CSI Food: Eliminatory my dear Watson

Andrew Hudson, Darren Saunders, Shevaun Paine and Maurice Wilson, ESR.

Identifying the source of an outbreak of foodborne disease, or the origin of a foreign object in food, is much like solving a detective mystery.

While ESR does have sophisticated machines and experienced scientists at its disposal, just like the TV series CSI, it is seldom the case that an incident can be "wrapped up" and the perpetrator brought to justice within 60 minutes. Rather more like the TV series, ESR provides the science that gives companies, the Ministry for Primary Industries (MPI) and the Ministry of Health (MoH) the information that they need to help them solve real life "cases".

This article provides a few vignettes, showing how science has been used to assist in tracking the sources of biological, chemical or physical hazards in foods. All of these examples are of incidents which occurred in New Zealand and include aspects of the detective work that went into their solution. Hopefully, too, there are some interesting points and lessons that can be taken from them.

The Sign of the Flour

(with apologies to Sir Arthur Conan Doyle)

As seekers of justice it is important not to hold pre-conceived notions about associations between foods and pathogens. This became all too evident when it was noted by ESR epidemiologists that there was an increase in cases of one type of salmonellosis in October 2008. So what's likely to be contaminated by Salmonella? The offending food in these cases often results from the organism getting into animal feed and hence the food. That means typically, at least in the international literature, pork, chicken or eggs (much less so in New Zealand though). "House to house inquiries", in the form of a case control study, pointed the finger of suspicion at the possibly ever-so-slightly-naughty practice of eating uncooked cake batter. So, with reference back to the usual suspects, that puts eggs in the frame.

But not this time. Using some more expert epidemiology, eggs were excluded. There was only one suspect left without an alibi: the flour. To seal the deal, Salmonella was isolated from unopened bags of flour from the homes of cases, bags of flour in commerce, and from samples taken at the flour mill. An enhanced DNA fingerprinting technique at our disposal was able to link Salmonella flour isolates to human cases.

A take home message from this outbreak was that extremely low concentrations of Salmonella cells can cause disease. The concentration found in the flour was 1 cell in 50-300g of flour, and so a routine investigation, in which 25g would be tested, would most likely have missed it. Low concentrations of Salmonella can cause disease, and this has been shown repeatedly (chocolate, peanut butter, flavoured chips……).

The Mouse of the Vegetables

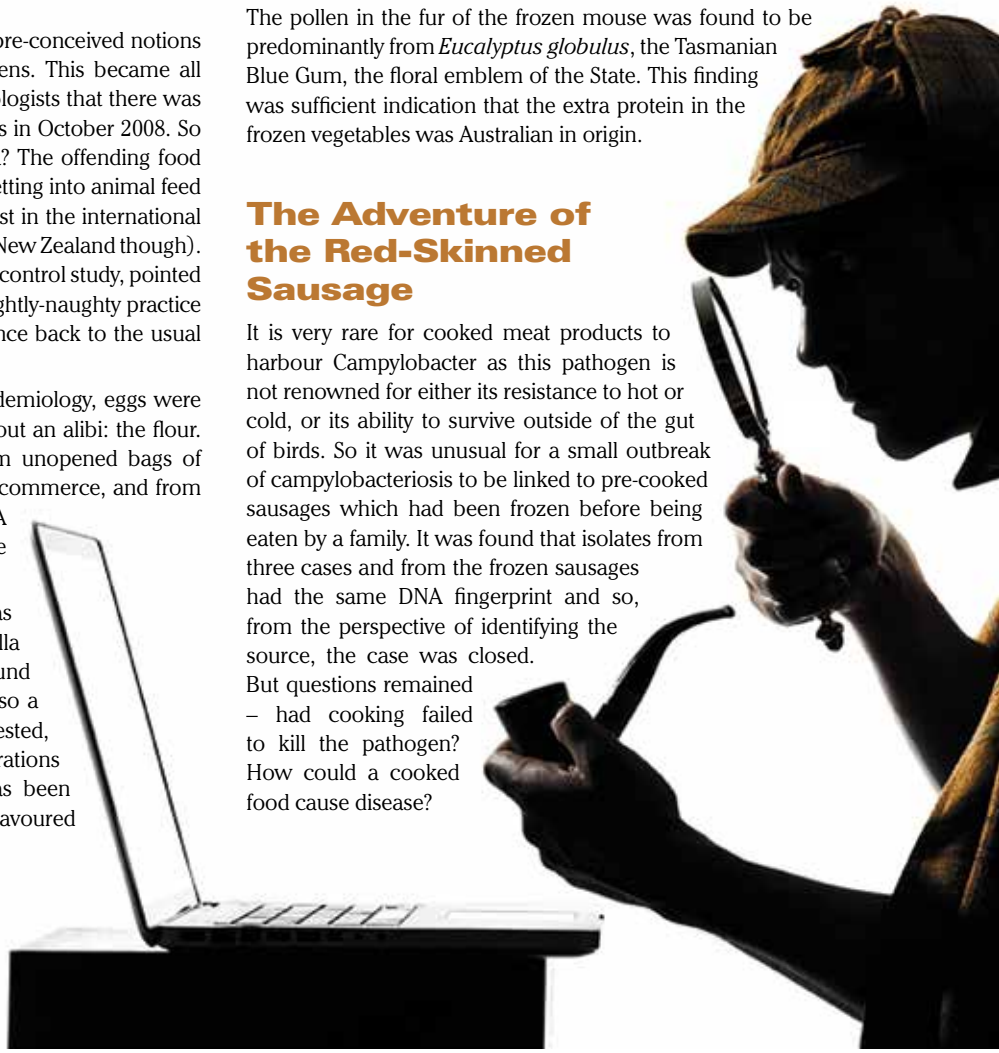
When the jar of olives spiced by the addition of a frog came into the laboratory, it was relatively straightforward to answer the key questions applicable to almost all food forensic investigation: What is the foreign matter? At what point, and how, did it become associated with the product? The foreign object was a frog of a species not found in New Zealand, but common to the olives' country of origin, Greece. Ockham's razor therefore dictated that the most likely explanation was that the frog became associated with the olives when they were packed in Greece.

However, foreign frogs or other fauna originating from a discrete geographic area in food are rare. More frequently occurring and less amphibious is the common house mouse (*Mus musculus*), which has been associated with humans from a time well before the dawn of recorded history. They have followed us over the earth, eating and fouling our food, and carrying disease. So, when partially vivisected mouse remains were found in frozen vegetables grown and processed in Tasmania, packed in New Zealand, opened and eaten in Christchurch, ascertaining where and when the mouse became associated with the vegetables was not as simple as declaring the mouse an Australian or New Zealand national based upon geographic range. *Mus musculus* gets around, so further forensic scrutiny was called for.

The laboratory had access to a palynologist, a pollen expert. The pollen in the fur of the frozen mouse was found to be predominantly from *Eucalyptus globulus*, the Tasmanian Blue Gum, the floral emblem of the State. This finding was sufficient indication that the extra protein in the frozen vegetables was Australian in origin.

The Adventure of the Red-Skinned Sausage

It is very rare for cooked meat products to harbour Campylobacter as this pathogen is not renowned for either its resistance to hot or cold, or its ability to survive outside of the gut of birds. So it was unusual for a small outbreak of campylobacteriosis to be linked to pre-cooked sausages which had been frozen before being eaten by a family. It was found that isolates from three cases and from the frozen sausages had the same DNA fingerprint and so, from the perspective of identifying the source, the case was closed. But questions remained – had cooking failed to kill the pathogen? How could a cooked food cause disease?



To investigate this further, we tested some of the frozen sausages a little more thoroughly. Firstly we tested a rinse of the sausages. Then the outsides of the sausages were heat-treated before testing the internal meat. We found Campylobacter on the outside of the sausages, but not the inside. The logical conclusion was that they had been cooked properly but externally re-contaminated prior to being sold. This impression was supported by observations of the shop made by Health Protection Officers (HPOs).

Another interesting point was that Campylobacter had been isolated from a frozen food involved in an outbreak. If that was not a first, then it was at least a rare event, and retail store that it can be worth testing frozen foods for this organism.

The Ham with the Twisted Listeria

Most people involved in the food industry will be aware of the problems posed by *Listeria monocytogenes* in ready-to-eat foods. An organism whose infections result in around a 20% case fatality rate is not trivial. But what if the same organism can cause two quite dissimilar types of disease? Will the food detectives be able to spot a change of MO? In our case yes, although we did benefit from a bit of "dumb luck". Having just written a short article on "febrile non-invasive gastroenteritis caused by *L. monocytogenes*" there could have been little better preparation for having four cases with exactly the right symptoms come to the notice of South Island HPO's. The ready-to-eat meats associated with these cases were tested with this knowledge in mind (a characteristic of this form of listeriosis is the very high concentration of the organism in food), and within a few days the outbreak had been identified and a recall implemented by the MoH.

The final Problem

Sometimes the obvious suspect turns out to live up to the stereotype, this one being a chicken product and campylobacteriosis. Following pre-Christmas functions, two groups of people became ill. The implicated food was chicken liver pâté. However, this was a little odd as, after all, it is made with cooked livers. Well, it seems not. There is a trend among chefs to cook chicken livers so that they are still "pink on the inside" before making the pâté. That would be fine if chicken livers were like steak, where contaminants are only on the outside, so that you can still have safe food that is essentially undercooked in the middle.

In some experimental work we looked at the presence and concentration of Campylobacter on the insides and outsides of chicken livers. Lo and behold we showed that the insides of the livers could be contaminated with the organism. So cooking until "pink in the middle" is unlikely to ensure that all organisms are killed. This leaves the chefs in a dilemma – is there a way of cooking them such that the eating qualities are maintained without transmitting disease. Well, yes there is, but that's another story.

So, whodunnit?

While investigation of food complaints may lack the salacious plot twists and drama of police investigations, many of the same forensic techniques are applicable. Food forensic examinations frequently require similar degrees of detective work and deductive reasoning. In many of these cases the good old (DNA) fingerprint has come to our aid and provided the proverbial smoking gun, but it is clear that you need to draw on evidence from multiple disciplines to crack cases wide open.