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Authors: Stabel, J. R., Palmer, M. V., and Whitlock, R. H.

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IMMUNE RESPONSES AFTER ORAL INOCULATION OF WEANLING BISON OR BEEF CALVES WITH A BISON OR CATTLE ISOLATE OF *MYCOBACTERIUM AVIUM* SUBSP. *PARATUBERCULOSIS*

J. R. Stabel,[1,3] M. V. Palmer,[1] and R. H. Whitlock[2]

[1] USDA-ARS, National Animal Disease Center, Bacterial Diseases of Livestock Research Unit, 2300 Dayton Road, Ames, Iowa 50010, USA

[2] New Bolton Center, Kennett Square, Pennsylvania 19348-1692, USA

[3] Corresponding author (email: jstabel@nadc.ars.usda.gov)

ABSTRACT: Paratuberculosis is endemic in domestic and wild ruminants worldwide. We designed the following study to compare host immune responses and pathologic changes in beef calves and bison calves after challenge with either a cattle or bison (*Bison bison*) strain of *Mycobacterium avium* subsp. *paratuberculosis.* In the first part of the study, six bison and six beef calves were orally inoculated with a cattle isolate of *M. avium* subsp. *paratuberculosis* over a 2 wk period. In the second part, an additional six bison and six beef calves were similarly inoculated with a bison strain of *M. avium* subsp. *paratuberculosis.* Throughout each of the studies, blood and fecal samples were taken monthly for a 6 mo infection period. Tissue samples were obtained at necropsy for culture and histopathologic analyses. Results from this study demonstrated that bison calves were more susceptible to tissue colonization than beef calves after challenge with the cattle isolate and, conversely, that beef calves were more susceptible to the bison strain of *M. avium* subsp. *paratuberculosis.* Although lesions were minimal they were most apparent in the jejunum and distal ileum. Interferon-gamma (IFN-γ) responses were noted in some calves by 1 mo postinoculation and were sustained longer in beef calves after challenge with the bison isolate. Antibody was not detected in either beef or bison calves during the 6 mo infection period. These results indicate that the host response to strains of *M. avium* subsp. *paratuberculosis* may differ between ruminant species.

Key words: *Bison bison,* cattle, immune responses, *Mycobacterium avium* subsp. *paratuberculosis,* pathology.

INTRODUCTION

Paratuberculosis (Johne's disease), a chronic enteritis caused by the acid-fast bacterium, *Mycobacterium avium* subsp. *paratuberculosis,* has been documented in domestic and wild ruminants worldwide. It has been reported in captive ruminants such as red deer (*Cervus elaphus*), fallow deer (*Dama dama*), sika deer (*Cervus nippon*), white-tailed deer (*Odocoileus virginianus*), tule elk (*Cervus elaphus nannodes*), and moose (*Alces alces*) maintained on game farms or in zoos (Soltys et al., 1967; Libke and Walton, 1975; Temple et al., 1979; Fawcett et al., 1995; Manning et al., 1998). However, free-ranging wild ruminants may present more of a problem in transmission of infection due to comingling of different species on pasture and sharing of water resources. Little is known about the potential for one ruminant species to act as a vector of transmission of infection to another species. Animal species as disparate as goats and camels that were comingled on a ranch in Kenya were found to be seropositive for *M. avium* subsp. *paratuberculosis* (Paling et al., 1988). More recently, an epidemiologic study in the Czech Republic demonstrated that wild ruminants such as red deer, roe deer (*Capreolus capreolus*), fallow deer and mouflon (*Ovis musimon*) were infected with *M. avium* subsp. *paratuberculosis* and that the infection source likely originated from two imported Holstein and Limousine cattle herds (Pavlik et al., 2000). It is also possible that the wild animals may become infected and act as reservoirs of infection for domestic livestock. A recent study described the experimental infection of young calves with a rabbit isolate of *M. avium* subsp. *paratuberculosis* (Beard et al., 2001). The virulence of the rabbit isolate was found to be comparable to that of the bovine isolate used in the

study. In addition, an isolate of *M. avium* subsp. *paratuberculosis* from bighorn sheep (*Ovis canadensis*) was used in experimental infection of elk (*Cervus elaphus nelsoni*), mule deer (*Odocoileus hemionus*), white-tailed deer and bighorn×mouflon hybrid sheep (Williams et al., 1983). All species became infected but only mule deer developed clinical signs of disease within the 12 mo period of the study.

Paratuberculosis has recently been reported in privately owned North American bison (*Bison bison*) on rangeland in the western United States (Buergelt et al., 2000). Although bison develop signs of unthriftiness and suffer from weight loss, other clinical signs of disease such as hypoproteinemia are lacking (Whitlock et al., 1999). In addition, it is difficult to culture *M. avium* subsp. *paratuberculosis* from the feces of infected bison for definitive diagnosis and serum antibody is negligible. This suggests that the host response to *M. avium* subsp. *paratuberculosis* in bison may differ from the host response in cattle. The objectives of this study were to evaluate potential differences in homologous and heterologous host responses to bison and cattle isolates of *M. avium* subsp. *paratuberculosis.*

MATERIALS AND METHODS

Animals

Weanling bison and beef calves were obtained from local farms in Iowa. The farms had no reportable incidence of Johne's disease in their herds within the last 5 yr and were assumed free of Johne's disease. Prior to acquisition and shipment to the National Animal Disease Center (NADC; Ames, Iowa, USA), calves were screened for *M. avium* subsp. *paratuberculosis* infection using the whole blood interferon-gamma (IFN-γ) assay. Only calves that had negative responses to both *M. avium* subsp. *paratuberculosis* antigens and *M. avium* purified protein derivative (PPD) were selected for use in the study. Bison or beef calves were each obtained from a single source. Calves were housed two animals per pen in a biosafety level 2 containment barn at the NADC. Upon receipt, calves were dehorned, wormed and acclimated to their diet and environment for 2 wk prior to initiation of the study. Calves were fed a mixed ration containing corn, wheat mids, and soybean meal for the term of the study.

Oral inoculation

Due to limited containment barn space the study was segregated into two phases, each with six bison calves and six beef calves. In the first phase of the study, calves were inoculated via stomach tube with live *M. avium* subsp. *paratuberculosis* isolated from mucosal scrapings from the ileum of a cow with clinical paratuberculosis. The bacterial isolate was propagated in M7H9 medium with OADC (Becton-Dickinson, Franklin Lakes, New Jersey, USA) and 2 mg/ml of mycobactin J (Allied Monitor, Fayetteville, Missouri, USA) and harvested in the log phase of growth. The bacteria were pelleted by centrifugation at 7,500 rpm, washed with 0.15 M phosphate-buffered saline (PBS; pH 7.4) two times and then resuspended in sterile PBS to a final concentration of 1×10^8 colony forming units (cfu/ml). Calves were inoculated with 60 ml of bacterial preparation on days 1, 2, 3, 7, and 14 of the study. In the second phase of the study, the protocol was the same except the isolate of *M. avium* subsp. *paratuberculosis* was obtained from a bison cow with clinical paratuberculosis.

Sampling

Blood and fecal samples were obtained from calves for three consecutive days prior to inoculation with the bacteria (days −3, −2, and −1) and then on days 7, 14, 21, and 28 of the study and every 30 days thereafter throughout the 6-mo infection period.

Fecal culture for *M. avium* subsp. *paratuberculosis*

Fecal samples (2 g) were processed by the NADC centrifugation and double-decontamination method previously described (Stabel, 1997).

Tissue culture and histopathologic analyses for *M. avium* subsp. *paratuberculosis*

Sections of duodenum, jejunum, ileum, and their associated lymph nodes were obtained at necropsy. Sections of cecum, spiral, transverse, and descending colon as well as colic, hepatic, and iliac lymph nodes were also taken. Portions of each tissue were weighed and homogenized in 0.75% hexadecylpyradinium chloride solution by use of a stomacher for 1 min and allowed to stand overnight to decontaminate the cultures. Dilutions of individual tissue homogenates were inoculated onto Herrold's egg yolk

medium (HEYM; NADC) containing 2 mg/L of mycobactin J. After 12 wk of incubation at 37 C, viable organisms were determined by counting the number of colonies on the agar slants.

Sections of tissue were fixed by immersion in neutral-buffered 10% zinc formalin. Tissues were routinely processed, embedded in paraffin, cut at 4–6 μm, and stained with hematoxylin and eosin (HE). Adjacent sections were cut from blocks containing tissues with lesions suggestive of paratuberculosis and stained by the Ziehl-Neelsen technique to visualize acid-fast bacteria. In the first phase of the study the following tissues were collected for microscopic analyses: palatine and pharyngeal tonsils; mandibular, parotid, medial retropharyngeal, tracheobronchial, mediastinal, superficial cervical, iliac, prefemoral, popliteal, and hepatic lymph nodes (LN); proximal, middle, and distal duodenum and associated lymph nodes; four sections of each of proximal, middle, and distal jejunum and corresponding jejunal lymph nodes; proximal, middle, and distal ileum and associated lymph nodes; ileocecal junction and ileocecal lymph node; cecum, proximal, middle, and distal spiral colon and associated colic lymph nodes; and transverse and descending colon. In the second phase of the study the following tissues were collected: mid-duodenum and associated lymph node; one section each of proximal, middle, and distal jejunum and associated lymph nodes; proximal, middle, and distal ileum and associated lymph nodes; ileocecal valve and ileocecal lymph node; cecum, spiral colon, and associated lymph node; transverse and descending colon; and hepatic and ileac lymph nodes.

Poymerase chain reaction analyses on fecal and tissue samples

Confirmation of colonies on agar slants was performed by polymerase chain reaction (PCR) analysis. Briefly, agar slants were flooded with 1 ml of sterile 1 mM Tris-0.05 mM ethylenediamine-tetraacetic acid (EDTA) buffer (pH 7.6) and slants were scraped. The solution was then decanted into a sterile microfuge tube and the tubes placed within a boiling water bath for 10 min to release DNA from the bacteria. After cooling to room temperature, tubes were briefly centrifuged at 14,000 rpm in a microfuge to pellet the bacterial debris. DNA (5 μl) was added to 45 μl of PCR mix containing: GeneAmp 10× PCR buffer II (Perkin-Elmer, Foster City, California, USA), 3.0 mM $MgCl_2$, 0.25 mM dNTPs, 0.6 mM primers, and 2 U of AmpliTaq gold. Primers sequences for the *M. avium* subsp. *paratuberculosis*-specific genetic element, IS900, were used in the reaction mixture as follows: 5′-CCGCTAATTGAGAGATGCGATTGG-3′, forward primer; and 5′-AATCAACTCCAGCAGCGCGGCCTCG-3′, backward primer, to yield a 229-bp product as previously described. Controls consisting of reaction mixture alone, positive control containing 1 μl of genomic DNA from *M. avium* subsp. *paratuberculosis,* and sample tubes with 5 μl of *M. avium* subsp. *paratuberculosis* DNA extracted from tissue samples were run following this protocol: 1 cycle at 94 C, 10 min; 50 cycles at 94 C, 1 min; 60 C, 30 sec; and 72 C, 1 min followed by a final extension cycle at 72 C, 10 min. Polymerase chain reaction amplicons and a 50–1,000 bp marker (BioWhittaker Molecular Applications, Rockland, Maine, USA) were then electrophoresed in a 4% NuSieve 3:1 Plus agarose gel (FMC Bioproducts, Rockland, Maine, USA) in 10× Tris-Borate-EDTA (1 M Tris-HCl, 0.9 M Boric acid, 0.01 M EDTA; GIBCO BRL) buffer at 65V for 1 hr. Gels were then stained with ethidium bromide, visualized, and photographed on a Bio-Rad Gel Doc 1000 Imager System (Hercules, California, USA).

Interferon-gamma analyses

One ml aliquots of whole blood obtained in sodium heparin tubes from each animal were pipetted into each of six wells of 24-well tissue culture plates (Becton-Dickinson, Franklin Lakes, New Jersey, USA). Blood samples were cultured alone (nonstimulated) or with concanavalin A or pokeweed mitogen (ConA, PWM; Sigma Chemical Co., St. Louis, Missouri, USA) at a concentration of 10 μg/ml. The nonstimulated culture was included to evaluate background absorbance in each sample. Samples were stimulated with ConA or PWM, T-cell dependent mitogens, as positive controls to provide information about the ability of the cells to respond to stimulation and secrete IFN-γ nonspecifically. Samples were also plated with avium purified protein derivative (AvPPD; Commonwealth Serum Laboratories, Victoria, Australia), bovine purified protein derivative (BoPPD; CSL), a johnin purified protein derivative (JPPD; National Veterinary Services Laboratories, Ames, Iowa, USA), or a whole cell sonicate of *M. avium* subsp. *paratuberculosis* (strain 19698, MpS; NADC, Ames, Iowa, USA). The MpS was prepared by sonication of 1-ml volumes of *M. avium* subsp. *paratuberculosis* (1×10^9/ml) in PBS at 25 W for 25 min, and a protein concentration was determined. Levels of AvPPD and BoPPD were added according to the protocol outlined in the commercial kit and an equivalent amount of JPPD and MpS was added to the appropriate wells.

Final concentration of JPPD or MpS in culture was 10 μg/ml. Cultures were incubated for 18 hr at 39 C in a 5% CO_2 humidified atmosphere. Plates were centrifuged at 500×G for 15 min and plasma was harvested from each well. Plasma samples were frozen at −20 C until analyzed for IFN-γ concentration by enzyme-linked immunosorbent assay (ELISA) using a commercial kit (Bovigam, BioCor, Omaha, Nebraska, USA). A sample was determined to be positive if the absorbance of the stimulated sample (either mitogen or antigen) was 0.100 abs units greater than the absorbance achieved for the nonstimulated control well for that animal. This classification of positive reaction was extracted from similar interpretations reported by researchers who have used the IFN-γ assay for detection of tuberculosis in cattle (Wood et al., 1992; Doherty et al., 1995).

Lymphocyte proliferation

Sixty ml of whole blood were obtained via jugular venipunture in sterile syringes containing 2× acid-citrate-dextrose (0.15 M sodium citrate, 0.05 M citric acid, 0.25 M dextrose). Blood was centrifuged at 1,800×G for 20 min at room temperature. Buffy coat fractions (5 ml) were removed from the interface and red blood cells were lysed. The cell pellet was resuspended in culture medium (RPMI-1640 containing 25 m*M* HEPES ([GIBCO], 2 m*M* L-glutamine, penicillin G (200 U/ml), and streptomycin sulfate [200 g/ml]) to a final concentration of 2×10^6 cells/ml. Cell viability was determined to be >95% by use of propidium iodide exclusion. Peripheral blood mononuclear cells were plated at a density of 2×10^5 cells/well in 96-well tissue culture plates in culture medium containing 10% FBS. Media for mitogenic stimulation contained 10 μg of ConA/ml, phytohemagglutinin (PHAP; Sigma)/ml, or PWM/ml. Medium for antigenic stimulation contained 10 μg of MpS/ml. Plates were incubated for 72 (mitogens) or 120 (MpS) hr at 39 C in 5% CO_2, then [^{3}H]methylthymidine (5 μCi/well) was added for an additional 18 hr. Cells were harvested on filtermats using a Tomtec harvester (Tomtec, Orange, Connecticut, USA) and counted on a Wallac 1450 Microbeta Plus liquid scintillation counter (Wallac, Gaithersburg, Maryland, USA). Results were expressed as counts per min.

Mycobacterium avium subsp. *paratuberculosis*-specific ELISA

Plates were coated with a whole cell sonicate preparation of *M. avium* subsp. *paratuberculosis* (cattle isolate) and incubated overnight in a humidified atmosphere at 4 C. After three consecutive washes with PBS containing 0.1% Tween 80 (Difco Laboratories, Detroit, Michigan, USA), plates were incubated for 30 min at 39 C with 1% gelatin to block nonspecific binding sites. Plates were then washed three times with PBS-Tween solution with 3 min soak periods between each washing. Test sera were diluted 1:400 in PBS, added to wells, and incubated for 1 hr at 39 C. Plates were washed 3× with PBS-Tween as described and incubated for 1 hr at 39 C with anti-bovine Ig (VMRD, Pullman, Washington, USA). After washing, anti-mouse Ig, biotinylated $F(ab')_2$ fragment (Sigma) was added to wells and incubated for 2 hr at 39 C. Plates were washed and streptavidin biotinylated peroxidase complex (Amersham, Piscataway, New Jersey, USA) was added to each well and incubated for 30 min at 39 C. Plates were washed a final time, and substrate solution (40 mM ABTS [2,2'-azino-di-3-ethylbenzthiozoline-6-sulfonic acid] in citrate buffer), pH 4.0) was added for a 10 min incubation at room temperature. Sera from a known negative and positive control animal were included in each assay run. Absorbance of test samples at 405 nm (test wavelength) and 490 nm (reference wavelength) was measured with a MR7000 Dynatech plate reader (Dynatech, Chantilly, Virginia, USA). A commercial ELISA test (IDEXX Corp., Westbrook, Maine, USA) was also run on serum samples taken on prechallenge days and 6 mo post-infection to compare to our in-house ELISA.

RESULTS

No overt signs of infection were noted in bison or beef calves throughout the 6 mo infection period regardless of inoculum administered. One bison calf in the second phase of the study was necropsied after 2 mo because of an unrelated sinus infection stemming from dehorning after arrival. Data from this animal were not included in the results of the study.

Shedding of viable *M. avium* subsp. *paratuberculosis* in the feces of infected calves was minimal in either phase of the study (results not shown). Inoculation with the cattle isolate of *M. avium* subsp. *paratuberculosis* resulted in two calves (one beef and one bison) excreting low levels of bacteria (<5 cfu/g) on days 7 and 60 of the study. In contrast, *M. avium* subsp. *paratuberculosis* was cultured only from

TABLE 1. Recovery of viable *Mycobacterium avium* subsp. *paratuberculosis* from tissues of beef and bison calves after 6 mo of infection with either cattle or bison isolate.

Tissue	Cattle isolate		Bison isolate	
	Beef calves	Bison calves	Beef calves	Bison calves
Duodenum/LN[a]	0/6	1/6	5/6	2/5
Proximal jejunum/LN	2/6	4/6	4/6	1/5
Mid-jejunum/LN	1/6	3/6	4/6	2/5
Distal jejunum/LN	3/6	5/6	5/6	1/5
Proximal ileum	0/6	0/6	0/6	1/5
Mid-ileum	0/6	1/6	3/6	0/5
Distal ileum	1/6	3/6	1/6	2/5
Ileal LN	0/6	0/6	6/6	2/5
Ileocecal LN	5/6	1/6	4/6	0/5
Ileocecal valve	0/6	1/6	1/6	1/5
Spiral colon/LN	2/6	2/6	6/6	3/5
Transverse colon	0/6	2/6	5/6	3/5
Descending colon	1/6	0/6	2/6	3/5
Colic LN	1/6	0/6	2/6	2/5
Hepatic LN	0/6	1/6	2/6	2/5
Iliac LN	0/6	3/6	2/6	2/5

[a] LN = lymph node.

two beef calves on days 14 and 60 after challenge with the bison isolate.

Results from the culture of *M. avium* subsp. *paratuberculosis* from tissues of calves are presented in Table 1. There were some distinct differences noted in the pattern of tissue colonization between beef and bison calves after inoculation with the two strains of *M. avium* subsp. *paratuberculosis.* After challenge with the cattle isolate, the jejunal region and the associated lymph nodes were most commonly colonized in bison calves (five of six calves) and less frequently in beef calves. In addition, the distal ileum was affected in 50% of the bison calves and only 17% of beef calves. The ileocecal LN was affected in five of six beef calves but only one of six bison calves after inoculation with the cattle isolate although the ileal LN was negative for both calf groups. Conversely, beef calves were more adversely affected after challenge with the bison isolate of *M. avium* subsp. *paratuberculosis* (Table 1). Colonization of the duodenum, jejunum, and mid-ileum was more significant in beef calves compared to bison calves. The ileal LN and ileocecal LN were colonized to a higher degree in beef calves than in bison calves. In addition, the spiral colon and transverse colon were culture positive for a majority of beef calves but only moderately affected in bison calves.

In general, the burden of infection within a tissue was relatively low (<10 cfu/g) with only a few organisms isolated on HEYM slants for each tissue. Some tissues had significantly higher colonization for some animals but this was a rare occurrence. Colonies from a majority of tissues appeared as tiny pinprick colonies on the medium after 12 wk of incubation regardless of isolate used for oral challenge or species of calf.

Gross lesions were not present in bison or beef calves from either phase of the study. Microscopic lesions consistent with paratuberculosis were few, but they were most common in bison inoculated with the beef isolate. Three of six (30%) bison inoculated with the cow isolate of *M. avium* subsp. *paratuberculosis* had microscopic lesions in the distal ileum, ileocecal valve, and at least one jejunal lymph node. One bison calf inoculated with the bison isolate had a microscopic lesion in the spiral colon. Jejunal lymph node lesions were composed of cortical infiltrates of small numbers of epithelioid macrophages and Langhan's type multinucleated giant cells. Similar lesions were present in submucosal lymphoid tissues of the distal ileum, ileocecal valve, and spiral colon. Acid-fast bacteria were not seen in any lesions.

Total lymphocyte proliferative responses to mitogens and *M. avium* subsp. *paratuberculosis* antigens averaged over each 6 mo phase of the study are presented in Figure 1. Baseline cell proliferation (NS) was higher for beef calves than for bison calves regardless of which bacterial inoculum was used for challenge. In contrast, T cell proliferative responses to ConA, PWM, and PHAP were significantly ($P<0.05$) higher for bison calves in both

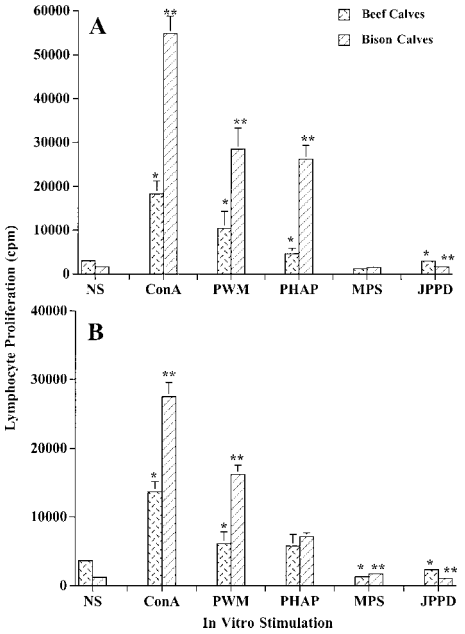

FIGURE 1. Effects of inoculation of beef or bison calves with a cattle isolate (A) or a bison isolate (B) of *M. avium* subsp. *paratuberculosis* on lymphocyte proliferative responses to either nonstimulation (NS), concanavalin A (ConA), pokeweed mitogen (PWM), phytohemaglutinin (PHAP), *M. avium* subsp. *paratuberculosis* sonicate (MpS), or johnin PPD (JPPD). Values are means±SEM, $n=6$; *$P<0.05$ denotes significance between beef and bison calves.

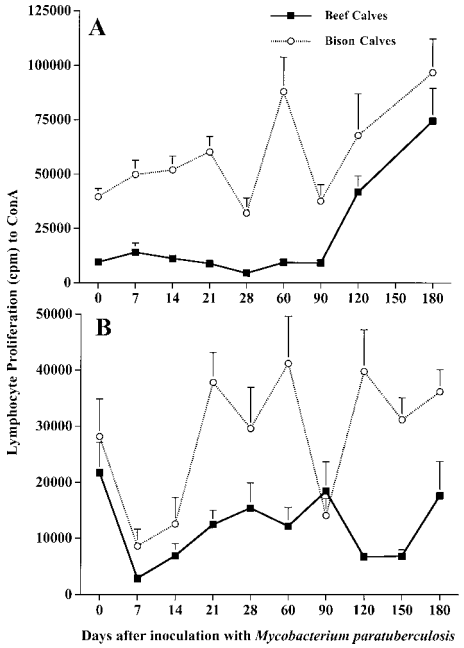

FIGURE 2. Lymphocyte proliferative responses to concanavalin A (ConA) stimulation at various time points after inoculation of beef or bison calves with either a cattle (A) or bison (B) isolate of *M. avium* subsp. *paratuberculosis*. Values are means±SEM; $n=6$.

parts of the study. Beef calves had statistically higher ($P<0.05$) antigen-specific responses to JPPD than bison calves throughout the study but response levels were not different than those observed for baseline proliferation so were unlikely to be biologically relevant.

Bison calves had significantly ($P<0.05$) higher proliferative responses to ConA at most bleeding times throughout both phases of the study (Fig. 2). This effect was particularly apparent in the first phase of the study when calves were challenged with the cattle isolate. Both groups of calves had a significant ($P<0.05$) decrease in ConA responsiveness within 7 days after challenge with the bison isolate of *M. avium* subsp. *paratuberculosis*, rebounding to pre-challenge baseline levels by day 28. Proliferative responses to PWM were also significantly ($P<0.05$) higher for bison calves on each sampling date in the study, regardless of bacterial inoculum used (Fig. 3).

The overall effects of calf species during the two 6 mo infection periods on the production of IFN-γ by peripheral blood mononuclear cells stimulated with T cell mitogens and mycobacterial antigens are presented in Figure 4. Disparate effects of calf species on IFN-γ production were noted with higher ($P<0.05$) ConA responses noted for beef calves and higher ($P<0.05$) PWM responses noted for bison calves in both phases of the study. Overall, antigen-specific IFN-γ production was higher ($P<0.05$) for beef calves compared to bison calves regardless of bacterial isolate used for challenge. Separation of MpS-stimulated IFN-γ production by bleeding date demonstrated similar responsiveness for bison or beef calves after inoculation with the cattle isolate of *M. avium* subsp. *paratuberculosis* over the 6 mo

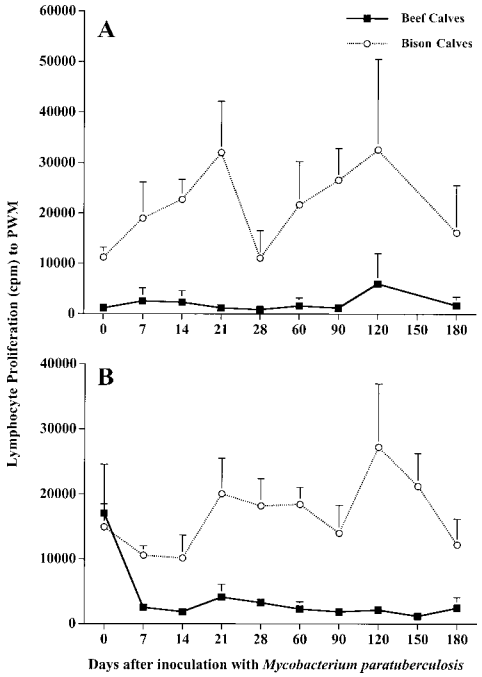

FIGURE 3. Lymphocyte proliferative responses to pokeweed mitogen (PWM) stimulation at various time points after inoculation of beef or bison calves with either a cattle (A) or bison (B) isolate of *M. avium* subsp. *paratuberculosis*. Values are means±SEM; n=6.

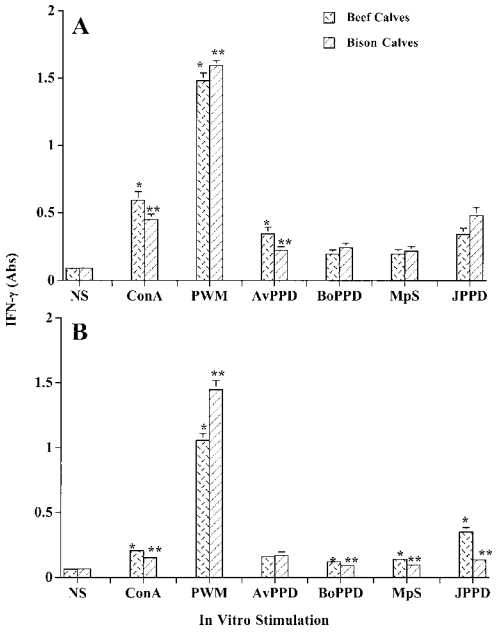

FIGURE 4. Effects of inoculation of beef or bison calves with a cattle isolate (A) or a bison isolate (B) of *M. avium* subsp. *paratuberculosis* (B) on interferon-gamma (IFN-γ) responses to either nonstimulation (NS), concanavalin A (ConA), pokeweed mitogen (PWM), *M. avium* PPD (AvPPD), *M. bovis* PPD (BoPPD), *M. avium* subsp. *paratuberculosis* sonicate (MpS), or johnin PPD (JPPD). Values are means±SEM, n=6; *$P<$ 0.05 denotes significance between beef and bison calves.

period (Fig. 5). Increased responsiveness to antigen stimulation was noted in both groups of calves by 60 days post-inoculation but appeared more consistent in bison calves. After inoculation with the bison isolate, beef calves had significantly ($P<0.05$) higher IFN-γ production to MpS at 4, 5, and 6 mo while bison calves remained relatively unresponsive. Similar responses were noted after stimulation of cells with JPPD (Fig. 6). After challenge with the cattle isolate, bison and beef calves had similar IFN-γ production over time with bison calves exhibiting an earlier response than beef calves (days 14–28). In addition, challenge with the bison isolate resulted in higher ($P<0.05$) IFN-γ production for beef calves from 2 mo post-inoculation through the end of the study. By 6 mo post-challenge with the cattle isolate, responses to either MpS or JPPD antigen stimulation were reduced in both groups of calves indicating a transient cell-mediated response to the cattle isolate.

There were no detectable serum antibodies (IgM, IgG) to *M. avium* subsp. *paratuberculosis* using our in-house ELISA for bison or beef calves during either of the 6-mo infection periods (data not shown). There was also no detectable antibody in either pre- or post-infection serum samples for bison or beef calves when measured by the IDEXX ELISA test.

DISCUSSION

Transmission of paratuberculosis between free-ranging wild ruminants and domestic ruminants may be a confounding factor in control of this insidious disease. One recent study demonstrated that a cattle isolate of *M. avium* subsp. *paratuber-*

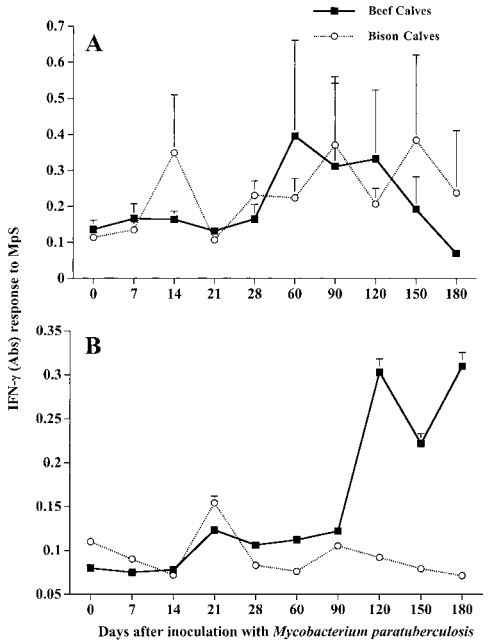

FIGURE 5. Interferon-gamma (IFN-γ) responses to *M. avium* subsp. *paratuberculosis* sonicate (MpS) stimulation at various time points after inoculation of beef or bison calves with either a cattle or bison isolate of *M. avium* subsp. *paratuberculosis.* Values are means±SEM; $n=6$.

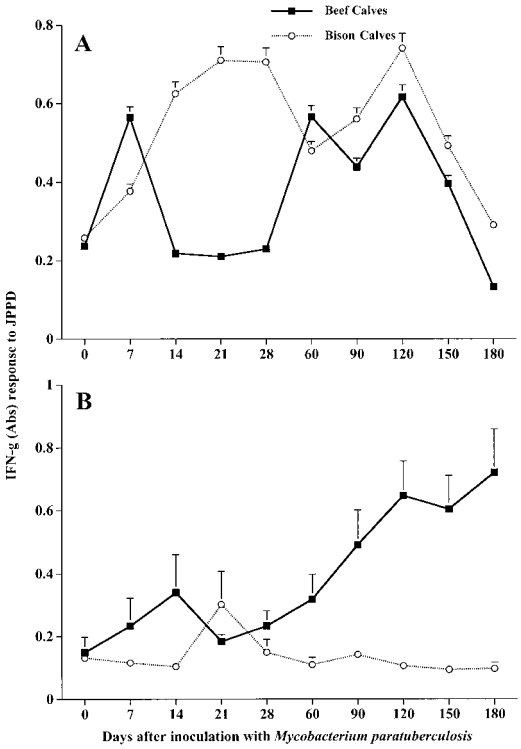

FIGURE 6. Interferon-gamma (IFN-γ) responses to johnin PPD (JPPD) stimulation at various time points after inoculation of beef or bison calves with either a cattle (A) or bison (B) isolate of *M. avium* subsp. *paratuberculosis.* Values are means±SEM; $n=6$.

culosis could infect wild ruminants such as red deer, roe deer, fallow deer, and mouflon (Pavlik et al., 2000). Interspecies transmission of *M. avium* subsp. *paratuberculosis* has been either suspected or confirmed by experimental infection in other species of ruminants (Chiodini and Van Kruiningen, 1983; Williams et al., 1983; De Lisle et al., 1993; Beard et al., 2001). A more recent documentation of *M. avium* subsp. *paratuberculosis* infection in bison posed the question of the source of infection (Pavlik et al., 1999; Whitlock et al., 1999). Did the strain isolated from the bison originate from cattle or was it a unique strain to bison? Strain analysis by restriction-length fragment polymorphism (RFLP) resulted in identification of several strain types (BC-1, CC-1 and ZC-18) in one infected bison herd, suggesting that multiple strains may be responsible for the infection (Pavlik et al., 1999; Whitlock et al., 1999). However, the majority of infected bison within the herd that were typed were classified as BC-1, which is a predominant cattle classification of *M. avium* subsp. *paratuberculosis.* Interestingly, cattle occupied the grazing lands prior to introduction of bison (Buergelt and Ginn, 2000a; Buergelt et al., 2000b). Restriction-length fragment polymorphism analyses of the bison and cattle isolates utilized in the present study were performed in our laboratory by a published procedure (Pavlik et al., 1999) and our results further substantiated that the isolates were identical and correctly classified as the BC-1 strain. Therefore, the bison isolate used to challenge calves was a bison-attenuated cattle strain. This information combined with the observation that clinical signs differ between bison and cattle infected with *M. avium* subsp. *paratuberculosis* poses a sec-

ond question. Is the host response to *M. avium* subsp. *paratubererculosis* different between bison and cattle? The data from the present study indicate that there are species differences in immunity to *M. avium* subsp. *paratuberculosis* infection.

Calves in the present study were only infected for 6 mo yet there were clear signs of subclinical infection within both beef and bison calves regardless of inoculum used. Fecal shedding was minimal but this was expected in a short-term experimental infection (Williams et al., 1983; Saxegaard, 1990). Culture of *M. avium* subsp. *paratuberculosis* from the tissues of infected calves was much more informative and delineated clear differences between beef and bison calves in the colonization of tissues. Challenge with heterologous isolates of *M. avium* subsp. *paratuberculosis* resulted in major differences in the susceptibility to infection between calf species with predominant colonization occurring in the jejunal tissues and associated lymph nodes. These results suggest that exposure of naive animals to isolates from different species may result in greater virulence.

Similar to microscopic changes seen in the present study, lesions in subclinically affected bison naturally infected with *M. avium* subsp. *paratuberculosis* are reported to be mild (Buergelt et al., 2000b). Using criteria proposed for the histomorphologic diagnosis of paratuberculosis, the lesions in all affected animals in the current study would be categorized as suspicious for paratuberculosis and mild in severity. Suspicious lesions are those in which noncaseating granulomatous inflammation consists of Langerhan's type giant cells and epithelioid macrophages, but acid-fast bacilli are not demonstrable (Buergelt et al., 2000b).

Bison calves had greater T-cell mediated lymphocyte responses than beef calves during the course of study regardless of inoculum used. Previously, it was shown that PBMC isolated from 18 mo old bison calves had higher proliferative responses to T-cell mitogens, ConA, and PHAP than age-matched beef calves (Stevens et al., 1997). The reason for the enhanced response is unknown but percentages of T and B lymphocytes and adherent cells appear to be similar between bison and cattle (Nagi and Babiuk, 1989). Beef and dairy calves similar in age to the group utilized in the present study could be expected to have a low level of immunologic maturity (Senogles et al., 1978; Person et al., 1983). There is no documented comparison on immunologic development in bison calves and beef or dairy calves. The efficiency in T cell responsiveness noted for the bison calves could explain some of the lack in clinical signs noted for naturally infected bison in the field compared to cattle harboring the same load of organisms.

Further measurement of cell-mediated immunity yielded disparate responses to T cell mitogens, ConA and PWM, in the IFN-γ assay. Higher ConA and lower PWM induction of IFN-γ by beef calves was consistent during both phases of the study, suggesting a true biologic response in these hosts during infection with *M. avium* subsp. *paratuberculosis.* It is not clear why bison calves would have higher proliferative responses to all T cell mitogens evaluated yet have lower ConA- and antigen-stimulated IFN-γ production. Perhaps the immune cell populations that are traditionally stimulated by ConA, PWM, and PHAP in cattle are different in bison, leading to a different paradigm of immune response between the two species of ruminants. There is no published record of measurements of immune cell phenotype and specific function of T cell populations (CD4+, CD8+, natural killer cells, γδ T cells) during disease states in bison that would help explain these data; however one study suggested that hematologic parameters are similar between bison and cattle (Miller et al., 1989). It is clear that PWM, a T-cell-dependent B-cell mitogen, was capable of inducing IFN-γ production by PBMC from bison calves. However, B cell function in bison has not been fully

evaluated either. Further work to delineate activated cell populations in bison is warranted.

The lack of serum antibody to *M. avium* subsp. *paratuberculosis* in either group of calves in both phases of the study was not surprising because the time after exposure to the live organism was relatively short. Antibody responses to *M. avium* subsp. *paratuberculosis* infection tend to appear in late subclinical and clinical stages of infection in most ruminant species (Stabel, 2000). We ran our in-house ELISA as it allowed us to differentiate between IgM (early antibody) and IgG (late antibody) responses although we saw no differences with either isotype in this study. Because we had no previous experience running bison serum samples in our laboratory we also used a commercial ELISA test that was proven effective in measuring *M. avium* subsp. *paratuberculosis*-specific antibodies in naturally infected bison (Whitlock et al., 1999). Similar absorbances were noted in beef and bison calves with the commercial ELISA but values were below the cutoff determined as positive by the manufacturer's instructions so these data were not reported.

In summary, challenge of bison and beef calves resulted in differing responses to the heterologous isolate of *M. avium* subsp. *paratuberculosis.* Beef calves had higher colonization of jejunal tissues after challenge with the bison isolate and bison calves had higher colonization after infection with the cattle isolate of *M. avium* subsp. *paratuberculosis.* Proliferative responses to T cell mitogens were also higher for bison calves throughout the study although IFN-γ production was higher for beef calves. In addition, clinical signs of disease in bison may be reduced compared to cattle because of enhanced T cell function allowing for containment of infection. Because the RFLP analyses showed that the isolates were identical strains (BC-1) this study demonstrates that cross-species transmission of *M. avium* subsp. *paratuberculosis* in bison is not only possible but also highly likely. Infection of bison may be a result of comingling of species within the ranchland or sharing water sources. Alternatively, infection may have occurred by placing bison on land that previously housed infected cattle because it is well known that *M. avium* subsp. *paratuberculosis* can survive for protracted periods up to 1 yr in the soil.

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