

Surveillance



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Welfare assessment of vertebrate toxic agents

Surveillance
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Indexation has been undertaken by Professor Neil Bruère, a distinguished veterinary scientist and Emeritus Professor of Veterinary Medicine from Massey University.

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Swine hepatitis E virus in New Zealand

Hepatitis E virus (HEV) infection is a major cause of epidemic and acute sporadic cases of hepatitis in human populations in many areas of Asia, Africa and Central America, where HEV is endemic. HEV infections are also seen in developed countries but generally in the form of sporadic acute or fulminant hepatitis⁽¹⁾⁽²⁾⁽³⁾. In New Zealand, two human cases of acute hepatitis have been reported as HEV antibody positive⁽⁴⁾.

The possibility of zoonotic HEV infections in humans from pigs was originally postulated after experimental infection of pigs with a human strain of HEV⁽⁵⁾. Studies in pigs and other domestic livestock and in feral rodents supported the existence of an animal reservoir for HEV⁽⁵⁾⁽⁶⁾⁽⁷⁾. Recently, direct evidence of zoonotic transmission was reported in humans who had consumed uncooked deer meat⁽⁸⁾ and uncooked pig liver⁽⁹⁾. The discovery of novel HEV variants from geographic areas not considered endemic for HEV is important in understanding the worldwide distribution of HEV infection as well as increasing awareness of HEV as a cause of acute hepatitis in these areas.

A survey was undertaken to determine the HEV status of New Zealand pigs, first by testing for HEV antibody in pig herds throughout the country to estimate the herd prevalence, then by attempting to amplify the HEV genomic sequence using the polymerase chain reaction (PCR).

Antibody to HEV was found in pig herds throughout New Zealand using the ELISA. Twenty of 22 herds (91%) were positive for HEV antibody.

Age-specific HEV seroprevalence in a high health status pig herd

Age groups	N tested	N positive (%)
Sows	24	18 (73)
20-week-old	25	24 (94)
one-week-old	23	12 (52.1)
Total	72	54 (75)

The ELISA was also used to measure the seroprevalence of HEV using 72 serum samples from one particular 'high health status' (HHS) pig herd. The highest prevalence in the herd was found in 20-week-old pigs (94%). Sows had a prevalence of 73%, and one-week-old piglets had a prevalence of 52.1% (see table).

Serum samples (n=10) collected from feral pigs from Auckland Island were negative. This population of pigs has been isolated from other pigs for approximately 200 years.

HEV RNA was amplified from faecal samples of ten- to 12-week-old piglets from the HHS herd. Sequence analysis of New Zealand swine isolates showed that they segregate with human HEV strains from non-endemic areas. The nucleic acid sequence identity between different New Zealand isolates is 99%.

This study shows that HEV is widespread in New Zealand pig

About 90% of 22 pig herds sampled throughout New Zealand were serologically positive for hepatitis E virus. The average ELISA seroprevalence in one 'high health status' herd was 75%. Hepatitis E viral RNA fragments were amplified from faecal samples of post-weaned piglets.

herds, with a pattern of seroprevalence similar to that previously described in Australia⁽¹⁰⁾. The fact that HEV infection is endemic in swine in countries such as Australia and New Zealand, which have a long history of animal quarantine, suggests that HEV has been present in an animal reservoir for many years. It is interesting that the Auckland Island pigs were free of HEV infection.

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Apicultural exotic disease surveillance

The report summarising the apicultural exotic disease surveillance activities for the year from 1 July 2003 to 30 June 2004 will appear in the next issue of *Surveillance*.

Undiagnosed haemolytic anaemia in young red deer

There are few reported cases of haemolytic anaemia and haemoglobinuria in farmed deer in New Zealand. Redwater associated with *Leptospira pomona* has been confirmed⁽¹⁾⁽²⁾. While there are few other infectious causes of haemolytic anaemia and haemoglobinuria potentially affecting deer in this country, there are a number of potential non-infectious causes including chemical and plant toxins, and various immunological, genetic and metabolic disorders⁽³⁾. There are also a number of haemoparasites not yet fully identified in deer in New Zealand, including *Anaplasma* spp, *Babesia* spp, and *Theileria* spp, that could potentially cause anaemia in cervid species.

This paper presents the clinical occurrence in a deer research herd of haemolytic anaemia and haemoglobinuria for which the known causes were excluded. It describes an investigation into the hypothesis, based on appearance of intra-erythrocytic protozoan-like bodies, that the cause may have been a *Theileria* organism.

Clinical observations and investigations

Case 1

On 23 March 1999, a four-month-old male red deer at the Massey University Deer Research Unit was observed to have red urine. It grazed perennial ryegrass/white clover pasture with ten similar-aged deer from which faecal samples were being collected regularly for experimental purposes. Although some members of the group had received dexamethasone to suppress immunity, the affected animal had not. Its temperature was normal, there was a mild increase in heart and respiration rates, and mucous membranes were slightly pale.

Erythrocyte numbers were slightly below the normal range ($8.8 \times 10^{12}/l$; normal $9.7-15.0 \times 10^{12}/l$), indicating mild anaemia. The leucocyte count was normal ($7.0 \times 10^9/l$; normal $2.3-8.6 \times 10^9/l$). Urinalysis confirmed the presence of haemoglobin. No leptospire were observed and leptospiral serology for serovars *pomona*, *hardjo* and *copenhageni* were negative. The deer was isolated, treated with oxytetracycline and recovered uneventfully shortly afterwards.

The history, clinical findings and laboratory tests thus failed to confirm any of the known causes of haemoglobinuria and intravascular haemolysis of red deer.

Case 2

On 25 April 1999, a five-month-old male from the same group as Case 1 was observed to be extremely weak and had severe respiratory dyspnoea. It had extremely pale mucous membranes, normal temperature and markedly elevated heart and respiration rates. Its blood was watery, and haematology showed severe anaemia (rbc $4.4 \times 10^{12}/l$; normal $9.7-15.0 \times 10^{12}/l$) and leucocytosis

Three deer were affected by acute haemolytic anaemia. Presumed intra-erythrocytic *Theileria* were identified in one animal using light and electron microscopy. However, PCR did not confirm this diagnosis and the cause of the anaemia remains undiagnosed.

(wbc $19.8 \times 10^9/l$; normal $2.3-8.6 \times 10^9/l$). The animal died shortly afterwards.

At necropsy there was minimal body fat. The lungs had multiple small, dark, firm lesions, which varied in size from 5-10 mm and were consistent with lungworm (these deer had not been treated for internal parasites because they were used as donors for faecal egg and larval cultures for research purposes). The abdominal contents were slightly yellow. The rumen was full of stalky grass. The contents of the small intestine were golden-yellow and mucoid. There were few faecal pellets in the distal colon. The spleen appeared slightly congested. Both kidneys were dark and the bladder was half-full with red urine.

Histopathology of kidney showed an acute haemoglobinuric nephrosis in which segments of cortical proximal tubules showed recent epithelial necrosis and extensive haemoglobin deposition. Many collecting tubules were also necrotic and contained sloughed epithelial cells with pyknotic nuclei. No leptospire were observed using silver stains. In the liver there was a pronounced dissociation of centrilobular hepatocytes. The lung showed severe alveolar oedema and accumulation of proteinaceous fluid within alveoli in some areas. Many pulmonary blood vessels contained fibrinoid and leucocytic thrombi.

Dark ground examination of urine was negative for spirochaetes and renal culture was negative for leptospirosis. The diagnosis was of acute haemolytic anaemia of unknown cause.

Case 3

At the same time a five-month-old female red deer grazing in a separate mob of approximately 60 weaners on the same deer research unit was found dead. This group of animals had no direct contact with the group containing Cases 1 and 2, but they had all been grazed together before weaning and had grazed some of the same pastures in the previous eight weeks.

At necropsy, the deer was thin and dehydrated with little internal fat. The liver was swollen with rounded edges; the kidneys were dark brown. The small intestinal content was watery but there were no mucosal lesions. The mesenteric lymph nodes were enlarged and dark yellow. The urinary bladder contained about 250 ml of red-tinged urine.

Histopathology revealed acute tubular nephrosis and large amounts of haemosiderin. No leptospire were visible using silver stains. The lungs were oedematous and there were areas of acute necrotising alveolitis associated with bacteria of mixed morphology. The spleen

was congested and showed lymphoid lysis, megakaryocytosis and prominent erythrophagocytosis. The liver showed centrilobular hepatic coagulative necrosis and erythrophagocytosis. These findings indicated acute intravascular haemolysis of unknown cause, and terminal aspiration pneumonia. Prominent erythrophagocytosis suggested a possible immune-mediated anaemia.

Further investigations

Despite lack of gross pathological evidence of leptospirosis, blood samples for serology were collected about six weeks later from six of the original experimental mob. All were titre-negative for *Leptospira hardjo* while titres to *L pomona* were 800 (n = 2), 1600 (n = 2) and negative (n = 2, including the recovered animal Case 1). Blood copper concentrations were normal. Gamma glutamyl transferase (GGT) was moderately elevated in one deer but normal in the remaining five. Facial eczema had been prevalent in the district during that autumn but the deer on the unit had shown no clinical signs.

Microscopic examination of a blood smear from Case 2 yielded a few cells with intra-erythrocytic bodies resembling an apicomplexan protozoal haemoparasite.

There are few reports of this class of parasite in New Zealand. *Theileria orientalis* is endemic in cattle in Northland. Infection is usually subclinical but it can cause illthrift and sub-optimal production sometimes associated with a mild regenerative anaemia⁽⁴⁾. Of two cattle herds investigated, the prevalence was 56% in one and less than 1% in the second. *Theileria orientalis* is a tick-borne protozoal parasite. The appearance of ticks on the Deer Research Unit had been confirmed for the first time during the summer immediately preceding this occurrence of haemoglobinuria. Their source was unable to be determined.

Attempts to demonstrate *Theileria*

Two nine-month-old male red deer from a property with no evidence of tick infestation were splenectomised and held in isolation. About two weeks later, 50 ml of blood was collected from Case 1 by sterile technique into heparin anticoagulant and was transfused into the jugular vein of one splenectomised recipient. Blood samples collected from five of that donor's in-contact deer and five deer from the group containing Case 3 were similarly collected, pooled and transfused into the other splenectomised recipient. No intra-erythrocytic inclusion bodies were seen in smears of any donor animal.

The splenectomised deer were held in isolation after blood transfusion. Temperature and clinical signs were recorded twice daily, and blood samples were collected for haematology and microscopic examination twice or thrice weekly. On days 11 and 12 post-transfusion, the deer that had received pooled blood developed a mild pyrexia (39.4-39.9°C) and a concurrent mucopurulent discharge from the right eye. Its temperature

dropped after 48 hours and the ocular discharge resolved without treatment. No other clinical abnormalities were observed. This animal was returned to pasture after 25 days.

PCR analysis for *Theileria* spp (International Livestock Research Institute Laboratory, Nairobi, Kenya) was conducted on methanol-fixed blood films from Case 2 (containing erythrocytes with inclusion bodies); on methanol-fixed blood films and frozen whole blood from splenectomised deer 13 and 16 collected six and 13 days after blood transfusion; and on methanol-fixed blood film and frozen whole blood from Case 1. This analysis used SSU 16S ribosomal RNA gene sequences, which should amplify any *Theileria* spp, along with specific probes for *T orientalis* and *T cervi*.

PCR tests indicated no evidence of *Theileria* spp.

Discussion

The investigation failed to determine a cause of the haemolytic anaemia. The microscopic appearance of cellular bodies within erythrocytes resembled *Theileria* spp, but PCR did not substantiate this.

There are a large number of *Theileria* spp that affect a range of ruminants. The incubation period for *Theileria* is nine-25 days. Transmission is by sporozoites in the saliva of ticks. Many infections are inapparent and can persist in the animal, usually as intra-erythrocytic piroplasms. Reduced immune competence could permit the asexual replication of merozoites normally present in macrophages to piroplasms that invade red blood cells in the host animal.

Theileria cervi has been observed as a relatively benign infection in white-tailed deer in the USA⁽⁵⁾⁽⁶⁾⁽⁷⁾. It has also been found in Axis and Sika deer⁽⁶⁾⁽⁷⁾⁽⁸⁾, but there is a suggestion that fallow deer may be more resistant⁽⁹⁾. Intra-erythrocytic piroplasms of *Theileria* spp were found in deer experimentally infected with the tick *Amblyomma americanum*⁽¹⁰⁾, with anaemia and deaths recorded in heavily tick-infested animals. The anaemia was possibly the result of dual tick and *Theileria* infection.

Adult ticks were observed in small numbers on a range of deer classes on the deer research unit during the preceding summer and were observed on deer in the mob containing Cases 1 and 2. This circumstantial evidence, together with the observation of the intra-erythrocytic protozoal-like organisms, justified the investigation into possible *Theileria* causation.

Transfusing blood into splenectomised animals is a standard way of replicating *Theileria*, since transfusion into normal healthy animals is often not successful in replicating the organism or producing clinical disease. The transfusion was an attempt to confirm the presence of an infectious disease and to increase the likelihood of detecting and identifying the presumed protozoal cause.

Samples were tested with a generic probe for *Theileria* spp and specific probes for *T orientalis* and *T cervi*. *Theileria orientalis* has

been found in cattle in New Zealand⁽⁴⁾, and the possibility of cross-species infection could not be discounted. *Theileria cervi* has been observed in North America and could potentially have been introduced into New Zealand in elk imported via Canada several years ago. *Theileria cervi* has been reported from elk⁽¹¹⁾. However, the negative PCR results suggest *Theileria* was not the causative organism. The cause of this haemolytic anaemia remains undiagnosed. Similar cases have been investigated and no protozoa detected. They will be reported separately in *Surveillance*.

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The diagnosis of Johne's disease

Test methods for Johne's disease are often insensitive, and tests based on cell-mediated immunity and some of the older serological tests lack specificity. Specificity problems arise from cross-reactions caused by subclinical infection with related *Mycobacteria*, particularly those belonging to the *Mycobacterium avium*/*Mycobacterium intracellulare* complex (MAC)⁽¹⁾⁽²⁾⁽³⁾⁽⁴⁾⁽⁵⁾. These organisms are common environmental organisms or occasional pathogens that cause subclinical infections in species that are also subject to Johne's disease. MAC organisms are closely related to the bacterium causing Johne's disease, previously known as *Mycobacterium paratuberculosis* but now classified as a subspecies of *M avium* and the name of *M avium* subsp *paratuberculosis* is now in common use⁽⁶⁾. The name *M avium* subsp *silvaticum* has been assigned⁽¹⁾ to a closely related organism mainly associated with wild birds, and *Mycobacterium avium* subsp *avium* is used for true avian organisms. The MAC group is diverse, consisting of at least 28 serotypes⁽⁷⁾. The MAC organisms, and even saprophytic *Mycobacteria* such as *Mycobacterium phlei*, have some antigenic epitopes in common with or closely related to those found on *M avium* subsp *paratuberculosis*. Therefore, when testing for Johne's disease, cross-reactions may occur in animals sensitised by *Mycobacteria* other than *M avium* subsp *paratuberculosis*.

The sensitivity of diagnostic tests for Johne's disease is affected by the inherent interactions between the immune system of the animal and the infecting *Mycobacterium*. The interaction is atypical and poorly understood. Until its complexities are better defined we depend on empirical observations of the interaction between organism and host. In the initial stages of infection the cell mediated immune (CMI) response is dominant. CMI responses can be detected by tests such as the intradermal delayed hypersensitivity test, lymphocyte stimulation test and the production of γ interferon in response to antigen. The responses tend to decline as the disease progresses. Conversely humoral responses are weak or absent in subclinically infected animals but become stronger in advanced stages of the disease⁽⁸⁾. This reciprocal relationship between CMI and humoral response is now widely accepted and is supported by sequential testing of experimental infections⁽⁹⁾.

The tissue response of the body also varies. In lepromatous (multibacillary) lesions large numbers of bacteria are present together with large numbers of epithelioid cells. Tuberculoid lesions contain few bacteria and few tubercle-like foci⁽¹⁰⁾. A lepromatous response is more common in animals that have poor resistance to the disease and exhibit poor CMI responses but are more likely to have a detectable antibody response. Animals with a tuberculoid response demonstrate stronger resistance to infection and tend to have stronger CMI reactions⁽¹⁰⁾⁽¹¹⁾⁽¹²⁾⁽¹³⁾⁽¹⁴⁾.

Laboratory confirmation of Johne's disease (paratuberculosis) is usually simple if there is clinical disease but difficult and unreliable in subclinically affected individuals. A suitable combination of test methods based on cellular immune response, serology, bacterial culture and histopathology will identify infected herds or flocks.

Test methods

The tests available for the diagnosis of Johne's disease include:

Tests based on the detection of organisms

- Microscopy of Ziehl-Neelsen (ZN) stained faeces or tissue smears
- Culture of organisms
- Traditional culture on mycobactin-containing media
- Radiometric methods
- Polymerase chain reaction (PCR) methods
- Combinations of culture and PCR

Tests based on CMI response

- Intradermal johnin (or avian tuberculin) test
- L migration test
- Intravenous johnin (or avian tuberculin) test
- Lymphocyte stimulation test
- γ Interferon test

Serological tests

- Complement fixation (CF) test
- Agar gel immunodiffusion (AGID) test
- Enzyme linked immunosorbent assay (ELISA)

Histological examination

Test accuracy

Sensitivity and specificity determine the accuracy of a test but their own accurate determination is seldom possible in practice. To determine the specificity, a large number of known non-infected animals must be tested. Identification of a suitable population is a complicated problem because the amount of sensitisation with other *Mycobacteria* will vary from one animal group or population to another and cannot be accurately measured or predicted. The proportion of animals in a population that have been exposed to *Mycobacteria* will influence the test's diagnostic specificity. Animals raised in a pathogen-free environment will yield specificity estimates different from what might occur in a field environment in which MAC organisms are common.

To determine the diagnostic sensitivity of a test, a large group of known infected animals must be tested. However, such a group may

be biased by containing a preponderance of clinically infected and few subclinically infected animals and therefore not represent a true population of infected animals. Figures quoted for sensitivity and specificity are therefore indicative rather than accurate and must be judged in relation to the manner in which they were determined and the characteristics of the population tested.

Identification of organisms

Microscopy

Microscopy of ZN stained faeces, gut mucosa or lymph node smears is useful for the rapid identification of the organism. However, some experience is necessary as only small bacilli of typical size and shape that are present in clumps must be identified as *M avium* subsp *paratuberculosis*. The sensitivity of the method is low and a diagnosis can be confirmed in only about one third of cases⁽⁴⁾. Saprophytic *Mycobacteria* and other acid-fast organisms are also seen in faeces and can be confused with *M avium* subsp *paratuberculosis*. Specificity of diagnosis therefore depends on the skill and experience of the operator.

Culture methods

Culture of organisms from faeces or tissues after pre-treatment to destroy contaminating organisms is traditionally done on a suitable medium with and without mycobactin⁽²⁾⁽³⁾⁽⁴⁾⁽¹¹⁾⁽¹⁵⁾. Slow growing acid-fast organisms that are mycobactin-dependent are classified as *M avium* subsp *paratuberculosis*. This method is regarded by many authors as completely specific. However, some other strains of *M avium* are also mycobactin-dependent in primary culture so absolute specificity is not assured, but specificity close to 100% can be assumed. The use of the IS900 gene probe to identify cultured *M avium* subsp *paratuberculosis* is being increasingly used to differentiate it from closely related organisms⁽²⁾⁽¹⁵⁾⁽¹⁶⁾. A method using 20, 10mer primers has also been used to sub-type *M avium* subsp *paratuberculosis* strains⁽¹⁷⁾.

The diagnostic sensitivity of culturing by traditional methods depends on the stage of the disease and the animal involved. In cattle, sensitivity has been given as 30-40% in subclinical cases and 60-92% in clinically affected animals⁽³⁾. The main drawback of culture for diagnosis is that it is slow. Cultures from cattle take up to 12 weeks to grow and *M avium* subsp *paratuberculosis* is subject to overgrowth by fast growing contaminants. Isolation of the strains that infect sheep is difficult and the sensitivity may be as low as 8%⁽¹⁸⁾ with some strains taking six months or more to grow. Radiometric culture methods, and combinations of culture with a PCR on the cultured material before colonies are visible to the eye, have been used to reduce the time delay⁽²⁾⁽¹⁹⁾⁽²⁰⁾⁽²¹⁾. Higher sensitivity is claimed for these methods than for traditional culture methods⁽²²⁾⁽²³⁾⁽²⁴⁾. The sensitivity of the radiometric method is claimed to be much higher than that for conventional culture methods when used for sheep⁽²⁰⁾. Concentration of organisms by filtration prior to culture doubled the number of positive cultures found⁽²²⁾.

Another approach to test sensitivity is to measure the analytical sensitivity. In the case of cultural methods, analytical sensitivity means the determination of the least number of viable organisms that can be detected by the test. Typically culture techniques can detect about 10³ organisms per gram of faeces (Anderson PR *et al*, according to⁽³⁾). Clearly when a technique has a limit to the number of organisms it can detect, the diagnostic sensitivity will not be ideal.

Polymerase chain reaction

In recent years a number of papers have been published on the use of the PCR test, especially since the suggestion that *M avium* subsp *paratuberculosis* might be the aetiological agent of Crohn's disease in humans⁽²⁵⁾. Modifications of basic PCR methods include nested primer PCRs⁽²⁶⁾⁽²⁷⁾, real time PCR⁽²⁷⁾⁽²⁸⁾, fluorescent PCR⁽²⁹⁾ and duplex PCR⁽³⁰⁾. Modifications to the methods of extracting DNA from faeces and tissues have also been developed⁽³¹⁾. The IS900 repetitive element is widely used as a probe for PCR tests for detection of *M avium* subsp *paratuberculosis* and has been successfully used in milk samples⁽³¹⁾⁽³²⁾. Although available as a kit it has yet to become a widely used diagnostic method for cattle. Presently available versions of the PCR test have been reported to be less sensitive than culture, with an analytical sensitivity of about 10³-10⁴ organisms per gram of faeces (Anderson PR *et al*, according to⁽³⁾). Several authors have reported a diagnostic sensitivity and specificity similar to that of culture⁽¹⁹⁾⁽²⁷⁾⁽²⁸⁾⁽³³⁾. A PCR test using nested primers achieved a high analytical sensitivity and some samples that contained as few as 112 colony-forming units of *M avium* subsp *paratuberculosis* per gram of faeces⁽²⁶⁾ gave positive results. The PCR method may be particularly useful in sheep where culture is insensitive and slow⁽³³⁾. The method has high specificity and results can be obtained rapidly but its potential for diagnosis of Johne's disease is yet to be fully realised.

Tests based on CMI response

Delayed hypersensitivity tests

Delayed hypersensitivity tests are generally performed using complex, mainly protein, extracts of either *M avium* subsp *paratuberculosis* or *M avium* subsp *avium* as antigen (allergen or sensitin). Reported differences in performance of the two products are more likely to be due to the way the products have been prepared and standardised than the subspecies of *M avium* from which they were derived. Both intradermal and intravenous tests have been used.

The intravenous test has been reported to be superior⁽¹⁾⁽³⁴⁾⁽³⁵⁾⁽³⁶⁾ but requires 20 to 30 times more johnin than the intradermal test and involves intravenous injection of the johnin then measuring the rectal temperature and/or the neutrophil/lymphocyte ratio six hours later. Because it is expensive and impractical, and the improvement in diagnostic accuracy is doubtful, the test is seldom used.

The intradermal johnin test has been widely used and is still prescribed for certification testing by some countries. However, the *OIE Manual of Diagnostic Tests and Vaccines* states that tests for delayed hypersensitivity are of limited value⁽⁴⁾. As far back as 1934 it was noted that the test failed to elicit reactions in 80% of clinical cases⁽³⁷⁾. By 1956 it was already known that skin sensitivity developed before symptoms appeared and animals often became anergic in advanced stages of the disease⁽³⁸⁾. A literature review in 1963 showed intradermal tests were unreliable⁽³⁹⁾. A 1984 review quoted 20 references on the intradermal johnin test but only one of them was written after 1972⁽¹⁾. Chiodini suggested the intradermal johnin test has a sensitivity of 54% and a specificity of 79%⁽¹⁾. Larsen reported that of 46 reactors, 15 developed disease and 21 excreted organisms while in 50 non-reactors ten developed disease and 20 harboured the organism⁽⁴⁰⁾. In another study, 78% of cattle that were eventually slaughtered for Johne's disease gave positive tests but 50% of animals in which the organism was never detected also reacted positively⁽⁴¹⁾. More recently it was reported that 55.6% of sheep with positive pathology gave positive reactions⁽¹²⁾. In another study, 58.1% of faecal culture positive animals were positive but 54.4% of faecal culture negative animals were also positive⁽⁴²⁾. In sheep 60% of faecal culture positives were identified by intradermal johnin testing⁽⁴³⁾. de Lisle *et al* described a test and slaughter programme in an infected herd, based on intradermal johnin and the complement fixation tests⁽⁴⁴⁾. The animals were tested eight times over a two-year period and reactors to either test were eliminated. Subsequently all animals were tested twice more and the entire herd was slaughtered. Histological and cultural examinations on the slaughtered animals showed 37 of the 102 animals examined were infected. The results of the final two tests showed the tests detected only two of the 37 infected animals. The testing programme detected clinical cases of Johne's disease, which were then culled, but failed to eliminate subclinically infected animals. Benedictus also found the test unsuitable as a diagnostic test in a control programme⁽⁴⁵⁾. In a review article, Collins stated that skin testing with johnin had not been successful and 'that the test should no longer be used for diagnosis, control or pre-purchase testing of animals for paratuberculosis'⁽²⁾.

Other review authors also suggested it is an unreliable diagnostic method⁽¹⁾⁽³⁾⁽⁴⁾. There has been no progress with the technique or allergens used for intradermal testing in the past 70 years and its use in its present form should be abandoned as a diagnostic test for individual animals. There may still be some justification for using it to detect sensitisation in a herd, but in this case the possibility of non-specific sensitisation must be carefully considered and more specific tests used to confirm a diagnosis.

In vitro cell mediated immune response tests

Several workers have investigated lymphocyte stimulation tests⁽¹⁶⁾⁽⁴⁶⁾⁽⁴⁷⁾. Results were not always encouraging and this, coupled with the complexity of the test as a diagnostic method, has

apparently discouraged its routine use. Investigation of tests based on the measurement of cytokines in response to antigen stimulation now appear most commonly in the literature. According to at least one author these tests are replacing intradermal skin tests⁽²⁾.

The γ interferon test was developed in Australia for testing cattle for tuberculosis and paratuberculosis⁽⁴⁸⁾⁽⁴⁹⁾⁽⁵⁰⁾. It is available in a commercial kit and is the most commonly used *in vitro* CMI test. Sensitivity has been reported as 71.8-93.3% in subclinically infected animals and 100% in clinically infected animals⁽⁵¹⁾. In sheep, when measured in histologically positive animals, the test had a sensitivity of 55.4% and a specificity of 93.9%, which is similar to the intradermal johnin test⁽¹²⁾. When used as a single test for predicting infection in individual animals the sensitivity varied from 50-75%, and when used in combination with the ELISA it correctly predicted 75% of cases⁽⁵²⁾. Both false-positive and false-negative results have been reported in goats⁽⁵³⁾. The test was recently reported to have a high sensitivity but that both false-positive and false-negative results occurred in cattle. The results, especially from younger cattle, tended to vary when tested sequentially⁽⁵⁴⁾. The test was more rapid and sensitive than the lymphocyte stimulation test⁽⁵⁵⁾. In experimentally infected calves, all had at least one positive test result, but control calves also had positive reactions and seven calves were also positive when bovine tuberculin was used as antigen⁽⁵⁶⁾. Prolonged γ interferon test responses were reported after vaccination of cattle with Johne's vaccines⁽⁵⁷⁾⁽⁵⁸⁾.

As with the johnin test, animals with tuberculoid lesions are more likely to respond positively in the γ interferon stimulation test than those with lepromatous lesions⁽¹²⁾⁽¹³⁾⁽¹⁴⁾. Cells from subclinically infected animals are more likely to respond to stimulation with *M avium* subsp *paratuberculosis* antigens than animals with clinical disease⁽⁵⁹⁾. Specificity may also be a problem since γ interferon was released in response to both paratuberculosis and bovine tuberculosis antigens⁽⁵⁷⁾ and negative controls sometimes gave positive results⁽⁵⁸⁾.

The γ interferon stimulation test is likely to suffer from similar drawbacks to the intradermal test. There seems little reason to hope that a new test technique is going to solve the problems of diagnosis when the same antigens are being used as in previously used tests. Problems of specificity and sensitivity will probably persist unless an immunodominant specific epitope can be found. It is not known why the CMI responses tend to decline as the disease progresses or why there is an apparent difference between animals with tuberculoid and lepromatous responses.

Serological tests

Complement fixation test

Twort and Ingram used the CFT for Johne's disease as early as 1913⁽³⁹⁾. Although early workers commented favourably⁽⁶⁰⁾ the test had poor specificity⁽⁶⁰⁾⁽⁶¹⁾. Despite numerous modifications, and the use of a variety of antigens, the test's reliability has improved little.

A culling programme based on intradermal johnin and CFT was unsuccessful⁽⁴⁴⁾. The CFT had a sensitivity of 17.9% in cattle shedding *M avium* subsp *paratuberculosis*⁽⁶²⁾. In another study the CFT had a sensitivity of 21.4% in subclinically infected animals and 83.4% in histologically confirmed clinical cases⁽⁶³⁾. Sensitivity was 54.6% in animals shedding the organism and 14.5% in infected animals that did not shed⁽⁶⁴⁾. In another study, 63.8% of 36 shedding cows were positive and 87.2% of non-shedders were negative⁽⁶⁵⁾. In sheep, three serological tests, including the CFT, had high sensitivity and specificity when tested in histologically positive sheep and non-infected flocks, respectively, but the results were variable and correlated poorly with the tests used in histologically negative sheep in infected flocks⁽⁶⁶⁾. The CFT is generally accepted as a good test only in clinically affected animals and becomes more reliable as the disease progresses.

Agar gel immunodiffusion test

The AGID has been widely used. Sensitivity and specificity have been variously reported as higher or lower than the CFT⁽⁶⁴⁾⁽⁶⁵⁾⁽⁶⁷⁾. However, reported differences in diagnostic sensitivity are easily influenced by the method and interpretation. Decreasing the cut-off point for a positive reaction increases diagnostic sensitivity but decreases diagnostic specificity, and vice versa. Therefore, claims of increased diagnostic sensitivity and specificity should be viewed sceptically and interpreted in relation to study design, test technique and cut-off points, and populations studied.

In cattle there is little to choose between the CFT and the AGID in terms of accuracy. In sheep the sensitivity of the AGID was reported as 38-56%⁽⁶⁸⁾ and 37.1%⁽⁶⁹⁾. A flock-level sensitivity of 61% and an equivalent specificity close to 100% have been reported⁽⁷⁰⁾. However, the AGID is difficult to perform and interpret when large numbers of tests are required. For these reasons the CFT is often preferred for large scale testing of cattle.

Enzyme linked immunosorbent assay

The ELISA is believed to be the best available serological test, particularly if cross-reacting antibodies have been removed prior to testing by adsorption with *M phlei* or extracts of it⁽⁷¹⁾. The specificity of the absorbed ELISA has been reported as 97-100% in both cattle and sheep⁽⁶²⁾⁽⁶³⁾⁽⁶⁶⁾⁽⁶⁸⁾⁽⁷²⁾⁽⁷³⁾⁽⁷⁴⁾⁽⁷⁵⁾⁽⁷⁶⁾⁽⁷⁷⁾. Sensitivity was 31-57%, but as with the other serological tests it increases as the disease progresses.

Histopathology

The histopathological examination of suitable tissues is highly specific in diagnosing Johne's disease. The method is mainly used for postmortem examination but mesenteric lymph node biopsies have been used⁽⁷⁸⁾. Histopathology has frequently been used as the gold standard against which to measure the efficacy of other tests. Sensitivity is regarded as high but its actual measurement is not possible. Clearly in some cases lesions are confined to small areas of the gut or to individual lymph nodes and may be missed if there has not been a comprehensive examination.

Conclusions

Clinical Johne's disease is easy to diagnose. Serology, culture, histology and smears can be used to confirm a clinical diagnosis. Diagnosis in subclinical cases is difficult and tests based on CMI responses may be more sensitive than serological tests. However, both CMI based tests and serology have sub-optimal specificity when animals have been exposed to other species of *Mycobacteria*. The most specific tests are faecal or tissue culture (or PCR) and the absorbed ELISA in live animals, and histology or culture from dead animals. The best test for individual live animals is a combination of a test based on CMI response and a test with high specificity. For this purpose the γ interferon test in combination with the absorbed ELISA has been used by several workers⁽¹²⁾⁽⁵²⁾. Detection of organisms by culture when time delays are not critical, and by PCR when faster results are required, are also highly specific test methods but lack sensitivity unless used as herd tests.

Diagnosis of infected herds is easier than diagnosis in an individual animal. When the objective is to identify non-infected herds or flocks and to maintain them free from the disease an appropriate combination of CMI tests, culture or PCR, absorbed ELISA and histological examination in culled animals should be carried out on suitable samples. Suspected non-specific test results should be confirmed by re-testing and the use of highly specific methods. If the purchase of non-infected animals is important, they should be acquired from herds identified as free from infection.

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The notation (Abstract PubMed) at the end of a reference indicates that the summary of the article was seen on the database of the National Centre for Biotechnology/National Library of Medicine at www.ncbi.nlm.gov/entrez/query.fcgi

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Libyostongylus douglassii in New Zealand ostriches

Libyostongylus douglassii (wireworm) is the cause of verminous gastritis in young ostriches. Young parasites burrow into proventricular glands and the submucosa of the proventriculus and gizzard. Adult worms live in the surface epithelium, suck blood and cause severe inflammation and anaemia. Affected birds develop gastric stasis and become anaemic, weak, emaciated and stunted. Chicks are the most susceptible to clinical disease, and severe losses may occur⁽¹⁾. Adult birds are less affected, especially if well fed.

Investigation

In April 2004 MAF investigated the first farm known to be affected by *Libyostongylus* (the index case) to describe the clinical disease and its likely distribution, means and time of entry, and to determine their significance to the ostrich industry. MAF and the ostrich industry identified methods for managing the parasite in commercial ostrich farms. A comprehensive review of *Libyostongylus* infection was commissioned by MAF and has been published on the MAF website⁽²⁾. The potential risks to kiwi, which is a native ratite, were assessed by MAF and the Department of Conservation (DoC).

Clinical disease

Mortality rates from *L. douglassii* on the index farm were estimated to be 50% in chicks, 20% in juveniles and 10% in adults. Chicks developed a 'hockey-stick' neck, indicating weakness, and became reluctant to move. Affected adults moved slowly, and became less aggressive. Severely infested birds became recumbent and died. Other clinical signs included anaemia, poor growth rates and decreased egg production. These signs had been present on the farm in 2003.

Distribution

The index farm was a large hatchery and breeding enterprise established in the central North Island in 1997 from a variety of local and imported ostriches. Because of the nature of the enterprise, there were many pathways by which other ostrich farms could have become affected. The farm received eggs from other properties for hatching. Chicks were dispersed to other farms for rearing. At three months of age the chicks were sent to 'finisher farms' and then to slaughter aged 12-14 months. Sixteen farms were identified as having sent live birds to the index farm, and 20 farms were known to have received live birds from the index farm. *Libyostongylus douglassii* infection and disease has subsequently been detected on two farms associated with this production system and on another farm in the North Island and one in the South Island.

Means and time of entry

In 1998, Cooke reviewed disease entities of farmed ratites in New

In April 2004, the nematode *Libyostongylus douglassii* was identified in clinically affected ostriches. The disease was confirmed by the morphology of adult worms, faecal culture, and examination of the proventriculus. The parasite is present in the North and South Islands. Surveillance will be enhanced in ostrich farms close to kiwi habitats.

Zealand⁽³⁾. She noted a case of air sacculitis associated with necrotic parasitic migration tracts. This was attributed to aberrant parasitic larval migration as there were no reports of internal parasites in New Zealand. Since the review, *Surveillance* magazine has reported a further 16 mortality events in chicks as summarised in the table. However, none has reported signs specifically suggesting *Libyostongylus* infection.

Ostrich farming is recorded in New Zealand as early as 1883, but modern ostrich farming commenced with the importation of eggs in 1994 and live birds in 1995⁽⁴⁾. *Libyostongylus douglassii* is likely to have been introduced with live birds imported since 1995. The last recorded importation of ostriches occurred in 1998. Infection may have been present on the index property since its establishment, or introduced subsequently from other farms infected by imported birds.

Libyostongylus infections are known to occur in many countries where ostriches are farmed, including Australia and North America⁽²⁾. The 1995 import health standards for ostriches coming from Australia⁽⁵⁾, Canada⁽⁶⁾, and the United Kingdom⁽⁷⁾ required a single treatment against internal parasites within five days of shipment. The treatment specified was an ivermectin based anthelmintic at a dose rate recognised as efficacious in ratites against cerebral nematodes (Australia and Canada protocols) or at a dose rate of 400 µg/kg bodyweight (United Kingdom protocol). This treatment may not have been completely effective for *Libyostongylus*⁽²⁾. In 1997, an import risk analysis concluded that *Libyostongylus* parasites could be introduced through importation of infested ratites of any age⁽¹⁾. The recommended safeguard was for live ratites to be treated with both fenbendazole and levamisole during pre-export quarantine⁽¹⁾.

Significance to industry

The initial entrepreneurial expansion of ostrich farming in the mid-1990s has settled into a market-related industry that reflects the value of products in developing markets. Export markets and related meat schedules have made ostrich farming viable in New Zealand. However, economic success depends on chick survival to slaughter, and poor chick growth rates and increased mortality are a significant production problem⁽⁴⁾. The advent of intestinal parasitism as a cause of chick mortality will now require intensive intervention to manage on-farm effects and spread between farms.

Libyostrongylus douglassii is not an Unwanted Organism under the Biosecurity Act 1993. Eradication was not considered technically feasible given the persistence of the organism in the environment (eggs can survive for about 30 months on pasture), and its probable time of entry and subsequent spread. Fact sheets to assist farmers and veterinarians have been published on the MAF website. Individual farmers and their veterinarians are investigating the efficacy of anthelmintic treatment regimens.

Significance to kiwi

As far as we know *L douglassii* is specific to the ostrich. However, MAF and DoC have considered the potential risk to kiwi. There are three groups of ratites: the ostrich, two rheas, and the emu-cassowary-kiwi cluster. Kiwi are probably closer to rheas than ostriches⁽⁸⁾. Nevertheless, as a precautionary measure it has been considered advisable to reduce the likelihood of the exposure of

kiwi to diseases of other ratites such as farmed ostriches and emu⁽¹⁰⁾. MAF and the Department of Conservation have identified ostrich farms near populations of native kiwi, and will develop a protective management protocol should any of these farms become affected by *Libyostrongylus* sp.

Ongoing surveillance

Countries where *Libyostrongylus* infection is common have reported infection only in ostriches. This parasite is therefore unlikely to pose a threat to kiwi. Nevertheless, as a precautionary approach, ostriches on the five farms that overlap kiwi habitats will be monitored for infection. Kiwi associated with a positive farm would be monitored for signs of infection. Enhanced passive surveillance will occur nationally through New Zealand's veterinary laboratories.

Mortality events in young ostriches in New Zealand reported in <i>Surveillance</i> from 1998 to July 2004		
Year reported	Case number	Details of reported cases of mortality events in young ostriches
1998	1	Several cases of fading ostrich chicks were reported affecting birds two to six months of age and involving inappetence and loss of condition. Affected birds usually had large amounts of impacted grass and/or stones in the gizzard and ventriculus, but no underlying disease was detected. The reasons for gastric stasis or impaction appeared to be management-related ⁽¹⁰⁾ .
	2	Mortality in chicks and young ostriches was reported in an ostrich hatchery. Necrotic lesions in the liver were noted on necropsy. Histology revealed necrotic tracts and surrounding granulomatous reactions with giant cells, suggestive of wandering parasite larvae ⁽¹¹⁾ .
	3	Sudden deaths occurred in two two- to three-week-old ostrich chicks. Necropsy of one showed a cloaca and bursae full of fluid and necrotic lesions in the liver. Histology revealed spreading necrotising lesions in the liver. <i>Salmonella</i> Typhimurium was obtained from the liver and cloaca ⁽¹¹⁾ .
1999	4	Eight of 12 seven-day-old ostrich chicks died over a 12-hour period following a shift from a hatching to a rearing shed. Petechial haemorrhages were present throughout the intestinal mesentery. A diagnosis of clostridial enteritis was made histopathologically ⁽¹²⁾ .
	5	Several of a group of three- to six-week-old ostriches became moribund and died after a day's illness. The predominant microscopic finding was acute multifocal hepatic necrosis with no visible aetiological agent ⁽¹²⁾ .
	6	A number of ostrich chicks one month in age were not growing well and a few died. Histology on the large intestine from one showed crypt destruction and collapse, with mild infiltrates of macrophages, lymphocytes, plasma cells and heterophils. A presumptive diagnosis of coronavirus infection was made since the lesions were very similar to those described with coronavirus infection in other species ⁽¹³⁾ .
2000	7	Conditions recorded in cases in which there were problems at the early stages of rearing included bacterial enteritis and bacterial hepatitis and often associated myocarditis. In one case of hepatitis, <i>Campylobacter jejuni</i> was isolated. In enteritis cases, the organisms involved included <i>C jejuni</i> , <i>Salmonella</i> Typhimurium and <i>Clostridium perfringens</i> ⁽¹⁴⁾ .
	8	Twenty of 100 five- to six-day-old ostrich chicks died after developing diarrhoea. Histology revealed acute enteritis associated with clostridia-like organisms and splenic lymphoid depletion, consistent with clostridial enteritis. <i>Clostridium perfringens</i> was isolated from an intestine sample. On a second ostrich farm, the same condition occurred in chicks three to four weeks old ⁽¹⁵⁾ .
	9	Three one-month-old ostrich chicks died suddenly. At necropsy the birds had numerous small white foci in the liver, which on histology were small foci of hepatic necrosis consistent with a bacterial septicaemia. On culture an alpha haemolytic <i>Streptococcus</i> species and <i>Candida albicans</i> were isolated.
	10	Histological examination of tissues from seven-day-old chicks that had acute diarrhoea revealed fibrinonecrotic bacterial enteritis. <i>Clostridium perfringens</i> and an alpha-haemolytic streptococcal organism most closely resembling <i>Streptococcus viridans</i> were isolated ⁽¹⁶⁾ .
2001	11	Cases of hepatitis around seven months of age reported with unknown cause ⁽¹⁶⁾ .
	12	Three hundred of 900 ostrich chicks died over a month. The deaths occurred at three weeks of age. A five-week-old bird killed for necropsy showed poor to moderate body condition with no body fat and the gastrointestinal tract almost empty. This suggested a nutritional problem but histology revealed severe atrophic enteritis caused by cryptosporidiosis ⁽¹⁷⁾ .
	13	A group of ostrich chicks progressed well for the first two to three weeks of life, then lost condition and died at four to five weeks of age despite apparently continuing to eat. Necropsy of one bird showed cachexia with severe serous atrophy of body fat reserves including that of the coronary groove. The proventriculus and gizzard were moderately filled with dry ingesta composed primarily of fine sand and small stones up to 3-4 mm in diameter and only a small amount of grass. The gizzard koilin layer was hypertrophic and the intestinal content was dry and pelleted. The findings are consistent with gastric impaction/stasis, a common problem in ostrich chicks up to a few months of age ⁽¹⁷⁾ .
	14	A one-month-old ostrich chick was found dead. Eleven other birds had died in the previous few weeks. At necropsy a thick white tenacious lining clung to the surface of the proventriculus. Histopathology revealed that the layer was a dense mat of fungal organisms consistent with <i>Candida</i> , confirming candidiasis ⁽¹⁷⁾ .
2002	15	One hundred of 920 ostrich chicks were euthanased because they had become unsteady on their legs and were losing weight, and many had diarrhoea, some with dysentery attributed to mixed bacteria including clostridia, possibly caused by a muddy environment and management stresses ⁽¹⁸⁾ .
2003	16	An ostrich farm on the East Coast of the North Island had a two-year history of tracheitis and oesophagitis ⁽¹⁸⁾ .

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Register of new host-parasite records

Exotic nematode in an alpaca

An *Ostertagia*-like nematode previously unrecorded in New Zealand, *Camelostrongylus mentulatus*, was recovered from compartment three of a dead 2.5-year-old alpaca in the Auckland area. Whilst considered to be primarily a parasite of camelids, *C mentulatus* has a broad host range throughout the rest of the world. In addition to alpacas, this includes camels, llamas, sheep, goats, antelopes, giraffes, gazelles and deer⁽¹⁾.



Figure 1: Spicules and bursa of a male worm of *Camelostrongylus mentulatus*

In some parts of South Australia it has been reported as being widespread in sheep⁽²⁾. It has also been suggested that *C mentulatus*, which parallels *Ostertagia* sp in pathogenicity and development in this host, may cause a comparable disease syndrome in the camel and llama⁽¹⁾⁽³⁾⁽⁴⁾.

Although showing a morphological similarity to *Ostertagia*, this parasite can, on closer inspection, be readily identified by its long slender spicules, which have pincer-like terminations and very obvious transverse striations along their whole length (Figure 1).

New species of *Trichuris*

A species of *Trichuris*, similar to *T discolor* in possessing a non-everted vagina in the female worm but different in having a tightly convoluted structure with a small egg chamber located approximately midway along its length, was recovered from the large intestine of a llama that died in Canterbury. This species was subsequently identified from published descriptions⁽⁵⁾⁽⁶⁾ as *Trichuris tenuis*, a parasite formerly unrecorded in New Zealand. While *T discolor* has previously been recorded in cattle in New Zealand⁽⁷⁾, and although both it and *T tenuis* have been recorded in llamas elsewhere, the latter species is considered to be the typical whipworm of camelids and primarily a camelid parasite⁽⁸⁾.

Oesophagostomum in a llama

The large intestinal nematode, *Oesophagostomum venulosum*, was also recovered from the llama referred to above. Although this species of *Oesophagostomum* is mainly a parasite of sheep and goats, in which it may occasionally be of some economic importance, it has also been recorded in a number of other hosts in New Zealand including cattle, deer, wapiti, chamois and tahr⁽⁹⁾. However, it has not been reported in llamas here before.

Wireworm in an ostrich

Large numbers of nematodes were recovered from the proventriculus of an adult male bird from a North Island ostrich

The following are some new host-parasite relationships recently recorded at Gribbles Veterinary Pathology laboratories or submitted to the author, for initial or second opinion identifications, by other veterinary diagnostic laboratories or persons in New Zealand.

farm that had experienced a series of deaths (Figure 2). These were identified as *Libyostrongylus douglassii*, the so-called ostrich 'wireworm'. *Libyostrongylus douglassii* is the most deleterious helminth of young ostriches with mortality rates occasionally greater than 50%⁽¹⁰⁾. The worms inhabit and physically occlude the ducts of the proventricular glands. This causes a compensatory production of thick mucus that impairs digestion and may lead to impaction. They also cause a diphtheritic proventriculitis, which is



Figure 2: Proventriculus of an ostrich with the koilin layer removed to reveal the presence of adult *Libyostrongylus douglassii*

commonly called 'vrotmaag' (rotten stomach) by producers in South Africa. Clinical signs of infection include general wasting, anorexia, anaemia and death⁽¹⁰⁾. Further information on this case is published on p14 in this edition.

Tapeworms in a blue duck

Cestode segments were recovered from the faeces of a blue duck (*Hymenolaimus malacorhynchus*) resident at a Christchurch conservation park. Unfortunately no scolex was present. However, based on the morphology of the proglottids (Figure 3), it was tentatively identified as a species of the genus *Sobolevicanthus*. This particular tapeworm has previously been recorded in a 'wild' duck,



Figure 3: Tapeworm proglottids recovered from the faeces of a blue duck

believed to be a mallard (*Anas platyrhynchos platyrhynchos*) or a grey (*Anas superciliosa superciliosa*), or a cross between the two, on Lake Wairarapa⁽¹¹⁾. Coincidentally, the scolex was absent also from the specimen recovered from this 'wild' duck⁽¹¹⁾.

Isospora in a kokako

Small numbers of coccidial oocysts were detected in the faeces of a kokako (*Callaeas cinerea*) from a Wairarapa wildlife centre. The subspherical oocysts lacked a micropyle and measured 22 x 19 µm. Following their sporulation they were identified as a species of either *Isospora* or *Atoxoplasma*. Neither of these apicomplexan parasites has been recorded in the kokako before.

Haematozoa in a North Island robin

Protozoan parasites suspected of being a species of *Haemoproteus* were recorded in a blood film from a North Island robin (*Petroica australis*) submitted to our Auckland laboratory. A *Haemoproteus* species, notably *Haemoproteus danilewskyi*, has been previously recorded in a number of other birds in New Zealand, including the blackbird, skylark and song thrush⁽¹²⁾, but not the North Island robin. Transmission of *Haemoproteus* infection is frequently by hippoboscids and such infections generally cause mild or inapparent clinical symptoms in birds⁽¹³⁾.

Capillaria in a shore plover

Small numbers of eggs of the nematode parasite *Capillaria* were detected in the faeces of a shore plover (*Thinornis novaeseelandiae*) held in a Wairarapa wildlife centre. Although known to be host to a number of ectoparasite infestations⁽¹⁴⁾⁽¹⁵⁾, this is the first occasion a helminth parasite has been reported in this bird.

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Welfare assessment of vertebrate toxic agents

To eradicate tuberculosis from livestock and feral vectors, and to protect our native fauna and flora, toxic agents and traps remain essential for vertebrate pest control in New Zealand. However, we have an ethical duty to use the most humane control methods available and to continue developing more humane methods.

The development of draft National Animal Welfare Advisory Committee (NAWAC) guidelines for testing traps (based on the ISO standard) has provided an objective process for assessing traps using pathological (eg physical injuries) and physiological (eg brain stem reflexes) measures, but no such guidelines have been available until now for assessing vertebrate toxic agents.

Our initial research looked at behavioural, biochemical and pathological changes in possums following poisoning with cyanide, 1080, phosphorus, cholecalciferol and brodifacoum. These studies were conducted with prior approval from the Landcare Research Animal Ethics Committee. We used this research, along with information from the literature, to identify some key welfare assessment principles.

Guidelines

The relative humaneness of vertebrate poisons can be assessed by the following five-step process⁽¹⁾.

1. Consider the capacity of the animal to suffer

Is the species capable of suffering? This is normally assumed in animal welfare science, and the Animal Welfare Act (1999) considers any mammal, bird, reptile, amphibian, fish, mammalian fetus in the last half of gestation, pre-hatched reptile or bird in the last half of development, any marsupial pouch young, and any octopus, squid, or crustacean capable of suffering. Further consideration should be given to whether animals are capable of experiencing particular forms of suffering, or whether there are aspects of the species' natural biology or individual characteristics that introduce or predispose it to certain welfare consequences.

Landcare Research has recently developed guidelines for assessing the relative welfare impact of vertebrate poisons on target pest species. The guidelines set out a five-step process that enables the comparison of type, degree and duration of welfare compromise between toxic agents.

2. Anticipate likely effects of the poison

Knowing the mode of action, cause of death, and effects in humans and other animals of a particular toxic agent can help in anticipating effects when designing experiments to assess humaneness. Vertebrate toxic agents, in contrast to traps, have diverse modes of action, and as a result the nature and duration of potentially painful and distressing effects are also extremely varied. The cause of death and modes of action have direct implications for animal welfare.

For example, a vertebrate toxic agent that interferes with energy metabolism could cause weakness then rapid death by cardiac failure, whereas one that interferes with blood clotting could cause a slower death through internal haemorrhage.

3. Determine the type, intensity and duration of effects, and the percentage of animals affected

To determine the degree of welfare compromise, the behaviour and pathology of poisoned animals need to be closely observed to describe the unpleasant effects caused by toxicosis. The aim is to develop a list of effects associated with each vertebrate toxic agent for each target species, through close observation of poisoned animals in cages or pens (at least in the first instance), to determine the proportion of animals experiencing adverse welfare effects (ie pain, fear, distress or suffering), and the time of onset and duration of those effects. The occurrence of undesirable effects is only important until the animal loses consciousness, after which it is considered no longer capable of suffering. This means that the time to death should not be used as the sole basis for welfare assessment.

Examples of degree of welfare compromise caused or indicated by several clinical signs of poisoning observed in possums

Effect	Minor	Moderate	Marked
Convulsions/seizures		Recovery from intermittent or short tonic or tonic/clonic convulsions	Recovery from regular or prolonged tonic or tonic/clonic convulsions
Vomiting	Occasional retching	Vomiting or high frequency of retching	
Breathing	Occasional abnormal breathing pattern	Prolonged abnormal breathing, or periods of laboured breathing	Prolonged laboured breathing
Bodyweight	Weight loss of <20% (severity would differ with species)	Weight loss of 20–30%	Weight loss >30%
Inactivity/lethargy	Mostly inactive with reduced awareness	Mostly prostrate or lying with reduced awareness	
Normal behaviour	Loss of normal behaviour patterns (eg grooming)		
Pathology	Lesions one or two areas, or short-term minor to moderate pain	Lesions three or four areas, or short-term severe pain, or long-term discomfort	Lesions areas, or causing long-term moderate to severe pain

4. Determine the degree of welfare compromise caused by each effect

The degree of welfare compromise or level of suffering caused by each effect is determined by the type, intensity and duration of the effect. This evaluation is based on an interpretation of the behaviour and pathophysiological effects in terms of animal welfare, based on a thorough knowledge of normal behaviour of the species concerned, and the welfare implications of similar effects of poisoning in other animals or humans. Because effects are so diverse, it is difficult to provide an exhaustive list of the welfare compromise caused by each but some examples are given (see table). Various descriptors could be used to define each degree. We have used 'minor', 'moderate' and 'marked', and have assumed that suffering increases with increasing magnitude of injury or change.

5. Assess the relative humaneness of the poison

Diverse effects and durations of effect make the assessment of humaneness from these data difficult. The guidelines recommend judging the relative humaneness of vertebrate toxic agents by listing and comparing (through expert opinion) the effects of each toxic agent, rather than making a judgement on the absolute humaneness of a vertebrate toxic agent. Incorporation of a list of several features allows consideration of all relevant information by knowledgeable experts. The percentage of animals affected and the type, intensity and duration of effects causing the suffering can be included. By fully describing the welfare implications for all the vertebrate toxic agents, the most serious areas of welfare compromise can be identified.

We have used this five-step process to make assessments of the relative humaneness of some commonly used vertebrate toxic agents in possums. For example, we found that in possums the first

sign of poisoning with cyanide was incoordination, followed by minor abnormal breathing in half the animals, prior to them becoming unconscious 6.5 minutes after ingestion of the cyanide⁽²⁾. Although convulsions were seen in 73% of possums, this was after they were unconscious. Thus we concluded there was potentially only 3 minutes of welfare compromise in possums following cyanide poisoning. In contrast, during brodifacoum poisoning in possums the first sign of illness occurred 13 days after consuming the poison. Such signs included changes in appearance and minor food intake reduction, followed by variable and minor effects (abnormal breathing, tremors, incoordination) and then lethargy and prostration in a third of animals for 4–5 days before death⁽³⁾. This indicated moderate welfare compromise (see table). Possums killed with brodifacoum showed widespread haemorrhages of varying severity, with all possums having at least one severe haemorrhage that would have caused or contributed to pain, distress or weakness. Consequently brodifacoum compromised welfare for 6–7 days before death.

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Quarterly review of diagnostic cases – April to June 2004

Cattle

Neospora **abortion** was diagnosed from tissues from a foetus aborted at six months of gestation by a Friesian cow from Taranaki. Fewer *Neospora* abortions have been seen this year than in previous years. Gribbles Palmerston North Laboratory made histological diagnoses in March, April and May and none after mid May. Diagnoses of placentitis – primarily **fungal abortion** – began in late May and continued to the end of the quarter. One foetus from the Taranaki region had multiple raised grey plaques over the skin surface, which histologically were hyperkeratotic with intra-epithelial pustules containing fungal hyphae. *Mortierella wolfii* was cultured from foetal stomach contents. This was the third abortion from this property in three days. The submitting veterinarian reported good quality hay was being fed.

There were a number of **abortion** outbreaks in dairy cattle in Otago-Southland in June. In one, approximately 12 of a large number of cows on a feeding pad aborted over two weeks. Although no specific agent was identified in the foetuses it was later found that the farmer had been mixing molasses with the piped drinking water supplied to the cows. This had turned the water into a brown sludge from which a heavy pure growth of *Klebsiella pneumoniae* was isolated. This opportunistic environmental pathogen has been reported as causing mastitis in housed cattle. After the water pipes had been cleaned out and fresh water supplied there were no new cases of abortion. In another outbreak in a small herd of 120 dairy cows on a crop and receiving hay, 15 cows aborted over a three-week period. Tests for *Neospora* and other infectious agents were negative. However, foetal tissues and placenta consistently showed histopathological changes typical of a **bacterial or mycotic abortion**. Fungal hyphae were identified in the liver of one aborted calf whose dam died a few days after aborting. A variety of Gram-negative, possibly enteric, bacteria but no fungi were isolated from the abomasal contents of some aborted foetuses. The hay had been brought in from a nearby deer farm and much was of poor-average quality with superficial spoilage and mould. After the poorer hay was discarded the number of abortions rapidly declined. Exposure to poor quality feed – usually hay – is becoming the major cause of abortion outbreaks in dairy cattle in the southern region.

A two-year-old Murray Grey cow from the Wairarapa became cast in the paddock and died later that evening. At postmortem there was haemorrhage from the mouth, nose, and eyes as well as in the kidneys and lung. Histological examination of liver, lung, kidney, heart, and intestine revealed a necrotising vasculitis with haemorrhage in all these tissues, consistent with a diagnosis of **malignant catarrhal fever**.

A herd of mixed-age Friesian cows in the Taranaki was grazing

Each quarter, Surveillance publishes a review of selected diagnostic cases handled by New Zealand's veterinary diagnostic laboratories owned by Gribbles Veterinary Pathology. These cases do not necessarily reflect the national disease profile but they do represent diseases of interest to the livestock industry or of significance to wildlife.

pasture supplemented with maize silage mixed with lime flour and sodium bicarbonate. Six cows became sick over a few days and three died. Serum calcium and phosphorus concentrations in samples from two recumbent cows were markedly decreased at 0.78 and 1.10 mmol/l (normal 2.12-2.62) and 0.22 and 0.61 mmol/l (normal 1.41-2.95), respectively. **Hypocalcaemia and hypophosphataemia** like this would induce recumbency because of inhibition of muscle function. In addition, glutamate dehydrogenase concentrations were elevated, indicating a degree of liver damage.

Several one-year-old Friesian bulls died on a Rangitikei farm. Postmortem of one animal showed severe abomasal ulceration and histology revealed severe diffuse almost full thickness necrosis. Perforating ulceration of the small intestine and necrosis of Peyer's patches with collapse of the lamina propria were all consistent with a diagnosis of **bovine virus diarrhoea mucosal disease** and a persistently infected animal.

A high non-pregnancy rate was investigated in a Taranaki dairy herd. Serum samples from five non-pregnant cows had negative *Neospora* titres and a sixth reacted at a 1:200 dilution, a minimal reaction not suggesting recent infection. BVD virus neutralising titres ranged from negative at <1:4 to strongly positive with two animals >1:4096. Three cows had high titres of 1:768, 1:1024 and 1:1536 indicating recent exposure to **BVD** virus. A negative titre means the animal has either never been exposed or is a persistently infected carrier. A BVD Ag ELISA on the cow with a titre of <1:4 was negative, indicating it had not been exposed.

A mature Friesian cow developed bilateral hyphaema. Haematology results were normal except for a severe **thrombocytopenia** with platelets $11.9 \times 10^9/l$ (normal 200-600). The practitioner reported similar symptoms and thrombocytopenia in a previous case. Possible causes of thrombocytopenia in cattle are acute BVD infection, bracken fern poisoning and bone marrow abnormalities. BVD antigen ELISA on blood was negative and there was no history of bracken fern exposure so the aetiology remains unresolved.

In late May a mob of two-year-old mixed breed cows from the Horowhenua had **photosensitisation** with swollen vulvae and hyperaesthesia leading to mania. Serum samples from four animals showed markedly elevated GLDH (up to 3037 IU/l; normal 8-41) and GGT (up to 564 IU/l; normal 9-39). The enzyme

concentrations reflect significant hepatocellular and less severe biliary damage. *Pithomyces* spore counts were low at the time and the cattle were grazing ryegrass and white clover pasture so the aetiology is unknown.

A mob of four-month-old Friesian cross heifers examined because of a history of diarrhoea and illthrift had no evidence of coccidiosis or gastrointestinal parasitism. A mean serum selenium concentration of 43.5 nmol/l (normal 140-1000) was consistent with **selenium deficiency**. Diarrhoea in selenium deficient herds is believed to be associated with retarded vitamin E absorption but the pathogenesis is not clear. Another mob of one-year-old Friesian bulls from Southern Hawke's Bay had a history of illthrift despite a thorough drenching history. A mean serum selenium concentration of 39 nmol/l confirmed selenium deficiency.

Hair samples from Galloway cattle were tested for **alpha mannosidosis**. The results of DNA testing showed that several cattle in this herd are carriers of the disease.

For the past few years a syndrome of **acute liver necrosis** has been seen in adult Friesian dairy cattle fed ryegrass straw on three different Canterbury farms. The problem recurred on one of the farms this year, affecting two cows in a group of 600. As in previous cases, the cows had been just dried off and were being fed only straw and little or no grass. The problem this year occurred within 24 hours of the cows being fed mouldy barley straw. A mycotoxin in the straw is postulated to be the likely cause. Histologically, the liver of one affected cow had severe diffuse acute coagulative necrosis.

In June, five of a group of 16 autumn-calved cows on a farm in South Otago were found dead and another two were showing the typical signs of **hypomagnesaemia**, confirmed by very low serum magnesium levels in the survivors. There was no information available about the type of feed these cattle received.

Acute **rumen acidosis** caused sporadic deaths of 16 adult dairy cows over a short period on a farm in Southland. The cows were on crop with access to grain but without proper conditioning to either feed. Diagnosis was confirmed by a low rumen pH in the one animal necropsied.

On a farm in west Otago, ten dairy cows being fed kale without supplements were found dead over a week. The rapid nitrate test on the crop was positive. **Nitrate toxicity** was confirmed by a quantitative test that showed the kale contained 1.7g of nitrate/kg of dry matter. At this concentration deaths could occur if kale were the sole feed. Hay was added to the diet and the deaths stopped.

On a Central Otago farm, 14 of a mob of 400 yearling Friesians were found dead one morning. They were being break-fed on Italian ryegrass supplemented with silage and hay. The only gross findings on necropsy of one animal were bloating and lots of fluid issuing from the nostrils. There were no toxic plants in the rumen contents nor growing in the paddock. Histological examination of a

range of tissues showed no significant findings and death from a clostridial disease was ruled out. As half the remaining dead animals were also bloated the cause of the deaths was most likely **bloat**. A new break of feed had been provided late in the afternoon the day before, much later in the day than usual. By this time the plants in the new break had already been severely frosted. It has been observed previously in this area of Otago that frosted Italian ryegrass appears to predispose stock to severe bloating.

Gangrenous ergotism was diagnosed in dairy cows on three farms in Southland and one in Otago in late June. Up to 40 cows were affected by a severe hindlimb lameness that initially manifested as a severe swelling of the hindlegs above the coronary band. This often progressed over two to three weeks to gangrene with sloughing of the skin and tissues distal to a distinct line around the mid-pastern. In most animals only the lower hindlegs were affected and in only one outbreak were the tails also gangrenous. The cows were often from mobs that spent long periods standing in muddy paddocks. The source of the condition was most likely the baleage or pit silage fed. In all cases it had been made in autumn from heavily seeded mature pastures. Ergotised ryegrass was found in baleage fed in two of the three outbreaks in which it was checked. As there is no treatment for this condition (apart from perhaps antibiotics to treat secondary invaders) many of the severely affected cattle had to be killed on humane grounds.

In a small Waikato herd of dairy calves, one animal became anorexic, weak and recumbent within 24 hours. At veterinary inspection the two-month-old heifer was moribund and was euthanased. Postmortem showed extensive friability and reddening of the abomasum and omasum, fibrin tags in the increased peritoneal fluid and marked swelling of walls of these organs. Crepitus was also a feature. The mucosae of the abomasum and omasum were ulcerated, reddened and swollen and there was haemorrhage in the gut lumen. Histologically, the abomasum and omasum were acutely and severely inflamed and oedematous and large bacterial rods consistent with clostridia were present in the interstitium of the mucosa and submucosa. The morphological diagnosis was severe acute **abomasal and omasal ulceration and necrosis**. No fresh tissue was received, but the organisms are presumed to be *Clostridium*, probably *perfringens* type A.

In August 2003, the Hamilton laboratory tested more than 1000 calf faecal samples to gauge the prevalence of **infectious agents in cases of calf diarrhoea**. The most common infectious agents recovered were rotavirus and Cryptosporidia. The results were:

	Total tested	Percent positive
Rotavirus	736	61
K 99 <i>E coli</i>	258	12
Coronavirus	35	11
<i>Salmonella</i>	736	10
<i>Cryptosporidium</i>	726	26

In one month the Hamilton laboratory had 43 submissions from groups of rising yearling calves with **illthrift**, often with **diarrhoea**.

Worms, yersiniosis and selenium deficiency were the most common diagnoses. Five cases had one or more animals with a FEC >500 epg or an increase in pepsinogen. Seventeen of the 43 submissions were tested for **worms**. Two of 18 cases tested had one or more animals with a faecal result for **coccidiosis** greater than 1+.

Yersiniosis was found in one or more animals in four of nine cases from which faecal samples were cultured. Selenium and copper analyses were the most frequent requests (23 groups). On nine farms the mean value for selenium was below the reference range, indicating **selenium deficiency**. Three of 23 groups had a mean copper or ferroxidase level below the reference range, confirming **copper deficiency**. None of 17 groups tested had low serum B12 levels. None of 13 groups tested contained a BVD antigen-positive animal but two of seven groups tested for BVD antibody had positive animals. GGT was requested on 13 submissions, and a total of 45 calves tested. In two groups, there was one calf with a high GGT (264 and 912 IU/l) suggesting **facial eczema**. Other diagnoses included one group with zinc toxicity and one animal had liver fluke eggs in its faeces.

Two deaths occurred in a mob of eight-month-old dairy calves in the Waikato. Postmortem showed severe abomasal haemorrhages and a large amount of abdominal fluid. The capsules of the kidneys were swollen. Other calves in the mob had black tarry faeces. Histology of the kidney revealed severe, generalised, chronic nephrosis and features consistent with **acorn poisoning**.

Deer

The carcasses of three five-month-old deer that died suddenly were diffusely jaundiced. Serology for *Leptospira pomona* of an in-contact herd mate revealed a microscopic agglutination titre (MAT) of >1:1600, confirming a recent **leptospirosis** infection. Tissues from a weaner deer on a Canterbury deer farm had histological evidence of haemoglobin in renal tubules and hypoxic necrosis of the liver. A silver stain revealed several scattered leptospiral organisms in the renal tubules. The farmer lost 16 weaners from a mob of 110 over a 10-14 day period. No further deaths occurred after the animals were vaccinated against leptospirosis.

Twenty of 450 weaner deer died over a short period on a Southland deer farm after an acute onset of depression and red urine that in some cases had the appearance of raspberry jam. Necropsy findings were consistently a mild haemolytic jaundice confirmed by histological examination of the kidney. Blood samples from a sick weaner showed a moderate azotaemia, a severe metabolic acidosis and a severe responsive anaemia. *Leptospira pomona* MAT titre from this animal was high at 1:800. Other deer in the mob also showed very high titres. The deer that died were from a mob of 300 home-bred deer that had been mixed a few days before with 150 brought in from a farm a few kilometres away. Serological testing of other age groups and other weaner mobs on the farm showed no evidence of previous exposure to leptospirosis so the infection was most likely introduced by the brought-in animals. Treatment of all

700 weaners on the farm with long-acting tetracyclines followed by vaccination of all age groups gave a rapid resolution of the outbreak.

Sheep

Thirty-six cases of **salmonellosis** were diagnosed throughout the Rangitikei, Wairarapa and Hawke's Bay regions in this quarter compared with 35 in the previous quarter. Persistence of disease into the late autumn and winter is unusual. Typical histories included diarrhoea, anorexia, depression and death. Postmortem findings included intestinal congestion, watery mucoid intestinal content and widespread haemorrhages. *Salmonella* Hindmarsh was cultured from the intestinal content of all affected animals. *Salmonella* Typhimurium was cultured from intestinal contents of three other ovine cases.

Necropsy of a ewe found dead showed a pericardial effusion and multifocal necrotic centres in the liver as well as adult liver fluke within bile ducts. Histologically there was marked disruption of the liver with an extensive area of fibrosis trying to wall off a central area of liquefactive necrosis associated with mineralisation. The histological findings supported a diagnosis of **Black disease**. *Fasciola hepatica* migrating through liver cause necrosis and induce an anaerobic environment. Toxins from sporulating *Clostridium novyi* kill the sheep.

A six-month-old Merino lamb developed ataxia before becoming paralysed. Examination of brain and spinal cord revealed a meningomyeloencephalitis involving the medulla oblongata, midbrain, colliculus, cerebrum and spinal cord. Characteristic neuropil necrosis and the presence of Gram-positive bacilli indicated **listeriosis** was the most likely aetiology. Extension of inflammation into the spinal cord is not commonly seen as most affected sheep present with circling, and only brain is collected.

Enteric listeriosis has occurred again this year in Canterbury sheep fed mouldy baleage. In one case, three weeks after the onset of enteric cases several ewes developed nervous signs and histological examination demonstrated lesions typical of **encephalitic listeriosis**. Poor quality baleage caused a number of small outbreaks of both enteric and encephalitic listeriosis throughout Otago and Southland. Most of the encephalitis cases did not exhibit the typical clinical signs of circling. Lateral recumbency, dullness or just 'found dead' were the major presenting signs.

Many deer farmers in southern New Zealand are diversifying into sheep because of the current poor returns for deer. A number of sudden deaths occurred in hoggets grazing on a deer farm in Southland. Necropsy of one animal showed a severely jaundiced carcass, very dark kidneys and a liver copper of 6000 nmol/kg confirming **chronic copper poisoning**. The farmer recalled that the paddocks on which the sheep were grazing had been topdressed with copper for the benefit of the deer on the farm.

Two of 60 Texel hoggets on a small holding in Southland were

presented with a hind limb ataxia that rapidly progressed to paralysis. A necropsy of one showed no gross findings but histological examination of the spinal cord and brain showed a mild lympho-plasmacytic meningoencephalitis and peripheral neuritis affecting the peduncles and all levels of the white matter tracts of the cord and extending into the spinal ganglia and peripheral nerves. No infectious agent was detected but the clinical signs and histological findings were similar to those reported in Britain in lambs with a presumptive *Sarcocystis* species infection and in the case reported the lambs were grazing pasture heavily contaminated with the faeces of foxhounds.

Goat

Culture of stomach contents from a goat foetus from Hikurangi revealed a heavy growth of *Bacillus licheniformis*. This agent is an uncommon cause of abortion, although the pathogenesis and lesions in pregnant cattle are well described (Agerholm JS, Jensen NE, Dantzer V, Jensen HE, Aarestrup FM. Experimental infection of pregnant cows with *Bacillus licheniformis* bacteria. *Veterinary Pathology* 36, 191-201, 1999).

Pigs

A Canterbury pig farm with 500 growers had a sudden increase in the number of deaths (up to 18 per week) and clinical examination revealed a wide discrepancy in the size of the pigs. Necropsy and histological examination of several pigs revealed severe lesions of **intestinal adenomatosis**.

Four four-week-old piglets from a single litter died over three to four days on a property at Ruawai in Northland. No gross lesions were seen at necropsy. The piglets had vomited before death. Histological examination of liver revealed extensive hepatocytic necrosis, with hepatocyte apoptosis, megalocytosis, karyomegaly, diffuse infiltrates of pleomorphic lymphoid cells, aggregates of neutrophils, and portal dissecting fibrosis. Numerous botryoid inclusion bodies were present. There was an erosive, necrotising gastritis in the fundus of the stomach. Mononuclear cells were present in the submucosa and muscular coat along with small numbers of botryoid inclusions. No lymphoid tissue was provided. Type IV **circovirus II infection** was diagnosed. Type IV infections are considered to be a late event in the pathogenesis of post-weaning multisystemic wasting syndrome (PMWS). This is the only example of type IV lesions seen so far in this country. The stomach lesions have not been reported previously in this country, but are apparently common in affected piglets in Canada.

Dogs

A litter of two-week-old St Bernard pups was experiencing continuing deaths. Of eight pups born, four died in the first few days. One survivor was twice the size of the others and growing well. The other three were presented to a veterinary clinic in respiratory distress and eventually were euthanased on humane grounds. At necropsy of one, abundant clear fluid poured from the

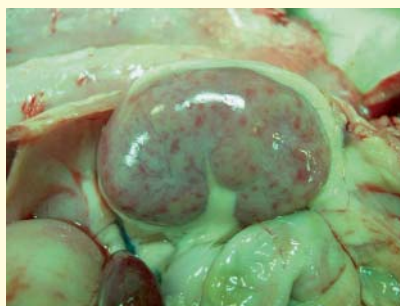


Figure 1: Haemorrhages on the surface of the kidney

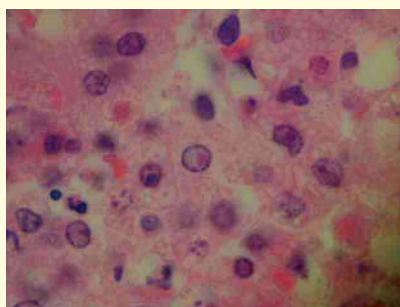


Figure 2: Adrenal cortical cells show eosinophilic intranuclear bodies marginating chromatin to the periphery of the nucleus

nose. The lungs were pale and firm with diffuse oedema and foamy fluid leaked from the cut surface. Both kidneys had multifocal random 1-3 mm haemorrhages on the cortical surface (see Figure 1) and severe multiple haemorrhages at the corticomedullary junction. Numerous ascarid nematodes filled the duodenum. Histological examination revealed multiple foci of necrosis in the renal and adrenal medulla and cortex. Glomeruli were often necrotic. Eosinophilic intranuclear bodies marginating chromatin to the periphery of the nucleus (Figure 2) were visible in adrenal cortical cells. In the lung multiple foci of acute coagulative necrosis mixed with fibrin were scattered through the alveoli, within alveolar walls, adjacent to bronchi and involving bronchioles. A single focal area of necrosis was visible in the granular layer of the cerebellum with individual cell necrosis and a light infiltrate of neutrophils. **Canine herpes virus** is widespread in the canine population, usually only causing disease in pups less than three weeks of age. Often disease develops if the pups become chilled. About a week later a litter of Huntaway pups also experienced losses confirmed as herpes virus.

A six-year-old female Labrador showed progressive muscle wasting and ataxia. Another dog had died the previous day. Serum biochemistry revealed elevated CK, AST and ALT and a stress hyperglycaemia. This would be consistent with **monensin toxicity**. Ionophores disrupt enzymes affecting transport of sodium, potassium and calcium across cell membranes leading to excessive uptake of calcium by the mitochondria, mitochondrial damage and ultimately muscle necrosis. Another possible diagnosis is MCPA (herbicide) toxicity.

A two-year-old female Huntaway-cross dog showed signs of depression. Haematology revealed a mild lymphocytosis, an eosinophilia, a hyperkalaemia, hyponatraemia (a ratio of 16.8.), azotaemia, hypercalcaemia and other changes. Taken together the finding were consistent with **Addison's disease**, for which an ACTH stimulation test is required for confirmation. The other possible differential is acute renal failure.

A mature farm dog was found dead and presented for necropsy.

Nothing was noted grossly but on histology the liver had moderate hepatocellular vacuolation of the periportal cells. The vacuoles were crisply edged consistent with lipid. On this basis, **phosphorus toxicity** was suggested. Tests of the fresh liver showed a level of 2100 mg/kg.

A five-month-old King Charles Spaniel was sick for two weeks with swollen submandibular lymph nodes, head tilt and incoordination. There was no response to antibiotics or steroids. Toxoplasma antibody titre was 1:4096. A few weeks later the titre had fallen to 1:2048. The neurologic signs persisted and wasting of the masticatory muscles developed. The pup was euthanased. At necropsy there were ulcers along the lateral aspect of the left side of tongue and left lip, severe atrophy of facial muscles with severe prominence of zygomatic arch. The lungs were rubbery and heavy with lots of foam in the trachea and bronchi. Protozoal cysts in the brain and heart were consistent with *Toxoplasma*, and malacia and demyelination in the brain was consistent with **distemper** infection.

Cats

An eight-year-old domestic long-haired cat from the Hutt Valley presented with a subdermal mass on the bridge of the nose. A fine needle aspirate revealed numerous degenerate neutrophils and large active macrophages. A ZN stain revealed dense clusters of mycobacteria. The pattern was not typical of cat leprosy (*Mycobacterium lepraemurium*), so an **atypical mycobacterial infection** (eg *M smegmatis*, *M fortuitum*) is likely. Culture is needed to differentiate the species of mycobacteria.

A nine-year-old castrated DSH cat in the Lower Hutt region was presented with a swelling on the nose and an ulcerated lesion on the lip. The lip lesion contained many neutrophils, small numbers of active mesenchymal cells and numerous *Cryptococcus* organisms. The nose lesion contained many mesenchymal cells, some neutrophils but only two suspect organisms were seen in three smears. This was probably an early lesion, or possibly the main infection had not been sampled.

A one-year-old male cat was treated for a suppurative lesion/cellulitis over the tail base and was re-examined six days later. There was swelling around the tail base, the tail appeared paralysed and blood was leaking from the nose and anus. The cat was febrile and jaundiced but not anaemic. However, there were marked increases in prothrombin time (PT), activated partial thromboplastin time (APTT) and thrombin time (TT) and a thrombocytopenia. Longer standing inflammation was indicated by a neutrophilia and monocytosis and toxic changes to neutrophils. Fine needle aspirates of a lymph node revealed a reactive lymphadenopathy. Biochemistry revealed elevated ALT, AST, and bilirubin. This is most likely a case of **disseminated intravascular coagulation** (DIC), secondary to septicaemia from the cellulitis around the tail base. An alternative diagnosis is severe liver disease or insufficiency leading to a lack of clotting factor production including fibrinogen and hence the marked increase in TT. The increased ALT may indicate primary hepatocellular damage but DIC and the formation of microthrombi may also have caused hepatic damage and increased liver enzymes. DIC is rarely diagnosed. Further tests include fibrinogen degradation products and bile acids to examine liver function further.

A mature Birman cat from Taupo fell ill suddenly and died shortly afterwards. At necropsy there was enlargement of most mesenteric nodes, which were suppurative and necrotic. The liver was also enlarged. Histology revealed acute necrosuppurative lymphadenitis and subacute cholangiohepatitis with microabscessation. The findings raised suspicion of salmonellosis, and culture turned up a heavy growth of *Salmonella Typhimurium*. Epidemiologically this was suspected to be type 160 associated with the salmonellosis of sparrows, but testing revealed it to be type 135.

Other

A daily death rate of 5% in a duck hatchery was investigated. Grossly affected birds had pericarditis and meningitis. *Pasteurella multocida* was cultured from the brain of two affected ducklings.

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Quarterly report of investigations of suspected exotic diseases

Exotic disease investigations are managed and reported by MAF's National Centre for Disease Investigation (NCDI). The following is a summary of investigations of suspected exotic disease during the quarter from April to June 2004.

Vesicular diseases ruled out

A veterinarian reported oral lesions in the mouth of a calf that had been salivating over the previous two days. The veterinarian described full epithelial thickness erosions on the hard palate and buccal mucosa, with no evidence of epithelial tags. Three other calves in the same paddock on the dairy farm were not affected. An AgriQuality investigating veterinarian ruled out exotic vesicular disease on clinical and epidemiological grounds. Blood testing for bovine virus diarrhoea (BVD) was negative for both antigen and antibody. The calf was treated with antibiotics and recovered within days.

A veterinarian reported a steer that had lost weight was scouring and had ulceration on the dental pad, hard palate and nostrils. It was one of a mob of 80 brought down from the back of the farm a week previously, when it was first noticed sick. No other animals in the mob were affected, nor other animals on the farm. A bull purchased about ten days previously was the only recent introduction to the farm, but it had had no contact with the mob of steers. Viral vesicular diseases were ruled out on clinical and epidemiological grounds. The farm had a history of BVD but blood tests were negative for both antigen and antibody. The condition resolved with antibiotic treatment, suggesting a bacterial cause.

Transmissible spongiform encephalopathy ruled out

The cases reported in this section are the more significant suspected transmissible spongiform encephalopathy investigations for the quarter. Numerous deer brains were examined, mostly from animals with illthrift. Two cases with inflammatory cell infiltrates of occasional blood vessels were seen, suspicious for, but not completely characteristic of, malignant catarrhal fever, and no neurological signs were reported. One other case, with generalised white matter spongiosis at the prescribed brain examination sites, was referred to MAF referral pathologist Dr Alastair Johnstone, who confirmed the lesion and considered it not suspicious for TSE but of unknown aetiology. Transmissible spongiform encephalopathy was ruled out in each case.

Twenty brains were examined from sheep with no neurological signs as part of a project with a major corporate farm. Scrapie was ruled out in each case.

Bovine brucellosis ruled out

Two bulls tested positive for *Brucella abortus* on the complement fixation test (CFT) during pre-export screening. ELISAs and *Brucella abortus* card tests were negative. The bulls were re-bled one month later and CFT was still positive for one animal. Three semen

samples collected from this bull on different occasions were negative for *Brucella abortus* on polymerase chain reaction (PCR) tests and bacteriology. The initial positive results were possibly caused by the low specificity of the CFT and cross-reactions with *Yersinia* or *E coli* species.

Equine viral arteritis and equine infectious anaemia ruled out

A Levin veterinarian submitted a serum sample to a New Zealand Veterinary Pathology laboratory to rule out exotic diseases after examination of a two-year-old horse with pitting oedema, affecting the ventral abdomen and limbs. The horse was lethargic and in poor body condition. The rectal temperature and mucous membrane colour and perfusion were normal. Serum biochemistry showed evidence of anaemia, hypoproteinaemia and liver damage. Equine infectious anaemia (EIA) gel diffusion tests and equine viral arteritis (EVA) serum neutralisation tests were negative. The hepatitis was treated with antibiotics and dietary management, and the ventral oedema resolved within three weeks.

A Palmerston North haematologist reported a case of anaemia and limb oedema in a ten-year-old home-bred gelding. The horse was dull with a reduced appetite, was pyrexia and had mild upper respiratory involvement. No other horses on the establishment were unwell. Routine haematology revealed a mild anaemia and an inflammatory leucogram, with normal biochemistry. Acute and convalescent blood samples taken eight weeks apart were negative for EVA and EIA using the virus neutralisation and gel diffusion tests, respectively. Haemoparasites were ruled out after negative blood film and molecular screening techniques. The horse made a full recovery within a few days. The horse is likely to have suffered a mild form of purpura haemorrhagica secondary to bacterial infection.

A pathologist phoned the 0800 number with a case of severe unresponsive anaemia in a horse that had polyuria and polydipsia, indicating a possible renal cause of bone marrow suppression. Paired sera tested negative for EIA on gel diffusion. The anaemia continued to worsen, precluding the horse from a racing career. After euthanasia, a necropsy at Massey University showed a large adrenal gland tumour, diagnosed as a histiocytic sarcoma, as well as secondary liver disease.

A veterinarian advised NCDI that the owners of a horse on a property investigated by MAF in 2000 had elected to euthanase the horse because of ill health. The previous investigation had ruled out exotic equine diseases, and this case was one of several given the

syndromic descriptor 'trumpet nose'. No aetiology for the condition has yet been ascribed. NCDI staff took the opportunity to investigate the animal in order to characterise the condition further. The horse was euthanased on farm and transported to Gribbles Alpha laboratory in Hamilton. Necropsy showed significant pathology involving and confined to the upper respiratory tract and draining lymph nodes, including severe sinus empyema; multifocal severe chronic rhinitis, pharyngitis and laryngitis; and chronic oedema of lips. These findings were confirmed by histology, which also revealed chronic fibrinopurulent cellulitis in the submandibular region, and chronic glomerulonephritis. The horse was seropositive to equine herpesvirus types 1 (weak) and 4 (strong). No viruses were isolated from any tissues. PCR assays on tissues including trigeminal ganglion, submandibular lymph node, and lung were positive for EHV types 2 and 5. Some tissues gave suspicious bands for EHV type 1. All were negative for EHV type 4 and equine adenovirus types 1 and 2. The initiating cause of the condition remains uncertain.

Taenia saginata investigated

A MAF Verification Agency veterinarian visited a Hawke's Bay property and conducted an interview to determine the possible origin of bovine cysticercosis in slaughtered cattle. Bovine cysticercosis is caused by *Cysticercus bovis*, the metacestode intermediate of *Taenia saginata*. The farm paddocks had been used by campers, who could have been a source of *Taenia* eggs, the infectious stage ingested by cattle from pasture. This report continues the investigation of a small cluster of bovine cysticercosis cases within this geographical region in the last 20 months.

In a separate but possibly related incident, a dairy cow was diagnosed at slaughter with bovine cysticercosis. An epidemiological examination revealed the animal had grazed near where foreign farm workers had been harvesting squash. Links between this team of workers and previous bovine cysticercosis case farms were tentatively established, suggesting that immigrant agricultural labour may be a pathway for *Taenia saginata* entry into New Zealand. Health screening, treatments and provision of appropriate field toileting facilities are biosecurity risk mitigation steps that should be considered.

Trichinella spiralis ruled out

A farmer contacted the NCDI with a request for testing for trichinellosis of pigs going for slaughter. The farm was under Restricted Place notice in 2001 when pigs were found to be infected with *Trichinella spiralis*. Specimens collected from three weaner pigs by AgriQuality and tested by Gribbles Veterinary Pathology were all negative on both histology and pepsin digest.

Brucella suis ruled out

Fifteen percent of pigs in a fattening unit failed to thrive. Animals examined at necropsy on two separate occasions showed severe lymphadenitis but no other gross pathology. There was potential for contact between wild and domestic pigs. *Brucella* PCR was

negative on lymph node tissues and EDTA blood. The ELISA for antibodies to *Brucella* species was negative on 25 serum samples. Histopathology was negative for PMWS. Virus isolation failed to detect pestiviruses. Most pigs made a full recovery and morbidity rates returned to normal. No diagnosis was established.

Brucella canis ruled out

A veterinary practitioner in Hunterville was presented with a five-year-old Huntaway dog suffering from a painful and enlarged testicle. The testicle was removed and *Brucella canis* ruled out on histology, which showed epididymal haemorrhage and fibrosis consistent with a sperm granuloma. This was most likely the result of trauma to the testicle leading to leakage of sperm into connective tissue and subsequent fibrosis. Bacteriology showed mixed growth including *E coli* and *Staphylococcus*. No *Brucella canis* was isolated. *Brucella canis* was excluded by a negative card agglutination test on a five-year-old Huntaway dog that had presented with an inflamed painful testis and mild pyrexia.

Exotic ticks identified

Three engorged brown dog ticks (*Rhipicephalus sanguineus*) were found in the pet transport area of Christchurch airport moments after a dog imported from Perth, Australia had passed through the area. A MAF Quarantine officer visited the dog at the residential address and found four more ticks on the animal. A veterinarian checked the dog for more ticks the following day and treated it with an acaricide. No further ticks were found at three monthly veterinary examinations and treatments. A PCR assay on the dog's blood one month later ruled out tickborne diseases including *Babesia gibsoni* and *Babesia canis*.

A forest researcher went on a training course, which included a fieldtrip, in the USA. On his return he found a tick attached to his leg. The tick was identified as a lone star tick, *Amblyomma americanum*, an exotic tick widespread in North America.

Newcastle disease and avian influenza ruled out

Avian influenza was ruled out by postmortem, histology and negative virus isolation in two turkeys that became ill and died within a 24-hour period. The turkeys were in poor condition and had rhinitis. No further deaths were observed over the ensuing three weeks. Avian influenza was considered as the turkeys were in contact with wild ducks.

Ten of 30 backyard pheasants died over a 24-48 hour period. All of the birds were found dead and subsequently buried, except for one that showed neurological signs including walking backward before it died. It was submitted to the regional laboratory for postmortem examination and sample collection for virus isolation and bacteriology at NCDI. Postmortem findings were unremarkable. Samples were passaged three times in embryonated chicken eggs and tested for haemagglutinating activity after each passage, with negative results. Microbiology yielded heavy mixed growth from all

organs but no significant isolates. No cause was determined, although possible aetiologies include Marek's disease and toxins.

Avian influenza and Newcastle disease were ruled out as the cause of sudden death in seven of 11 backyard chickens. The birds showed signs of polydipsia and anorexia just prior to death. A poultry specialist visited the property and examined the dead birds. The crops were distended with green, red and purple fluorescent watery material. The owner explained that the birds had been fed 'play-dough' the evening before the deaths. A presumptive diagnosis of salt poisoning was made.

IBD seropositivity investigated

Ten sera from a poultry farm tested in the poultry industry Infectious Bursal Disease (IBD) Eradication Scheme were submitted to NCDI by the Poultry Vet Services Laboratory after two had tested positive using the IBD IDEXX ELISA. The ten sera were tested with the virus neutralisation test (IBD VNT), and the two ELISA-positive sera returned test values of $>1:128$ and $\leq 1:32$. An AgriQuality veterinarian visited the farm and found no clinical evidence of virulent IBD. A 30-week-old bird submitted for postmortem showed no evidence of bursal atrophy. Three months later each flock on the farm was sampled. Of 135 birds tested, 12 were positive on the IDEXX ELISA. Of 72 submitted to IBD VNT, 69 had titres of 1:8 or less, there were two titres of 1:16, and one of 1:32. This low seroprevalence several months after the initial test was considered strong evidence that infection with virulent IBD had not occurred. The cause of the serological titres to the IDEXX ELISA and IBD VNT is not known. Previous studies at NCDI have estimated the specificity of the IDEXX ELISA when applied as a diagnostic test to New Zealand poultry as 87.5% (52 positives from 364 sera from IBD-negative flocks), indicating the results from this investigation are within the expected performance of this test applied in these circumstances. The IBD VNT is considered highly specific, although no definitive cut-off point has been established. In the same NCDI study, all 364 sera from IBD-negative flocks had very low titres ($<1:2$ up to 1:8) (Stanislawek W, unpublished).

Libyostrongylus douglassii confirmed in ostriches

The nematode *Libyostrongylus douglassii* was identified in ostriches on a farm in Reporoa. Suspicion was raised after screening of samples by Gribbles Alpha, and Phil McKenna, Gribbles Veterinary Pathology Palmerston North, confirmed the identification by larval culture and adult morphology. This is the first identification of this organism in New Zealand. A severe clinical picture with high morbidity and mortality was observed on the farm. The parasite is a well known pathogen of commercial significance overseas, where it may occur in dual infestations with *Libyostrongylus dentatus*. Dual infestations have not been detected in this investigation. Early indications are that the infestation has been present for many years, with opportunity to become widely distributed. A second farm, in

Hawke's Bay, associated with the index case by bird movements has been confirmed positive for the parasite. Infestation has also been diagnosed on a third farm, in Bay of Plenty. Diagnosis can be by larval culture of faeces or from fresh and formalin fixed proventriculus collected at postmortem or slaughter. The hardiness of the organism and the belief that it is already widespread have kept response options in the ostrich farming sector to the provision of advice on farm surveillance and biosecurity, and measures to break the lifecycle and prevent build up of heavy environmental contamination. Concerns about a hypothetical but unknown threat to kiwi remain, and MAF is consulting Department of Conservation on this issue.

In a separate investigation, a Fairlie ostrich farmer reported symptoms in his flock consistent with *Libyostrongylus* infestation. The farm operates a hatchery that is also used by Department of Conservation and private aviculturalists. Faecal egg counts carried out by the MAF reference pathologist on samples from three adult ostriches were all nil, indicating no evidence of infestation in these birds.

Psittacine poxvirus ruled out

A Whangaparaoa veterinarian presented with a featherless lorikeet phoned the 0800 number to report suspected psittacine poxvirus. The bird was submitted to an Auckland laboratory for necropsy and collection of specimens for histology and virology. The provisional diagnosis of psittacine beak and feather disease was confirmed by histology and PCR testing at Massey University. There were no lesions of psittacine poxvirus seen at postmortem or on histology. Psittacine beak and feather disease is caused by a circovirus endemic in captive parrots in New Zealand but has only recently been found in wild parrots. The potential impacts of psittacine beak and feather disease on New Zealand's wild native parrots – kaka, kea, kakariki and kakapo – are not known.

Red blood cell inclusions in a North Island robin

A pathologist reported piroplasm-like structures in a blood smear from a North Island robin (*Petroica australis*). The sample was collected as part of a surveillance programme conducted by the Department of Conservation. An avian specialist examined the sample and found the erythrocyte parasites were most likely to be the gametocytes of *Haemoproteus* spp. *Haemoproteus* are generally considered to have low pathogenicity although they have been reported to cause haemolytic disease in pigeons and quail.

European foulbrood ruled out

A member of the public reported sudden death of hives, and suspected European foulbrood. An experienced beekeeper found that both hives were dead with few bees left but the brood showed no evidence of foulbrood. The hives had not been treated for varroa and were located in an area where varroa infestation has been previously reported. Infestation with varroa was considered the cause of the hive deaths.

Varroa in the South Island investigated

A beekeeper reported finding an unusual mite in a brood frame. The apiary was located at Pleasant Point in the South Island, an area not known to be affected by varroasis. The mite was sent to the MAF National Plant Pest Reference Laboratory (NPPRL) for identification. The mite was a female pollen mite – *Melittiphis alvearius*. Pollen mites are found on the European honeybee, *Apis mellifera*, or in its hives. The mite is not a parasite or predator but rather a scavenger of pollen in the hives. This mite has been previously recorded in New Zealand.

A Westport beekeeper found mites in an apiary and suspected *Varroa destructor*. He contacted AgriQuality, who in turn contacted MAF. NPPRL staff identified *Melittiphis alvearius*, a non-parasitic mite.

A *Varroa destructor* mite was identified on a sticky board submitted by a beekeeper in Oxford, North Canterbury. Testing was undertaken within the MAF exotic bee disease surveillance programme. Movement controls were imposed and surveillance in spatially associated and contact apiaries undertaken. Within a few days, a further varroa mite was found by the laboratory, this time on the outside of a sealed bag containing a sticky board from an apiary in Murchison. The two samples were unrelated except that both were read at the same MAF laboratory. Movement controls were imposed and surveillance undertaken around the Murchison apiary. By mid-July, no further mites had been detected despite substantial surveillance. Laboratory cross-contamination has been

accepted as the likely explanation for the Murchison result. The source of the mite in Oxford has not yet been identified.

Exotic fish viruses ruled out

Approximately 80 dead porcupine fish (*Allomycterus jaculiferus*) washed up on Wellington beaches. The condition of the collected tissues did not allow histopathological interpretation. Pooled tissue virus isolation was negative on two separate occasions, ruling out infectious haematopoietic necrosis and infectious pancreatic necrosis. A possible explanation is dumping of by-catch from a fishing vessel.

Brucella abortus seropositivity in Hector's dolphin

The Hector's dolphin is the rarest dolphin in the world and is only found off the coast of New Zealand. The opportunity to undertake a range of serological and PCR tests in three dolphins arose during satellite tracking research. Serum was collected and tested for *Calicivirus* (negative), *Brucella abortus* (one positive) and influenza virus (negative). Seropositivity to the *Brucella abortus* ELISA was observed in one dolphin. PCR for *Brucella* spp will be attempted to clarify the interpretation of this result.

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