

ANIMAL HEALTH LABORATORY

The AHL seeks to use innovative diagnostic approaches, and as part of this commitment recently acquired a next generation sequencing (NGS) machine. This machine allows simultaneous sequencing of all DNA present in a sample, producing information on the identity of all organisms present whether they be plant, animal, pathogen, or a mixture of many organisms. NGS was recently used in an outbreak of coldwater disease in South Island salmon hatcheries. MPI was concerned that this represented an outbreak of a new disease. The cause of this disease, the bacterium *Flavobacterium psychrophilum*, had not been previously identified in New Zealand, although other *Flavobacterium* spp. have been known to be present in NZ for some time. The NGS instrument enabled AHL to sequence the entire genome of the New Zealand outbreak strain. Based on this evidence and low mortalities, *F. psychrophilum* was considered unlikely to be a recent incursion. This new technology shortens turnaround times, and this has already proved invaluable during diagnostic, investigation and response work.

Supporting active surveillance programmes

Laboratory testing carried out by AHL supports a number of surveillance programmes to confirm the continuing absence of specific pathogens such as arboviruses and TSE. Testing also monitors the presence of avian influenzas in wild ducks, and the presence of subtypes that could pose a possible risk to commercial poultry industries.

In addition, AHL performs specific pathogen testing requested by stakeholders and clients, such as testing of bees from the Pacific Islands as part of a monitoring programme for bee health.

Facilitating trade

The AHL functions as the national veterinary reference centre for New Zealand and exists to produce credible and authoritative documentation to facilitate and support New Zealand's primary industries export/import trade. During 2012, more than 2000 submissions from a wide variety of sources produced nearly 14 500 specimens, which were the subject of almost 31 500 diagnostic tests carried out at AHL in support of investigations of exotic veterinary diseases, responses and active surveillance.

The Animal Health Laboratory (AHL) is part of the Ministry for Primary Industries' Investigation and Diagnostic Centre and Response Directorate. The laboratory is responsible for rapidly and accurately diagnosing exotic, new and emerging diseases of animals and plants assisting to maintain the safety of New Zealand's agricultural and horticultural industries and the environment.

Our testing also serves as a resource for passive surveillance, to give continuing assurance to our trading partners for our claim of freedom from specific unwanted diseases.

The AHL facilities operate to the ISO/IEC 17025 and AS/NZS 2243.3 standards and we are annually audited by IANZ to maintain our international accreditation status for a wide range of tests.

Much of the AHL testing is either not available elsewhere in New Zealand or requires specialist expertise and facilities. We carry out testing in PC2 and enhanced PC3 laboratories to ensure the appropriate levels of biosecurity containment when testing for exotic organisms and also when such organisms are being used during inter-laboratory proficiency testing schemes, which are a requirement of our IANZ accreditation.

Throughput

The AHL is a centre of science excellence and maintains its accreditation and certification under the MPI Export Laboratory Programme, the Laboratory Approval Scheme (Animal Products Act 1999), IANZ and by operating to the exacting standards of AS/NZS 2243.3 and ISO/IEC 17025.

The laboratory divides its work among four science disciplines: virology, immunology, bacteriology and aquatic animal diseases. Each team consists of expert senior scientists, scientists and technicians capable of carrying out the complex analyses and investigations that are presented to us on a daily basis.

We maintain about 450 test methods, many of which are uniquely offered by us within New Zealand. These tests range from classical and well-established techniques, for example virus isolation, virus neutralisation, ELISA

and microscopy/pathology, to molecular analysis and state of the art technologies, such as real-time PCR and NGS and bioinformatic analysis. Some testing cannot be offered within New Zealand, and AHL manages the subcontracting of the work overseas to accredited reference laboratories.

Table 1 shows the number of tests processed by the Animal Health Laboratory (AHL) in the 2012 calendar year. About 31 500 tests are processed each year. The AHL is not a commercial laboratory, but does charge for the work conducted to support trade, on a cost-recovery basis.

Supporting incursion investigations

During 2012 the AHL performed nearly 5000 diagnostic tests in support of incursion investigations by its three laboratory teams – bacteriology and aquatic animal diseases, immunology and virology. Examples of some of the wide variety of incursion investigations that occurred during 2012 are provided below. In all instances the presence of an exotic or new to New Zealand disease could not initially be excluded by Incursion Investigators. Therefore, the possibility of exotic disease was investigated using a combination of expertise across teams and a variety of traditional and contemporary analytical techniques. Usually exotic or new to New Zealand disease were ruled out but on rare occasions they were confirmed, and in as short a timeframe as possible. This helped manage incursions quickly and effectively.

BEE PATHOGENS

Investigations were carried out into suspected European foulbrood, Israeli acute paralysis virus, deformed wing virus, Kashmir bee virus and *Nosema* species.

AQUATIC

Oysters from a North Island harbour were found to be positive for OsHV-1. A salmon mortality event in South Island was investigated, with AHL laboratory staff sampling in the field to exclude the following pathogens: infectious salmon anaemia, *Aeromonas salmonicida*, *Yersina ruckeri*, viral haemorrhagic septicaemia, infectious haematopoietic necrosis, *Renibacterium salmoninarum*, iridovirus and totivirus. Unexplained lesions and mortalities in lampreys from South Island river systems were investigated but no causal infectious disease was identified. *Flavobacterium psychrophilum*

TABLE 1: SUMMARY OF TEST NUMBERS AND DESCRIPTION OF WORK CONDUCTED BY AHL, 2012

PURPOSE OF TESTING (NOT ALL TESTING IS DEFINED HERE)	NUMBER OF ACCESSIONS MANAGED, SAMPLES RECEIVED, TESTS PERFORMED	DESCRIPTION OF WORK
Exotic diseases and pests (investigations to rule in or rule out)	Exotic disease investigation Accessions: 214 Samples: 1 853 Tests: 827	(1) Tests to rule out the presence of specific exotic pathogens, e.g., bee viruses, classical swine fever in pigs, and <i>Bacillus anthracis</i>
Definitive diagnostic tests	Diagnostic Accessions: 127 Samples: 1 074 Tests: 1 676	(2) Diagnostic tests (3) Fish pathology (4) Identification of reptiles and amphibians found as hitchhikers on imported goods.
Reference work/test validation	Accessions: 3 Samples: 1 217 Tests: 1 217	Mostly confirming diagnoses using capability that is not available elsewhere.
Surveillance Projects (Crown-funded)	Accessions: 142 Samples: 1 866 Tests: 6 929	Surveillance on TSE, arbovirus and avian influenza in wild birds.
Import/export/trade	Accessions: 1 045 Samples: 5 361 Tests: 9 122	(1) About half of our work relates to certification for import and export, maintaining overseas trade for primary industry. (2) Trade in companion animals and animal travel overseas (e.g., dogs, cats, parrots and racehorses). (3) Quality assurance reference testing for industry partners.
Artificial breeding (AB)	Accessions: 164 Samples: 626 Tests: 861	More than 10 percent of our testing is for AB purposes, e.g., tests for bovine viral diarrhoea (BVD type 2) by virus isolation on bull semen; infectious bovine rhinotracheitis (<i>Brucella abortus</i>) and leptospirosis by MAT test on serum.
Quality Assurance	Quality control – external Accessions: 101 Samples: 611 Tests: 625 Quality Control – internal Accessions: 110 Samples: 1 207 Tests: 4 991	The ISO/IEC 17025 certification requires that we ensure our testing is robust, and we achieve this by subscribing to 56 inter-laboratory proficiency testing programmes through at least 11 international authorised reference partners worldwide. New ILPT programmes are regularly subscribed to as our capability extends to be prepared to detect a greater range of pathogens. This is in addition to our extensive programme of internal QC/QA testing.

was isolated for the first time in New Zealand following salmon mortalities in a hatchery. Using next-generation sequencing it was determined that the strain was likely to have been present in New Zealand for a long time.

AVIAN

A variety of samples from poultry were tested for avian influenza, Newcastle disease, West Nile virus and exotic *Salmonella* serovars. All exotic agents were ruled out. Suspected outbreaks of avian botulism were investigated in wild birds; all samples were negative for exotic disease agents but avian botulism was confirmed in one outbreak. Further investigations were performed into pet bird mortalities. Testing was performed for avian influenza, paramyxovirus type 1, beak and feather disease virus, proventriculus dilatation disease, avian polyomavirus, psittacine herpes virus and *Salmonella* spp. Only beak and feather disease virus and *Salmonella* Typhimurium were identified, both of which are endemic.

BOVINE

Investigations required testing for anthrax, brucellosis, chlamydia, *Listeria* spp, *Mycoplasma* spp, *Pasteurella multocida*, bovine herpes viruses (BHV-1, -2 and -5), bovine viral diarrhoea, orthopoxvirus and *Theileria* spp. Several vesicular disease exclusions were also performed. Since September 2012 a number of outbreaks of regenerative anaemia in cattle have been linked to *Theileria orientalis* Ikeda strain, which has not previously been reported in New Zealand. Sporadic outbreaks of peritonitis and pleuritis caused by *Pasteurella multocida* continue to be identified in calves.

CAMELIDS

Alpaca samples were tested for *Brucella* spp. and *Mycoplasma haemolamae*. In animals with anaemia, wasting and deaths, *M. haemolamae* was identified in one animal without symptoms, by PCR at a subcontracted laboratory. *M. haemolamae* had not previously been reported in New Zealand.

CANINE

Investigative testing was performed for agents including canine distemper virus, *Leptospira* spp, *Babesia* spp. and Q fever. Testing performed as part of a suspect *Babesia* investigation brought to light the use of a fraudulent test result by an individual trying to bring a dog into New Zealand from Asia. Testing by AHL prevented a

border biosecurity breach and the animal in question was returned to its country of origin.

CERVINE

An investigation into lesions on deer antler buds revealed infection with New Zealand red deer parapoxvirus.

EQUINE

Horses were investigated for vesicular stomatitis virus and equine infectious anaemia virus. No exotic infectious agents were identified.

FELINE

Lyme disease was ruled out in a cat whose owner contracted lyme disease in Australia.

LEPORINE

Following an investigation of a rabbit with liver lesions, a diagnosis of hepatic coccidiosis was made but *Francisella tularensis* was excluded.

OVINE

Sheep were tested for scrapie, bluetongue virus, *Mycoplasma capricolum*, Maedi/Visna, sheep pox, Q fever, brucellosis and *Chlamydia* spp. Unexplained ovine abortion events potentially linked to *Helicobacter/Flexispira* infection continue to be investigated.

PORCINE

Pigs were tested for trichinella, sarcocystis, *Taenia* spp, coronaviruses, porcine boca virus, torque teno sus virus, pestivirus, pig parvovirus, influenza A, *Brucella suis* and *Mycoplasma* spp.

REPTILIAN

During the year several samples were submitted from captive tuatara and coastal bearded dragons with skin lesions. An emerging fungal pathogen, the *Chrysosporium* anamorph of *Nannizziopsis vriesii* (CANV), was isolated from a number of these skin lesions. This was a first report of CANV in New Zealand.

Exotic disease preparedness

The AHL has made further enhancements to preparedness for exotic disease investigations and responses. Significant effort has been invested in advancing preparedness for foot and mouth disease (FMD), including preparation of the AHL FMD laboratory plan, training of another

AHL scientist in FMD diagnostics at the World Reference Laboratory in Pirbright, UK (three AHL scientists have attended this training course over the last three years) and facilitation of participation in FMD proficiency testing schemes that will begin this year.

National bio-containment project

The high-level containment laboratory at Wallaceville, operated by the Ministry for Primary Industries, conducts daily diagnostic testing of cases of suspect exotic animal diseases not found in New Zealand, such as avian influenza and anthrax. It is also used by ESR for diagnosis of high-risk human diseases such as pandemic influenza, polio and SARS. The laboratory offers the highest level of containment for this type of work in New Zealand, and is therefore the only laboratory suitable for this vitally important role.

The current facility was built in 1999, is nearing the end of its design life and is getting too small. A new laboratory is in the planning phase to ensure we can continue this essential work and keep pace with changing international regulations. These changes will not result in any new operational activities.

The new facility must be designed to meet international standards for safety and containment and meet operational science needs. We will be working with a range of internationally experienced designers and engineers over the next six months to develop a draft design and put forward an indicative business case for consideration at the end of 2013. Further design work and a detailed business case are expected to be completed in the middle of 2014. If approved, construction will start in April 2015 and the new laboratory will be completed by 2017.

National and international connections

This year New Zealand Veterinary Pathology hosted the annual meeting of the New Zealand Veterinary Laboratory Network in Palmerston North. The meeting was attended by representatives from AgResearch,ASUREQuality, Gribbles, MPI, NZ Veterinary Pathology, Poultry Veterinary Services and Tegel. A new attendee was welcomed: Dr Julie Collins-Emerson from IVABS, Massey University.

AHL experts represent New Zealand on the following multinational animal disease working groups:

- International Veterinary Biosafety Workgroup, an international working group involved in setting standards for high containment veterinary laboratories world-wide – Joseph O’Keefe;
- FluLabNet, an EU-organised collaborative network on influenza – Wlodek Stanislawek;
- Sub-committee of Animal Health Laboratory Standards, an Australian and New Zealand committee that seeks to protect market access for animals and animal products by applying internationally accepted best practice, especially for emergency animal disease diagnosis and management – Wendy McDonald; and
- Sub-committee of Aquatic Animal Health Standards, an Australian and New Zealand committee that provides technical advice on aquatic animal health issues in support of policy planning – Brian Jones.

Staffing and structure

TABLE 2: STAFFING AND STRUCTURE

Director, Investigation and Diagnostic Centres	Veronica Herrera (Wellington)
Animal Health Laboratory Manager	Joseph O’Keefe
Bacteriology and Aquatic Animal Diseases	
Manager	Wendy McDonald
Aquatic Animal Diseases Principal Adviser	Brian Jones
Fisheries Forensic Analysts	Graeme Bremner, Philip Clarke
Scientists	Jenny Draper, Hye Jeong Ha, Sharon Humphrey, Cara Brosnahan
Technical staff	Taryn Haydon, Henry Lane, Katy Booth
Immunology	
Team Manager	Richard Spence
Immunology Principal Adviser	Reinhold Kittelberger
Scientists	Rick Clough, Rudolpho Bueno
Technical staff	Michaela Hannah, Richard Swainsbury, Courtney O’Sullivan
Technical Resource Co-ordinator	Judy Jenner
Biosafety Officer	Kanishka Fernando
Virology	
Manager	Grant Munro
Scientists	Wlodek Stanislawek, David Pulford, Della Orr, Edna Gias
Technical staff	Joanna Hamilton, Mike Hansen, Ickel Marie Bueno, Mary-Ann Tuboltsev, Oliver Quinn, Smriti Nair
Containment Laboratory	
Supervisor	Vacant
Quality Assurance	
Adviser	Irina Bolotovski

STAFF PUBLICATIONS IN SCIENTIFIC AND TECHNICAL JOURNALS

Kittelberger R, O'Connor K (2012). Differences in performance of an *Ehrlichia canis* immunofluorescence antibody test in two dog populations. *Surveillance* 39(1), 12–13.

Kittelberger R (2012). Novel prion protein in BSE affected cattle in Switzerland - Letter to the Editor. *Emerging Infectious Diseases* 18(5), 890–892.

Watts J, Kittelberger R (2012). Transmissible spongiform encephalopathies (TSE) surveillance programme. *Surveillance* 39(3), 27–28.

Lee E, Kittelberger R, George S, Green O (2012). Arbovirus surveillance programme in New Zealand. *Surveillance* 39(3), 29–30.

Keeling SE, Johnston C, Wallis R, Brosnahan C, Gudkovs N, McDonald WL (2012). Development and validation of real time PCR for the detection of *Yersinia ruckeri*. *Journal of Fish Diseases* 35(2), 119–125.

Collins RA, Armstrong KF, Meier R, Yi Y, Brown SDJ, Cruickshank RH, Keeling SE, Johnston C (2012). Barcoding and border biosecurity: Identifying cyprinid fishes in the aquarium trade. *PloS ONE* <http://dx.doi.org/10.1371/journal.pone.0028381>. Accessed 2012

Stanislawek W, Frazer J, Rawdon T, McFadden A, Tana T (2012). Avian influenza surveillance programme. *Surveillance* 39(3), 20–23.

McFadden AMJ, Tisdall DJ, Hill FI, Otterson P, Pulford D, Peake J, Finnegan CJ, La Rocca SA, Kok-Mun T, Weir AM (2012). The first case of a bull persistently infected with Border disease virus in New Zealand. *New Zealand Veterinary Journal* 60(5), 290–296.