HUMANE EUTHANASIA OF BATS FOR PUBLIC HEALTH RABIES TESTING July 2014

The Michigan Rabies Working Group (RWG) is an interagency group that was formed in the late 1990's and whose members represent the Michigan Departments of Health and Human Services, Agriculture and Rural Development, and Natural Resources, the United States Department of Agriculture (USDA), Michigan State University, local public health agencies, animal control officers, wildlife enthusiasts, and animal welfare organizations. This group meets regularly to discuss rabies issues in Michigan. Providing guidance for the safe and humane euthanasia of bats for public health testing became a recent priority for the RWG. The objectives of this document include:

- Describe, in brief, the epidemiology of rabies in the U. S. and Michigan
- Discuss the public health concerns when bats are found in and around human dwellings
- Describe how to safely collect a bat for rabies testing
- Provide information about safe, humane, and effective euthanasia techniques in bats
- Provide information about packaging and shipping specimens for rabies diagnostic testing.

About Rabies in the U.S. and Michigan

Rabies is a preventable viral disease transmitted primarily by the bite of an infected mammal. Untreated, rabies has the highest case fatality rate of any infectious disease known, being virtually 100% fatal once symptoms begin. Any mammal can be infected with rabies, but following the implementation of rabies vaccination of domestic dogs in the 1950's, wild mammals have become the primary reservoir for this virus in the United States. Wild animals accounted for 92% of all animal rabies cases reported in 2012¹. The rabies reservoir species in the U.S. include bats, raccoons, skunks, and foxes. In Michigan, bats are the primary reservoir species, with a focus of skunk-strain rabies in the southeast and "thumb" area of the state. The Centers for Disease Control and Prevention has an excellent Rabies Website at http://www.cdc.gov/rabies/. In addition, current information about rabies in Michigan can be found at http://www.michigan.gov/rabies.

Bats and Rabies

It is estimated that less than 1% of the bats in the wild are infected with the rabies virus. However, on average, 4-6% of the bats that are submitted for public health testing (found in and around people's homes) to the Michigan Department of Health and Human Services Bureau of Laboratories (MDHHS BOL) are infected. The reason for the higher rate of positive bats in public health testing is that sick bats are more likely to display unusual behavior, such as an inability to fly, and end up potentially exposing humans and unvaccinated pets to rabies. Bats can pose a particular difficulty for public health and animal control agencies. While human rabies cases are rare in the U. S. (1-2 per year on average), the majority are caused by bat strains of rabies. In many of these cases, there has been no known contact or bite from a bat. Even seemingly insignificant bat bites can transmit rabies. The current ACIP Recommendations for Human Rabies Prevention—United States, 2008² describes a bat exposure as either direct contact with a bat, or "finding a bat in the same room as a person who might be unaware that a bite or direct contact had occurred (e.g., a deeply sleeping person awakens to find a bat in the room or an adult witnesses a bat in the room with a previously unattended child, mentally disabled person, or intoxicated person). These situations should not be considered exposures if rabies is ruled out by diagnostic testing of the bat". Thus, when a bat needs to be tested because of public health concerns, it must be euthanized in such a way as to preserve the brain for diagnostic rabies testing.

Collection of a Bat for Rabies Testing

Because bats are one of the natural reservoirs of the rabies virus, it must be assumed that all bats may be infected. This presents a risk to the personnel who are involved in collecting, euthanizing and preparing rabies specimens for testing. Collection of a bat should occur in such a way as to avoid contact with the bat. If a bat is present in someone's home and you cannot rule out the possibility of exposure, do not kill or free the bat. The bat should be gently collected pending consultation with the local public health agency. There are precautions that should be taken to capture the bat safely. Tools you should have available include:

- Leather work gloves
- Small box or coffee can
- Piece of cardboard
- Tape

Once the bat lands, wearing gloves, approach slowly and place a box or can over it. Slide the piece of cardboard under the container, trapping the bat inside. Securely tape the cardboard to the container and punch small holes (less than ½ inch in diameter) in the container for the bat to breathe. **Contact your local public health or animal control agency** to discuss whether the bat should be tested for rabies and to receive instructions on which steps need to be taken next. Placing the container with the bat in a cooler with ice may reduce the bat's activity level and make it easier to handle prior to euthanasia, if rabies testing is determined to be necessary.

Providing for Safe, Humane Bat Euthanasia

Because any bat may be infected with rabies, **all personnel handling bats for euthanasia should be vaccinated against rabies.** In addition to being vaccinated, the Michigan RWG recommends that personnel involved in euthanizing bats must be properly trained in the use of common euthanasia techniques. Personnel must be provided with appropriate personal protective equipment (PPE), depending on the method of euthanasia utilized, which may include protective clothing, gloves, mask, and eye protection. Caution and extra care should be used if these ideal circumstances cannot be met while performing euthanasia on a bat that must be tested. When using gaseous agents, care must be taken to protect personnel (proper equipment, ventilation and/or scavenging system).

The primary reference regarding humane euthanasia of animals is the "AVMA Guidelines for the Euthanasia of Animals: 2013 Edition"³. In addition, the American Association of Zoo Veterinarians has published "Guidelines for Euthanasia of Nondomestic Animals"⁴. While there

is little objective information in the literature regarding humane techniques for euthanasia in bats, the basic principles of euthanasia apply. Death must be as painless and distress free as possible. Any technique chosen must induce loss of consciousness as quickly as possible, followed by cardiac and respiratory arrest. The technique must also be reliable, irreversible, and most importantly, safe for humans.

The AVMA Guidelines for the Euthanasia of Animals classify effective euthanasia methods as "acceptable", or "acceptable with conditions". <u>Acceptable methods</u> are those that "consistently produce a humane death when used as the sole means of euthanasia." <u>Methods acceptable with conditions</u> are those that "require certain conditions to be met to consistently produce humane death, may have greater potential for operator error or safety hazard, are not well documented in the scientific literature, or may require a secondary method to ensure death.³" A number of methods may be utilized to achieve humane euthanasia in bats. Optimal methods provide for as little contact as possible with the bat. **Bats should NEVER be handled with bare hands.** The following is a list of agents that may be used to accomplish euthanasia in bats:

Methods Acceptable or Acceptable with Conditions for the Euthanasia of Bats

Inhalation Agents

Methods Acceptable with Conditions:

- Inhalant anesthetics (liquid) e.g. halothane, enflurane, isoflurane, sevoflurane, methoxyflurane, desflurane
- Carbon dioxide (CO₂ at 70% or higher concentrations) compressed gas cylinders are the only acceptable source. Insectivorous bats may be resistant, so this method should be paired with other methods that ensure death, such as an injectable barbiturate.⁴
- Carbon monoxide (CO) more dangerous to personnel, requires quality equipment

Injectable Agents

Acceptable Methods:

- Barbiturates intravenous or intraperitoneal (Limitation: DEA controlled substances, must be licensed to administer)
- Dissociative agent combinations, such as ketamine/diazepam or ketamine/xylazine – intravenous or intraperitoneal (Limitation: DEA controlled substances, must be licensed to administer)

Methods Acceptable with Conditions:

• 70% Ethanol – intraperitoneal. Only acceptable for bats the size of a mouse or smaller. Studies have shown that 500µl (0.5 ml) of 70% ethanol was effective in causing respiratory arrest in mice in less than three minutes.⁵

Physical Methods

Physical methods such as cervical dislocation or decapitation are acceptable euthanasia methods for small mammals, but are **unacceptable** for the euthanasia of bats due to the concern for rabies

exposure. Thoracic compression (the application of pressure to the chest to restrict respiration) was previously considered acceptable with conditions for euthanasia of small mammals by AVMA. However, based on the new AVMA Guidelines, this is now considered unacceptable unless the animal is deeply sedated or insentient for other reasons.

Unacceptable Methods for the Euthanasia of Bats

Unacceptable Inhalation Agents:

- Exhaust fumes from an idling gasoline internal combustion engine (unacceptable due to production of additional gases leading to inadequate concentrations of CO gas and inadequate cooling of the gas)
- Diethyl ether (flammable and explosive)

Unacceptable Physical Methods:

- Blunt force trauma (may destroy the brain)
- Drowning (inhumane)
- Gunshot (may destroy the brain)
- Thoracic compression (inhumane, unless sedated or insentient)
- Freezing (inhumane formation of ice crystals on the skin and in tissues may cause pain or distress)
- Cooling to induce torpor followed by freezing (inconsistent torpid bats may awaken from torpor when subjected to freezing or subfreezing temperatures)⁶

Examples of Recommended Euthanasia Techniques

The overarching goal of this document is to provide for the safe, humane and effective euthanasia of bats for public health testing purposes. With that goal in mind, the following are examples of techniques that meet these criteria. These are not meant to be exhaustive. The AVMA Guidelines³ recognize the inherent lack of control over free-ranging wildlife, and acknowledge that the quickest and most humane means of terminating the life of free-ranging wildlife in a given situation may not always meet all criteria established for euthanasia. Moreover, the methods selected will often be situation specific, as a means of minimizing potential risks to the animal's welfare, personal safety, and potential infectious disease concerns. The Guidelines specifically state that "human safety is of utmost importance for all euthanasia procedures".

Persons who are trained and knowledgeable about euthanasia techniques may have developed their own effective methods to achieve humane euthanasia in bats. Any technique used to euthanize bats should be safe for the personnel performing the task, and produce as quick and humane a death as possible in the animal, given the circumstances. The following techniques are provided as simple examples for those who may lack the experience with this species but are willing and able to perform this task.

1. **CHAMBER ANESTHESIA/EUTHANASIA:** A bat collected in the manner outlined above may be placed inside an anesthesia induction chamber without the need to remove

the bat from the container. Ensure that there are sufficient holes in the cardboard to allow for anesthetic gas to get in. Seal and fill the chamber with an inhalation agent such as one listed above. Turn off gas and leave the bat inside the chamber to allow sufficient time for the anesthetic gas to cause euthanasia. Alternatively, the bat may be left in the chamber for sufficient time to achieve anesthesia, whereby it can be removed from the chamber and container and injected intraperitoneally with either a barbiturate euthanasia solution or 70% ethanol. If CO_2 is used to induce anesthesia in a bat, it should be followed by an injectable euthanasia agent to assure death.

2. **CONTAINER EUTHANASIA:** A cotton ball soaked with one of the above listed liquid inhalation anesthetic gases can be placed inside a sealable heavy-duty ziplock plastic bag, along with the collection container, and left to allow sufficient time for the anesthetic gas to cause euthanasia. Alternatively, the bat may be left in the sealed bag for sufficient time to achieve anesthesia, whereby it can be removed from the bag and container and injected intraperitoneally with a barbiturate euthanasia solution or 70% ethanol.

Intraperitoneal Injection Technique

Administration of an injectable euthanasia solution into the peritoneum (free space in the abdomen) of a bat should be performed in a manner similar to the technique used in mice and other small mammals. Once the bat is anesthetized, use gloved hands to restrain the bat with the abdomen facing up. Draw an imaginary line between the bat's knees, and insert the needle at a 30 degree angle along that line on the bat's right side, close to midline. Advance your needle about ½ centimeter into the abdomen. Draw back on the needle. If fluid enters the syringe, pull back needle, reposition and draw back again. If no fluid is aspirated in the syringe, inject the euthanasia solution⁷.



Figure 1: Intraperitoneal injection site for a bat

Euthanasia and Humane Killing

According to the AVMA Guidelines, euthanasia is defined as, "a method of killing that minimizes pain, distress, and anxiety experienced by the animal prior to loss of consciousness, and causes rapid loss of consciousness followed by cardiac or respiratory arrest and death.³" The AVMA Guidelines state that, "free-ranging animals may need to be killed quickly and efficiently in ways that may not fulfill the criteria for euthanasia established by the Panel on Euthanasia.³" Every attempt should be made to terminate a bat's life with an acceptable euthanasia method performed by an experienced person. However, the Michigan Rabies Working Group recognizes that there may be very specific instances in which the use of humane killing, rather than euthanasia, may be required. Humane killing is defined as, "killing performed in a manner that minimizes animal distress, but may not meet the requirements of euthanasia due to situational constraints.³" Regardless of the method chosen, the priority for public health agencies is to preserve the animal's brain for diagnostic rabies testing.

Packaging and Shipping Specimens for Rabies Diagnostic Testing

At no charge, the MDHHS BOL provides complete shipping units for submitting rabies specimens. Units can be requested by the following methods: call (517) 335-9040 and ask for Unit #47, fax a Shipping Requisition form (DCH-0568) to 517-335-9039 or send an email to MDHHSlab@michigan.gov. Directions on packaging and shipping can be found on the BOL website at http://www.michigan.gov. Directions on packaging and shipping can be found on the BOL website at http://www.michigan.gov/mdhhslab. Navigate to Providers/Lab Services/Specimen Submission. Acceptable specimens include whole bats or the head (or brain-including both sides of the cerebellum and brain stem) of larger mammals. Specimens should be refrigerated prior to shipping. Specimen must be thawed before testing can proceed, causing a delay in test completion. A Rabies Test Requisition form (DCH-1053) should be completed and included with the specimen. Shipping information is on the form.

Specimens can be delivered to the lab 24/7. Recommended methods include Fed Ex or United States Postal Service (USPS) for "overnight delivery". Specimens shipped "overnight delivery" will be delivered on the next <u>business</u> day. It is best to avoid having specimens in transit over a weekend. Specimens collected on a Friday may be refrigerated and shipped on Monday, if results are not critical. Specimens can also be delivered to the laboratory by public or private couriers or private citizens. Testing is available on the weekends by special arrangement with MDHHS at 517-335-8165 Monday through Friday, and 517-335-9030 nights and weekends. Emergency testing is reserved for human exposures when timely results will prevent unnecessary post-exposure treatment. When specimens need to be delivered to the lab on a Saturday, the submitter must request "**Saturday Delivery**" from either Fed Ex or the USPS.

REFERENCES

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