Stoat pathogen survey, submission and testing protocol

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1. Purpose

The purpose of this protocol is to provide instructions for collection and testing of specimens and reporting of results required to complete a survey of pathogens associated with disease in stoats *(Mustela erminea)* in New Zealand.

2. Scope

This protocol applies to personnel collecting and submitting specimens, conducting necropsies and diagnostic tests and reporting results.

3. Co-ordination

The handling of samples, submissions and collation of results will be handled by a single individual who will ideally have experience and training in veterinary pathology and diagnostics (the "project manager"). The project manager will have discretion to decide what testing is appropriate and who the testing should be sub-contracted to. The project manager should be contacted in the first instance for queries about individual submissions or the study in general.

4. Submissions

4.1 SELECTION OF ANIMALS FOR INCLUSION

Animals and specimens for inclusion in this study should originate from stoats displaying signs of disease, or those suspected of being diseased, as a result of some pathogen. Normal healthy animals or those known to be suffering from some non-infectious disease (e.g. nutritional deficiency, toxicity, trauma) should be included if necessary to establish normal ranges (e.g. clinical pathology values, histology of normal tissues, etc.).

4.2 SUBMISSION OF SAMPLES AND/OR ANIMALS

When a sick or suspected sick stoat is discovered, the project manager should be contacted to discuss submission details and to provide background infor-

mation. This is essential to ensure detailed information is obtained for each submission to the study.

Suitable organisations for submission of specimens are appended.

In cases where the sick stoat(s) is no longer required, it should be humanely killed. Ideally this should be achieved by the humane administration of a drug overdose (e.g. barbiturate). Killing the stoat by other means (e.g. a blow to the head) is likely to damage tissues making later diagnosis more difficult. If possible, a blood sample should be collected into EDTA (lavender top) and plain (red top) tubes and sent with the carcass (see reference list below). If there is any evidence of discharges (runny eyes, discharging wounds, etc.), swabs should be collected into both bacterial and viral transport media (if available). In some cases the project manager may request that specific tissues are sent rather than the whole carcass.

Blood smears are best made at the time of collection. Place a small drop of blood at one end of a glass slide and use the edge of a second slide to spread the blood out across the surface of the first. Air dry for a few minutes before packaging.

The carcass, blood tubes and any other collected material should be carefully sealed in a plastic bag. If more than one animal is submitted, samples should be clearly labelled, indicating which samples pertain to which animals.

The carcass should not be frozen except if specifically requested. If storage prior to shipment can not be avoided, samples should be stored in a fridge at 4°C. Freezing of the carcass is likely to seriously reduce the chances of isolating viral agents, but may not have any effects on examination for parasites (Peuser 1996).

All submissions should include a completed submission form (attached). This should include as much detail of clinical symptoms and the history of the illness as possible, to assist in the diagnosis.

4.3 TRANSPORT TO LAB/PROJECT MANAGER

Killed stoats, swabs blood tubes and smears should be sealed into separate plastic bags and packed in ice in a suitable container (e.g. a chilly bin). The specimens should be sent by overnight courier to the project manager or to an address provided by him/her.

Alternatively, the stoat could be submitted directly to a local veterinary laboratory where necropsy facilities are available. Such submissions should be organised in advance through the project manager.

5. Necropsy and laboratory testing

5.1 NECROPSY

Wherever possible, investigation of each carcass submitted should include a full necropsy and examination for parasites (both ectoparasites and gastrointestinal parasites). A clinical pathology screen should be completed if unclotted blood, serum, smears or other suitable samples are available. Further investigation is at the discretion of the pathologist conducting the investigation and is likely to depend on preliminary assessment of each case. In many cases it may be possible to store swabs, fresh tissue samples, etc at -70°C until histology or other investigations have been completed.

5.2 LABORATORY TESTING

Gross and microscopic examination

Relevant publications are listed below. The dearth of information on this topic may require some preliminary work with clinically normal animals. Wax embedded tissues (for histology) should be catalogued and stored for later use.

Pathogen isolation

Bacterial culture can be performed using standard techniques. Virus isolation can be performed using a variety of standard laboratory cell lines (e.g.Vero, BHK-21), canid and fetid derived cell lines (MDCK, NLFK) or specific mustelid cell lines (MV 1 Lu mink cells, MiCll mink cells, Mpf ferret cells). The latter are available through the American Type Culture Collection at http://www.atcc.org.

Serology

Tests may be performed for specific agents known to infect ferrets either in the natural environment or experimentally. Such agents would include canine distemper virus, influenza virus, Aleutian disease virus and *Neospora caninum*. Alternatively, serological assays can be performed seeking new agents where group reactive antigens are known to exist. Examples would include adenovirus, paramyxoviruses (e.g. measles), and enteroviruses.

In either case, serum should be catalogued and stored to produce a serum bank for future reference.

Molecular assays

Group-specific polymerase chain reaction assays exist for many families of micro-organism and are presented in the scientific literature. They are appli-

cable to fresh tissue, various fluids (e.g. blood, urine, CSF) and wax embedded tissues. Prolonged exposure of tissue samples to formalin (greater than 24 hours) markedly reduces the sensitivity of these assays.

6. Reporting

Each submission should be assigned a unique identification to allow archived material to be referenced later. Recorded details should include:

- history,
- source location,
- physical characteristics, sex, maturity, etc.,
- clinical details,
- gross pathology,
- microscopic pathology,
- other laboratory results (e.g. bacteriology, parasitology, haematology).

At the completion of the project the collected data should be published to ensure the information and conclusions are available in future. A database of archived materials (histology slides, wax embedded tissues, serum, frozen tissue samples) should be established.

7. Cost estimates

The following represent estimates of costs (excl. GST) per case, based on current commercial rates at veterinary diagnostic laboratories. It is expected that necropsy and possibly histopathology would be charged for each case, but other charges would depend on the findings of these initial investigations.

Pathology

Histopathology	$65
Necropsy (1/2 hour)	$60

Bacterial and viral culture, antigen detection

Bacterial culture and typing	$40
Virus isolation (per cell line)	$50
Electron microscopy	$50
PCR (depends on agent)	$50-$150

Serology

Leptospirosis (MAT)	$9
Neospora	$ 15
Serum neutralisation test (viral)	$15-$40
Other serology (CFT, ELISA)	$9-$15

Parasitology

Ectoparasite exam and faecal egg count	$25

8. Appendix

Alpha
14 Ellis St
Hamilton
Ph 07 846 2266

AgriQuality
P O Box 98-905
Manakau City
Ph 09 262-7350

Institute of Veterinary, Animal and Biomedical Sciences
Massey University
Private Bag 11-222
Palmerston North

LabNet
Puddle Alley
Mosgiel
Ph 03 489-4600

Lab Works
P O Box 113
Lincoln University
Lincoln
Ph 03 325-3636

STOAT SURVEY PROJECT SUBMISSION FORM

Project Manager
Telephone
Fax

Address (street and postal)

Sender=s name and address

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Telephone ( ) .....

Fax ( ) .....

Reports to:

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Date specimens collected: .....

Date of despatch: .....

Date received:

Species: .....

Sex: ..... Adult/Juvenile:.....

Investigation / submission number

Location code: .....
(Agribase No., and/or XY co-ordinates)

Owner:.....

Property location and type: .....

.....

Number and type of specimens

Comments from specimen collector/sender:

Signature of sender:
.....