



Standard Operating Procedures for detecting and reacting to incidents of health risks for and die-offs in Saiga antelopes and other wildlife in Kazakhstan



Preface

In 2010 and 2015 mass die-off events have been observed in Saiga antelope of the Ural and Betpak-Dala populations in Kazakhstan. In intervening years, smaller die-offs of hundreds to a few thousands of animals have also been observed. These are the first such reported incidents after the dramatic decline in numbers in the 1990s, which led to the current status of a critically endangered species. Only a few thousand animals were left in 2003. Hunting of Saiga antelopes is forbidden and the species is protected by international conventions. Mass die-off events receive a lot of attention nationally and internationally, however, they always seem to arrive somehow unexpected, which was the case in 2010-2011, leading to little coordinated and late reactions from various institutions, governmental departments, laboratories, and NGOs. All parties involved were in some way responsible for the investigation, but their individual reactions were neither coordinated nor appropriate to identify the definite cause of these earlier incidents.

The government of Kazakhstan and other stakeholders have therefore initiated a process for improved reaction to such incidents in order to minimise the risks for the health of saiga, other wild and domestic animals, and human health. This is based on regular biological and health monitoring of the population especially during aggregation and calving in spring.

As documents from Soviet time show, mass die-offs happened in some years with an irregular pattern and can consequently also be expected in the future. Monitoring will increase the sensitivity to abnormal mortality and will help to ensure, that reaction to these incidents is fast, effective and involves institutions and experts working in an interdisciplinary manner. However, this process needs to be formally adopted by the Government of Kazakhstan and an appropriate unit of experts set up to ensure effective and timely reaction to any unusual mortality event affecting wildlife and specifically saiga antelope.

These Standard Operating Procedures (SOP) are meant to provide the framework for an improved reaction on disease outbreaks in wildlife, from the discovery of the outbreak to the final diagnosis and provision of information to the public. They should be followed by all involved organisations and agencies and thereby improve the efficiency of their actions.

The SOP have initially been produced as a result of two workshops in Kazakhstan in 2013, involving various veterinary authorities, ministries and researchers from Kazakhstan, and further developed in 2016. Towards the end of 2016, the Association for the Conservation of Biodiversity of Kazakhstan compiled the final version, consisting of the main procedures and several annexes.

The development of these Standard Operating Procedures has been made possible through financial support by the Food and Agriculture Organisation of the UN (FAO) and by the Convention on the Conservation of Migratory Species of Wild Animals (CMS) through the Saiga Conservation Alliance. Additional support was provided by Fauna & Flora International, Frankfurt Zoological Society and the Royal Society for the Protection of Birds in the framework of the Altyn Dala Conservation Initiative.

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Some annexes of this document have been developed on the basis of “Necropsy of Wild Animals” by Munson L. (University of California, 2004).

Standard Operating Procedures for detecting and reacting to incidents of health risks for and die-offs in Saiga antelopes in Kazakhstan

Step	Action
1. System of monitoring, detection and confirmation of die-off events	
a. Monitoring for the early detection of die-off events (<i>routine work, permanently</i>)	<p>1. Inclusion of the following indicators into the reporting system of rangers of PAs, State Enterprise “Okhotzooptom”, and hunting areas, using the questionnaire for wildlife rangers for observations of dead animals (Annex 1):</p> <p>a. Level of natural mortality of the population (collection of data on all observations of dead animals, including from causes other than disease).</p> <p>b. Unusual cases of singular mortality.</p> <p>c. Observation of signs, which are typical for causes of die-offs or particularly dangerous diseases.</p> <p>2. Data to be provided monthly to the Rapid Response Unit, weekly during the calving period in May, or immediately in case of meeting criteria in 1.b. below.</p> <p>3. Encouragement of local people to give information on observed carcasses to rangers of nature protection agencies.</p> <p>4. Veterinary services inform the state agency responsible for wildlife and/or the Rapid Response Unit monthly about the epidemiological situation among livestock regarding diseases, which might pose a risk to Saiga antelopes in their distribution range.</p>
b. Criteria, which serve as a signal for the initiation of investigatory activities	<p>1. Doubling of cases of singular mortality from the background normal.</p> <p>2. Group mortality with a concentration of at least 3 carcasses in the field of view of the observer.</p> <p>3. Observation of certain signs in living or dead saigas, which are typical for previous die-offs or particularly dangerous diseases (as listed in Annex 2).</p>
c. Scientific observation and investigation of saiga calving	<p>1. Conduction of a complex expert monitoring at the time of saiga calving, including some members of the Rapid Response Unit (RRU), including temporarily setting up video cameras.</p> <p>2. Provision of information about monitoring results, collected by other groups working at the area, to representatives of the Rapid Response Unit (RRU).</p>
d. Assignment of responsibilities in the case of detection of a die-off incident and reaction on it.	<p>1. Detection: State Enterprise “Okhotzooptom”, PAs, hunting areas, local population, scientific expeditions, individual experts, tourists, or any other persons observing unusual saiga disease or</p>

Information exchange.	<p>mortality.</p> <p>2. Initial diagnosis: local veterinary services, representatives of the Rapid Response Unit.</p> <p>3. Reporting: outcomes of point 1 and point 2 are reported to responsible governmental agency CFW MoA RK.</p> <p>3. Reaction: CFW MoA RK initiates complex work of Rapid Response Unit in its full composition according to regulations about the RRU in Annex 3.</p> <p>4. Reaction: a governmental inter-departmental commission (representing at least, but not exclusively: CFW MoA RK, Committee of Veterinary Control and Surveillance MoA RK, Committee of Science MES RK, Ministry of Emergency Situations) acts as defined in these procedures in cooperation with and supporting the action of the Rapid Response Unit.</p> <p>5. Information exchange: CFW of MoA RK – main authority, responsible for information exchange and coordination of all involved parties, acting according to this Standard Operating Procedures, step 7.</p>
2. Actions in case of risks for saiga health	
a. Immediate actions	<p>1. No initiation of any actions prior to the identification of the general type of die-off (infectious disease, toxic poisoning, etc.) among saiga.</p> <p>2. Initiation of surveillance among domestic livestock and preventive measures (in the event of an initial differential diagnosis which includes reportable severe infectious disease, limit free grazing and transport of livestock until the circumstances of the incident are clarified).</p> <p>3. After clarifying a differential diagnosis of the disease causing die-off, act according to the characteristics of the possible infectious or toxic agents and likely drivers or co-factors.</p>
b. Responsibility for planning and conduction of actions	Appropriate measures are undertaken interactively by veterinary services and departments of the CFW MoA RK among domestic livestock and wild animals respectively.
3. Investigation	
a. Collection of data and additional information	<p>1. The investigation is conducted by the Rapid Response Unit, consisting of experts with different expertise, using a complex approach in accordance with approved procedures and international standards (Annex 3).</p> <p>2. Diverse retrospective information should be promptly made available to the Rapid Response Unit.</p> <p>3. The investigation should be based on reliable (quantitative) data and analysis.</p>
b. Participants in the investigation and timeframe	1. The investigation is conducted by the Rapid Response Unit (RRU), consisting of experts, who have been given this role by the

	<p>responsible authorities in accordance with the approved regulation for the RRU in Annex 3.</p> <p>2. The duration of the RRU's work is determined by the circumstances and results, being received during the investigation.</p>
c. Sampling of pathological material for laboratory analysis	<p>1. The investigation and sampling for laboratory analyses is done by the Rapid Response Unit according to standard procedures and to written protocols (Annexes 4).</p> <p>2. Sampling of pathological material is conducted by veterinary services being a part of the RRU in accordance with the standard necropsy or live sampling protocol (Annexes 5.1-5.4), guaranteeing a sufficient amount of quality material for investigative clinical pathology (serology, microbiology, parasitology, histopathology, toxicology, clinical chemistry). It is necessary to take 4 samples each: 1 for storing it as a reference, 3 for different, independent, official, diagnostic laboratories.</p>
d. Measures for biological security of the staff	<p>Means for individual protection should be available in sufficient amount and means to exclude Anthrax (field diagnosis system should be provided). In case of suspect anthrax cases only trained staff should undertake investigation, and necropsy should be undertaken only if examination of stained peripheral (capillary) blood smear is negative.</p>
e. Measures of biosafety	<p>Measures for biological safety are implemented according to appropriate protocols (example in Annex 6) and depending on the level of hazard.</p>
f. Storage and transport of samples to the laboratory	<p>1. Samples of biological material should be stored according to standard procedures allowing a reliable, high quality diagnosis.</p> <p>2. The transport is done following a protocol agreed within the RRU, appropriate to the investigation and differential (different pathogens and samples can require different preservation and storage methods) and using biologically secure packaging in order to prevent distribution of the infection. Field missions should ensure a cold chain (+4 degrees) at a minimum and if possible availability of liquid nitrogen transport dewars of 35 liters and/or appropriate transport medium for samples to preserve organisms for culture or other diagnostic techniques where un-degraded DNA is vital.</p>
g. Laboratories	<p>Laboratories, to which samples are taken, should be selected in advance and informed about the amount and kind of material (list and storage preservation methods agreed in advance).</p>
4. Further measures against an epidemic	
a. Measures for biological safety, which can be undertaken at the time of the investigation	<p>1. Further activities for disease control are conducted by veterinary or public health services depending on the diagnosis.</p> <p>2. Horns from carcasses of saiga males are collected and</p>

	<p>registered for disposal, overseen by the CFW MoA RK.</p> <p>3. The approach for dealing with carcasses has to be selected taking into consideration the circumstances of the die-off and the diagnosis. Veterinary regulations exist for disposal of carcasses from certain specified diseases and where practicable these should be followed and where impracticable an agreed alternative method should be devised with the veterinary authorities.</p> <p>4. In the case of carcasses posing negligible onward infectious or toxicological risks, as determined by the RRU in conjunction with veterinary authorities, consideration should be given to not burying carcasses (for instance, if differential diagnostic indicating a non-infectious disease epidemic or observation of carcasses 1 week after death unless an anthrax outbreak where the carcass and soil contaminated by the carcass should be burnt).</p>
b. Controlling access to outbreak site	1. Access to the outbreak site is controlled by rangers of the State Enterprise Okhotzoprom outside and protected area rangers inside protected areas and granted only to representatives of the RRU, governmental commission, or others, authorised by the CFW MoA RK.
5. Rescue of newly born saiga calves	
a. Assessing feasibility and catching	<p>1. If the outbreak happens around the time of calving, the RRU assesses the need and feasibility for actions to rescue saiga calves when at the site of the outbreak (taking into account the age of the calves, the type of infection, biological security, etc.). Further steps follow only if assessment result is positive.</p> <p>2. PA rangers and rangers of State Enterprise Okhotzoprom (with support from NGOs, volunteers) set up short-term enclosures and bring saiga calves to them. Feeding is organised following standard procedures (based on experience of breeding centre of agricultural university “Zhangir Khan”) for about 2 weeks, before releasing calves to the wild.</p> <p>3. On capture the calves should receive a long acting dose of broad spectrum antimicrobial drugs, with drug choice taking account of any preliminary diagnosis.</p>
6. Processing of results of the investigation and laboratory results	
a. Responsibility for an overall assessment of evidence, received as a result of the investigation. Reporting.	<p>1. The RRU is responsible for the complex investigation and provides a complete report to the governmental inter-departmental commission, including an overall analysis of the results of the epidemiological investigation and laboratory diagnostics.</p> <p>2. A preliminary conclusion about the proximate cause(s), and a further report on the drivers and co-factors for the die-off, sufficiency of information and evidences, and further actions is adopted jointly by the governmental commission under the chair of the CFW MoA RK.</p>

7. Exchange and distribution of information

a. Distribution of information

1. In case of an incident raising significant public interest, an inter-agency communication group is founded (including relevant NGOs, if needed), in order to manage information and organise public relations; this communication group is chaired by the MoA RK.
2. Official information about the progress of the investigation into the reasons for the die-off is provided by the press service of the MoA RK or as agreed by the communication group.
3. Information provided by other sources, including members of the RRU, cannot be regarded as official.

Questionnaire for description of animal carcass

General data:

Observer's name:

Employer/affiliation/ contact information:

Date:

Time:

Please describe weather conditions for past week (estimate time of last rainfall)! Note if conditions are typical for the time of year or unusual in any way.

Coordinates:

Oblast:

Rayon:

Selskiy okrug:

Please describe the location (nearby landmarks, villages, relief, water bodies, vegetation)! Is the carcass located in any particular place in relation to such features?

Is there livestock in the area (if yes, what kind and how far away (km)?):

What observations of live, wild animals you have made in the area (species?, when?, where?, what number?)?

Species found dead:

Number of carcasses found? How far away from each other (meters; km)?

(If further two carcasses of the same species are in your field of view, you should immediately inform the Committee of Forestry and Wildlife MoA and local veterinary service!)

Did you find any carcasses of other species?

Yes

No

If yes, which, how many and where?

If near villages, were there observations of disease in livestock?

Yes

No

If yes, which diseases and/or which symptoms were observed?

Description of the carcass (as found in the field without moving it):

In what position did you find the carcass (please take photo!)?

Is there evidence of the animal struggling before death with crushing of vegetation and soil disturbance?

Yes No

If yes, which?

Is the carcass bloated?

Yes No

Does the hair strip easily and is the skin discoloured on the abdomen?

Yes No

Are the eyes moist?

Yes No

Do you detect any smell?

Yes No

If yes, which?

Are there any maggots in the oral cavity, eyes, nose, and/or anus?

Yes No

If yes, describe them and estimate number and size of each maggot in mm.

Try to naturally bend the joints of the legs. Are they stiff and do not bend?

Yes No

Can you see diarrhoea or discharges (eye, nose, mouth, urogenital tract)?

Yes No

If yes, describe volume, colour, consistency and where observed!

Is there blood?

Yes No

If yes, where is it coming from, and is it clotted or not (fluid)? Which colour does it have?

Is there sign of pending or recent calving? (e.g. swollen, bruised perineum, sign of calf, swollen mammary glands, colostrum or milk, placenta)

Yes No

If yes, which?

Can you see any signs of an outside force, which might have killed the animal (bites, bullet holes, ...)?

Yes No

If yes, which?

Can you see signs of scavenging?

Yes No

If yes, from which scavengers?

Please estimate, how many days ago the animal died!

What is the estimated age of the animal?

Calf <1 month Juvenile <9 months
 Young Adult >9 months Adult >2 years
 Mature Adult >3 years Aged adult >4years

Sex:

Male Female

Is the soil and vegetation dry or wet?

Dry Wet

Is the hair of the carcass dry or wet?

Dry Wet

Is the ground under the carcass dry or wet?

Dry Wet

Have you observed any other changes in the carcass?

If the animal will not receive a post mortem examination, please turn the carcass over (while protecting yourself from direct contact with it) and note any observations!

If you see animals ill, but alive, please watch them for some time and describe in detail the symptoms (please take photo and video records)!

Reaction in case of observation of animal carcasses

Short information for wildlife rangers

Background

Early reporting of an outbreak is essential for a rapid and effective response. Cause of death of an animal can be due to injury (e.g. predation, birthing trauma, fracture), acute poisoning or metabolic or digestive disorder, or other chronic or acute disease. Multiple deaths in a location can be due to an outbreak (epidemic) of disease and these can be due to acquired infections (e.g. bacteria, virus, vector borne parasite or combination) or non-infectious (commensal parasite turned virulent, plant factor or environmental toxin, digestive or metabolic disorder) disease. Some infections are transmissible to humans or other animals and all sudden death cases should be considered hazardous unless the risk has been assessed by a professional qualified to determine the likely cause of death.

DO NOT ATTEMPT TO CUT OPEN A CARCASS IN THE FIELD. IF BLOOD IS PRESENT IN NATURAL ORIFICES OR BLEEDING VISIBLE DO NOT HANDLE THE CARCASS AND ASSUME IT IS ANTHRAX UNTIL PROVEN OTHERWISE.

Post mortem examination should be done by a professional as soon after death as possible as the pathological diagnosis is less easy as time passes, as bacterial invasion of the body from the gut and decomposition commences within seconds of death. This does not mean old carcasses are not of some diagnostic value and all information possible should be obtained.

First responders to a carcass should record detailed information of the first observations, in order to allow for an appropriate reaction to the outbreak. A proper description of the situation in the field can build the basis for a decision about the initiation of a complex investigation of the incident and even potential countermeasures and actions for biosafety.

In most cases, wildlife rangers will be the first to observe carcasses, which are not located close to any settlement. Therefore, it is crucial that they follow a certain protocol in the case of an observation of a dead animal, both wild and domestic. This will maximise information gained and effective control.

Routine for wildlife rangers:

- During your regular patrols, you should always pay attention to any dead animals, which you find along the way.
- Try to look for them also in far distance using binoculars or by looking for groups of scavengers in the air.
- Try to change patrol routes and use also roads, which are rarely used, to decrease the chances of missing dead animals in your patrolling area.

What to do when a dead animal is found?

- Be aware that the animal might have died due to a disease, which is also dangerous for humans or livestock, and that you might inadvertently carry the pathogen on your body, clothes or vehicle to your home or community, putting their lives or livelihoods at risk. Therefore avoid direct contact with the carcass.
- Never change anything at the scene, especially the position of the carcass. Do not cut into the carcass!
- Record the coordinates of the location.
- Describe the scene using the special questionnaire. This questionnaire should be part of your report to your main office.
- If...
 - ...the carcass looks normal (i.e. not thin, unthrifty, traumatised) and the death appears sudden ASSUME IT IS ANTHRAX AND A HAZARD or
 - ...you can see from the location of the carcass a further two carcasses or more of the same species or
 - ...you see obvious signs of diarrhoea or
 - ...you can see discharges (if blood ASSUME IT IS ANTHRAX)

...you have to notify as soon as possible the Committee for Forestry and Wildlife of the Ministry of Agriculture and the local veterinary service. Stay in the area, guide veterinary service personnel to the location and stay available to answer questions.

- If the carcass looks suspicious to you and is located near a human settlement, keep people, dogs and livestock away from it until the area is declared safe following formal investigation. If the carcass is found near a potentially contaminated water body, keep livestock away from it.
- If you do not observe anything of the above mentioned, you can continue your patrol after filling out the questionnaire as part of your report to your main office. If you come to a village in the surrounding area, please inform the local veterinarian about your observations.

Anthrax

Anthrax can affect animals and humans. It can lead to death.

Signs, which might indicate Anthrax, are:

- Blood coming out of the carcass, especially if dark and not clotting normally
- Spongy swellings (other than bloat) under the skin, especially around the neck and chest
- Swollen neck or head in carnivores
- Even in the absence of these signs, it is not possible to exclude anthrax until blood has been examined by a professional, so assume the worst and do not handle or open the carcass until anthrax is formally ruled out

Regulations for a Rapid Response Unit reacting on cases of disease and mortality in wildlife

Mission

Establishment of a rapid response unit (RRU) for investigating saiga mortality: This unit should be comprised of a group of scientific experts and advisers (national and international) of various disciplines available to react anytime in the event of a disease epidemic or mass die-off in Saiga antelopes, in any of the three populations in Kazakhstan.

RRU composition: A core team should be ready on 24 hour notice for immediate departure to the place of the incident in order to initiate a fast and thorough investigation including sampling and laboratory analyses. Supporting experts are called in as necessary and issues unfold to undertake specialised studies relating to the syndrome.

RRU coordination: The RRU should be logistically and financially supported from all relevant ministries and agencies and well coordinated by one of them in order to produce a reliable and high-quality conclusion, enabling appropriate measures to be taken against an epidemic among wildlife, domestic animals and humans.

General principles for the Rapid Response Unit (RRU)

- The RRU is responsible for disease outbreak investigation in all populations of Saiga antelope and other wildlife species in Kazakhstan.
- The RRU has an independent, inter-ministerial and departmental status and works independently.
- The RRU acts strictly according to the officially approved Standard Operating Procedures.
- The Committee of Forestry and Wildlife of the Ministry of Agriculture (CFW MoA RK) has the coordinating and administrative role in the RRU.
- The RRU is activated by the CFW MoA RK.
- The RRU is on site of the incident 36 hours after activation.
- All equipment (see attached list) needed for investigation and sampling at the site should be pre-prepared and ready to be taken at any time
- Local veterinary services and wildlife rangers from State Enterprise Okhotzoprom support the RRU in its work, especially concerning logistics (transport and accommodation).
- The RRU takes samples to the appropriate approved laboratories for analysis.

- At the end of the investigation, the RRU provides a preliminary diagnosis and all relevant information to the inter-departmental commission, dealing with the incident.
- Information is exchanged and distributed according to the Standard Operating Procedures.
- International experts can be brought in ad-hoc to support the work of the RRU in order to get additional expertise in the group.

Composition of the Rapid Response Unit

The experts in the RRU should cover various disciplines to tackle the complex issues associated with wildlife disease outbreaks. They are selected according to their field of work and previous experience and training as well as personal characteristics. Additional to their usual field of expertise, the members of the RRU should be trained on main aspects of saiga ecology and generally diseases in wildlife and reactions to it. Such training should also contain a practical component conducted at a potential site of an incident.

The following table shows the composition of the RRU, which is recommended for an outbreak investigation. The pool of people, who could be activated, should at least contain 2 experts for each of the following positions in order to guarantee that an interdisciplinary team can be constituted rapidly in any and every investigation.

Expert	Usual place of work	Responsibilities
Administrator/ coordinator	Committee of Forestry and Wildlife MoA RK	Representative of the responsible state agency, the Committee of Forestry and Wildlife of the MoA, coordinating activities, organising logistics, solving issues with local administration
Veterinarians (3 teams with 3 experts - one veterinary pathologist, one wildlife veterinarian with appropriate training, and one livestock veterinarian)	National veterinary reference centre (plus others, if officially members of RRU)	Investigation of ill animals or carcasses, sampling according to protocols
Expert on disease ecology	independent (anti- plague stations?)	Identification of ecological links, which might play a role in the disease outbreak
Expert on saiga ecology / zoologist	independent (Institute of zoology, ACBK?)	Investigation of ill animals and other animal species in the area
Geobotanist	independent (Institute of botany?)	Investigation of vegetation at the site of the outbreak or die-off (and, if possible, along the path the animals used to this site)

Pasture expert	Independent (Institute of botany?)	Assessment of condition of pastures at the die-off site, possible contacts between livestock and wildlife, and effects of specific plant species on livestock and potentially wildlife
Ecotoxicologist / biochemist	independent	Taking samples from environmental components for toxicological analysis
Local veterinarian	Local veterinary station	Providing information on the condition, vaccination, distribution of domestic livestock in the area
Physicist/radiologist	Kazakh agricultural university (Astana)	Taking samples and radiological examinations at the outbreak and die-off site
Meteorologist	Met Station locally, Kazgidromet	Evaluation of unusual weather conditions before and during the outbreak

Important issues to be solved

1. A decision should be passed by the government, adding to the rules for reactions on emergency situations the inter-ministerial and departmental cooperation to support this Rapid Response Unit.
2. Financial resources are needed to fund the investigations of the RRU, especially to cover expenses related to the field investigations. Various possibilities should be checked for funding this.
3. Funding needed to set up the RRU (training, equipment).

Outbreak investigation protocol

Submitter's name:

Affiliation:

Address:

Telephone:

E-mail:

Date of completion of form:

Date and time of investigation:

Date of first report of mortality event (and subsequent events):

Disease onset: (The best estimate of when the outbreak started. Describe the most decomposed carcasses in terms of presence of larvae, dryness, skin and bone. Was disease observed before the deaths, and for how long before the first deaths?)

Number of investigators:

Total time taken for investigation:

Weather before and during investigation:

Specific die-off location (attach photos if applicable)

Oblast:

Rayon:

Selskiy Okrug:

Latitude/longitude (degrees, minutes, seconds):

Environmental factors: (Record conditions over the period leading up to the outbreak such as storms, precipitation, temperature changes, or other changes that may contribute to stress e.g. food shortage, water availability, presence and relative abundance of insect vectors, recent use of pesticides. Also any unusual coloration in water, or moulds on vegetation, or unusual plants in the area.)

Species affected: (The diversity of species affected may provide clues to the disease involved. Include domestic livestock, small and large mammals, birds)

Vector presence and density: mosquitos, biting flies (horsefly, biting midge, ...) and ticks should be recorded

Age/sex: (Any selective mortality related to age and sex. Reproductive status of females, i.e. pregnant, giving birth, with calves at foot; deaths in calves and its timing relative to mothers; whether males affected and how many relative to females)

Morbidity/mortality/alive: (Ratio of sick animals to dead to live animals.)

Known number of dead animals: (Number actually counted.)

Estimated number of dead animals: (Consider removal by scavengers or other means.)

Observations of the carcass(es): (Any unusual behaviour and physical appearance. Signs of struggle before death, e.g. flattened vegetation, scuffed ground? Discharges, if so describe orifice, nature and volume; wounds, skin and hair condition, teeth condition. Bloat or rigor mortis. Swellings or discoloration. Condition of calf and placenta if born or stillborn. Attach photos if applicable.)

Population and species at risk, but currently unaffected: (Number of animals of each species in the area that could be exposed to the disease. Include domestic livestock.)

Population movement: (Recent changes in the number of animals on the area and their source or destination, if known. Occupation of this area by relevant species in recent years. Consider asking rangers, local people etc.)

Problem area description: (Land use, habitat types, and other distinctive features. Any unusual features such as flooding, unusual plants, discoloration of water or plants. Attach photos if applicable)

Comments: (Additional information/observations that may be of value such as past occurrences of disease in area. Sickness or death in domestic or other wild animals or people.)

(Adapted from Appendix A, Field Manual of Wildlife Diseases, USGS, 1999)

Necropsy Report – Cover Sheet

Name of prosector

Address of prosector

Location of carcass

SPECIES

ID no.

WEIGHT

SEX

DATE OF DEATH

DATE OF NECROPSY

HISTORY: (briefly summarize clinical signs, circumstances of death):

SHIPPING TISSUES: PLEASE OBTAIN PROPER CITES AND EXPORT PERMITS BEFORE SHIPPING TISSUES BETWEEN COUNTRIES. After 72 hrs in fixative, ship tissues in a leak-proof container in adequate formalin to keep tissues moist.

Necropsy report – examination worksheet

PROSECTOR:

GENERAL CONDITION: (Nutritional condition, physical condition, position of organs, costal pleura, diaphragm)

Neonates: examine for malformations (cleft palate, deformed limbs etc).

SKIN: (Including pinna, feet, condition of fur, intactness of the skin, subcutaneous loose and connective fat tissue, insect bites)

MUSCULOSKELETAL SYSTEM: (Bones, joints, muscles)

BODY CAVITIES: (Fat stores, abnormal fluids)

Neonates: assess hydration (tissue moistness)

HEMOLYMPHATIC: (Spleen, lymph nodes, thymus)

RESPIRATORY SYSTEM: (Nasal cavity, larynx, trachea, lungs, regional lymph nodes)

Neonates: determine if breathing occurred (do the lungs float in formalin?)

CARDIOVASCULAR SYSTEM: (Heart, pericardium, great vessels)

DIGESTIVE SYSTEM: (Mouth, teeth, esophagus, stomach, intestines, liver, pancreas, gall bladder, mesenteric lymph nodes, content of stomach and intestines). Neonates: is milk present in stomach?

URINARY SYSTEM: (Kidneys, ureters, urinary bladder, urethra)

REPRODUCTIVE SYSTEM: (Testis/ovary, uterus, vagina, penis, prepuce, prostate, mammary glands, placenta)

ENDOCRINE SYSTEM: (Adrenals, thyroid, parathyroids, pituitary)

NERVOUS SYSTEM: (Brain, spinal cord, peripheral nerves)

SENSORY ORGANS (Eyes, ears)

PRELIMINARY ANATOMOPATHOLOGICAL DIAGNOSES:

LABORATORY STUDIES: (List, for example, bacterial and viral cultures submitted and results, if available)

(Adapted from Munson L., Necropsy of Wild Animals, University of California)

Checklist for fixed tissue

All tissues listed below should be preserved in 10% buffered formalin (one part by volume of tissue to 10 parts by volume of fixative). Tissue samples should not be thicker than 0.5 cm. A sample of **all** lesions seen and of **all** listed tissues should be included:

- 1) Salivary glands
- 2) Oral and pharyngeal mucosae
- 3) Tonsil
- 4) Tongue (cut across tip)
- 5) Trachea
- 6) Lungs (specimens from several lobes)
- 7) Thyroid and parathyroid glands
- 8) All major lymph nodes (cervical, mediastinal, bronchial, lumbar, mesenteric etc. sectioned to 1 cm³)
- 9) Thymus
- 10) Heart (sections from auricles and ventricles and from valves)
- 11) Liver (three representative specimens, including bile duct and gall bladder)
- 12) Spleen (representative cross-section including capsule)
- 13) Oesophagus (representative section: 3 cm)
- 14) Stomach (several specimens from all areas)
- 15) Intestines (3 cm representative specimens from each region)
- 16) Omentum (specimen of 3 cm)
- 17) Adrenal glands (whole gland cut in half)
- 18) Kidney (1 cm³ from cortex and medulla of each kidney)
- 19) Urinary bladder (ureters and urethra, cross-section of bladder, including mucosa; 2 cm sections of ureters and urethra)
- 20) Uterus and ovaries: if possible the complete uterus (plus contents) should be preserved with ovaries attached. Uterine horns should be incised to allow entry of fixative. If this results in a specimen which is too bulky, representative samples of the cervix and uterine wall should be preserved. Ovaries should be preserved whole or, if large, should be cut transversely.
- 21) Testes (0.5 cm³ section of each testis, including capsule)
- 22) Epididymis (representative sample)
- 23) Prostate gland (whole gland or, if large, representative 1 cm³)
- 24) Eyes (whole eye, with sclera incised to allow entry of fixative)
- 25) Brain (cut in half, with one half preserved in buffered 10% formalin and the other half preserved for virology and toxicology)
- 26) Spinal cord (sections from cervical, thoracic and lumbar regions)
- 27) Diaphragm and skeletal muscles (representative samples of major muscle groups)
- 28) Bones (sawn section of femur including marrow)
- 29) Skin (section of abdominal skin, lip and ear pinna)
- 30) Neonates (umbilical stump and surrounding tissues should be preserved)
- 31) Blood smear (fixed in methanol)

Equipment checklist

A necropsy kit should always include the following items:

Protective clothing

- 1) rubber boots or rubber overshoes
- 2) rubber or plastic gloves
- 3) rubber apron
- 4) overalls (boilable)
- 5) face mask including goggles to cover eyes

In case anthrax is suspected, complete protective clothing and equipment is needed, for instance as described in http://www.who.int/csr/resources/publications/anthrax_web.pdf

Necropsy documentation

- 1) photo-/ video camera
- 2) field notebook and pen/pencil
- 3) necropsy protocols
- 4) Satellite navigator (GPS)

Specimen containers and sampling equipment

The following list of equipment is necessary for sampling (sufficient for at least 30 carcasses):

- 1) sterile disposable 5 ml syringes and sterile needles (20 g)
- 2) culture tubes with sterile swabs
- 3) universal plastic sealing pots / tubes (about 100 pieces)
- 4) microscope slides in box
- 5) sterile Universal bottles
- 6) sterile blood tubes (120 plain tubes, 30 EDTA / heparin)
- 7) plastic bags with closure tops (Whirlpack or Ziploc type)
- 8) heavy duty plastic sealing tape
- 9) 300 ml wide mouthed glass or plastic jars
- 10) measuring tape or ruler
- 11) rubber or plastic gloves (and talc)
- 12) aluminium foil
- 13) rabies kit (World Health Organization) (or drinking straw in a small jar of buffered glycerine)
- 14) labels, string, waterproof marker pen/pencil

Post-mortem equipment

The minimum requirements for conducting a safe and satisfactory field post-mortem examination are as follows:

- 1) curved knife for skinning
- 2) straight, pointed knife for dissection
- 3) 25 cm rat-toothed forceps
- 4) 15 cm pointed forceps
- 5) 15 cm dissecting scissors
- 6) sterile scalpel and blades
- 7) enterotome
- 8) bone saw
- 9) large pair of bone forceps or bone-cutting shears

- 10) axe
- 11) sharpening stone and steel
- 12) spring balance to weigh to 10 kg
- 13) block and tackle
- 14) nylon rope
- 15) small gas or alcohol burner for sterilising instruments.

The kit may be packed in a stout, heavy, wooden box.

Alternative to the list above: big or small anatomical set of instruments.

Transport equipment

- 1) an insulated, plastic cooler box
- 2) leak-proof, screw cap, plastic containers
- 3) absorptive packing material
- 4) string and heavy duty plastic sealing tape
- 5) sterile buffered 50% glycerine (see Appendix I for formulation)
- 6) 'easy blood' (see Appendix I for formulation)
- 7) 'blue ice' freezer packs (pre-frozen).
- 8) field vehicle refrigerator (if possible)
- 9) liquid nitrogen dewar (if possible)

Fixatives

The following fixatives are used:

- 1) 10% buffered formalin (see Appendix I for formulation)
- 2) 100% acetone for cytology (caution! fire hazard!)
- 3) 70% alcohol for parasites
- 4) paradichlorobenzene

Disinfection materials

Disinfection materials include the following:

- 1) jerry can of water
- 2) a plastic bucket and brush
- 3) soap
- 4) nailbrush and towel
- 5) borax
- 6) 5% formalin
- 7) sodium hypochlorite (0.5%)
- 8) 70% ethyl alcohol for disinfecting instruments
- 9) sodium carbonate (5%)
- 10) Gel or cream for the hands (after treatment with 70% alcohol)
- 11) Cotton wool, paper tissues

Others

- 1) Field microscope with a mirror for a light source or adapted for car batteries (A field microscope will permit assessment for anthrax before opening a carcass).
- 2) Centrifuge, portable and working on 12V car battery (if available)
- 3) Set of staining solutions: Gram solution, Giemsa's solution (for colouring blood smears, microscopy and identifying pathogens of anthrax)

(Adapted from Munson L., Necropsy of Wild Animals, University of California)

Protocol for safe handling and disposal of carcasses

1. All dead animals should be handled only while wearing gloves; this includes carrying of dead animals, during necropsy procedures, and the dressing out of carcasses. There are several types of gloves to choose from, including leather, rubber, and latex gloves. Rubber or latex gloves are preferred due to their low cost, wide availability, and ease of disinfecting (latex gloves are disposable).
2. The carcass should be placed in a plastic body bag and sealed as soon as possible. If a zoonotic disease is suspected (i.e., rabies, tularaemia), it is recommended to double bag the carcass.
3. Avoid direct contact with the dead animal's body fluids (i.e., blood, urine, faeces). If contact does occur, wash the skin area contacted with soap and water as soon as possible.
4. Avoid contact with the dead animal's external parasites (i.e., fleas and ticks). If possible, spray the carcass with a flea & tick spray prior to handling it. If pesticide poisoning is suspected as the cause of death and laboratory testing is to be performed on the animal's tissues, avoid spraying the carcass as it will interfere with laboratory results.
5. Proper disposal of the carcass (incineration, burying, etc.) is critical to prevent exposure of other wildlife and humans to disease. Three common effective methods of carcass disposal are: incineration, burying, and rendering. Incineration is the preferred method to use when the carcass is diseased; however, it can also be the most expensive. An acceptable alternative is to bury the carcass. The carcass should be buried at least 4 feet deep and covered with lime to discourage scavengers from uncovering and consuming it.
6. Persons who have direct contact with wildlife, especially carnivorous animals, on a regular basis are highly recommended to receive the rabies pre-exposure vaccination series. The pre-exposure series consists of a total of three vaccinations and is highly efficacious in preventing rabies. It is also recommended to have a rabies antibody titre tested every two years to determine the level of protection.

*(Adapted from California Department for Fish and Wildlife, Wildlife Investigations Lab;
<https://www.wildlife.ca.gov/Conservation/Laboratories/Wildlife-Investigations/Monitoring/Protocols>)*