Immunological and Molecular Characterization of Susceptibility in Relationship to Bacterial Strain Differences in *Mycobacterium avium* subsp. *paratuberculosis* Infection in the Red Deer (*Cervus elaphus*)

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Johne’s disease (JD) infection, caused by *Mycobacterium avium* subsp. *paratuberculosis*, represents a major disease problem in farmed ruminants. Although JD has been well characterized in cattle and sheep, little is known of the infection dynamics or immunological response in deer. In this study, typing of *M. avium* subsp. *paratuberculosis* isolates from intestinal lymphatic tissues from 74 JD-infected animals showed that clinical isolates of *M. avium* subsp. *paratuberculosis* from New Zealand farmed red deer were exclusively of the bovine strain genotype. The susceptibility of deer to *M. avium* subsp. *paratuberculosis* was further investigated by experimental oral-route infection studies using defined isolates of virulent bovine and ovine *M. avium* subsp. *paratuberculosis* strains. Oral inoculation with high (10⁹ CFU/animal) or medium (10⁷ CFU/animal) doses of the bovine strain of *M. avium* subsp. *paratuberculosis* established 100% infection rates, compared to 69% infection following inoculation with a medium dose of the ovine strain. The high susceptibility of deer to the bovine strain of *M. avium* subsp. *paratuberculosis* was confirmed by a 50% infection rate following experimental inoculation with a low dose of bacteria (10⁵ CFU/animal). This study is the first to report experimental *M. avium* subsp. *paratuberculosis* infection in red deer, and it outlines the strong infectivity of bovine-strain *M. avium* subsp. *paratuberculosis* isolates for cervines.

Paratuberculosis, or Johne’s disease (JD), caused by *Mycobacterium avium* subsp. *paratuberculosis*, represents a mycobacterial disease of major importance to the livestock farming industry after the elimination of bovine tuberculosis. The economic impact of JD on sheep and cattle farming has been recognized for some time (17, 24), leading to major efforts towards the development of improved means of management and control of the disease in these domesticated ruminant species. Recently, JD has also been identified as an emerging problem in deer farming (20, 22).

In deer, as in cattle and sheep, JD presents as a chronic inflammatory disease of the lower intestinal tract, which can lead to loss of condition due to impaired gut digestive and absorptive functions. In severe cases, mortality can occur in clinically affected adult animals. However, in contrast to the case for cattle and sheep, JD may often be diagnosed in yearlings and can cause death in deer less than 1 year old (21). Primary diagnosis of JD in farmed deer is based on the detection of *M. avium* subsp. *paratuberculosis* shed in feces, milk, or semen or on postmortem examination of affected gastrointestinal tract tissues, such as epithelial and subepithelial tissues of the small intestine, especially the lower part of the jejunum, ileum, and ileocecal junction region and its associated draining lymph nodes (2). However, improved and more precise in vivo immunodiagnostics tests are currently being developed for the early identification of *M. avium* subsp. *paratuberculosis* infection in deer (16). Furthermore, preliminary studies on the feasibility of prophylactic vaccination against JD in deer have been undertaken (23).

The emerging problem of JD in farmed deer is underscored by the fact that little is known about *M. avium* subsp. *paratuberculosis* infection dynamics in this species. In particular, little is known about the pattern of immunological reactivity in *M. avium* subsp. *paratuberculosis*-infected deer that would be relevant to the development of improved immunodiagnosics or vaccine disease management tools. In other *M. avium* subsp. *paratuberculosis*-susceptible ruminant species, such as cattle, controlled experimental infection studies using defined isolates of *M. avium* subsp. *paratuberculosis* have identified both cell-mediated and humoral immune reactivity (32). *M. avium* subsp. *paratuberculosis*-specific peripheral blood CD4⁺ lymphocytes capable of secreting gamma interferon (IFN-γ) were activated, along with the production of immunoglobulin G (IgG) class antibodies specific for mycobacterial surface glycolipids. Such responses have been reported to become identifiable at approximately 20 weeks after experimental infection in cattle, emphasizing the chronic subclinical nature of the disease (32). In contrast, nothing is known of the kinetics of immune reactivity following controlled *M. avium* subsp. *paratuberculosis* infection in deer, nor are the patterns of cellular and humoral immunological reactivity well defined.

Recent advances in molecular typing have facilitated the identification of different *M. avium* subsp. *paratuberculosis* isolates. Through the use of IS900 restriction fragment length polymorphism (RFLP) and/or IS1311 PCR-restriction enzyme

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were experimentally infected orally with defined numbers of *M. avium* subsp. *paratuberculosis* organisms obtained from homogenized gut lymphatic tissues (4) as follows: high-dose bovine-strain group (n = 16), 10^7 CFU strain 614; medium-dose bovine-strain group (n = 16), 10^6 CFU strain 564; medium-dose ovine-strain group (n = 16), 10^5 CFU strain JD3; and low-dose bovine-strain group (n = 16), 10^3 CFU strain 564. In each case, infection inocula were administered as four one-quarter doses of the total challenge dose, given on each of four sequential days.

Animals were subsequently maintained on open pasture in separate paddocks. In addition, a fifth group of animals (n = 17) was used as a sentinel control group; these animals were not inoculated with *M. avium* subsp. *paratuberculosis* but were maintained in the same paddock as the deer inoculated with the high-dose bovine strain for the duration of the study.

At intervals of 6, 12, 18, 24, 30, 36, and 44 weeks postinoculation, 20-ml heparinized blood samples were drawn from manually restrained animals by jugular venipuncture into evacuated tubes. At 44 weeks, all animals were euthanized humanely using a captive bolt stun gun. Following exsanguinations, the intestines were removed from below the abomasum through to the rectum. Samples were taken from serial sections of the ICLN and ICV. Tissues were examined first macroscopically and then histologically to determine the grade of pathology at a microscopic level. Histological lesions were graded on a scale of 0 to 3 using the Perez classification (29) but without identifying the subtypes within each pathology grade. Based on the gross lesions and histopathological grading, animals were assigned a numerical disease score on a grading scale of 0 to 3 (0, no pathology; 3, severe pathology) to categorize the pathology found in individual animals. Additionally, samples of ICLN and ICV were homogenized and aliquots plated onto Middlebrook 7H11 agar for the bacteriological culture identification of *M. avium* subsp. *paratuberculosis*.

**Molecular typing of *M. avium* subsp. *paratuberculosis* strains.** Seventy-four isolates of *M. avium* subsp. *paratuberculosis*, from 10 different properties, were cultured from cervine intestinal (mostly ileo-cecal) lymph nodes. Approximately 1 g of lymph node tissue was homogenized in sterile, distilled water using a pestle and mortar and examined macroscopically for acid-fast organisms by Ziehl-Neelsen staining. Of this homogenate, 250 μl was cultured onto Middlebrook 7H11 agar (Difco) with and without Mycobactin J (Allied Medical, 0.1 μg/ml) and in a third vial containing Mycobactin J, vancomycin (50 μg/ml), nalidixic acid (50 μg/ml), and amphotericin B (50 μg/ml). Vials were incubated for up to 20 weeks at 37°C in a 5% CO2 atmosphere. Growth was checked microscopically by Ziehl-Neelsen staining. Prior to PCR analysis, isolates were heat killed at 90°C for 1 h.

DNA was extracted from mycobacterial pellets for PCR typing by standard methods (5, 7). Extracted samples were resuspended in 50 μl of sterile, distilled water. PCR to detect IS900 was performed with the primers P90 (5'-GAA GGG TGT TCG GGG CGG CCG TGT CCT AGG) and P91 (5'-GTC GGC TCT GTG GTG AA) and M94 (5'-H11032-GAA GGG TCT GCA TCG TCG ACA GTG TG) and in a third vial containing Mycobactin J, vancomycin (50 μg/ml), nalidixic acid (50 μg/ml), and amphotericin B (50 μg/ml). Vials were incubated for up to 20 weeks at 37°C in a 5% CO2 atmosphere. Growth was checked microscopically by Ziehl-Neelsen staining. Prior to PCR analysis, isolates were heat killed at 90°C for 1 h.

**Isolation and preparation of *M. avium* subsp. *paratuberculosis* for experimental infection.** Two inocula were prepared directly from lymph nodes of a clinically affected merino sheep (no. JD3) (4) and a clinically affected red deer (no. 564). These clinically diseased animals were euthanized, and in addition to the lymph nodes taken to harvest *M. avium subsp. paratuberculosis* organisms, fresh and fixed samples were taken for culture, histopathological examination, IS900 PCR, and IS1311 PCR-REA to confirm the diagnosis and identify the strains. The JD3 strain was confirmed as an “ovine” strain, and the 564 strain was confirmed as a “bovine” strain. An estimate of the number of organisms present in each tissue homogenate was made by microscopic counting under phase contrast prior to dosing the animals. CFU of bacteria were confirmed retrospectively by plate culture. There were consistently low levels of bacterial contamination when *M. avium subsp. paratuberculosis* was obtained directly from lymphatic tissues, recovered positively from animals at necropsy. These two strains of *M. avium subsp. paratuberculosis* (JD3 and 564) were used to experimentally challenge deer by the oral route in this study.

**Experimental infection, longitudinal blood monitoring, and necropsy.** Eighty-one deer were randomly assigned to one of five groups. Four of these groups

**MATERIALS AND METHODS**

**Ethical approvals.** The animal experiments carried out in this study were approved by the Invermay AgResearch Animal Ethics Committee (INV607/03).

**Farming set and collection of field samples.** A total of 74 infected red deer (*Cervus elaphus*) originating from 10 farms throughout the South Island of New Zealand were subjected to gross pathological examination at necropsy. These farms were noncontiguous, and some were separated by more than 400 km. None of the farms involved shared property boundaries. Samples of the jejunal lymph nodes and the ileocecal lymph node (ICLN), as well as tissue sections of jejunum, ileum, and ileocecal valve (ICV), were taken for bacteriological culture and histological examinations.

**Experimental animals.** A group of 81 newly weaned 4-month-old red deer (average weight at commencement of study, 50 kg; range, 41 to 60 kg) were obtained from the AgResearch Invermay Deer Farm, which had a (average weight at commencement of study, 50 kg; range, 41 to 60 kg) were obtained from the AgResearch Invermay Deer Farm, which had a

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Serological tests for anti-*M. avium* subsp. *paratuberculosis* IgG1 antibodies. A standard enzyme-linked immunosorbent assay (ELISA) protocol (11) with modifications for use in deer (13) was used in this study, with variations in antigens and antibodies used as stated. Ninety-six-well microtiter plates (Maxisorp; Nunc Products, Denmark) were coated with 50 μl of either of the following two target antigens at a final concentration of 5 μg/ml in carbonate buffer, pH 9.6: *paratuberculosis* protoplastic antigen (PpAg) (obtained from Allied Monitor Inc., Fayette, MO) or purified protein derivative of *M. paratuberculosis* (PPDp) (obtained from CIDC Lelystad, The Netherlands). After incubation overnight at 4°C, unbound antigen was removed from the plates by washing six times. Antibody binding was visualized using a polyclonal goat anti-

 primerase derivative of [3H]thymidine as described previously (13). Beta emission was measured as mean counts per minute using a liquid scintillation counter.

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<th>Tissue</th>
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*Total number of animals with infection in ICN and/or IVC.

Significantly lower incidence of culture-confirmed infection in deer inoculated with the low dose of the bovine strain of *M. avium* subsp. *paratuberculosis* (*P* = 0.02) or with the medium dose of the ovine strain of *M. avium* subsp. *paratuberculosis* (*P* = 0.043) compared to animals inoculated with either the high or medium dose of the bovine strain (Fisher's exact test).

RESULTS

Infection dynamics: experimental infection studies with deer. At postmortem, pathological and histological investigation identified that 59 out of 81 deer had pathology consistent with *M. avium* subsp. *paratuberculosis* infection, which was subsequently confirmed as positive by culture. Twelve of 81 animals were culture positive in the absence of identifiable pathology, and 10 of 81 animals remained noninfected and nondiseased. Among the different infection subgroups, viable *M. avium* subsp. *paratuberculosis* organisms were isolated from the intestinal tissues of 16/16 of animals infected with either the high or medium dose of the bovine strain and from 8/16 animals infected with the low-dose of the bovine strain (Table 1). All 17 of the in-contact sentinel were also confirmed to be culture positive for *M. avium* subsp. *paratuberculosis*. Among the animals inoculated with the ovine strain of *M. avium* subsp. *paratuberculosis*, 11/16 were culture positive at necropsy.

Longitudinal patterns of immune reactivity. In deer that were experimentally infected with *M. avium* subsp. *paratuberculosis*, antigen-specific LT and IFN-γ responses were first apparent at 12 weeks postinfection (Fig. 1). Overall CMI responses were highest among deer that received the high- or medium-dose inocula of the bovine strain of *M. avium* subsp. *paratuberculosis*. LT responses in both the groups receiving both the high and medium doses of the bovine strain peaked at 18 weeks and then declined; IFN-γ responses in the medium-dose bovine-strain group followed a similar pattern, while IFN-γ responses in the high-dose bovine-strain group rose progressively until week 36 postinfection. Deer infected with the low dose of the bovine strain of *M. avium* subsp. *paratuberculosis* showed a lesser degree of CMI reactivity, with LT responses first apparent at 12 weeks but IFN-γ responses not apparent until week 30 postinfection. The deer which were maintained in contact with the group infected with the high dose of the bovine strain of *M. avium* subsp. *paratuberculosis* showed a pattern of immune reactivity similar to that of the animals infected with the low dose of the bovine strain of *M. avium* subsp. *paratuberculosis*, with LT responses first apparent at 12 weeks but IFN-γ responses not apparent until week 30 postinfection. Deer infected with the ovine strain of *M. avium* subsp. *paratuberculosis* showed a pattern of immune reactivity similar to that of the animals infected with the low dose of the bovine strain of *M. avium* subsp. *paratuberculosis* compared to animals infected with either the high or medium dose of the bovine strain (Fisher's exact test).
36 and then rose to a magnitude similar to that observed in the animals infected with the medium dose of the bovine strain. Deer infected with the ovine strain of *M. avium* subsp. *paratuberculosis* remained serologically nonreactive throughout the study.

**Immunological responses in relation to disease severity.** In order to fully understand how immunological responses relate to disease status, a further analysis was undertaken by first categorizing animals according to disease severity and then comparing several immune parameters across these groupings. Using these criteria, deer presenting with severe pathology constituted 16% of the animals (13/81); medium-grade pathology, 14% (11/81); low-grade pathology, 43% (35/81); infected and no pathology/culture positive, 15% (12/81); and uninfected, no pathology/culture negative, 12% (10/81).

LT responses were largely independent of disease severity (Fig. 3) whereas IFN-γ responses showed a clear relationship to disease severity; animals exhibiting severe pathology had the strongest IFN-γ secretion, and IFN-γ responses generally lowered with declining disease severity. Serological reactivity followed a similar (but more exaggerated) pattern as cytokine secretion, with IgG1 antibody responses to both PpAg and PPD-*j* strongly evident in deer exhibiting severe pathology and decreasing incrementally in association with decreasing severity of disease. Chronologically, increased LT responses were first evident among *M. avium* subsp. *paratuberculosis*-infected deer (all disease severity groups) at 12 weeks postinfection, while IFN-γ secretion and IgG1 antibody responses were delayed, becoming first apparent only at 18 weeks (and only in the high- and medium-pathology groupings at this time point) (Fig. 3). Uninfected animals had low levels of CMI, with LT reactivity at 12 to 18 and 42 weeks postchallenge and low levels of IFN-γ at 38 to 42 weeks.

**DISCUSSION**

The present study has identified that red deer are particularly susceptible to infection with strains of *M. avium* subsp. *paratuberculosis* that can be identified by IS1311 PCR-REA as having the bovine genotype. Further, the ensuing patterns of immunological reactivity were most vigorous and diverse (CMI and humoral reactivity) following experimental infection with a defined virulent bovine strain of *M. avium* subsp. *paratuberculosis*. A total of 74 tissue samples, derived from New Zealand farmed deer with JD lesions and culture-confirmed *M. avium* subsp. *paratuberculosis* infection, were typed by IS1311 PCR-REA analysis. All 74 isolates proved to be bovine strains of *M. avium* subsp. *paratuberculosis* based on the identification pattern of three bovine-strain-specific fragments in Hinf1-digested products (data not shown). These results suggest that on-farm disease in deer, at least in New Zealand, is predominantly due
to cross-infection with *M. avium* subsp. *paratuberculosis* isolates of bovine strain specificity.

While it has been suggested previously that deer might be equally susceptible to both bovine and ovine strains of *M. avium* subsp. *paratuberculosis* (6, 8, 9), there have been few independent verifications of this, and reviews commonly cite an instance from 1993 in which three cervine-derived isolates exhibited IS900 RFLP banding patterns similar to those generated by ovine-strain isolates in New Zealand (9). Whittington et al. summarized some documented occurrences in ruminants of strains of *M. avium* subsp. *paratuberculosis* as determined by IS900 RFLP analysis (34). Of 33 cervine-derived *M. avium* subsp. *paratuberculosis* isolates described (20 from New Zealand [9], 11 from South America [26], and 2 from Denmark [31]), only 3 had been reported to exhibit RFLP banding patterns similar to those of ovine strains of *M. avium* subsp. *paratuberculosis*. In the same report Whittington et al. confirmed that in a comparison of 297 *M. avium* subsp. *paratuberculosis* isolates which were strain typed by both IS900 RFLP and IS1311 PCR-REA there was complete concordance between these two typing approaches with respect to ovine/bovine genotype (34). Additionally, Collins et al. have reported complete agreement between RFLP typing and an alternative, also PCR-based, typing method (6). Pavlik et al. (28) and Machackova et al. (20) have strain typed more than 110 isolates of *M. avium* subsp. *paratuberculosis* from wild cervids, including red (*Cervus elaphus*), fallow (*Dama dama*), and roe (*Capreolus capreolus*) deer, by IS900 RFLP analysis. All cervine isolates from red, fallow, and roe deer resembled bovine-type *M. avium* subsp. *paratuberculosis* RFLP patterns except for a single isolate from a fallow deer which returned an “intermediate” strain type (20). In separate studies, 40 *M. avium* subsp. *paratuberculosis* isolates from a herd of farmed red deer in the Czech Republic also demonstrated bovine-strain banding patterns (21), as did a single fallow deer isolate of *M. avium* subsp. *paratuberculosis* from Spain (1) and three *M. avium* subsp. *paratuberculosis* isolates recovered from red deer in Italy (27). Thus, while deer may become infected with ovine strains of *M. avium* subsp. *paratuberculosis* (6, 9), this appears to be the exception rather than the rule. This is especially striking for New Zealand deer farms, where many properties have mixed animal grazing systems involving deer and sheep and many sheep properties have endemic ovine-strain *M. avium* subsp. *paratuberculosis* in their flocks.

Based on the findings obtained following typing of deer isolates of *M. avium* subsp. *paratuberculosis*, a controlled infection experiment was set up to specifically address the issue of the comparative susceptibilities and immune reactivities of red deer to bovine or ovine strains of *M. avium* subsp. *paratuberculosis*. In common with previous reports of experimental *M. avium* subsp. *paratuberculosis* infection in cattle (32), the data obtained showed that deer infected with bovine strains of...
M. avium subsp. paratuberculosis developed vigorous M. avium subsp. paratuberculosis-specific CMI responses which were first evident at 12 weeks postinfection. The onset of peripheral blood immune reactivity in deer is more rapid than that reported for cattle (32), and the course of clinical JD in deer has been reported to be more rapid than that observed in cattle (22), together suggesting that M. avium subsp. paratuberculosis infection in deer progresses more acutely than the equivalent disease in cattle.

In the present study, not only were deer susceptible to infection with high \((10^9 \text{ CFU/animal})\) or medium \((10^7 \text{ CFU/animal})\) doses of the bovine strain of M. avium subsp. paratuberculosis, but 50% became infected when the challenge inoculum was reduced to \(10^3 \text{ CFU/animal}\) (low dose). In contrast, deer inoculated with \(10^7 \text{ CFU/animal}\) of the ovine strain of M. avium subsp. paratuberculosis had a 69% infection rate. These results together suggest greater susceptibility of deer to establishment of infection with a bovine strain of the pathogen. CMI (LT and IFN-\(\gamma\)) and antibody responses were vigorous in deer following infection with high or medium doses of the bovine strain of M. avium subsp. paratuberculosis, while the equivalent (medium-dose) ovine strain of M. avium subsp. paratuberculosis only induced noticeable LT reactivity, with little observable IFN-\(\gamma\) induction or antibody production. Mixed CMI and antibody responses characterize disease caused by M. avium subsp. paratuberculosis in cattle (30). A limited LT response in the absence of IFN-\(\gamma\) and antibody (as observed in deer inoculated with sheep strains of M. avium subsp. paratuberculosis) might instead represent the development of protective immunity, possibly involving Th1-type pathways of immune reactivity.

The immunological reactivity of deer to the bovine strain of M. avium subsp. paratuberculosis was dose dependent, with both CMI and antibody responses strongest in deer infected with the highest dose \((10^9 \text{ CFU/animal})\). Both CMI and antibody responses appeared to peak and then decline throughout the course of infection, but with different kinetics; CMI responses generally peaked at 12 to 18 weeks and then declined, while antibody responses peaked at 24 weeks. The early onset of CMI reactivity followed by seroconversion is a noted feature of chronic mycobacterial infection in ruminants, especially deer (12, 14), and has been noted specifically for M. avium subsp. paratuberculosis infection in cattle (3) and sheep (30). Khalifeh and Stabel have previously described a decline in the M. avium subsp. paratuberculosis-specific CMI response among chronically infected cattle, coincident with progression from subclinical to clinical disease (18). In sheep, Kurade et al. reported a decline in CMI reactivity concomitant with a rise in serological reactivity at approximately 20 weeks postinfection in animals experimentally infected with M. avium subsp. paratuberculosis, suggesting a change in the predominant immune phenotype around this time (19). Similarly, we have described
that peripheral blood LT responses in *M. avium* subsp. *paratuberculosis*-inoculated sheep remain low for the first 6 months and then increase beyond 8 months in line with increased IgG1 antibody production and sharply decreased *M. avium* subsp. *paratuberculosis*-specific IFN-γ production (4).

Control of JD in farmed ruminants is dependent on detection and removal of subclinical disease in infected animals. We have recently developed a serological screening tool which detects subclinical JD infection in deer (16). In the present study the pattern of immunological reactivity in deer experimentally infected with *M. avium* subsp. *paratuberculosis* was analyzed in the context of the clinical outcome following experimental infection. LT responses were shown to be common to all *M. avium* subsp. *paratuberculosis*-exposed deer, regardless of disease outcome at necropsy. While LT appears to have limited value as a stand-alone test for the diagnosis of active *M. avium* subsp. *paratuberculosis* infection in deer, it is possible that LT combined with other components of the immunological response could form the basis of a composite immunodiagnostic test, similar to the one used for the diagnosis of *M. bovis* infection in farmed deer in New Zealand (13, 15).

In contrast to LT, both IFN-γ production and IgG1 anti-*M. avium* subsp. *paratuberculosis* antibody reactivity were shown to correlate with disease severity, with responses strongest in the high- and medium-grade pathology groups. Serological reactivity to *M. avium* subsp. *paratuberculosis* surface glycolipids has been shown previously to be a feature of active *M. avium* subsp. *paratuberculosis* infection in cattle (32), and the IgG1 response to PPD and protoplastic antigens (PpAg) shown here correlates with the onset of disease and clinical pathology in deer. IgG1 serological reactivity to PpAg appears to be capable of differentiating between lesion negative/infected animals and lesion negative/uninfected animals at time points between 24 and 44 weeks postinfection, identifying subclinically affected animals as shown previously in naturally infected deer.

In summary, we have reported here that isolates of *M. avium* subsp. *paratuberculosis* from clinical cases of JD in New Zealand farmed deer were exclusively of the bovine strain genotype; moreover, deer experimentally challenged with the bovine strain of *M. avium* subsp. *paratuberculosis* more readily established infection and developed a more pronounced immunological response than animals inoculated with the ovine strain of *M. avium* subsp. *paratuberculosis*. While the ovine strain of *M. avium* subsp. *paratuberculosis* is still infectious to red deer, the susceptibility of deer to this strain appears to be lower, and the repertoire of immunological reactivity as a consequence of infection is more limited. The bovine strain of *M. avium* subsp. *paratuberculosis* used in the current study was isolated from infected deer lymphatic tissue, and the ovine strain was isolated directly from sheep. Consequently, it is debatable whether the increased virulence was due to host modification of the bacterial phenotype or to intrinsic genotypic virulence of the strain. In practical terms, it is likely that bovine strains of *M. avium* subsp. *paratuberculosis* will be of more economic relevance to the commercial farming of red deer, and the range and magnitude of immune reactivity suggest that it may be possible to develop improved diagnostic screening tools for bovine strains of *M. avium* subsp. *paratuberculosis* infection and JD in deer. In contrast, infection with ovine strains of *M. avium* subsp. *paratuberculosis* in deer is more insidious and appears to be less amenable to immunodiagnosis.

Results from the current study suggest that farmed deer may represent an important natural reservoir of *M. avium* subsp. *paratuberculosis* infection which poses a threat to other domesticated livestock and wildlife.

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