J. Comp. Path. 2014, Vol. 151, 410–419

Available online at www.sciencedirect.com

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EXPERIMENTALLY INDUCED DISEASE

Virulence of Two Strains of *Mycobacterium bovis* in Cattle Following Aerosol Infection

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Summary

Over the past two decades, highly virulent strains of *Mycobacterium tuberculosis* have emerged and spread rapidly in man, suggesting a selective advantage based on virulence. A similar scenario has not been described for *Mycobacterium bovis* infection in cattle (i.e. bovine tuberculosis). An epidemiological investigation of a recent outbreak of bovine tuberculosis in a USA dairy indicated that the causative strain of *M. bovis* (strain 10-7428) was particularly virulent, with rapid spread within the herd. In the present study, the virulence of this strain (10-7428) was directly compared in the target host with a well-characterized strain (95-1315) of relevance to the USA bovine tuberculosis eradication programme. Aerosol inoculation of 10^4 colony forming units of *M. bovis* 95-1315 ($n = 8$) or 10-7428 ($n = 8$) resulted in a similar distribution and severity of gross and microscopical lesions of tuberculosis as well as mycobacterial colonization, primarily affecting the lungs and lung-associated lymph nodes. Specific cell-mediated and antibody responses, including kinetics of the response, as well as antigen recognition profiles, were also comparable between the two treatment groups. Present findings demonstrate that *M. bovis* strains 95-1315 and 10-7428 have similar virulence when administered to cattle via aerosol inoculation. Other factors such as livestock management practices likely affected the severity of the outbreak in the dairy.

Published by Elsevier Ltd.

Keywords: bovine tuberculosis; immunity; *Mycobacterium bovis*; virulence

Introduction

Mycobacterium bovis, a member of the *Mycobacterium tuberculosis* complex, has a wide host range compared with other species in this disease complex. The organism is infectious to man and is the species most often isolated from tuberculous cattle. In 1917, the United States Department of Agriculture (USDA) initiated a federal bovine tuberculosis eradication programme with significant contributions from state and local authorities as well as producer groups (Palmer and Waters, 2011). Over the past century, the programme has relied primarily on slaughter inspection to identify bovine tuberculosis-affected herds, as well as test and cull strategies or isolation of infected cattle (i.e. the 'Bang method') and movement/border testing policies for the control of bovine tuberculosis. Consequently, disease prevalence in the USA has diminished dramatically from an estimated individual animal prevalence of 5% in 1917 to an approximate herd prevalence of <0.0001% in 2011 (Palmer and Waters, 2011; K. Orloski, USDA Animal and Plant Health Inspection Services [APHIS], Veterinary Services, personal communication). Complete eradication of bovine tuberculosis within the USA, however, may be difficult due to ongoing importation of tuberculous cattle from Mexico (McCluskey *et al.*, 2014), spillover from a wildlife reservoir host (i.e.

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0021-9975/$ - see front matter
http://dx.doi.org/10.1016/j.jcpa.2014.08.007

white-tailed deer, *Odocoileus virginianus*) in Michigan (Schmitt *et al.*, 1997), continued detection of *M. bovis* in captive cervids with transmission to cattle (Waters *et al.*, 2011b) and infrequent interherd spread of the disease within the cattle population in the USA (Miller and Sweeney, 2013).

The prevalence of bovine tuberculosis in England and Wales has been steadily increasing since 1979, despite compulsory testing and an active control campaign (Abernethy *et al.*, 2013). Approximately 10% of English cattle herds were under movement restrictions due to tuberculosis in 2010, with ~25,000 cattle slaughtered at a cost of £91 million (Defra Publications, 2011; Anonymous, 2013). The steady rise in prevalence in Great Britain has been attributed to the failure of the current tuberculin test and slaughter strategy and the presence of a wildlife reservoir (i.e. the Eurasian badger, *Meles meles*) (Defra Publications, 2011; Karolemeas *et al.*, 2011). Bovine tuberculosis also persists, although generally at low levels, within other European Union countries, despite concerted control efforts (Reviriego Gordejo and Vermeersch, 2006). In New Zealand, the presence of another wildlife reservoir (i.e. the brushtail possum, *Trichosurus vulpecula*) seriously hinders bovine tuberculosis control efforts (Ryan *et al.*, 2006). Thus, bovine tuberculosis remains an important animal health problem in developed countries and new tools for control of the disease in wildlife and/or domestic livestock, such as vaccines and improved diagnostic tests, are desperately needed.

An epidemiological investigation was recently conducted by USDA APHIS Veterinary Services on a tuberculosis-affected dairy herd in Colorado, USA, as well as on numerous other dairy herds across the USA determined to be at risk due to animal movement from the affected herd following an epidemiological investigation (i.e. including trace-in and trace-out investigations) (Francisco *et al.*, 2014). Several aspects of this particular outbreak were unique when compared with other tuberculosis-affected herds detected within the past 30 years in the USA. The within-herd prevalence was substantially higher (11.1%) than that typically detected within the USA (average over the past decade is <2%) and ante-mortem test response rates were also particularly high (i.e. 32% for caudal fold test [CFT], 18% for CFT followed by comparative cervical test [CCT] in series and 21% for CFT followed by interferon [IFN]-γ release assay in series) when compared with typical response rates (Francisco *et al.*, 2014). Additionally, numerous calves were infected and at least one cow had supramammary lymph node lesions. One year prior to the outbreak, all adult cattle >2 years of age within this herd were screened for bovine tuberculosis and all were negative. Together, these findings indicate that *M. bovis* infection spread rapidly within the herd. Genotyping indicated that infected animals were colonized with an *M. bovis* strain unique to other currently defined strains within the USA. The USDA APHIS National Veterinary Services Laboratories' designation for this isolate was *M. bovis* 10-7428.

For cattle infection trials involving experimental challenge to evaluate candidate vaccines and diagnostic strategies, it is critical to select strains of virulence representative of relevant field isolates as well as of geographical and epidemiological significance. Strain virulence significantly influences the outcome of pathogenesis, diagnostic and vaccine efficacy studies with *M. tuberculosis* (Gagneux and Small, 2007; Kato-Maeda *et al.*, 2012; Via *et al.*, 2013); however, few studies have been performed to directly compare the virulence of geographically relevant strains of *M. bovis* in cattle. In one such study, Palmer *et al.* (2002) demonstrated that *M. bovis* 95-1315 has a similar level of virulence in cattle compared with *M. bovis* HC2005T (an isolate obtained from the tracheobronchial lymph node from a dairy cow in Texas, USA). Thus, 95-1315 was selected as a relevant strain for direct comparison with 10-7428 for virulence in cattle. The comparator strain, 95-1315, was isolated from a white-tailed deer in Michigan, USA, in 1995 (Schmitt *et al.*, 1997) and has been used in numerous experimental infection trials (Palmer *et al.*, 2002) and is endemic in Michigan (north east corner of the lower peninsula) white-tailed deer and cattle (i.e. in deer and cattle in the same area). Therefore, the objective of the current study was to compare the recently recognized *M. bovis* 10-7428 (a strain of particular interest to regulatory officials given its perceived high virulence in the field) with a recognized virulent strain (*M. bovis* 95-1315) to specifically address the question of whether a highly virulent strain had emerged within the USA.

Materials and Methods

Mycobacterium bovis *Aerosol Challenge*

Two strains of *M. bovis* were used for challenge inoculum: 95-1315 (Schmitt *et al.*, 1997) and 10-7428 (Francisco *et al.*, 2014; Colorado dairy isolate). Low passage (≤3) cultures of both strains were prepared using standard techniques (Larsen *et al.*, 2007) in Middlebrook 7H9 liquid medium (Becton Dickinson, Franklin Lakes, New Jersey, USA) supplemented with 10% oleic acid–albumin–dextrose complex (OADC) plus 0.05% Tween 80. Holstein steers