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Review

Contributions of biotechnology to the control and prevention of brucellosis in Africa

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Zoonotic diseases such as brucellosis have a major impact on the health and economic prosperity of the developing world. Recent advances in our understanding of brucellosis and new developments in diagnostics and vaccine technology provide unique opportunities for biotechnology companies in developing countries to make an essential contribution to the control of this disease.

Key words: Brucellosis, biotechnology, zoonosis, livestock, public health, diagnostics, vaccine.

BACKGROUND

Health of both human and animal population is pivotal recognised for economic development, prosperity and stability. The burden of infectious diseases affects health and reproductivity of livestock, thereby greatly reducing its value and opportunities for trade. Zoonotic diseases like brucellosis are not only of veterinary importance but may also severely affect human health, contributing to morbidity and reduction of working capacity with concomitant loss of income. Brucellosis has been reported from almost all countries in Africa (Refai, 2002). A recent study identified brucellosis in sub-Saharan Africa as a major priority for control and prevention through its impact on multiple livestock species including cattle, goats, sheep and pigs, its widespread distribution and its debilitating effect on man (Perry et al., 2002).

Brucellosis is prevalent in all major livestock production systems in sub-Saharan Africa, yet its presence often remains unrecognised through lack of awareness by both veterinarians and health care staff and absence of accessible laboratory diagnostic facilities. As a consequence brucellosis remains a largely neglected disease with little attention to control and prevention except in South Africa where a successful control policy has been instigated (McDermott and Arimi, 2002). This is based on vaccination programmes combined with test and slaughter policies (Emslie and Nel, 2002). Preliminary data suggests that the incidence of brucellosis is highest in pastoral production systems where large numbers of animals mix and lowest for confined farms. Bovine brucellosis seems to be more common than ovine brucellosis, however this may be an artifact reflecting the serological testing of livestock species. Much less is known of the prevalence in man and of the effect on human health in this region of the world. Provision of improved diagnostics is crucial to enable such investigations to be undertaken (Muriuki et al., 1997).

BRUCELLOSIS

Brucellosis is one of the most important bacterial zoonosis worldwide (Young, 1995). The aetiological agents are gram-negative coccobacillae belonging to the genus *Brucella. Brucella melitensis, B. abortus* and *B. suis* have small ruminants, cattle, and pigs respectively as their principle hosts. Transmission from infected livestock to man can either be direct through contact with infected material, or indirect through consumption of animal produce. The epidemiology of brucellosis is complex. Important factors that contribute to the

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prevalence and spread in livestock include farming system and practices, farm sanitation, livestock movement, mixing and trading of animals, and sharing of grazing grounds (Kadohiri et al., 1997; Omer et al., 2000; Kabagambe et al., 2001). Further complications arise through wild animal reservoirs which may also carry and transmit the disease (Godfroid, 2002). *Brucella* has a low infectious dose (10 organisms of *B*. *melitensis* are sufficient to cause infection in man), making infection a genuine risk to those occupationally exposed such as farmers, veterinarians and butchers and to the public through the consumption of contaminated unprocessed milk, milk products and meats. Abortion materials characteristically contain high numbers of brucellae and consequently pose significant infection risks if not properly handled and disposed of. Similarly, environmental contamination contributes to further spread among animals. Infected non-pregnant livestock may not demonstrate clinical signs of infection, which together with the complex epidemiology makes the control and prevention of this disease challenging.

In livestock, *Brucella* results in abortion, reduced fertility and weak offspring. In addition, other more specific problems such as hygromas in cattle, or orchitis and spondylitis may be seen in swine. In man, the disease may affect almost any organ and causes a variety of problems, which if not treated early may lead to severe and prolonged disability (Corbell, 1997). Illness caused by *B. melitensis* generally is more prolonged and more severe and debilitating than illness caused by *B. abortus* or *B. suis*.

DIAGNOSIS

Diagnosis of brucellosis however is often difficult to establish, largely through similarity with clinical presentations of other infections prevalent in sub-Saharan Africa such as malaria. Therefore laboratory testing is an absolute prerequisite for a proper diagnosis of human brucellosis and for detection and confirmation of brucellosis in animals. Laboratory diagnosis of brucellosis in animals or man may be achieved either through blood culture or serological testing. Cultivation requires containment level three facilities that are rarely available in developing countries while classical serological tests may give inconsistent results when not performed by experienced staff. Poor reproducibility has been demonstrated with a frequently used serological screening test, the Rose Bengal test (RB), when performed at different study sites (Maichomo et al., 1998). Specificity issues have also plagued the RB test. Consequently, positives should be confirmed in a more specific test such as the serum agglutination test, complement fixation test, or the enzyme linked immunosorbent assay (Omer et al., 2001; Al Dahouk et al., 2003). These assays ideally should be done in a well-equipped laboratory with suitably trained staff. New diagnostic developments such as hand-held polymerase chain reaction machines offer promising new opportunities for the development of both bed-side diagnostics and pen-side tests for brucellosis (Emanuel et al., 2003). New developments in serological test design already have led to new diagnostic tests for human brucellosis (Orduna et al., 2000; Smits et al., 2003). Of these the *Brucella* IgM/IgG flow assay for the serodiagnosis of human brucellosis is specifically designed for user-friendliness and speed (Smits et al., 2003; Irmak et al., 2004), and potentially can be converted to a field test for veterinary use.

DISEASE CONTROL

Although controlled or eradicated in a number of developed countries, re-introduction of brucellosis remains a constant threat, while in others, especially in the developing world, this disease continues to exert its devastating impact perpetuating poverty. Despite tremendous efforts and financial investments, many European Mediterranean countries have yet to eradicate this disease. Many factors, in particular the types of husbandry system, may have contributed to the failure to effectively control the disease in these countries. The re-emergence of brucellosis as a major veterinary and public health problem in the former Soviet Republic during the past decade through a weakening of the veterinary system and transition from large government controlled farms to small-scale private farming, further emphasises the essential role of a continued and co-ordinated control effort. The transmission and spread of brucellosis is affected by a variety of factors and good knowledge of these is essential to the success of a control policy (Reviriego et al., 2000; Bikas et al., 2003; Minas et al., 2004). In general, prevalence of brucellosis usually is higher and control more problematic in pastoral or migratory populations, practiced by a significant proportion of the agricultural population of Africa.

Vaccination of livestock is crucial to the control of brucellosis. Effective reduction of disease prevalence in livestock through mass vaccination eventually will also lead to a reduction of brucellosis in the human population. However, vaccination alone is not sufficient and should be accompanied by other measures such as restriction of animal movement and trade, culling of infected animals and improved farm sanitation to reduce the further spread of disease. In addition, a surveillance system is essential to control the efficacy of control measures and to identify outbreaks at an early stage. Clearly the control of brucellosis requires significant efforts both in terms of human and financial resources and time. In Argentina and other countries in South and Central America, brucellosis has been recognised as a disease problem since the 19th century, but in spite of control efforts starting in Argentina in 1932, the disease still is not considered to be controlled in this country

(Samartino, 2002). Despite the bleak situation outlined above, in resource poor countries control measures provided that they are adapted to the local situation and supported by the local population and instigated together with improved diagnostics, could provide immediate cost-effective benefits (Roth et al., 2003). Demonstration of the cost-effectiveness of control measures is an essential prerequisite to gain acceptance and sustainability of such efforts.

Veterinary vaccines for brucellosis are available for brucellosis in cattle and in small ruminants (Schurig et al., 2002). The attenuated live *B. abortus* S19 vaccine is the recommended vaccine for bovine brucellosis (Nicoletti, 1990). The attenuated live *B. melitensis* Rev-1 vaccine is recommended for goats and sheep (Elberg and Faunce, 1957). These attenuated strains are still smooth and consequently their use results in positive serology that can be confused with naturally infected animals. The live rough strain *B. abortus* 45/20 reverts to virulence in vivo and was subsequently used as a killed vaccine. Protective effect was limited and consequently its use should be avoided. Newly developed vaccines such as the *B. abortus* RB51 vaccine provide promising alternatives but require more extensive field studies and experience to establish its safety and efficacy. A safe and effective vaccine for brucellosis in man does not exist despite considerable efforts.

SUSTAINABLE DISEASE MANAGEMENT

Ideally, effective control of brucellosis should be through a combination of improved diagnosis, vaccination and treatment, together with measures to increase awareness, and improved farm sanitation and food hygiene. Collectively these will increase the effect of control measures and lessen the burden of disease. An integrated disease education and community participation program may assist achievement of this goal. Traditional beliefs and habits may interfere with disease prevention and prohibit its acceptance due to lacunas in disease and health knowledge. Awareness of the cause of this disease and knowledge of measures for prevention and resulting benefits of this can be provided through such a program, creating a positive attitude towards disease prevention. A disease education and community participation program will promote involvement, encourage acceptance thereby increasing the efficacy of control measures. For instance, in the absence of a strong government and means of enforced vaccination, the instigation of other control measures will depend in the voluntary acceptance from livestock owners. They may not be willing, or reluctant to co-operate in the absence of incentives or awareness of health and financial benefits. Disease education will provide information on the benefits of disease control and stimulate community participation. Good knowledge of local factors contributing to the spread and transmission of the disease is vital when evaluating the effectiveness of the disease control measures. This can be obtained through epidemiological investigations and interviewing healthcare workers, veterinarians and risk groups.

Recently McDermott and Arimi (2002) summarised epidemiological findings for brucellosis in sub-Saharan Africa. Brucellosis is common in cattle but less well studied in small ruminants. Bovine brucellosis prevalence rates ranging from 3.3% for the Central African Republic (Nakoune et al., 2004) to as high as 41% for Togo have been reported (Domingo, 2000). Values falling within this range were reported for Chad (Schelling et al., 2003), Sudan (El-Ansary et al., 2001), Eritrea (Omer et al., 2000), Tanzania (Weinhaupl et al., 2000), Burkina Faso (Coulibaly and Yameogo, 2000), Ghana (Turkson and Boadu, 1992; Kubuafor et al., 2000), Mali (Tounkara et al., 1994), Nigeria (Ocholi et al., 1996) and Zimbabwe (Mohan et al., 1996). In goats, a prevalence of 4% has been reported from Sudan (El-Ansary et al., 2001), while in Uganda 2% were positive (Kabagambe et al., 2001). Herd prevalence is usually higher.

Human brucellosis has been poorly studied in Africa. Seroprevalence of 3.8% has been reported in nomadic pastoralists from Chad (Schelling et al., 2003). Occupational contacts, including butchers, slaughterhouse workers, milkers, and cow attendants in one state in eastern Sudan revealed 1% were infected (El-Ansary et al., 2001). In contrast, slaughterhouse workers in Djibouti gave 6.5% positive (Chantal et al., 1996) and high-risk groups from Eritrea showed a seroprevalence between 3.0% and 7.1% (Omer et al., 2002). Studies of febrile patients in a large hospital in Kampala, the capital of Uganda, yielded 13.3% (Mutanda, 1998), while in eastern Nigeria 5.2% were seropositive (Baba et al., 2001).

More detailed investigations have shown that the seroprevalence of brucellosis in cattle is closely related to the husbandry system with greatest risk for dairy cattle associated with mixed-breed herds in the state of Asmara in Eritrea (Omer et al., 2000). Other risks included use of hired caretakers, keeping sheep in addition to goats, free browsing for goat herds in eastern and western Uganda (Kabagambe et al., 2001), and features of pastoral managements such as extensive grazing for cattle herds in Kenya (Kadohira et al., 1997). Factors like nomadism, traditionalism with as an example sharing of males for breeding purpose. Education level and disease knowledge, animal trade and vaccination status have been identified in other studies (Mikolon et al., 1998; Lithg-Pereira et al., 2003). The transmission and the risk of disease in the human population is generally closely related to the presence of brucellosis in livestock, professional engagement with animal raising and food production and sanitairy conditions at the working place or food hygiene and food habits. Risk factors for having brucellosis have been investigated in detail in different

countries (Bikas et al., 2003; Al-Shamahy et al., 2000; Gotuzzo et al., 1987).

Clearly the epidemiological information and our understanding of brucellosis in Africa are growing. Major lacunas in our knowledge are the presence of brucellosis in small ruminants, the significance of human brucellosis and the relative contributions of the various animals species to infection in man. Nevertheless, the available information highlights the urgent need for a control policy to drastically curtail the negative public health and economic effects of this disease. The impact of brucellosis affects both public health and livestock, consequently, effective control is best delivered through a unified approach involving both medics, scientists and veterinarians. Co-ordination of both health scientists and veterinarians is crucial because although brucellosis affects human health and economic prosperity, as a zoonosis, control should target the disease reservoir in animals. Beyond those involved with livestock, those involved in processing animal produce such as milk need to adopt control measures such as pasteurisation. To be effectively achieved control measures will require full co-operation and hence the benefits should be clearly demonstrated and communicated. Collectively, epidemiological information together with demonstration of the cost-effectiveness of brucellosis control, can be used to set priorities and influence policy.

FUTURE DEVELOPMENTS IN DIAGNOSTICS

Biotechnology can make important contributions to the control and prevention of brucellosis. First, there is an urgent need for affordable, rapid (bed-side and pen-side) diagnostics permitting decentralised brucellosis testing. Secondly, there is a need for cheap and well-validated vaccines that do not interfere with diagnostic tests. At present, diagnostic testing is often not performed because expertise and laboratory facilities are not available or laboratory testing is performed, but with considerable delay through requirements to submit samples to a central laboratory with results being available often only after days or even weeks. Diagnostic delays results in increased opportunities for spread of disease, hampering control efforts. New developments in test design and format such as fluorescent-polarization based assays (Dajer et al., 1999; Nielsen et al., 2001), polymerase chain reaction based assays (Al Dahouk et al., 2004), electronic noses (Pavlau and Turner, 2000; Turner and Magan, 2003) and lateral flow assay devices (Smits et al., 2003), provide new opportunities for the development of simple, rapid and affordable tests for infectious diseases that may be used outside the established laboratory. These developments provide new opportunities for biotechnical companies in developing countries for test development and marketing.

VACCINES – POBLEMS AND PITFALLS

Existing vaccines induce high antibody levels against the lipopolysaccharide antigens of brucellae, which are the basis of serodiagnostic assays, consequently resulting in positive serological tests. A rough vaccine strain based on the rifampicin-resistant mutant *B. abortus* RB51 does not have this problem, however, its efficacy in non-bovine species has been questioned. Vaccine production in developing countries provides an important role for biotechnology companies. Furthermore, with the availability of genome sequences (DelVechio et al., 2002a; Paulsen et al. 2002) the prospect of the development of an effective acellular vaccine has become a step closer. Here the challenge is to provoke a good Th1 response that will result in protective immunity. Post-genomic approaches may also help with selection of better antigens for test development, possibly able to distinguish between immune responses following either natural infections or vaccination (DelVechio et al., 2002b; DelVechio et al., 2002c). Biotechnology entrepreneurship is rapidly growing in the developing world and offers a means of making a real contribution to the economic growth of these countries (Tonukari, 2004).

CONCLUSION

Accumulating epidemiological evidence emphasises the need for brucella control in sub-Saharan Africa. Control of brucellosis in other situations has highlighted the importance of detailed knowledge of local epidemiology and community support for effective control. Demonstration of the cost-effectiveness of control is essential to underpin policy changes and full community participation. Being a zoonosis, vaccination of livestock is pivotal in the control of this disease. Existing vaccines are beneficial, but also have problems, however, these can be successfully used in control programs. New knowledge and biotechnological developments bring an effective acellular vaccine a step closer. Similar technological advances have enabled the development of simple, rapid and user-friendly diagnostics suitable for de-centralised testing. De-centralised testing is essential for rapid diagnosis and early instigation of disease control measures. This could also offer sensitivity and specificity permitting enhanced monitoring and surveillance in countries with a poorly developed infrastructure.

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