CERTIFIED CWD SAMPLER TRAINING GUIDE

Missouri Department of Agriculture

Missouri Department of Conservation

Serving nature and you

RECOMMENDED EQUIPMENT -

Extraction Spoon
Permanent Marker
10% Formalin Jars
Submission form
Rubber Boots

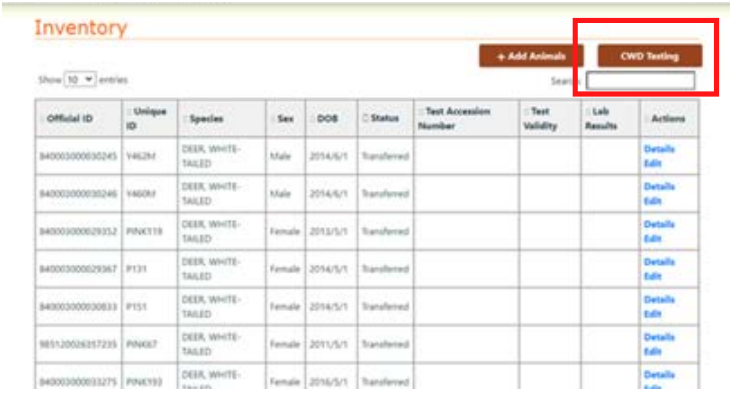
Safety goggles
Bleach
Gloves
Electrical tape
Cutting board

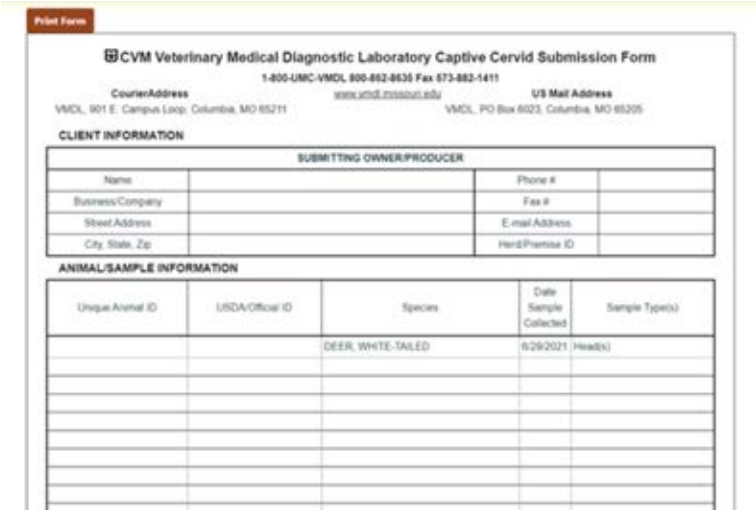
Scalpel
Forceps
Scissors
Knife
Gallon Ziploc Bags

Whirl-Pak's
Icepacks
Coolers
Apron

STEP 1: PRINT SUBMISSION FORM -

- Using your MDC login - log into the MDC Confined Wildlife Information System Software.
- Go to "Wildlife Inventory" in the top right corner.
- From here you will click “CWD Testing” at the top right.





- You can add a new CWD Test Submission from this page on the top right.
  - *Note: Only animals who have been marked dead will show up to be selected for sampling.*
- Fill out, submit, and print the sampling form.
- **For a step by step guide look at the Confined Wildlife Information System manual.**

LAB CHOICES -

Big Game Hunt Preserve:

- MU (able to run ELISA and IHC)
- MDA (able to run ELISA only)
- NVSL (able to run ELISA and IHC)
- Other diagnostic laboratories (contact prior to sampling to verify ability to submit)

Class III Wildlife Breeders in CWD program:

- MU (able to run ELISA and IHC)
- NVSL (able to run ELISA and IHC)
- Other diagnostic laboratories (contact prior to sampling to verify ability to submit)

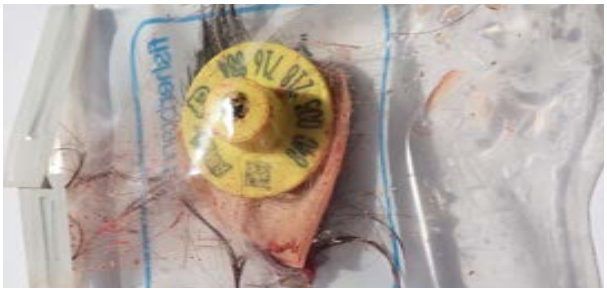
STEP 2: - PREPARATION -

- Label 3 Whirl-Pak's and 1 formalin jar with producer name, **Official ID** number, date, etc.
- Put on gloves, apron, goggles, and rubber boots.
- Prepare solution of 9 parts water and 1 part bleach for clean-up.
- Prepare scalpel, forceps, cutting board, extraction spoon (optional), knife (optional), and scissors (optional).

STEP 3: COLLECTING IDENTIFICATION

Collection options (choose 1):

- To collect the ear tag, you will want to remove the Official ID with a 1" x 1" piece of ear attached for each animal sampled. Place in Whirl-Pak. **Do not place in formalin.** Set aside.
- If animal is a trophy animal, you may avoid cutting the ear by doing the following:
  a. Remove the Official ID and/or MDC leg seal.
  b. Collect the Official ID and/or MDC leg seal and an alternate piece of skin (we suggest the leg or hock area).
  c. Place ID(s) and skin into one Whirl-Pak. **Do not place in formalin.** Set aside.
- If you are submitting a microchip, you must place the microchip along with the surrounding skin in a Whirl-Pak. **Do not place in formalin.** Set aside.

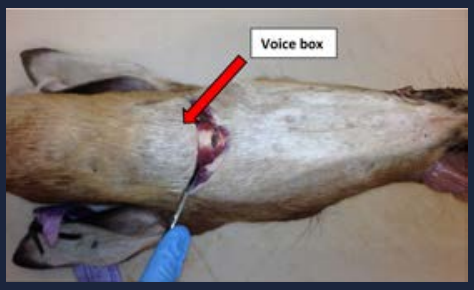


STEP 4: INITIAL CUT

- Position deer with underside of neck facing upwards and nose pointing away from you.
- Feel along deer's neck for its voice box. The voice box is the largest, most prominent lump that you will feel.
- In front of the voice box there will be an indent. Make your first cut here.

Non Capped

Capped



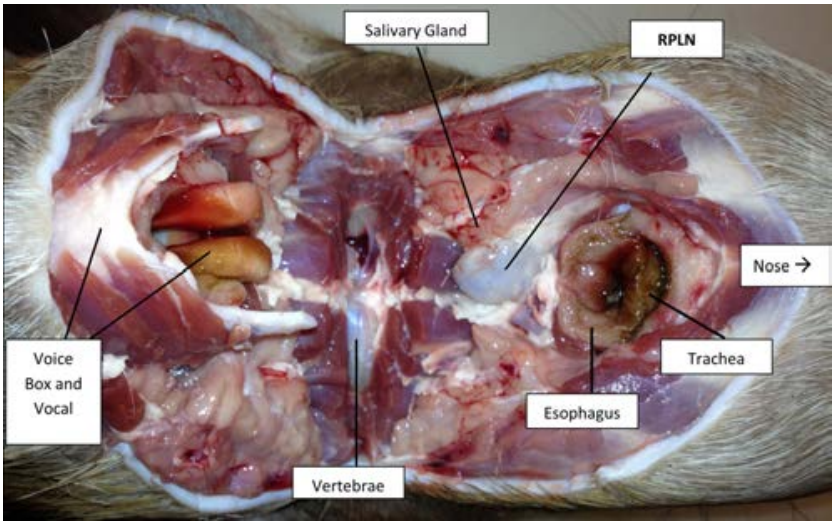
Non Capped

Capped

- Cut down along the jawbones. Angle the cut back on each side toward the ears.
- Cut all the way down to the bones of the vertebrae and the base of each ear.

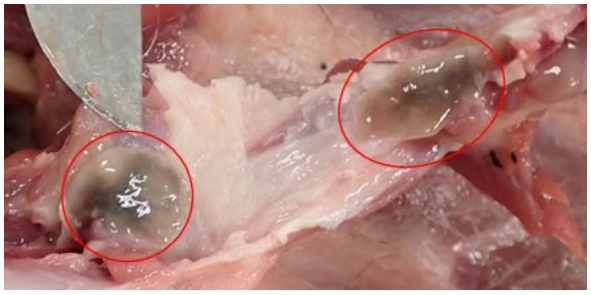
STEP 5: IDENTIFY THE RETROPHARYNGEAL LYMPH NODES

- **Retropharyngeal Lymph Nodes** (RPLN's) will be located nestled below the **esophagus** and **trachea**. There will be one on the left and one on the right.
- The **lymph nodes** will have a bean-like shape.
- If you are unable to locate the RPLN with the initial incision, try cutting out tissue going in the direction of the deer's nose. If you are still not able to locate the RPLN try cutting in the opposite direction.
- Grasp the RPLN with your forceps and, using a scalpel, begin to cut the other tissues away.



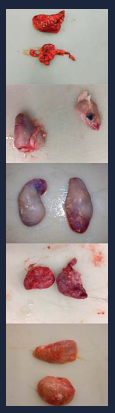
STEP 6: VERIFYING TISSUE -

- The easiest way to determine if the tissue you sampled is lymph will be by pinching the tissue between your index finger and thumb. Lymph nodes feel firm and hold their shape - if your tissue is not firm it is likely not a lymph node. Most often people accidently sample salivary tissue. Salivary tissue is bumpy and easily spread with the side of your scalpel.
- The second way to determine if the tissue you sampled is lymph would be to cut into the lymph node. Cutting into the lymph node should be avoided but is sometimes necessary if you are unsure which tissue you are looking at. Typically, the inside of a lymph node will have branching from the center outward. The center and these branching structures will typically be a different color shade than the surrounding lymph tissue (see right).



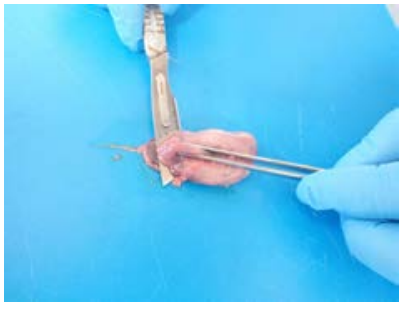
LYMPH NODE VARIETIES

Lymph nodes can vary in color and size depending on the age, sex, and state of decomposition of the deer. Lymph node color can range from extremely pale pink to extremely dark brown/red. Sometimes they can even be green!

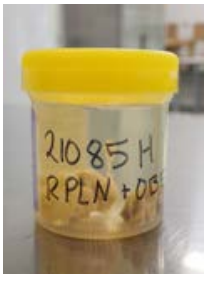


STEP 7: PREPARATION FOR SUBMISSION OF RETROPHARYNGEAL LYMPH NODES

- To prepare the retropharyngeal lymph nodes for submission you must cut each lymph node in half longitudinally.

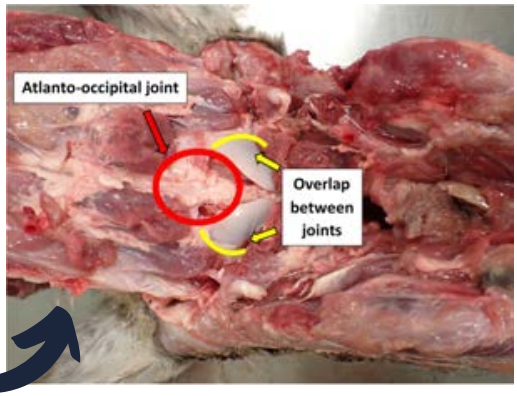


- **Fixed:** *[Formalin]* Place half of the left lymph node and half of the right lymph node in 10% buffered formalin jar. Set aside.



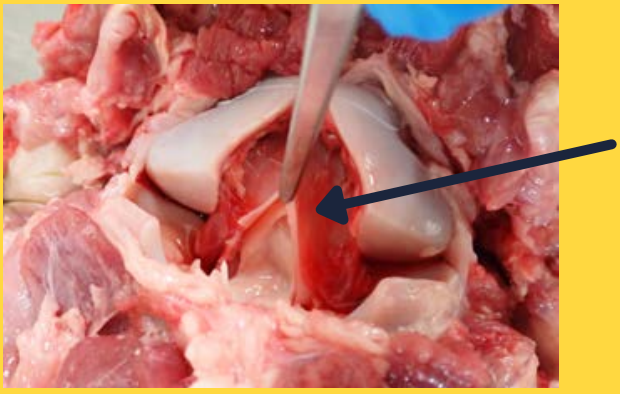
- **Fresh:** *[Refrigerated or frozen]* Place half of the left lymph node and half of the right lymph nodes in one Whirl-Pak. This should be your second Whirl-Pak. Set aside.

STEP 8: ACCESSING THE OBEX



- Extend your existing cuts made from sampling the lymph nodes until you expose the joint juncture between the skull and first vertebrae (atlanto-occipital joint). This will be a smooth, hard, gray structure with overlap of the joint juncture on each side (overlap hidden under tissue in photo).
- Within this joint juncture, between the vertebrae and the base of the head, there will be a circular opening. This is where the spinal cord will be located. To get to the spinal cord you will need to open the joint juncture.
- You will need to extend this opening by severing the connective tissue. Using your scalpel blade, sever the connective tissue between the vertebrae that make up this circular opening. This should allow the joint juncture to open revealing the spinal cord.
- Place your hand on jaw and neck of deer and firmly press down to further expose the brain stem and spinal cord.

STEP 9: FREEING THE OBEX



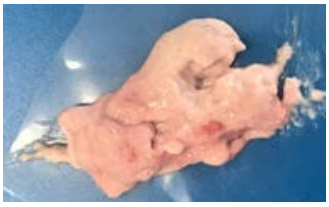
- Use a clean pair of forceps and scalpel or extraction spoon begin gently releasing the brain stem from the bone. This will allow you to reach as deep in the joint as possible. Deep in the joint is where you will sever the brain stem.
- Insert the scalpel or spoon into the circular opening until the curved part of the spoon or scalpel is entirely inside the canal.
- Next, angle the scalpel or spoon sharply down and move it right and left to cut the brain stem away from the brain.
- Gently pull out the brain stem, including the **obex**, in a scooping motion, keeping it intact (see left).

IMPORTANT NOTE-

Unless you are sampling a recently deceased deer, more often than not you will find the obex to be more decomposed than what is pictured in Step 10 Option 2.

STEP 10 OPTION 1: PREPARATION FOR SUBMISSION

(FOR MDA SPRINGFIELD LAB)

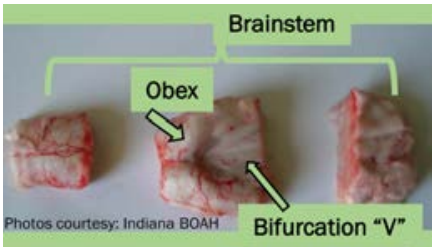


Option for Big Game Hunt Preserve only:

- Once the obex is removed, turn it over, and the "V" should be visible.
- Submit the whole sample in one Whirl-Pak (fresh).
- ELISA only.
- Best tissue for Elk and Red Deer.

STEP 10 OPTION 2: PREPARATION FOR SUBMISSION

(FOR MU OR NVSL LAB)



- Once the obex is removed, turn it over, and the "V" should be visible.
- Make two incisions above and below the V.
- Place the middle section in formalin jar (fixed).
- Place outer trimmings in one Whirl-Pak (fresh).

STEP 11 FOR OPTION 1:- SUBMISSION OF OBEX-

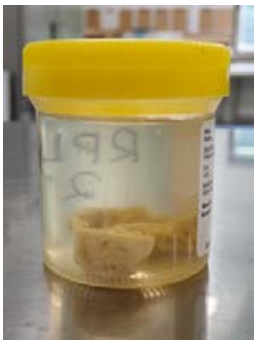
(TO MDA SPRINGFIELD LAB)

- **Fresh:** Place the obex in one Whirl-Pak.

STEP 11 FOR OPTION 2:- SUBMISSION OF OBEX-

(TO MU OR NVSL LAB)

- **Fixed:** *[Formalin]* Place the middle piece of obex in the same formalin jar you used for the RPLNs.
- **Fresh:** Place the trimmed obex pieces (the outer pieces) in one Whirl-Pak.



STEP 12: STORING SAMPLES -

- Place obex, lymph node, and ear tag Whirl-Pak's into one larger bag. Seal bag shut.
  - Samples from each animal must be packaged in separate plastic bags (1 bag per animal).
    - Don't pool samples, as they could contaminate one another in a container/bag.

Class III Wildlife Breeders enrolled in Herd Certification Plan have 7 days to submit their samples taken by either a certified sampler or an accredited veterinarian.
Procedure:

- Refrigerate the fresh tissues (the lymph nodes, the obex, and the ear tag) for up to two days.
- If you freeze the tissues, do not thaw, and refreeze.
- Keep the formalin jar out of the refrigerator to allow the tissue to get "fixed".
- Package the samples by following **Step 14** and ship to either the University of Missouri Veterinary Diagnostic Laboratory or NVSL using the form available here: https://www.aphis.usda.gov/library/forms/pdf/VS_Form10_4.pdf

Big Game Hunt Preserves may submit samples to the Missouri Department of Agriculture lab (Springfield) or the University of Missouri Veterinary Diagnostic Laboratory.

- Tests of the lymph nodes will be run using ELISA testing and if there is a suspect result then it will be cleared or confirmed by running an IHC test.
  - If the sample goes to MU, the IHC test will be done at the MU laboratory.
  - If it goes to MDA Springfield lab, the sample will be forwarded to NVSL by the Springfield laboratory for IHC testing.
  - If there is any question regarding the sample, the tests may be repeated at NVSL. Genetic work will need to be done at NVSL.
- It is recommended that samples be handled as described for breeders.
  - If BGHP wish to freeze tissues to submit a weeks' worth of samples, the fresh tissues may be frozen, and the formalin kept at room temperature.
  - It is recommended that samples be shipped weekly. The Wildlife Code allows for submitting within 30 days, but this is not recommended. If DNA samples are kept by the Big Game Hunt Preserve, please retain some form of ID with that sample.

STEP 13: CLEAN UP AND DISPOSAL

- Clean sampling area with a bleach/water solution that is 9 parts water and 1 part bleach. Let blead sit for 15 minutes before rinsing.
- Scrub instruments and rinse, then soak them in the bleach solution for 15 minutes.

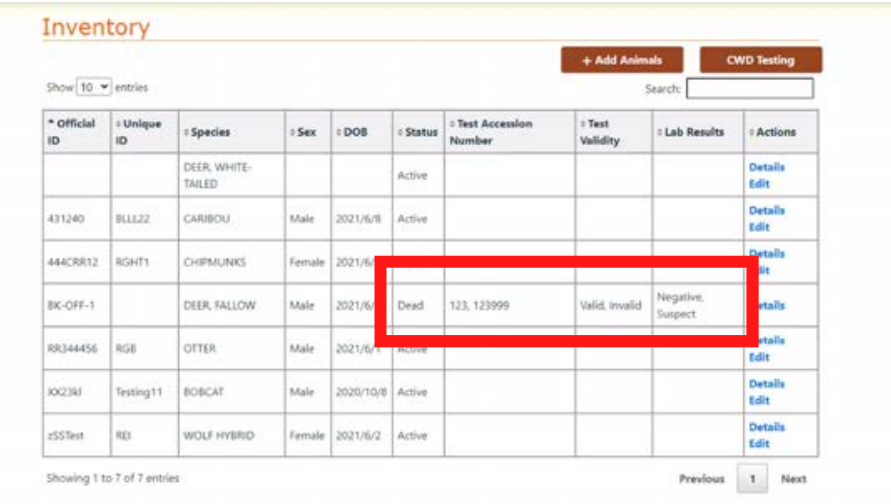
STEP 14: PREPARING FOR SHIPMENT

- When you are ready, you will place the Whirl-Pak's and formalin jar in a larger bag and place that bag inside the shipping cooler.
- Line the bottom of the shipping container with absorbent material (eg. newspaper or paper towels).
- Place frozen ice packs in a re-sealable bag on top of absorbent material.
- Fill extra space with more absorbent material to prevent shifting during transit.
- Include the laboratory submission form in a sealed plastic bag.
- Close Styrofoam lid and seal the box.
- It is best to ship as soon as possible to get your results sooner and prevent tissue decomposition.

SHIPPING DESTINATIONS-

- Veterinary Medical Diagnostic Laboratory
  University of Missouri:
  901 E. Campus Loop
  Columbia, Missouri 65211-0001
  Phone: (573) 882-6811
- Missouri Department of Agriculture Animal Health Veterinary Diagnostic Laboratories:
  701 North Miller Avenue
  Springfield, Missouri 65802-6460
  Phone: (417) 895-6861
- United States Department of Agriculture Animal and Plant Health Inspection Service National Veterinary Services Laboratories:
  P.O.Box 844,
  1920 Dayton Ave
  Ames, IA 50010
  Phone: (515) 337-7514

STEP 15: CHECK /ENTER YOUR LAB RESULTS



To check lab results:

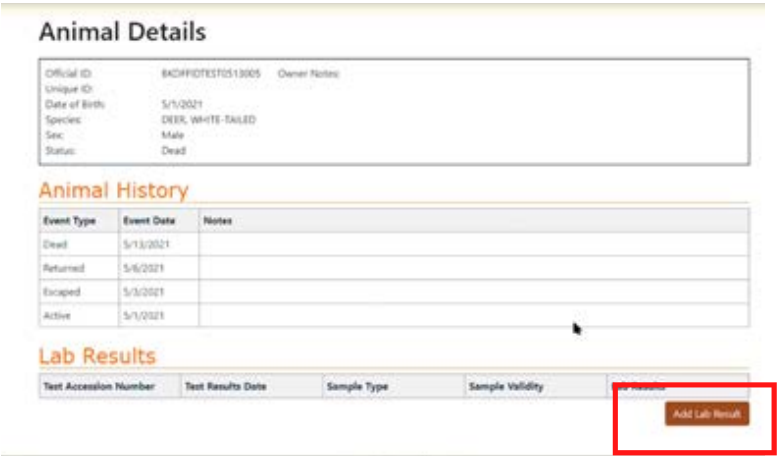
- Lab results will be documented (listed separated by comma, in order) under the “Test Accession Number”, “Test Validity”, and “Lab Results” column.

WHEN TO MANUALLY ENTER LAB RESULTS

- Always: when you receive results from NVSL.
- Always: when you receive results from another lab not listed.

To manually enter your lab results:

- Under "Wildlife Inventory" select the animal you would like to add results for and click "Details" to the right.
- Under the "Lab Results" section, enter the appropriate lab results and upload a pdf of the lab form showing the results.



DEFINITIONS

C

Caudal point: Away from the head; At the level of the spinal cord, caudal indicates the direction that points down toward the feet.

Cerebrospinal fluid (CSF): A clear, watery fluid that fills the ventricles of the brain and the subarachnoid space around the brain and spinal cord.

E

Esophagus: A relatively straight, muscular tube through which food passes from the pharynx to the stomach.

F

Fixed sample: A sample that is placed in 10% formalin.

Formalin: A colorless solution of formaldehyde in water, used chiefly as a preservative for biological specimens.

Fresh sample: A sample that is refrigerated or frozen. These samples are placed in Whirl-Pak's.

L

Lymph node: Lymph nodes are small structures that work as filters for harmful substances. They contain immune cells that can help fight infection by attacking and destroying germs.

O

Obex: The most caudal point within the fourth ventricle of the brainstem, as it narrows and communicates with the central canal of the spinal cord.

Official ID: A numbering system for the official identification of individual animals in the United States. The AIN contains 15 digits, with the first 3 being the country code (840 for the United States), the alpha characters USA, or the numeric code assigned to the manufacturer of the identification device by the International Committee on Animal Recording. (Tags from the Missouri Department of Agriculture)

R

Retropharyngeal Lymph Nodes (RPLN): Paired groups of cervical lymph nodes located in the suprahyoid portion of the retropharyngeal space.

T

Trachea: Also known as the windpipe, a cartilaginous tube that connects the larynx to the bronchi of the lungs, allowing the passage of air, and so is present in almost all air-breathing animals with lungs.

U

Unique ID: A farm style tag with the Premise ID on it and/or a system the producer prefers to visually identify animals in the herd.

V

Ventricle: A hollow part or cavity in an organ. The ventricles of the brain are a communicating network of cavities filled with cerebrospinal fluid (CSF) and located within the brain parenchyma.

Vertebrae: Each of the series of small bones forming the backbone, having several projections for articulation and muscle attachment, and a hole through which the spinal cord passes.

W

Whirl-Pak: Whirl-Pak® sample bags are strong, sterile, and leakproof. Made of polyethylene, they are designed to carry solids or liquids to a laboratory for testing.

#

10% Formalin Jar: Laboratory specimen jar filled with 10% Neutral, Phosphate Buffered Formalin.

MISSOURI - DEPARTMENT OF - AGRICULTURE -

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MISSOURI - DEPARTMENT OF - CONSERVATION -

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