

DIAGNOSIS MURDER



In September 2000 Ian Lowther, a UK national, was sentenced to life imprisonment for a murder he committed 23 years earlier. His conviction was made possible by advances in biotechnology which allowed forensic scientists to match his DNA to samples found on the victim's body. Since then, DNA profiling has become standard crime scene practice.

One of the companies that has pioneered the application of molecular biology, and which is a major supplier of DNA test kits to the US police, believes that the same know-how can help veterinarians and farmers. Life Technologies is a global company which already supplies a wide range of biotechnical solutions to diagnostic laboratories, mainly for human healthcare and food safety testing. Now, the company believes that animal health could benefit from that same expertise.

"Diagnostic technology has advanced in leaps and bounds over the last few years," says European Professional Service Veterinarian, Christina Boss.

"Thanks to improved molecular tests, we can now identify animal pathogens much more quickly and reliably than ever before, and detect infections at a much earlier stage. So veterinarians and farmers can make disease and herd management decisions with much greater confidence than before."

One of the gene technologies which is proving most useful on-farm is the PCR (polymerase chain reaction) test. Each test contains a specific strand of genetic material which complements a section of the RNA or DNA from a specific pathogen. When the sample is repeatedly heated and cooled (so-called thermal cycling), the polymerase enzyme copies the specific gene code and effectively

amplifies it. So even a small amount of the target pathogen in the sample can be detected.

Like most diagnostic tests, PCR is carried out under laboratory conditions, and requires a specialist processing machine and a series of different reagents. However, most testing facilities will have access to the required equipment and be able to carry out PCR tests.

Since PCR was the subject of the Nobel Prize in 1993, the technique has been enhanced and commercialised to the point where results can be obtained in a matter of hours. So-called rapid PCR tests are now available for a number of animal diseases.

In Germany, Life Technologies has just been given FLI (*Friedrich-Loeffler-Institut*) approval for a rapid PCR test for MAP (*Mycobacterium avium* ssp. *Paratuberculosis*) - the cause of Johne's disease in cattle. The test can produce a result in just one day, compared to several weeks for conventional laboratory culture.

The speed and accuracy of PCR means that the test can be used as an integral part of biosecurity, herd surveillance, disease control and even disease eradication.

No longer should the veterinarian be left wondering 'Who dunnit?'

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THE SAMPLING NUMBERS LOTTERY

One of the most valuable benefits of modern diagnostics is that they can be used to assess the disease status of whole herds or groups of animals, as part of a disease eradication programme, a disease-free certification system, or disease control programme. The question is: how many samples do you have to take to find if a certain pathogen is present or absent?

Calculating the optimum (or, more accurately, the minimum) sample size for a given group of animals can be done using statistical probability based on distribution. It depends on the size of the group, the prevalence of infection, the sensitivity and selectivity of the diagnostic test being used, and how the information is going to be used (eg for control, eradication etc).

Luckily, the veterinary epidemiologists have done much of the groundwork and there are a number of software programs that will do the maths for us. For example, if the expected prevalence of disease is 5% and there are 500 animals in the group, then samples need to be taken from 56 different animals in order to be 95% certain of getting at least one positive result. However, it is worth noting that the sensitivity and specificity of a given diagnostic test can vary considerably depending on the prevalence of the disease.

In some circumstances, pooled samples taken from a group of animals may be tested for the presence of pathogens as the first step in a screening program. Recent studies have shown that oral fluids collected using cotton 'chewing' ropes in pig pens can be used to test for the presence of a number of viral pathogens, including PRRSV, SIV and PCV2, in groups of pigs. Any groups that

test positive can then undergo individual tests to identify which animals are carrying the pathogen.

Pooling samples can provide a practical, cost-effective and non-invasive way of screening large numbers of animals for disease, but this approach is not suitable for all types of diagnostic test or all pathogens. Test results from pooled samples are validated against test results from individual samples to ensure that the veterinarian will get the same information at the herd level as with the individual samples.

Existing epidemiological calculators are not appropriate for deciding sampling sizes for oral fluids, but researchers are working on new calculators which will provide recommendations for this new approach to screening.

When diagnostics are being used to certify the disease-free status of a group of animals, then sample size is an even more critical factor. Even if half the group is tested, can we ever be certain that the other half does not contain an infected animal? Again, we have to rely on the statistics of probability.

Modern diagnostics are increasingly allowing us to test samples from more than one animal and for more than one pathogen at a time, and thus allowing herd health to be monitored over time. However, it is still essential to take the right number of samples from the right animals. The best advice is: if in doubt, ask your diagnostic laboratory for guidance.

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THE VALUE OF DIAGNOSTICS



The articles in this series will take a closer look at modern diagnostics and how they can help beef, dairy, swine and poultry farmers and veterinarians optimise their businesses.

Infectious disease continues to be the biggest cause of economic loss for livestock farmers. At a time when input costs are high and margins are being squeezed, losses due to culling, poor growth, variability, and treatment need to be minimised if businesses are to realise their full potential.

The financial benefits of a better understanding of disease status, along with the advent of new diagnostic tests based on molecular technology, has brought about a significant shift in the way diagnostics are being used by livestock farmers. The role of diagnostics has expanded from disease management into business management.

The speed and reliability of molecular tests means that diagnostics are increasingly being used to prevent and even predict disease issues in groups of animals.

Their ability to detect infections before clinical signs have developed has given buyers an opportunity to screen new additions to their herds and thus enhance biosecurity; it has also provided an effective way of identifying and removing those animals with persistent, sub-clinical infections which continue to infect other members of the herd or flock.

The days when farmers and veterinarians had to wait days or even weeks for test results are fast disappearing. Many of the new generation of tests can be conducted within hours, allowing management decisions to be made quickly – infected animals to be culled or quarantined – before issues get any worse.

As well as being fast, the test results are very reliable: accurately diagnosing the

presence of infection with low levels of false positive or false negative results. So farmers can take herd management decisions knowing that they are not wasting time or money. The tests are also very specific, allowing the identification of different pathogen subtypes – again allowing a more precise management decision to be made.

Sensitive and specific diagnostic tests are essential for disease surveillance and to support cost-effective and targeted deployment of control measures.

Judicious use of diagnostics not only helps reduce the disease impact on individual animals but can also be used to develop strategic disease prevention programmes for entire herds, or even entire regions. For example, vaccination schedules can be based on sound herd disease risk profiles, and disease eradication schemes implemented.

The ability to identify emerging diseases and detect changing patterns in existing diseases on a global scale is also a valuable tool for what is an increasingly global industry.

The growing availability and usefulness of modern diagnostic tests is reflected by the increasing availability of diagnostic laboratory services provided by universities, the state or independent commercial companies. This is also a reflection of the potential for the tests to improve not only animal health but also business performance.

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CUTTING COSTS



Not so long ago veterinarians spent a lot of time trying to convince their clients of the benefits of prevention rather than cure, and in particular the economic benefits of vaccination. However, vaccination is like insurance: if you don't have to make a claim, you always wonder if you really needed it.

The development of more reliable diagnostic tools over recent decades has provided a more concrete basis on which to build a vaccination strategy with a more predictable return on investment. Modern diagnostics are able to identify specific pathogen subtypes based on genetic sequencing. Accurate and specific information of this sort can ensure that the most appropriate vaccine and vaccination schedule is applied for an individual group of animals, so producers can be reassured that the money they spend on prevention is less likely to be wasted.

Appropriate vaccination will reduce the amount of money that has to be spent on treatment, and in particular antimicrobial drugs. At a time when farmers are under pressure from government agencies and consumers to reduce their usage of these drugs, the benefits go beyond the purely financial.

The ultimate aim is to achieve a disease-free herd or flock and then keep it that way by careful surveillance and good biosecurity. Modern diagnostics can help in both situations. Regular monitoring of the environment and the animals themselves can identify disease risks before infection occurs and before any clinical signs develop. Rapid test results, such as those obtained from real-time PCR (polymerase chain reaction), can be used to test new additions to the herd, and those animals that have been transported – reducing the need for lengthy quarantine and the risk of disease being introduced.

Although vaccination, surveillance and good biosecurity can undoubtedly reduce disease risk,

there will always be times when individual animals become sick and require treatment. Here again, diagnostic tests can help to identify the causative agent and thus allow the most effective treatment to be administered quickly. In the past, the time taken to culture samples from animals meant that a best-guess treatment may have been started initially while the test results were awaited. By the time the results were back, the issue may well have been decided one way or the other anyway.

The advent of tests based on molecular biology, such as real-time PCR, means that some pathogens can be identified in little over 24 hours – and with great accuracy. So the most appropriate treatment can be given very early on and thus increase the chances of a successful outcome, not only for the individual animals but also for the group as a whole, as the risk of a bigger disease outbreak is reduced.

Strategic vaccination, targeted treatment and regular surveillance are essential cornerstones of our increasingly productive food animal production systems. They are able to significantly improve animal health and welfare, and reduce disease-related losses and production variation. Appropriate use of modern diagnostic tools can optimise herd health management based on these principles, and make sure that the money that producers spend on disease control is minimised and not wasted.

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THE RIGHT TEST?



The introduction of routine serological tests in diagnostic laboratories provided veterinarians with a valuable tool for diagnosing and tracking a wide range of common infections in production animals. In particular, the technology provided information about infections caused by viruses, which are extremely difficult to identify by culturing them under laboratory conditions. But has serology been replaced by newer, molecular technology?

Serological tests, such as ELISA, rely on the fact that viruses and other pathogens are recognised as 'foreign' by the immune system and stimulate the production of specific antibodies. The antibodies not only help the immune system to deal with the first infection, but they remain at a low level as sentinels to identify future attempts by the same pathogen. Serological testing can not only identify an acute infection, and track its progress, but can also show whether an individual animal or herd has been exposed to infection in the past.

Although serology is undoubtedly a useful diagnostic tool, it does have a number of drawbacks. It takes time for the body to generate an immune response to infection, and so serology may be of limited use in the early stages of infection. In some infections the immune response is faster and longer lasting than others; in some there may be very little response. This kind of knowledge is essential when ordering tests and interpreting results.

Vaccination works by stimulating the production of antibodies to a specific pathogen or pathogens, and thus raising the level of protection against infection. In many cases serological tests cannot differentiate between vaccinated animals and those that have been exposed to natural infection. So when new arrivals are tested, it may be impossible to know for sure if they have been infected or vaccinated. The exceptions are those diseases where marker vaccines are available, for examples for IBR in cattle.

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PCR-based diagnostic tests detect the presence of the pathogen itself, or rather its nucleic acid, and so can detect infection at a very early stage before antibodies are produced. As long as there are no virus particles left, vaccinated animals will test negative with PCR. However, so will those animals that have been exposed to infection in the past, but which are no longer viraemic.

Some infections produce only a short period of viraemia, and so PCR-based tests have to be applied at the right phase of infection to be useful.

It is clear that both serological tests and those based on molecular technology have a role to play in food animal production. ELISA (the most practical serological tool) is an excellent method for herd management over time; Real-time PCR can be the right tool for analysis in the acute phase for individual animals because of its higher sensitivity. The key is knowing the advantages and drawbacks of each approach and thus the most appropriate technique to apply in each situation. In some cases, a combination of both may provide the most accurate and cost-effective means of protecting and improving animal health.

New tests and techniques are continually being developed and validated, so it is always worth seeking the advice or confirmation from your testing laboratory. A good laboratory will be happy to provide advice and guidance on testing for specific diseases, and help with the interpretation of results.

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EMERGING DISEASES



The increasing demand for animal protein and the rapidly growing markets in Latin America and Asia are driving an increase in the production and trade of food animals globally. Many experts believe that these conditions will increase the risk of pandemics, the emergence of diseases in new locations and the development of novel genetic forms of existing pathogens. West Nile virus in European horses, Blue Tongue and Schmallenberg viruses in production animals, and a novel strain of E. coli O104:H4 bacteria in the food chain in Germany last year, are perhaps examples of this theory in practice.

The emergence of a new or unexpected disease is always a potential threat to food production systems based on the intensive rearing practices. The only defence is to isolate and characterise the new pathogens as quickly as possible and then develop preventive measures, such as new vaccines, changes in husbandry, or restrictions on movement. A few years ago, this would have taken a considerable amount of time, and may even have been impossible.

The good news is that the development of new molecular technology techniques has meant that emerging pathogens can now be identified and characterised very quickly. For example, it took just three days to identify the German E. coli strain and reveal that it was a new type that had never been seen before in humans. Once a genome is characterised, it is possible to look for clues to the origin of the pathogen, and to develop a specific course of action. The German E. coli was traced back to central Africa, and it was known that this particular strain would not respond to antibiotics. In fact, using antibiotics was only likely to make the infection worse.

The genome of the Schmallenberg virus was sequenced within a week of the first case being reported in Germany in November 2011. A real-time PCR diagnostic test was developed and made commercially available in a matter of months by Life Technologies.

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The genetic changes which make the PRRS virus so difficult to control in swine herds, are another example of how molecular technology can be used to our advantage. Life Technologies is currently working on a project to characterise the virus genome in more detail and thus open up new and more effective options for both diagnostics and vaccines. In the future swine veterinarians may be able to offer farmers a specific vaccine which is matched exactly to the indigenous virus strain which is causing outbreaks on their farm. They will also have a better understanding of the reservoir of infection and be able to adjust vaccination to match any changes in virus type.

This very precise, prophylactic approach to animal health could be applied to many common infections in cattle and poultry, as well as swine. It has the potential to reduce our reliance on antibiotics as a means of maintaining the health and productivity of food animals, and as such would no doubt be welcomed by farmers, consumers and legislators.

Our ability to respond rapidly and precisely to disease threats by sequencing genomes and developing fast, reliable diagnostics, may prove increasingly valuable to keep production animals healthy and to safeguard our global food supply in the future.

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LESS IS MORE



Cattle, pigs and poultry are all vulnerable to diseases caused by a combination of several viral, bacterial or parasitic pathogens. Bovine respiratory disease for example may be associated with some or all of the following: BRSV (bovine respiratory syncytial virus), PI3 (parainfluenza 3), BVDV (bovine viral diarrhoea virus), adenovirus, IBR (infectious bovine rhinotracheitis), *Mycoplasma bovis*, *Pasteurella multocida*, *Mannheimia haemolytica*, *Histophilus somni*, lungworm and *Aspergillus*. A similar range of pathogens can threaten swine and poultry.

Although the presentation may make a clinical diagnosis relatively straightforward, profiling the exact pathogens involved is essential in order to decide on the best long-term herd management, such as the most effective vaccination programme. Even if the list of possible pathogens can be reduced to the six or eight most likely, that still means six or eight separate samples and six or eight separate diagnostic tests. A labour intensive and potentially costly process.

In an ideal world veterinarians would be able to send an appropriate sample to a laboratory and ask for a 'respiratory complex' test to see which of the common respiratory pathogens are present. This form of syndromic panel testing is high on the agenda at Life Technologies, which is applying its considerable knowledge of modern diagnostic tools to develop multi-pathogen tests for common production animal disease complexes.

The task is not an easy one. PCR devices are like washing machines, with different programmes/temperatures to suit different pathogen tests; the multivalent test has to be able to combine several different tests, but all using the same programme. In addition, the more tests you put together in one

system, the more likely they are to interact with one another and thus lose sensitivity and specificity.

However, the company has managed to overcome the practical issues in the testing system and successfully developed a panel test that can screen for bacteria which are responsible for abortion in ruminants. In just two hours the single test can detect the presence of one or more of eight different pathogens that are commonly associated with abortion, including *Anaplasma phagocytophila*, *Coxiella burnetii*, *Listeria monocytogenes*, *Leptospira pathogens*, *Campylobacter fetus*, Bovine herpesvirus type 4, and Chlamydia and Salmonella species. It means that instead of taking eight separate samples and conducting eight separate tests, just one sample from the placenta or cervix can help with the differential diagnosis in cases of abortion.

Multiplexing diagnostics is at the forefront of diagnostic technology and in the near future veterinarians are likely to benefit from more and more tests that will help to simplify diagnosis in multi-pathogen diseases. The development of multivalent diagnostic tools is definitely a case of 'Less is More'.

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