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Katherine Clift

The importance of biosecurity

Welcome to the December 2007 edition of Surveillance. The importance of biosecurity, of which surveillance for pests and diseases is an integral part, has been highlighted recently by the equine influenza outbreak, with its associated containment and control efforts, in Australia.

New Zealand and Australia have close ties in primary industries, as the recent New Zealand response to the outbreak of equine influenza in Australia has shown. Horses that had entered New Zealand from Australia within the month prior to the detection of the Australian outbreak were traced and assessed, using information provided by both the New Zealand horse industry and Australian authorities. All horses were found to be free of equine influenza. Further information on the investigation can be found in this edition of Surveillance. A number of MAF Biosecurity New Zealand staff with various areas of expertise have recently visited Australia to assist with disease control efforts and benefit from participating in a large response. Lessons learned from the Australian outbreak will be watched with interest in New Zealand.

The equine influenza outbreak in Australia also provides a timely reminder to consider how New Zealand might respond to an incursion of a disease or pest. MAF Biosecurity New Zealand is currently seeking comment on two documents that relate to this issue – a discussion paper exploring joint decision-making and resourcing between agricultural industries and government for preparedness activities (such as surveillance, response readiness and biosecurity planning) and incursion responses, and a draft policy for responding to pests and diseases. Agreements between industry and government for responding to pests and diseases exist in Australia, and one such agreement has been invoked in responding to equine influenza. Further information on the discussion document and draft response policy, including information on how to provide comment, is included in this edition of Surveillance.

Although equine influenza has been of increased interest recently, work has been continuing in other areas, including on the Biosecurity Surveillance Strategy. After taking into account areas identified for improvement in the review of the current state of biosecurity surveillance, and undertaking research into various topics that could contribute to improving biosecurity surveillance, drafting of a framework for the future of biosecurity surveillance has commenced. Public comment on the draft of the Biosecurity Surveillance Strategy will be sought in mid to late 2008.

Katherine Clift
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Managing the risk of equine influenza in horses imported from Australia during the 2007 Australian epidemic

When notified on 25 August 2007 that equine influenza had occurred outside of quarantine in Australia, MAF Biosecurity New Zealand immediately banned importation of horses from Australia. Horses imported from Australia during the previous 24 days, and their contacts, were examined and tested, as were any horses with respiratory disease. No horses had clinical signs consistent with equine influenza and all horses tested were negative by polymerase chain reaction.

Equine influenza (EI) is a respiratory disease of horses and other Equidae (donkeys, mules and zebras)[1][2][3]. EI is caused by influenza A viruses from the family Orthomyxoviridae. There are two recognised subtypes – H7N7 (type one) and H3N8 (type two) – and strain differences within subtypes. Viruses from subtype one have not been in general circulation since the late 1970s and probably persist only at a low level in some parts of the world. However, type two remains a significant cause of respiratory disease in horses worldwide[1]. Predominant clinical signs include a fever and a cough at rest. EI is highly contagious, and in a naïve horse population, such as that in New Zealand, outbreaks spread rapidly affecting horses of all ages. The disease is characterised by high morbidity and low mortality.

On 17 August 2007, EI was suspected in a clinically sick horse at the Eastern Creek Quarantine Station, Sydney, New South Wales (NSW). The disease was confirmed on 20 August and MAF Biosecurity New Zealand (MAFBNZ) notified of this case on 23 August 2007. Sick horses were also seen on 22 August 2007 outside of quarantine at the Centennial Parklands Equestrian Centre (CPEC) adjoining Centennial Park in central Sydney. These horses were confirmed on 24 August 2007 as having EI[4] and MAFBNZ notified of the outbreak on 25 August 2007.

Prior to this incursion, Australia and New Zealand were the only major horse breeding countries of the world to have remained free of EI[1]. Because of the relatively free movement of horses between Australia and New Zealand before notification of the EI outbreak, it was important to assess whether equine influenza virus (EIV) had been inadvertently introduced into New Zealand.

On the day of notification MAFBNZ responded by stopping all imports of horses from Australia and increasing border controls for horse equipment and horse semen. That same day the Exotic Disease Response Centre (EDRC) was set up at the Investigation and Diagnostic Centre (Wallaceville, Upper Hutt). At this time the date of EIV release from Australian quarantine was unclear but considered unlikely to have occurred before 1 August 2007.

Consequently, the objective of the EDRC was to determine the EI status of all equine imports from Australia during August 2007.

This article outlines the investigation undertaken by operational staff at the EDRC to achieve the objectives described above.

Methods

Casing and tracing

An EDRC casing and tracing task group was given the objective of collecting the details of all horses imported from 1 August 2007, including their current New Zealand locations (Figure 1). A complete list of imports was obtained from several sources: MAFBNZ import group, trans-Tasman import companies, and lists supplied from Equestrian Sports New Zealand, New Zealand Thoroughbred Racing and Harness Racing New Zealand.

The team commenced phoning New Zealand owners of imported horses on the day MAFBNZ was notified of the Australian outbreak. Horse owners were asked to provide details of the disease status of the imported horse and all in-contact horses. The horse owner was also asked voluntarily to separate the imported horse/s and any in-contacts from other horses, and remove them from paddocks adjoining the boundary fence. They were advised that a veterinarian

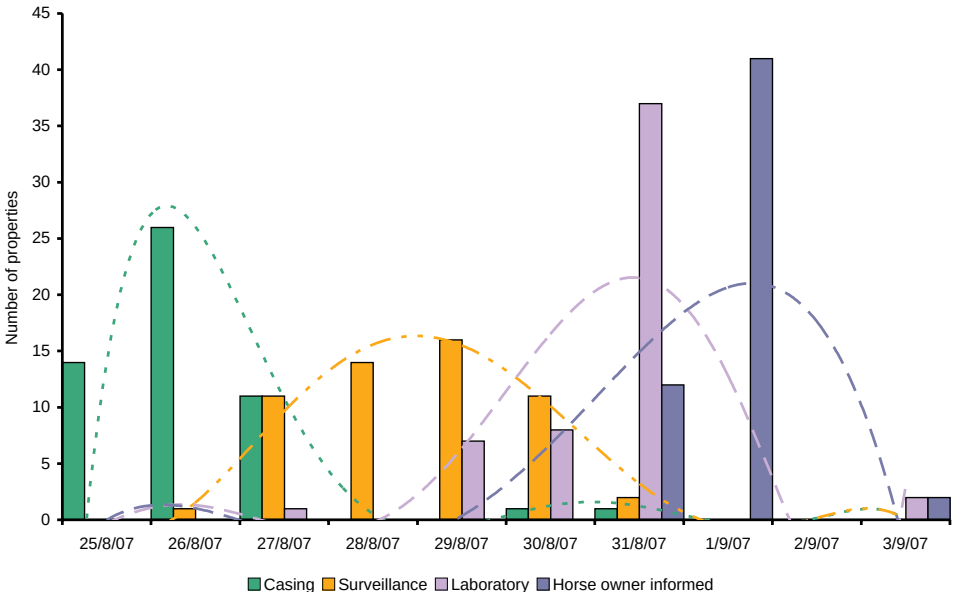

Figure 1: The cascade of operational activity carried out between 25 August and 3 September 2007, as part of MAFBNZ equine influenza response

would visit the property in the forthcoming days.

Tracing was also undertaken to clarify the location of source farms in Australia, the network of contacts within Australia and New Zealand, and infected zones in Australia.

Risk management

Criteria were developed to evaluate the risk posed by imported horses and the property visits were prioritised accordingly.

The period considered to be of greatest risk was the immediate 10 days before MAFBNZ was notified (15–25 August 2007). This was based on the assumption that an infected horse would not be infectious beyond 10 days and that infected horses imported more than 10 days earlier (1–14 August 2007) or their in-contacts would have displayed clinical signs of EI.

Risk was stratified into three levels based on the date the horse was imported and whether it and associated horses showed clinical signs. When information from tracing projects became available, the potential for contact between imported horses and infected Australian horses was also assessed.

The three risk levels were defined as:

- high risk: imported horses, or in-contact horses, with respiratory signs;
- medium risk: healthy horses imported from Australia in the previous 10 days, ie between 15 and 25 August;
- low risk: healthy horses imported from Australia between 1 and 14 August.

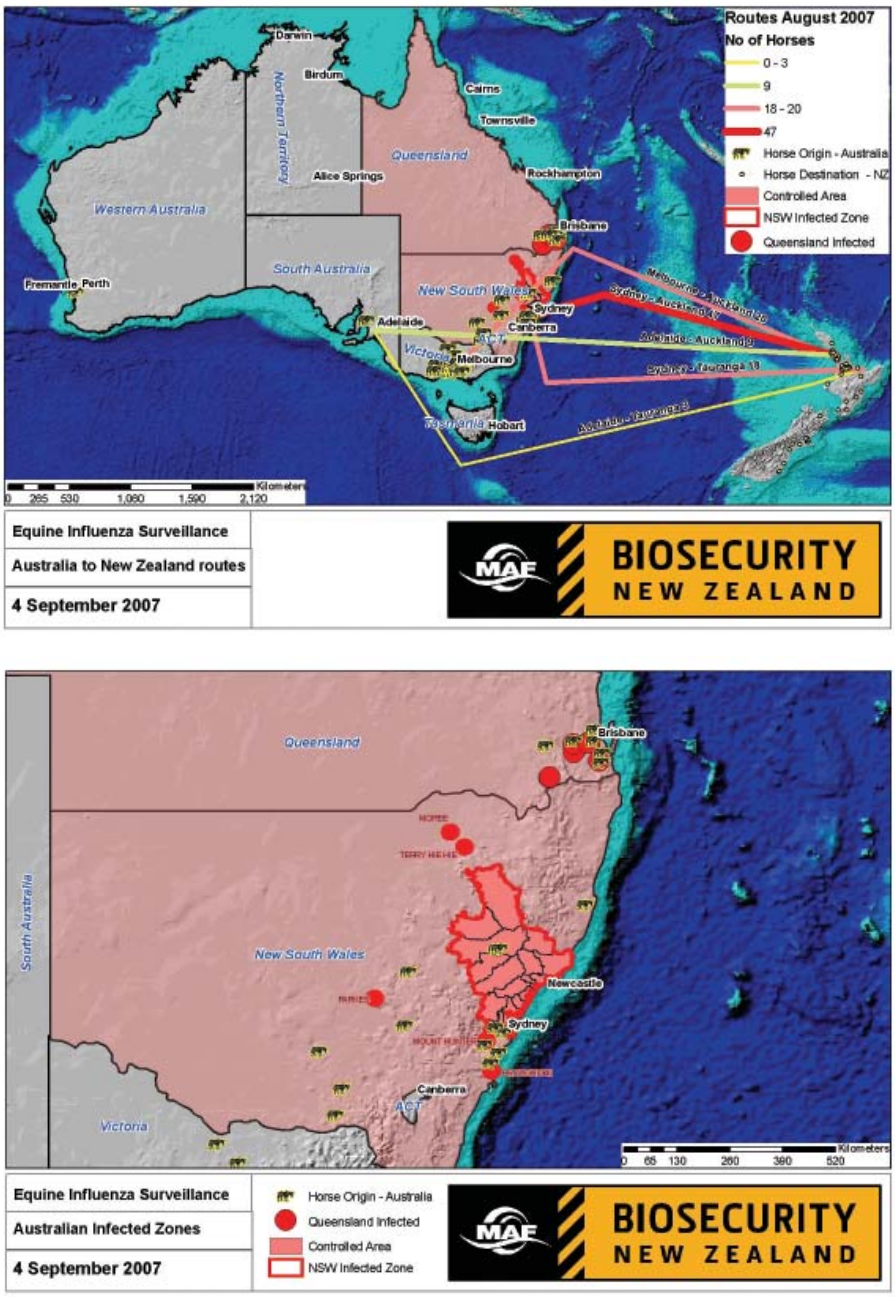

Figure 2: Map A. The Australian origins and New Zealand destinations of horses imported during August 2007. Map B. The Australian origins of imported horses in relation to known (at 4 September 2007) equine influenza infected properties (maps provided by Quenten Higgan, AsureQuality)

Surveillance

The New Zealand EI response plan completed by MAFBNZ and ratified by the New Zealand Equine Health Association (NZEHA) in 2006 was used as the basis for procedures and data collection forms for farm visits[5]. AsureQuality (Hamilton, New Zealand) veterinarians trained in biosecurity procedures were used for all visits to medium and high risk farms. Private veterinary practitioners were enlisted to carry out visits to low risk farms. Surveillance visit tasks are detailed below.

- High risk (imported horses and in-contacts tested): collection of epidemiological data, vaccination history, clinical examination, serology (haemagglutination inhibition [HI] test), collection of nasal swabs for the real time reverse transcriptase TaqMan® polymerase chain reaction (RRT-PCR) for influenza A, +/- virus isolation.
- Medium risk (imported horses and in-contacts tested): collection of epidemiological data, vaccination history, clinical examination, serology (HI), collection of nasal swabs for influenza A RRT-PCR, +/- virus isolation.
- Low risk (imported horses tested): collection of epidemiological data, clinical examination, serology (HI).

Nasal swabs were tested using the influenza A RRT-PCR, which

targets the matrix gene of influenza A viruses[6]. Positive controls for both EI subtypes were used as part of the testing procedure.

Confirmatory testing for any samples that gave positive or suspicious results in the screening test included further testing using a conventional nested PCR targeting the matrix gene of influenza A viruses[7] followed by sequencing of PCR product and virus isolation in embryonated hen eggs.

Serological testing involved an in-house HI test against EI subtype two using the protocol recommended by the OIE Manual for Diagnostic Tests and Vaccines (2007). Samples with titres to H3N8 were also tested against subtype one. Where there was no history of vaccination, a plan was put in place to test a second serum sample 14-21 days later to check for a fourfold rise in HI titre.

Test interpretation

A case definition was developed for cases of EI and suspect cases. Suspect cases were defined as: imported horses or in-contacts demonstrating clinical signs consistent with EI; or horses with nasal swabs positive for EI using RRT-PCR; or unvaccinated horses serologically positive using the HI test. A case was defined as being positive to conventional PCR with confirmatory sequencing; positive by virus isolation; or having a fourfold rise in HI titre over a two- to three-week period.

As EI vaccination is common worldwide, and many horses imported into New Zealand have travelled to countries where the disease is endemic, it was necessary to assess whether a positive titre to type two in the HI test indicated true exposure to field strains of EIV or resulted from previous vaccination. Criteria used to assess the significance of the result and the need for further investigation included:

- presence of titres to both HI subtypes and titres suggestive of an immune response to EI vaccine antigen[8];
- vaccination status of the HI-positive horse (determined from vaccination records, country of birth, country of origin);
- prevalence of HI-positive horses in the same import consignment and on the New Zealand destination property;
- presence of respiratory disease consistent with EI;
- prevalence of respiratory disease in the import consignment, and in imported and non-imported horses on the New Zealand destination property;
- the Australian origin of imported horses in relation to known EI infected properties;
- presence of EIV RNA detected by RT-PCR, in nasal swabs collected from any of the horses imported from Australia during the month of August or an in-contact horse tested.

Results

A total of 16 consignments, involving 96 horses, was imported from Australia between 1 and 25 August 2007. Fifty-five horses arrived prior to 15 August 2007 and 41 thereafter. The last import consignment was on 20 August 2007. The majority of imported horses (85/96; 85%) were of Australian origin. Eleven horses were transiting through Australia, having originated from Germany (two), USA (two), Singapore (one) and Hong Kong (six). As part fulfilment of New Zealand import requirements these horses had completed two weeks post-export quarantine in Sydney. Horses were imported by three air freight companies and one sea freight company (Figure 2, map A). The median number of horses per consignment was five (mean = 6, range 2–18). The horses were delivered to 41 North Island and 12 South Island properties (Figure 3). The locations of the Australian properties of origin in relation to known areas of infection are shown in Figure 2, map B.

In addition to imported horses, the EDRC was notified of two properties whose horses had respiratory disease during the response (report cases). On examination, the clinical signs in these horses were not consistent with EI. No clinical signs in imported and in-contact horses were consistent with EI.

Results of serology and PCR testing are presented in Table 1. All 88 high- and medium-risk horses were negative to EI by RRT-PCR. Sampling and PCR testing was sufficient to detect EIV with a 95% confidence assuming 50% of a consignment of six horses were excreting EIV and the sensitivity to detect EIV was 68% (given sampling technique, sample handling and the test sensitivity of the EI PCR; calculated using FreeCalc[9]).

Sera from 15 of 131 (11%) horses tested using the EI HI test had titres to type one and/or type two EI antigen. Seropositive horses were present in seven of 16 (44%) consignments. Case records showed that all of the EI seropositive horses had been vaccinated previously.

All veterinary visits and transfer of samples to the Animal Health Laboratory were completed by 1 September 2007. Between 31 August

Risk group	Description	Farms	Imported horses	In contact horses or horses reported with respiratory signs	RRT-PCR	HI
High	Imports (15/8/07 to 25/8/07) with respiratory disease and in-contacts	15	26	14	0/39	3/40
High	Report cases	2	0	10	0/3	0/10
Medium	Imports (15/8/07 to 25/8/07) and in-contacts	18	38	11	0/44	6/49
Low	Imports (1/8/07 to15/8/07)	20	32	-	0/2	6/32

and 3 September all owners were informed that horses had tested negative to EI by influenza A RRT-PCR. The EDRC was stood down following reporting of the final test negative results on 3 September 2007. The timeline for surveillance tasks is shown in Figure 1.

Discussion

Data gathered during this investigation provided evidence that horses imported from Australia during August 2007 did not introduce EI to New Zealand. No imported horses or associated horses presented clinically with signs consistent with EI and nasal swabs from all horses tested were negative to EI by the influenza A RRT-PCR.

Negative PCR results and the absence of clinical signs of EI in any of the imported horses suggested that the HI titres observed in 11% of the horses tested were unlikely to have arisen from recent exposure to EIV. Examination of case records indicated that all the seropositive horses had been vaccinated for EI subtypes one and/or two previously. All but two of the 15 seropositive horses had HI titres to both type one and type two EI antigen; this is consistent with a vaccine response, as antigen from both subtypes is often included in vaccine formulations. With some of the modern EI live vaccines only subtype two is included[1]. The HI titres for horses tested during this response had a similar range and distribution to those described by Horner and Ledgard[8] in a random survey of New Zealand horse sera. In Horner and Ledgard's survey all positive titres were determined to have arisen from EI vaccination. No clustering of seropositivity within import consignments was apparent in the current investigation.

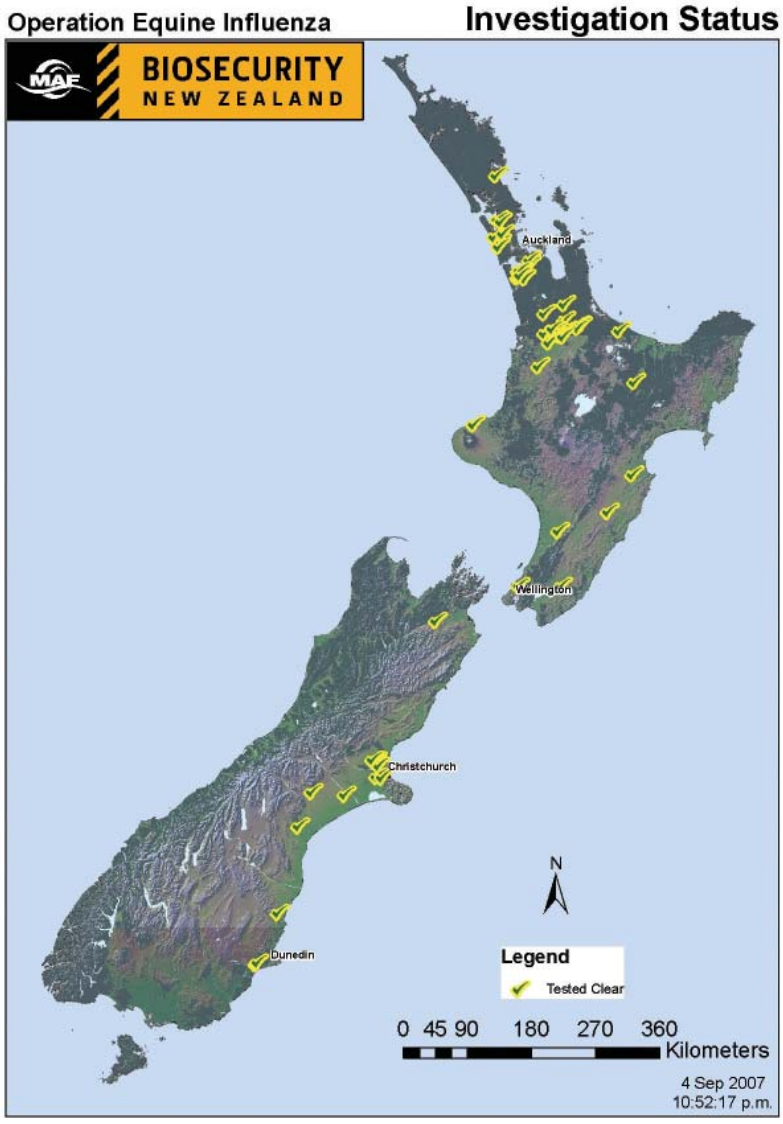

Figure 3: The New Zealand location of horses imported from Australia during August 2007. All horses tested negative to equine influenza

Initially Australian authorities identified 8 August 2007 as the most likely start of the risk period. MAFBNZ took the precaution of adding a buffer period and investigated the EI status of all horses imported from 1 August 2007. This approach proved to be well founded as Australian epidemiological assessment indicated that the entry of EIV into NSW may have occurred as early as 3 August 2007[4]. Immediate voluntary quarantine of imported horses, and prioritising veterinary visits based on risk, allowed efficient processing of horses while mitigating potential transmission of EI had it been introduced.

The cost of the introduction of EI to New Zealand would likely be substantial. A cost-benefit analysis is presently being carried out by the New Zealand Institute for Economic Research to assist with quantifying the impacts.

In its media release of 27 August 2007, the Australian Racing Board estimated that the thoroughbred horse industry contributes approximately $7.7 billion to the Australian economy. The EI outbreak in Australia is likely to affect this contribution and that from other sectors of the horse industry. There is likely to be an indirect cost to the New Zealand horse industry through disruption of horse racing and breeding programmes.

The threat of EIV introduction remains a constant risk to the New Zealand horse population. Debate on the type of quarantine procedures for horse imports from Australia into New Zealand is likely to ensue if endemic infection occurs in Australia. Adopting stringent pre- and post-quarantine controls between the two countries would affect the current relatively free trade of horses. About 1,350 horses were transported from Australia to New Zealand by air between June 2006 and June 2007 by major horse transport companies. About 230 horses were imported by sea freight in the same period (Bruce Graham, NZEHA, 2007, personal communication).

As a result of the Australian incursion MAFBNZ has identified the need to review several aspects of EI response policy including the use of vaccination (pre-emptive or in response to an incursion). Other ongoing work includes a cost-benefit analysis of maintaining the

current quarantine measures for horse diseases between Australia and New Zealand, a review of import health standards and biosecurity measures of quarantine facilities relating to EI, and consideration of the availability of quarantine facilities if there were a need to adopt quarantine procedures for horse imports from Australia.

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Responding to pests and diseases – release of two documents for consultation

MAF Biosecurity New Zealand (MAFBNZ) has released its draft 'Policy for responding to pests and diseases (risk organisms)' for comment. The policy sets out what the Crown will do and what people can expect of responses to pests and diseases. The policy's focus is on roles and decision-making, using a consistent approach across all sectors, no matter how big or small the response may be.

The policy emphasises that MAFBNZ will continue to respond where organisms pose nationally significant threats to New Zealand's people, environment and economy, with the aim of achieving the best overall outcome for New Zealand. A greater emphasis has been placed on preparing for specific pests and diseases and on long-term management where eradication may not be feasible.

Alongside this, a group of representatives from primary production sectors and MAFBNZ have been working for more than two years on new ways for Government and industries to work together to address biosecurity threats more effectively. A public discussion paper, 'Joint decision-making and resourcing for readiness and incursion responses', has been released and outlines a framework for joint decision-making and resourcing for readiness and incursion responses that directly impact on primary production industries.

The framework is based on the idea that those directly benefiting from an activity should be involved in decision-making and, in return, contribute to the direct costs of that activity. This approach would give industry more say in which pests and diseases we should plan for, including how to mitigate the potential impacts of, and better respond to, pests and diseases of common concern.

Both the framework and the process for developing the framework represent a major shift in culture – one from MAF being the decision-maker and consulting with industry, to one of joint decision-making by consensus. If adopted, this approach would not, however, change MAF's responsibilities for pests and diseases that are not covered by a joint agreement with primary production organisations. In the absence of any joint government–industry agreement the standard approach set out in the new 'Policy for responding to pests and diseases (risk organisms)' applies.

Comments and formal submissions on both documents are due 14 December 2007. Copies of the draft policy and discussion paper, including information on how to make a submission, are available from the MAFBNZ website (www.biosecurity.govt.nz) under the strategy and consultation section.

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New Zealand's contribution to explaining the pathogenesis of atypical scrapie

New Zealand's freedom from TSEs of livestock is the reason negative control material is sought from us for evaluation of new TSE tests. The hypothesis that the recently recognised brain condition currently referred to as atypical scrapie may be just a spontaneous degenerative condition has aroused international interest in determining whether atypical scrapie also occurs in TSE-free countries such as New Zealand.

New Zealand is free from the transmissible spongiform encephalopathies (TSEs) of livestock. This country has never had a case of bovine spongiform encephalopathy (BSE) or chronic wasting disease (CWD) of deer. There have been two incursions of scrapie in 1952–54 and 1976–77. Both occurred in imported animals. The first occurrence was in 1952 in sheep imported from the United Kingdom. This outbreak was stamped out by 1954 through the slaughter of all in-contact sheep and resting or de-stocking pastures.

In 1976–77 there was a second incident in which scrapie was detected in imported sheep while they were still in quarantine on an off-shore island. This outbreak was eradicated by stamping-out and total de-stocking of the island. It has never been re-stocked. The reasons why scrapie was not imported with the founding sheep breeds during the early settlement of New Zealand have been discussed by Bruere[1]. It is this long history of freedom from TSEs that is the primary reason why brain material from New Zealand cattle, sheep and goats has been sought for use as negative control material in the development of rapid tests for BSE and scrapie.

For the purpose of clarity, throughout this article scrapie will be referred to as classical scrapie to clearly distinguish it from a novel neurological condition of older sheep that has been dubbed "atypical scrapie".

Atypical scrapie is a newly described condition of sheep[2]. The name arose because the condition was first detected during screening for classical scrapie but the laboratory results of the testing were "atypical" or unclassifiable. The test results have demonstrated that the condition is clearly neither BSE in sheep nor classical scrapie, but the significance of the findings is not at all clear. It is likely this classification will be refined as our understanding is enhanced by research studies and further experience gained during surveillance activities. There are no recognised human health or food safety risks associated with atypical scrapie (or classical scrapie).

Atypical scrapie has never been detected in New Zealand.

All countries that have conducted large scale screening of the healthy slaughter sheep population for TSEs have detected atypical scrapie, including countries that are free of classical scrapie[3][4]. The frequency of detection is approximately 0.07% of rapid tests conducted and appears remarkably consistent across countries[5]. Another significant source of case material is fallen stock[6][7][8]. A minority of cases have been detected in animals showing clinical signs of neurological disease[2][9][10]. Whether these clinical cases are later detections of the same underlying pathology or whether they represent a different condition remains to be determined.

Most cases of atypical scrapie occur in older animals with 60% of cases being older than five years and the youngest reported to have been 30 months[8]. Almost all occur as single cases and, even when the entire flock is sampled, no further cases are usually found. Where further cases were found, the flock size was proportionately larger[8].

Over 90% of all atypical scrapie cases have been detected with the BioRad Platelia rapid test or its successor the BioRad TeSeE ELISA in obex tissue. This is the site most suitable to detect classical scrapie or BSE in small ruminants, for which European surveillance programmes were aiming. On the other hand, the highest concentrations of abnormal prion protein for atypical scrapie are found in the cerebellum[2], although there seems to be greater variability in neuro-anatomical distribution of the atypical scrapie prion protein[11] than is seen in classical scrapie.

Even when using obex tissue rather than cerebellum, the BioRad TeSeE ELISA was able to detect numbers of atypical scrapie cases similar to the numbers of classical scrapie cases. Other screening tests failed to detect comparable numbers of atypical scrapie cases during scrapie surveillance in the UK. The limited evaluation data available for other screening tests for the detection of atypical scrapie[12][13] suggest that by using cerebellum and optimised conditions, some of these test methods are able to achieve higher sensitivities. Based on the limited evaluation data, some of these methods have already been recommended for atypical scrapie testing[12][13]. The next evaluation round for TSE screening methods planned by the European Food Safety Authority will more comprehensively evaluate the use of such test methods for the detection of atypical scrapie in small ruminants. The sheep and goat brain samples from New Zealand are expected to be used in this evaluation round as negative controls.

Experimental transmission of atypical scrapie by intracranial inoculation of brain homogenate from such cases has been successful in ovinised transgenic mice[14] and sheep of the same genotype[15]. In both cases of experimental transmission, the incubation period has been far shorter than the typical reported age of affected sheep. There is no evidence that suggests natural transmission occurs among sheep. Some authors[16] have hypothesised that the observed epidemiology is most consistent with a spontaneous aetiology, while others[14] have argued an infectious aetiology.

Because of New Zealand's freedom from TSE diseases of livestock,

the New Zealand Ministry of Agriculture and Forestry (MAF) has on several occasions supplied brains from sheep and cattle for use as negative control material for the evaluation of TSE rapid tests. New Zealand has been the major supplier of negative control material for test evaluation. For example, in 2001 and 2002 a total of 1,084 sheep caudal brain stem samples were provided to the European Commission for use in diagnostic test evaluation. One thousand New Zealand sheep brain and lymphoid tissue samples from this shipment were used in the two European small ruminant TSE test evaluations in May and September 2005[12][13]. No TSEs were detected in these samples, even though several of the evaluated test methods would have detected atypical scrapie if it were present, including the already mentioned BioRad TeSeE ELISA. MAF is again preparing to provide further sheep brains to the European Commission. It is proposed to send a maximum of 1,000 brain stems and cerebellums together with up to 200 whole brains from sheep over three years of age, as well as up to 300 whole goat brains.

If correct, the spontaneous aetiology hypothesis of atypical scrapie raises some challenging issues for New Zealand. For example, one would expect all sheep flocks throughout the world to be equally at risk of developing the condition. There might be variations in frequency, due to differences in the proportions of different genotype(s), but ultimately the condition would be expected to manifest itself in any sheep flock of sufficient size. Detection of a single case in New Zealand would be suggestive of, but not proof of, a spontaneous aetiology.

Finding abnormal prion proteins in a national flock with a long history of freedom from classical scrapie would also raise significant questions with respect to explaining and understanding the behaviour of these prion proteins in this flock, versus the behaviour of prion proteins in flocks in which classical scrapie disease develops.

If all tests are negative, this would be two consecutive shipments in which no cases of atypical scrapie have been detected using highly sensitive tests that would be expected to detect the condition if it were present. This would provide some support for the alternative hypothesis that atypical scrapie is naturally infectious.

Because of the mounting evidence that classical scrapie and atypical scrapie are distinct conditions of differing pathogenesis, the World Organisation for Animal Health (OIE) has recently convened an ad hoc group to examine both. If there is consensus that atypical scrapie is significantly different from classical scrapie, we expect the existing OIE Standard for Scrapie will be amended to adequately distinguish the two conditions.

There is much that remains uncertain about atypical scrapie.

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Reports from New Zealand Food Safety Authority

National Chemical Residue Programme 2005/2006

The New Zealand Food Safety Authority (NZFSA) operates a risk-based National Chemical Residue Programme that randomly samples urine and blood from live animals, and animal products at the point of harvest.

The National Chemical Residue Programme (NRP) includes all classes and species of cattle, sheep, deer, pigs, goats, horses, wild animals, broilers, ostriches and emus, honey and farmed salmon. The dairy National Chemical Contaminants Programme (NCCP) monitors raw milk and colostrum at the farm prior to consolidation and dilution, through to transportation and processing.

Both programmes test for a wide range of contaminants and agricultural compounds, including antibacterial and antibiotic substances, banned or restricted substances, endo- and ecto-parasiticides, persistent organochlorines (including dioxin), vertebrate poisons and heavy metals.

Regulatory action is required for any products that exceed the maximum permissible levels (MPLs) although the actions may be quite different in different cases. MPLs are action limits set under the Animal Products Act and are used as triggers to ensure that animal products meet market access requirements (including for the New Zealand market). No MPL exceeds an MRL set under the Food Act and so compliance with MPLs automatically ensures compliance with MRLs.

Sampling years for all programmes are now aligned for the same report periods. Results for the year 1 July 2005 – 30 June 2006 are presented in Table 1.

The NRP found 10 samples of marker tissue that exceeded the MPL. These were from:

- two pigs, for carbadox;
- one horse, for moxidectin;
- one horse, for doramectin;
- one horse, for flubendazole;
- one pig and one lamb, for levamisole;
- one pig, for flumethrin;
- one broiler, for nicarbazin;
- one bobby calf, for sulphonamides.

Investigation showed that none of these events was systemic in nature.

Some fish samples were tested for mercury, as shown in Table 2. Advice on consumption based on these results has been added to the NZFSA fish advisory for pregnant women – the highest exposure risk group. This advisory outlines different types of seafood, and the recommended intake per week, for pregnant women who wish to derive the benefits of seafood while limiting their exposure to methylmercury. The advisory can be found on NZFSA's website.

NZFSA surveys various aquatic species each year. The results will be added to the existing data set and used to update the NZFSA advisory.

Primary product group	Number of	Number of sample compound combinations[1]	Number of samples with detections above the New Zealand threshold	% of samples that contained residues below the regulatory limit
National Chemical Residue Programme				
Farmed mammals	4,211	52,009	9[2]	99.79%
Ostriches and emus	70	492	0	100%
Honey	42	525	0	100%
Farmed salmon	5	32	0	100%
Broilers	134	3,129	1	99.25%
National Chemical Contaminants Programme				
Raw milk and colostrum	360	86,546	6[3]	99.31%

1. The overall number of sample-compound combinations is not precise, as some procedures can test for many different compounds on the one sample. Sometimes these compounds are from one compound class (eg synthetic pyrethroids), or from one activity class (eg antibiotics). More compounds of an activity class can be detected than are specified for reporting.
2. Two samples were in horse liver, a tissue not normally eaten, and a default MPL was set (muscle from these horses would have conformed if tested). Each of the nine nonconforming samples had only one residue detected.
3. This figure (six) was for colostrum only; there were **no** detections in raw milk.

Species	No. of samples
Kahawai	60
Ribaldo	60
Albacore tuna	33

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Bovine cysticercosis

Postmortem examination of adult cattle at slaughter premises continues to detect, on rare occasions, suspicious lesions potentially caused by *Cysticercus bovis.* This report contains information for an 18-month period from 1 January 2006 to 30 June 2007 because of a change in the annual reporting period for this parasite.

From 1 January to 31 December 2006 there were nine submissions of suspect *C bovis* lesions to the animal diagnostic laboratory approved by NZFSA.

Three lesions remained suspicious for *C bovis* after histological examination.

In the period 1 January to 30 June 2007 there were five submissions of suspect *C bovis* lesions. Two lesions remained suspicious for *C bovis* after histological examination.

Products from carcasses with such lesions are treated by freezing or cooking, or are excluded from the human food chain.

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Varroa management programme report

The previous annual report on the Varroa National Pest Management Strategy (NPMS) appeared in this publication in June 2006[1]. In that month, an incursion of *Varroa destructor* was detected in Nelson in the South Island by MAF Biosecurity New Zealand (MAFBNZ) and the Varroa NPMS, during combined testing under the Varroa NPMS and the Surveillance Programme for Exotic Diseases of Honey Bees.

In addition to collecting samples for laboratory examination for exotic pests and diseases, the testing programme used Apistan miticide strips and sticky boards applied for 24 hours to collect mites falling from adult bees. The sensitivity of the test applied to a specific hive allows very low levels of mites to be detected, and a near absolute population estimate of mites in a hive can be made from counting the numbers of mites adherent to sticky boards[2]. The sensitivity of the surveillance programme is influenced by the clustered nature of varroa-infested apiaries in a new incursion[3], together with differing risk of exposure of apiaries to an incursion.

A model for planning surveillance therefore had to accommodate this non-random distribution with reasonable use of resources. The Varroa NPMS had been instituted to detect an incursion of varroa in the South Island early enough that eradication could be attempted. It is possible that varroa had reached Nelson in the year the NPMS was put in place as delimiting surveys demonstrated spread had already occurred. Measures to limit further spread were instituted while the feasibility of local eradication was evaluated. MAFBNZ did not attempt eradication, largely because it was unable to access legally a suitable toxin for use in remote baiting of feral colonies. The beekeeping industry arranged transport of most varroa-infested hives to the North Island. Additional infested areas were detected in August 2006 and efforts to eliminate varroa locally were concluded in January 2007. Continuing operations under a MAFBNZ incursion response comprise movement controls and extension work with beekeepers with the two objectives of slowing the spread of varroa through the South Island, and assisting beekeeping enterprises to adapt management practices to the presence of varroa.

The Varroa Agency Incorporated (VAI) funded its NPMS surveillance through a levy on hives and with additional funding from regional government. In consultation with stakeholders, the VAI took the decision in October 2007 to disestablish its surveillance programme under the NPMS, and the NPMS itself is expected to be revoked. However, the Surveillance Programme for Exotic Diseases of Honey Bees will continue to sample apiaries across the North and South Islands for pests and pathogens not known to be present in New Zealand.

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Quarterly review of diagnostic cases – July to September 2007

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.

New Zealand Veterinary Pathology

Cattle

A single four-week-old calf from a mob of 40 in the Waikato was found circling and having seizures, and died soon after. Blood lead analysis revealed levels of 1.8 mg/l, consistent with **lead toxicity**. In another case, a four-week-old calf from the Manawatu was euthanased after exhibiting a sudden onset of nervous signs. Liver analysis revealed lead levels of 8.8 mg/kg. In cattle, levels higher than 4 mg/kg are suggestive of toxicity.

A cow from the southern Waikato that aborted recently had a *Leptospira pomona* microagglutination titre of ≥1:1600. Bovine virus diarrhoea (BVD) antigen testing and *Neospora* immunofluorescent antibody (IFA) testing were negative. *Leptospira pomona* was considered the likely cause of the **abortion**.

A group of calves in the Bay of Plenty had increased mortality and morbidity. One calf died and two others were severely depressed, with apparent blindness and stiff neck. Cytology of cerebrospinal fluid from the dead calf revealed a moderate septic meningitis with numerous intracellular bacilli present. Culture of the cerebrospinal fluid produced a pure growth of alpha-haemolytic *Streptococcus*, confirming **streptococcal meningoencephalitis**.

A yearling bull in the Manawatu died suddenly, with few premonitory signs. Necropsy by the field veterinarian showed severe thickening of the oesophageal and oral mucosa. Histology revealed severe ulcerative oesophagitis with numerous bacterial colonies present in the necrotic tissue. There was also marked vasculitis, and numerous PAS-positive fungal organisms were present within vessels. The lungs had a severe mycotic pneumonia. Numerous renal infarcts were present. Lesions were consistent with **disseminated fungal disease**, perhaps compounded by necrobacillosis. The lesions probably arose from systemic spread of a mycotic or necrobacillary rumenitis, secondary to carbohydrate overload.

A group of yearling Jersey bulls in the Waikato exhibited illthrift. Faecal egg counts and BVD antigen testing gave no significant results, but *Yersinia pseudotuberculosis* was cultured from the faeces of four animals. **Yersiniosis** was diagnosed.

Five of 50 cows in the Waikato died suddenly after being shifted to a new paddock. Plant material from the paddock and rumen content from one of the dead cows had detectable levels of cyanide. **Cyanide toxicity** (from ingestion of cyanogenetic plants) was diagnosed. In a similar case, one paddock on a property in the Waitomo area was associated with deaths each spring. Postmortem of a cow that died in the paddock showed moderate amounts of 'swamp grass' in the rumen. Rumen contents were positive for cyanide, suggesting this was another case of cyanide toxicity.

A property in the Northern Waikato had about 10 yearling heifers die in the space of six to eight weeks. Necropsy of several showed severe oesophageal and oral ulceration, enteritis, abomasitis and colitis. Histologic findings revealed marked enteritis with cryptal and Peyer's patch necrosis, consistent with BVD. BVD antigen testing on spleen was positive in three animals tested. On two of these animals, sequencing to type the BVD virus identified it as BVD type I. This ruled out the possibility of type II acute BVD. **Mucosal disease** was diagnosed. It appears that the property experienced infection of naïve pregnant cows with BVD virus, resulting in the birth of a group of persistently infected calves. These animals later (as yearlings) experienced infection by a cytopathic strain of the virus, resulting in an outbreak of clinical mucosal disease. A similar situation seems to have arisen on a few other farms in the area this year; on one property in the Waikato four of a group of poorly doing yearling heifers were persistently infected with the BVD virus.

A group of four-week-old calves in the Bay of Plenty had severe diarrhoea. Rotavirus and *Cryptosporidium* tests were negative but culture for *Salmonella* produced ***Salmonella* Ruiru**. This is an unusual isolate in New Zealand and is only occasionally isolated from diarrhoeic calves and adult cattle.

Sheep

Routine scanning of a Texel cross flock in the Waikato revealed that 10% of the animals were not pregnant. The scanner believed half were carrying dead fetuses. Three ewes were culled and the laboratory found the uteri of two contained dead and autolysed fetuses suspended in abundant necrotic and suppurative material. The other uterus had retained fetal membranes with a suppurative endometritis. Culture of samples from all three uteri featured a heavy growth of *Arcanobacterium (Actinomyces) pyogenes*. From one of the animals the culture was pure but in the others it was mixed with environmental contaminants. Selective culture and/or enrichment for *Listeria*, *Salmonella* and *Campylobacter* was negative. Histology revealed all animals had a marked suppurative endometritis with bacterial colonies visible. Early embryonic death caused by ***Arcanobacterium* (*Actinomyces*) *pyogenes*** was diagnosed.

Several deaths occurred in a mob of sheep in the Nelson area over the course of one week after feeding on poor quality balage. All the dead animals were found near the water supply in the paddock. Necropsy of one ewe showed marked pulmonary congestion with myocardial petechiation. The intestine appeared grossly inflamed and there were no solid faeces in the distal colon. Histologic examination revealed an acute suppurative gastroenteritis with abomasal infarction and terminal septicaemia. Culture of the mesenteric lymph node yielded a heavy growth of Gram-positive rods consistent with ***Listeria*** spp.

A mob of 40 ewes in the Waitomo area experienced five abortions over a short space of time. Histology on three aborted lambs revealed a marked suppurative placentitis with a suppurative fetal pneumonia in all cases. Culture of stomach content from two lambs produced a heavy growth of ***Listeria ivanovii***.

Deer

Twelve of a group of 300 wapiti and wapiti crosses in the Bay of Plenty died over a three-week period, after exhibiting illthrift and marked weight loss. The submitting veterinarian necropsied a 10-month-old wapiti and noted marked emaciation with a lack of peritoneal fat stores. Mesenteric lymph nodes were prominent and the small intestinal wall was moderately thickened. Histology revealed marked mucous metaplasia with lymphocytic abomasitis and numerous nematodes embedded in the mucosa of the abomasum. The small intestine featured a marked granulomatous enteritis and lymphadenitis. **Ostertagiasis** with concurrent **Johne's disease** was diagnosed.

An adult deer from a farm in the Taupo area with a history of sudden death with diarrhoea was necropsied by the submitting veterinarian, who observed changes in the lung consistent with **lungworm**. Moderate numbers of nematodes consistent with *Dictyocaulus* spp were observed histologically in the bronchi. The intestines were too autolysed for histologic assessment but culture of intestinal content indicated concurrent infection with ***Yersinia pseudotuberculosis***.

Alpacas

An alpaca from the Blenheim area had been born with a wry nose, had poor skeletal development and swelling around the fetlocks. Serum phosphate levels were 0.75 mmol/l. Normal serum phosphate level for this species is approximately 1.9–3.4 mmol/l. This hypophosphataemia, together with the clinical signs, is consistent with a diagnosis of **rickets**.

A three-month-old female cria from the South Waikato presented with severe diarrhoea. Faecal egg count was 1,275 eggs/gram, and *Yersinia pseudotuberculosis* was isolated from the faeces, suggesting concurrent **gastrointestinal parasitism** and **yersiniosis**.

Pigs

Sudden death of four 12-week-old pigs occurred in a 30-sow piggery. The pigs were thin and severely icteric. On histological examination the most significant abnormalities were present in the liver, which had a marked chronic vacuolar hepatopathy with single cell necrosis. These findings were considered most consistent with **porcine circoviral infection**.

Horses

A 12-year-old shire gelding in the Central Plateau region exhibited sudden onset of profuse salivation and lethargy. The horse recovered rapidly one or two hours later after it was moved from its grazing paddock. The paddock was considered a risk for arsenic contamination because of its location in a volcanically active area. The owner's dog had died suddenly two days earlier with a similar syndrome of profuse salivation. Analysis of sediment from a puddle in the paddock revealed an arsenic level of 13.7 mg/kg. The highest admissible level of arsenic in general foodstuffs for people is 0.2 mg/kg. This suggests the levels in the paddock may have been high enough to cause **arsenic toxicity** in the horse.

An adult horse in the Bay of Plenty exhibited weight loss and diarrhoea. ***Salmonella* Typhimurium** was isolated from the faeces, confirming salmonellosis. In a similar case, an adult horse in the King Country had severe diarrhoea with rapid weight loss, and was also positive for *Salmonella* Typhimurium.

Two foals from a property in the Waikato exhibited coughing and had a mucopurulent nasal discharge. Cytology on a transtracheal wash from one revealed severe septic inflammation with neutrophils containing coccobacilli. Culture of transtracheal wash samples from both foals yielded a heavy growth of ***Rhodococcus equi***.

Dogs

A two-month-old Rhodesian ridgeback in the South Auckland area presented with severe haemorrhagic gastroenteritis. Eighteen pups on the same premises were at risk. The sick pup had large numbers of coccidial oocysts in the faeces, and faecal culture was positive for *Campylobacter coli*. A parvovirus antigen test on faeces was negative. Concurrent **coccidiosis** and **campylobacteriosis** were diagnosed.

Birds

An eight-year-old cockatoo had recently been re-homed and was inappetent and very thin. A mildly increased CK of 1,394 IU/l (normal up to about 400), and an AST of 483 IU/l (normal range 40-300) were considered to reflect muscle damage from recent handling. Serum zinc levels were 46 µmol/l, which equates to 3 ppm. **Zinc toxicity** in cockatoos has been associated with serum zinc levels greater than 2 ppm. In another case, a two-year-old galah presented with lethargy. The bird had been chewing on its cage and x-rays revealed radio-dense fragments in its gizzard. The bird had a serum zinc level of 214 µmol/l (approximately 14 ppm), well above the toxic range for this species. Analysis of paint flakes from the galah's cage revealed that the paint contained 2,150 mg/kg of zinc, suggesting the paint was zinc based and the cause of the toxicity.

A two-year-old pigeon in Wellington presented with severe weight loss. Smears made from a crop wash revealed numerous flagellate organisms amongst abundant keratinised squames. **Trichomoniasis** was diagnosed.

A nine-year-old parrot was taken to a veterinarian in Canterbury to have a wing clip. The beak was noted to be slightly deviated. The next morning, the owner found the bird depressed and vomiting and it died shortly after. Necropsy revealed numerous white foci over the liver and spleen. *Yersinia pseudotuberculosis* was isolated, suggesting septicaemic **yersiniosis** as the cause of death.

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Gribbles Veterinary Pathology

Cattle

Through the spring period, once calf rearing is underway, diarrhoea emerges as a major limitation to successful rearing. During the first week of life, **K 99 *E coli*** is the primary pathogen causing problems. In a shed of 300 Jersey calves in the Wairarapa, 15 had profuse, watery diarrhoea and four died. Diarrhoea developed within the first 24 hours of life, and most calves recovered after intense fluid therapy. A faecal sample from a four-day-old calf was positive for K99 *E coli* on an ELISA.

During the first month of life, rotavirus and cryptosporidia are frequent causes of calf diarrhoea. In a case from Manawatu, six Friesian calves died, and six more from a mob of 50 were ill. The calves developed light green, bloody diarrhoea and died quickly. Moderate to heavy numbers of **cryptosporidia** were detected on stained faecal smears.

Another more pathogenic cause of calf scours is ***Salmonella* Typhimurium**. In one Hawke's Bay case, six 14-day-old Friesian-cross calves from a shed of 71 stopped drinking. The next day three were dead with evidence of diarrhoea staining the perineum. Moderate to heavy growth of *Salmonella* Typhimurium was cultured from the faeces of each dead calf. In another case in Hawke's Bay, from a shed of 100 Friesian calves, four had diarrhoea and two were dead. *Salmonella* Typhimurium was cultured from intestinal content of one dead calf.

From six to eight weeks of age onwards, **coccidia** can cause bloody mucoid diarrhoea. In a mob of 236 Friesian calves in Hawke's Bay, 50 had diarrhoea and 20 died. Moderate to heavy numbers of coccidial oocysts were visible in faecal samples from three of four calves.

As calves are released into paddocks around the rearing pens and become more inquisitive, **lead toxicity** is often seen. Three calves died from a mob of 40 Friesian/Jersey cross calves in Taranaki. Two were found dead and the third was seen convulsing before death. The calves had access to an old battery, rubbish in a dump and flaking paint. Liver lead concentrations were 16.1 mg/kg. Tissue lead concentrations greater than 5 mg/kg are considered toxic. In another Taranaki case, two one-month-old Jersey calves were found dead with access to lead paint. Blood lead concentrations were 2.1 mg/l. Blood lead concentrations greater than 0.3 mg/l are considered toxic.

From a mob of 45 Friesian yearlings in the Wairarapa, three were recumbent, dyspnoeic, frothing at the mouth and eventually died. Histological examination of the lungs revealed a severe fibrinosuppurative and necrotising **bronchopneumonia** associated with culture of *Histophilus somnus* and *Arcanobacterium pyogenes*.

Four yearling heifers in a mob of 221 died suddenly, two within 24 hours and another two the following day, after an injection of copper. Histological examination of tissues from one animal revealed massive acute hepatic necrosis consistent with acute **copper poisoning**.

The brains and spinal cords from female one- to two-week-old Jersey calves had low to moderate numbers of distended myelin sheaths, some containing macrophages, in the lateral margin of the reticular formation in the section cut through the cerebellar peduncles. Similar mild changes were also seen in the dorsal white matter in the obex. The spinal cord of one calf had distended myelin sheaths in the dorsal white matter tracts. Occasional macrophages were present in these spaces. No changes were detected in the other calf. Similar changes had been seen in a related calf last year. There is a possibility this is an inherited **demyelinating disease**.

Pigs

Thirty Landrace cross weaner pigs had died over a period of six months on a 100-sow Taranaki pig farm. Typically the weaners faded and died. The farm's worming programme was irregular. A 5 kg piglet submitted for necropsy was in poor condition with ulcerations over bony prominences, and numerous 5–15 mm crusts on the skin of the lower limbs and ventral abdomen. Fat reserves were completely metabolised. Large numbers of coiled nematodes dilated a reddened caecum and colon, consistent with severe *Trichuris* **nematodiasis**.

Horses

Two histologically confirmed cases of **equine herpes virus abortion** occurred on a property in Canterbury. Seven of 14 mares aborted and the abortions occurred in two clusters three weeks apart – four initially and three later.

Three sporadic cases of **equine herpes virus abortion** and one suspect case occurred at different studs in the Waikato. The three typical cases all had foci of necrosis in the livers and intranuclear inclusions in hepatocytes. The suspect case had no inclusions.

A three-week-old Arab foal died after developing pneumonia. It had consolidated areas of lung at post mortem. The lymph nodes were difficult to find. Histologically the lymph nodes were small and had no lymphoid follicles. No lymphoid follicles were present

in the spleen. Tissue from the thymic area was largely fat with one small nodule of mononuclear cells. The lung had multiple foci of suppurative inflammation containing large colonies of bacteria that seemed to be centred on bronchioles and contained a mixture of cocci and filamentous bacteria. There was loss of bronchiolar epithelium and two possible intranuclear inclusions indicating an adenoviral infection. Severe generalised suppurative pneumonia and necrotising bronchiolitis with severe lymphoid hypoplasia was diagnosed. This is diagnostic of severe **combined immunodeficiency** in Arab foals. There was some evidence of an adenoviral infection that commonly occurs in these foals.

Dogs

Two histologically confirmed cases of **post-vaccinal distemper** have been seen in dogs from Canterbury during the last 12 months. The first was in a three- to four-month-old Collie cross whose clinical signs developed within days of vaccination. The second case occurred in October this year after a booster dose of the vaccine. Both cases were from the same practice and subsequent enquiries revealed the two dogs were littermates although they had different owners. Considering how widely used these vaccines are it seems likely there is some host peculiarity that made these dogs susceptible to the vaccine-induced disease.

A five-year-old spayed Poodle bitch undergoing immunosuppressive therapy (azothiaprine and dexamethasone) for immune-mediated haemolytic anaemia was biopsied and found to have a necrotising **hepatitis** associated with protozoan parasites resembling *Neospora*, *Hammondia* or *Toxoplasma*. *Toxoplasma* was ruled out using immunohistochemistry. PCR for *Neospora* and *Hammondia* is pending.

A three-year-old spayed Rottweiler bitch was presented with lethargy and fluid distension of the abdomen. Cytology on the fluid revealed an eosinophilic effusion. The eosinophil count in the peripheral blood was 10.1 x 10^9/l (0.1 – 1.5 x 10^9/l), suggesting a **hyper-eosinophilic syndrome**. Immunosuppressive therapy with steroids reduced the eosinophil count and the abdominal effusion.

Cats

Two separate cases of fat-associated **dermal mycobacteriosis** were seen in domestic cats from the Hobsonville area of Auckland. The cats were aged nine and 12 years and had disseminated lesions involving the pannicular fat of the dorsum and the sternum.

Fish

A freshly dead 22.4 kg yellowtail **kingfish** (*Seriola lalandi*) from a fish farm in Northland was presented for necropsy. The fish was from one tank of a brood stock nursery, which had been experiencing illthrift and **sporadic losses**. No gross lesions were found. Histology of the gastrointestinal tract revealed large numbers of superficial intracellular apicomplexan parasites resembling Epi-eimeria in the mucosa of the atrophic and inflamed intestine. No other lesions were found. Subsequently, as part of the same investigation, a number of young grower fish of the same and different species were examined and found not to have the infection. The losses in the brood stock were from only one of a number of tanks. The investigation continues.

Different species of **aquarium fish** from a zoo collection in Auckland were reported to be showing unusual swimming behaviour and increased mortality, suspected to be caused by chronic marine tuberculosis and sporadic infections. A platy that died was examined postmortem. No gross lesions were seen but small numbers of **microsporidian** sporocysts were found at the centre of granulomata in the brain when examined microscopically. The phylum Microsporidia is a mixed group of poorly understood intracellular parasites affecting invertebrates, a few lower vertebrates and humans. They are often an incidental finding in aquarium fish but when they affect the nervous system can be responsible for spinal deformity and swimming irregularities.

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Quarterly report of investigations of suspected exotic diseases

Exotic disease investigations are managed and reported by MAF Biosecurity New Zealand's Investigation and Diagnostic Centre (IDC) Wallaceville. The following is a summary of investigations of suspected exotic disease during the period from July to September 2007.

Vesicular disease ruled out

A veterinarian alerted the Investigation and Diagnostic Centre (IDC) to a suspected vesicular disease after observing erosive lesions in a pet four-month-old female kunekune pig. The pig had been admitted to the veterinary clinic earlier that day, recumbent, showing signs of abdominal pain, low temperature, injected mucous membranes, and erythematous skin lesions on the distal parts of all four legs. The veterinarian euthanased the pig later that day, after it started to have seizures, and noticed the oral lesions. Vesicular disease was ruled out by an AsureQuality initial investigating veterinarian (IIV) who examined the animal and determined the oral erosive lesions to be traumatic in origin.

A veterinarian reported mouth and tongue erosions in four dry cows from a mob of 250 on an Otago dairy farm. All four animals had normal rectal temperatures. An IIV found the mouth lesions were thickened, pyogranulomatous erosions affecting the tongue, buccal area or hard palate. No feet or teat lesions were identified. The animals were in good body condition and were eating well. All other stock were unaffected. The last movement of stock onto the property was six weeks earlier when six in-calf heifers arrived. The IIV ruled out exotic vesicular disease on clinical and epidemiological grounds. The lesions were considered typical of actinobacillosis. Their precipitating cause appeared to be trauma either from feeding kale with woody stalks, or from stones ingested with grass silage fed from a pad with a poorly prepared stony surface.

A single dairy cow on a Taranaki farm was found by a veterinarian to have three ulcers on its tongue and nasal planum. An AsureQuality IIV found the animal had a normal temperature, was not lame and there were no lesions on the feet or udder. No other cattle on the farm were affected, and there was no relevant importation pathway. An exotic vesicular disease was ruled out on clinical and epidemiological findings.

Atypical scrapie ruled out

Histological examination by MAF Biosecurity New Zealand's expert reference pathologist of the brain of a sheep that had died after displaying progressive nervous signs, could not rule out atypical scrapie. Fixed brain material sent for immunohistochemistry to the Veterinary Laboratories Agency, Weybridge, UK, had no evidence of PrP immunolabelling, allowing both scrapie and atypical scrapie to be ruled out.

Equine influenza ruled out

A veterinarian contacted the MAF Biosecurity New Zealand (MAFBNZ) 0800 hotline after a client, who had recently collected their USA-bred miniature pony mare from Karaka quarantine facility, reported the horse had developed a mild nasal discharge. As part of enhanced quarantine measures instituted since the equine influenza (EI) outbreak in Australia, a nasal swab from this horse (along with 12 others that came in to Karaka from Kentucky, USA on 25 August) was tested for EI by PCR at IDC Wallaceville, prior to release from quarantine. All results were negative. The entire quarantine cohort of 13 horses had been clinically normal during their two-week quarantine at the Karaka facility, apart from one that had transient travel sickness on arrival. Under the direction of the Incursion Investigator, an equine veterinarian visited the miniature horse and found no signs consistent with EI, only a mild serous nasal discharge with no cough or pyrexia. A second deep nasal swab tested for EI by molecular methods at IDC Wallaceville, again gave negative results.

Brucella canis ruled out

A veterinary pathologist contacted MAFBNZ after histological examination of fixed testicular material from a three-year-old dog revealed a fibrinosuppurative orchitis and epididymitis with cellulitis. The dog had presented with incontinence, lumps in the epididymis as well as two scrotal plaques. *Brucella canis* was ruled out after serum sent to IDC Wallaceville tested negative to the *Brucella canis* card agglutination test.

Leishmania spp ruled out

An 11-year-old spayed female Sheltie tested positive for antibodies to *Leishmania* spp during routine pre-export testing to meet Australian import health standards. The immunofluorescent antibody test (IFAT), carried out by IDC Wallaceville but subcontracted to VetPath Laboratories Western Australia, returned a positive titre of 1:128. The dog was born in Texas USA where she lived for four years before moving to New Zealand in 2000. A local veterinarian working under the direction of the Incursion Investigator found the dog to be healthy with no clinical abnormalities consistent with *Leishmania* infection. Routine haematology and biochemistry analyses showed no abnormalities. A subsequent molecular assay on EDTA blood at Acarus Laboratory, Bristol University, UK, was negative for *Leishmania* spp DNA. A second serum sample tested for *Leishmania* spp antibodies by IFAT at Texas Veterinary Medical Diagnostic Laboratory (TVMDL), USA, gave negative results. *Leishmania* IFAT is known to cross-react

with *Trypanosoma cruzi* (Chagas disease) antibodies. *Trypanosoma cruzi* is endemic in parts of the USA including Texas. IFA testing for *T cruzi* at TVMDL (the *T cruzi* IFAT does not cross-react with *Leishmania* antibodies) also gave negative results. Repeat testing of both serum samples with a newly implemented *Leishmania* IFAT at IDC Wallaceville gave negative results. The investigation concluded that the initial result was a false-positive reaction caused by a non-specific reaction or a cross-reaction with other components or agents in the blood.

Coccidiomycosis ruled out in an imported dog

A bulldog imported from Arizona, USA, had a non-healing wound since its importation in November 2006. The wound was considered to be a possible infection with the fungus *Coccidioides immitis*. The dog had a low CFT titre of 1:4 at the time of importation, and at the time of writing had negative titres on both the complement fixation and agar gel immunodiffusion tests. The case has been referred to a small animal medicine specialist to attempt to find an alternative diagnosis.

Rhipicephalus sanguineus confirmed

A veterinarian reported a client who had found a tick on the family dog recently imported from Brisbane, Australia. MAF's reference expert identified the tick as a partially engorged female brown dog tick (*Rhipicephalus sanguineus*), which was estimated to have been attached for five or six days. This timeline fits with the tick having been picked up in pre-import kennels where the dog spent the 10 days before departure. The dog is recorded as having been treated with fipronil (spot-on application) seven days before leaving, in accordance with import health requirements, and was checked by an experienced quarantine officer on arrival at Christchurch. IDC has checked import measures and overseen testing and control measures applied to all in-contact cats and dogs. Serum and EDTA blood from the dog was tested by IFAT for antibodies to *Babesia gibsoni* and a molecular test for *Babesia* spp DNA, with negative results. This case appears to highlight the difficulties associated with arrival inspection as a risk mitigation measure, and the efficacy of fipronil as a spot-on application. MAFBNZ border standards group is examining dog import inspection and treatment procedures, and following up with Australian authorities. *Rhipicephalus sanguineus* has caused temporary infestations in North Island houses on three occasions and is the most commonly intercepted tick. It could establish, especially in northern parts of the North Island, or in heated houses (where temperatures are suitable) in other parts of the country. *Rhipicephalus sanguineus* is not known to have established in New Zealand.

Avian influenza and Newcastle disease ruled out

A MAFBNZ Quarantine Officer alerted the IDC to a foreign pigeon captured from a vessel originating from Zhoushan, China. Four pigeons had landed on the vessel during bad weather five days before the vessel entered the Nelson port. The approximate position of the vessel when birds landed was 240 nautical miles west of New Caledonia and 600 nautical miles east of Australia. The captured bird was euthanased and a postmortem undertaken at IDC Wallaceville. The bird had a low grade hepatitis with multiple foci of inflammation in which heterophils and occasional histiocytes were prominent. Several sections of trematode worms were present in the small intestinal lumen. Newcastle disease and avian influenza were ruled out subsequent to negative PCR tests on a range of tissues. The other three pigeons were not recovered.

A hobby poultry keeper contacted MAFBNZ after noting increased mortality through autumn in his flock of about 50 birds. Ten to 15 birds died during this period following several days of anorexia, lethargy and dropped wings. A specialist avian veterinarian conducted a postmortem on two birds and considered erythroblastosis (a disease in the leucosis complex) to be the underlying cause. Samples from two other birds tested negative for coccidial oocysts and intestinal nematode eggs. Unfortunately, no material was fixed for histology. Since the initial report in early May, only one further bird had died by the time the investigation was closed in late July. Highly pathogenic notifiable influenza and velogenic Newcastle disease were ruled out on clinical, pathological and epidemiological grounds.

MAFBNZ was contacted after school children found about 15 dead and dying house sparrows in their playground. An AsureQuality field officer was contracted to collect the affected birds and euthanase any still alive. Necropsies and histology on a full range of tissues from six birds at IDC Wallaceville were unremarkable. Samples tested by real time influenza A and Newcastle disease (ND) RT-PCRs were negative for ND. One duplicate from each pool gave a suspicious result for influenza A. Further testing using H5, H7 and nested PCRs, to eliminate the possibility of low viral RNA yield in the samples, gave negative results for both samples. Samples were also negative after two passages for virus isolation. No *Salmonella* species were isolated from pooled liver and intestines.

Avian pneumovirus ruled out

As part of routine export testing carried out at IDC Wallaceville, five of 30 25-week-old chickens tested positive to avian pneumovirus (APV) using the IDEXX ELISA. None of the birds exhibited signs of respiratory disease that might suggest infection with APV. APV was ruled out following negative immunofluorescent antibody tests (NVSL, Ames, IA, USA).

Infectious bursal disease ruled out

A flock of layers was investigated following detection, through routine infectious bursal disease (IBD) surveillance, of two birds with positive IBD VNTs. Titres were low (1:64 and 1:16). There was no clinical evidence of IBD in the flock. Additional sera were collected from all sheds on the property and 20 young sentinel birds were introduced into the shed containing VNT positive birds. The introduced birds subsequently tested negative to IBD by PCR.

Serological investigation of the flock revealed that 37% of a further 200 birds were positive to the IBD ELISA but only 1.4% were positive to the VNT. The serological results were not consistent with IBD infection in the flock or vaccination. A project is underway at the IDC Wallaceville to develop a more specific VNT for IBD.

A specialist poultry veterinarian reported to MAFBNZ a cluster of cases involving broilers with bursal atrophy, increased mortality and cellulitis. Swabs and tissue specimens were submitted to the IDC to rule out an exotic viral aetiology. Cloacal swabs and spleen were negative on PCR testing for IBD. Newcastle disease was ruled out on PCR tests of the liver, spleen and kidney. A cytopathic virus detected by virus isolation in the bursa and spleen of one bird was identified by electron microscopy as an adenovirus. It tested negative by haemagglutination inhibition (HI) test for type III adenovirus – the cause of egg drop syndrome. This adenovirus is therefore likely to be type I or type II. Histopathology of the lung, liver, spleen, bursa and thymus was not conclusive for inclusion body hepatitis. Examination of the mortality rates for all sheds in the production system indicated that the mortality was increased in the first week and from 28 days onwards. This trend was consistent in the last two production runs but not in the production figures prior to April 2007. Mortality figures include culls, dead birds and moribund birds. Two farmers interviewed perceived the largest category of increased mortality to result from leg problems, mainly femoral head necrosis, a condition of chickens and turkeys that may be associated with several different bacterial infections such as staphylococci, *E coli* or streptococci. Predisposing factors for femoral head necrosis include immunosuppressive viruses such as IBD virus, chicken anaemia virus and non-infectious bone pathologies such as hypophosphataemic rickets. The combination of an adenovirus isolation from the spleen and bursa, postmortem reports of small bursas and splenomegaly, and increased prevalence of femoral head necrosis and cellulitis, does indicate that an immunosuppressive condition may be involved. Further work will be undertaken to type the adenovirus and improve the sampling strategy to define the condition.

Avian blood parasites investigated

A pathologist reported suspicious intracytoplasmic inclusion bodies in a blood smear from a New Zealand crimson rosella. The blood smear was submitted as a routine check before the bird was admitted to Auckland Zoo. Review of the smears by Massey University pathologists ruled out blood parasites. A staining artefact may have been present as no inclusion bodies were observed in a second blood smear made several days after the first.

Tracheal mites ruled out

A beekeeper reported the loss of one to three hives per apiary in about 18 apiaries. The interviewing apiary officer suspected starvation and robbing of the hives as the most likely cause of hive loss. However, as a precaution, bees from three hives were tested for tracheal and external mites (including *Tropilaelaps* species). No tracheal mites were found in any of the 22 bees dissected. Varroa mites and a small number of other mites were found: *Tyrophagus communis* (mould mite – Acaridae, one mite), *Carpoglyphus lactis* (dried fruit mite – Carpoglyphidae: Acari, two mites), and *Melittiphis alvearius* (melittiphis mite – Laelapidae: Acari, one mite). These other mites are of no pest significance to honey bees; they are scavengers on waste products in the hive (pollen, honey, fungal growths). All are regularly found in samples from MAFBNZ's apiculture surveillance programme.

Gobio sp investigated

A vial apparently containing two small fish preserved in formalin was delivered to IDC Tamaki with no accompanying paperwork, return address or identification of sender. IDC Wallaceville sent the fish to NIWA to the Marine Invasives Taxonomic Service (MITS), which identified *Gobiopterus semivestita* (syn *G semivestitus*). This species is a small, tropical freshwater fish from northern Australia and Papua New Guinea and was first found by the Department of Conservation in a small tributary of the Ngunguru River (near Whangarei, Northland) several years ago. Its origin in New Zealand is unknown; it may occur here naturally and been undetected, it may have arrived in New Zealand via natural dispersal or it may have been introduced via shipping. As this species is reportedly already established in New Zealand and has been here for several years, no further action was required

Exotic oyster ruled out

A member of the public reported an unusual looking oyster growing on the hull of their recreational vessel moored at Gulf Harbour Marina, Whangaparaoa, Auckland. The vessel had not been moved in the past six months. The report was emailed to the Ministry of Fisheries and passed on to the Animals and Marine Incursion Investigation Team. A specimen sent to the MITS was identified as the Pacific oyster *Crassostrea gigas*. Its unusual shell morphology was probably the result of growing in a low energy (few waves or little current) environment. As this species was introduced to New Zealand many years ago and is well established and widely farmed commercially, it is of no biosecurity concern.

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Animal Disease Emergency

To report suspected exotic diseases in animals, please phone toll free, all hours 0800 809 966

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