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NEW SOUTH WALES

# ANIMAL HEALTH SURVEILLANCE

Information contributed by staff of the Livestock Health and Pest Authorities and the Department of Primary Industries

## Eradication of a highly pathogenic avian influenza outbreak

A highly pathogenic avian influenza (HPAI) outbreak was eradicated from NSW between October and December 2013.

Competent veterinary authorities the world over control virulent avian influenza virus, because death rates from this virus in poultry can approach 100% and it can also cause death and illness in humans.

When HPAI virus (subtype H7N2) swept through a poultry farm in NSW in October 2013 it killed thousands of hens in a few days. It would have killed more birds, but it was stopped by the stamping out policy implemented by the owners and by the NSW DPI–LHPA Alliance. It spread to a second layer farm before it was eradicated.

The first infected premises were producing eggs from both free-range and caged hen operations. The farm also had a feed mill supplying pig premises and other poultry premises. The mill operated under biosecurity procedures that had proved effective in a previous HPAI incident in NSW (reported in *Animal Health Surveillance News 2013/1*).

Wild ducks frequented dams on the property, but the hens' drinking water came from treated town supplies—not from dams. Replacement laying hens were supplied from a biosecure farm 60 km away, and there had been no recent introduction of hens.

The second infected premises were located 35 km from the first. This was a caged-layer farm consisting of two sheds located close to each other. Clinical signs appeared on the second farm 10 days after the hens on the first farm had been diagnosed with HPAI. Only one of the sheds on the second farm had been affected by the time eradication started.

Clinical signs in the birds on the first farm included decreased feed intake and sudden death. The daily death rate quickly escalated above the norm for the farm.

There was no evidence of respiratory disease, conjunctivitis, or coughing. At necropsy there was a fibrinous perihepatitis (inflammation of the coating of the liver) and pericarditis (inflammation of the fibrous sac around the heart). Small haemorrhages were found in many organs—especially the heart and kidney. The spleen and wattles were swollen. The trachea was normal, but internal egg laying

## *In this issue!*

*The combs are blue and pale in these birds infected with avian influenza virus. Photo C Holland*

(indicating inflammation of the oviduct) was common.

The clinical signs of the birds on the second farm included facial swelling, blueness and mottling of the comb, and lethargy. The death rate rapidly increased over several days.

Testing of cloacal swabs from both farms by real-time polymerase chain reaction at the Virology Laboratory at Elizabeth Macarthur Agricultural Institute (EMAI) identified infection with an H7 avian influenza virus. These results were confirmed at the Australian Animal Health Laboratory (AAHL) at Geelong and the virus was typed as H7N2. Nucleic acid sequencing of viruses isolated at AAHL confirmed that they were from the Australian H7 lineage, not exotic strains.

The most likely pathway of infection on the first farm was via wild ducks that transmitted LPAI (low pathogenic avian influenza) H7N2 to the free-range flock. This could have occurred from direct or indirect contact (for example, through flies) with duck faeces. No AI viruses were detected in the wild ducks sampled in the area, but this does not exclude them as a source of the virus. Alternatively, the virus could have been introduced from other wild birds.

Once in the free-range poultry, the virus probably mutated to HPAI and disease expression escalated. The virus then quickly spread to the caged-layer sheds on the same farm, possibly via contaminated cardboard egg cartons or on workers or equipment. It also could have spread by aerosol, even though the caged and free-range operations were 600 to 700 m apart.

Although feed for the second farm was sourced from the first one, it is unlikely that this feed carried the virus to the second farm. The feed mill operations delivered feed to many properties before the disease was found and the two properties quarantined, but these other properties remained disease free. On the second farm, clinical signs started in a discrete group of cages, suggesting focal introduction rather than initial widespread dissemination throughout the flock. Response staff concluded that contaminated

cardboard egg trays were the most likely means of spread from the first to the second property. Each farm used its own trays to transport eggs to the grading plant, but the trays from both farms were returned in the same vehicle at the end of processing. Sequencing data indicated that the virus that infected the second premises was identical to the virus from the first infected premises.

The eradication program used the nationally pre-agreed AUSVETPLAN *Disease Strategy: Avian Influenza* (2011). It comprised the key elements of quarantine, movement control, surveillance, valuation, destruction, disposal, decontamination and proof of freedom.

Carbon dioxide gassing in purpose-built containers was the chosen method of destruction. It was humane and rapid. Staff used off-site heat treatment, on-site composting and off-site landfill to dispose of carcasses, manure and eggs.

Fortunately there were no other commercial poultry farms and only very small backyard poultry flocks in the two 10 km-radius Control Areas. Opportunities for lateral wind-borne spread to other poultry were therefore negligible.

A significant number of traced premises had been in contact with the infected properties through truck movements, people, eggs and potentially contaminated equipment or materials. These premises were observed, sampled and tested according to a surveillance schedule before being cleared. The pullet-rearing operation associated with the first farm was intensively surveyed and remained disease free. This demonstrated that the biosecurity procedures operating at this level at this farm were effective.

Epidemiologists conducted a comprehensive risk assessment of the feed in the feed mill and concluded that it was not contaminated with the virus. However, the feed mill was not permitted to start operating again until all the poultry sheds had been decontaminated and other biosecurity procedures were in place to ensure that the mill remained isolated.

After decontamination and disinfection, the first premises were left vacant for 21 days and then restocked with sentinel birds. The second farm chose to restock with sentinels only and placed 200 birds in each shed. The first farm partly restocked some of its sheds and placed sentinel birds in others. Cloacal and tracheal swabs and blood samples were collected 21 days later for testing. The birds all tested negative and the properties were declared free of avian influenza.

Compensation at market value was paid to the owners for the destruction of their birds and eggs. The estimated cost of the outbreak was about five million dollars and will be shared by the owners, the federal government, state governments, the Australian chicken meat industry and the Australian egg industry.

**For further information contact Therese Wright, Manager Animal Biosecurity Services and Response, Biosecurity NSW, NSW DPI Orange on (02) 6391 3351.**

## Pacific Oyster deaths at Port Stephens: another emerging disease?

Throughout 2013, NSW DPI received a number of reports of diploid and triploid Pacific Oysters, and some Sydney rock oysters, that were dead or dying in the Port Stephens estuary. (Diploid Pacific oysters are standard oysters with two sets of chromosomes. Triploid oysters, on the other hand, have triple chromosome sets; because they are sterile they grow faster and fatter.)

From November to the end of December 2013, investigators sent 12 different submissions to EMAI's State Veterinary Diagnostic Laboratory for diagnosis. The major laboratory findings were:

- All submissions tested negative for POMS (Pacific oyster mortality syndrome), which has decimated the Pacific oyster industry in the Georges River and Hawkesbury River estuaries.
- Affected oysters had inflammation or ulceration, or both, in the gut and on external surfaces.
- The gut was filled with undigested food and haemocytes (the oyster equivalent of blood cells).
- Isolates of several species from the Vibrionaceae family of bacteria were obtained from two submissions; the significance of the presence of these bacteria is being further investigated.

However, EMAI specialists could not immediately make a diagnosis. In December, staff began a structured program to sample Pacific oysters and water quality, temperature and salinity within Port Stephens. This study was designed to obtain new samples of affected oysters, exclude known pathogens, and identify any elusive potential pathogens responsible for the extensive oyster deaths.

Limited data describing the environmental conditions during late 2013 suggest that conditions may have been stressful at times, with high air temperatures and short periods of reduced salinity. However, no specific

environmental factors adequately explain the losses observed. The cause of the deaths has still not been determined.

**For further information contact Melissa Walker, Strategy Leader Aquatic Biosecurity, Animal Biosecurity and Welfare Branch, NSW DPI, on 02 4916 3911.**

## Heliotrope poisoning (pyrrolizidine alkaloid toxicity) in dairy heifers

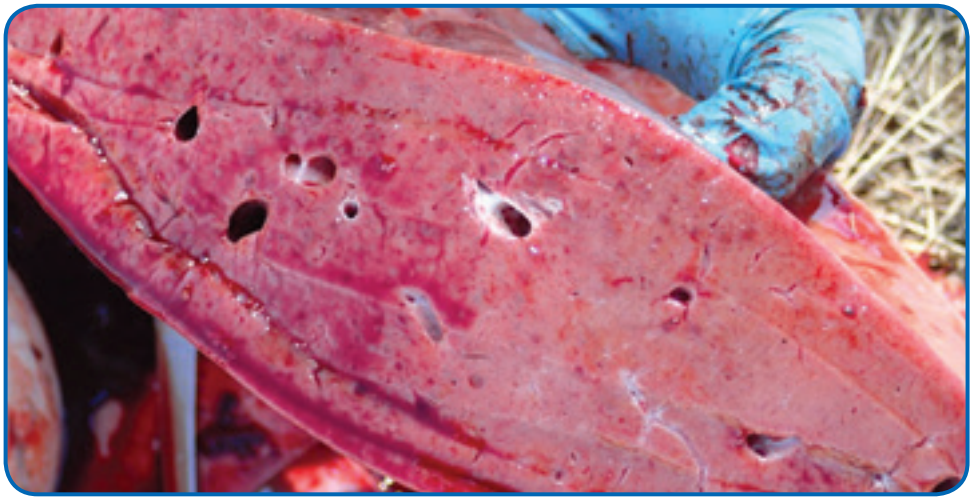
The Riverina LHPA and private vets worked together to diagnose pyrrolizidine alkaloid toxicity in a group of 60 autumn-calving Holstein dairy heifers in southern NSW.

The cattle had been fed lucerne hay that had been inadvertently contaminated with heliotrope (*Heliotropium europaeum*, known as common heliotrope, potato weed or blue weed). The hay had been fed in racks for several weeks, but when the heifers started to lose condition it was withdrawn.

The clinical disease started after calving, some 5 months after the cattle had had access to the contaminated hay.

A total of 40 heifers died, at a regular rate of one or two a week over an 8-month period. This was accompanied by a drop in milk production, ill thrift

*Pyrrolizidine alkaloids can cause photosensitivity, in this case on the 'white' skin on the backline of an affected heifer. Photo D Salmon*



*Liver of a heifer affected by pyrrolizidine alkaloid toxicity, showing enlarged, fibrotic bile ducts. Photo D Salmon*

*Common heliotrope contains toxic pyrrolizidine alkaloids. Photo D Salmon*

and weight loss, skin lesions and neurological signs such as reduced awareness, wandering and blindness.

Necropsy of three heifers revealed heart enlargement and an increased amount of pericardial fluid. The liver was slightly rounded and enlarged. The cut surface revealed thickened and fibrous bile ducts. The gall bladder was enlarged, and sometimes there was mild yellowing of the omentum (the membrane surrounding the abdominal organs) and the gastrointestinal tract.

Histopathological examination showed fibrosis and proliferation of the bile ducts (biliary hyperplasia), enlargement of liver cells (megalocytosis) and mild karyomegaly (enlarged nuclei) in the liver cells. The pathologist said that the chronic liver damage suggested pyrrolizidine alkaloid toxicity or exposure to aflatoxin.

At the same time, laboratory tests ruled out bovine ephemeral fever, liver fluke, and the possibility that the heifers were persistently infected carriers of bovine viral diarrhoea virus. Biochemistry on the blood from 10 heifers in the group indicated liver damage.

Common heliotrope is toxic to livestock. Although it occurs commonly in predominantly winter rainfall areas, heliotrope also depends on summer rainfall for development. Such conditions occur regularly in the Riverina, where heliotrope covers several thousand square kilometres.

Heliotrope, like Paterson's curse (*Echium plantagineum*), contains pyrrolizidine alkaloids that produce toxic metabolites when ingested. These metabolites inhibit the division of liver cells, resulting in liver damage and chronic, fatal liver toxicity.

Cattle are 30 to 40 times more susceptible than sheep or goats to pyrrolizidine alkaloid poisoning. Calves and younger cattle are more susceptible than older cattle.

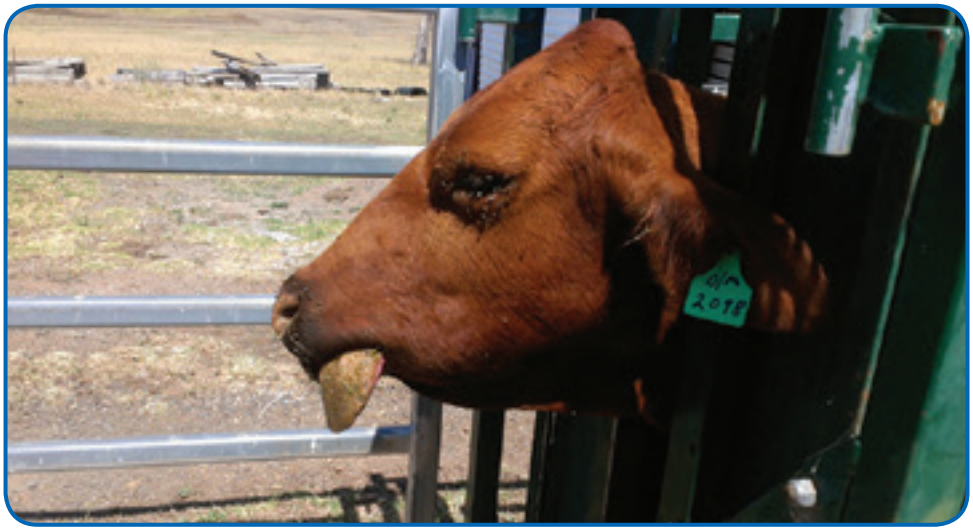
Pyrrolizidine alkaloids are most toxic when heliotrope is consumed while green. However, heliotrope is still toxic after it is used to make silage or dried in hay; moreover, it is apparently more palatable in these forms than when it is green and fresh.

Once severe liver fibrosis occurs the changes are irreversible and are usually fatal. Even with supportive therapy, the disrupted hepatic blood flow and the inability of the remaining liver cells to divide stops the liver from regenerating. The outlook is therefore poor, and slaughter should be considered.

**For further information contact Dan Salmon, Senior District Veterinarian, Riverina LHPA, on (03) 5881 1055.**

## Middle ear abscess in a cow

One cow out of a herd of 20 recently purchased Santa Gertrudis cows was presented with massive weight loss 8 weeks after calving (from fat score 3 to fat score 1). The cow was depressed and ate with its head stretched out to one side. There were signs of facial paralysis on the right side



*A middle ear abscess caused partial tongue paralysis. Photo R McKinnon*

of the face, with inability to blink on that side. When the tongue was pulled out it stayed that way if pulled to the right. The cow was also sham drinking.

The cow was euthanased. At necropsy, a grape-sized abscess was found in the middle ear. Infection of the middle ear usually arises from an infection in the pharynx that ascends via the eustachian tube.

**For further information contact Bob McKinnon, Senior District Veterinarian, Central North LHPA Tamworth, on (02) 6762 2900.**

## Pulsed field gel electrophoresis, a definitive tool for molecular surveillance of veterinary pathogens

Pulse field gel electrophoresis (PFGE) is a powerful genotyping tool that can be used for both research and diagnosis. This article explains how our EMAI researchers have been using PFGE for disease surveillance.

PFGE is used to separate what otherwise appear to be the same, or similar, organisms in bacteriological or serology tests into genetically unique groups known as *pulsotypes*.

Whole intact DNA from bacterial cells is extracted into an agarose gel. Then, the DNA is digested by using specific enzymes to break it down into fragments of various lengths.

Specialised electrophoresis equipment is used to resolve the DNA fragments into pulsotypes that show up like DNA-based bar codes.

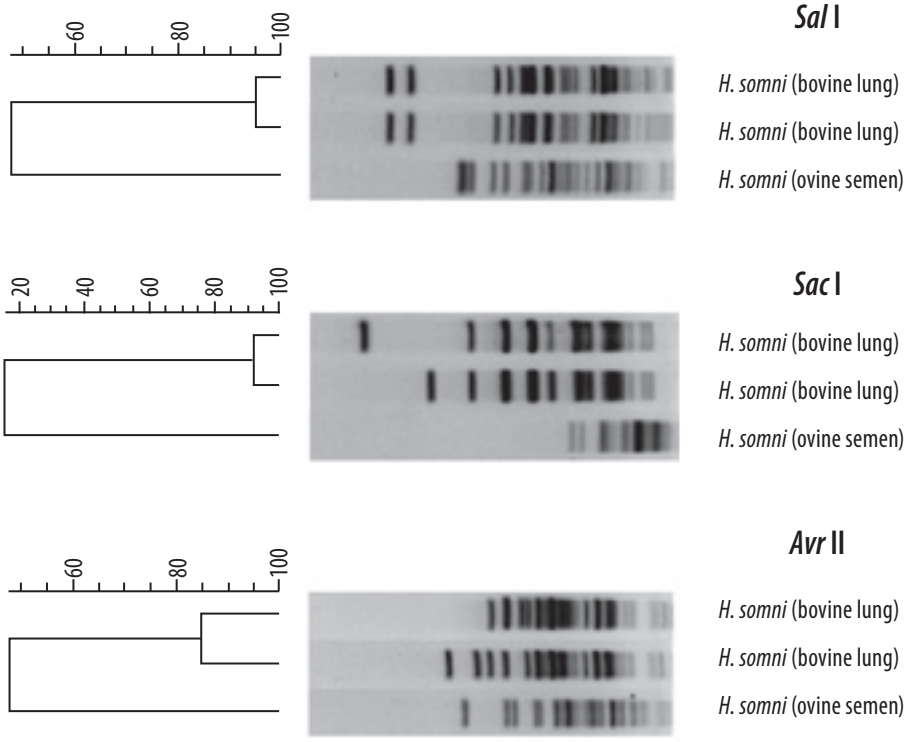
Pulsotypes from standardised gels can then be easily compared within and across gels to generate phylogenetic trees, depending on the degree of relationship between them. (Phylogenetics is the study of how organisms are related during evolution.)

EMAI researchers have used PFGE to study the distribution of pulsotypes of a *Salmonella* Litchfield serotype of bacteria in feral pigs in northern Australia. The question for the researchers was: Are the pulsotypes common through all home ranges or social groups, indicating that they are easily transmitted, or are they contained and therefore not able to spread easily? The findings, yet to be published, may help to determine patterns of transmission of a range of diseases of feral pigs, including exotic diseases.

Similarly, research has also shown that the bacterium *Histophilus somni* can comprise different pulsotypes that may behave differently in different species.

*Histophilus somni* is a highly contagious bacterium that causes acute, often fatal pneumonia, meningoencephalitis (inflammation of the linings of the brain), myocarditis (heart muscle inflammation) and arthritis in cattle. It also causes polyarthritis and occasional abortions in sheep, although this is apparently a different syndrome from that seen in cattle. Isolates from sheep were until recently reported as *Histophilus ovis*. However, *H. ovis* has been reclassified taxonomically as *H. somni* because the two organisms are bacteriologically indistinguishable.

PFGE on three *H. somni* isolates was able to show that they were different pulsotypes. Two of the isolates originated from cattle lungs and one from sheep semen. They generated unique pulsotypes with two enzymes (*Sac* I and *Avr* II). The third enzyme (*Sal* I) could not differentiate between the cattle lung isolates but could separate these from the isolate from the sheep




*Three isolates of the bacterium Histophilus somni originating from cattle lungs and sheep semen were pulsotyped by using three enzymes, Sal* I, *Sac* I *and Avr* II.

semen. The PFGE technology gives greater information on the 'bacterial culprit' than would be obtained from culture alone.

PFGE remains the definitive gold standard strain-typing technique used in outbreak investigation and surveillance. Veterinary diagnostic laboratories throughout Australia routinely culture and isolate organisms of veterinary importance but do little more than report the result beyond the species or subspecies.

Unlike culture, PFGE can be used to further classify within serotypes in a way that has yet to be extensively applied to veterinary pathogens in Australia. PFGE could be used to further investigate organisms epidemiologically, providing information vital to surveillance.

Furthermore, there is increasing scientific evidence of the relationship between the genetic makeup of bacteria that are important in veterinary science and their resistance to antibiotics. PFGE might be able to help Australian livestock industries to use antibiotics appropriately.

**For further information, contact Ian Marsh, Research Officer, Animal Biosecurity Surveillance and Research Unit, NSW DPI Menangle, on (02) 4640 6502.**

## *Getting information on animal diseases*

This surveillance report can convey only a very limited amount of information about the occurrence and distribution of livestock diseases in New South Wales. If you would like more specific information about diseases occurring in your part of the state, contact your local Livestock Health and Pest Authorities District Veterinarian or Departmental Regional Veterinary Officer.

***For statewide information, contact the Department of Primary Industries Animal and Plant Biosecurity Branch in Orange on (02) 6391 3237 or fax (02) 6361 9976.***

For more information on national disease status, check the National Animal Health Information System (NAHIS) via the internet at:
http://www.animalhealthaustralia.com.au/status/nahis.cfm

This is a report under the Animal Disease Surveillance Operational Plan, Project 8, 'Reporting for Animal Disease Status in NSW'.

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***Copies of NSW Animal Health Surveillance reports are available on the internet at: http://www.dpi.nsw.gov.au/newsletters/animal-health-surveillance***



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