TRANSLOCATION PROTOCOL FOR GUNNISON'S PRAIRIE DOGS IN ARIZONA

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Nongame Wildlife Branch, Wildlife Management Division
Arizona Game and Fish Department

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INTRODUCTION

All of Arizona's native wildlife, including federally listed threatened and endangered species, are protected under the general provisions of Arizona Revised Statutes, Title 17. The Arizona Game and Fish Department (Department) includes the Gunnison's Prairie Dog (GPD) on the Species of Greatest Conservation Need Tier 1C (AGFD 2012). The list provides policy guidance on management priorities only, not legal or regulatory protection.

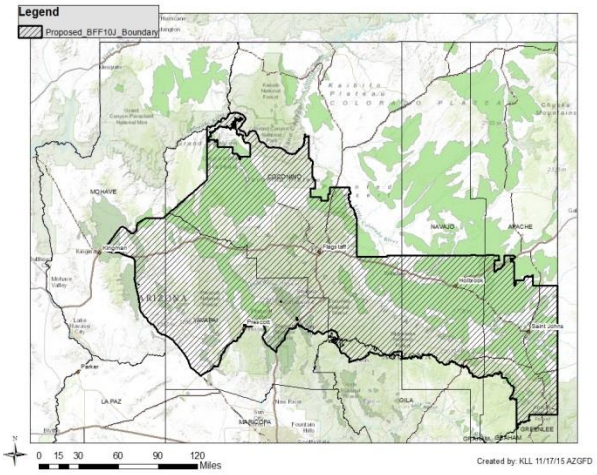

Figure 1: Gunnison's Prairie Dog Range Map excluding tribal lands.

GPD persist throughout the grasslands of northern Arizona (Figure 1). In 2007, the Department began conducting GPD Occupancy Surveys to establish a long-term data set across the entire range of GPD in conjunction with Colorado, New Mexico, and Utah. This monitoring program is part of a multi-state Conservation Assessment to evaluate the range-wide status of the GPD and evaluate impacts to the species, which resulted from a 2004 petition to list the species under the Endangered Species Act (Forest Guardians 2004). GPD monitoring is critical to determine the stability of the population (including the goal of maintaining populations across 75% of the historic range which is in the Interagency Management Plan for Gunnison's Prairie Dogs in Arizona), the presence of disease, and the potential for future black-footed ferret release areas (Underwood 2007). While recent surveys show the status of their population remains relatively stable (Hicks 2014 unpublished) population fluctuations occur due to weather (i.e. drought) and disease epizootics (i.e. plague).

In some areas GPD are considered pests (e.g. urban areas, competition with livestock), or colonies are located in areas to be developed. The Department allows for the hunting of GPD through Commission Rule (R12-4-304) and the seasons established within Commission Order 14. In addition, the animal is readily eradicated in urban settings by licensed pest control businesses. While these lethal methods solve an immediate perceived problem, translocation of these animals can be an alternative method to eradication.

To maintain a stable GPD population and to increase the opportunities for black-footed ferret reintroduction, the Department will use translocation as a population management technique to

achieve both of those goals. This document provides guidance on, and a protocol for, a GPD translocation by taking into consideration the purpose for the action, required permitting, the humane treatment of the animals, and the risk of disease transfer. To increase the success of the translocation, and most importantly, to reduce the risk of spreading disease, all steps of this translocation protocol will be followed by the translocating party, and coordinated with the Department.

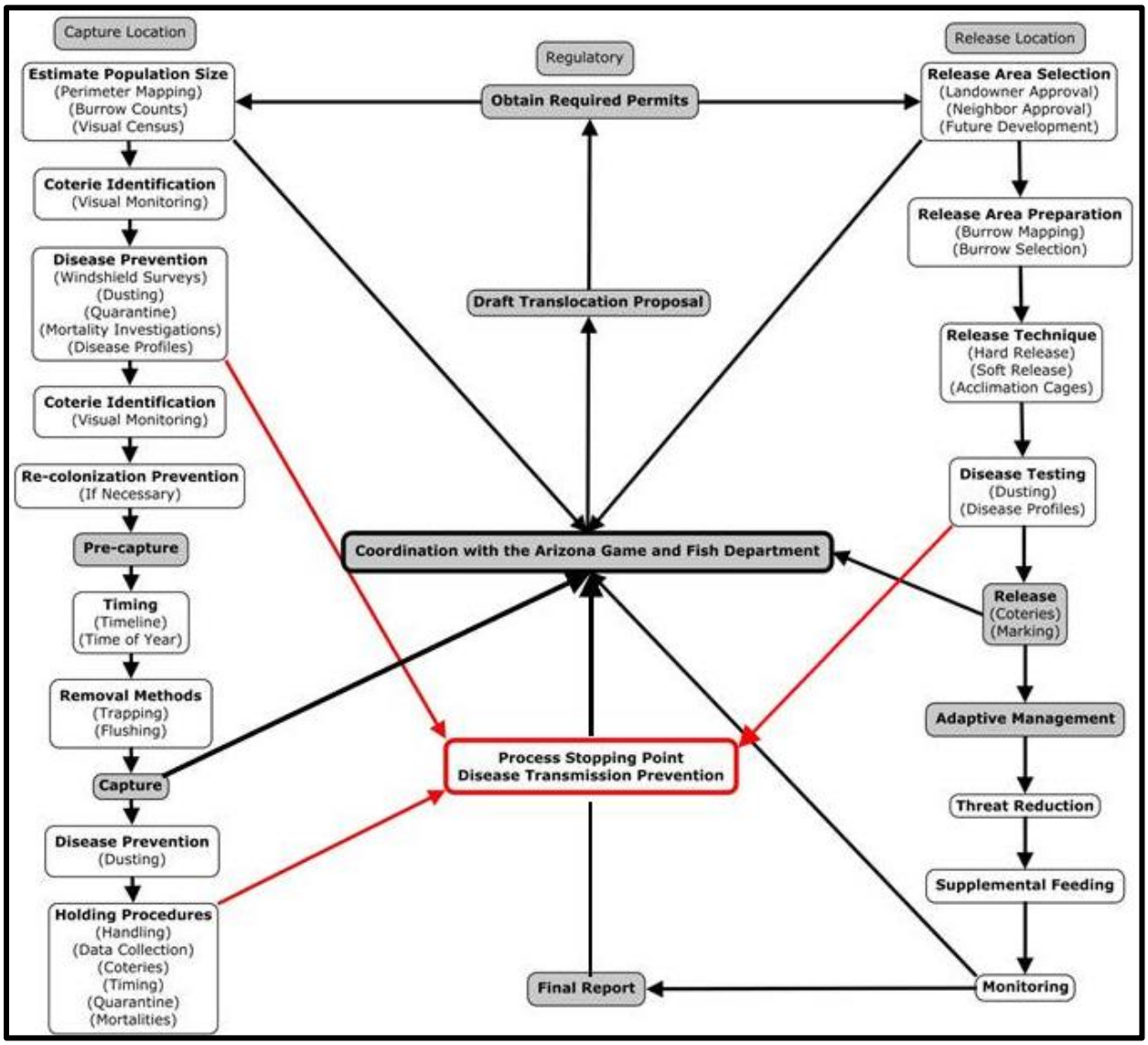

Figure 2. Arizona Game and Fish Department's Translocation Process.

PRE-TRANSLOCATION PROCESS

REGULATORY

Department Coordination

For all translocations, the Department will require the proper coordination and consistent communication throughout the entire process (Figure 2). For efficiency, pre-translocation coordination and communication should occur to ensure the Department supports the project. Commission Rule (R12-4-418) mandates that the translocation is in the best interest of the wildlife, and requires a proposal to permit the project. Post-permitting coordination and communication will occur prior to capture, after release, and through the annual report required for the license.

Required Licenses

In Arizona, wildlife are managed by the state and can only be taken as prescribed by the Arizona Game and Fish Commission (A.R.S. §17-102). The Department authorizes the translocation of wildlife through either a Scientific Collecting License (R12-4-418) or a Wildlife Service License (R12-4-421).

A Scientific Collecting License (R12-4-418) allows an individual to take, possess, and transport live wildlife only if:

1. The license is for the purpose of wildlife management; gathering information valuable to the maintenance of wild populations; the advancement of science; or promotion of the public health or welfare;
2. The license is for a purpose that is in the best interest of the wildlife or the species, will not adversely impact other affected wildlife in this state, and may be authorized without posing a threat to wildlife or public safety;
3. The license is for a purpose that does not unnecessarily duplicate previously documented projects; and
4. The applicant has submitted an acceptable typewritten, computer or word processor printed, or legibly handwritten project proposal as part of the application form.

A Wildlife Service License (R12-4-421) allows an individual or company to capture, remove, transport, and relocate to the wild designated live wildlife if the wildlife causes a nuisance, property damage, poses a threat to public health or safety, or if the health or well-being of the wildlife is threatened by its immediate environment.

Translocation Proposal

Commission Rule requires the Department to approve a proposal that addresses all activities included in the Translocation Checklist (Appendix A). In addition to the Checklist, the proposal must also have an estimated timeframe for each activity. Deviation from the identified timeframes must be coordinated with the Department to ensure the best interest of the wildlife.

Minimum Standards for Translocations - The Department shall establish minimum experience and proficiency standards for the persons conducting the translocation of GPD to better

implement the best management practices for capture, transport, marking, and release of wildlife. Any applicant for either a Scientific Collecting License or a Wildlife Service License for the translocation of GPD must demonstrate through resume and/or reference:

1. Experience conducting two trapping events with mammals of similar size that were captured and released. When an applicant only assisted (i.e. was not the responsible party for the capture), they must demonstrate experience with a minimum of four trapping events (captured and released) with mammals of similar size including at least one event that captured GPD.
2. Proficiency in the proposed trapping techniques. Applicant must justify their proficiency and describe trapping technique in detail including methods or techniques to prevent injury, harm, or predation.
3. Experience releasing non-target animals captured using the proposed trapping technique.
4. Experience marking a minimum of 40 small mammals with proposed marking technique, if auxiliary marking is proposed.

Additionally, the Department recommends a tetanus vaccination for all participants of the translocation, due to the possibility of injury from GPD bites and from handling metal traps.

Translocation Experts - Not every individual has the appropriate experience to meet the Minimum Standards described above. However, opportunities exist to gain experience through Department licensed individuals and organizations. The Department can help facilitate communication with these individuals and organizations.

CAPTURE LOCATION

Population Estimate

Estimating the population will require a combination of perimeter mapping and visual surveys. An accurate population estimate helps determines the appropriate acreage needed to accommodate the colony at the release location.

Visual Surveys – Visual surveys are used to estimate population size. Visual surveys are simply a count of the number of GPD visually observed. Observations are completed from a location (e.g. blind, or truck) or a distance that does not disturb the colony when colonies are most active. Surveyors should avoid conducting surveys during extreme weather conditions (i.e. heavy rain, high winds, extreme heat). Visual observations begin when GPD emerge from their burrows in the morning, and continue for four hours. Optics are required with a 10 to 15 power pair of binoculars and 20 power spotting scope recommended equipment. A large colony is surveyed in sections from a single location. Sections can be separated by topography, size, visual obstruction, flags, or stakes to delineate.

Perimeter Mapping - Perimeter mapping determines the acreage of the colony by walking the outer edge of the colony with the tracks function on a Global Positioning System (GPS) (Figure 3). While mapping, the surveyor ensures all active burrows are within the perimeter by using binoculars to scan ahead. Burrows with questionable activity outside the perimeter are investigated to determine whether to include. When doing so, pause the tracks function, determine whether the burrows are included, and then resume walking the perimeter from the point where the tracks function stopped. Alternatively, a preliminary perimeter survey could be conducted to determine the location of all active burrows prior to initiating the GPS tracks function. However, this will increase the effort. The surveyor ends at the starting point, enabling the GPS to calculate acreage. Computer software (e.g. ExpertGPS or ArcGIS) can also be used to calculate acreage.

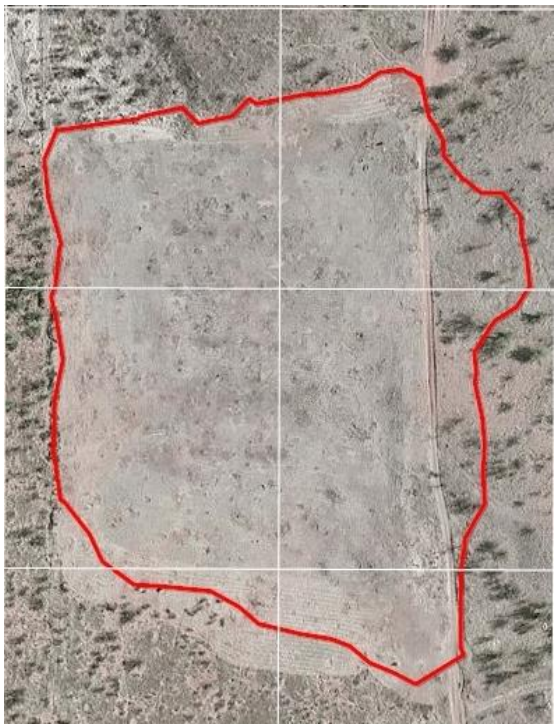

Figure 3: GPD colony with perimeter delineated.

Burrow Counts

Map the release site holes with GPS in order to identify suitable release holes in advance and recreate the geographic layout of the capture site at the release site (maintain existing coteries from capture site and release neighbors by neighbors). Locate, count, and map at least 25-30 good (open) abandoned release holes per 100 prairie dogs. Burrow counts are also a useful indicator of the number of traps needed to cover an entire colony.

Coterie Identification

Translocations are more successful when coteries (i.e., family groups) are moved together. Identifying coterie boundaries can be very simple or take several days of observation depending on the size of the colony. Similar to visual survey, the colony is divided into sections. A map depicting active burrows is useful to identify boundaries.

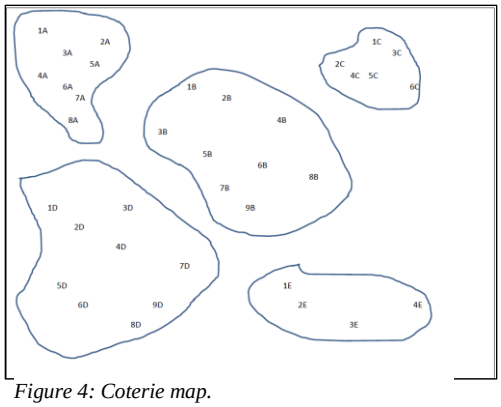

Figure 4: Coterie map.

The behavior of each individual that emerges from a burrow is monitored. Observers will document other burrows it may investigate and interactions with other GPD. A "kissing" or any other friendly behavior indicates these individuals belong to the same coterie. Chasing, alarm calls, or fighting indicates they are not part of the same coterie.

Mapping the burrows of coterie members allows for the delineation of coterie boundaries (Figure 4). Once all coteries are identified, flags are positioned around the

burrows to delineate coterie boundaries. Flags should be color coded or marked to distinguish among coteries. One flag is used for each burrow, but extra flags should be available for replacement.

Disease Prevention

Plague exists throughout GPD habitat in Arizona. To prevent the spread of plague, a license requirement will entail monitoring for the presence of plague following the Sylvatic Plague Monitoring Protocol (Appendix B), as well as treatment (e.g., burrow dusting) to reduce the proliferation of plague prior to and during the translocation.

Point Count Surveys – Point count surveys will be done daily for two weeks prior to the translocation to ensure there is no evidence of a plague epizootic. Evidence of an epizootic includes observation of dead animals above ground, recently active burrows collapsing or becoming overgrown with vegetation, and/or a trend of greatly reduced population numbers over time.

Figure 5: Prairie dog burrow dusting.

Dusting - Dusting the source colony burrows with DeltaDust or Deltamethrine will occur at least two weeks prior to trapping but at most six months to eliminate any fleas (Figure 5). Habitat dusting involves spraying an insecticide directly into a burrow cavity ensuring a 20 meter buffer from any water source.

Quarantine - The source colony will be self-quarantined prior to translocation. Self-quarantine is not an exclusionary quarantine, but rather a post-dusting two week daily monitoring period to document any mortalities. Evidence of an epizootic include dead animals above ground, recently active burrows collapsing or becoming overgrown with vegetation, and a trend of reduced population numbers over time. If mortalities occur, the translocation process will cease, pending the results of disease testing.

Figure 6: Dead prairie dog.

Mortalities - If plague is detected during quarantine, no translocations will occur until subsequent testing is negative for plague. In addition, necropsies will be completed by the Department on all mortalities (Figure 6), unless cause of death can be definitively determined. All carcasses will be placed in two zip-lock bags and labeled with GPS coordinates at the point of discovery, date, type of animal, name of the collector and who they are affiliated with, and a brief description of how the animal was found (Appendix C). Ideally, a carcass will be chilled, but not frozen. If shipping will not occur within 48 hours, freezing is acceptable.

Disease Profiles - The Department will construct a disease profile for both the source population and the release location prior to translocation. The profile will consist of an assessment of the

disease potential, habitat similarities and conflicts, and other species inhabiting the area, including predators and competitors.

Re-Colonization Prevention

If removal is due to nuisance issues, then modification (i.e. barriers or fences) to capture location will occur to prevent re-colonization. Generally, nuisance colonies will not be removed unless there are plans to implement re-colonization prevention measures.

RELEASE LOCATION

Release Area Selection

The release area must be at least five kilometers from the capture location to prevent re-colonization, and located at least 250 meters from an existing colony to prevent territorial disputes. There may be exceptions if there are barriers or natural topography that would prevent colonies from intermingling

Landowner Approval - The release area must have land-owner and Department approval. Coordination with the Department is required, as the Department may have specific areas pre-selected for translocation.

Neighbor Approval - Once land-owner approval is obtained, the possibility of a translocation will be discussed with neighboring land-owners, unless the release area is at least five kilometers from the property boundary. If the neighboring land-owner disapproves, then prevention measures are necessary (e.g. barrier, fences) to avoid dispersal onto this property prior to release, or a new release site should be selected.

Future Development - The potential release area should be investigated for future conflicts. Proposed development or change of land ownership could conflict with the intent of the translocation effort.

Release Area Preparation

Release locations will have variable habitat conditions that influence the suitability of the area. The amount of preparation depends on the number and/or condition of existing burrows. In addition, vegetation may need to be mowed, lowered, or removed (e.g. removal of junipers) to accommodate the new colony.

Burrow Selection - Each release area will be assessed to determine the presence and condition of any existing burrows. Open burrows can be used if the vegetation and debris is cleared away from the entrance, a determination is made that the burrow is not partially collapsed (using a long trowel, stick, or pole), and to the extent possible ensure no other wildlife occupies the burrow. Additional affirmation of non-use by other wildlife should be made at the time of release. At abandoned colonies, historical evidence of occupancy may exist, but the burrows may be collapsed leaving only a dirt mound. Collapsed burrows will need to be excavated using an auger and/or artificial burrows installed (i.e. excavation, nest pens, and acclimation cages; see Release Technique below).

Burrow Mapping - If the release area is an unoccupied colony with an existing burrow system, it will be mapped to ensure an appropriate number of suitable burrows exist, and to recreate the geographic layout of the capture site at the release site (i.e. maintain existing coteries and to release neighboring coteries accordingly). A minimum of 25-30 suitable abandoned burrows should be available per 100 GPD.

Release Technique

Applicants seeking authority to translocate GPD will need to describe the type of release method, and justify the use of that method in the proposal. There are two types of release method, hard and soft.

Hard Release - A hard release can be used when there is an existing burrow system in good condition (i.e. requires minimal or no work to prepare for the release) where the animals are simply released into the burrow without acclimation cages restricting their movements.

Soft Release - A soft release is required when releasing into a burrow system in poor condition (i.e. collapsing burrows) or into artificial burrows where acclimation cages are installed.

Acclimation cages are necessary in partially collapsed or artificial burrows to prevent immediate dispersal, but are not necessary in pre-existing burrows in good condition. Acclimation cages are constructed according to the specifications provided in Roe and Roe (2004) and Long et al. (2006) (Figure 7). Nest box(es) may be connected to the acclimation cage with artificial tunnels. Acclimation cages are left in place for a minimum one week after translocation. Supplemental feeding is required (see Post Translocation Process).

Figure 7: Acclimation cage.

Disease Prevention

Disease prevention at the release location will help ensure the survival of the released animals. Steps to prevent disease transmission to released GPD are critically important at abandoned colonies as the possible cause of abandonment was likely due to disease. The Department will require Dusting and Disease Profiles at the release location as described in the Capture Location section.

TRANSLOCATION PROCESS

PRE-CAPTURE

Timing

While it is preferable to capture, transport, and release the animals within the same day to reduce stress and prevent the likelihood of escape, logistically it may not be possible. GPD may be kept

for up to three days in kennels as long as bedding (timothy hay), food, water and tubing (or other hiding cover) is provided daily.

Timeline – A schedule that outlines the different phases of the capture effort (pre-baiting, trapping days, holding time, and translocation dates) should be established. . The schedule will include frequency of trap checks and time of day/temperature when trapping will end to prevent heat mortalities.

Time of Year - Trapping can begin a minimum of two weeks after pups emerge (mid-June), and continue until two months prior to hibernation (mid-September). Extraordinary circumstances may require earlier/later trapping date, but only at the Department's discretion.

Removal Methods

There are two methods for removing GPD; trapping and flushing/sudsing. Trapping is the Department's preferred method as it reduces capture mortality and is more effective. Flushing/sudsing is used when time is of concern (i.e. development occurring), but this method requires extensive equipment, training, and/or professional services. A combination of both trapping and sudsing may be used if complete eradication of the colony is warranted (i.e. construction, pest elimination).

Trapping – Trapping entails the use of live traps such as Tomahawk or Havahart traps that have a 6-8 inch square door and are 18-24 inches long. Traps are placed on level ground 1-2 meters from an active burrow with the entrance of the trap facing the burrow, and strategically placed to trap coteries. Each trap will have a flag or tag with a unique number and letter to identify the coterie. For example, with ten traps in one coterie, each trap would receive a unique number ranging from 1-10 and the coterie would be letter A. Therefore, each flag would be labeled 1A, 2A, 3A, etc. Alternatively, a coterie identifying system could be prepared using GIS in a map of the colony that labels each burrow with a unique number and letter. Bait is placed on and/or behind the trap treadle and with a small trail of bait from the treadle to the entrance of the burrow. Bait is replenished as necessary. Traps are visually monitored and checked regularly to prevent predation and overheating of trapped animals. Trapping will cease during inclement weather conditions and will be closed at the end of each day.

Flushing/Sudsing – Flushing/sudsing is the use of suds (i.e. water and soap) to flush the animals from the burrows. Individuals are captured by hand as they emerge and placed in a carrier. A water tanker and a pump that can provide suitable pressure to create foam are needed. Animals captured this way are treated with a saline solution to rinse soap and dirt from the eyes and towel dried. Prior training or experience with this process is required.

Pre-Baiting

Pre-baiting is a technique used to accustom the animal to enter the traps through the use of baits (i.e. sweet feed, sunflower seed, and/or peanut butter), but without trapping. Trap doors are open and clipped temporarily to prevent the premature capture of GPD. The length of the pre-baiting period is correlated to the length of time allotted for the animals to be trapped. An adequate pre-baiting period is 5-10 days prior to trapping.

CAPTURE

Disease Prevention

Dusting - Each captured individual must be dusted or sprayed with an insecticide (i.e. Permethrin) to prevent the transportation of fleas.

Holding Procedures

Handling - Upon capture, a cotton or burlap sheet is used to cover the trap to reduce stress on the animal during transport and processing. Prior to removing a trap, a label is affixed to the trap with the respective flag identification number and symbol to identify coteries. While processing, the animals are placed into a cone-shaped handling bag or held securely with thick leather welding gloves. Handlers must use gloves, long sleeves, long pants and apply insecticide to exposed skin.

Data Collection – Each individual will be examined to assess the sex and age (i.e. juvenile or adult) to separate adult males and maintain pups with adult females. Data on each individual, including capture location, holding cage, release burrow, release date, age, sex, and any identifying tags/marks will be recorded.

Marking - For post-release monitoring, it is optional to uniquely mark each GPD to monitor the status of the translocated population. Any four marking methods can be used either exclusively or in combination.

- Two uniquely numbered metal ear tags can be affixed on each ear at the base (Monel Small Animal Ear Tag 1005-1; National Band and Tag Co., Newport, KY).
- Color-coded ear tags can be used to help identify individuals with binoculars or remote cameras (Nelson and Theimer 2012).
- Passive Integrated Transponder (PIT; 134.2 kHz; Biomark, Inc., Boise, ID) can be injected subcutaneously on the back of the neck.
- Hair dyes can be used to paint a unique number on each side of the animal (Revlon Colorsilk black permanent hair color; Revlon, Inc., New York, NY).

Holding and Transportation - It is important to minimize the amount of handling and time spent in captivity. If the animals will be released on the same day they were captured, they may be transported to the release location in the trap, holding cage, or pet carrier. If holding the animals, all animals will be placed in holding cages or pet carriers with other animals from their coterie. Cages or carriers will be kept in shaded areas to reduce stress and prevent overheating until the animals can be released. All held animals will be provided daily food, water, and timothy hay, tubing, or some other material to reduce stress. GPD may be kept for up to three days in kennels.

Quarantine - If unexplained mortalities occur during trapping, all activities will cease and any animals held in captivity will be quarantined until necropsies and disease testing are performed. The Department will determine whether the translocation will continue based upon test results.

RELEASE

Coteries – To the extent possible, affirmation of non-use by other wildlife should be made prior to release. All GPD will be released by gloved hand directly into the burrows with others from their coterie. Each burrow will be marked to identify and release additional coterie members on subsequent trapping days. Pups are released with adults at an average of four prairie dogs per hole; range of one adult male, 1- 4 pups, and adult females released per hole.

POST TRANSLOCATION PROCESS

ADAPTIVE MANAGEMENT

Threat Reduction

Post-translocation monitoring will provide the data necessary for the Department to implement any necessary threat reduction measures. Dusting the burrows annually will reduce the threat of disease. In some cases the Department may impose a hunting closure and/or conduct predator control at the translocation site.

Supplemental Feeding

Supplemental feeding provides the necessary food and water to the translocated animals while they become acclimated to the release location. For a soft release, supplemental food and water must be provided on a daily basis during acclimation and for a minimum of one week after the acclimation cages have been removed. For a hard release, supplemental food must be provided every day for a minimum of one week, then every other day for an additional week. Supplemental food may include: carrots, corn-on-the-cob, apples, sweet feed, sunflower seeds, and grass hay (not alfalfa hay). To ensure the success of the colony post-release, it may be necessary to provide additional supplemental food during times of severe drought.

Monitoring

Post-release monitoring should occur once-a-month until hibernation, and at least once in the spring after hibernation. Monitor release site for prairie dogs and signs of activity, digging, expansion, mortalities, and predators. Perimeter mapping and density estimates may be useful to monitor population changes of the translocated colony over time.

FINAL REPORT

After all activities are completed, a final report of activities and data must be submitted to the Department.

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APPENDIX A: TRANSLOCATION CHECKLIST

Gunnison's Prairie Dog Translocation Checklist
Arizona Game and Fish Department

Action			Date	Completed
Pre-Translocation	Regulatory	Department Coordination		
		License		
		Translocation Proposal		
	Capture Location	Population Estimate		
		Coterie Identification		
		Disease Prevention		
		Re-colonization Prevention		
	Release Location	Release Area Selection		
		Site Preparation		
		Release Technique		
		Disease Prevention		
Translocation	Pre-capture	Timing		
		Removal Methods		
		Pre-baiting		
	Capture	Disease Prevention		
		Holding Procedures		
	Release	Maintain Coteries		
Post-Translocation	Adaptive Management	Threat Reduction		
		Supplemental Feeding		
		Monitoring		
		Final Report		

APPENDIX B: SYLVATIC PLAQUE MONITORING PROTOCOL

(Luce, R. J. 2003. A Multi-State Conservation Plan For The Black-tailed Prairie Dog, Cynomys ludovicianus, in the United States – an addendum to the Black-tailed Prairie Dog Conservation Assessment and Strategy, November 3, 1999.)

Appendix D

Black-tailed Prairie Dog Sylvatic Plague Monitoring Protocol

Since its documented appearance in wild rodents on the Pacific Coast of North America in the early 1900s, sylvatic plague has spread eastward to approximately the 103rd Meridian, affecting sciurid and cricetid rodents, insectivores, lagomorphs, carnivores, and humans (bubonic plague) (Barnes 1982, Cully 1993). Prairie dog species are extremely susceptible to this typically flea-borne disease and may serve as "amplifying hosts" (Barnes 1993).

Plague epizootics may originate from focal areas, with possible maintenance in non-focal areas between epizootics. During epizootics, plague can spread over great distances and in the process affect humans, most often during and shortly following epizootics (Cully 1993). Several wildlife species are considered enzootic or maintenance species for sylvatic plague, meaning individuals have some or considerable resistance to the disease. Examples include the California vole (Microtus californicus) in San Mateo County California, kangaroo rats (Dipodomys spp.), deer mice (Peromyscus maniculatus), and northern grasshopper mice (Onychomys leucogaster) (Cully 1993).

In the past, plague has been monitored for the protection of human health and conservation of prairie dog populations for ecosystem values, particularly protection of reintroduced populations of black-footed ferrets. As part of a range-wide commitment to black-tailed prairie dog management, the Interstate Prairie Dog Conservation Team is developing this plague protocol to monitor and react to the threat of sylvatic plague on a range-wide basis.

Application of Deltadust Insecticide, a prophylactic treatment for flea control in burrows, is sometimes used prior to prairie dog relocation into plague-affected colonies (Dave Seery, pers. comm.) This technique may have limited applicability for flea control in other situations and is the only active treatment method currently available.

Sylvatic plague surveillance methods are summarized below.

Technique	Description
"Windshield surveys"	General observations of prairie dog towns to detect die-offs, with followup evaluations needed to confirm cause and status. Coordination with health professionals, field personnel, and private landowners will be important to achieve a valid sample of colonies statewide
Collection and analysis of dead prairie dogs	Prairie dogs often die in burrows, but a small percentage of those exposed to plague die above-ground and can be picked up if colonies are regularly surveyed for dead and dying prairie dogs
Collection and analysis of fleas from prairie dog burrows	This technique has had widespread use as a surveillance technique for human health concerns. It is a part of the Shirley Basin/Medicine Bow black-footed ferret plague contingency plan in Wyoming (Luce and Oakleaf 1994). Young et al. reported on using this technique on Fort Belknap Agency, Montana, and the Pueblo Chemical Depot in central Colorado

Technique	Description
Collection of blood samples from members of Order Carnivora, especially coyotes and badgers	Although such species as badgers and coyotes can become infected with plague, their primary role in the disease cycle is the transport of plague-infected fleas (Poland and Barnes 1979 cited in Gage et al. 1994). Nobuto blood-sampling papers have been used extensively, since the technique does not require access to refrigerators and requires only 0.2 ml of blood (Wolff and Hudson 1974, Gage et al. 1994). This technique has recently been used in association with black-footed ferret reintroduction, either via collection of blood samples from live animals, dead animals collected for this purpose, or animals killed during animal damage control activities (Anderson et al. no date, Williams et al. 1998, Matchett 2001). In addition, black-footed ferrets captured for removal of radio collars, for implantation of transponder chips, or for canine distemper vaccination can be bled for disease analysis samples. This technique can easily be incorporated into blood collection for other purposes, such as genetic analyses (NPWRC 1999).
Collection of blood samples from domestic dogs	Barnes (1982) reported using domestic dogs as sentinels for exhibiting antibodies to plague. This technique has been effective on Native American reservations in the Southwest to detect seroconversion before plague was observed in rodents or humans.
Collection of blood from potentially resistant small mammals	Certain rodent species appear to be resistant to plague and may serve as maintenance or enzootic hosts that maintain plague between epizootics (Cully 1993, Gage et al. 1994). The Wyoming Game and Fish Department has monitored small mammals for plague seroconversion in Shirley Basin, Wyoming (Luce et al. 1994, 1996, 1997). Trapping efforts focused on deer mice and grasshopper mice, with the assumption that active plague would be detectable by antibodies produced during the short life span of these rodents. These investigations detected a relationship between seroprevalence of plague in deer and grasshopper mice and status of white-tailed prairie dog populations in Shirley Basin.

ACTIONS:

1. State wildlife agencies will initiate a public information program to inform landowners, hunters, and other members of the public concerning the need to notify the agency of die-offs of prairie dogs or ground squirrels.

2. State wildlife agency prairie dog coordinators, in cooperation with state public health officials, will take the lead to inform state Department of Agriculture, USDA-Wildlife Services, NRCS, veterinarians, and local government personnel that deal with animal control, or have regular contact with landowners and the public, of the need for reporting die-offs.

3. State wildlife agency prairie dog coordinators, in cooperation with state public health officials, will take the lead in providing information and training for state Department of Agriculture, USDA-Wildlife Services,

NRCS, veterinarians, and local government personnel that deal with animal control, on protocols for collection of dead prairie dogs and ground squirrels, packaging and record keeping.

The CDC and Wyoming State Veterinary Laboratory (WSVL) both have extensive experience conducting disease surveillance in wild mammals. CDC does not charge for diagnostic services, but has limited laboratory capacity. The eleven black-tailed prairie dog states will use CDC, individual state diagnostic labs, or WSVL diagnostic services for examination of prairie dog and ground squirrel carcasses for disease detection. Although other laboratories can provide a similar service as the WSVL, there would be a significant advantage in having all of the diagnostic examination done at a lab that is familiar with the procedures, will produce consistent results, and will report them state by state for the eleven states as the WSVL has done for black-footed ferret reintroduction sites for several years. In addition to testing for plague, specimens will be tested for tularemia, pasteurellosis, undetected poisoning, drowning, and predator kill.

4. State prairie dog coordinators will develop windshield survey routes throughout the prairie dog range to be conducted annually by wildlife agency or other personnel in each county, or smaller unit where prairie dogs occur, during March and April. Windshield surveys will follow the CDC protocol (Enscore pers. comm.)(Appendix 1). Significant decline in any colony or complex should be immediately reported to the state prairie dog coordinator.

In the event of a suspected die-off (if a windshield survey route reports a significant loss of prairie dogs or ground squirrels), the state will implement the plague contingency plan immediately (Appendix 2).

A. Make local inquiries to determine whether or not the colony was poisoned, or whether mortalities were due to heavy shooting

B. If neither shooting nor poisoning occurred, the colony or complex should be searched for prairie dog and ground squirrel carcasses as soon as possible after discovery of the population decline. Carcasses should be handled in the field according to protocol (Appendix 2)

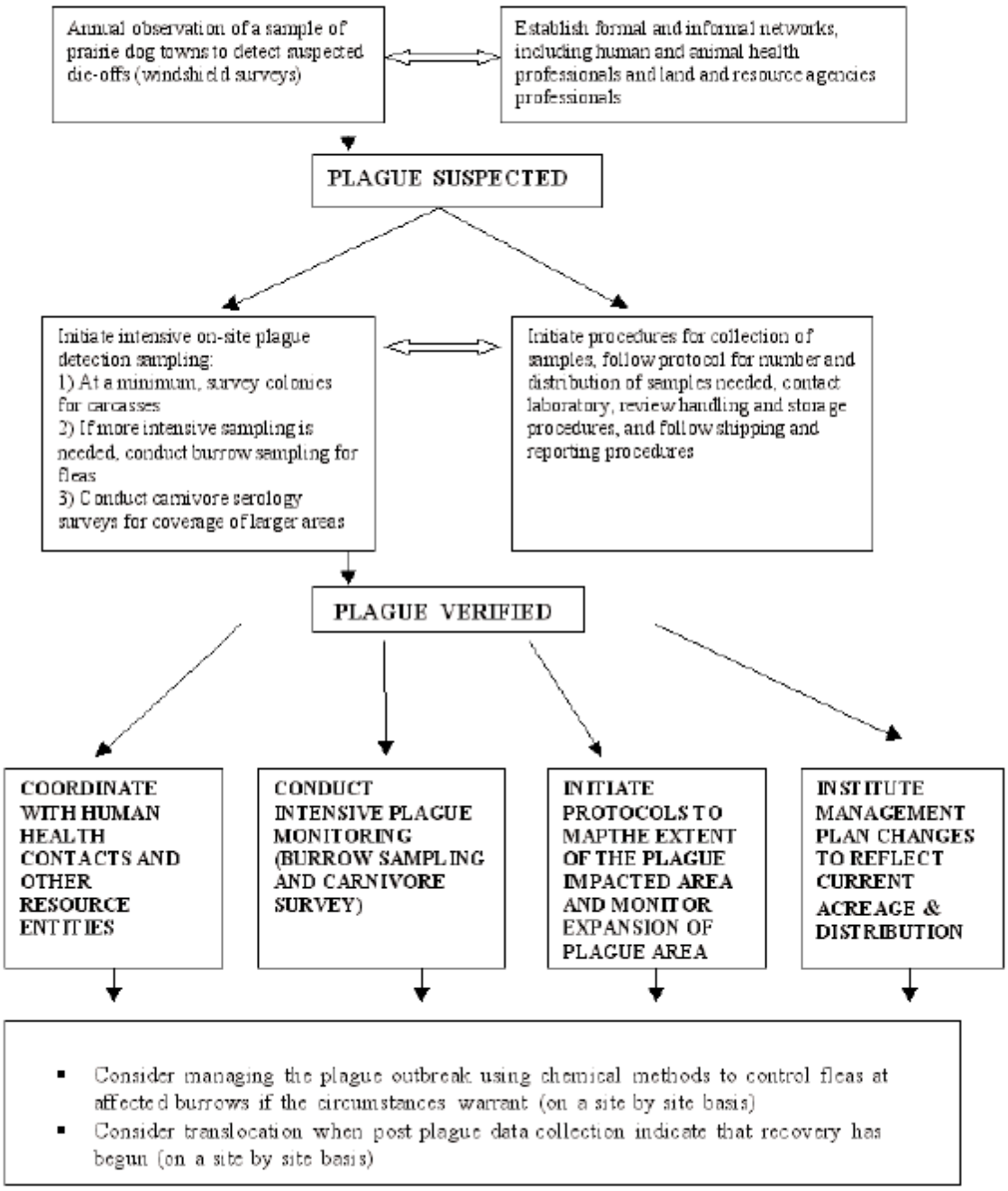
C. In the event that carcasses cannot be found, and the disappearance of prairie dogs is verified as recent, burrow swabbing should be conducted to collect fleas according to CDC protocol (Appendix 3)

6. If plague is verified, the prairie dog coordinator, in cooperation with state public health officials and CDC, should immediately notify, and make plague contingency recommendations to, the following: landowners and wildlife agency personnel in the affected area, state Department of Agriculture, USDA-Wildlife Services, NRCS, veterinarians, and local government personnel that deal with animal control, and the general public through local media sources.

7. Post-plague monitoring of prairie dog colonies should be conducted annually in March or April to document the rate of re-colonization and verify occupied acreage. Initial monitoring, which will take place from one to several years, should consist of windshield surveys. When visual surveys indicate prairie dog colonies are recovering, a quantitative survey method should be initiated. The recommended method, due to widespread use, particularly on black-footed ferret reintroduction sites, is transecting using the Biggins method (Biggins et. al. 1993) that equates active and inactive burrow densities to population density.

8. The prairie dog coordinator and the prairie dog working group should evaluate the extent of the impact of the epizootic as it effects the acreage and distribution objectives in the management plan. The group should determine whether or not there is a need to modify prairie dog management in the plague area, and potentially elsewhere in the state, if occupied acreage is below the objectives in the state management plan.

Plague Monitoring Framework



Appendix 1

Centers for Disease Control
Procedure for Visual Evaluation of Prairie Dog Colonies for Plague in the Southwestern United States

Citation: Enscore, R. personal communication. Undated. Centers for Disease Control and Prevention, NCID, Division of Vector Borne Infectious Diseases, Plague Section, Fort Collins, Colorado. 3pp.

A. HEALTHY COLONY

OBSERVATION: The vast majority of burrows show signs of recent use, unless it has rained within the past 24 hours – in which case the colony should be reexamined following a period of at least 24 hours without precipitation. Active prairie dogs are observed during periods of acceptable weather conditions. Only a relatively few (<10%) burrow openings appear inactive (lack of disturbed dirt, presence of cobwebs or wind-blown vegetation over the entrance). An occasional carcass or dried bones may be present as a result of non-plague death or predation.

EVALUATION: Unless recently (days) introduced, plague is not likely to be present. Fleas are not likely to test positive.

SAMPLE RECOMMENDATIONS: No samples recommended.

B. DEAD COLONY

OBSERVATION: The colony appears completely inactive. Burrows show no signs of recent use (re-examine if it has rained within 24 hours). An occasional desiccated carcass and bones may be present, and have likely been scavenged.

EVALUATION: 1) Make inquiries to determine if the colony was poisoned. This is especially likely if it appears that dirt was shoveled into the burrows. If there is no evidence of poisoning and the food supply appears ample: 2) it is likely that plague or some other zoonotic disease killed the colony. An experienced observer can usually make an estimate (recently, 1 season, or 2 seasons) on how long the colony has been inactive by considering the soil type and degree of burrow degeneration.

SAMPLE RECOMMENDATIONS: Sample only if there is no evidence of poisoning. A recent (same season) die-off might produce many fleas through burrow swabbing. Older die-offs will likely produce few or no fleas. Typically, many burrows (dozens or even hundreds) may be swabbed with only a few producing flees. If burrowing owls are using the inactive burrows, small black stick-tight fleas may be present in large numbers (in contrast to the larger, reddish-brown prairie dog fleas). Fresh or desiccated prairie dog carcasses may also be collected for analysis.

C. SCATTER PATTERN:

OBSERVATION: Inactive burrows constitute an unusually high (typically 20-90%) percentage of the total burrows. Active burrows however are clearly evident and active prairie dogs are observed during periods of acceptable weather. Active and inactive burrows are scattered amongst each other in no particular pattern (see below), keeping in mind that family units may have multiple burrow openings and hence an inactive unit may produce a small cluster of 2-5 inactive burrow openings. An occasional carcass (fresh or desiccated) and bones may be present.

EVALUATION: Several scenarios could account for these observations – and more than one scenario may be in play at the same place and time. Presented in order of likelihood: 1) Make inquiries to determine if the colony was poisoned. This is especially likely if it appears that dirt was shoveled into the burrows. This scatter pattern could be produced if the application of poison was scattered and not comprehensive, 2) If there is no evidence of poisoning, assess the available food supply. Such a pattern of death could also be attributable to a population crash as a result of lost carrying capacity of the site or over-population, 3) If there is no evidence of poisoning or population crash, hunting by humans or excessive predation by carnivores or birds of prey are highly likely. Human hunting usually produces physical evidence such as footprints, tire tracks and spent ammunition shells. Depending upon the local culture, human hunters may collect their prey (many Native American groups regard prairie dogs as a delicacy) or leave it for scavengers. Experienced observers can often spot carnivore tracks and recognize hunting and attack patterns in these tracks near burrow entrances, 4) Finally, a zoonotic disease could be responsible, but given this mortality pattern, a disease with a lower mortality rate than plague is more likely.

SAMPLE RECOMMENDATIONS: If there is no evidence of poisoning, population crash, or excessive human hunting: collect fleas by swabbing burrows – especially inactive burrows – and collect fresh or desiccated prairie dog carcasses if available.

D. DEAD ZONE

OBSERVATION: Within an otherwise healthy appearing colony, there is a zone of inactive burrows. This zone may encompass a relatively small or large proportion of the colony, and may be located anywhere in the colony. Eventually it spreads to encompass a section of the colony and appears to be spreading, along a discernable line of demarcation over the remaining section of the colony. Experienced observers can often clearly distinguish and mark (flagging tape) this demarcation line between active and inactive regions. Marking allows for periodic re-examination to assess the rate of spread and facilitates sampling. Fresh or desiccated carcasses may be present. Near the demarcation line, recently inactive burrows may reveal the odor of decaying carcasses and flies may be common at burrow entrances.

EVALUATION: 1) There is a high probability that plague is active in such a colony. Although other zoonotic diseases are possible, plague is most likely, 2) Depending upon the location of the dead zone with respect to other human activity (homes, barns, etc.) poisoning is also a possibility and should be investigated.

SAMPLE RECOMMENDATIONS: Collect fleas by swabbing burrows immediately along both sides of the demarcation line, concentrating a majority of your efforts immediately along (within 10meters) the inactive (dead) side of the line. Fleas are likely to be numerous. You may wish to apply extra insect repellent but be extremely cautious not to directly or indirectly get repellent on your burrow swab! (If this happens: discard it, wash your hands, and start with a new one). If others in a group are getting fleas and you are not, and you are swabbing essentially the same area, you likely have repellent on your swab. Collect any available rodent carcasses (fresh or desiccated, prairie dog or other rodent) for testing.

Additional Notes: Please include GPS coordinates for all samples. One set of coordinates per colony is acceptable. Specify the type of inactivity pattern noted for each sampled colony: dead colony, scatter pattern, dead zone. Analysis of samples from "dead zone colonies" will receive laboratory priority.

The above activity patterns are typical for the warm months. Visual examination during winter months is more difficult due to decreased daily activity among even healthy animals.

Appendix 2

Field Procedures for Collecting and Handling Carcasses as Diagnostic Specimens

1. Search prairie dog colonies systematically using walking or 4-wheeler transects spaced at about 50 meters.

2. When a carcass is discovered, ascertain if possible, whether or not the animal was shot. If mortality by shooting is confirmed there is no need to collect the specimen.

3. Before you collect a carcass, prepare a tag with the following information: species, date, location (both legal description and UTM is recommended), name of collector, agency or affiliation of collector, telephone number and address of collector, brief description of circumstances for collection.

4. When collecting a carcass, the collector should wear leather or latex gloves, and a long sleeved shirt or jacket that is tight at the wrist, to ward off fleas.

5. Invert a one-gallon plastic ziplock freezer bag over your hand, grasp the carcass in your hand, quickly fold the bag over the carcass, roll the bag on the ground, away from your body, to expel the air, and seal the ziplock.

6. Immediately place in a second ziplock bag, put in the tag, roll and seal the second bag.

7. As soon as possible after collection, freeze the specimen.

8. Sample Size:

   1) If specimens are from a single sample area (one prairie dog colony or area) collect as many specimens as is practical up to 15, but initially ship only the freshest five specimens to the diagnostic lab.

   2) Freeze the additional specimens that were collected, up to ten, and save for further testing needs, depending upon the results from the testing of the first five specimens. Keep the samples until notified by the WSVL or other lab that results were obtained form the first five samples and that the additional specimens will not be needed.

9. Ship the frozen specimen to WSVL, CDC, or designated lab.

   (DO NOT USE UPS). U.S. Postal System or FEDEX can ship carcasses that are sealed in plastic bags and a cardboard box. Their regulations require:

   1) Carcasses must be individually labeled and bagged in watertight bags (minimum triple bag in ziplocks)

   2) Placement of absorbent packing material around the carcass (crumpled newspaper, etc.)

   3) Use of approved laboratory shippers or hard-sided containers, adequately taped closed

4) Marking of the container with "Biomedical Material" label (for U.S. Postal Service) or shipped as hazardous material by Federal Express (requires a special form and should be labeled as Diagnostic Biomedical Material on the form. Labels and forms may be obtained from the U.S. Postal Service or Federal express.

5) Carcasses should be frozen or packed with frozen ice packs (no wet ice).

10. Cost: WSVL cost for testing for plague, tularemia, pasteurellosis, undetected poisoning, and predator kill is a maximum of $60.00 per specimen. CDC testing is free but the Ft Collins laboratory has limited capacity and can handle no more than 50 specimens per year.

11. Contact before shipping:

Dr. Beth Williams
Wyoming State Veterinary Lab
1174 Snowy Range Road
Laramie, WY 82070
307-742-6638

or

(Shipment by U.S. Postal System)
CDC/Bacterial Zoonoses Branch
c/o Mr. Leon Carter
P.O.Box 2087
Ft. Collins, CO 80522

(Shipment by FEDEX)
CDC/Bacterial Zoonoses Branch
c/o Mr. Leon Carter
Rampart Road (CSU Foothills Campus)
Fort Collins, CO 80521

Appendix 3

Centers for Disease Control
Procedure for Flagging (Swabbing) Rodent Burrows

Citation: Gage, K. Personnel Communication. Undated. Centers for Disease Control, Ft. Collins, CO. 3pp.

Leon Carter: 970-221-6444 (Biologist, Diagnostic and Reference Section - Responsible for handling specimens and doing much of the plague-associated laboratory work at CDC.)
Ken Gage: 970-221-6450 (Plague Section Chief - Responsible for CDC's plague surveillance and control program. Trained as medical entomologist/zoologist)
Rusty Enscore: 970-221-6452 (Environmental Health Specialist IV, Plague Section - Registered Sanitarian)
John Montenieri: 970-221-6457 (Biological Technician, Plague Section - GIS specialist)

Some important flea vectors of plague infest rodent species that live in burrows. Although these fleas usually can be found in abundance on live hosts, they also can be collected by a procedure known as burrow flagging or burrow swabbing.

This procedure requires:

1) **Burrow swabbing device** consisting of a flexible cable, wire, or strong rubber hose with spring-loaded clip attached to the end. We prefer a steel plummer's "snake" that has an alligator clip screwed on the end as a means of attaching the flag. A simple burrow swab can be made by attaching a flag to the end of a piece of wire (about the thickness of a coat hanger), but this primitive swab allows only the top 2 or 3 feet of a burrow to be swabbed and will miss some fleas. Despite the shortcomings of the latter technique, it can be useful when die-offs are encountered unexpectedly and more sophisticated means of swabbing fleas are not available.

2) **Flags** consisting of white flannel cloth squares (approx. 25 cm2 or 10 in2). We prefer white flannel because it is easier to see the fleas on white cloth than on cloths of other colors. Flannel is better than most other cloths because of its deep nap, which increases the likelihood that fleas will continue to cling to the cloth flag after it is removed from the burrow.

3) **Plastic bags** (approx. 20-40 cm2 or 8-15 inches)(Zip-loc type are best)

4) **Insect repellent** (DEET) to spray on clothes and exposed skin on arms, legs, etc. Although this is recommended for safety reasons, care must be taken not to apply repellents to hands because the repellent is likely to transfer to the flagging material, thus preventing fleas from jumping onto the flag. Note: Clothing also can be treated with permethrin-containing sprays but these sprays should not be applied directly to the skin.

Procedure:

1. Attach a flag to the clip on the end of the burrow swab.

2. Force the flag as far as possible down the burrow. The fleas confuse the flag with their normal host and cling to it as it passes through the burrow.

3. Slowly withdraw the flag from the burrow after approximately 30 seconds.

4. Quickly place the flag in a plastic bag.

5. Seal the bag to prevent the fleas from escaping.

6. Keep track of the number of burrows swabbed so that a burrow index can be calculated.
Burrow index = no. fleas collected/no. burrows sampled - This value often increases dramatically during die-offs among prairie dogs, rock squirrels, California ground squirrels, or other ground squirrel species)

7. Place another flag on the swab and repeat steps 1-6 for each burrow.

8. Transport flags back to laboratory in the plastic bags. Keep the bags in a reasonably cool place to prevent dessication of the flea samples (Yersinia pestis is very susceptible to death by dessication) or death of the plague bacilli due to excessive heat (remember pick-up hoods can get very hot in direct sunlight! Fried samples will come back negative for plague everytime!).

9. Place bags in freezer overnight to kill the fleas.

10. Place the flags and loose contents of the plastic bags in a white enamel pan. Fleas may be picked from the flags and bottom of the pan with forceps.

11. Place fleas in vials containing 2% saline and a very small amount of Tween-80 detergent (<0.0001% of solution). Remember the detergent is added to reduce surface tension and allow the fleas to sink to the bottom of the vial. Too much detergent will kill the plague bacteria and prevent successful isolation. Fleas can be submitted in 2% saline without Tween-80, but an effort should be made to submerge the fleas. If the fleas have been killed by freezing, this should not be a problem. Although not recommended for routine collecting, some investigators occasionally remove live fleas directly from the flags and place them in vials of saline. Live fleas placed in saline containing the Tween-80 detergent will be unable to float on the surface of the liquid, thus ensuring that they will drown soon after being placed in the saline. Without the detergent, surface tension can become a problem because the numerous bristles and setae found on fleas enable them to remain afloat on the surface of saline. This can be a potential safety problem because floating fleas often survive shipment and arrive at the laboratory ready to jump onto lab personnel. Rapid freezing of the fleas obviously eliminates this problem, but adding Tween-80 to the saline also helps reduce the growth of fungi on flea samples. Dead fleas trapped in the surface tension at the air-saline interface rapidly become overgrown with fungi making identifications more difficult.

12. Vials containing 2% saline and fleas can be shipped to CDC for taxonomic identification and analysis of the fleas for Yersinia pestis infection. The fleas can be shipped at ambient temperature in the vials of 2% saline. For best results, ship the specimens as soon as possible because the fleas will start to decay soon after collection. Be sure and double wrap the vials in a leak-proof material and then place them in a crush-proof box or metal mailing tube for shipment to CDC.

13. CDC Address: (Shipment by U.S. Postal System)
CDC/Bacterial Zoonoses Branch
c/o Mr. Leon Carter
P.O.Box2087
Ft. Collins, CO 80522

(Shipment by FEDEX)
CDC/Bacterial Zoonoses Branch
c/o Mr. Leon Carter
Rampart Road (CSU Foothills Campus)
Fort Collins, CO 80521

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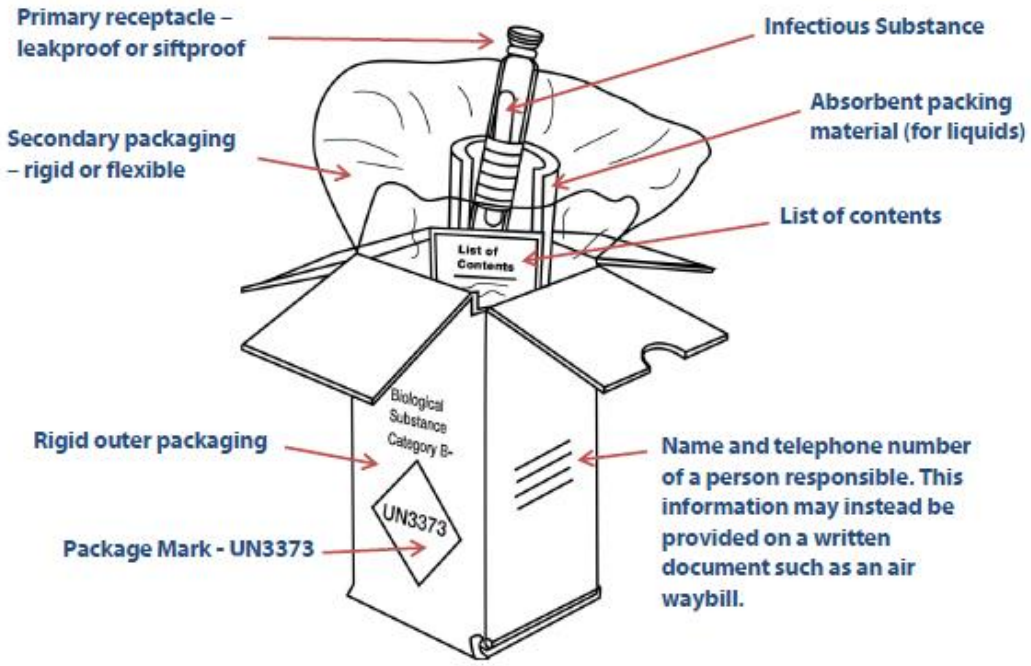
APPENDIX C: SHIPPING HAZARDOUS MATERIALS

Packaging Division 6.2 Materials

Biological substance , Category B, UN 3373



Center for Surveillance, Epidemiology, and Laboratory Services
Laboratory Training Branch

CDC