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Tuberculosis in Cattle and Its Control: Limitations to the Use of the Interferon- Gamma Assay in Attested Herds

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Introduction

The Interferon-gamma (IFN-γ) assay, as performed on blood samples taken from cattle in herds currently undergoing a *Mycobacterium bovis* breakdown is now approved by the European Commission for use as an ancillary test for the detection of *M. bovis*- infected cattle that are not responsive to the single comparative intradermal tuberculin test (SICTT). The reasons why the assay requires to be conducted on freshly collected heparinised whole blood are summarised in this report.

Materials and Methods

Paired blood samples of heparinised blood were taken from 29 SICTT-positive cattle and 60 non-reactor cattle undergoing IFN-γ tests as part of routine surveillance. All samples were assayed at 8 hours and again at 24 hours post-collection, using avian and bovine tuberculins. At *post-mortem* examination, 6 out of the 29 reactor animals displayed gross visible lesions consistent with tuberculosis. No *post-mortem* data were available for the non-reactor animals.

Results and Discussion

The optical density readings of these assays are presented in Figure 1. These demonstrated a considerable decline in the magnitude of both readings when the assay was delayed for 24 hours as compared to 8 hours in both groups of cattle. When the assay interpretation was applied to each animal, 22 (75.9%) of the 29 reactor cattle remained positive at 24 h. In contrast, just 31 (51.7%) of the 60 non-reactor cattle remained positive. As the interpretation of the IFN-γ assay is based on a comparison of the levels of response to the two PPDs, such a decline led to IFN-γ assay responsive animals being wrongly identified as non-responsive when the assay was delayed for 24 hours. Consequently a proportion of *M. bovis* infected animals in these high risk herds might not have been identified by this means. Furthermore, this is even more likely to be the case when the animals under test are in a currently attested herd in which the prevalence of tuberculosis is low and the responsiveness to tuberculins as measured in optical density readings in the IFN-γ assay is of a low order.

The sensitivity of the IFN-γ assay matches that of the SICTT, provided the assay technique is standardised and provided the sample is presented for assay within 10 hours of collection. As there is a considerable loss of sensitivity due to decay of the relatively small amounts of IFN-γ released in the course of the assay, it is not suitable surveillance purposes in attested herds. However the assay is of considerable value in the investigation of chronically restricted herds.

The sensitivity of the combined tests, when used in parallel, approaches 97 *per cent* in such herds.

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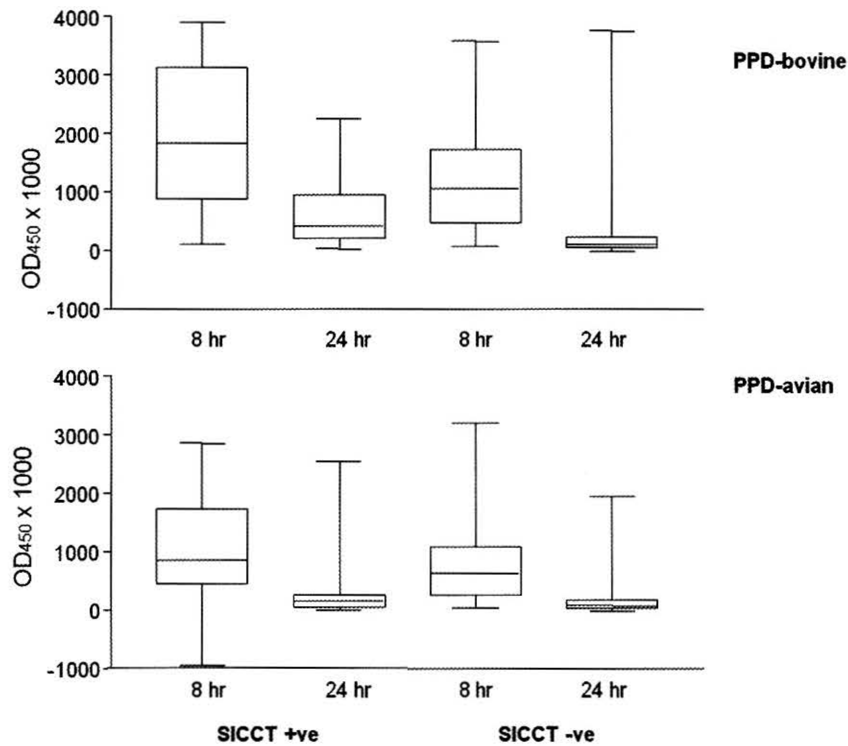

Figure 1. IFN-γ responses of SICTT +ve (N=29) and SICCT –ve (N=60) cattle when blood was cultured with PPD-bovine and PPD-avian at 8 h and 24 h post collection.

The assay, when used in this manner, provides a means of controlling and eliminating *M. bovis* infection from herds in which such infection would otherwise remain intractable over a prolonged period. The specificity of the assay is of the order of 95 *per cent* in cattle from unexposed herds: again, this renders the assay unsuitable for surveillance purposes on a national or regional scale. This lack of specificity can be improved by the use of ESAT-6 and other *M. bovis*-specific antigens which are now available.

Consequently and despite these limitations, the IFN-γ assay, when used strategically in known *M. bovis* infected herds, provides a valuable means of identifying animals that would otherwise remain in these herds as an unidentified source of the microorganism. The results of the assay can be optimised by ensuring that factors that adversely affect its perfomance, such as a delay in the processing of blood samples, are avoided.