Scarlet "Nurse" Health Stamp

New Vaccines and Tests for Tb and Yersiniosis in Deer

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Summary

- Control of Tb using efficient diagnosis is the most important challenge currently facing NZ deer farmers. A new blood test (BTB) has been developed in our laboratory which, when used in parallel with skin testing (ST), provides improved precision for Tb diagnosis. It is sensitive (95%) in diagnosing Tb and specific (98.6%) in excluding non-specific reactions. New Zealand now leads the world in technology for Tb diagnosis in farmed animals.

- Studies on Tb infected animals with BTB have identified not only disease, but certain animals with immune reactivity, which appears to be protective. Vaccines (BCG) are now being tested in deer to see if comparable levels of artificial protection can be established. Early results are promising, but vaccine development for Tb will be costly and may not provide the ultimate solution.

- We have developed a vaccine for Yersiniosis (Yersiniavax) which protects "at-risk" weaner deer and is being tested in field trials this year. If successful, a new vaccine for yersiniosis will be available next year.

Introduction

Immune reactivity to infection is considered to be the most important factor in resistance to disease. The catastrophic consequence of failed immunity is seen in the unique susceptibility of AIDS patients to infection and cancer. Generally, resistance to disease is controlled by the genetic makeup of the individual, but environmental factors such as nutrition, climate, or handling stress, cause increased susceptibility to infection. The immune reactions which occur in infected animals, as their natural response to control disease, have been used indirectly to diagnose infectious disease in many species.

Immunological tests have been widely used in "Test and Slaughter" programmes to control important animal diseases, such as brucellosis and tuberculosis. Until recently, the tests used to diagnose disease have been relatively simple, though somewhat imprecise. Test programmes have tolerated wastage of test positive non-diseased animals (False (+)), while some diseased animals remained undetected (False(-)) with the tests. Studies in the area of immunodiagnosis have concentrated on immune reactivity in disease, even though it is well known that many animals exposed to infection never develop disease and may produce protective immunity.

Vaccination has been widely used in preventive programmes (prophylaxis) against many infections for centuries. The ultimate example of the success of vaccines is seen in the use of human smallpox vaccines, which "eradicated" smallpox from humans worldwide since the 1970's. Success with other diseases has been less impressive, with infections often controlled, rather than eradicated.

The real challenge for immunology in the 90's is to accurately chart pathways of immune reactivity associated with disease (diagnostics) and to separate them from protective immunity (prophylactics). Only then can we have efficient disease diagnosis and generate protection against infection using vaccines.

Tuberculosis

Diagnostic Tests

Success in controlling an infection in domestic animals is influenced by:

1. Effective antibiotic treatment.
2. Accurate diagnosis and removal of diseased individuals.
3. The use of vaccines to prevent reinfection.

While antibiotic treatment of humans with tuberculosis is widely used to control disease and its spread from individuals, this approach is not appropriate for domestic animals. Triple antibiotics, given orally each day for a period of nine months, are required to eliminate the bacterium which causes tuberculosis. This is unacceptable, considering management problems and cost, for Tb control in farm animals. It has, however, been used to manage Tb in infected monkey colonies in zoos, where controlled levels of antibiotics can be administered daily.

Accurate diagnosis and slaughter of Tb infected animals has been the main strategy used for Tb control in farmed animals. Ideally, isolation of the infectious organism provides the classical diagnostic test for a given disease. However, because the bacteria which causes Tb in animals (*Mycobacterium bovis*) grows very slowly inside cells, they are difficult, if not impossible, to isolate routinely from specimens obtained from living animals. The result is that indirect tests for disease must be used to identify infected animals. Animals which are exposed to infection mount an immune response, and these immune markers can be measured as an indicator of disease. The resulting immunity may involve one of two pathways; a cellular response which produces specific immune cells (lymphocytes); or soluble serum protein molecules (antibodies) which react specifically with the infectious organism.

Since 1908, the skin test has been used to measure cellular immunity to Tb in humans and cattle. This test involves the injection of soluble extracts (antigens) of Tb bacteria (*M.bovis*) into the skin (intradermally). Migration of immune cells into the injection site produces skin thickening two or three days later. While the skin test (ST) is an acceptably **sensitive** marker (80%) of disease as a herd test, it is not **specific** in distinguishing between reactivity to *M.bovis* and other species of related *Mycobacteria*, especially *M.avium*, which produce reactions in animals. The ST produces up to 80% (False +) reactions, due to *M.avium* or other related bacteria found in the soil or water.

To circumvent False (+) problems, a comparative cervical skin test (CCT) has been developed for deer. While the CCT has improved specificity [low False (+)], and the test salvages animals with reactivity due to *M.avium*, it has poor sensitivity [high False (-)] to diagnose true *M.bovis* (False -). Unless there is strong evidence that there are high levels of non-specific (*M.avium*) reactivity in a herd, and no evidence of *M.bovis*, CCT must not be used because of its inability to **accurately** diagnose Tb.

Shortcomings in this skin test for Tb in deer resulted in our being commissioned in 1985 to look for an alternative laboratory test. This work has resulted in the development of a blood test for Tb (BTB) which provides a new weapon in the fight against the disease. The BTB measures cellular immune reactivity (lymphocyte transformation - LT), antibody reactivity (ELISA) and inflammation (INF), using a complex range of tests carried out on deer blood samples. The combined results are used to diagnose disease caused by *M.bovis* or non-specific reactions due to *M.avium*. Bloods (30,000) have been processed from almost 1,000 NZ deer herds; mostly from ST(+) animals. The performance of BTB is compared with ST & CCT in Table 1. These results show that the BTB is superior to skin tests because it retains both high levels of sensitivity (94%) and specificity (98.6%) for Tb diagnosis in deer. It also has the advantage that it can detect exposure to *M.bovis* in deer three months earlier than ST, and especially because it is very sensitive in identifying ST(-) 'anergic' animals, which harbour serious Tb lesions but remain undetected by a skin test.

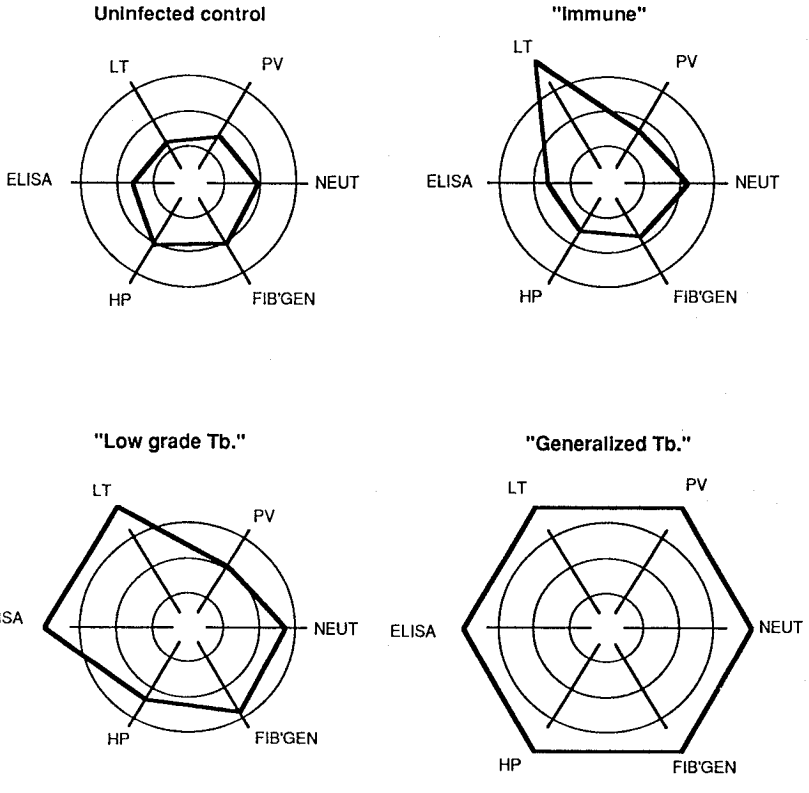
Table 1: Performance tests for *M.bovis* infection in deer.

	Sensitivity	Specificity	Limitations
Skin Test (ST)	80-85%	98%	Must be given intradermally
Comparative Cervical (CCT)	30-80%	98.5%	Difficult to (a) administer (b) read
Blood Test (BTB)	95%	98.6%	Technically complex
ELISA	87%	97%	

The experiments carried out to develop and validate the BTB have used slaughter data from more than 3,000 deer to establish a database for Tb diagnosis. Studies have also been carried out on infected groups of experimental animals, sampled repeatedly throughout the year. The slaughter results, and patterns of immune reactivity in animals sampled repeatedly, have been vital, not only in establishing a diagnostic test for disease, but also in giving us an insight into reactivity in animals which have been exposed to Tb but do not develop disease. We consider that such animals may have developed protective immunity, which could be the key target for activation using vaccines.

Patterns of BTB reactivity in seriously diseased, moderately diseased, and lesion free animals, are shown in Figure 1. These results confirm that the patterns of reactivity vary between diseased and lesion free animals, raising the hope that it may be possible to look to vaccines to provide immune protection against Tb.

Fig 1: Radial plot showing levels of **Inflammation** [neutrophils (Neut), Fibrinogen (FIB'GEN), Haptoglobin (HP), Plasma viscosity (PV)], **Antibody** (ELISA) and **Lympohocyte Transformation** (LT) in diseased, immune and non-infected deer. The inner circle represents two standard deviations below the mean value for the species. The outer circle represents two sd above the mean with the mean being represented by the circle that lies between these two limits.



Vaccination

Vaccines are accepted as the cheapest means for disease control and their widespread use has controlled many important diseases of animals and man. They come in different forms, ranging from killed micro organisms to live organisms or antigenic extracts. Depending on the pathway of protective immunity required for a specific disease, it is necessary to use tailor-made vaccines to induce appropriate patterns of immunity. Live, laboratory strain bacterial (attenuated) vaccines have been used to control brucellosis in farmed animals. Non-toxic extracts (toxoids) are also widely used to control clostridial disease (blackleg, tetanus, pulpy kidney) in farmed animals. The general principle is that live organisms are required to induce cellular immunity, while killed organisms, or soluble extracts, can be used to evoke antibody mediated protection (clostridial disease).

Tuberculosis is a very complex, chronic, intracellular bacterial infection, and most evidence suggests that cellular immunity is fundamental in the development of protection against Tb. BCG has been widely used as a human live Tb vaccine for many years. There is strong evidence that killed vaccines do not provide protection against Tb, so any programme for development of vaccines for Tb in animals must start by considering live vaccines, such as BCG. Major considerations dictate that such a vaccine must:

1. Induce immune protection.
2. Produce no unacceptable side effects.
3. Be non-infectious for "in-contact" animals.
4. Be acceptable in the food chain.

Animal vaccines for Tb which require a live vaccine present a special challenge, because Tb not only infects animals but is also an important disease in humans. Any vaccine must, therefore, have proven efficacy in the host animals and be safe, so that it is not transferred to "in-contact" animals and cannot become an unacceptable residue in the food chain.

However, animal studies present us not only with a challenge, but also with a unique opportunity for Tb vaccine development. Our ability to carry out detailed studies on tuberculosis, and to selectively slaughter animals, provides us with special insight into the disease in individual species, such as cattle or deer. The extensive studies carried out recently to develop the BTB for Tb diagnosis in deer have allowed us to identify patterns of immunity typical of the **disease** (Fig. 1c, d) and have identified patterns of immunity which may be **protective** (Fig. 1b).

A preliminary experiment carried out using different combinations of BCG to vaccinate deer has provided us with important results which will influence the course of future work.

Patterns of immune reactivity found with different BCG vaccines are given in Table 2. These results show that good levels of immunity can be induced with vaccines and that live BCG, alone, invokes non-specific reactivity and no antibody; a response typical of animals exposed to *M.avium*. Our BTB studies suggest that animals which have high levels of *M.avium* reactivity under field conditions have increased levels of resistance to *M.bovis*. In contrast, animals vaccinated with BCG in oil produce *M.bovis* specific cellular reactivity, an antibody typically found in diseased animals. Increased inflammation, following skin testing in animals given BCG in oil, produces a response which typifies natural Tb disease in deer.

Table 2: Patterns of reactivity in BCG vaccinated yearling deer.

	Type of vaccine		
Reaction	Live BCG	Live BCG in oil	Killed BCG in oil
LT *M.bovis*	+	+++	+++
LT *M.avium*	+++	+	+
Antibody	-	+++	+++
Inflammation post skin test	-	+	+++

A more recent, still to be completed, experiment shows that young animals (three to four months old) produce an active response to vaccines, leading us to anticipate that it may be possible to vaccinate animals pre-weaning. If vaccines are to be of value in the field, they must produce acceptable levels of reactivity in juvenile animals, which are especially susceptible to natural infection.

While results from the initial experimental work using BCG in deer are promising, and merit more detailed analysis and further comprehensive studies, Tb vaccination for animals must be seen, at best, as a long term solution. Should an acceptable vaccine be developed, it will involve a considerable investment to prove that it is safe and effective, and that it does not interfere with routine tests used to diagnose natural disease. Vaccines would need to be used selectively to control disease only in herds with a "high-risk" from infection in Tb

"endemic" areas. The major priority in the immediate future is to use the most efficient diagnostic tests to clear Tb from within the farm fence, before concerning ourselves with vaccines to prevent Tb reinfection.

Yersiniosis

Test or Vaccination?

Unlike Tb, yersiniosis in deer provides totally different management challenges. Yersiniosis in deer is caused by *Yersinia pseudotuberculosis*; an acute infection caused by extracellular multiplication of the bacterium in the gut of the affected animals. If diagnosed early, the non-diseased animals can be readily treated by antibiotics (tetracycline) and fluid replacement therapy. The disease usually affects young (weaner) animals, and susceptibility is increased by stresses such as are found in weaners exposed to transport, relocation, altered or insufficient diet, and severe climatic changes. Vaccines would be used primarily to protect weaners exposed to stress and considered to be "at risk" from yersiniosis. Protection is likely to be mediated by antibodies, so killed bacteria, or antigenic extracts, should be sufficient to produce protection in "at-risk" animals.

The ability to use killed, whole bacteria, has meant that there are no real problems with safety or public health, so we have been able to concentrate on issues such as acceptable "side-effects" and immune protection, using killed "yersiniavax".

Table 3: Protective effect of yersinia vaccine in experimentally infected fawns exposed to stress.

	Number with Clinical Symptoms
Vaccinated	9/60 (15%)
Control	18/60 (30%)

* Both the incidence and severity of disease was significantly lower in the vaccinated animals.

Studies carried out over the past three years at Invermay suggest that we now have an acceptable, safe, and protective "candidate" killed vaccine for yersiniosis in young deer. No significant side effects are seen with the vaccine and levels of protection (Table 3) obtained suggest that this vaccine will significantly reduce the incidence and severity of yersiniosis in weaner deer. Field studies are underway this year to confirm the efficacy of this vaccine under natural conditions. Should these prove consistent, the yersinia vaccine should be available to the deer industry in the immediate future.

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