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Investigation into infectious bursal disease seropositivity on two commercial free-range layer properties

An investigation of seropositive results to an infectious bursal disease ELISA on samples from free-range layer poultry from two properties found no clinical, pathological or molecular evidence of infectious bursal disease. A cross-reacting non-virulent infectious agent was considered the most likely cause of the seropositive results.

Infectious bursal disease (IBD) is an acute, highly contagious viral infection of young chickens⁽¹⁾. In 1993, a mild strain of IBD virus was introduced into New Zealand⁽²⁾. The relatively small proportion of infected farms, and the confirmed presence of a strain of low pathogenicity, stimulated the Poultry Industry Association of New Zealand and the Egg Producers Federation of New Zealand to propose a Pest Management Strategy under the Biosecurity Act 1993⁽³⁾. The poultry industry subsequently eradicated infection from all known positive farms and the last flock seropositive to IBD was detected in January 1999⁽⁴⁾.

The poultry industry currently operates a 'Country Freedom Quality Plan' for IBD surveillance and flock accreditation. Only exotic strains of IBD are notifiable in New Zealand under the Biosecurity Act 1993. As it is impossible to distinguish clinically, pathologically or serologically between exotic low-virulence strains of serotype 1 infectious bursal disease virus (IBDV) and the strain previously seen in New Zealand, all suspect cases of IBD have to be reported to the MAF Investigation and Diagnostic Centre (IDC). The IDC has previously investigated two flocks in which seropositives were detected by the industry scheme⁽⁵⁾⁽⁶⁾. Both flocks were free of IBDV.

In March 2005, Poultry Vet Services, one of two laboratories that perform the ELISA screening (FlockChek®, IDEXX Laboratories) for the industry IBD surveillance programme, reported ten seropositive results from a sample of ten birds on each of two properties. Both properties were free range layer operations located in the same region. Both properties had given a clear test under the industry scheme in November 2003. Epidemiological investigation determined that the two properties purchased birds from the same contract rearer. There were disease conveyor links between the two properties as they had shared equipment in the past.

Cross-sectional survey

To verify the ELISA results and quantify the seroprevalence, 60 additional blood samples from each property were submitted to the IDC for Virus Neutralisation Testing (VNT)⁽⁷⁾. As the VNT has no designated cut-off, an arbitrary titre of 1:16 ($\log_2 4$) was chosen based on previous IDC investigations⁽⁵⁾⁽⁶⁾⁽⁸⁾. The sample from property A showed a seroprevalence of 0.21 (95% confidence interval 0.11, 0.31; binomial approximation) and property B a seroprevalence of 0.25 (0.14, 0.36). The distribution of titres is shown in Figure 1. The median titre for property A was 1:96 and

property B was 1:128. Stratification of results by shed demonstrated that the seropositive birds were almost exclusively confined to a single shed on each property, with a prevalence of 0.6 (0.39, 0.81) in the single shed on property A, and 0.74 (0.54, 0.93) in the shed on property B. Both sheds contained the youngest birds on the properties: 26-week-old and 20-week-old layers, respectively.

Both farms received 16-week-old birds from the same contract rearer. This property was investigated and 30 birds bled from each shed including the sheds known to have supplied the layer properties under investigation. All sheds were found to be seronegative for IBD.

As all birds on both farms were over 16 weeks old, direct IBD antigen detection or virus isolation was considered to have low sensitivity in determining farm disease status. A sentinel bird investigation was therefore undertaken.

Sentinel bird investigation

The aim of the sentinel bird investigation was to determine whether IBDV was present in the sheds containing the youngest birds on both farm A and farm B.

Sample size determination

Sentinel sample size was calculated using FreeCalc, version 2 (software developed by Cameron 1998) and was based on a sensitivity of antigen detection set at a conservative 70% and a prevalence of 20% infected birds. Type 1 and type 2 errors were both set at 5%. This calculation assumes random mixing of the sentinel broilers with the existing population in the shed. This assumption was violated as the sentinel broilers had to be housed six or seven to a cage for welfare reasons (Figure 2). To overcome this limitation the sample size chosen was aimed at ensuring the sentinel

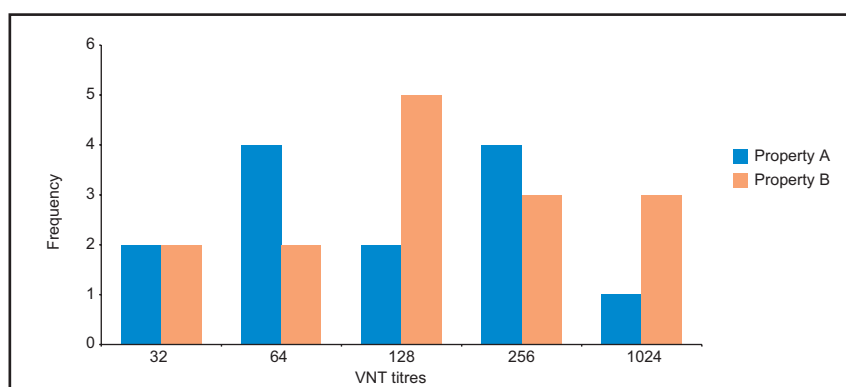


Figure 1: VNT titres from seropositive layer birds from properties A and B (sampled before the sentinel bird investigation)

birds became an accurate representation of the existing bird population, and there was effective contact between the birds and any virus present.

Effective contact

IBD virus persists well in litter⁽⁹⁾ so infectivity of litter would be high. Effective exposure to litter and fomites was maximised by placing the cages in direct contact with the flock birds, and use of floor feeding of the sentinels. Environmental contact was enhanced by eye-dropping the birds on two occasions with a suspension of litter (50 g) in antibiotic broth (100 ml). The eye-drop method was chosen as it is the suggested method for the infection of three- to five-week-old susceptible chickens in the preparation of agar gel precipitin positive control antigen⁽¹⁰⁾.

Each of the two affected sheds contained approximately 400 layer birds. Twenty 20-day-old broilers were placed in each shed and ten additional broilers were placed with two small groups of Muscovy ducks (12 birds) and guinea fowl (two birds) and pet Seabright bantams (three birds) on property B. These birds would have fallen outside the industry IBD testing scheme.

Sampling and identification of sentinels

Prior to being moved to the farms, the sentinel broilers were sampled and tested for IBD using both the ELISA and VNT. Each sentinel bird was identified by a wing tag (Allflex® button tag).



Figure 2: Sentinel birds housed six to a cage showing close contact with flock birds

Six days after placement in the affected sheds, five sentinel birds from each shed were blood sampled, killed by cervical dislocation and necropsied. Samples of cloacal bursa, thymus, spleen, intestine (with lymphoid tissue), liver, kidney and skeletal muscle were fixed for histopathological examination. Fresh bursa, spleen and caecal tonsil were collected into viral transport medium for RT-PCR and virus isolation.

After 21 days, repeat blood samples were taken from the remainder of the sentinels for serology and the birds were killed and necropsied. Samples were again taken for histology, RT-PCR and virus isolation.

Additional sampling of layer flock for RT-PCR

A sample size of 60 layer birds from each of the two affected layer sheds was calculated using FreeCalc, version 2. This assumed a prevalence of 5% shedding birds, that RT-PCR has a 95% sensitivity and 100% specificity, alpha and beta errors set at 5%. Cloacal swabs were taken from the birds for IBD antigen detection using RT-PCR. The swabs from each shed were pooled in lots of ten, and placed in transport media.

Results

None of the sentinel birds on property A showed clinical signs attributable to IBD during the observation period.

On property B, two birds died in the second week after being pecked by a layer that broke into their cage. Some of the birds also suffered what appeared to be an outbreak of coccidiosis, and the birds were treated with toltrazuril (Baycox) at 1 ml/litre drinking water on days 7, 8, 18 and 19. Despite this, two birds died on day 20, and the balance of the sentinel birds housed in the layer shed on property B were in poor body condition after 21 days. It is believed that this was also caused, in part, by suboptimal performance of the feeding system. The poor condition of the birds was reflected in the size of their bursas and thymuses, which were poorly developed in most birds. The sentinels housed with the ducks, Seabrights and

Table 1: Summary statistics from sentinel bird ELISA readings on day 21 sampling

		Number	ELISA(positive)	Range S/P	Median S/P	Mean S/P
Property A		15	2	0.05-0.24	0.13	0.14
Property B	Housed with layers	11	7	0.06-0.67	0.24	0.29
	Housed with non-layers	5	1	0.03-0.31	0.05	0.12
	Overall	16	8	0.03-0.67	0.2	0.24

Table 2: Bursal sizes at necropsy

			Bursameter values - range	Mean Bursal size
Farm A		Day 6	4-6	5
		Day 21	4-6	5
Farm B	Housed with layers	Day 6	5-6	5
		Day 21	3-4	3
	Housed with non-layers	Day 6	4-5	5
		Day 21	5-6	5
Overall		Day 6	4-6	5
		Day 21	3-6	4

guinea fowl on property B were in good body condition.

Serology

Serological tests used were the FlockChek® ELISA (IDEXX Laboratories, USA) and VNT⁽¹⁰⁾. All sera collected from the sentinel birds at day 0 (entry) and day 21 were tested with both assays. At the six-day sampling the sentinel birds were tested with the ELISA only and all were negative at an S/P ratio of 0.2, the manufacturer's recommended cut-off. At 21 days, when the remaining sentinel birds were sacrificed, 13% (2/15) of sentinel birds from property A, 64% (7/11) of sentinel birds in the layer shed from property B and 20% (1/5) of sentinel birds from property B placed with the Muscovy ducks and guinea fowl had seroconverted on the ELISA.

At the 21-day sampling the entire population of sentinel birds showed an increase in ELISA values (Figure 3). There is a statistically significant difference in the ELISA values between initial and 21-day samples ($P=4.3 \times 10^{-8}$) calculated for sentinel birds housed in the layer sheds. There was no significant difference between farms. The median S/P value at 21 days was 0.13 for property A and 0.2 for property B, the range of S/P values was positively skewed for property B. Table 1 shows the day 21 ELISA summary statistics for all sentinel birds, including those in the layer sheds and those placed with the ducks and guinea fowl on property B.

Sera collected at day 0 all had titres of <1:2 to the VNT. Day 21 VNT results showed a unimodal distribution of low titres, which were positively skewed for property B (Figure 4). The median titres were 1:3 for property A, 1:8 for property B sentinel birds located in the layer shed and 1:4 for property B sentinel birds placed with the Muscovy ducks and guinea fowl.

RT-PCR

The RT-PCR utilises two pairs of primers designed to amplify the segment A gene. The assay was developed using virus isolates recovered during the first outbreak of IBDV infection in New Zealand⁽¹¹⁾. Sentinel bird tissues used were bursa, spleen and caecal tonsil collected at 6 and 21 days and pooled for each bird.

All RT-PCR results were negative. The result from the RT-PCR performed on the pooled cloacal swabs from the layer birds was similarly negative as was the RT-PCR performed on the eye-drop suspension.

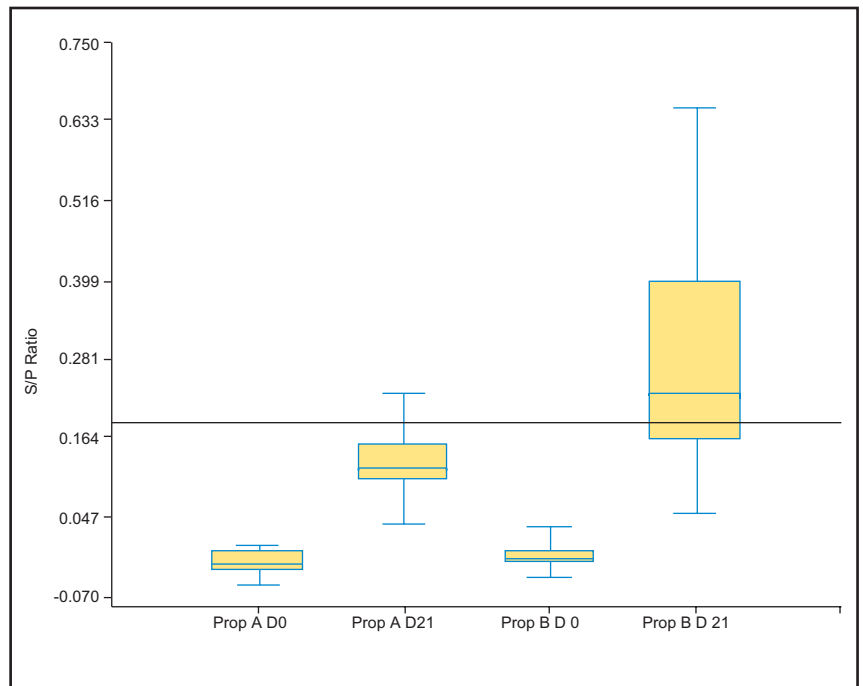


Figure 3: Paired (day 0 and 21) IDEXX ELISA S/P ratios for property A and property B. Horizontal line indicates cut-off at 0.2 (property B: sentinels housed with layer birds only)

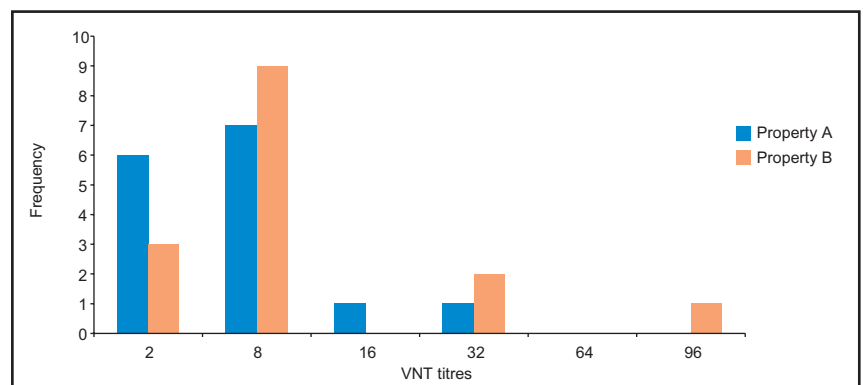


Figure 4: Day 21 VNT titres for sentinel birds on properties A and B (property B: sentinels housed with layer birds only)

Pathology

Bursal size was recorded at necropsy using a Bursameter (Solvay Animal Health) and values ranged from 3-6 across the two properties (Table 2). Each point on the Bursameter is 1/8 inch, therefore a bursa with a score of 4 has a diameter of 1/2 inch or about 13 mm. Due to feeding differences the condition of sentinel birds on property B was generally poor compared with property A and this was reflected in the size of the bursas and thymuses, which were poorly developed.

None of the acute, subacute or chronic lesions associated with IBD were recognised on histopathological examination of tissues from the sentinel birds.

Virus isolation

Sentinel bird samples were passaged three times in primary chicken fibroblast cells. No IBDV was isolated from any of the samples. This was confirmed by RT-PCR conducted using pass 3 material. Adenovirus was isolated from six samples and confirmed by electron

microscopy. Adenoviruses are frequently isolated from poultry in New Zealand.

Discussion

The aim of the investigation was to determine whether IBDV was present on farms A and B. Appropriately aged sentinel birds were introduced to the sheds so that presence of IBD antigen or live virus could be demonstrated using RT-PCR or virus isolation. Bursal tissue could also be collected to assess any characteristic pathogenic effects at necropsy and using histopathology.

Seroconversion in the sentinel populations indicates that the sentinel birds were effectively exposed to the same agent as the older layers. A degree of seroconversion occurred with both the ELISA and the VNT. The two assays showed moderate agreement to a *Kappa* statistic ($Kappa=0.55$) at the recommended cut-off (S/P ratio 0.2) for the ELISA and at a cut-off titre of 1:4 for the VNT. Selection of other cut-off points for the VNT reduced the level of agreement. Agreement between the assays beyond chance would strengthen the hypothesis that IBD is present on the two properties only if the two tests were independent. As both assays detect antibodies induced by group antigens at the VP2 (40KD) gene⁽¹⁾ cross reactivity is likely and therefore the tests are not likely to be completely independent. A single cross-reactive agent could then account for the low seropositive results that occurred in both assays.

The distribution of VNT titres found in the two layer sheds shows lower values than expected after field exposure to IBDV or vaccination. Antibody levels are normally very high, with VN titres of greater than 1:1000 common⁽¹⁾. Similarly, the 21-day samples taken from the sentinel birds show low ELISA S/P ratios. The median S/P value from property B is 0.24 and is very close to the cut-off; the median value from property A is 0.13, well below the cut-off. Clinical trials suggest that both the VNT and ELISA readings would be much higher at three weeks post exposure⁽¹²⁾⁽¹³⁾.

The isolate of IBD obtained from domestic poultry in New Zealand between 1993 and 1998 was of low virulence⁽¹⁴⁾⁽¹⁵⁾. However, gross and histopathological changes to bursas were obvious. The sentinel birds on properties A and B showed no histopathological lesions consistent with IBD at the 6 and 21 day sampling times. This effectively rules out the low virulence New Zealand IBDV. It does not rule out the possibility of an exotic non-pathogenic IBDV. However, RT-PCR was negative on all samples tested. Segment A genes are common to all types of IBD so this test would be highly sensitive. The presence of IBDV was therefore ruled out.

Conclusion

No clinical or pathological evidence of IBD was found on either of the properties as a result of this investigation. Molecular techniques and virus isolation did not demonstrate IBD virus or antigen. Epidemiological investigation demonstrated links between the two properties; this and the strong serological clustering within one shed on each property suggested the involvement of an infectious agent.

The high seroprevalence of low level titres to the VNT in the layer birds, and the low ELISA and VNT titres found on paired serology in the sentinel broilers, indicate that in this case the seropositivity results from a lack of test specificity. Biosecurity New Zealand determined that IBDV was unlikely to be present on either property A or property B and that the seropositive results were most likely due to a cross reacting non-virulent infectious agent.

References

- (1) Lukert PD, Saif YM. Infectious Bursal Disease. In: Saif YM (ed). Diseases of Poultry. Pp 161-79. Blackwell Publishing, Ames, Iowa, 2003.
- (2) Thompson J. Suspected exotic disease investigations. *Surveillance* 21(1), 9-10, 1994.
- (3) O'Neil BD. Chief Veterinary Officer's Annual Report 1995 - Disease and pest control. *Surveillance* 23(3), 15-9, 1996.
- (4) Ryan T, Leong R, Diprose B. Country-freedom plan for infectious bursal disease: a producer-led national disease control programme. *Surveillance* 27(4), 3-5, 2000.
- (5) Stone M. Quarterly report of investigations of suspected exotic diseases. *Surveillance* 31(3), 26-9, 2004.
- (6) Stone M. Quarterly report of investigations of suspected exotic diseases. *Surveillance* 31(4), 35-8, 2004.
- (7) OIE. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. Infectious bursal disease, part 2: Section 2.7.1, 2004.
- (8) Stone M. Quarterly report of investigations of suspected exotic diseases. *Surveillance* 30(3), 32-5, 2003.
- (9) Benton WJ, Cover MS, Rosenberger JK, Lake RS. Physicochemical properties of the infectious bursal agent (IBA). *Avian Diseases* 11, 438-45, 1967.
- (10) OIE. Terrestrial Animal Health Code. Infectious bursal disease 2: 817-832, 2004.
- (11) Tham KM, Young LW, Moon CD. Detection of infectious bursal disease virus by reverse transcription-polymerase chain reaction amplification of the virus segment A gene. *Journal of Virological Methods* 53, 201-12, 1995.
- (12) Marquardt WW, Johnson RB, Odenwald WF, Schlotthober BA. An indirect enzyme-linked immunosorbent assay (ELISA) for measuring antibodies in chickens infected with infectious bursal disease virus. *Avian Diseases* 24, 375-85, 1980.
- (13) Nicholas RAJ, Reed NE, Wood GW, Hebert CN, Muskett JC, Thornton DH. Detection of antibodies against infectious bursal disease virus: a comparison of three serological methods. *Research in Veterinary Science* 38, 189-92, 1985.
- (14) Motha J. Characterisation of infectious bursal disease viruses isolated in New Zealand. *Surveillance* 23(4), 26-7, 1996.
- (15) Chai YF, Christensen NH, Wilks CR, Meers J. Characterisation of New Zealand isolates of infectious bursal disease virus. *Archives of Virology* 146, 1571-80, 2001.

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Investigation of a case of human echinococcosis on the Chatham Islands

An investigation into the possible origin of a case of hydatids in a man from the Chatham Islands concluded that the infection was probably acquired before 1990 and that the Chatham Islands appear to be free of *Echinococcus granulosus*.

Echinococcosis is a zoonotic infection caused by adult or larval (metacestode) stages of cestodes belonging to the genus *Echinococcus* in the family Taeniidae. *Echinococcus granulosus* has a worldwide geographic distribution on all continents. A few islands are now free (Iceland, Greenland) or 'provisionally free' (New Zealand, Tasmania, southern Cyprus)⁽¹⁾.

An official campaign to control *E granulosus* was introduced in New Zealand in 1959⁽²⁾. The last outbreak involving fertile hydatid cysts was diagnosed in 1995 from sheep on Arapawa Island⁽³⁾ and New Zealand's 'provisional freedom' from hydatids was declared in 2002⁽⁴⁾.

The whole of New Zealand remains subject to a controlled area notice⁽⁵⁾, which states that:

- (i) The slaughter of ruminants and pigs at home killing facilities within the controlled area shall be conducted within a dog-proof enclosure in such a manner as to ensure that raw offal is not accessible to dogs.
- (ii) Owners shall control their dogs at all times in such a manner as to prevent them from having access to the raw offal of ruminants and pigs.
- (iii) The offal of ruminants and pigs shall be cooked by boiling for a minimum of 30 minutes before feeding to dogs within the controlled area.

All species of *Echinococcus* are notifiable organisms in New Zealand under the Biosecurity Act 1993. There is ongoing passive surveillance for metacestodes at slaughterhouses as all animals slaughtered for human consumption are inspected postmortem. Any suspected cyst is submitted to an approved regional laboratory and any confirmed case reported to Biosecurity New Zealand, Investigation and Diagnostic Centre (IDC) and an investigation initiated.

Humans are an aberrant or dead-end host in the life cycle of *E granulosus* as, under normal circumstances, they play no part in transmission of the parasite. Under the Health Act 1956, hydatid disease is an infectious disease notifiable to the Medical Officer of Health.

Case description

In September 2005, the Hawke's Bay Medical Officer of Health reported a case of hydatids in a 41-year-old farm worker undergoing treatment at Christchurch Hospital. Three interlinked cysts, of diameters 7 cm, 7.5 cm and 5 cm, were reported in his liver. The cysts were described as active based on magnetic resonance imaging carried out on two occasions, one month apart. During this time

there was enlargement of the cysts from a combined maximum dimension of 18 cm to 19.7 cm. There had been cyst rupture into the intrahepatic ducts. No calcification was reported. Microscopy confirmed the diagnosis as *Echinococcus granulosus* but ageing the cyst accurately was not possible. Descriptive histopathology was not undertaken.

Investigation

The IDC investigation first attempted to establish the date at which the patient was infected and determine whether this date was before or after the last recorded animal hydatids outbreaks in the early 1990s. *Echinococcus granulosus* cysts have a variable growth rate. An ultrasound study of 66 patients in Turkana, Kenya, categorised viable cysts as slow, moderate and rapid growing⁽¹⁾. Classification in a rapid or moderate growth category would place the New Zealand patient's infection date after 1995, whereas slow growth would place it well before this date.

Given the reported active nature of the cysts, and the possibility infection could have occurred after 1995, together with New Zealand's recent declaration of 'provisional freedom' from hydatids following a 40-year eradication campaign, an investigation was undertaken with the following objectives:

- establish a possible origin of infection,
- investigate any risk activities that could maintain an *E granulosus* transmission cycle,
- attempt to age the cysts more precisely.

A key question was if the parasite had survived the hydatids control programme, or if the parasite had been reintroduced by live animal imports, could a cycle of infection between dogs and sheep have been maintained on the Chatham Islands.

A patient interview was arranged to obtain an occupational history including exposure to dogs, and the risk factors that may have led to infection. Arrangements were made to have all available fixed tissues (no fresh tissues were available) from the patient submitted to a MAF reference pathologist, and a recognised expert in hydatids, for descriptive histopathology and an attempt to age the cyst.

Patient interview

An interview with the patient established that he had never been overseas and, except for three years on a farm in Taupo, had spent his entire working life on the Chatham Islands. The farm in Taupo, where he had worked between 1989 and 1992, was considered low risk as it had a regular dog worming programme and adequate procedures for the disposal of dead stock and offal during that

period. Since 1992 the man had worked on 32 farms on the Chatham Islands. On five, he had been employed for considerable periods of time. He had visited the other 27 farms for short periods when working as a shearing contractor. The listed properties also included his home property and immediate neighbours.

A questionnaire for each of the five main farms collected the following information: owner and property details; property type; dates of employment; animal demographic data; dog management, including whether the dogs could scavenge dead stock, were fed uncooked offal or were used for hunting; worming schedules; kennelling history; whether animals were slaughtered for home consumption and dog food; disposal methods for offal and dead stock; whether animals were sent to commercial abattoirs, and if so where. Basic data including owner details, animal demographics and dates of employment were collected for the 27 secondary properties.

All the properties identified had both dogs and sheep. There was a reported lack of compliance with the existing hydatids Controlled Area Notice on many properties.

Chatham Islands

The Chatham Islands lie 800 km east of the New Zealand mainland and consist of two inhabited islands – Chatham and Pitt – and many smaller outlying islands within a radius of 40 km. There are approximately 700 inhabitants, with 50 of those living on Pitt Island. Farm level demographic data are shown in the table. There are approximately 40,000 sheep on the islands (source: AgriBase). The islands have no licensed slaughterhouse and more than 20,000 sheep and lambs are sent to New Zealand each year (Lynch D, Ovis Management Ltd, personal communication) through the ports of Timaru and Napier. Most are sold through saleyards as store sheep, as the loss in condition that results from the four-day sea voyage means they are unsuitable for immediate slaughter.

Animal demographic data for the Chatham Islands				
	Farms with sheep	Farms identified by patient with dogs and sheep	Dogs registered (main island and Pitt Island)	Dog numbers on identified farms
Totals	47	32	456	208

Targeted surveillance

Because of the reported viability of the patient's cysts, the uncertainty around their aging, the possibility that the date of infection could have been after the last recorded outbreak of *E granulosus* in animals and the reported lack of compliance with conditions of the controlled area notice, a targeted survey of dogs on the island was undertaken.

The sampling frame used was the dog registration database maintained by the Chatham Islands Council. There were 456 dogs registered and compliance with registration requirements was reported to be high after a recent reduction in the cost of registration together with the efforts of the island animal control

officer. Non-probability purposive sampling was undertaken of all 208 dogs owned by the 50 dog owners on the 32 properties identified in the patient's occupational history. An IDC incursion investigator visited the Chatham Islands and, with the assistance of the council animal control officer, obtained serum and faecal samples from 196 of the 208 dogs. The council dog registration database proved to be accurate and complete.

Tests

During the New Zealand hydatids control campaign, surveillance and diagnosis of infection in dogs was achieved by direct visualisation of worms following an arecoline purge. As arecoline is no longer registered for use in dogs, and because of the variability of its effect and poor diagnostic sensitivity, the coproantigen ELISA was selected as the primary screening test.

Coproantigen(s) highly specific for the genus *Echinococcus* can be detected by antibody capture ELISA in dogs experimentally infected with *E granulosus* by five to ten days after infection and does not depend on the presence of eggs. Faecal antigen conversion to negative status occurs within five days of praziquantel treatment⁽⁶⁾. An advantage of this test is that faecal samples can be taken from the ground or directly from the dog's rectum.

The coproantigen ELISA has a reported sensitivity of 70-87% and specificity over 96%⁽⁷⁾⁽⁸⁾⁽⁹⁾.

Two coproantigen capture ELISAs were set up at the IDC: an AgResearch ELISA (AR-ELISA) and the Bommeli Echinotest ELISA (Bom-ELISA). The AgResearch ELISA had not been used for many years. Nevertheless, reagents were available and the ELISA was re-activated. This ELISA uses a coproantigen-specific rabbit antibody as a capture antibody, a coproantigen-specific sheep antibody as a second antibody and an anti-sheep IgG-HRP conjugate. It is a monophasic ELISA.

The Bom-ELISA uses a coproantigen specific polyclonal antibody as capture antibody, in parallel with a non-specific polyclonal antibody as control antibody, and a coproantigen specific polyclonal antibody conjugated to HRP as secondary antibody. It is a biphasic ELISA. The Bom-ELISA is no longer manufactured but kits were still available in limited numbers.

Faecal samples that tested positive by either of the coproantigen capture ELISAs were tested for the presence of *E granulosus* DNA by a conventional PCR⁽¹⁰⁾. DNA was extracted from faecal sediment using the QIAamp DNA Stool Mini Kit (QIAGEN). The PCR primers selected amplify a recently discovered 269-base pair tandem-repeat DNA sequence (Genbank locus DQ157697), which is highly specific to and abundant in the *E granulosus* genome. The repeat comprises 1.25% of the genome amounting to 6,900 repeat copies. Faecal samples were tested in duplicate, along with four negative controls and four positive controls (from experimentally infected dogs), plus two non-template controls. PCR products were separated by agarose gel electrophoresis and stained with ethidium bromide.

Consultation with a number of international hydatids specialists established that serology is not currently used for diagnosis of *E granulosus* in dogs and no commercial kits were available. Serological testing appears to lack specificity in dogs exposed to other intestinal parasites, for this reason the serum samples collected on the Chatham Islands were not tested.

Results

Of the 196 faecal samples obtained, four contained insufficient material for testing. Of the remaining 192 samples, 165 were tested in both ELISAs, 189 were tested in the AR-ELISA and 168 in the Bom-ELISA.

Several cut-offs were calculated for the AR-ELISA based on the mean +2 standard deviations (SD) and mean +3SD. One sample was considered positive. When this sample was not included in the cut-off calculations, two more samples were positive. All three samples tested negative in the Bom-ELISA (using the manufacturer's cut-off). In the Bom-ELISA two samples were positive and these were negative in the AR-ELISA. Three control samples from experimentally infected dogs tested positive in both ELISAs.

In both ELISAs, the absorbance readings of the samples were relatively high, suggesting non-specific reactions. In the AR-ELISA the average OD was 0.509. Of the samples tested, 56.6% exhibited ODs above this, 30.2% were between 0.400 and 0.500, and 13.3% below 0.400. In the Bom-ELISA, 11.3% of the samples showed OD values >1.000 in the non specific control wells, and 29.8% had OD values between 0.500 and 1.000 in the control wells.

The five dogs that had given the ELISA positive results were re-sampled by the Chatham Islands animal control officer. None was reported to have been wormed between sampling dates. Both the original and fresh samples were retested with both ELISAs. The original faecal samples gave test results consistent with those described above, with both ELISAs. The repeat faecal samples from all five dogs tested negative with both ELISAs.

PCR was performed on the repeat faecal samples only, due to insufficient material left from the original sampling. Each of the five dog faecal samples was tested in duplicate, along with four negative controls and four positive controls (from experimentally infected dogs), plus two non-template controls. All PCR products were separated by agarose gel electrophoresis and stained with ethidium bromide.

DNA bands of expected size (269 and 558 base pairs, representing 1 and 2 tandem repeat units) on agarose gels were produced by PCR products amplified from DNA extracted from the faeces of all the experimentally infected dogs but not from any of the five ELISA positive dogs. These results suggest that no *E granulosus* DNA was present at detectable limits in the five samples of dog faeces from

the Chatham Islands. It also suggests that the coproDNA extraction protocol was effective. To verify that the DNA bands produced from the experimentally infected dogs' faeces were amplified from the desired target, the DNA was excised from the gel and sent for DNA sequencing (Waikato DNA Sequencing Facility, University of Waikato). BLASTN analysis determined that the most significant alignment was to *E granulosus* repeat region sequence of 269 base pairs (gb/DQ157697), indicating that the primers used do specifically target the repeat sequence.

Histology

Histology slides from the patient's cyst were examined by an expert veterinary histopathologist, who did not identify any viable cells of the internal germinal layer of the parasite in any of the sections, and found no characteristic brood capsules or protoscolices (Figure 1). Many of the cyst walls contained focally extensive areas of degeneration with calcification and leucocyte infiltration. The laminated structure identified the cysts as echinococcal, but no evidence of viable parasitic infection could be found in the organism remnants. In most places the pericystic fibrotic tissue was dense, often infiltrated by leucocytes and histiocytes, and in places calcified. While this allowed the lesion to be classified as chronic, accurate aging was not possible.

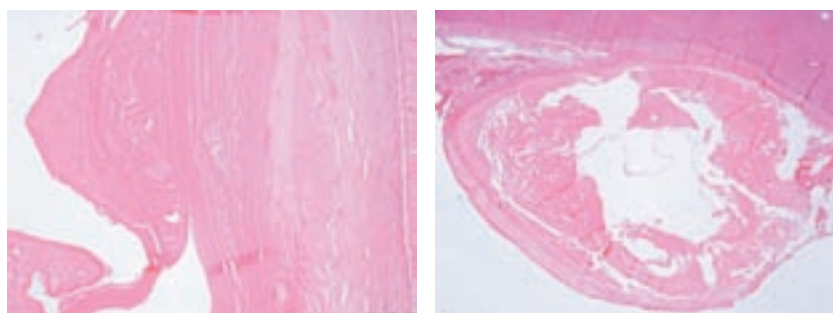


Figure 1: Cross sections of the hydatid cyst wall showing the cyst wall (left) and absence of viable cells in the internal germinal layer (right) (courtesy Alastair Johnstone)

Discussion

The IDC laboratory considered the 192 dog faecal samples tested to be negative, based on identification of experimentally infected dogs in both ELISAs and lack of confirmation of positives in one ELISA by the other ELISA. Given the reported test sensitivity of 70% and specificity of 96%, the finding of five positive samples is enough to show the population is negative for hydatids at the 95% level of confidence given a minimum disease prevalence of 2%. As the survey was targeted at farms considered to represent a higher risk of exposure for the patient to *E granulosus* (compared with a random sample of farms) the level of confidence may be higher than this.

The five dogs with positive ELISA results were followed up by repeat faecal sampling. All showed negative results to both coproantigen ELISAs and the PCR assay. As the dogs had no anthelmintic treatment between sampling times this allows a high degree of confidence that all five dogs are negative for *E granulosus*. The

initial ELISA results are assumed to be false-positives due to test non-specificity. There are no published validity data or other reports in the literature on PCR assays using the primers employed in this investigation.

Geographical coverage of the island was comprehensive, with farms visited and dogs sampled from all corners of the island and a substantial area of the body of the main island (Figure 2). Dogs on Pitt Island belonging to two families identified at the patient interview were also sampled. The targeted survey is a geographically representative sample of the total Chatham Islands dog population, and of the dog population resident on the 46 recorded properties with sheep, which represent the host population at highest risk of infection.

Further, as the MAF pathologist's histological examination of the patient's cyst showed no viable contents, this strongly suggests the cyst is chronic and that the patient was infected before 1990.

Test results and geographical coverage of the targeted survey together with the cyst histology present strong evidence that the Chatham Islands are free of *E granulosus*. This investigation is further evidence of New Zealand's commitment to the maintenance phase of eradication from *E granulosus*.



Figure 2: The Chatham Islands showing the locations of dogs sampled

Acknowledgements

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References

- (1) Eckert J, Gemmell MA, Meslin FX, Pawowski ZS. WHO/OIE manual on echinococcosis in humans and animals: a public health problem of global concern. P 265, 2001.
- (2) Lawson JR. Hydatid disease and sheep measles: the history of their control and the economics of a recent change of control policy. New Zealand Journal of Zoology 21, 83-9, 1994.
- (3) Pharo H, van der Logt P. Hydatids diagnosed on Arapawa Island. Surveillance 24(2), 8-9, 1997.
- (4) Pharo H. New Zealand declares 'provisional freedom' from hydatids. Surveillance 29(3), 3-7, 2002.
- (5) Belton D. Declaration of a Controlled Area. 1204, Hydatids, 1945, 2001.
- (6) Deplazes P, Gottstein B, Eckert J, Jenkins DJ, Ewald D, Jimenez Palacios S. Detection of *Echinococcus* coproantigens by enzyme-linked immunosorbent assay in dogs, dingoes and foxes. Parasitology Research 78, 303-8, 1992.
- (7) Allan J C, Craig PS, Garcia Noval J, Mencos F, Liu D et al. Coproantigen detection for immunodiagnosis of echinococcosis and taeniasis in dogs and humans. Parasitology 104, 137-355, 1992.
- (8) Craig PS, Gasser RB, Parada L, Cabrera P, Parietti S et al. Diagnosis of canine echinococcosis: comparison of coproantigen and serum antibody tests with arecoline purgation in Uruguay. Veterinary Parasitology 56, 293-301, 1994.
- (9) OIE. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, OIE, 2004.
- (10) Abbasi I, Branzburg A, Campos Ponce M, Hafez SKA, Raoul F et al. Copro-diagnosis of *Echinococcus granulosus* infection in dogs by amplification of a newly identified repeated DNA sequence. American Journal of Tropical Medicine and Hygiene 69, 324-30, 2003.

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Quarterly review of diagnostic cases – October to December 2005

Each quarter, *Surveillance* publishes a review of selected diagnostic cases handled by New Zealand's diagnostic laboratories. These cases do not necessarily reflect the national disease profile but they do represent diseases of interest to the livestock industries or of significance to wildlife or companion animals.

Gribbles Veterinary Pathology

Cattle

A two-month-old Jersey calf from Taranaki had been weak and not thrived since birth despite intensive nursing from a committed owner. After prolonged periods of recumbency, the calf was euthanased. Gross lesions were confined to the heart. The aorta bifurcated about 2 cm from the outlet of the left ventricle, forming a major persistent artery. The pulmonary artery emerged from the right ventricle but was also markedly dilated. A **congenital cardiac defect** consistent with duplicated major trunks was diagnosed.

A four-month-old Friesian heifer calf on a Wairarapa dairy farm died suddenly. Necropsy showed a severe bilateral diffuse pneumonia. Neutrophils mixed with necrotic debris and fibrin expanded most alveoli and bronchi, with necrosis of the respiratory epithelium. *Haemophilus* species were cultured from the fresh tissue suggesting ***Haemophilus pneumonia***, although environmental factors and viral infection may also have played a role.

An adult dairy cow had an abscess-like lesion about 10 cm in diameter on the side of the face that extended to the ventrolateral aspect of the ear. The lesion was reported to be present for only a few weeks. A fine needle aspirate collected a small amount of purulent material but when opened the mass had necrotic tissue inside. Histology confirmed a diagnosis of **squamous cell carcinoma** with extensive coagulative necrosis, rather than an abscess.

Neurological disease in young calves through the late spring period can be associated with lead toxicity, bacterial meningitis or thiamine deficiency. A three-month-old calf developed convulsions and opisthotonus before becoming recumbent and dying. Histological examination of the brain revealed neuronal necrosis in a linear pattern through mid gyri. Blood vessel endothelium in these areas was hypertrophied. These findings are typical of **polioencephalomalacia** associated with thiamine deficiency.

Several properties from different districts around the country have suffered early summer losses from **polioencephalomalacia**. This year the losses started after an unusually mild spring and affected older mature cattle as well as young yearling stock.

Polioencephalomalacia was diagnosed in several calves by brain histological examination. In one case a Friesian from a group of illthrift calves in the Taranaki region showed nervous signs and died; a second calf died without showing clinical signs. The calf also had low serum copper levels. In a second case in the Manawatu

region, several six-month-old calves were listless and recumbent, some scouring and some developed opisthotonus. Response to vitamin B1 injections was poor in some calves. Histological examination of the brain of one calf euthanased in extremis revealed marked polioencephalomalacia. The severity of the lesions explained the poor response to treatment. The calf also had histological evidence of enteritis with lesions typical of yersiniosis. Other calves in the group had low serum selenium.

A bull from the Rangitikei district was found down and in discomfort alongside two other dead bulls, and later died. On postmortem the rumen was distended and empty intestines had blackish discoloration. There appeared to be a lot of cerebrospinal fluid when the head was cut off. The four remaining animals were shifted and two days later another was found dead and autolysed but the brain was removed. Another bull was unwell and blind. The bulls were grazing a paddock with maize stubble that had been oversown with oats and with many different weeds but no obvious access to lead. Blood lead concentrations of 1.6 mg/l and 0.83 mg/l (toxic > 0.3) indicated **lead poisoning**.

Deaths occurred in calves on two properties in the Waikato. On the first, nine of 40 died about one month before more sudden deaths. Some calves had respiratory signs then diarrhoea but recovered. Two calves were bloated. At postmortem one carcass was very pale. On the second property ten of 45 calves died, all in the six- to eight-week age range. They were noted coughing. At necropsy, petechial haemorrhages were seen on nearly all organs, intestines were discoloured and there was consolidation of lung tissue. Histology indicated **monensin poisoning**, after feeding of excess quantities of monensin.

Over the course of a month, a mature Friesian cow in the Hawke's Bay region developed wheals in the skin of the caudal udder, upper flank and over the trunk. Histological examination of one lesion revealed a **cutaneous lymphosarcoma**. These are usually associated with the sporadic form of the disease not in association with EBL virus. However, EBL serology was advised to confirm this. A two-year-old Friesian cow from an Otago dairy farm developed multiple, hairless, raised, crusty, circular skin lesions resembling ringworm over the head, neck and flanks with higher numbers around the anus. The cow was slightly thin with a mild pyrexia but was still eating and drinking. A biopsy of a typical lesion revealed ulceration of the epidermis and a dermis infiltrated by masses of neoplastic lymphocytes confirming cutaneous lymphosarcoma.

Exposure to fathen (*Chenopodium album*), a common oxalate-containing weed, caused a severe hypocalcaemia in a mob of 600 dairy cows in Southland. Two cows were found dead and 40 showed severe milk fever signs but responded to intravenous calcium. Serum calcium concentrations were very low in the affected cows tested. The mob was on a once/day, 16-hour milking cycle and had last been milked at 7.00 pm then placed on a large recently grassed paddock. Unfortunately much of the new grass had failed to strike for some reason and the paddock had become heavily infested with fathen. The cows with **fathen poisoning** were found next morning.

A cow on an Otago dairy farm developed a large growth between the claws of one foot and became very lame. A biopsy showed histological changes and large numbers of silver positive bacteria with spirochaete morphology, consistent with **papillomatous digital dermatitis**. The disease was first reported in this country in 2004 in a bull from a herd of 500 dairy bulls near Palmerston North (Vermunt JJ, Hill FL. Papillomatous digital dermatitis in a Holstein-Friesian bull. *New Zealand Veterinary Journal* 52, 99-101, 2004).

A brain submitted to the Christchurch laboratory from a feedlot heifer with nervous signs had typical randomly scattered necrotic lesions consistent with thrombotic meningoencephalitis (TME). The microscopic lesions were typical. *Haemophilus somnus* is commonly recovered from cases of pneumonia in calves in New Zealand but the TME form of the disease has been reported only once in a calf on pasture in this country. TME is mainly a feedlot disease and in this Canterbury feedlot the bacterium causes both TME and fibrinous pleuropneumonia.

Sheep

Four of 60 ewes on a Taranaki farmlet were thin and underweight. There had been regular deaths of ewes and lambs after lambing. The sheep were regularly wormed but otherwise neglected. Necropsy on one very thin ram that died showed minimal fat reserves, abundant fluid in the abdomen and a shrunken liver. Histological examination revealed pathology consistent with chronic biliary damage, most likely associated with previous sporidesmin toxicity, leading to **chronic liver disease** and ascites.

A mature Corriedale ram died suddenly on a Rangitikei hill country property. Bacterial culture of faeces resulted in a heavy growth of *Salmonella Hindmarsh*. The infection probably led to acute septicaemia and rapid death.

Two-month-old lambs on a Wanganui sheep farm were not thriving. All had diarrhoea yet the ewes were in good condition. Five lambs were submitted for postmortem. Intestinal samples examined histologically revealed *Moniezia expansa* tapeworm segments in the small intestinal lumen. In addition, large numbers of coccidia were expanding enterocytes, some as oocysts and some as megaloschizonts, surrounded by extensive areas of eosinophilic necrosis. Liver copper concentrations of flock mates were insufficient as well, indicating a **multifactorial illthrift** problem.

Two lambs in north Otago died two weeks after mulesing. Both were Merinos and were weak before death. They had enlarged dark spleens on postmortem. Haematology showed marked anaemia with signs of regeneration. The blood smears revealed numerous small round pink-blue structures suspicious for *Eperythrozoon ovis* (*Mycoplasma ovis*) organisms. The association of the structures with the surface of red cells is suggestive of *M ovis* infection. Anaemia in Merino lambs after mulesing has been reported previously (Gill JM. *Surveillance* 26(2), 6-7, 1999).

Several deaths in three- to four-month-old lambs were diagnosed as *Leptospira interrogans* serovar pomona infections. In one case from Taranaki, 60 of 3,000 lambs of four months of age died over a period of days without previous clinical signs. Histological examination revealed lesions typical of acute infection with *L interrogans* serovar pomona. In another case in the Dannevirke region, six lambs were found dead, two were weak and recumbent. Serology revealed a high titre of 1/1600 antibodies to *L interrogans* serovar pomona. Histology confirmed the diagnosis. In a case in the Hawke's Bay region, 15 lambs died in two days. Diagnosis was made on serology and histology. The cases tended to coincide with periods of heavy rain during which creeks flooded and puddles in which *Leptospira* organisms from contaminated creeks could concentrate formed in paddocks.

A mob of 1200 ewes and their unweaned lambs on a Southland sheep farm were placed on a paddock of new grass that also had a large amount of oxalate-containing fathen (*Chenopodium album*). Within three days the grass was gone and the fathen was being eaten. Thirteen ewes were found dead and 15 in a recumbent position resembling milk fever responded to intravenous calcium. The mob was removed from the paddock and no more animals were affected. The unweaned lambs were not affected and there were no after-effects of **fathen poisoning** in the affected ewes.

A mob of 600 ewe hoggets and their eight-week-old unweaned lambs on a South Otago sheep farm were yarded for drenching. Five days later the lambs started dying and over the next few days 30 were found dead or dying. The live affected lambs examined were recumbent but could stand and walk if lifted to their feet. Their rectal temperatures were high (41°C) and they were slightly bloated. Necropsy showed no significant lesions apart from a mild acute bronchopneumonia of the apical lobes of the lungs of some lambs necropsied. Culture of liver and lung from one lamb grew a heavy pure growth of *Mannheimia haemolytica* and confirmed the septicaemic form of this disease. The recent yarding probably spread the bacterium to the susceptible lambs. No ewe hoggets were affected.

A Selwyn district Canterbury flock had a group of 300 four-week-old, crossbred lambs of which 100-200 were lame and some had died. A swab of a joint from one lamb grew *Streptococcus dysgalactiae*. Because of the numbers involved, this case was notified to IDC and the same bacterium was recovered from several other

lambs. Although normally considered a mastitis pathogen of cows, this bacterium has been recorded previously as a cause of **polyarthritis** in lambs. Treatment with penicillin gave immediate improvement.

Goats

Clinical cases of bloat caused sporadic deaths in young goats on a farm in the Waikato. The abomasum of one animal examined histologically showed autolytic changes in the mucosa but the lumen contained characteristic packets of *Sarcina* organisms. This organism has been recognised in North America as a cause of bloat in milk-fed kids (DeBey BM, Blanchard PC, Durfee PT. Abomasal bloat associated with *Sarcina*-like bacteria in goat kids. Journal of the American Veterinary Medical Association 209, 1468-9, 1996). *Sarcina* is an abomasal gas-producing organism that has also been associated with bloat and abomasal ulcer in lambs (Vatn S, Tranulis MA, Hofshagen M. *Sarcina*-like bacteria, *Clostridium fallax* and *Clostridium sordellii* in lambs with abomasal bloat, haemorrhage and ulcers. Journal of Comparative Pathology 122, 193-200, 2000). It has been reported in South Island lambs (Surveillance 31(1), 21-5, 2004).

Deer

Three stags developed acute anaphylactic reactions two or three minutes after injection of xylazine for debriding. Two responded to adrenaline treatment, the third died. Histological examination of the laryngeal tissues revealed a heavy infiltrate of submucosal eosinophils confirming death from anaphylactoid reaction. Such **xylazine reactions** are usually a delayed-type hypersensitivity. Increased genetic susceptibility, possibly from inbreeding, and variation in the drug formulation are being investigated.

Alpaca

A one-year-old female alpaca from the Wellington region had been losing weight slowly, then was found dead. The liver was pale and swollen on necropsy, and histological findings were consistent with a diagnosis of hepatic **lymphosarcoma**. Sections of kidney revealed a mild suppurative nephritis.

Dogs

A miniature Schnauzer from Wanganui had a caesarean operation, then four days later developed anorexia, lethargy, diarrhoea and respiratory effort. She was feeding seven puppies. Haematology showed a severe non-regenerative anaemia that was Coombs positive, indicating an **immune mediated haemolytic anaemia** (IMHA). Routine biochemical examination showed increased liver enzymes, increased creatine kinase and a mild stress-induced hyperglycaemia. A liver aspirate indicated a suppurative cholangiohepatitis. There were small numbers of intracellular Gram-positive large rods. A spore forming rod was also seen. The cause of the RBC destruction is often never found in IMHA but may be primary, or secondary resulting from infectious, neoplastic and other causes. Coombs positive IMHA has been reported in

horses as a result of *Clostridium perfringens* infection and this is also suggested in dogs. The reason for the presence of the bacteria is not known. Clostridia multiply in anaerobic environments, possibly caused in the liver by lobe torsion or a thrombus following surgery resulting in devitalised tissue. Prednisolone treatment for IMHA and broad spectrum antibiotics resulted in improved demeanour and the ALT decreased two days later.

A four-month-old female Scottish terrier from Wanganui was in poor condition despite eating well. Routine biochemistry revealed slightly low urea of 2.2 mmol/l (ref range 2.5-8.4) and albumin 27 g/l (ref range 28.7-38.7). Pre-prandial serum bile acid values were 79 µmol/l and post-prandial 245 µmol/l, indicating severe hepatic dysfunction suggesting a possible **portal shunt**.

After three dogs from the Wellington region had been swimming in the local river, one young dog collapsed and died. Necropsy revealed little except for ingestion of muddy algae-contaminated water and copious amounts of airway froth. Histology similarly revealed little. Stomach contents and river water samples were positive for two neurotoxic cyanotoxins: anatoxin-a and homoanatoxin, both powerful depolarising neuromuscular blocking agents that are rapidly absorbed when ingested orally. They mimic the effect of acetylcholine and act at both the nicotinic and muscarinic receptors. Thus **cyanotoxin poisoning** was the cause of the dog's death and raised alarm over possible human health risks. The regional council and district health boards acted swiftly to erect warning signs along 20 km of the river. Anecdotal evidence suggests four more dogs were poisoned in the same area about the same time after swimming and drinking the water. (With thanks to Susie Wood, Cawthron Institute.)

Cats

A 15-year-old castrated male cat from the Manawatu showed weight loss and polyphagia and had numerous cutaneous masses over the body. Aspirates of two masses yielded mostly blood but also showed macrophages containing needle- or spicule-like structures that did not stain with normal Romanowsky stains. Modified Ziehl Neelsen staining of the most cellular of the three aspirates revealed numerous long slender acid-fast organisms. The most likely cause of the lesions is *Mycobacterium lepraemurium*, a common infection causing painless skin nodules. It cannot be cultured using conventional techniques. Cats may be infected with *M bovis*, *M avium*, *M smegmatis* and culture is needed for a diagnosis. The cat was euthanased and demonstration of *Mycobacteria* by the modified ZN stain confirmed **cutaneous mycobacterial infection**.

A nine-year-old female cat from the Wairarapa had a history of chronic vomiting over six months. On endoscopy the pyloric region of the stomach appeared inflamed. A blood sample showed the cat to be hyperthyroid and it had a neutropaenia with toxic changes, consistent with acute inflammation. Cytology on a scraping of stomach wall revealed large numbers of bacteria of a mixed population of rods, cocci and many spirochaetes, which were

demonstrated by Leishmans stain to be *Helicobacter*-like organisms. Several *Helicobacter* species may infect cats and may not all be pathogenic. *Helicobacter* have been found in apparently normal cats in small numbers but the large numbers in this case suggest they were significant. In cats *Helicobacter felis* may cause a lymphocytic plasmacytic gastritis and destruction of the parietal cells. The ***Helicobacter*-like infection** and the hyperthyroidism were probably unrelated. *Helicobacter* change the environment in the stomach, and this may have allowed growth of the other bacteria that were seen cytologically.

Birds

Two clinically normal **North Island brown kiwi** in a capture facility in South Waikato were screened for pathogens. Faecal samples from each bird yielded *Salmonella* Typhimurium. The same birds were found to be carrying *Salmonella* in November 2004 when a screen picked up *Salmonella* Typhimurium phage type 135 in one and *Salmonella* Saintpaul in the other. The same strains were confirmed in this case.

A commercial breeder of **rainbow lorikeets** in the Waikato lost several adults sporadically over the winter months. *Yersinia* was cultured from faeces. By November the losses had increased with several birds dead at a time. Necropsy of one showed the carcass was in good condition but dehydrated and there was marked splenomegaly and hepatomegaly. Culture of the intestine yielded a pure growth of *Salmonella* Typhimurium. *Salmonella* Typhimurium was isolated from the liver of a second bird, suggesting an outbreak of septicaemic **salmonellosis**. Further investigation revealed that a clutch of duck eggs had been left in the bottom of the aviary to go rotten. The lorikeets played in and around the eggs, whereas other birds in the same environment ignored them and did not suffer mortalities. This may have been the source of the *Salmonella*. Losses stopped after cleaning and disinfection of the premises.

A 29-year-old **tawny frogmouth** that had anorexia and weight loss was treated, hospitalised for three months, was well for two months but then became ill again and died. At postmortem a 15 mm diameter cystic mass was attached to the liver and there appeared to be visceral gout. Histology revealed pathology in the liver, heart, lungs, spleen, kidneys, intestines and brain. Protozoa consistent with toxoplasma were detected in the intestinal wall, heart and brain.

Plasmodium relictum was confirmed in an adult female **blue penguin** (*Eudyptula minor*), one of six at the Auckland Zoo euthanased because of chronic weight loss, anorexia and weakness. There was a history of prolonged stress because of work on the exhibit. The penguin was in poor body condition and histology revealed protozoal schizonts in endothelial cells, hepatocytes and histiocytes of the lung, liver and spleen. PCR on frozen liver and formalin-fixed, paraffin-embedded lung, liver and spleen was positive for *Plasmodium relictum*.

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New Zealand Veterinary Pathology

Cattle

Two calves started coughing within three days of arrival at a property in Northland. Within three weeks one had died and the other had a temperature of 39.5°C and rough lung sounds. Lesions in the lungs of the dead calf and the presence of two lungworm larvae in the pulmonary parenchyma allowed a diagnosis of **lungworm hypersensitivity**. Lungworm larvae migrate through the lungs about five to seven days after ingestion, provoking a severe interstitial inflammatory reaction after about the tenth day. This suggests the calves may have been infected with lungworm on the new property, but pre-existing or concomitant viral pneumonia cannot be ruled out.

A Manawatu property mentioned in the previous quarterly review of diagnostic cases (Surveillance, December 2005) continued to have problems with **mycotic rumenitis and abomasitis** in calves. The cows calve in three groups through the year: autumn, spring and late summer. A calf affected in December was assumed to be from the late summer calving group, whereas earlier calves affected were born in the spring. This calf had been treated with injectable oxytetracycline early in the course of disease, which was believed to be septicaemia. It developed diarrhoea within three days and died within ten days of presentation despite treatment with fluids. This calf had similar histopathology to that in the earlier calves, with the major finding a severe, multifocal ulcerative fungal abomasitis with fibrinoid vascular necrosis and thrombosis. Fibrinoid vascular necrosis was also present in the rumen, and the calf also had an acute fibrinous interstitial pneumonia and pleuritis. An attempt to isolate the fungal agent from the lesions was unsuccessful. The cause and predisposing causes of the mycotic infections are unknown but extensive use of antibiotics on the farm may potentiate the growth of fungi.

Three yearling heifers in the Taupo district were found dead following access to an open bag of selenium prills. Analysis of livers from two heifers revealed selenium concentrations of 214,000 and 312,000 nmol/kg (>15,000 nmol/kg considered toxic) confirming **selenium toxicity**.

A number of cases of **copper toxicity** have occurred in cattle recently. In a typical case one cow died suddenly in a Northland herd and another went off milk. The cow had a subnormal temperature, dehydration, tachycardia and anaemia. The main changes on biochemistry were a severe cholestatic hepatopathy and a markedly elevated serum copper concentration of 104 µmol/l (reference range 8.0-25.0). This cow and a third subsequently died. Kidney copper concentrations were 260 µmol/kg and 180 µmol/kg (>157 µmol/kg regarded as toxic). Inaccurate in-line dispensing of copper sulphate resulted in toxicity.

A weaner calf from the Hamilton area died suddenly after exhibiting severe respiratory distress. On necropsy the calf had severe haemothorax. There was a history of possible exposure to rat bait

which had been laid in the area. A coagulation panel revealed a severely prolonged prothrombin time, confirming anticoagulant **rodenticide toxicity**.

A dairy farm in the Levin area experienced increased mortality in scouring calves. Histological examination revealed a relatively mild enteritis but culture of intestinal content yielded a growth of **Salmonella Heidelberg**.

A group of six- to seven-week-old dairy calves at pasture in the southern Wairarapa developed weakness and blindness. Six calves became recumbent and died. Blood lead levels were 0.6 mg/ml (normal 0.1-0.2 mg/ml), consistent with **lead toxicity**. The source of the lead was not identified.

Sheep

Five deaths were reported over ten days in a group of six-month-old lambs on a southern Rangitikei property. A lamb that was euthanased had a temperature of 41°C and appeared bloated. On postmortem, mucopurulent material was found around the brain. Histological abnormalities included a severe acute suppurative meningitis, choroiditis, and ventriculitis, as well as an acute interstitial pneumonia. Small colonies of Gram-negative coccobacilli were visible. No suitable fresh specimens were available for culture but histologic findings were considered to be most consistent with *Histophilus ovis* infection. *Histophilus ovis* can cause small outbreaks of meningitis and septicaemia in weaned lambs. *Histophilus ovis*, *Haemophilus agni* and *Haemophilus somnus* have been shown to be the same species of bacterium. Based on recent 16S rRNA and rpoB gene sequencing, they have been tentatively assigned to a new genus and the species referred to as ***Histophilus somni*** (Angen Ø, et al. Proposal of *Histophilus somni* gen. nov., sp. nov. for the three species *incertae sedis* '*Haemophilus somnus*', '*Haemophilus agni*' and '*Histophilus ovis*'. International Journal of Systemic and Evolutionary Microbiology 53, 1449-56, 2003).

Alpaca

An adult alpaca from the Bay of Plenty region presented with crusting around all four feet. Examination of a skin scraping revealed numerous *Chorioptes* spp mites. **Chorioptic mange** has not previously been reported in alpacas in New Zealand.

Horses

Blood from a foal from the Auckland region that was in poor condition had markedly elevated liver enzymes and albumin levels. Haematology revealed a monocytosis and a hyperfibrinogenaemia. The biochemistry and haematology results were consistent with an inflammatory hepatopathy, probably bacterial in origin. Microagglutination tests for *Leptospira interrogans*, including types *copenhageni* (*icterohaemorrhagiae*), *pomona*, and *hardjo* were submitted. The *Leptospira copenhageni* titres were over 1/1600, and the other titres were negative, suggesting recent infection with ***Leptospira copenhageni***.

Dogs

A 13-week-old pup from the Wellington area presented with a mixed small and large bowel diarrhoea. The faeces showed no evidence of parasitism but ***Yersinia enterocolitica*** was cultured. Cultures for *Salmonella* and *Campylobacter* were negative. *Yersinia enterocolitica* can be isolated from the faeces of both normal and diarrhoeic dogs. In this case, its isolation was considered significant in the absence of other known pathogens and in the presence of consistent clinical signs.

A ten-year-old entire male dog presented with a very large (20 cm diameter) polycystic abdominal mass. Histology revealed it was in the kidney and was composed of large cystic spaces lined by cuboidal epithelium, separated by abundant, densely cellular mesenchymal tissue that had some features of low grade malignancy. Renal **nephroblastoma** was diagnosed. Nephroblastomas arise from the embryonic remnants of the metanephric blastema and are more common in males. They are more common in juvenile dogs but have also been described in adult and aged dogs.

A one-year-old Kelpie cross pup from the Christchurch area presented with skin lesions on the face, feet, tail base and the tip of the tail. The lesions had been waxing and waning over about six months, and antifungal and antibiotic treatments had given no significant improvement. **Dermatomyositis** was diagnosed on the basis of skin biopsy and the classical clinical presentation. Canine dermatomyositis is only rarely diagnosed in New Zealand. Overseas it is most common in collies and shelties but this is not the case in New Zealand. Dermatomyositis is inherited as an autosomal dominant with variable expressivity. The different breed distribution in New Zealand may be because the original dogs of the collie and sheltie breeds brought to this country were not carriers of the gene.

Cats

A two-year-old domestic shorthaired cat from the Wellington area had a firm nodular dermal mass along the ventral midline of the jaw between the mandibles. Histologic examination revealed a nodular granulomatous dermatitis with intralesional acid-fast bacilli. Culture at NCDI yielded a growth of ***Mycobacterium bovis***.

A seven-year-old domestic shorthaired cat from the Auckland region had a history of recurrent dermatitis. Histologic examination revealed a marked pyogranulomatous dermatitis and a single long Demodex mite was visible in one hair follicle. Feline **follicular demodecosis** was diagnosed. Feline follicular demodecosis is typically caused by *Demodex cati*.

A 12-year-old domestic shorthair cat presented with firm swelling of four toes on three feet. The swellings were dark fleshy material that protruded around nails, with some haemorrhage and a purulent discharge. Histological findings were consistent with metastatic carcinoma. Bronchogenic carcinomas in cats commonly metastasise to the toes, and may present initially as nodules affecting the toes because the primary tumours tend to be clinically silent. **Metastatic**

bronchogenic carcinoma was considered the most likely diagnosis.

An eight-year-old cat from the Rotorua area presented with recurring fever of unknown origin. *Yersinia pseudotuberculosis* was cultured from the blood. **Septicaemic yersiniosis** is uncommon in cats but has been described previously.

Birds

About 15 Indian **ringneck parrots** living in an outdoor aviary in the Christchurch region died over the course of one month. Necropsy of one showed an enlarged liver with several pale spots over the margins of liver lobes. Histological examination revealed widespread lesions characterised by numerous large 5-20 µm basophilic smudgy inclusions, mostly within vascular endothelium and free within blood vessels. Many of the larger basophilic inclusions contained numerous small 1 µm diameter bodies. The spleen, sinusoids of the liver, vascular endothelium of the myocardium and brain, and vascular endothelium of the intestines, ventriculus and proventriculus were affected. Lesions were considered to be most consistent with **avian malaria**, which has been described as far south as Christchurch in both native and non-native bird species. It is caused by the protozoal parasite

Plasmodium spp, most likely *Plasmodium relictum*. The vector is the exotic mosquito *Culex quinquefasciatus*, which has expanded its range in New Zealand since first introduced 30 years ago.

A silver laced **Wyandotte** hen from the Auckland region was submitted for postmortem after being ill for two weeks with neurological signs including weakness, loss of balance and ataxia. The owner had recently lost four birds with similar clinical signs. Postmortem showed splenomegaly and moderate hepatomegaly. Histology revealed infiltrates of neoplastic lymphocytes in the sciatic and spinal nerves, small intestine, proventriculus, spleen, liver, lung and brain. **Lymphoma** was diagnosed. Lymphoid leucosis was considered the most likely aetiology as it tends to affect mature birds, as compared with Marek's disease.

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Quarterly report of investigations of suspected exotic diseases

Exotic disease investigations are managed and reported by MAF's Investigation and Diagnostic Centre (IDC) Wallaceville. The following is a summary of investigations of suspected exotic disease during the period from October to December 2005.

Exotic vesicular disease ruled out

A veterinary practitioner found erosive mouth lesions in three of 154 six-month-old beef steers. An Initial Investigating Veterinarian (IIV) found the steers recumbent with opisthotonus, extensor rigidity and muscle spasms consistent with tetanus. The group had been recently castrated using double-rubber rings and had not received tetanus vaccine. The remainder of the animals were in good health. The mouth lesions were superficial (1 mm) erosions with no under-running or epithelial tags, and were present in the premandibular gingiva and rugae of the hard palate. The tongue and feet were not affected. Scabs consistent with sunburn were present on the muzzle. The IIV decided no exotic disease was involved and made a preliminary diagnosis of tetanus with possible bovine viral diarrhoea virus (BVDV) involvement.

A veterinary practitioner detected erosive teat, muzzle and nasal lesions in a recently calved Friesian dairy cow. An IIV found the cow was in poor condition but had a normal rectal temperature. There was a shallow healing lesion on a teat, two raised healing lesions on the muzzle and a single small erosion in the nostril area. The tongue and feet were not affected. No similar lesions were found in three other adult cows examined because of a bilateral mucoserous nasal discharge. Other stock on the property were healthy. The IIV diagnosed bovine papular stomatitis, ruling out exotic vesicular disease on clinical and epidemiological grounds.

A veterinary practitioner reported an 18-month-old cattle beast with extensive erosions/ulcers of the entire dorsal and ventral surfaces of the tongue and around and within both nares. No other animals in the herd were affected. An investigating veterinarian concluded that no vesicular disease was present. Whole blood gave a negative polymerase chain reaction (PCR) for malignant catarrhal fever, and serum ELISA was negative for bovine virus diarrhoea (BVD) antigen.

A veterinary practitioner from Pukekohe reported a dairy herd with a high prevalence (30-40%) of extensive crusty skin lesions on the caudal udder. The lesions extended slightly laterally but did not include the teats. No oral or foot lesions were reported and there was no drop in milk production. The high prevalence, lesion description and location are suggestive of bovine herpes mammillitis but cowpox and pseudocowpox are also possible causes. No viral particles were observed on electron microscopy (EM) of epidermis and crust samples. The samples were passaged twice in different cell lines and no cytopathic effect was observed, and staining revealed no inclusion bodies or evidence of herpes virus. The lesions resolved uneventfully. Exotic vesicular disease was ruled out on clinical and epidemiological grounds.

Transmissible spongiform encephalopathy ruled out

The case reported in this section is the most significant suspected transmissible spongiform encephalopathy (TSE) investigation for this quarter. During the quarter 861 cattle, 16 sheep, one goat and 179 deer brains were submitted, under the TSE surveillance incentive programme, to MAF approved veterinary diagnostic laboratories. All were negative for TSEs.

A pathologist found spongiform lesions of the white matter in brain tissues from a three-year-old Friesian that was a non-responsive downer cow after calving. Her condition was provisionally attributed to complications arising from facial eczema earlier in the year. The serum gamma glutamyl transferase level in April 2005 was 2122 iu. The location of the lesions and a negative Prionics western blot test ruled out TSE.

Bovine virus diarrhoea type II ruled out

Two calves with haemolytic disease, petechial haemorrhages of the mucosa and severe thrombocytopenia tested positive for BVD antigen. As exotic BVD type II virus infection was considered a possible differential, blood and fresh tissue were tested and found positive for BVD virus by cell culture passage and PCR. Virus isolation and bacterial culture were negative. Sequence analysis of the BVD virus DNA showed a high homology (95-99%) with published sequences of BVD virus type I.

Anthrax ruled out

A veterinary practitioner from Ashburton examined a cow in extremis. The cow had calved unassisted that day, had a rectal temperature of 40°C and extensive subcutaneous emphysema of the perineum extending forward to the costal arch and including gas gangrene in all four quarters. No *Bacillus anthracis* was cultured from milk samples or seen on blood smear. A heavy mixed growth of *Streptococcus uberis*, *E coli* and *Clostridium perfringens* type A was cultured from milk samples, and PCR for *Clostridium perfringens* was also positive. Post calving clostridial disease due to *Clostridium perfringens* was diagnosed as the likely cause.

Exotic *Salmonella* phage types detected

A Gribbles pathologist reported a scouring calf with a mixed enteric infection including rotavirus and cryptosporidium, and a *Salmonella* Typhimurium not previously reported in animals in New Zealand. The farmer collected the faecal sample when around 70 of 250 end-of-season calves were scouring, with 10% mortality. Environmental

Science and Research (ESR) identified *Salmonella* Typhimurium phage type RDNC-Feb 05 (RDNC = 'Reacts but does not conform to a known phage type pattern'), a pattern recognised in five human isolates in New Zealand since February 2005. Two human isolates were fully susceptible to antibiotics but the bovine isolate was resistant to streptomycin, sulphonamides and tetracycline. These three antibacterials are most commonly associated with resistance in animal and environmental isolates, with a prevalence of between 2.1% (sulphonamides and tetracycline) and 3.3% (streptomycin) of all similar isolates tested at ESR in 2004. This pattern of multiresistance was identified in 1.2% (3/242) of the same group of 2004 isolates. The clinical syndrome was considered typical of a mixed enteric infection in young calves, associated with a build up of environmental contaminants through the calving season and suboptimal husbandry.

Gribbles Veterinary Laboratories reported a *Salmonella* Typhimurium not reported previously in cattle. Exotic species of *Salmonella* are unwanted organisms under the Biosecurity Act 1993. Phage typing at ESR identified *Salmonella* Typhimurium phage type RDNC-Sept05 from scouring calves on two south Taranaki properties that obtained calves from slaughter premises. The scouring occurred one week after purchase and was not reported in other species or age groups on the properties. The mortality rate was about 15-20%. One farm experienced a concurrent rotavirus infection.

Tissues from a scrapie positive sheep investigated

Gribbles Veterinary Laboratory, Auckland, has an ongoing contract to supply a histopathology service to the Falkland Islands but receive only fixed tissues (in 10% formalin). Fixed lung tissue from a seven-year-old ewe that had weight loss, pruritis and ataxia was unremarkable on histology. CNS tissue from the same animal, submitted by the Falkland Islands Department of Agriculture to the Veterinary Laboratories Agency (VLA), Weybridge, England, was positive for atypical scrapie (Epstein V, Pointing S, Halfacre S. Atypical scrapie in the Falkland Islands. *Veterinary Record* 157, 667-8, 2005). Gribbles had disposed of the 'wet' fixed tissue through a commercial waste destruction company, which used Rotaclave – autoclave, 135°C at 8 bar pressure for min 35 minutes, and a deep burial sanitary landfill. IDC directed the disposal of the remaining paraffin blocks by returning them to the Department of Agriculture on the Falkland Islands.

Echinococcus granulosus ruled out

The Hawke's Bay Medical Officer of Health reported hydatids in a patient in Christchurch Hospital with cysts in the liver. *Echinococcus granulosus* was ruled out on the Chatham Islands after targeted surveillance with faecal sampling of dogs, and histopathology of the cysts. More details of this investigation are presented in this edition of *Surveillance*.

Mycoplasma mycoides mycoides (Large Colony) in sheep investigated

A veterinary practitioner reported an outbreak of polyarthritis in three- to four-week-old lambs on a south Canterbury farm, most cases occurring after tail-docking. Ewes were unaffected, but 1-50% of the lambs had clinical lameness, depending on the mob. Lambs coped with the lameness and remained in good condition. Joint samples from one lamb gave no bacterial growth. Postmortem of two other lambs showed the arthritis was typical of the fibrinous arthritis in the earlier cases, and a third had a purulent arthritis. *Streptococcus dysgalactiae* was isolated from the two cases of fibrinous arthritis, and *Fusobacterium necrophorum* from the purulent arthritis. *Mycoplasma* culture was negative and *Chlamydia psittaci* was ruled out on histology, which identified large numbers of Gram-negative rods in the synovium of two lambs, which was at variance with the culture results from one. *Fusobacterium necrophorum* is a recognised cause of polyarthritis in lambs in New Zealand. *Streptococcus dysgalactiae* has not been reported here as a cause of polyarthritis, but is the most common isolate in Northern England and Scotland. Molecular sequencing identified *Streptococcus dysgalactiae* subsp *dysgalactiae*. *Streptococcus dysgalactiae dysgalactiae* may be carried in the mammary gland and lower reproductive tract, and has been isolated from biting flies. In the UK the condition has been associated with contaminated lambing environments. The epidemiology is not fully understood. In bovine mastitis, the organism has characteristics of both a contagious and an environmental pathogen.

An investigation was initiated after one of ten repeat export tests for MmmLC had a Complement Fixation Test (CFT) that was anti-complementary. The initial export test had similarly been anticomplementary. A farm visit was undertaken and a third serum sample collected along with vaginal, nasopharyngeal and ear swabs from the ewe. The third serum sample was also anticomplementary. The swabs taken from all three locations were positive for MmmLC by real-time RT-PCR. PCR products were confirmed by DNA sequencing to be MmmLC. Nested CAP21 PCR results were confirmed by restriction enzyme digestion to be MmmLC. Despite DNA evidence, isolation was negative. There was no history of infectious disease consistent with MmmLC infection in the ewe tested, the in-contact mob or the farm. Adult sheep had a low prevalence (<0.1%) of mastitis and no arthritis.

Equine influenza ruled out

A veterinarian reported a horse experiencing severe respiratory signs with depression, fever (40.5°C) and purulent bilateral nasal discharge. An investigating veterinarian found no clinical disease in four in-contact horses examined and there was no history of respiratory disease in six horses from contiguous properties. This horse died 48 hours after first clinical signs. Exotic equine diseases were ruled out on clinical and epidemiological evidence. Serology

was negative for equine influenza using the haemagglutination inhibition (HI) test.

Equine viral arteritis or equine infectious anaemia ruled out

A Gribbles pathologist reported a Standardbred gelding with pyrexia, marginal anaemia, and ventral oedema of the sheath and abdomen. Horses were moved regularly from the Canterbury premises for breeding, training and racing. Blood film and molecular screening for haemoparasites was negative. Equine infectious anaemia (EIA) and equine viral arteritis (EVA) were excluded using the gel-diffusion (AGID) and virus neutralisation (VNT) tests, respectively, performed on acute and convalescent samples. The horse made a full recovery over a few days.

Gribbles Veterinary Pathology Auckland reported a pregnant Clydesdale mare, recently imported from Australia, with mild pyrexia (38.5°C), conjunctivitis and a slight serous nasal discharge. No illness was observed in the in-contact horses. Acute and convalescent blood samples allowed EVA to be ruled out by VNT, EIA by AGID, EHV4 by ELISA, and EI A1 and A2 by HI test. The horse made a full recovery and was still in foal following this investigation.

Three positive VNT results for equine viral arteritis (titres: 19 July, $\leq 1/8$; 25 July, $1/4$; 1 August, $1/4$) in a four-year-old Thoroughbred gelding were concluded to be false positives. An investigation of risk contacts (one stallion; two mares mated within three weeks; 22 of 43 other young horses present at the same training facility; 13 of 27 horses on a neighbouring property where some mating occurred) failed to identify any other VNT positive animals. Two of the original positive samples were negative and one suspicious following a retest of stored serum at an overseas OIE reference laboratory (titre: 19 July, $1/4$; 25 July, $<1/4$; 1 August, $<1/4$). Cut-off points used to define a positive test varied between laboratories; $1/4$ was considered suspicious rather than positive by the OIE reference laboratory.

A pathologist reported an eight-year-old Standardbred mare with mild anaemia and an inflammatory leukogram. A blood smear did not reveal any blood parasites. A VNT was negative for EVA and the AGID test was negative for EIA, performed on acute and convalescent samples. A year earlier the mare had had a hysterectomy for a pyometra. An echographic image showed an enlarged cystic ovary, which was surgically removed. The mare made a slow recovery.

An Auckland pathologist reported a five-year-old Thoroughbred with severe anaemia (PCV 0.08). No overseas travel or contact with imported horses was reported. EIA was ruled out on negative acute and convalescent AGID tests one month apart.

A Gribbles pathologist reported a 16-year-old Clydesdale stallion with chronic weight loss, anaemia and an abdominal effusion.

Haematology demonstrated a moderate to severe anaemia and an inflammatory leukogram. Biochemistry showed a marked hypoalbuminaemia and hyperglobulinaemia. The effusion was a transudate. EIA virus was ruled out using the gel-diffusion (Coggins) test on serum. The horse showed a gradual improvement following antibiotic treatment.

A Gribbles pathologist reported anaemia in an 18-month-old Thoroughbred of New Zealand origin. There was no history of contact with imported horses in the six weeks before sample submission or after. Paired serum samples two weeks apart were negative for EIA using an AGID test. A blood smear did not reveal any blood parasites.

Contagious equine metritis ruled out

A veterinarian reported a maiden mare that developed a profuse discharge six days after mating, and signs consistent with contagious equine metritis (CEM). Swabs from the cervical mucosa yielded a heavy growth of *Streptococcus equi* spp *zooepidemicus* with a light growth of the same organism from the clitoral sinus and fossa. No *Taylorella equigenitalis* was isolated. There were no clinical signs of CEM or venereal disease in seven other mares on the same stud all served by the same stallion this season. The owner indicated a possible source of iatrogenic infection. The mare was treated with trimethoprim sulfadoxine and prostaglandins, and the infection cleared ten days later.

Equine viral meningoencephalitis ruled out

A Gribbles pathologist reported histological findings suggestive of a viral meningoencephalitis in a yearling Thoroughbred colt that died within a few hours of showing severe discomfort with profuse sweating, and an elevated heart rate and temperature. Necropsy findings included congested lips and gums with extensive petechial haemorrhages in the mucosa, ecchymotic haemorrhages in visceral tissues and numerous multifocal petechial haemorrhages on the brain surface. MAF's reference pathologist examined brain sections and determined the most likely aetiology as acute bacterial septicaemia with terminal disseminated intravascular coagulation. The mononuclear cells initially believed to represent lymphocyte cuffing were confined to the cerebellar parenchyma immediately beneath the meninges and were identified as residual stem cells. Immature neutrophils, including myelocytes and metamyelocytes, were also identified in many blood vessels throughout the brain and meninges. Culture showed no *Salmonella* species, only a heavy growth of *Escherichia coli* and light growth of *Streptococcus equi* subsp *zooepidemicus*, considered to be contaminants. Virus isolation identified no cytopathic or haemagglutinating viruses.

Brucella abortus ruled out

A pathologist from New Zealand Veterinary Pathology reported fistulous withers in an 18-year-old gelding that was presented with

a swollen painful discharging wither area. *Brucella abortus* was ruled out by competitive *Brucella abortus* ELISA and PCR for *Brucella* spp. No *Brucella* was isolated from swabs.

Brucella suis ruled out

A gilt was ELISA positive for *Brucella suis* in routine pre-export testing. The farm of origin had high herd health status with no clinical disease suggestive of brucellosis. Another sample one week later gave a weak positive to *Brucella suis* ELISA. A repeat sample one month after the first was negative. The positive reaction was likely to have been a cross-reaction from an enteric infection with *Yersinia enterocolitica*. The export of 150 gilts to Tahiti continued, with the false seropositive animal remaining in New Zealand.

Exotic nematode in camelids investigated

Routine faecal egg count examinations of 33 llamas and alpacas from a Canterbury property showed unusual worm eggs from four llamas and two alpacas. A MAF reference parasitologist identified the eggs as likely to be from *Lamanema chavezii*, a host-specific pathogenic nematode of South American camelids. Confirmation has not been possible as no adult worms were collected in post-drench faecal examinations, and no molecular tests are available for this worm. There were no clinical manifestations in the herd, and no recent or indeed direct import pathway identified. Routine anthelmintic treatments are effective against *Lamanema chavezii*, with no resistance reported in the literature.

Brucella canis ruled out

Brucella canis was ruled out by a negative *Brucella canis* card test on serum from a ten-year-old golden retriever that was depressed and inappetent and had a firm right testicle. Cytology from a testicular aspirate showed degenerate neutrophils and suppurative inflammation.

A Gribbles pathologist received serum samples from a dog with orchitis. The three-year-old dog was a resident of the dog colony at Massey University for five or six months of the year. There was no reported contact with imported dogs. The dog had a painful swollen testicle, exercise intolerance and increased speed of body temperature rise on the treadmill. The dog was segregated from the dog colony during testing and treatment. A serum sample was negative on the *Brucella canis* card test. An ultrasound examination diagnosed a testicular abscess. The dog responded well to antibiotic therapy.

A veterinarian reported orchitis in a huntaway working dog from a farm in the Masterton district. There was reportedly no contact with imported dogs. The dog had a painful, swollen left testicle and pyrexia. A serum sample was negative on the *Brucella canis* card test. The dog responded well to antibiotic therapy.

A Huntly veterinarian reported a six-year-old dog with unilateral testicular and epididymal changes consistent with *Brucella canis*. The

dog underwent unilateral orchidectomy. *Brucella canis* was ruled out on a card (rapid agglutination) test.

A Gribbles pathologist reported a necrosuppurative orchitis in a seven-year-old Labrador. The dog was born in New Zealand and had not been used for breeding. *Brucella canis* was ruled out on a negative card test and negative *B canis* PCR.

Ehrlichia canis ruled out

A dog was positive to the immunofluorescent antibody test (IFAT) for *Ehrlichia canis* on two occasions during routine pre-export testing. The dog was born and bred in New Zealand, had not been overseas and had no history of health problems. *Ehrlichia canis* was ruled out following a negative PCR test.

Ehrlichia canis infection was suspected in a three-year-old bitch with petechial bleeding on all mucous membranes. Clinical pathological features of *Ehrlichia canis* infection such as anaemia, thrombocytopenia, neutrophilia and eosinophilia were present. The dog's owners had been in Vanuatu three weeks earlier. No morulae or other *Ehrlichia canis* stages were identified in a buffy coat blood smear. A PCR and IFAT were negative for *Ehrlichia canis*. The dog made a full recovery with treatment.

A beagle dog presented to the Institute of Veterinary Animal and Biomedical Sciences (IVABS) Massey University with a history of inappetence and general malaise, died. Histological examination found lesions consistent with an immune-mediated vasculitis. The lesions and their distribution were typical of several immune mediated conditions such as 'beagle pain syndrome'. Screening for exotic organisms such as *Rickettsia* and *Ehrlichia* using special staining was negative. *Ehrlichia canis* was excluded by IFAT.

Canine heartworm ruled out

New Zealand Veterinary Pathology reported a 15-month-old dog with a history of uveitis and an eosinophilia. The dog had been imported from Australia as a month-old pup. Heartworm was ruled out following a negative Knots test and a negative antigen ELISA.

Myxomatosis ruled out

Several members of the public noted rabbits dying (about ten seen dead or dying) in the Nelson region over two months. The rabbits had respiratory distress, with tachycardia, lethargy and a white mucoid discharge around the eyes. The most significant postmortem findings were congestion of the lungs and hyperaemia of subcutaneous fat. Pharyngeal swabs and lung tissue samples were culture negative for *Pasteurella* species. Rabbit haemorrhagic disease (RHD) was diagnosed following a positive PCR test on liver, spleen and serum from a dead rabbit. Myxomatosis was ruled out after diagnosis of the endemic RHD.

Exotic tick investigated

A small tick removed from a person's head two days after they returned from Sydney was lost. The next day IDC was notified.

The household had a dog and a cat. The cat could have been in contact with the tick. An AgResearch entomologist was consulted and the owner instructed to vacuum-clean the areas in the house where the tick was lost and to discard the vacuum-cleaner bag. All in-contact clothing was washed and the dog and cat were treated with an acaricide for two months. The risk of a tick (possibly an *Ixodes* species) population establishing in the house is considered extremely low.

A practitioner diagnosed hypomagnesaemia in beef cattle showing neurological signs at a livestock sale in Christchurch, and noted ticks on the animals' ears. A MAF reference parasitologist identified the ticks as *Haemaphysalis longicornis*, the New Zealand cattle tick.

Avian influenza ruled out

A member of the public reported two of her poultry had died with oedema of the comb and wattles. Postmortem of one showed enlarged liver, thymus and spleen. Bacterial culture yielded a heavy mixed growth from swabs of cardiac tissue, a light mixed growth from the lungs and no growth from the thymus. No *Pasteurella multocida* was cultured. Virus isolation for avian viruses was negative. The cause of death was considered to be a localised bacterial infection of the heart.

Avian influenza and Newcastle disease ruled out

The owner of 80 Muscovy ducks reported 20 ducks died over four to five weeks. The ducks were free ranging and were fed barley and some table scraps. All ducks examined appeared underweight. Two ducks that had been off colour were slightly ataxic and had subnormal rectal temperatures, and were euthanased. No gross pathology was observed. Histology gave no cohesive explanation for the ducks' poor condition. There was no evidence of avian influenza or Newcastle disease. *Salmonella* Typhimurium phage type 160 and *Streptococcus suis* were isolated from the cloaca and pharynx, respectively, from bird one. *Aeromonas hydrophila* group 2 was isolated from the pharyngeal swabs from both birds and *Yersinia enterocolitica* biotype 1A from the pharyngeal swab of bird two. The cause of the deaths is uncertain but likely to be multifactorial including inadequate nutrition.

Over a two-month period half of a population of 12 backyard layer chickens died after developing cyanotic combs, gaping, diarrhoea, gradual inappetence and stopping laying. A sick bird that was euthanased had a heavy lice infestation. Microbiology was negative for *Salmonella* and *Campylobacter*. The only histological finding was highly oedematous lung tissue with protein-rich fluid filling air capillaries, alveolar ducts and peribronchiolar connective tissue in some areas, but no signs suggestive of AI virus infection. Exotic viral diseases were ruled out on clinical signs and histopathology. The cause of the oedema is not known.

An Auckland primary school caretaker reported approximately 60 wild birds dying over six weeks. No definitive diagnosis was

reached, although *Salmonella* Typhimurium DT160 was suspected. Only one bird, a thrush, was found in sufficiently fresh condition for laboratory testing. Culture from the liver was negative for *Salmonella*, but positive for *Escherichia coli*, *Enterococcus* species and *Alcaligenes faecalis*. Histopathologically, only non-specific lesions were noted.

On separate occasions over three weeks, a member of the public noted three birds displaying neurological signs. The last bird seen was euthanased. There were no significant findings on gross pathology, histopathology (including the brain) and no isolation on culture of the liver. The cause of the signs is not known and the informant has seen no further sick or dying birds.

Exotic viruses were ruled out by laboratory investigation of a thrush that died suddenly. A number of similar deaths were reported to MAF. A veterinarian found no gross abnormalities. Histological examination revealed multifocal necrosuppurative hepatitis consistent with salmonellosis although culture was unsuccessful.

Exotic mites ruled out

A member of the public reported imported feather dusters from an Auckland retail outlet had what was believed to be small eggs attached. A MAF reference parasitologist found live Lepidoptera larvae and a small number of *Mallophaga* (chewing lice). The larvae were identified as *Tineola bisselliella*, the cosmopolitan webbing clothes moth. The lack of development suggested these were of New Zealand origin and probably infested the dusters during storage before sale. The lice belonged to three species – *Goniodes dissimilis*, *Lipeurus caponis* and *Menopon gallinae* – all typically found on birds. All had been dead for some time, possibly from treatment of the birds for parasites in their country of origin or after fumigation as specified by the MAF import health standard for the importation of feathers.

Pacheco's disease ruled out

Approximately six birds died over eight weeks in two adjacent aviaries, each containing around 50 birds of various species. All affected birds were lethargic and 'fluffed up' and were sick for about two weeks before death. All birds were wormed and fed a balanced diet. A veterinarian examined a dead juvenile Barraband parakeet and postmortem showed only that the bird was in very poor body condition. Only fixed liver and pancreas were available for histology. A PCR test on fixed liver tissue was negative for polyoma virus and negative for Pacheco's disease.

Macaw wasting disease ruled out

An imported Macaw was reported with clinical, radiological and gross pathological signs of Macaw wasting disease. Also known as proventricular dilatation syndrome (PDS), this has an uncertain, although probably viral, aetiology. It has not been reported in New Zealand and is an unwanted organism. Clinical signs and proventricular enlargement or dysfunction raise suspicion of PDS.

The disease has spread around the world and affects many species of parrots with moderate consequence and a high likelihood of entry where live parrots are introduced. It is characterised by involvement of central and peripheral nervous tissues with lymphoplasmacytic inflammatory infiltrates. Definitive diagnosis requires demonstration of characteristic lymphoplasmacytic infiltrates in the ventriculus, proventriculus, crop and brain. In the present case the key microscopic changes were absent. No alternative diagnosis was made. Other aetiologies considered included upper gastrointestinal tract infection, gastrointestinal obstruction, neoplasia, trauma, mal-assimilation disorders, malnutrition and lead toxicity.

Exotic *Salmonella* in an iguana investigated

New Zealand Veterinary Pathology and the Auckland Zoo reported *Salmonella enterica* subspecies *enterica*, serovar Mount Pleasant, had been cultured from a cloacal swab from a juvenile green iguana (*Iguana iguana*). This serovar has not previously been identified in New Zealand. ESR made the identification following pre-purchase examination before sale to the Auckland Zoo. The source of infection was likely to be from the parent iguanas, other lizards on the same property or the food. The juveniles were reported to have been fed faeces from the adults to promote colonisation of intestinal flora. The parent iguanas had died and were burnt. Flukers® crickets imported as lizard food from the United States were negative for *Salmonella*. Lizards on the property yielded two other exotic serovars of *Salmonella enterica*, Onderstepoort and Biljmer. A technical review of the relevance of *Salmonella* Mt Pleasant showed there is little published information. There was no apparent illness in the juveniles but the adults were reported to have died suddenly with diarrhoea. As there were no reports of *Salmonella* Mt Pleasant being pathogenic in reptiles, and the only reported case of pathogenic effects was in humans, the previously exotic *Salmonella* serovar was not considered to be an unwanted organism and MAF restrictions were lifted.

Tracheal mites of honey bees ruled out

A private apiary consultant reported adult bee deaths in a number of apiaries visited on behalf of beekeepers providing pollination services to Bay of Plenty kiwifruit and avocado orchardists. There were no problems with spring build-up of the hives, and the problems were only noted in early November. Pesticide residue testing identified low levels of iprodione, a fungicide considered to have low toxicity for bees. Exotic causes of adult honey bee mortality were ruled out after testing of samples from four apiaries gave negative results for *Acarapis woodi* (tracheal mite) and targeted exotic external mites including *Tropilaelaps clareae*. Examinations identified a single oribatid mite (sub-order Cryptostigmata) that is a detritus feeder and general hive scavenger, considered of no pest significance to bees.

Small hive beetle ruled out

A Cromwell apiarist reported beetles in his honey house to an AgriQuality Apiary Advisory officer (AAO). It was uncertain whether damage to honey combs was associated with the beetles or an incidental finding. The beetles were identified as *Dermestes lardarius* (Coleoptera: Dermestidae), the larder beetle, which occurs in New Zealand, eats many animal and plant products and can damage timber structures. They are also reported to 'cannibalise' wasps.

A Hamilton AgriQuality AAO reported a beetle-like parasite on a honey bee from Helensville. The 'beetle' was identified as the head of a worker ant from the Family Formicidae. Ants frequently visit flowers to collect nectar. The bee and ant must have been visited the same flower at the same time. It is unclear who decapitated the ant and how.

European foulbrood ruled out

A scientist from HortResearch suspected European foulbrood when typical brood signs were noted in a beekeeper's hives. The samples grew no Gram-positive lanceolate cocci typical of *Melissococcus pluton*. PCR testing was also negative. The brood signs were probably caused by parasite mite syndrome; low levels of *Varroa destructor* were present.

An AgriQuality AAO in Hamilton reported suspect European foulbrood in a single hive belonging to a Drury beekeeper. Submitted larvae were negative for EFB PCR. No *Melissococcus pluton* was isolated and no Gram-positive lanceolate cocci typical of *M. pluton* were seen. Signs were likely to be those of half-moon syndrome.

Exotic bee mites were excluded by laboratory examination of a sticky board from a hive with adult bees dying in large numbers. A mite from the hive was identified as *Melittiphis alvarius*, a scavenger of pollen. The hive was also negative to European foulbrood on culture and PCR. Hive collapse had most likely occurred through queen failure.

Perkinsus marinus ruled out

A diver collected, from Moa Point, Wellington, several paua that had nodules between the foot and the mantle. The paua were returned to their shells and frozen. *Perkinsus marinus* was ruled out by histology. The small ovoid lesions showed no histological abnormalities, except for a widespread mycosis. This may be related to a recognised mycosis causing shell disease in paua and common in the Wellington region.

Betanodo virus ruled out

A fish expert detected vacuolation of the brain of juvenile Yellowtail Kingfish (*Seriola lalandi*) showing neurological abnormalities from

a farm in Northland. Exotic viral encephalopathy and retinopathy, caused by Betanodo virus, was one of the differential diagnoses. No gill lesions or other abnormalities were detected at post mortem examination of ten affected and five unaffected fish. Histology, virus isolation, Betanodo virus PCR and *Brucella* PCR of brain specimens were all negative. Although morbidity rates were low, new cases kept occurring. Three weeks later a further 20 fish were submitted for testing. Again histology and Betanodo virus PCR and virus isolation were negative. Mortality levels returned to normal after three weeks. Environmental factors are a possible cause of the neurological abnormalities.

Seasquirt (*Styela clava*) investigated

On 8 September 2005, *Styela clava* (clubbed tunicate) was identified from Viaduct Harbour, Auckland, and in Lyttelton Harbour on 3 October 2005. Subsequently, *Styela clava* was confirmed retrospectively occurring in August 2002 and May 2002 in the Auckland region and Lyttelton Harbour, respectively. However, it may have been present up to seven years before this. A Biosecurity New Zealand led response to determine the spread of *Styela clava* surveyed 25 sites nationwide. *Styela clava* was detected at low densities in two other sites: Tutukaka and Lyttelton marina. Survey sites were selected according to risk, based on proximity to infested sites, volumes of maritime traffic, tracings from infested sites, and proximity to high value areas (such as aquaculture management areas). Biosecurity New Zealand also embarked on a public awareness campaign to encourage the public to report any sightings of *Styela clava* and to promote vessel cleaning, which lessens the risk of spread. A fact sheet was produced for general marine users, a waterproof identification card for divers and newspaper advertisements in daily newspapers. A number of finds in the Hauraki Gulf have been reported as a result of this publicity.

Mortality event in tuatua investigated

A mortality event of tuatua (*Amphidesma subtriangulatum*) shellfish in Christchurch was hypothesised to have an environmental aetiology. No pathological features attributable to an infectious process were detected following histological examination of specimens.

Exotic diseases of mussels ruled out

A member of the public reported vesicles filled with clear fluid on the mantle and viscera of mussels collected from a reef at Kakanui, Otago. The mussel viscera also appeared smaller than usual and dull in colour. Lesions were noted after the mussels had been boiled before eating. Histology identified considerable sloughing of the epithelium in the digestive diverticulae in all samples, and inflammatory foci associated with re-absorption of gametes in many mussels examined. The epithelial sloughing in the digestive diverticulae did not involve the digestive tract or digestive ducts and was considered to result from infection with a known picornavirus. There was no suggestion of an exotic pathogen, or any issue of public health significance.

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