Antibody Tests for Identification of Mycobacterium bovis-Infected Bovine Herds

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Antibody in sera from 560 cattle of tuberculosis (TB)-infected and TB-free herds was investigated by competition and indirect enzyme-linked immunosorbent assays using bovine purified protein derivative tuberculin as the antigen. Antibody was detected in sera from both types of herd, with a widely overlapping range of titers. However, a “tail” of high-titered sera was observed for the distribution of data for only those cattle from TB-infected herds.

Mycobacterium bovis is the etiological agent of bovine tuberculosis (TB), a serious problem of both public health and paramount economic importance in large areas of the world (9). The standard method for diagnosis of bovine TB is the tuberculin intradermal test (IDT) with purified protein derivative (PPD) tuberculin. Antibody to mycobacterial antigens was investigated with various rates of success, since the humoral immune response to M. bovis is late and irregular during the course of disease (6, 12). Therefore, diagnostic tests based on antibody response alone show poor efficiency in the detection of TB-infected animals on an individual basis.

In this study, a new diagnostic scheme, which aims at the detection of a defined distribution of the antibody responses in the cattle population, is proposed; this should allow a correct assessment of the TB status of bovine herds. The 560 serum samples examined in this study were obtained from 283 adult animals of TB-infected herds undergoing eradication and 277 samples examined in this study were obtained from 283 adult animals of TB-free herds; on the whole, serum samples were collected on 34 farms located in three provinces of the Lombardy and Emilia regions of Northern Italy. Antibody to M. bovis was investigated by two enzyme-linked immunosorbent assay (ELISA) formats, after adsorbing bovine PPD tuberculin onto the solid phase. In the indirect ELISA, PPD antigen was reacted with 1:8 and 1:40 diluted sera and antibody was revealed by a rabbit, anti-bovine immunoglobulin G, alkaline phosphatase-conjugated antiserum; antibody activity was expressed in terms of final optical density (OD). In the competition ELISA, antibody activity in 1:2 and 1:4 diluted sera was expressed in terms of percent competition against a rabbit, biotinylated, anti-bovine PPD antiserum; rabbit antibody was revealed by a rabbit, anti-bovine immunoglobulin G, alkaline phosphatase conjugate.

Antibody-positive animals were detected in both infected and uninfected cattle herds. In particular, the antibody profiles of TB-infected and TB-free herds largely overlapped (Fig. 1); both ELISA formats showed poor efficiency in the detection of single TB-infected animals, and the discriminating power of the antibody tests was not improved by limiting the analysis to IDT-positive animals of the infected herds. No significant differences between the sensitivities and specificities of indirect and competition ELISAs could be shown. This is substantiated by the similar fractions beneath the receiver operating characteristic (ROC) curves (2) (Fig. 2). The area beneath the curve is proportional to the discriminating power of the test. In fact, by means of the ROC curves it is possible to determine a cutoff point for the readout value that corresponds to the highest accuracy of discrimination between infected and noninfected animals on the basis of a preestablished parameter of classification; this parameter was either the herd of origin (infected or uninfected) or the IDT status of the animals (positive or negative). Interestingly, the indirect ELISA format was able to detect a “tail” (about 6%) of high-titered sera for the distribution of data for antibody-positive animals, which was peculiar to TB-infected herds only. On the basis of the distribution of the antibody responses, shown by the histograms (Fig. 1), there is evidence that the detection of some antibody titers of $\geq 1.2$ OD units by the indirect ELISA (at serum dilution 1:40) can be correlated with active, ongoing infection at the herd level. The prevalence of high-titered sera was lower when antibody was investigated by the competition ELISA. In this respect, we can speculate that the competition ELISA was not as discriminating because of (i) a limited range of epitopes recognized by the rabbit antiserum and/or (ii) a poor average affinity of bovine antibody to PPD tuberculin.

In order to confirm the specificity of the antibody titers revealed by the two ELISAs, we performed some immunoblotting assays on antibody-positive sera from TB-infected and TB-free herds; these assays were performed with secreted and somatic, sonicated antigens of M. bovis, prepared as previously described (5). Most sera were positive in such immunoblotting assays against somatic and/or secreted antigens of M. bovis, showing products with the following molecular masses: 23, 27, 33, 38, 60, 66, 71, 74, 80, 97, and 101 kDa. Furthermore, the reaction patterns observed on the immunoblots indicated that infected cattle tend to mount a stronger, more regular response to somatic (mainly 27-, 66-, and 74-kDa) proteins than to secreted proteins; such a bias was not shown by antibody-positive sera from noninfected cattle (Fig. 3).

Our results confirm that the antibody response to M. bovis in cattle cannot be relied upon for the detection of single infected animals (6, 12). Such an antibody response usually arises in the presence of generalized TB, whenever moderate bacterial pay-loads are available for processing. This has been confirmed by experimental infections of cattle with high and low infectious doses of M. bovis, in that the former bring about a much earlier
antibody response (11). However, with regard to the drawbacks of the current immunological techniques (3, 4, 10), any further contribution to such techniques may be of use to the purpose of an earlier and more accurate diagnosis of TB. Our results also confirm that a detectable antibody response to M. bovis can be induced by related organisms; these may be other mycobacteria or even other gram-positive bacteria (7, 8). On the basis of our results, we suggest that an antibody test (indirect ELISA) can help define the TB status of bovine herds with a status previously characterized as dubious by IDT and/or gamma interferon (1, 13) methods. In this respect, we suggest the investigation of antibody in the 6 to 8 weeks which usually elapse between two intradermal tests; the latter could even be instrumental to the diagnosis because of the booster effect on the antibody response in infected animals (14). As for

FIG. 1. Distribution of antibody titers in the cattle population under study. Shown are the results of competition ELISAs (serum dilution, 1:2 [A] or 1:4 [B]) and indirect ELISAs (serum dilution, 1:8 [C] or 1:40 [D]). Grey bars, noninfected cattle; black bars, infected cattle. Abbreviations F(%), percent frequency in the population; % INH, percent OD inhibition.

FIG. 2. ROC curves. Shown are the results of competition ELISAs (serum dilution, 1:2 [A] or 1:4 [B]) and indirect ELISAs (serum dilution, 1:8 [C] or 1:40 [D]). Abbreviations: S, sensitivity level (range, 0 to 1); FPF, false-positive fraction (range, 0 to 1).

FIG. 3. Immunoblotting of sera from TB-infected cattle. Sonicates of M. bovis AN5 were electrophoresed and blotted onto an Immunobilon P membrane. They were reacted with sera from TB lesion-positive (lanes 1 to 7) or noninfected (C−) cattle. Abbreviations: Ag, total staining of proteins in the gel; Std, molecular mass standards.
the evaluation of the antibody test, the detection of some high-titered, antibody-positive sera would confirm an ongoing *M. bovis* infection in the herd; another antibody test 3 to 4 weeks later could provide further evidence of infection, as a result of an increased prevalence of such high-titered sera. This outcome, possibly associated with a persistent dubious result by IDT, should be viewed as conclusive evidence of infection at the herd level.

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