Pinilla), University of Illinois Urbana-Champaign, Urbana IL; USDA APHIS (Pepin)

First manifesting in 2019, SARS-CoV-2 has been found in multiple species, including dogs, cats, minks, and cervids. Multiple regions in the United States, including Illinois, have identified SARS-CoV-2 infections in the wild white-tailed deer (WTD) population. The seroprevalence in Illinois has been estimated at 6.9%; with an overall seroprevalence of 40% estimated across 4 different states. Although the exact path for spillover into wild cervids is unknown, the virus has been shown to replicate within and spread among WTD. There is concern that the virus may spill back into the human population from a wildlife reservoir, potentially following viral evolution. However, transmission requires contact between WTD and humans. The aim of this crosssectional survey was to understand the frequency and type of close contact between the general public in the state of Illinois and WTD, and to identify populations at highest risk for such contact. The survey was distributed electronically using convenience sampling, recruiting participants from list serves, social media, and community partners or extension liaisons. Questions addressed frequency and distance of contact with WTD, encompassing live animals and bodily fluids. Summary statistics were stratified by regions of Illinois, property type, and demographics, and logistic regression was used to identify risk factors. The survey is part of a broader study that seeks to establish whether disease spread in wild cervids correlates to disease spread in humans. We hypothesize that Illinois residents surrounded by deer habitat, and in occupations that involve cervid exposure, will have higher risk close contact with WTD.

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Interactions between 11KT, gonadal composition, brain, and behavior in *A. ocellaris* undergoing sex change

Monica Shotwell, Emma Ibanez, Gabriel Graham, Meghan Connolly, Justin S. Rhodes

College of Veterinary Medicine, University of Illinois, Urbana-Champaign (Shotwell), Department of Psychology, University of Illinois, Urbana-Champaign (Ibanez, Graham, Rhodes), Neuroscience Program, University of Illinois, Urbana-Champaign, (Connolly, Rhodes)

Amphiprion ocellaris clownfish exhibit protandrous sex change from male to female. Clownfish live in small hierarchical groups with only a single reproductive pair and up to several nonbreeding subordinate males. The female is the largest and dominant fish. When a female is displaced, the male changes to female, while the largest subordinate completes the pair. Similarly, when males are paired together, the largest changes sex to female, and the smaller stays a male. There is much to learn about the mechanisms behind sex change regarding involvement of the brain, gonads, and circulating sex hormones. The purpose of this study was to examine the composition of the gonads, sex steroid hormone levels, cellular composition of the brain, and behavior in clownfish undergoing protandrous sex change. Sex change was induced in 10 fish by pairing males together or removing the female. At 6 months, blood was taken to measure sex steroids. The fish were then euthanized for analysis of gonadal composition and brain gene expression. The body was sectioned and stained with H&E. Gonadal composition was determined by outlining ovarian, testicular, and connective tissues using Adobe Photoshop. Preliminary results show 3 fish changed sex as indicated by the presence of vitellogenic oocytes in the gonads or complete loss of testicular tissue. This was associated with low plasma levels of 11-ketotestosterone (11KT) and female-typical behavioral displays. The remaining fish displayed male-typical behavior and 11KT levels and had varying levels of testicular tissue. Analysis of the telencephalon is currently underway using single nuclei RNA sequencing. Results will provide new insight into the neuroendocrinology of sex change in clownfish.

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Progesterone does not antagonize estrogen action in reducing Kisspeptin expression

Kayla Tando, Behrokh Marzbanabbasabadi, Po-Ching Patrick Lin, CheMyong Jay Ko

Department of Comparative Biosciences University of Illinois Urbana-Champaign, Urbana, IL, USA(Tando, Lin, Ko), Amol University of Special Modern Technologies, Amol, Iran (Marzbanabbasabadi)

Kisspeptin (Kiss1), a neuropeptide that controls GnRH secretion, is expressed by Kiss1 neurons which are found mainly in the anteroventral periventricular nucleus (AVPV) and arcuate nucleus (ARC) of the hypothalamus, influencing estrous cyclicity, and also in the amygdala, influencing sexual behaviors. Estrogen induces apoptosis of Kiss1 neurons and progesterone (P4) is known to antagonize effects of estrogen. Prior unpublished studies have shown a neonatal estrogen injection disrupts estrous cycling and causes infertility in adult canines while a neonatal estrogen/P4 injection does not affect cycling but causes infertility. In this study, estrogen and P4 were given to neonatal female rats to evaluate effects on estrous cycling and Kiss1 expression in the AVPV, ARC and amygdala. We hypothesize estradiol benzoate (EB) treated rats will have reduced hypothalamic Kiss1 expression and abnormal estrous cycling while EB+P4-treated rats will have normal hypothalamic Kiss1 expression and estrous cycling but decreased Kiss1 expression in the amygdala. On postnatal day (PND) 1.5, groups were injected with EB, EB+P4 co-treatment, or a control vehicle. Estrous cyclicity was monitored during PND 40-49 and PND 61-70. At PND 70, samples of AVPV, ARC, amygdala, uterus and ovary were collected. Anovaginal distance (AVD) and vaginal opening size were also evaluated. The EB group mainly exhibited proestrus while the EB+P4 group fluctuated between proestrus, diestrus and metestrus. AVD was consistent among all groups. EB-treated and EB+P4-treated groups had smaller vaginal openings and smaller ovaries. Both the EB and EB+P4-treated groups had reduced Kiss1 expression in the AVPV, ARC and amygdala compared to controls. EB and EB+P4-treated rats did not have corpora lutea upon ovarian histological evaluation while controls had multiple corpora lutea. The study is ongoing and will contain behavioral testing data in future trials. Results of this study may have implications regarding neonatal sterilization of animals.

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Transition to Captivity: Genetic Changes in the Domestication of the Red Fox (*Vulpes vulpes*)

<u>Victoria L. Wills</u>, Jennifer L. Johnson, Amber M. Zillinger, Elysia Goodson, Anastasiya Sushkova, Miranda Hamilton, Lyudmila N. Trut, Halie Rando, Anna V. Kukekova.

Department of Animal Sciences, University of Illinois at Urbana Champaign (Wills, Johnson, Zillinger, Sushkova, Hamilton, Rando, Kukekova), College of Veterinary Medicine, University of Illinois at Urbana Champaign (Wills), Institute of Cytology and Genetics of the Russian Academy of Sciences (Trut)

The domestication of red foxes (Vulpes vulpes) has been occurring for 125 years due to fur farming in Eastern Canada. The original farm fox stock came from local wild foxes, but only some successfully transitioned to captive life, resulting in selection for foxes with genetic variants favorable to captivity. Two genomic regions on fox chromosome 4 (region 25 and region 37) were highlighted in previous studies comparing contemporary wild and farm-bred foxes. To further characterize these regions, we genotyped wild fox populations from Ontario, Newfoundland, Maryland, and England and farm foxes from Canada, Poland, Nebraska, Iowa, and Russia with 17 indel markers from region 25 and 12 indel markers from region 37. The genotypes were then used to determine haplotypes in each region and calculate differences in haplotype frequencies between wild and farm populations. If haplotypes under selection in farm foxes are identified, the frequencies of these haplotypes will also be compared between conventional farm bred foxes and populations of tame foxes developed in the Farm-Fox experiment to test if the same genomic regions were important for early fox domestication and selecting foxes for friendly behavior. The markers for these candidate regions together with mitochondrial DNA will also be used to evaluate farm fox admixture in wild fox populations. Specifically, we will compare Canadian wild foxes from wild and suburban areas to test whether farm haplotypes are present in these populations and associated with a habitat. We expect that genetic analysis of regions 25 and 37 in contemporary foxes will provide an insight into the history of fox domestication and pinpoint genes important for fox adaptation to captivity.

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