Diseases and pathogens of stoats and other wildlife in New Zealand

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Diseases and pathogens of stoats and other wildlife in New Zealand

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A B S T R A C T

Introduced stoats (*Mustela erminea*) threaten the persistence of several endangered bird species in New Zealand. Potential agents for the biological control of stoats may be identified by surveying naturally occurring disease. We conducted a histopathological survey of disease and a culture and polymerase chain reaction (PCR) survey of the arthropod-borne bacteria *Bartonella* in free-living stoats in New Zealand. Of 60 stoats, 38 (63%) exhibited inflammation of the lung tissue and 20 (30%) showed signs of inflammatory liver disease. We found no evidence of nematode parasitism in lungs or livers and parasitism of the intestines was rare (7%). In 16 (27%) stoats there were no significant pathological lesions. Using culture and PCR analysis we failed to detect *Bartonella* in any of 167 specimens collected from 11 species. Two stoats showed signs of infection by a bacterium closely related to *Yersinia pestis*. The high incidence of pneumonia among New Zealand stoats suggests the possibility that their pulmonary immune system is compromised in some way and we recommend the identification of the organisms causing the disease using culture and PCR techniques. The apparent absence of *Bartonella* suggests that this organism could be a candidate vector for some form of biological control.

Keywords: bird conservation, biocontrol, *Mustela erminea*, pest management, wildlife disease, New Zealand.

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1. Introduction

Introduced mammals are probably the gravest threat to New Zealand's biodiversity. Predators such as stoats (*Mustela erminea*), weasels (*M. nivalis*) and ferrets (*M. furo*) are a particularly serious problem for native birds, since many of New Zealand's endemic species have evolved in the absence of predators (King 1984). Although weasels have apparently never established themselves in great numbers in New Zealand, stoats and ferrets are now widespread and, depending on food availability, can be locally abundant. The impact of stoats, in particular, is being felt by several endangered species and there has been considerable expenditure by DOC on large-scale culling of stoats with the aim of reducing stoat predation (McDonald & Murphy 2000). Given that conventional methods of stoat control are limited to trapping and poisoning, and consequently have very high recurring costs, there are moves towards identifying more cost-effective methods of long-term and widespread control of stoat predation.

In its May 1999 budget, the New Zealand Government allocated an extra NZ$ 6.6M over 5 years to DOC for the development of an integrated stoat control research programme. The vision of this Stoat Research Programme is: 'That stoats will no longer be a threat to indigenous biodiversity'

In the first round of funding aimed at realising this vision, several review and screening projects were commissioned. One (McDonald & Larivière 2001a, b) reviewed all the available information about the diseases of stoats and closely related mustelids. A second project (McDonald et al. 2000, 2001) examined British stoats for signs of disease. We are now reporting the results of a third project undertaken with the aim of detecting signs of disease in stoats caught in New Zealand. There is surprisingly little information about the diseases and pathogens of stoats, either in New Zealand or elsewhere (McDonald & Larivière 2001a, b). Studies of the general biology of stoats in New Zealand have provided basic data on the prevalence of certain parasites, such as the nematode worm *Skrjabingylus nasicola*, and various ectoparasites, notably fleas (e.g. King & Moody 1982).

Our research has concentrated on two areas. First, we conducted a survey of naturally occurring disease, by investigating histopathological signs of disease, particularly inflammatory responses to infection. Comparable British studies (McDonald et al. 2001) concluded that British stoat populations were remarkably healthy, since 61% of stoats exhibited no significant pathological lesions at the microscopic level. Among these British samples, we identified nematode parasitism in the intestines of 14% (6/44) stoats and in the lungs of 11%. We also identified pulmonary granulomatous inflammation associated with bacterial infection in 11% (5/44) and blood-filled cavities in the livers of two individuals. Second, we undertook a survey of the incidence of carriage of the arthropod-borne bacterium *Bartonella* in New Zealand stoats. In a comparable British study, we identified *Bartonella* parasitism in 73% (33/45) of

stoats. Bacteria such as *Bartonella* may not cause serious pathogenic responses in their hosts, though they can be highly transmissible and very widespread (e.g. Breitschwerdt & Kordick 2000). Such organisms may represent candidate vector organisms for biological control agents.

1.1 OBJECTIVES

The objective of our project was to provide basic information on the incidence of disease in free-living stoats and other wildlife in New Zealand. This information was intended to inform decisions about the future direction of research into the biological control of stoats in New Zealand. We examined two main areas:

- A histopathological survey of naturally occurring disease in stoats and other wildlife.
- A survey of the incidence of *Bartonella* in stoats and other wildlife.

Each of these two areas is dealt with separately throughout this account, although there is a degree of overlap between the two investigations.

2. Methods

2.1 ANIMAL ETHICS

The samples used in this study were obtained from DOC and Landcare Research staff, hunters and other members of the public who trapped or shot the animals as part of their normal pest control procedures. Trapping of stoats was undertaken using permitted Fenn Mark IV or similar steel spring traps that are designed to kill the animal (Bateman 1971). For further details of stoat control regimes see McDonald & Murphy (2000). Some additional stoat and weasel samples were collected during programmes of research undertaken by Landcare Research, which were governed by their own ethical procedures.

2.2 SAMPLE COLLECTION

Samples were collected between 3 October 2000 and 13 June 2001 from a range of locations in New Zealand and also incidentally to a contemporary live-capture programme undertaken by Landcare Research.

2.3 HISTOPATHOLOGICAL SURVEY

Immediately after retrieval by the trapper or hunter, tissues were extracted and fixed in 10% neutral-buffered formalin solution. The range of tissues collected included brain, heart (myocardium, epicardium and endocardium), lung, kidney, liver, mesenteric lymph node, thymus, spleen, stomach, duodenum, pancreas and colon. Samples were transported to Bristol, UK for analysis. A haemotoxylin and eosin (H&E) stained section was prepared for each tissue and was examined by light microscopy for pathological change. Where indicated, further specialised stains, Gram, Periodic Acid Schiff (PAS) and Ziehl Neelson (ZN), were applied in order to determine whether there were specific micro-organisms associated with the granulomas.

2.4 INCIDENCE OF *BARTONELLA*

Blood samples were collected in EDTA Vacutainer tubes and frozen at −20ºC or −80ºC where possible. Samples were transported to Bristol, UK on dry ice.

Cultivation of *Bartonella* spp. was attempted from all suitable samples. For 162 samples, an aliquot of 200 µL (or all the sample if its total volume was less than 200 µL) of blood was inoculated onto a blood agar plate (Columbia agar base containing 10% whole defibrinated horse blood). Plates were then incubated for up to 35 days at 37°C in a moist, 5% CO_2 atmosphere. Plates were checked after 3 days' incubation, then once a week, for signs of growth or contamination. Plates that became heavily contaminated were discarded and, provided this contamination appeared before day 24 after inoculation, a repeated culture attempt was made using a fresh aliquot of blood. Samples that failed to yield any visible growth on plates after 30 days' incubation were considered negative, and plates were discarded.

The preparation of samples for polymerase chain reaction (PCR)-based testing and the testing itself were carried out under conditions specifically designed to minimise the risks of contamination or cross-contamination of samples. For 167 samples, DNA was extracted from a 50-µL aliquot of each blood sample for use as template in a nested PCR reaction, targeting a fragment of the citrate synthase gene (*gltA*) of *Bartonella* spp.

DNA extracts were prepared using an alkaline hydrolysis protocol. A 450-µL aliquot of a 5% ammonium solution was added to an Eppendorf tube containing each blood aliquot. Tubes were sealed, vortexed, then heated to 100°C for 20 min. After a brief centrifuge, the tubes were opened and returned to the heating block for a further 20 min, during which time approximately half the volume evaporated. Tubes were then closed and centrifuged at 13 000g for 10 min, then 5 µL of supernatant was removed and diluted in 45 µL of sterile, distilled water. Five microlitres of this dilution were added to a 50-µL PCR mix comprised of 25 µL of 2 × PCR mastermix (ABGene, Epsom, Surrey, UK), 1 µL of each primer (cs440p and cs1137r, synthesised by MWG Biotech, Milton Keynes, Bedfordshire, UK) at a concentration of 10 pmol/µL, and 18 µL of sterile, distilled water. Each mix was subjected to a thermal cycle consisting of 40 repetitions of 96°C for 10 s, 55°C for 10 s, and 72°C for 40 s. After

completion of this cycle, 1 μL of the PCR mix was removed for use as template in a 30-μL volume nested reaction comprising 15μL of 2 × PCR mastermix, 1 μL of each primer (cs781f and cs1137r) and 12 μL of sterile distilled water. This second reaction mix was then subjected to the same thermal cycle as described above. Following completion of this thermal cycle, 10 μL of each mix were mixed with 2 μL of a solution containing 33% (v/v) glycerol and 1% (w/v) xylene cyanol, loaded onto a 1% agarose gel, then electrophoretically resolved at 100 V for 20 min.

The presence of amplification products was determined by UV-illumination of gels following their staining with ethidium bromide. Blood samples were processed using this approach in batches of 18. Negative (n = 4) and positive (n = 1) control blood samples were processed concurrently, and each PCR batch also incorporated a reagent control (i.e. no template was added). The appearance of a product in any of the negative controls, or the failure of any positive control to yield a product, led to the batch being repeated.

The nucleotide base sequences of all amplification products obtained were determined by direct sequencing using the same primers (cs781f and cs1137r) as used for amplification reactions. Samples were submitted to the University of Dundee Sequencing Facility for sequence determinations. For each product analysed, complete sequences were assembled and verified using Align Plus 4 software (Scientific and Educational Software, Durham, North Carolina, USA). Comparison of assembled *gltA* sequences with those previously obtained for *Bartonella* species and other bacteria (e.g. from GenBank) was performed using Align Plus 4 software or using National Centre for Biotechnology Information BLAST (NCBI, US National Library of Medicine, Bethesda, Maryland) (http://www.ncbi.nlm.nih.gov/BLAST/).

3. Results

3.1 HISTOPATHOLOGICAL SURVEY

Ninety-four samples from ten species were analysed (Table 1). On the whole, preservation of tissue structure was remarkably good, considering that samples from many of these animals may not have been fixed for a significant period after death. Samples from the intestinal tract were most affected by autolytic change, but in most cases the ‘ghost outline’ of the underlying tissue permitted some analysis of tissue structure. A summary report on the individuals examined is appended to this report (Appendix 1) and a full account is available (from R.A.M) on request. Summarised comments on the appearance of the major organ systems are given below.

TABLE 1. SUMMARY OF THE FINDINGS OF THE HISTOPATHOLOGICAL SURVEY (NUMBERS OF INDIVIDUALS IN EACH CATEGORY).

SPECIES	NO. SHOWING INFLAMMATORY DISEASE				NO. WITHOUT SIGNS OF INFLAMMATORY DISEASE	NO. EXAMINED
	LUNG	LIVER	GUT	OTHER ORGANS		
Stoat (*Mustela erminea*)	38	18	8	6*	16	60
Weasel (*Mustela nivalis*)	2	1	1			3
Ferret (*Mustela furo*)	1					1
Cat (*Felis catus*)	1				2	3
Rabbit (*Oryctolagus cuniculus*)				1*	1	2
Brown hare (*Lepus europaeus*)	1				4	5
House mouse (*Mus musculus*)	5	2	4		1	6
Ship rat (*Rattus rattus*)	1	1		1*		2
Kiore (*Rattus exulans*)	2	1				2
Brushtail possum (*Trichosurus vulpecula*)	3	1		1*	6	10

* Six stoats were affected by diseases of other organs, including: heart (3 cases of myocarditis, 1 myocardial degeneration), pancreas (2 cases of pancreatitis), brain (1 case of meningitis, 1 gliosis in hindbrain). One rabbit was affected by splenitis. One ship rat by myocardial degeneration. One possum by myocarditis.

3.1.1 Immune and haemopoietic systems (spleen, thymus and mesenteric lymph node)

A longitudinal section of spleen was included in virtually all stoat tissue selections. These all had a remarkably similar appearance and the spleen was invariably markedly active. The white pulp (lymphoid) areas were extremely prominent, often with very large secondary follicles dominating. Red pulp was not often severely congested, but was always very cellular. The cellular content of red pulp included lymphocytes and plasma cells, while megakaryocytes were often prominent indicating extramedullary haematopoiesis. Also of note in the red pulp, was the presence of a population of very large, blastic, round cells with an open-faced nucleus and prominent nucleolus. These cells may have been lymphoblastic. In one or two spleens this population dominated, and the cells were so large and pleomorphic in appearance that in any other species their presence may have suggested neoplasia.

The samples generally included a mesenteric lymph node. Again, these were all very similar in appearance, consistent with marked immunological activity of the gut. There were very prominent secondary follicles with germinal centres and mantle zones, frequent paracortex hyperplasia and dilated medullary sinuses with sinus histiocytosis.

3.1.2 Brain

Sections of brain were taken from 24 of the stoats. These invariably included the hindbrain, and in many cases cerebellar tissue was also present. Cerebral tissue was only included in one case. The brain was histologically normal in the

majority of stoats, with no evidence of lesions consistent with viral infection (e.g. distemper virus). In one stoat (9) there was a focal, granulomatous meningitis that affected the meninges ventral to hindbrain, and in another stoat (8) there was mild focal gliosis within the parenchyma (Appendix 1).

3.1.3 Heart

Myocardial tissue was invariably normal (including endocardium and epicardium). In two stoats (15, 178) there was evidence of a mild and focal myocarditis—generally involving a mixed mononuclear cell infiltrate.

3.1.4 Lung

Most lungs had evidence of either focal or diffuse congestion/haemorrhage and this was interpreted to be an agonal change. Most stoat lungs (n = 33) also had evidence of diffuse or local interstitial pneumonia that involved infiltration of neutrophils and macrophages (pyogranulomatous). In 5 cases, there was additional involvement of the bronchi with neutrophilic exocytosis into the bronchiolar lumena (bronchopneumonia). Some lungs had evidence of bronchial associated lymphoid tissue (BALT) aggregates, but this was not a consistent feature.

Parasitic larvae were not identified within the pulmonary tissue. There were occasional focal and well-defined microgranulomas—in one stoat (84) one of these was centred upon fragments of aspirated ingesta (plant/insect) so the cause was apparent. Two further stoats (102, 154) with pulmonary granulomas had a large, crescentic, translucent structure at the centre reminiscent of the adiaspores of the fungus *Chrysosporium*. Other granulomas had no apparent cause.

3.1.5 Gastrointestinal tract

The stomachs examined, although frequently autolytic, were generally histologically normal. In most cases, only the glandular stomach was examined, but sometimes a portion of that part of the stomach that is lined by squamous epithelium was also present. In six stoats, there were small focal granulomatous infiltrates at the base of the glandular mucosa, sometimes extending into the muscularis mucosa. In several of these cases there were parasitic larvae in the centre of these aggregates, suggesting that these lesions were attributable to larval migration.

Duodenal tissue was identified by the inclusion of pancreas at the same level. There was often mucosal autolysis, but in many cases there was clear evidence of normal villus structure in the ‘ghost’ tissue remaining. Parasites were infrequently observed. In a few cases, there were larvae embedded within the lamina propria, but adult worms were never identified in the lumen. Sections of the ileum often had mucosal autolysis but probably had a normal underlying structure. The majority of ileum sections included very active Peyer’s patches. Occasional caecal sections also had active lymphoid tissue. Sections of the colon were also largely normal.

Pancreatic tissue was generally normal (both exocrine and endocrine). Stoats have very prominent islets relative to some species. In two stoats (8, 15) there was evidence of interstitial pancreatitis. In one of these animals this was a focal change, but in the other this was a diffuse lymphocytic infiltrate that was restricted to the interstitial and periductal tissues, without significant change to the exocrine tissue.

3.1.6 Liver

Liver samples were generally histologically normal. Seven livers had evidence of diffuse hepatocyte vacuolation. Other livers had focal (centrilobular) vacuolation of hepatocytes. This is a non-specific change that may have a number of different causes (e.g. chronic anaemia, metabolic, starvation, etc.). Occasional portal areas had a mild, mononuclear cell infiltration but this was rarely above what would be considered background in other species. Several livers had small parenchymal (mid-zonal) foci of hepatocyte degeneration and mixed inflammation (focal hepatitis), but these rarely appeared significant. Two stoats (141, 179) had evidence of vascular telangiectasis, and in one of these cases there was a relatively large area of hepatocyte loss, blood pooling and surrounding granulomatous inflammation.

3.1.7 Kidney

At least one entire hemisection of kidney was examined in almost all stoats. No significant abnormality was found in any sample. One kidney (in stoat 93) had evidence of a single small focus of interstitial lymphoid aggregation. In most species, this would be considered an incidental finding, so no importance was ascribed to it. Occasionally, a section of adrenal gland was included adjacent to the kidney, and where present this was always normal.

3.1.8 Testes

Testicular structure was generally normal; in most cases a cross-section of epididymis was also included on the slide. In the majority of stoats, there was no evidence of active spermatogenesis within the testes, and the epididymal lumena were devoid of spermatozoa.

3.1.9 Ovary

Ovarian tissue was histologically normal where present, including the fallopian tube. Follicular structures were often present.

3.1.10 Other comments

One stoat (15) was of particular interest. This individual exhibited granulomatous plaques covering a number of abdominal viscera, with granulomatous disease of the lung and meninges. This animal appeared to have a systemic granulomatous inflammatory disease.

3.1.11 Other species

Weasel

Two of the three weasels examined exhibited interstitial pneumonia and granulomatous inflammation similar to that observed in stoats. The third weasel exhibited evidence of parasitic larvae within the intestinal mucosa and diffuse hepatocyte vacuolation.

Ferret

The single ferret examined showed signs of mild interstitial pneumonia.

Cat

One of the three cats had minor, focal interstitial pneumonia.

Rabbit

Of two rabbits, one had no significant abnormality while the second had a single focus of granulomatous inflammation within the spleen.

Hare

Four of the five hares examined had no significant lesions. One animal had parasitic enteritis.

House mouse

Only one of six mice examined had no significant lesions. Four had interstitial pneumonia, one showed minor inflammation of the lung. Three of these also had duodenal nematode parasites and two showed signs of hepatocyte vacuolation. One mouse with pneumonia and duodenal parasites also had a granuloma around a section of aspirated material. In one case there was a fragment of ingested material, suggesting that the lesion was secondary to traumatic penetration of the gastric mucosa. Five of the six livers appeared to have asynchrony of nuclei—some giant nuclei and some binucleate cells.

Ship rat

One rat had interstitial pneumonia. The other showed both hepatocyte vacuolation with nuclear asynchrony in the liver and focal myocardial degeneration.

Kiore

Both kiore had interstitial pneumonia. One of the two showed hepatocyte vacuolation while the other had some binucleate cells in the liver.

Possum

Of the ten possums, six had no significant lesions. One of these six was carrying a well-formed foetus. One had hepatocyte vacuolation and three had focal interstitial pneumonia. One of these latter three animals also had a focal granulomatous myocarditis.

3.2 INCIDENCE OF *BARTONELLA*

A total of 167 blood samples were tested for the presence of *Bartonella* spp. These samples were collected from 94 stoats, 25 ferrets, 16 cats, 12 possums, 6 house mice, 5 brown hares, 2 weasels, 2 hedgehogs, 2 kiore, 2 rabbits, and 1 brown rat. Although PCR-based detection was attempted on all 167 samples, culture attempts were possible on only 162 samples due to their small volume. Of the 162 attempted blood cultures, 52 were lost to either bacterial or fungal contamination. Of these, 47 were collected from stoats, four from cats and one from a ferret. *Bartonella* spp. were not found in any of the 110 samples tested using culture or any of the 162 samples tested using PCR.

PCR-based methods yielded amplification products on three occasions. For one male stoat (114, Grebe Valley, 17 Jan 01), a very weak band was repeatedly observed on the agarose gels used for evaluation of amplification products. However, attempts to obtain enough of this sample to form a suitable template for successful nucleotide base sequence determination failed. Two other male samples, (124, 125, both Grebe Valley, 18 Jan 01), yielded amplification products. Nucleotide base sequence determination of these products was possible and indicated that they possessed an identical 330 base pair nucleotide base sequence (Fig. 1). Comparison of this sequence with those of *gltA* sequences derived from *Bartonella* spp. indicated a significant degree of heterology (e.g. *B. henselae*, Fig. 1). Typically, the *gltA* fragments of *Bartonella* spp. differ from one another by up to 15%, whereas the sequence obtained from these two samples differed from all *Bartonella* spp. by at least 30%. In order to determine the identity of the most likely source of this amplification product, the *gltA* sequence obtained was translated into a predicted GltA amino acid sequence (length = 108 amino acids), and this sequence was used in a BLAST search of the GenBank database. As indicated in Fig. 2, the query GltA sequence shared 93% identity/95% similarity with the GltA of *Yersinia pestis*. These values compare with scores of 76% identity/87% similarity with the GltA of *B. henselae*.

4. Discussion

4.1 HISTOPATHOLOGICAL SURVEY

As in the previous survey of British stoats (McDonald et al. 2000, 2001), many of these individuals had histologically normal tissues and were likely to have been in good health at the time of death. Many of the observations made on the tissue of healthy animals are comparable to those discussed in detail by McDonald et al. (2000, 2001). The consistently marked activity of the spleen and the prominent lymphoid areas of the spleen suggest that stoats have a functional systemic immune system and a strong systemic humoral (antibody) response. In common with the British samples, no lesions of the brain were observed that would have been consistent with infection by distemper. However, recent

```
5′ - CGAAGCCTGCTTGCGGATGCTCGAAGAGATCAAAACCGTAGAGCATATTCC
5′ - TGAAGCATGCCTAAAAATGTTACAAGAAATAGGTTCTGTTGAAAGAATTCC

TGAGTTTATTAATCGCGCTAAAGATAAAAACGACTCATTCCGTCTGATGGGCTTTGG
TGAATTCATTGCACGTGCAAAAGATAAAAATGATTCTTTCCGCCTTATGGGTTTTGG

TCACCGCGTTTATAAAAATCATGACCCGCGAGCCACAGTCATGCGCGAAACCTGCCA
TCATCGAGTCTATAAAAATTATGATCCACGCGCAAAAATCATGCAACAAACCTGCCA

TGAAGTTCTCACCGAACTGGGC---CTAAACGACAGTTTGTTAGAAGTGGCTATGGA
TGAGGTTTTAAAAGAATTGAACATTCAAAATGATCCACTTCTTGATATTGCTATCAC

GTTAGAACGCATCGCATTAAACGACCCATACTTTATTGAGAAAAAACTGTATCCAAA
GCTTGAAAATATTGCTCTAAATGATGAATATTTTATTGAAAAAAAACTTTACCCTAA

CGTGGACTTCTATTCAGGTATCATACTGAAAGCCATGGGAATTCCATCAAC – 3′
TGTCGATTTCTATTCTGGCATTACATTAAAAGCTCTAGGATTTCCAACAGA – 3′
```

Figure 1. Alignment of the nucleotide base sequence obtained from amplification of partial *gltA* derived from samples 124 and 125 (top row of paired lines) with that of *Bartonella henselae* (bottom row). The two sequences differ at 109 of 330 sites, and thus share only 67% similarity.

1. Alignment of query sequence with ‘best matching sequence’ obtained from *Yersinia pestis* GltA.

Identities = 101/108 (93%), Positives = 104/108 (95%)

```
Query: 1
EACLRMLEEIKTVEHIPEFINRAKDKNDSFRLMGFGHRVYKNHDPRATVMRETCHEVLTELGLNDSLLEVAMELE
EACL+MLEEIKTVEHIPEFI_RAKDKNDSFRLMGFGHRVYKN+DPRATVMRETCHEVLELL__N+SLLEVAMELE

RIALNDPYFIEKKLYPNVDFYSGIILKAMGIPS
_IALNDPYFIEKKLYPNVDFYSGIILKAMGIPS


Subject: 269
EACLKMLEEIKTVEHIPEFIRRAKDKNDSFRLMGFGHRVYKNYDPRATVMRETCHEVLEELKLNNSLLEVAMELE
NIALNDPYFIEKKLYPNVDFYSGIILKAMGIPS
```

2. Alignment of query sequence with *Bartonella henselae* GltA.

Identities = 83/109 (76%), Positives = 96/109 (87%), Gaps = 1/109 (0%)

```
Query: 1
EACLRMLEEIKTVEHIPEFINRAKDKNDSFRLMGFGHRVYKNHDPRATVMRETCHEVLTELGL_NDSLLEVAMEL
EACL+ML+EI_+VE_IPEFI_RAKDKNDSFRLMGFGHRVYKN+DPRA_+M++TCHEVL_EL_+_ND_LL++A+_L

ERIALNDPYFIEKKLYPNVDFYSGIILKAMGIPS
E_IALND_YFIEKKLYPNVDFYSGI_LKA+G_P+


Subject: 270
EACLKMLQEIGSVERIPEFIARAKDKNDSFRLMGFGHRVYKNYDPRAKIMQQTCHEVLKELNIQNDPLLDIAITL
ENIALNDEYFIEKKLYPNVDFYSGITLKALGFPT
```

Figure 2. BLAST protein-protein output files resulting from search of GenBank database for a best match to the putative amino acid sequence derived from the amplification products obtained in this study.

serological investigations of New Zealand stoats has revealed the presence of distemper antibodies in 2 of 32 stoats collected from Canterbury in 2001 (T. Zheng, pers. comm.). Aspirated and ingested material appear to cause occasional problems for stoats in both Britain and New Zealand. This is to be expected, given the predatory nature of the species and the fact that they spend so much time underground (McDonald et al. 2001).

Two cases of blood-filled cavities in the liver were observed in stoats collected from the Grebe Valley and from Reefton. The Reefton individual latter died in captivity at Landcare Research, Lincoln, and similar observations of liver disease were recorded by their vet (R. Fairley, report to J. Duckworth, pers. comm.). The observations made on these specimens were similar to peliosis-like lesions observed in two stoats collected in Britain. At the time, we suggested that the British samples may have been secondarily exposed to rodenticides and this remains a possibility for the New Zealand specimens (McDonald et al. 1998; Murphy et al. 1998).

Despite these similarities, our current findings differed to a surprising extent from the British survey (McDonald et al. 2001). In particular, we detected pneumonia of varying degrees of severity, while this was not detected in any of the 44 British stoats. While this is a screening study, it is tempting to speculate on the implications of these findings. The widespread incidence of pneumonia suggests that the pulmonary immune system of stoats in New Zealand may be compromised in some way. This invites speculation that such a weakened system may be particularly susceptible to attack by some putative, lethal biocontrol agent. The cause of the high incidence of pneumonia could not be detected by histopathological techniques, but is likely to be microbial, either viral, bacterial or mycoplasmal. In the development of a control agent, the identification of the organism causing the inflammation observed here is an obvious first step. Thereafter, a search for an organism, or strain of the same infecting organism, that invades hosts through the lungs would be particularly useful.

There are numerous potential causes of pneumonia in mustelids (Fox 1998) and evidence of viral-bacterial synergism in the symptoms of severe pneumonia (Jakeman et al. 1991). Mink (*M. vison*) and ferrets are also susceptible to avian influenza (Buchmann et al. 1995; Englund & af Segerstad 1998). McDonald & Larivière's (2001a, b) review of diseases of stoats and related mustelids, identified a range of diseases where horizontal transmission may occur through the respiratory tract, perhaps the best known of which are tuberculosis and paratuberculosis caused by *Mycobacterium* spp. (Ragg et al. 1995; Beard et al. 1999).

While intestinal and pulmonary parasitism was relatively commonplace among British stoats, it was rare in these samples. In our previous reports (McDonald et al. 2000, 2001), we advocated an investigation of the diversity and particularly the epidemiology of nematode parasites of stoats. This may yet prove to be of value, though it would again be contingent on the acceptability of introducing new parasites to New Zealand from Britain. The evidence derived from these two studies does not, however, detract from the potential importance of the genus-specific and comparatively well-known nematode, *Skrjabingylus nasicola*, as a candidate vector organism. This value is particularly clear since this species is already present in New Zealand.

Examination of tissues from a range of other New Zealand wildlife provided an interesting comparison to samples from the stoats. A similar range of lesions were present in these other species: of particular note is the comparable prevalence of interstitial pneumonia of varying severity in the house mice, possums and the cat. The wide range and frequency of lesions observed in the small number of house mice sampled is also noteworthy given the small sample size.

This survey has provided confirmation of a range of conditions affecting stoats in New Zealand. The high frequency of pneumonia among stoats in New Zealand is perhaps the most significant finding of the study. While this is an unexpected finding it holds significance for the direction of future research in this field. If lethal biological control is adopted as a target for the stoat control programme, then this might be profitably directed at organisms that invade the stoat's respiratory system. Therefore, identification of the cause of pneumonia among New Zealand's stoats should be considered a priority.

4.2 INCIDENCE OF *BARTONELLA*

McDonald et al. (2000) suggested that *Bartonella* was likely to be present in wild stoats and other mammals living in New Zealand. This suggestion was made on the basis of our finding that *Bartonella* DNA was present in 73% of the British stoats tested and Britain being known to be the source for New Zealand stoats. While we are not aware of any other investigations of the prevalence of *Bartonella* spp. specifically in stoats in New Zealand, *B. henselae* has been recorded in 17% (8/48) of domestic cats in the Auckland area (Joseph et al. 1997). There are also large populations of free-living rodents all of which are hosts to numerous ectoparasites (Tenquist & Charleston 2001) that may act as vectors for *Bartonella* spp.

In the light of our earlier findings in British stoats (McDonald et al. 2001), it is somewhat surprising that none of the animals collected in New Zealand were found to harbour *Bartonella* spp. Previous studies of a wide range of mammalian hosts have more often than not detected bacteraemia due to *Bartonella* spp. Of the species tested here, *Bartonella* spp. have previously been associated with cats, house mice, rats and rabbits with, typically, at least 10% of animals tested yielding evidence of infection. In the current survey, the sample sizes for these non-mustelid hosts were small (e.g. n = 16 for cats) thus, our failure to detect infection cannot be considered in any way indicative that New Zealand populations of these species are free from *Bartonella* infection.

Among the mustelids, two species, stoats and ferrets, were sampled in relatively large numbers (94 and 25 animals respectively) in this study. Our failure to detect *Bartonella* spp. in these samples is, therefore, probably a reliable indication that *Bartonella* parasitism does not occur in these species in New Zealand. Previous work using a PCR-based approach for the detection of *Bartonella* spp. did find evidence of infection in stoats in Britain, although these results could not be verified by cultivation of infecting bacteria (McDonald et al. 2000).

The apparent absence of infection in New Zealand is intriguing. Although this incongruence may be due to methodological failings in either survey, there are also several possible theoretical explanations. The distribution of *Bartonella* parasitaemia in subpopulations of host species is known to be uneven. Studies on brown rats in the USA found that in some populations almost all individuals sampled were infected, whereas in others no animals were infected (Ellis et al. 1999). Thus, although some UK stoat populations are infected, others may be infection-free. Animals transported to New Zealand may have been drawn from infection-free populations. Alternatively, in several host species, temporal differences in prevalence have been observed. In Europe, at least, these differences appear to have an annual cycle with the highest levels of *Bartonella* infection occurring during the summer and early autumn, thereby coinciding with peaks in ectoparasitic infestation and host population density. The dynamics of natural *Bartonella* infections, if there are any, remain unknown in New Zealand samples, thus the suitability of the timing of their collection is uncertain (the majority of samples in the current study were collected during the first six months of 2001, corresponding to the summer and autumn in New Zealand).

The detection of organisms possessing a GltA fragment most similar to that of *Yersinia pestis* was surprising as the PCR primers employed have, to date, proven specific for the genus *Bartonella* or closely related species. That the GltA sequence was 93% identical to that of *Y. pestis* does not, however, indicate that the organism infecting the rodents was *Y. pestis* itself. Nothing is known about GltA diversity between *Yersinia* and closely related species, but, among *Bartonella* species, GltA sequences vary by about 6% to 9% (R. Birtles, unpubl. data). If we assume that *Yersinia* species are as phylogenetically diverse as *Bartonella* species, then finding a difference of 7% between the stoat-infecting organism and *Y. pestis* may well indicate that the former is a different member of the genus *Yersinia*. *Yersinia pestis* has been recorded in black-footed ferrets (*Mustela nigripes*) (Williams et al. 1994). *Yersinia pseudotuberculosis* has also been previously isolated from a wide number of wild animal species including the mustelids, otter (*Lutra lutra*), martens (*Martes foina* and *M. americana*), polecat (*Mustela putorius*) and mink (Wetzler 1981; Nikolova et al. 2001). *Y. pestis* is naturally maintained in rodents, which act as enzootic reservoirs for plague in certain parts of the world, but other species, including the recognized pathogens *Y. pseudotuberculosis* and *Y. enterocolitica*, are also frequently obtained from rodents and larger wild animals worldwide. *Y. enterocolitica* is of particular interest in New Zealand, since it causes significant public health problems and has an incidence of 14 cases per 100 000 per annum. (Crump et al. 2001). It is transmitted through faecal contamination of water supplies, and has a major reservoir among wild pigs (*Sus scrofa*).

The apparently high prevalence of *Bartonella* infection among stoats in Britain was in marked contrast to the organism's apparent absence from New Zealand. Assuming that suitable arthropod vectors are present in New Zealand, it seems likely that introduced *Bartonella* spp. would spread and establish themselves among New Zealand stoats. In the event that an introduced bacterial vector for a vectored-immunocontraceptive is desirable, then *Bartonella* may be a suitable candidate. This would require more detailed studies of the epidemiology of this infection among wildlife where it is endemic.

4.3 RECOMMENDATIONS

To develop the identification of agents suitable for the biological control of stoats in New Zealand, we recommend the following actions, in descending order of priority:

- Investigation of the causes of pneumonia among New Zealand stoats by culture and broad-spectrum PCR from fresh lung material.
- Investigation of the pulmonary immune system of New Zealand stoats.
- Identification of candidate biocontrol organisms and/or strains that infect the respiratory tract of mustelids.
- Field and laboratory investigation of the ecology and epidemiology of *Bartonella* and their arthropod vectors in stoats in Great Britain.

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Appendix 1

HISTOPATHOLOGICAL FINDINGS FROM EXAMINATION OF SAMPLES FROM 94 INDIVIDUAL ANIMALS OF 10 SPECIES. (SEX AND DATE OF COLLECTION IS NOTED WHERE KNOWN. MISSING NUMBERS CORRESPOND TO ANIMALS FOR WHICH ONLY BLOOD SAMPLES AND NOT TISSUE SAMPLES WERE AVAILABLE. LOC. —LOCATION, COL.—COLLECTOR).

ID	SPECIES	SEX	DATE	LOC.	COL.	COL. CODE	MAJOR HISTOPATHOLOGICAL FINDINGS
1	Stoat	M	12-Apr-01	Mt White	JD	177	Focal (traumatic?) gastritis. Diffuse interstitial pneumonia
2	Stoat	M	21-Mar-01	Central Otago	JD	171	Diffuse pyogranulomatous interstitial pneumonia
3	Stoat	M	12-Apr-01	Mt White	JD	175	Mild focal pyogranulomatous interstitial pneumonia
4	Stoat	F	12-Apr-01	Mt White	JD	178	Mild to moderate interstitial pneumonia
5	Stoat	M	12-Apr-01	Mt White	JD	176	Interstitial pneumonia. Diffuse hepatocyte vacuolation
6	Stoat	F	24-Jan-01	West Coast	JD	D2	Pyogranulomatous interstitial pneumonia. Granulomatous peritonitis
7	Stoat	M	30-Jan-01	Scargill	JD	143	Subtle focal hepatitis
8	Stoat	M	29-Jan-01	West Coast	JD	149	Pyogranulomatous bronchopneumonia Slight focal hepatitis. Slight focal gliosis in hindbrain Mild focal interstitial pancreatitis
9	Stoat	M	1-Feb-01	Cass	JD	154	Focal granulomatous meningitis. Diffuse interstitial pneumonia. Single pulmonary granuloma (fungal?). Diffuse hepatocyte vacuolation
10	Stoat	F	24-Jan-01	West Coast	JD	D1	Pyogranulomatous interstitial pneumonia
11	Stoat	M	31-Jan-01	Scargill	JD	138	Focal peribronchiolitis
12	Stoat	M	29-Jan-01	Scargill	JD	152	Diffuse pyogranulomatous bronchopneumonia with prominent bronchial associated lymphoid tissue (BALT)
13	Stoat	M	1-Feb-01	West Coast	JD	155	Splenic lymphoblastic proliferation. Focal pyogranulomatous interstitial pneumonia
14	Stoat	F	21-Feb-01	Tekapo	JD	165	Pyogranulomatous interstitial pneumonia. Diffuse hepatocyte vacuolation
15	Stoat	F	24-Jan-01	West Coast	JD	123	Systemic granulomatous/lymphocytic inflammatory disease: interstitial pneumonia, interstitial pancreatitis, myocarditis, hepatitis
16	Stoat	M	29-Jan-01	West Coast	JD	147	Parasitic granulomatous focus stomach wall, Pyogranulomatous interstitial pneumonia (focal)
17	Stoat	M	29-Jan-01	West Coast	JD	148	Diffuse pyogranulomatous interstitial pneumonia
18	Stoat	F	29-Jan-01	Scargill	JD	144	Focal pyogranulomatous interstitial pneumonia. Portal lympho- -cytic hepatitis. Gastric submucosal granulomatous focus
19	Stoat	F	12-Apr-01	Scargill	JD	139	Multifocal pyogranulomatous bronchopneumonia. Single focus duodenal nematode larvae. Focal slight hepatitis
20	Stoat		18-May-01	Eglinton	IY	1	No significant abnormalities
21	Weasel		18-May-01	Eglinton	IY	2	Diffuse interstitial pneumonia
22	Weasel		18-May-01	Eglinton	IY	3	Interstitial pneumonia and pulmonary granulomas
23	House mouse		23-May-01	Hollyford	IY	4	No significant abnormalities
24	House mouse		23-May-01	Hollyford	IY	5	Mild inflammatory change in lung, stomach and colon
25	Kiore		29-May-01	Hollyford	IY	6	Macrovesicular hepatocyte vacuolation. Diffuse interstitial pneumonia
26	Ship rat		29-May-01	Hollyford	IY	7	Diffuse interstitial pneumonia
27	House mouse		30-May-01	Hollyford	IY	8	Diffuse interstitial pneumonia. Diffuse macrovesicular hepatocyte vacuolation. Nematode in duodenal lumen

ID	SPECIES	SEX	DATE	LOC.	COL.	COL. CODE	MAJOR HISTOPATHOLOGICAL FINDINGS
28	House mouse		30-May-01	Hollyford	IY	9	Diffuse interstitial pneumonia. Duodenal nematode
29	House mouse		30-May-01	Hollyford	IY	10	Interstitial pneumonia (focal). Aspiration pulmonary granulomas. Duodenal nematodes
30	House mouse		30-May-01	Hollyford	IY	11	Diffuse interstitial pneumonia. Hepatocyte vacuolation. Gastric mucosa granuloma (embedded ingesta)
31	Kiore		31-May-01	Hollyford	IY	12	Diffuse interstitial pneumonia
70	Cat		23-Apr-01	Gordonton	DP		Minor interstitial pneumonia
71	Hare		23-Apr-01	Gordonton	DP		No significant abnormalities
72	Hare		23-Apr-01	Gordonton	DP		No significant abnormalities
73	Hare		23-Apr-01	Gordonton	DP		No significant abnormalities
74	Possum		23-Apr-01	Gordonton	DP		Diffuse macrovesicular hepatocyte vacuolation
75	Possum		23-Apr-01	Gordonton	DP		No significant abnormalities
76	Rabbit		23-Apr-01	Gordonton	DP		No significant abnormalities (tissues autolysed)
77	Ferret	M	21-May-01	Rotoiti	GT	F7	Mild interstitial pneumonia
81	Cat	F	18-May-01	Rotoiti	GT	C30	No significant abnormalities
84	Stoat	M	21-Jan-01	Grebe Valley	DAN	34	Diffuse interstitial pneumonia. Single aspiration granuloma
85	Stoat	F	21-Jan-01	Grebe Valley	DAN	35	Focal pulmonary congestion/haemorrhage (agonal?)
88	Stoat	M	23-Jan-01	Grebe Valley	DAN	44	Granulomatous pulmonary focus
89	Stoat	M	23-Jan-01	Grebe Valley	DAN	45	Focal pulmonary congestion/haemorrhage (agonal?)
91	Stoat	M	23-Jan-01	Grebe Valley	DAN	47	Interstitial pneumonia. Single granulomatous focus
92	Stoat	F	24-Jan-01	Grebe Valley	DAN	48	Focal pulmonary congestion/haemorrhage (agonal?)
93	Stoat	F	24-Jan-01	Grebe Valley	DAN	49	No significant abnormalities
94	Stoat	M	24-Jan-01	Grebe Valley	DAN	50	No significant abnormalities
95	Stoat	F	24-Jan-01	Grebe Valley	DAN	51	Interstitial pneumonia
96	Stoat	M	24-Jan-01	Grebe Valley	DAN	52	Diffuse pulmonary congestion/haemorrhage. Intestinal nematode larvae
97	Stoat	M	25-Jan-01	Grebe Valley	DAN	54	Interstitial pneumonia. Evidence of feathers in intestinal lumen
98	Stoat	F	26-Jan-01	Grebe Valley	DAN	56	No significant abnormalities
99	Stoat	F	26-Jan-01	Grebe Valley	DAN	57	Interstitial pneumonia
100	Stoat	M	27-Jan-01	Grebe Valley	DAN	58	Diffuse microvesicular hepatocyte vacuolation
101	Stoat	F	28-Jan-01	Grebe Valley	DAN	59	Diffuse interstitial pneumonia
102	Stoat	M	20-Jan-01	Pig Creek	DAN		Diffuse interstitial pneumonia. Solitary pulmonary granuloma (fungal?)
115	Stoat	M	18-Jan-01	Grebe Valley	DAN	13	Diffuse microvesicular hepatocyte vacuolation
116	Stoat	F	18-Jan-01	Grebe Valley	DAN	14	No significant abnormalities
117	Stoat	M	18-Jan-01	Grebe Valley	DAN	15	No significant abnormalities
118	Stoat	F	18-Jan-01	Grebe Valley	DAN	16	Multifocal (lymphocytic) hepatitis
119	Stoat	F	18-Jan-01	Grebe Valley	DAN	17	Diffuse microvesicular hepatocyte vacuolation
120	Stoat	M	18-Jan-01	Grebe Valley	DAN	18	No significant abnormalities
127	Stoat	M	19-Jan-01	Grebe Valley	DAN	25	No significant abnormalities
131	Stoat	M	20-Jan-01	Grebe Valley	DAN	29	No significant abnormalities
132	Stoat	F	20-Jan-01	Grebe Valley	DAN	30	Diffuse hepatocyte vacuolation. Single focus mononuclear hepatitis
133	Stoat	M	20-Jan-01	Grebe Valley	DAN	31	No significant abnormalities
141	Stoat	M	28-Jan-01	Grebe Valley	DAN	60	Interstitial pneumonia. Focal pulmonary granulomatous infiltrate. Hepatic necrosis/haemorrhage with granulomatous border (peliosis-like)
142	Stoat	F	28-Jan-01	Grebe Valley	DAN	61	No significant abnormalities
143	Stoat	F	28-Jan-01	Grebe Valley	DAN	62	No significant abnormalities
144	Stoat	M	28-Jan-01	Grebe Valley	DAN	63	Diffuse interstitial pneumonia
148	Stoat				DAN		No significant abnormalities
149	Stoat				DAN		No significant abnormalities

ID	SPECIES	SEX	DATE	LOC.	COL.	COL. CODE	MAJOR HISTOPATHOLOGICAL FINDINGS
151	Stoat				DAN		No significant abnormalities
152	Stoat				DAN		No significant abnormalities
161	Rabbit	M	22-Apr-01	Paeroa	DP		Focal pyogranulomatous splenitis
162	Hare	F	23-Apr-01	Paeroa	DP		Parasitic enteritis
163	Possum	F	24-Apr-01	Paeroa	DP		No significant abnormalities
164	Possum	M	25-Apr-01	Paeroa	DP		No significant abnormalities
165	Possum		26-Apr-01	Paeroa	DP		Focal interstitial pneumonia
166	Possum	F	27-Apr-01	Paeroa	DP		Focal interstitial pneumonia
167	Possum	F	28-Apr-01	Paeroa	DP		Focal interstitial pneumonia. Focal granulomatous myocarditis
168	Hare	M	29-Apr-01	Paeroa	DP		No significant abnormalities
169	Cat		3-Oct-00	Hamilton	RM		No significant abnormalities
170	Possum		3-Oct-00	Hamilton	RM		No significant abnormalities
171	Possum		3-Oct-00	Hamilton	RM		No significant abnormalities
173	Possum	F	17-Oct-00	Hamilton	RM		No significant abnormalities. Carrying well-developed foetus
174	Stoat	F	4-May-01	Mt White	JD	174	Gastric submucosal granuloma. Diffuse pyogranulomatous interstitial pneumonia
175	Stoat	F	13-Jun-01	West Coast	JD	120	Gastric submucosal parasitic granuloma. Focal pyogranu--lomatous pneumonia
176	Stoat	F	4-May-01	West Coast	JD	159	Hepatocyte vacuolation and very mild focal hepatitis. Diffuse pyogranulomatous bronchopneumonia. Focal myocarditis
177	Stoat	F	13-Jun-01	Reefton	JD	187	Diffuse pyogranulomatous interstitial pneumonia. Focal pyogranulomatous hepatitis
178	Stoat	M	29-May-01	Reefton	JD	D5	Focal interstitial pyogranulomatous pneumonia. Diffuse hepatocyte vacuolation. Focal granulomatous myocarditis (minor). Splenic haemosiderin stores prominent
179	Stoat	M	11-May-01	Reefton	JD	D4	Focal pyogranulomatous interstitial pneumonia. Hepatic telangiectasia, blood-filled spaces, fibrosis
180	Weasel	F	7-May-01	Canterbury	JD		Parasitic larvae duodenal mucosa. Diffuse hepatocyte vacuolation
181	Ship rat	F	30-May-01	Hollyford Valley	IY		Focal myocardial degeneration. Diffuse hepatocyte vacuolation