Immune Responses in Cattle Inoculated with *Mycobacterium bovis*, *Mycobacterium tuberculosis*, or *Mycobacterium kansasii*

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Running Head: Immune responses to mycobacteria
Abstract

Cattle were inoculated with *Mycobacterium bovis*, *Mycobacterium tuberculosis*, or *Mycobacterium kansasii* to compare antigen-specific immune responses to varied patterns of mycobacterial disease. Disease expression ranged from colonization with associated pathology (*M. bovis*), colonization without pathology (*M. tuberculosis*), to no colonization or pathology (*M. kansasii*). Delayed type hypersensitivity and interferon-γ responses were elicited by each mycobacterial inoculation; however, responses by *M. bovis*- and *M. tuberculosis*-inoculated animals exceeded those of *M. kansasii*-inoculated animals. Specific antibody responses were detected in all *M. tuberculosis*- and *M. bovis*-inoculated cattle three wks after inoculation. From 6 – 16 wks after *M. tuberculosis* inoculation, antibody responses waned whereas responses persisted with *M. bovis* infection. With *M. kansasii* inoculation, initial early antibody responses waned by 10 wks after inoculation and then increased 2 wks after injection of purified protein derivative for skin test at 18 wks after challenge. These findings indicate that antibody responses are associated with antigen burden rather than pathology, cellular immune responses to tuberculin correlate with infection but not necessarily with pathology or bacterial burden, and exposure to mycobacterial antigens may elicit an antibody response in a pre-sensitized animal.
INTRODUCTION

Tuberculosis (TB) in humans and animals may result from exposure to bacilli within the Mycobacterium tuberculosis complex (i.e., M. tuberculosis, M. bovis, M. africanum, M. pinnipedi, M. microti, M. caprae, or M. canetti) (8). Despite ~99.95% sequence identity (12), M. bovis and M. tuberculosis exhibit distinct differences in virulence and host adaptation. As compared to M. tuberculosis, experimental M. bovis infection of mice or rabbits results in more severe pathology and shorter mean survival times (9, 17, 18). Mycobacterium tuberculosis is primarily a human pathogen that demonstrates a high level of attenuation in cattle (as reviewed by Francis, 1947 (10)) whereas M. bovis has a wider host range affecting many domesticated and free-ranging mammals as well as humans. Prior to initiation of control and eradication campaigns in the early to mid 1900’s, M. bovis infection accounted for up to 30% of human tuberculosis cases, primarily being transmitted to humans by consumption of un-pasteurized dairy products and contact with infected livestock. Control efforts including slaughter surveillance and test / cull campaigns have dramatically reduced the prevalence of M. bovis infection in domestic cattle herds, thereby, reducing the spread of M. bovis to humans. However, in developing countries, M. bovis infection of humans persists as a serious and relatively common zoonosis (16).

Mycobacterium kansasii, while not a member of the M. tuberculosis complex, may cause disease in otherwise healthy humans, albeit infrequent, that is clinically indistinguishable from M. tuberculosis infection (1, 3). As with humans, M. kansasii infection of cattle is uncommon; however, it is occasionally associated with granulomatous lesions within lymph nodes and the respiratory tract of cattle (B. Harris, unpublished observations). Of particular relevance for tuberculosis diagnosis, M. kansasii infection/sensitization may elicit responses to antigens generally considered tuberculosis-specific such as ESAT-6, CFP-10, and MPB83 (2, 30, 35).
With experimental *M. bovis* infection of cattle, levels of MPB83-specific antibody correlate with disease severity, bacterial burden, and specific cell-mediated immune responses (15, 33). With this particular scenario, disease severity (i.e., pathology) and bacterial burden are intimately linked; thus, it is difficult to define a potential correlation of a particular immune response to either readout independently. *Prior studies have demonstrated that virulent and attenuated strains of* *M. bovis* *induce similar delayed type hypersensitivity responses in cattle; however, only the virulent* *M. bovis strain induces a persistent IFN-γ, IL-2, and antibody response* (34). The objective of the present study was to compare mycobacteria-specific immune responses to patterns of mycobacterial disease expression in which mycobacterial burden is uncoupled from pathological changes.

Disease expression patterns included: persistent colonization with associated pathology (i.e., *M. bovis* infection), colonization without associated pathology (i.e., *M. tuberculosis* infection), and no colonization or pathology (i.e, *M. kansasii* infection). Antigen-specific immune responses were evaluated for correlation to manifestations of disease expression.

**MATERIAL AND METHODS**

**Calves, challenge inoculum, and necropsy.** Friesian or Friesian cross castrated male calves of ~6 months of age were obtained from bovine TB-free herds and housed within either biosafety level-3 facilities at the Veterinary Laboratory Agencies (VLA), Addlestone, UK (*M. bovis* - and *M. tuberculosis*-inoculated animals) or biosafety level-2 facilities at the National Animal Disease Center (NADC), Ames, IA, USA (*M. kansasii*-inoculated animals). All cattle experiments were cleared by local ethical review and animals procedures performed in accordance with either British Home Office requirements (i.e., A(SP)A, 1086; VLA studies) or according to institutional guidelines and approved animal care and use protocols (NADC studies). Treatment groups included: *M. tuberculosis*-inoculated (*n = 5*), *M. bovis*
inoculated (n = 5), and *M. kansasii*-inoculated (n = 4) calves. Inoculation of *M. tuberculosis* [2.8 x 10⁶ colony forming units (CFU)] and *M. bovis* (1.0 x 10⁶ CFU) was by direct instillation of the challenge inoculum into the trachea of sedated calves as described (5, 27). Inoculation of *M. kansasii* (4 x 10⁸ CFU) was by direct instillation of the inoculum into the tonsilar crypts of sedated calves as described (19). In general, these two routes of inoculation with tuberculous mycobacteria to cattle (i.e., intratracheal and intratonsillar) result in similar disease severity and infection (5, 20, 27). Whole blood in heparin was collected at 0, 2, 4, 6, 8, 10, 12, and 16 wks after challenge for IFN-γ assays and serum for antibody detection assays at 0, 3, 6, 7, 9, 10, 12, 13, 16, and 18 wks (slight variations in collection time points dependent upon treatment groups and assay) after challenge. The 100-fold larger dose of *M. kansasii* was based upon prior studies (19) and the relative low virulence of *M. kansasii* as compared to *M. bovis* and *M. tuberculosis*.

The *M. bovis* field isolate AF2122/97 was grown to mid-log phase in Middlebrook 7H9 media (Becton, Dickinson and Company (BD), Oxford, UK) supplemented with 10% (v/v) Middlebrook acid-albumin-dextrose-catalase enrichment (BD), 4.16g/l sodium pyruvate (Sigma-Aldrich Co., Poole, UK) and 0.05% (v/v) Tween 80 (Sigma-Aldrich) and stocks stored frozen at -80°C. Stocks of *M. tuberculosis* H₃₇R₅ were prepared in the same way except that the 7H9 media was supplemented with 0.2% (v/v) glycerol (Sigma-Aldrich) rather than sodium pyruvate. The *M. kansasii* field isolate 03-6931 [subtype 1 based on 16S-23S rRNA sequence (1, 30)] was grown to mid-log phase in Middlebrook’s 7H9 media supplemented with 10% oleic acid-albumin-dextrose complex (Becton Dickinson, Franklin Lakes, NJ) plus 0.05% Tween 80 (Sigma Chemical Co., St. Louis, MO) and stocks stored frozen at -80°C. Enumeration of bacterial stocks (CFU/ml) was by serial dilution on modified Middlebrooks 7H11 agar (11). In addition, the virulence of *M. tuberculosis* and *M. bovis* strains was confirmed in guinea pigs prior to the start of the cattle challenge experiment (data...
not shown). The *M. kansasii* strain used in the present study was isolated from a tuberculous lesion (i.e., pyogranuloma) detected within an adult cow upon routine slaughter surveillance. The *M. kansasii* isolate had 100% identity with the subtype 1 genotype, most commonly associated with disease in immune competent humans (30).

**Post mortem examination.** Approximately 16 – 18 wks after inoculation, all cattle were euthanized by intravenous injection of sodium pentobarbital and examined. Various tissues were collected (tonsil, lung, lung-associated lymph nodes, and head-associated lymph nodes) for bacteriologic culture (described below) and microscopic examination including Ziehl-Neelsen staining for acid-fast bacilli as well as hematoxylin and eosin staining for morphologic assessment of tissues as described (4, 31). All major visceral organs were visually inspected for lesions and tissues collected for further microscopic and bacteriologic assessment when warranted. Lungs were examined externally and sliced into 0.5- to 1-cm-thick sections for detection of visible lesions. Lymph node tissues were sliced into thin sections (1 to 2 mm thick) and examined for the presence of visible lesions. The severity of the gross pathological changes was scored using a semi-quantitative scoring system as previously described (29). In summary, each of 7 lung lobes was scored from 0 - 5 depending on the number of lesions and extent of pathology observed, 0 being no pathology and 5 being extensive gross-coalescing lesions. The lymph nodes of the upper and lower respiratory tract were similarly scored but using a score of 0 - 3. The scores of the individual lung lobes were added to calculate the lung score and of the lymph nodes to calculate the lymph node score. Both lymph node and pathology scores were combined to determine the total pathology score per animal.
**Bacterial enumeration.** Tissue sections collected at post mortem from lymph node and lung samples were individually homogenized in 5 ml of sterile PBS using a rotating-blade macerator system. Enumeration of CFU/sample was by inoculating modified 7H11 agar plates with 50µl of tissue homogenate and counting colonies after incubation at 37°C for 4-6 wks (11). A semi-quantitative bacterial burden score of 0 - 4 was determined for each tissue sample where 0 = no colonies, 1 = 1-10 CFU, 2 = 11-100 CFU, 3 = 101-1000 CFU and 4 > 1000 CFU per sample. The score for all tissues was combined to provide a total bacteriology burden score per animal. All culture positive samples from the *M. tuberculosis* infected animals, and a representative sample from 4 of the *M. bovis* infected cattle, were submitted to the VLA Molecular Strain Typing Laboratory to confirm strain identity by spoligotyping. Strains were spoligotyped according to the method of Kamerbeek et. al. (14) with minor modifications (6).

**Tuberculin Skin Test Procedures.** For VLA studies (i.e., *M. bovis*- and *M. tuberculosis*-inoculated animals), skin tests were performed as specified in the European Economic Community Directive 80/219EEC and amending directive 64/422/EEC, annex B. For NADC studies (i.e., *M. kansasii*-inoculated animals), skin tests were performed as specified in the USDA, Animal and Plant Health Inspection Service, Uniform Methods and Rules for Bovine Tuberculosis Eradication circular (APHIS 91-45-011 (24)). Approximately 16 wks after inoculation, skin thickness was measured with calipers immediately prior to *M. bovis* PPD administration and 72 hrs after injection. **Skin test was applied to the mid-cervical region or caudal fold for VLA and NADC studies, respectively.** Data are presented as the change in skin thickness (mm) from pre-injection measurements (mean ± SEM).
Interferon-γ assay. Duplicate 250µl heparinised whole blood aliquots were distributed in 96-well plates with *M. bovis* PPD (10µg/ml, VLA or Prionics Ag, Schlieren, Switzerland) or no antigen and incubated at 37°C/5% CO2 for 20 hours. IFN-γ concentrations in stimulated plasma were determined using a commercial ELISA-based kit (Bovigam™, Prionics Ag). Absorbencies of standards (recombinant bovine IFN-γ; Endogen, Rockford, IL) and test samples were read at 450 nm using an ELISA plate reader (Molecular Devices, Menlo Park, Calif.). Duplicate samples for individual treatments were analyzed and data presented as optical densities at 450 nm of the response to *M. bovis* PPD minus the response to no-antigen (mean ± SEM).

Lateral-flow assay. The VetTB STAT-PAK™ (Chembio Diagnostic Systems, Inc., Medford, NY) was performed as described for use with samples from cattle (33). Briefly, the lateral flow device consists of a plastic cassette containing a strip of nitrocellulose membrane impregnated with test antigen (i.e., MPB83, ESAT-6, and CFP-10) and laminated with several pads made of glass fiber and cellulose. Thirty µl of sera and 3 drops of sample diluent are added sequentially to the sample pad. As the diluted test sample migrates to the conjugate pad, the latex particles conjugated to antigen bind antibody (IgM, IgG, and IgA), if present, thus creating a coloured immune complex. This complex flows laterally via capillary forces across the nitrocellulose membrane impregnated with specific antigen and binds to the immobilized antigen producing a visible blue band. In the absence of specific antibody, no band is visible.

Multi-antigen print immunoassay (MAPIA). MAPIA was performed as described for use with samples from cattle (30, 33). Briefly, *M. tuberculosis*-complex antigens were immobilized on nitrocellulose membrane strips, blocked for 1 h with 1% nonfat skimmed
milk in PBS with 0.05% Tween 20 and then incubated for 1 h with serum samples diluted 1:40 in blocking solution. After washing, strips were incubated overnight with peroxidase-conjugated Protein G (Sigma) diluted 1:1000, washed, and developed with 3,3′,5,5′-tetramethyl benzidine (TMB) (Kirkegaard & Perry Laboratories Inc., Gaithersburg, Md).

Responses to individual *M. tuberculosis* complex antigens are identified by MAPIA.

**Serum IgG ELISA to MPB83 and *M. bovis* culture filtrate.** Antigen specific IgG was measured in serum from clotted blood as described previously (28). Briefly, Nunc Maxisorp ELISA plates (Nunc, Roskilde, Denmark) were coated with either recombinant MPB83 (0.1 µg/ml, Lionex Diagnostics and Therapeutics GmbH, Braunschweig, Germany) or a *M. bovis* AN5 culture filtrate previously prepared at VLA (1 µg/ml). Serum samples were diluted 1:100 prior to addition to pre-coated and pre-blocked assay plates. **No-antigen coated wells were used to ensure the absence of non-specific responses and pre-challenge responses** were used for comparison to specific responses after challenge. Total IgG was detected using a horseradish peroxidase-conjugated sheep anti-bovine IgG (AbD Serotec, Oxford, UK) with data expressed as optical densities at 450 nm (mean ± SEM).

**Serum Ig ELISA to *M. bovis* lipoarabinomannan (LAM).** ELISA to LAM was performed as described (30, 33). Briefly, Immulon II 96-well microtiter plates (Dynatech; Chantilly, Va.) were coated with LAM-enriched mycobacterial antigen (8 µg, prepared as described, (32)). Serum samples were diluted 1:100 prior to addition to pre-coated and pre-blocked assay plates. Total Ig was detected using a horseradish peroxidase-conjugated goat anti-bovine IgG heavy and light chains (Kirkegaard & Perry Laboratories Inc.). Data are presented as Sample / Positive (S/P) ratios (mean ± SEM). S/P ratios of test samples were calculated from absorbency values using the formula: sample – negative control / positive
control – negative control. **Positive and negative control sera of known reactivity were obtained from previous studies.**

**Statistics.** Data were analyzed by analysis of variance followed by Bonferroni multiple comparisons test or Student’s *t* test using a commercially available statistics program (Prism 4.0, GraphPAD Software, La Jolla, Ca.).

**RESULTS AND DISCUSSION**

**Disease Progression.** Granulomatous lesions containing acid-fast bacilli were detected in lungs as well as lung- and head-associated lymph nodes from 5/5, 5/5, and 3/5 *M. bovis*-infected animals, respectively (Table 1). Lesions with acid-fast bacilli were not detected in any *M. tuberculosis* or *M. kansasii*-inoculated animals (Table 1). *Mycobacterium bovis* was isolated by culture in 5/5 animals whereas *M. tuberculosis* was isolated in 3/5 animals inoculated with the respective mycobacteria. **In the *M. bovis*-infected cattle, *M. bovis* was isolated by culture from lung-associated lymph nodes and lungs from 5/5 animals, and from medial retropharyngeal lymph nodes from 3/5 cattle. In the *M. tuberculosis*-infected cattle, *M. tuberculosis* was isolated by culture from lung-associated lymph nodes in 3/5 animals, and from medial retropharyngeal lymph nodes from 1/5 cattle.**

Analysis of spoligotype patterns confirmed that mycobacterial strains isolated from tissues matched the respective challenge strains (i.e., H37Rv for *M. tuberculosis* and AF2122/97 for *M. bovis*, data not shown). *Mycobacterium spp.* were not detected in *M. kansasii*-inoculated animals. The mycobacterial burden differed (P < 0.05) between challenge species (Table 1), with *M. bovis* > *M. tuberculosis* > *M. kansasii*. 
Cell-mediated responses. Injection of M. bovis PPD for skin test (at ~16 wks post inoculation) resulted in palpable reactions in all calves regardless of mycobacterial treatment; however, differences were detected with responses (mean ± SEM) by M. tuberculosis- (22.6 ± 5.1) and M. bovis- (12.8 ± 1.9) inoculated cattle exceeding (P < 0.05) responses by M. kansasii- (4.5 ± 0.3) inoculated cattle. Similarly, IFN-γ responses to M. bovis PPD were elicited in all calves regardless of mycobacterial treatment. Two wks after inoculation, mean IFN-γ responses did not differ (P > 0.05) between treatment groups. From 4 wks until the end of the study, mean IFN-γ responses to M. bovis PPD by M. tuberculosis- and M. bovis- inoculated cattle exceeded (P < 0.05) that of M. kansasii-inoculated cattle (Figure 1).

Throughout the study, IFN-γ responses to M. bovis PPD did not differ (P > 0.05) between M. tuberculosis- and M. bovis-inoculated cattle. Patterns of IFN-γ responses to ESAT-6/CFP10 antigens were similar to that of responses to M. bovis PPD (data not shown; 30). Thus, delayed type hypersensitivity and IFN-γ responses did not correlate with disease progression differences detected between M. tuberculosis- and M. bovis-inoculated cattle. Likewise, transient IFN-γ responses may be elicited to mycobacterial species (e.g., M. kansasii) that are efficiently cleared by cattle. It is interesting to note that IFN-γ response kinetics towards challenge with all three pathogens develops very similar up to two wks post-infection, after which time-point the responses in the M. kansasii-infected calves were curtailed and remained at a significantly lower level throughout the rest of the observation period. One caveat concerning this observation is that M. bovis antigens were used for the assay; thus, responses may be indicative of early cross-reactive responses. Two wks post-infection is most likely the time point when a developing cellular immune response becomes effective, which appears to be capable of eliminating M. kansasii, whilst unable to fully control M. bovis and to a lesser extent, M. tuberculosis infection. This response kinetic is very similar to that observed after mouse aerosol infection in which M. tuberculosis is
exported to the regional lymph nodes for development of the initial primary response (7, 21, 26). This delayed immune kinetic in mice is associated with a slow and delayed migration of parenchymal dendritic cells to pulmonary lymph nodes (26). It would be of interest to conduct similar studies with *M. kansasii* to see if the migration kinetics is identical to its more virulent cousins, *M. tuberculosis* and *M. bovis*.

**Antibody responses.** Specific antibody responses were detected in all *M. tuberculosis*- and *M. bovis*-inoculated cattle three wks after inoculation (Table 2). At 12 and 16 wks after inoculation, IgG responses to MPB83 and *M. bovis* culture filtrate by *M. bovis*-inoculated cattle exceeded (P < 0.05) respective responses by *M. tuberculosis*-inoculated cattle (Figure 2). Antibody levels to MPB83 persisted from 3 – 16 wks after challenge with the *M. bovis* group whereas responses to MPB83 by *M. tuberculosis*-inoculated cattle waned from 6 – 16 wks after challenge (Figure 2). Using a non-quantitative assay (i.e., MAPIA), 3/4 *M. bovis*-inoculated cattle responded to MPB83 by 3 wks after challenge and 4/4 at all other subsequent time points whereas 3/5, 4/5, 4/5, and 3/5 *M. tuberculosis*-inoculated cattle responded to MPB83 at 3, 6, 9 and 12 wks after challenge. *Mycobacterium kansasii* inoculation elicited LAM-specific IgG: pre-inoculation responses (S/P, mean ± SEM) were 0.12 ± 0.13 versus 1.01 ± 0.28 at 7 wks after inoculation (P < 0.05). Initial early responses waned by 10 wks after challenge to 0.50 ± 0.20 and then increased (P < 0.05) 2 wks after injection of PPD for skin test at 18 wks after challenge to 1.47 ± 0.26. Prior to skin test, only 2/4 *M. kansasii*-inoculated calves had antibody specific to MPB83/ESAT-6/CFP10 (i.e., VetTB STAT-PAK™) whereas after skin test, all 4 animals had responses to this antigen cocktail (Table 2).

The strong and persistent generation of MPB83-specific IgG following infection with *M. tuberculosis* is an interesting observation given that *in vitro* *M. tuberculosis* expresses...
very low amounts of MPB83 compared with *M. bovis* (35). Potent immune responses to

MPB83 have previously been observed in *M. tuberculosis*-infected mice (13) and it is also

upregulated in *M. tuberculosis* infected macrophages (i.e., *in vitro*) (22, 23). Together with

the present findings of MPB83-specific responses by *M. tuberculosis*-inoculated cattle, these

data support the notion that MPB83 expression is up-regulated by *M. tuberculosis* *in vivo*.

**Immune response / disease progression associations.** As indicated in the early 1900’s (25),

administration of culture-derived *M. tuberculosis* (in this case - H$_{37}$R$_v$) to healthy cattle elicits

an immune response without expression of disease (i.e., pathology); thus, the rationale for

early 20$^{th}$ century studies evaluating live *M. tuberculosis* as a vaccine for bovine tuberculosis.

With the present study, *M. tuberculosis* was detected in 3 of 5 inoculated animals upon

necropsy, ~16 wks after inoculation. MPB83-specific antibody was detectable early after

challenge in all 5 *M. tuberculosis*-inoculated animals - yet these responses waned to pre-

inoculation levels by the end of the study (Figure 2A). Thus, with experimental *M.

tuberculosis* H$_{37}$R$_v$ infection of cattle, antibody responses are associated with mycobacterial

burden rather than pathology. A wane in the early MPB83-specific antibody response likely

followed the clearance of *M. tuberculosis* from the host. In contrast, persistent *M. bovis*

infection resulted in lesions and sustainable specific antibody responses (Figure 2). Together,

these findings indicate that antibody responses upon mycobacterial infection are indicative of

antigen load rather than mycobacterial-induced pathology.

Inoculation of *M. kansasii* elicited LAM- (4/4) and MPB83- (2/4, as detected by

MAPIA) specific responses. Injection of PPD for skin test boosted both LAM- and MPB83-
specific responses in all four animals, as previously detected with *M. bovis* infection of

cattle (33). Mycobacteria were not isolated upon necropsy and lesions were not detected.

These findings demonstrate the potential for correlation of specific antibody responses to a
CONCLUSIONS

With this study, cellular immune responses correlated with mycobacterial infection but not necessarily with pathology or bacterial burden. Present findings highlight several scenarios for interpretation of antibody responses by cattle to mycobacterial exposure. 1) The classic model in which infection (e.g., *M. bovis* infection of cattle) is not controlled by the host resulting in significant pathology and mycobacterial burden. With this scenario, the association of antibody responses to pathology (15) is likely coincident to antigen burden. 2) An antigen load model in which antibody responses are positively correlated to antigen burden. With *M. tuberculosis* H₃₇R₅ infection of cattle, as the mycobacterium is cleared - antibody responses wane. With *M. bovis* infection of cattle, as infection progresses and the mycobacteria persist - antibody responses also persist or increase. 3) The sensitization model in which exposure to a mycobacterium induces an antibody response (e.g., *M. kansasii* inoculation) which wanes over time; yet, the response may be boosted significantly by re-exposure to mycobacterial antigens (e.g., PPD) or other live mycobacteria.

Good and reliable models of human latent tuberculosis are not readily available for support of both applied (e.g., vaccinology and diagnostic studies) and basic (e.g., immunopathogenesis and bacterial pathogenesis) tuberculosis research. An animal model where different infection outcomes can be modelled with organisms with overlapping antigen repertoires, like the three organisms studied here, would be of advantage. *Mycobacterium bovis* infection of cattle could be considered as a model of clinical tuberculosis, whilst *M. tuberculosis* H₃₇R₅ infection could be akin to latent, subclinical tuberculosis. Infection with *M. kansasii*, on the other hand, might be a model for human tuberculous infection that has
been successfully cleared from its human host. Therefore, we propose that these three infection systems could be developed into useful models mimicking different stages of human tuberculosis infection.

ACKNOWLEDGMENTS

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REFERENCES


Figure 1. Interferon-γ responses upon experimental inoculation of calves with *M. bovis* (n = 5), *M. tuberculosis* (n = 5), or *M. kansasii* (n = 4). Data are presented as optical densities (mean ± SEM) at 450 nm of the response to *M. bovis* PPD minus the response to no-antigen (ΔIFN-γ). * Differs (P < 0.5) from the response by *M. bovis* and *M. tuberculosis*-inoculated animals at the same time point.

Figure 2. Antibody responses upon experimental inoculation of calves with *M. bovis* (n = 5) or *M. tuberculosis* (n = 5). Data are presented as optical densities (mean ± SEM) at 450 nm of the response to MPB83 (panel A) or *M. bovis* culture filtrate (panel B). * Differs (P < 0.5) from the response by *M. tuberculosis*-inoculated animals at the same time point.
Table 1. Disease expression upon mycobacterial inoculation.

<table>
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<tr>
<th>Group</th>
<th>Gross Pathology</th>
<th>Cultureb *</th>
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<tbody>
<tr>
<td><em>M. bovis</em></td>
<td>All positive</td>
<td>27.2 ± 7.3</td>
</tr>
<tr>
<td>(n = 5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>M. tuberculosis</em></td>
<td>All negative</td>
<td>13.9 ± 5.5</td>
</tr>
<tr>
<td>(n = 5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>M. kansasii</em></td>
<td>All negative</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>(n = 4)</td>
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<td></td>
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*a* Gross lesions were confirmed as tuberculous upon histologic evaluation and culture of lungs as well as lung- and head-associated lymph nodes.

*b* Cumulative score (mean ± SEM) based on a ranking of total CFU/plate of lung and lung-associated lymph node homogenates at highest dilution. Mycobacteria were not detected in any tissues collected from *M. kansasii*-inoculated animals. **Mean scores reflect results from each animal within treatment groups, including culture negative animals.**

* Results differ (P < 0.05) according to mycobacterial treatment with *M. bovis* > *M. tuberculosis* > *M. kansasii*-inoculated animals.
Table 2. Kinetics of serum antibody response to MPB83/ESAT-6/CFP-10 as detected by VetTB STAT-PAK™ in cattle during experimental mycobacterial infection

<table>
<thead>
<tr>
<th>Group</th>
<th>Weeks after Inoculation&lt;sup&gt;a&lt;/sup&gt;</th>
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<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td><em>M. bovis</em>&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0/4</td>
</tr>
<tr>
<td><em>M. tuberculosis</em></td>
<td>0/5</td>
</tr>
<tr>
<td><em>M. kansasii</em></td>
<td>0/4</td>
</tr>
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<sup>a</sup>Data are presented as the # positive / # tested.

<sup>b</sup>For *M. kansasii*-inoculated cattle, responses at 18 wks were 2 wks after injection of PPD for skin test.

<sup>c</sup>One of the *M. bovis* infected cattle was euthanized at week 6 and therefore kinetics data was only available for 4/5 original study animals.

na – Animals in *M. tuberculosis* and *M. bovis*-inoculated groups were euthanized 16 wks after inoculation; thus, samples were not available for analysis at this time point.
Figure 1.
Figure 2

A. IgG to MPB83

B. IgG to *M. bovis* Culture Filtrate

Weeks Relative to Challenge