ASSESSING NEONICOTINOIDS EXPOSURE IN FREE-RANGING WHITE-TAILED DEER IN MINNESOTA

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SUMMARY OF FINDINGS

Recent research has raised concerns about potential adverse effects of neonicotinoid exposure in white-tailed deer (*Odocoileus virginianus*) including reduced survival and productivity. To assess whether free-ranging deer in Minnesota are being exposed to neonicotinoids, we solicited deer hunters to voluntarily submit spleens from harvested animals during the 2019 deer hunting season. Interested hunters signed up to receive a sampling kit (n=1836) and were asked to submit the spleen of their harvested deer and also report the sex, age, date of harvest, and kill location. Hunters could also submit a tooth sample if they wanted their harvested deer aged. A total of 770 spleens and 517 tooth samples were submitted by hunters throughout Minnesota and 29 additional spleens were collected by agency staff from opportunistic deer (agency culling and sick deer). A subsection of each collected spleen (n=799) was submitted to the Ecdysis Foundation (Estelline, SD) for analyses of neonicotinoid exposure; results are expected to be returned by late summer 2020.

INTRODUCTION

With the rising use and popularity of neonicotinoid pesticides in agricultural practices, there are increasing concerns over the potential adverse impacts of these pesticides on wildlife. Neonicotinoids, including imidacloprid, thiamethoxam, and clothianidin, can negatively affect bees and other pollinators through decreased winter survival (Dively et al., 2015), and reduced reproductive success (Laycock et al., 2012). However, less is known about the potential impacts on large mammals, such as white-tailed deer (*Odocoileus virginianus*). With much of Minnesota in an agricultural region, Minnesota deer are likely exposed to various levels of neonicotinoids depending on their location.

Neonicotinoid pesticides are insecticides that are applied through seed treatments, in foliar sprays, applied granularly to pastures, and injected into trees to target destructive insects. They are most commonly used in agricultural practices but also can be found in some residential applications such as gardens, turfs, and animal practices. Five compounds of neonicotinoid insecticides are currently used in the United States, they include: acetamiprid, clothianidin, dinotefuran, imidacloprid, and thiamethoxam (Environmental Protection Agency, 2019). Neonicotinoids act as a neurotoxin by binding to acetylcholine receptors. One advantage of neonicotinoids is their specificity to receptor sites on insects making them less toxic to mammals. By 2008, neonicotinoids accounted for 80% of the seed treatments, and 24% of all insecticide use globally. In North America, of the 133 million acres of corn, soybean, wheat, cotton, and sorghum treated with insecticides, over 98% were treated with neonicotinoids (Minnesota Department of Agriculture, 2016). Furthermore, Main et al. (2014) found canola, wheat, soybeans, corn, barley, field peas, dried beans, and oats were the most common crops treated with neonicotinoid seed treatments in the prairie ranges of Canada.

The most common neonicotinoids used in Minnesota are clothianidin, thiamethoxam, and imidacloprid (Minnesota Department of Agriculture, 2016). In 2016, Minnesota Governor Mark

Dayton passed an executive order stating that a verification of need by the agricultural producer is required to use neonicotinoid pesticides (Executive Order 16-07, 2016). Minnesota was the first state in the U.S. to require such reporting. However, the Minnesota Department of Agriculture (MDA) does not have the authority to regulate treated seeds. This means that the use of neonicotinoids in seed treatments is difficult to track. According to an MDA document (2016), “almost all corn seed and 20% of soybeans treated outside of Minnesota’s borders and shipped into the state for planting is not tracked”.

A recent study conducted in South Dakota was designed to identify the effects of neonicotinoids on captive white-tailed deer by adding controlled amounts of imidacloprid to their water source (Berheim et al., 2019). Berheim et al. (2019) found imidacloprid exposure in all groups of deer, including the control group. Furthermore, as imidacloprid levels in the spleen increased, fawn survival, thyroxine levels, jawbone lengths, body weights, and organ weights decreased suggesting imidacloprid could negatively impact deer population performance. Archived liver and spleen samples from free-ranging deer in North Dakota were also tested with the same procedure and results showed imidacloprid levels were 2.8 times higher in the liver and 3.5 times higher in the spleen in the free-ranging deer than the captive deer in their study (Berheim et al., 2019).

OBJECTIVES

1. Assess the feasibility of working with deer hunters to obtain biological samples from free-ranging white-tailed deer for disease screening and collecting relevant metadata for those samples.
2. Estimate exposure of neonicotinoids in white-tailed deer across different regions of Minnesota.
3. If high levels (>0.33 ng/g) of neonicotinoids are discovered in white-tailed deer, provide a basis to direct future research on potential impacts of neonicotinoid exposure on fawn survival and recruitment.

METHODS

Our study area consisted of all deer permit areas (DPAs) in the state of Minnesota. DPAs were categorized based on estimated use intensity of agricultural neonicotinoid products (Figure 1). We determined neonicotinoid intensity by looking at 3 major crops commonly treated with neonicotinoids in Minnesota: corn, soybeans, and wheat. We calculated the individual acreage of these 3 major crops using the 2019 cropland data layer (USDA-NASS, 2019) within each DPA using the deer permit areas layer (Minnesota Department of Natural Resources, MNDNR, 2019). We then summed the acreage of each crop type to calculate a total crop acreage. Total crop acreage was then divided by the total acreage of the deer permit areas and multiplied by 100 to calculate percent coverage of these 3 combined crops. DPAs were divided into one of the 3 following categories classified by percent coverage of these row crops: heavy use (≥66% crop coverage), moderate use (33-65% crop coverage), and little to no use (0-33% crop coverage). We designed these categories (i.e. bins) to capture a gradient of potential exposure across Minnesota (Figure 2).

Hunter Recruitment

We used multiple methods of communication and outreach to recruit hunters and gain project interest. We contacted deer hunting groups to inform them of the study, and solicit their members to assist in sample collection. These hunting groups included the Minnesota Deer Hunters Association (MDHA), Bluffland Whitetail Association (BWA), Quality Deer Management Association (QDMA), and Backcountry Hunters and Anglers (BHA). We also collaborated with tribal biologists (Fond du Lac Band, Grand Portage Band, and 1854 Treaty Authority Band) to encourage tribal hunters to participate in sample collections. The Minnesota chapter of The Wildlife Society (TWS), and MNDNR wildlife staff also received emails detailing study information and how they could participate and were asked to promote the project through their organizations. The project was featured in the MNDNR's monthly Deer Notes newsletters. Deer hunters who provided an email address to the electronic licensing service (ELS) database also received an email blast. This was designed to reach audiences not connected with deer hunting groups or other targeted organizations. Finally, staff participated in radio interviews and newspapers articles about the project when opportunities arose. Interested participants signed up through an online form that collected their name, email address, physical address, and which DPA they hunted most frequently.

Sample Collection

Our goal was to collect 200 samples from the little to no use category, 200 samples from the moderate use category, and 400 samples from the heavy use category for 800 total samples. We began collecting samples at the start of regular firearms deer season (9 Nov 2019) and stopped collecting samples at the end of archery season (31 Dec 2019).

All hunters that agreed to participate in sample collection were mailed a small sampling kit. Kits were pre-labeled with an ID and preassigned to an individual based on the online form. Each kit contained: a detailed sampling protocol, 1 cold pack, 1 sealable sample bag (spleen), 1 ziplock bag, 1 coin envelope (tooth), and 1 return shipping box with prepaid postage and insulated insert. The hunter was instructed to collect the spleen, and record date of harvest, DPA of harvest, age class (fawn, yearling, adult), sex, and any additional comments on the data label. A link to an instructional video was included in the protocol to describe the location, texture, and color of a spleen in a freshly harvested animal. Images and written descriptions were also provided in the instructional protocol for hunters unable to access the video. Interested hunters were asked to provide a front incisor from their deer and include it with the shipment to be aged via cementum annuli at no cost to the hunter as an additional incentive.

Once a successful hunter collected the samples and recorded the data, they were instructed to ship their samples using the provided shipping box and prepaid label. Sample boxes arrived at the Wildlife Health Program (WHP) office in Forest Lake, MN daily, were immediately placed in a chest freezer, and were stored at -20°C. Once the hunting season ended, all samples were verified to ensure spleen tissue and 4 subsamples (2.5cm x 2.5cm) were collected after allowing spleens to thaw overnight. We archived 3 subsections and sent 1 subsection to the lab for processing. Scissors were sanitized between each spleen with 70% ethyl alcohol (EtOH) to prevent cross-contamination. Cardboard boxes were used as cutting surfaces and were replaced after each sample was processed. The subset samples were then organized by sample ID and refrozen. If the hunter submitted a tooth, staff ensured the root was intact prior to sending to the lab.

Spleen samples were then shipped to Ecdysis Foundation (Estelline, SD) where they will be tested for the presence of imidacloprid using enzyme-linked immunosorbent assay (ELISA). A subset of positive samples (up to 100) from the ELISA screening will be submitted to Michigan State University Veterinary Diagnostic Laboratory (Lansing, MI) to differentiate between pesticide compounds using mass spectrometry. Teeth were shipped to Matson's Laboratory (Manhattan, MT) for cementum annuli aging process to estimate age for each specimen.

RESULTS

A total of 1,836 kits were shipped to 1,756 project participants. Of the 1,836 kits shipped, 1,190 were requested in the little to no use category, 417 were requested in the moderate use category, and 191 were requested in the heavy use category; 38 participants did not list the area they were going to hunt in. An additional group of interested hunters (n=86) continued to inquire about the project past the kits distribution deadline; they did not receive a kit but will be sent a final report once test results are returned and data is aggregated. Successful hunters returned 798 kits to MNDNR. Upon initial examination, 774 kits contained a spleen sample. Of the returned kits, 569 contained a tooth; however, 52 teeth were unsuitable for lab submission due to missing the root. Four spleen samples were omitted due to incorrect or insufficient data. In total, 770 spleen samples submitted were by participating hunters for a 42% rate of return on all disseminated kits. To reach the goal of 800 samples, an additional 28 samples were collected from the agency culling efforts in southeast Minnesota, as well as 1 sample from a sick deer that was taken by wildlife staff in the north metro area. In March 2020, 799 spleen samples and 517 tooth samples were shipped to respective labs for analysis. Currently, lab results for both spleen and tooth samples are pending and are expected to be returned late summer 2020.

Of the 799 spleens, 497 came from the little to no use of neonicotinoids category (248% of goal), 220 came from the moderate use of neonicotinoids category (110% of goal), and 82 came from the heavy use of neonicotinoids category (20% of goal) (Figure 3).

DISCUSSION

Our efforts to engage the deer hunting community to participate in this project were successful. While we reached the overall sample goal of 800 spleens, we did not achieve the exact sampling distribution we had hoped for, especially in the heavy use category. We likely fell short of our 400-sample goal in the heavy use category due to the low numbers of kits (n=191) requested by hunters in that category. The vast majority of the samples provided by hunters were the correct tissue, with only a 3% error rate. Communication to project participants seemed to be effective using a broad approach; however, the ELS email list likely reached the largest group, with 93,602 emails sent. The online sign-up form allowed staff to easily track interested participants, especially those who requested multiple kits. By pre-assigning kits to hunters, there was less data entry or effort required by the hunter and MNDNR, which potentially increased data quality. The final key item to the project's success was the short video created to show hunters how to identify and remove a spleen. This video, paired with the photos and written descriptions in the instruction packet, likely played a large role in the hunters successfully collecting the proper tissue. However, more information should have been provided to hunters regarding proper tooth extraction.

All lab results are currently pending and expected by late summer. Results have the potential to be delayed further due to the COVID -19 pandemic; however, at this time it is believed the lab will still be able to follow the timeline. Once results are received from the lab, they will be analyzed and a short project summary will be written. This summary will be distributed to all project participants, whether or not they submitted a completed kit. In addition to the summary, successful participants will receive results on their individual deer including neonicotinoid levels and deer age.

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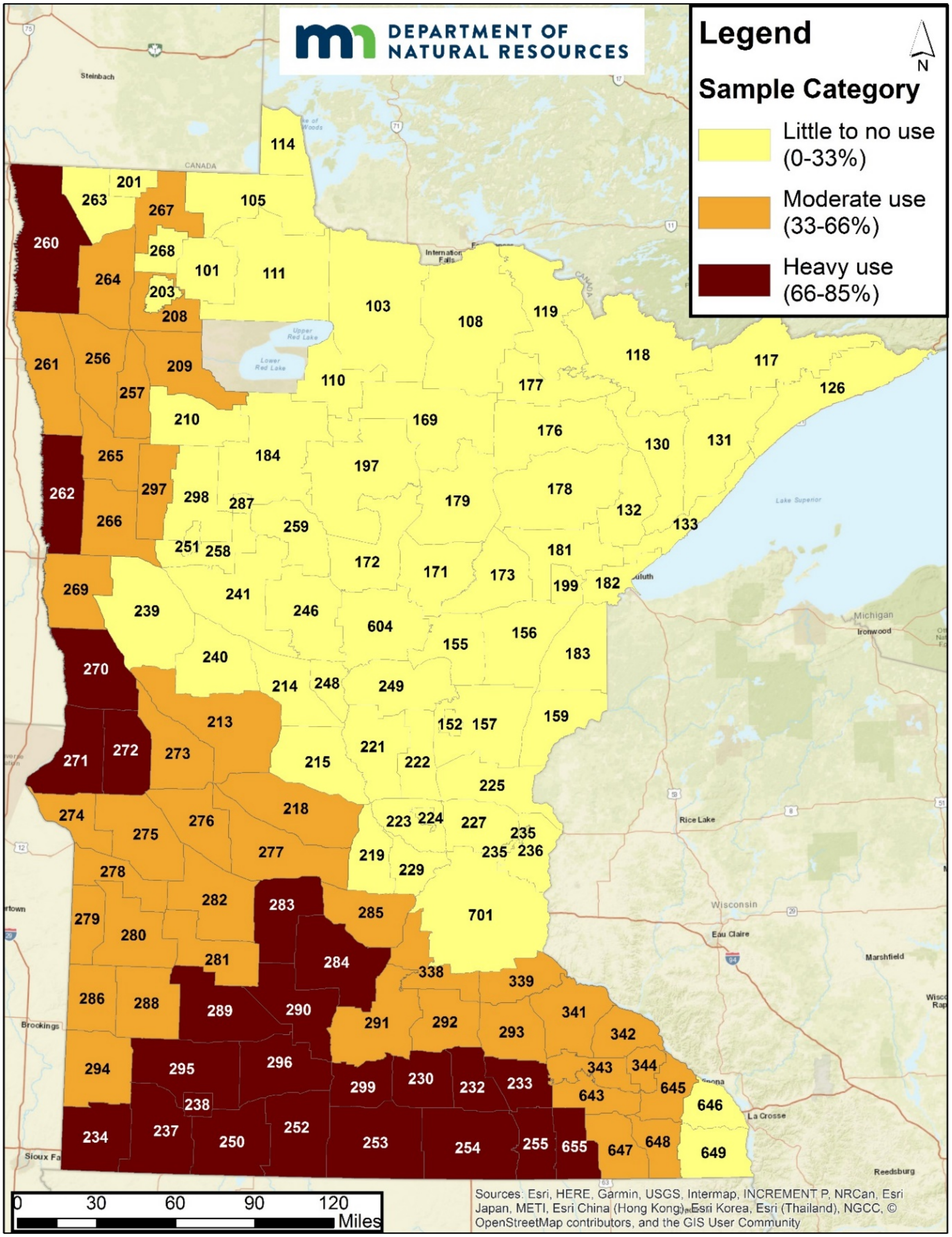

Figure 1. Minnesota Deer Permit Areas (DPA) 2019 depicting the 3 neonicotinoid use categories based on percent crop coverage, including little to no use (33-66%), moderate use (33-66%), and heavy use (66-85%).

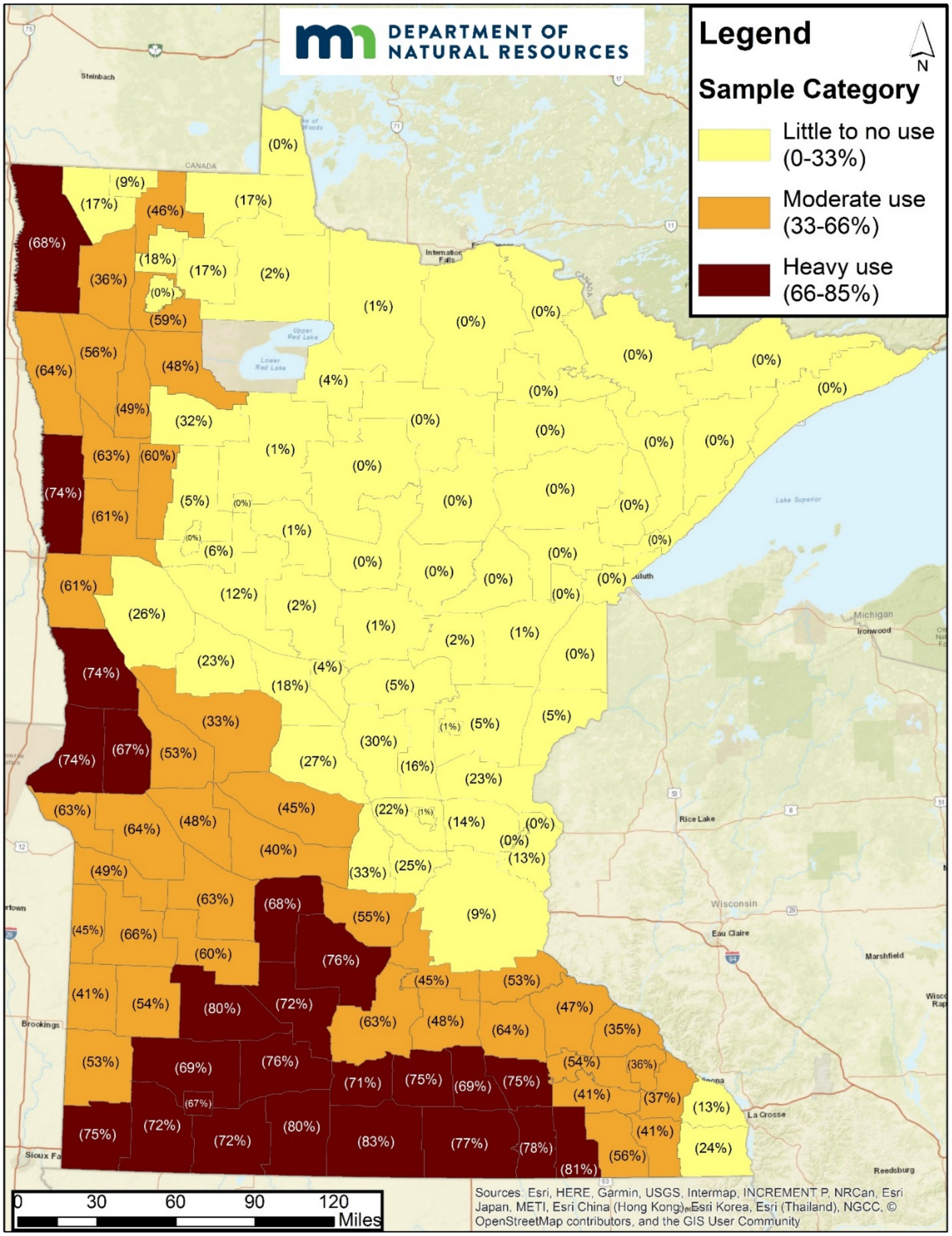

Figure 2. Minnesota Deer Permit Areas (DPA) 2019 depicting the percent crop coverage, ranging from 0 to 83 percent and binned into three usage categories.

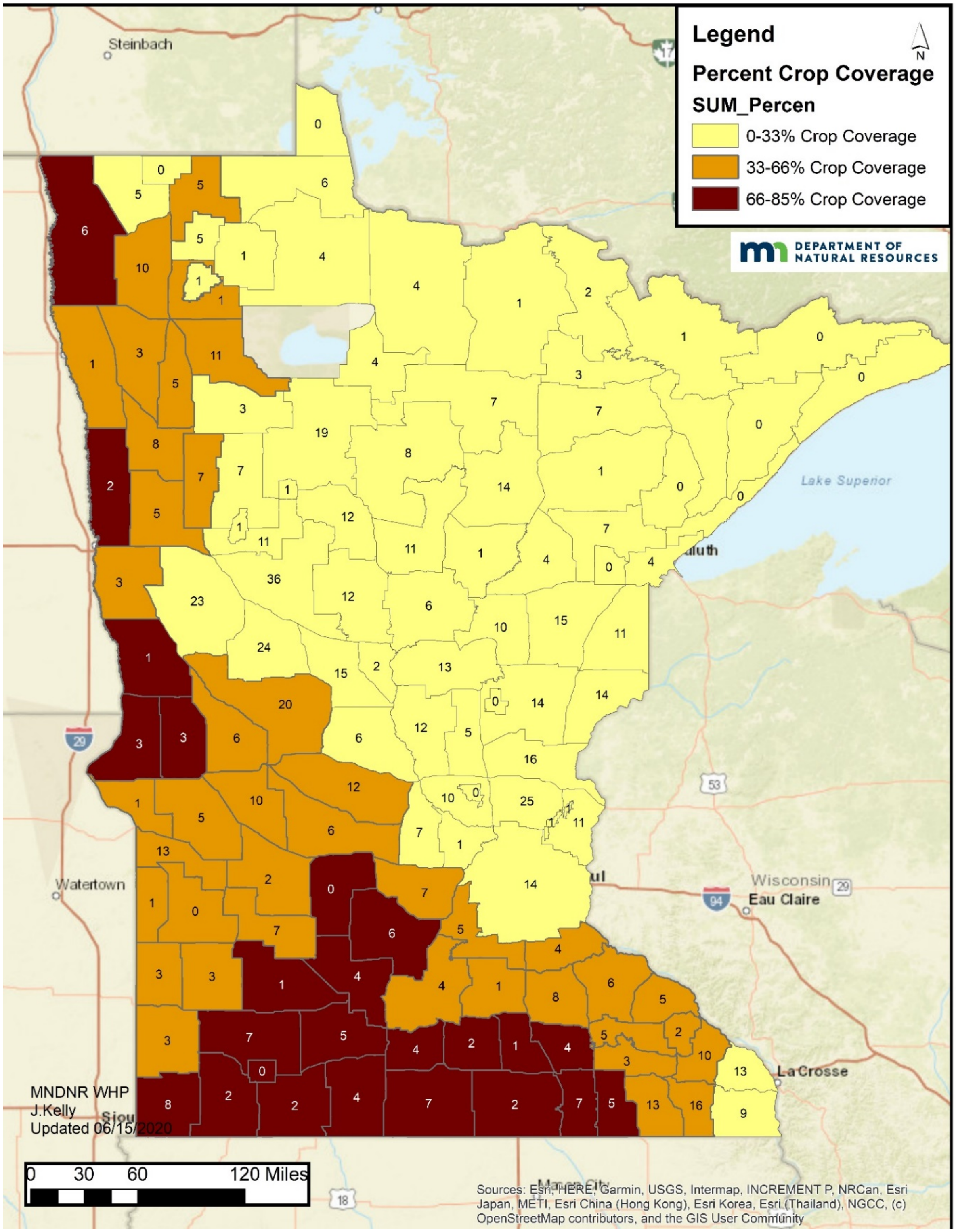

Figure 3. Spleen samples (n = 799) collected by Deer Permit Area (DPA) and crop coverage category (little to no use = 497, moderate use = 220, and heavy use = 82). Minnesota 2019.