TEST AND SLAUGHTER FOR DISEASE MANAGEMENT

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INTRODUCTION

Diagnostic tests are used to identify diseased animals which must then be treated or culled in disease managment programmes. Under most circumstances the infective pathogen can be isolated and cultured from the host thus confirming, with 100% specificity, the presence of the infectious agent. Such an approach is feasible with acute diseases, caused predominantly by pyogenic or enteric bacteria which result in extracellular infections. They are easily cultured *in vitro* using blood, tissue or faecal specimens obtained from the live animal. Generally such diseases are responsive to antimicrobial therapy or preventable by prophylactic vaccines. They cause intercurrent problems and require precise management responses to avoid long term complications.

The second category of infection involves chronic or latent infection by organisms which produce less morbidity or mortality in groups of animals, but which have an important impact on the disease-free-status of herds within the context of a National Disease Control Programme. These diseases usually involve intracellular pathogens, which are difficult to culture, not amenable to therapy, and for which there may be no acceptable vaccine. Key examples of these diseases in domestic livestock include the bacterial disease caused by *M.bovis* (tuberculosis) and viral diseases such as foot-and-mouth disease, rabies and bluetongue.

Choice of Test

In diseases where there is an inability to isolate the infectious agent from specimens obtained from living animals, indirect immunoassays are used to reach a diagnosis. Classically such immunological tests involve a single assay system deemed to have the best precision in diagnosing individual infected animals. Immunoassays may involve intradermal skin tests to evaluate cellular immunity, or agglutination, precipitation, complement fixation, immunofluorescence, enzyme linked immunosorbent assay (ELISA) or immunohistochemical testing for antibodies. To further add to the variety of assays, when one considers precipitation tests alone, they may be further subdivided into precipitation-in-gel, counter electrophoresis or western blot analysis. Each test measures a different immune parameter and reflects different levels of precision to evaluate the quality or quantity or immune factors. So far there are no absolute immune markers of disease and it is often impossible to distinguish between disease and naturally acquired protective immunity.

Ultimately test precision is measured by the **sensitivity** (%) of a test to detect all infected animals and its **specificity** (%); which is a measure of the ability to exclude non-infected animals. Significant variations in test precision will occur depending on the parameters chosen within a given test. The example of the diagnostic precision of the ELISA test for Tb is shown in Figure 1. In this case enzyme labelled antiglobulin reagents are used which react with deer immunoglobulin to detect deer antibody to *M.bovis*. If they are altered they may produce dramatic differences in the diagnostic outcome. Using an antiglobulin reagent which is polyvalent, and detects IgG, IgM, IgA antibody levels to Bovine

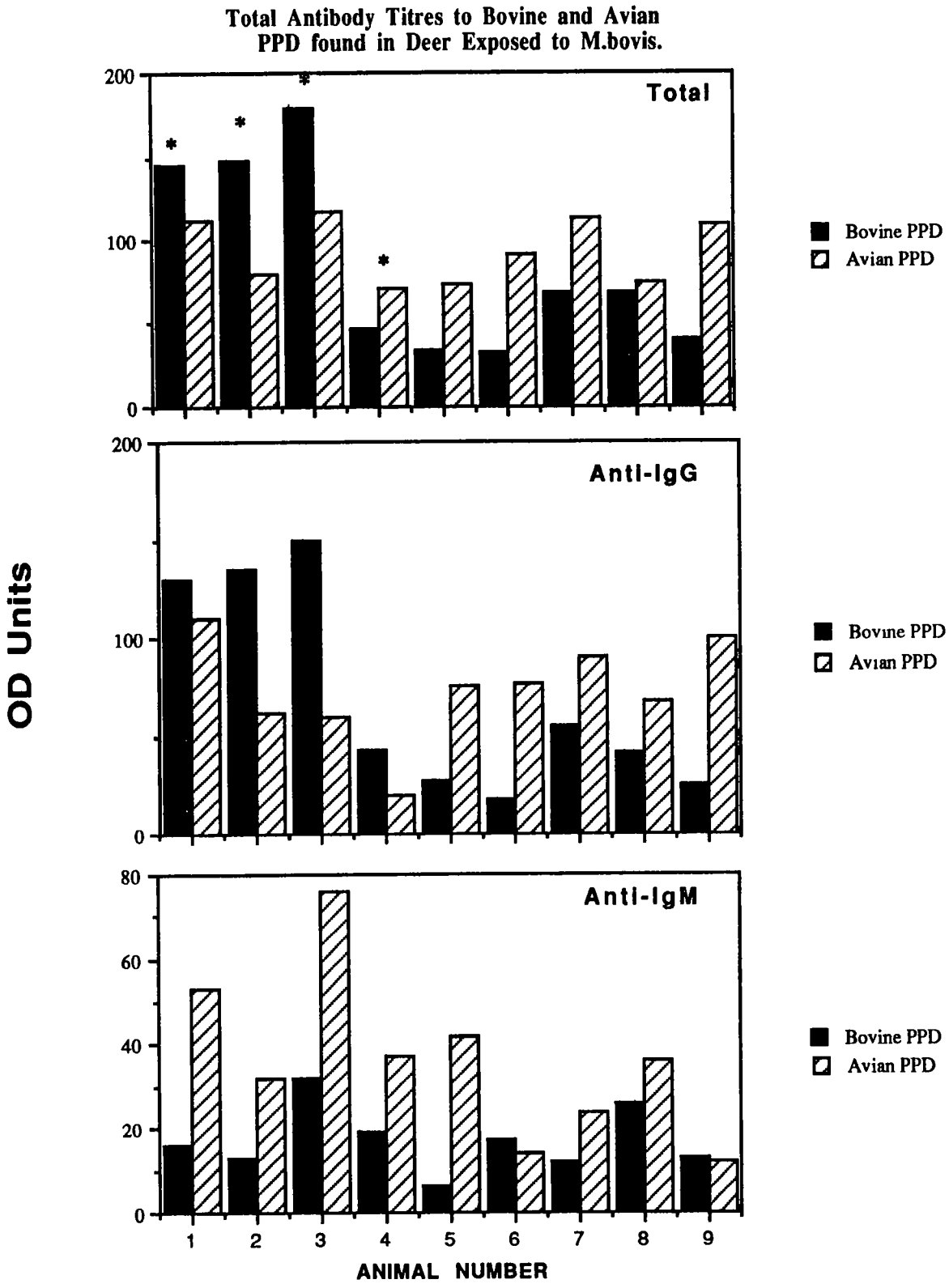

FIGURE 1: Sera from 9 animals were tested in the ELISA to determine the levels of antibody to bovine PPD (black bars) and avian PPD (hatched bars). Animals 1-4 were found to have tuberculous lesions at necropsy. The first graph shows the total levels of immunoglobulin against each antigen. The second graph shows the levels of PPD specific, antibody of the IgG isotype and the third graph shows the levels of PPD specific IgM.

If the response to bovine PPD > avian PPD by more than 5 ELISA units the response is considered positive.

(M.bovis PPD) or Avian (M.avium-PPD) antigens, the test identifies 3/4 (75%) infected deer (1-3 marked*). A specific mouse monoclonal antibody which reacts only with deer IgG detects 4/4 (100%) of the infected animals (B>A). A monoclonal antibody directed against IgM; the early phase antibody which has a broader specificity for infectious antigens, does not detect any (0/4) of the infected animals and incriminates non-diseased animals (No.6 and No.9) as potentially diseased [False (+)]. This example shows that when different standards are applied, using a single assay provides a variable and sometimes totally unacceptable outcome.

A second example, given in Table 1 shows how the diagnostic precision of a given assay (ELISA) may be altered using results with combinations of antigens rather than that obtained with a single antigen. Here, different combinations of complex antigens (PPD) or pure peptides (MPB70) are used as target antigens to detect disease specific antibodies to M.bovis in Tb infected deer.

Table 1.
Performance of Composite ELISA Testing for Tb Diagnosis.]

Antigen	Test Performance	
	Sensitivity	**Specificity**
PPD-B	82%	78%
PPD-B v's PPD-A	59%	82%
MPB70	53%	98%
Composite (A-B-MPB)	86%	98%
Number Tested	58	51

Sensitivity values obtained in M.bovis infected herds.
Specificity values obtained in M.bovis free herds.

The results show that if the complex antigens from M.bovis (PPD-B) alone are used the test is 82% sensitive and 78% specific for disease diagnosis. By using comparative assays involving complex antigens from M.bovis (PPD-B) and M.avium (PPD-A), sensitivity decreases significantly (59%) but specificity increases to 82%. Using a purified M.bovis specific (MPB70) peptide alone, sensitivity decreases to 53% but specificity increases to 98%. However, when a composite ELISA assay is used which measures the relative response to PPD-B, PPD-A and MPB70 is used, the diagnostic precision of ELISA is increased, with test sensitivity at 86% and specificity at 98%; a very acceptable diagnostic assay for herd screening, especially since it detects the vast majority (>95%) of seriously diseased animals.

COMBINED IMMUNOLOGICAL TESTS

Immunoassays are often used to screen large populations of animals. Whereas a single test used optimally to provide a composite result will increase diagnostic precision, different tests used in combination will provide a further increase in the levels of validity for accurate disease diagnosis within a national herd programme (Griffin et al 1990). The outcome using a single skin test (ST) with a test performance of 85% sensitivity and 98.5% specificity is given in Table 2. Accepting that 100,000 animals have been tested with ST and disease incidence is at a level of 0.3% the test (ST+) will identify 255/300 infected (True+) animals but produce False (+) results in 1245 disease-free (True -) animals. Slaughter of all ST(+) animals gives a positive predictive diagnosis of 17%. When a second, quite different , test such as the lymphocyte transformation assay (LT) is used to further test all ST (+) animals and select reactors [ST (+), LT(+)] for slaughter, then the predictive value changes markedly. Accepting a sensitivity of 94% and a specificity of 98.6% for Tb diagnosis with LT, the combined tests will diagnose Tb in 240/255 of the ST (+) disease animals and salvage 1222/1245 of the non-disease animals. In this case the positive predictive value of the combined tests is 91%.

Table 2
PREDICTIVE VALUE OF SINGLE AND COMBINED TESTS IN 100,000 ANIMALS.

	True (+)	True (-)	Total	Predictive Value
ST	255	1245	1500	17%
ST + LT	240	23	263	91%

The management outcome is that a single ST(+) and slaughter will identify 85% of disease animals but cause unnecessary slaughter of 1245 animals. By contrast the combined tests (ST+, LT+) will diagnose 80% of the infected animals but waste only 23 non-diseased animals. The reality of such an example is seen in National Deer Herd data for NZ deer between 1985 and 1990 (Corrin et al 1987, Carter 1990) where 10,272/573,136 animals reacted to the ST alone in 1989. Use of combined tests in 1989 reduced the reactor rate to 2091. 8171 more animals were salvaged using combined tests to define a reactor than if a single test (ST) had been used. The blood tests for tuberculosis (BTB) (Griffin *et al* 1987, 1989) is a composite test of which the LT is but one part. Detection of antibody to M.bovis by ELISA is another component and has been shown to have a sensitivity of 86% (Table 1). If all ST negative animals in herds known to contain disease are screened using the cheaper ELISA, then a further 38 diseased animals will be found meaning that 278/300 diseased animals are detected. Thus in order to salvage 1222 false positive animals only 22 diseased animals remain undiagnosed whereas using the ST alone 45 remain undiagnosed and 1222 animals are needlessly slaughtered. Thus there are benefits to be gained, in terms of both disease control and economics, by using the two tests in conjunction.

COST-BENEFIT OF TEST AND SLAUGHTER

The immediate costs involved in a test programme involve the expenses due for conducting the test. While some countries offer free testing for notifiable disease in domestic animals, the New Zealand deer industry is required to underwrite all direct costs due for tests.

A major cost in a test programme involves financial losses due to slaughter of test positive animals on farm or through processing plants. Diseased animals may be condemned or their meat discounted (5$ v's $2 per kg). All animals slaughtered as test positive reactors are devalued. While the identification and removal of infected stock is of benefit to the farmer and can readily justify costs incurred, slaughter of non-diseased (False +) stock represents an unacceptable loss without any obvious benefit.

Apart from direct costs incurred through testing and losses through slaughter, a further consideration involves gain/loss through scientific **information** concerning the disease status of a herd. Accepting that animals with ST(+) reactions have a very low true reactor rate (<20%) and an even lower lesion rate (<10%), the majority of ST(+) animals may either not have been exposed to M.bovis, nonspecific sensitization, or have been exposed to M.bovis but not have developed disease (Griffin et al 1988).

Skin test and slaughter as the sole means for disease control is severely limited in providing adequate information because the findings are only relevant for test animals which have confirmed M.bovis at slaughter. Animals with no disease (NVL - no visible lesion) may be presumed to be free from exposure to M.bovis giving a very false impression of the risk of Tb, when disease is present at a low incidence within a herd. When ancillary tests such as the BTB are used to retest ST(+) animals they provide extra information which gives a much more accurate impression of the disease status of these animals. As an example, if 10 ST(+) animals all show non-specific sensitisation to M.avium on BTB testing it can reasonably be assumed that the animals are disease free and confidence is gained that the herd has a low risk of M.bovis infection. By contrast, if 10 ST(+) animals show 2/10 BTB(+) for M.bovis at retesting, then disease risk is immediately identified. If the 2 ST(+) BTB(+) animals have lesions then disease is confirmed at slaughter. However, should the 2 reactor [ST(+), BTB(+)] animals show no evidence of M.bovis at slaughter, there remains the real possibility that these animals are 'sentinels' which have been exposed to M.bovis but have not developed disease. This alerts the owner to the prospect that other False (-) ST animals may have remained undetected within the herd. Extra caution can then be applied at subsequent tests to identify residual diseased animals. Caution will not cause the introduction of infection into a disease-free herd but it will greatly improve the likelihood of detecting disease when present.

Use of combined tests to stratify animals as having **reactions** or as being true **reactors**, allows for a more selective approach to slaughter or salvage False (+) reaction animals. Understanding the specificity and level of reactivity in reactor animals provides valuable extra information as to the disease status of test positive animals, and by inference the risk of residual disease in the remainder of the herd. The latter point should be stressed as until a diagnostic test with 100% sensitivity is developed, a clear test or destruction of all positive reactors gives no guarantee against residual infection remaining undetected. Because BTB provides a quantitative measure of disease reactivity it has a predictive value in stratifying animals as diseased or reactive (NVL) and can identify the prospect of active disease or infection in individual animals due for slaughter. It can also indicate the likelihood of undetected disease remaining within a herd.

At each slaughter episode the farmer should know if the objective is to identify a diseased, exposed (immune) or non-specifically sensitised animals which should be lesion free. Knowledge improves control, and together they advance the likelihood of accurate and cost effective disease exclusion from a herd.

CONCLUSIONS

When immunodiagnostic tests are used for disease diagnosis or exclusion, it is vital to select relevant diagnostic tests which maximise disease detection. Having chosen relevant tests it becomes important to standardise the parameters within the test to optimise its validity. Different tests used in combination, have a much higher predictive value for accurate diagnosis than single tests and allow for maximal disease diagnosis and salvage of uninfected stock. Rather than use traditional single test and slaughter for disease control we can now use different tests in combination to provide more accurate disease diagnosis and allow for selective cost-effective slaughter of animals. With the sophisticated range of immunodiagnostic tests currently available and our understanding of modern immunology we must produce new technology which can accurately diagnose or exclude specific infectious diseases. We need to understand more about the immune parameters which characterise the disease reaction and to distinguish between these and the patterns of protective immunity found in non-diseased animals, which have been exposed to infection. The use of vaccines to generate protective immunity give us an additional probe to clarify aspects of immunity and protective immunity.

The outcome must be; "do not use test and slaughter but replace it with a combined test and selective slaughter programme."

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