Recent advances in brucellosis

The United Kingdom was declared officially free of bovine brucellosis caused by *Brucella abortus*, the only indigenous form of the disease, in 1981. Since then, the disease has also been eradicated from Australia, Canada and New Zealand and is now uncommon in most European Countries and the USA. On the other hand, with the relaxation of state control the disease is increasing in several countries of the former USSR in which it was previously well controlled. *B. melitensis* infection is still widespread around the Mediterranean littoral and has recently undergone a resurgence in Malta. It has also established itself as a major problem in cattle in Israel and countries of the Arabian peninsula, in which it was formerly confined to sheep and goats.

Transmission to man still occurs through consumption of unpasteurised dairy produce, direct contact with infected animals, or indirectly by environmental exposure. Human cases are occasionally imported into the UK and other countries that are free of indigenous infection. Diagnosis is not difficult if the level of suspicion is high and the presentation is typical, but the varied and sometimes misleading manifestations of localised, sub-acute or chronic infection, mean that cases may be misdiagnosed [1].

Although *B. melitensis* accounts for most recorded cases, *B. abortus* and *B. suis* cause substantial morbidity in countries in which they persist in domestic animals, notably in Asia and Latin America. *B. canis* rarely causes overt human disease, and *B. neotomae* and *B. ovis* have not been identified as causes of disease in man. Anecdotal evidence suggests that strains of *Brucella* species that have been isolated from marine animals in the UK and USA [2, 3] may be pathogenic for man.

Molecular genetics

The average size of the genome is $2.37 \times 10^3$ MDa, with a DNA G + C content of 58–59 mol % All types show > 95% homology in DNA–DNA pairing studies, justifying the nomination of *Brucella* as a monospecific genus [4]. However, the suggestion that all types should be regarded as biovars of *B. melitensis* has met with resistance on practical grounds and the old nomenclature has been retained. Seven biovars are recognised for *B. abortus* (1–6 and 9), three for *B. melitensis* (1–3), and five for *B. suis* (1–5) [5]. The other species have not been differentiated into biovars although variants exist. The strains isolated from marine animals form a separate group and should be designated as a new nomenclature.

Restriction fragment patterns produced by infrequently cutting endonucleases support the differentiation of the nomen species [6]. Restriction endonuclease analysis has generally been unsuccessful for strain differentiation, but amplification and sequencing of selected genes has provided evidence of polymorphism in a number of genes including *omp* 2, *dnaK*, *htr* and *ery* (the erythulose-1-phosphate dehydrogenase gene) [7–9]. The *omp* 2 gene is believed to determine dye sensitivity, one of the traditional typing methods for bovar differentiation. Its polymorphism and the capacity for post-translational modification of its product may explain the tendency for variation in dye sensitivity patterns even within nomen species. A 7.2-kb deletion in the *ery* gene in *B. abortus* strain 19 [9] may explain the erythritol sensitivity of this strain which is a major factor in its attenuation.

The genome of *Brucella* contains two chromosomes of 2.1 and 1.5 Mb, respectively. Both replicons encode essential metabolic and replicative functions and hence are chromosomes, not plasmids [10, 11]. Natural plasmids have not been detected in *Brucella*, although transformation has been effected by wide host range plasmids following conjugative transfer or electroporation [12].

Antigenic structure

The major surface antigen of smooth phase strains is a lipopolysaccharide (S-LPS) with a lipid A containing two types of aminoglycose; distinctive fatty acids, but excluding β-hydroxymyristic acid; a core region containing glucose, mannose and quinovosamine; and an O chain comprising a homopolymer of c. 100 residues of 4-formamido-4,6-dideoxymannose which are linked predominantly α-1,2 in A epitope dominant strains, but with every fifth residue linked α-1,3 in M dominant strains. Types that express both epitopes to a dominant strains. Types that express both epitopes to a similar extent produce both LPS types [13]. The presence of 4-amino,4,6-dideoxymannose in the LPS is also responsible for the antigenic cross-reactivity between *Escherichia hermanni*, *E. coli* O157, *Salmo-
nella O30, Vibrio cholerae O1, and Yersinia enterocolitica O9 LPS [13].

Numerous protein antigens have been characterised. Some are recognised by the immune system during the course of infection and are potentially useful in diagnostic tests. The ribosomal proteins L7/L12 stimulate cell-mediated responses and elicit delayed hypersensitivity responses as components of brucellins [14]. They seem to be important in the induction of protective immunity [15].

Mechanisms of pathogenicity

The S-LPS is a major determinant of virulence and dominates the antibody response. It is the main component responsible for conferring incomplete and short-term protection against infection in passive transfer experiments with monoclonal and polyclonal antibodies [16]. The elimination of virulent brucelae depends upon activated macrophages and hence requires development of Th1 type cell-mediated immunity. Brucella LPS is a relatively poor inducer of interferon-γ and tumour necrosis factor-α, both essential for the elimination of the organism [17, 18]. Unusually, it is an effective inducer of interleukin 12, which stimulates Th1 type responses and is closely correlated with interferon-γ production. Other important virulence factors include: the production of adenine and guanine monophosphate which inhibit phagocyte function [19]; Cu-Zn superoxide dismutase which is believed to eliminate reactive oxygen intermediates in phagocytes [20]; and a number of stress-induced proteins which promote survival within macrophages [21].

Diagnosis

The clinical picture is often misleading and the disease may present with gastrointestinal, respiratory, dermal or neurological manifestations as well as those considered more typical of brucellosis [1, 22]. Diagnosis should always be supported by laboratory tests. Blood culture is often effective during the acute phase, but allowance should be made for the relatively slow growth of the organism; the lysis concentration method gives the best results [23]. Presumptive identification is made on the basis of morphological, cultural and serological properties. Reliance should not be placed on commercial gallery-test systems as they may misidentify Brucella spp. as Moraxella phenylpyruvica, with serious consequences [24]. Polymerase chain reaction (PCR) methods with random or selected primers need standardisation and further evaluation, especially for chronic disease [25]. Similarly, antigen detection methods are potentially useful but have not been validated. Enzyme immunoassay (ELISA) is widely used for serological diagnosis. IgA and IgG antibodies seem the most useful indicators of active infection [26, 27]. Western blotting against selected cytoplasmic proteins may allow differentiation of active from past or subclinical infection [28].

Treatment and prevention

The recommended treatment for acute brucellosis in adults is rifampicin 600–900 mg and doxycycline 200 mg daily for a minimum of 6 weeks [29], although the combination of intramuscular streptomycin with an oral tetracycline is claimed to give fewer relapses [30]. There is evidence of antagonism between rifampicin and tetracyclines, but the two regimens give very similar results if adequately prolonged. Quinolones in combination with rifampicin also seem to be effective [31]. Controlled clinical trials with other antibiotics, including new macrolides, have either given inferior results or have been too limited for proper evaluation. Meningo-encephalitis or endocarditis require combination therapy with rifampicin, a tetracycline and an aminoglycoside. Rifampicin is the treatment of choice for uncomplicated disease in children; co-trimoxazole is an alternative but is less effective.

A safe and effective vaccine for use in man is not yet available, but recent work on purE mutants of B. melitensis has given encouraging results in animals and may lead to clinical trials [32]. Prevention is dependent upon the control of the disease in animal hosts, effective heat treatment of dairy produce and hygienic precautions to prevent occupational exposure.

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