Preliminary data of different methods for the indirect diagnosis of *Mycobacterium bovis* infection

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SUMMARY

We compared the response induced by QuantiFERON-TB Gold antigens to that obtained with the Intradermal Comparative Tuberculin Test and BOVIGAM assay. Our results showed that the QuantiFERON-TB Gold technique used in humans could also be applied for the diagnosis of TB infection in cattle.

KEY WORDS: Tuberculosis, *M. bovis*, QuantiFERON, BOVIGAM, ICTT

Received January 17, 2011 *Accepted* February 16, 2011

The predominant immunological response in *Mycobacterium bovis*-infected cattle is affected by T lymphocytes (de la Rua-Domenech *et al*., 2006). Ante mortem tests of cellular immunity are very important for the control of bovine tuberculosis (TB) since they can identify *M. bovis*-infected animals very early. To make ante mortem diagnosis there are two tests: Intradermal Comparative Tuberculin Test (ICTT) and BOVIGAM assay. The ICTT is the international standard for the indirect diagnosis of bovine TB in cattle. It is based on the elicitation of a delayed-type hypersensitivity response to the intradermal injection of purified protein derivative (PPD), both avian and bovine PPD. These are crude protein extracts from supernatants of mycobacterial cultures (Monaghan *et al*., 1994). The BOVIGAM assay detects the amount of interferon-γ (IFN-γ) released from blood samples stimulated with avian and bovine PPD and with a negative control (nil antigen) (Wood *et al*., 2001). Both tests may be not specific since their results may be positive in non-infected animals exposed to the antigens of environmental mycobacteria. Several proteins can increase the specificity of the ante-mortem diagnosis of bovine TB, as ESAT-6 and CFP-10. They are encoded by the RD1 region of difference, present in *M. bovis* and in the other components of *Mycobacterium tuberculosis* complex and in a few species of non-tubercular mycobacteria (Pai *et al*., 2006). These antigens are used in the QuantiFERON TB Gold (QFT-G) immunoassay based on the release of IFN-γ, which allows the diagnosis of TB infection by *M. tuberculosis* complex in humans.

The purpose of this study was to examine if the standard method of QFT-G can also be applied in cattle. To verify this, we compared the response induced by ESAT-6 and CFP-10 to that obtained with the ITCC and BOVIGAM in two groups of cattle. The first group (Group I) tested consisted of 26 animals. They formed a herd located in Central Sardinia, where in recent years several outbreaks of bovine TB were identified, this group was considered at high risk of TB. The second group (Group II) tested consisted of 20 animals forming a herd located in Northern Sardinia in an area free from bovine TB, this group was taken as control. ICTT was performed on each animal using 0.1 mL (5000 IU) of bovine tuberculin and 0.2 mL (5000 IU) of avian tuberculin, as prescribed by

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regulations currently in force in Italy (Lauzi *et al*., 2000).

A heparinized blood sample was collected from each animal just before the ICTT, and was used in the IFN-γ test for bovine TB. The BOVIGAM assay was performed according to the instructions of the kit itself. To assess the production of IFN-γ induced by ESAT-6 and CFP-10 antigens the protocol indicated in the kit of the QFT-G was followed. The amount of IFN-γ was determined by ELISA, concerning BOVIGAM assay, the samples were considered positive when OD_{450nm} bovine PPD-Nil antigen was ≥0.1 and OD_{450nm} bovine PPD-avian PPD was ≥0.1. The cut-off of positivity for ESAT-6 and CFP-10 was ≥0.35 IU/ml and the results were scored using software provided by the QFT-G kit. Statistical analysis was performed with Fisher's exact test.

All the cattle belonging to the two groups tested were ICTT negative (Table 1). BOVIGAM assay was positive in six animals of Group I and in three animals of Group II (Table 1).

A positive response to the ESAT-6 and CFP-10 antigens was observed in two animals of Group I (both BOVIGAM positive) while no positivity was found in Group II (Table 1).

The statistical analysis showed no significant differences between the ICTT test and the response to the ESAT-6 and CFP-10 either in Group I (p=0.4902) or in Group II
(p=1). A significantly different response between ICTT and BOVIGAM was found in Group I (p=0.0226), whereas no difference was observed between the two tests in Group II (p=0.2308). No significant difference between BOVIGAM and ESAT-6 and CFP-10 was registered in the two groups of cattle tested (p=0.2485 for Group I and p=0.2308 for Group II).

A limitation of our study was that we were not able to isolate any strain of *M. bovis* from animals in Group I as they have been slaughtered and it was not possible to obtain the biopsy specimens. Another limitation of our work was the low number of cattle that we analyzed but, in our study, we examined a farm (Group I) that was considered at risk of bovine tubercular infection and the control group (Group II) was selected with approximately the same number of animals. Despite these limitations our data showed that the QFT-G technique used in humans could also be applied for the diagnosis of TB infection in cattle since no statistically significant difference was observed in the performance of the three assays used. A significantly different response between ICTT and BOVIGAM was found in Group I (p=0.0226). False negative tuberculin reactions can occur in cattle subjected to the skin test for a variety of reasons (de la Rua-Domenech, R *et al*., 2006) and consequently its sensitivity may be less than that of the other assays BOVIGAM or QFT-G that may be able to detect a substantial proportion of infected cattle that escape detection by the tuberculin test.

BOVIGAM gave a larger number of positive results in Group I than that obtained with the antigens of QFT-G. This difference might be due either to a greater sensitivity of the BOVIGAM, or to the different conditions of the methodology used, or to its lower specificity (due to cross-reactivity), compared to the ESAT-6 and CFP-10. It would be very important to continue this study in a larger number of samples to establish if the use of both BOVIGAM test and specific antigens might be an effective tool for ante-mortem diagnosis of bovine tuberculosis.

The use of the ESAT-6 and CFP-10 antigens could provide a greater accuracy and specificity in detecting TB infection in cattle, compared to the

TABLE 1 - *Results of ICTT, BOVIGAM and ESAT-6/CFP-10 antigens of QFT-G in Group I and in Group II.*

	ICTT Group I	*ICTT Group II*	*BOVIGAM Group I*	*BOVIGAM Group II*	*ESAT-6 CFP-10 Group I*	*ESAT-6 CFP-10 Group II*
Positive	0	0	6	3	2	0
Negative	26	20	20	17	24	20
Total	26	20	26	20	26	20

tests based on the response induced by the stimulation with PPD. In fact it can score false positive results due to atypical mycobacteria recovered from mammals (de la Rua-Domenech *et al*., 2006). In our work, in Group II, three cattle were positive to the BOVIGAM. Since this group was not at high risk of TB because it is located in an area TB-free, the positive results could be attributed to a cross reactivity with non-pathogenic environmental mycobacteria. The use of the ESAT-6 and CFP-10 antigens would also be very interesting also for its economical impact. In fact as a result of the greater specificity of these antigens, their use might avoid the economic losses resulting from the slaughter of the animals positive to the stimulation induced by tuberculin, but appearing to be healthy both in anatomical and pathological examinations.

ACKNOWLEDGMENTS
This study was funded by grant of RAS 2009.

REFERENCES

DE LA RUA-DOMENECH R., GOODCHILD A.T., VORDERMEIER H.M., HEWINSON R.G., CHRISTIANSEN K.H., CLIFTON-HADLEY RS. (2006). Ante mortem diagnosis of tuberculosis in cattle: a review of the tuberculin tests, gamma-interferon assay and other ancillary diagnostic techniques. *Research in Veterinary Science*. **81**, 190-210. Epub 2006 Mar 2. Review.

LAUZI S., PASOTTO D., AMADORI M., ARCHETTI I.L., POLI G., BONIZZI L. (2000). Evaluation of the specificity of the gamma-interferon test in Italian bovine tuberculosis-free herds. *The Veterinary Journal*. **160**, 17-24.

MONAGHAN M.L., DOHERTY J.D., COLLINS J.F., KAZDA, QUINN P.J. (1994). The tuberculin test, Veterinary Microbiology. **40** (1-2), 111-124.

PAI M., KALANTRI S., DHEDA K. (2006). New tools and emerging technologies for the diagnosis of tuberculosis: part I. Latent tuberculosis. *Expert Review of Molecular Diagnostics*. **6**, 413-422.

WOOD P.R., JONES S.L. (2001). BOVIGAM: an in vitro cellular diagnostic test for bovine tuberculosis. *Tuberculosis (Edinburgh)*. **81** (1-2), 147-155.