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How do you get the Rose Bengal Test at the point-of-care to diagnose brucellosis in Africa? The importance of a systems approach

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ABSTRACT

Brucellosis is a major neglected zoonotic disease, whose burden both in animals and humans is severely under-reported. Diagnosis in humans identifies cases in order to treat the disease at the individual level. In animals diagnosis is implemented at the population level in the context of appropriate control or eradication strategies. Molecular and bacteriological diagnosis are rarely undertaken in sub-Saharan Africa, at least outside research projects, due to cost, skills and laboratory infrastructure issues. The brucellosis toolbox contains a wide range of serological tests, but the perfect test for use in animals and humans respectively does not exist. Drug and diagnostic discovery for the neglected zoonoses are notoriously poor, and there is limited investment interest in developing new tools for brucellosis diagnosis. But are current tools being used to their full capacity? The rose Bengal test (RBT) stands out as an efficient, practical and very cheap test adapted for use in the resource-poor context. In this paper, we argue that a social science or system's approach to explore the practicality of improving diagnostic capacity at the point-of care in high-risk brucellosis areas of rural Africa may be a step towards solving the issue of under-diagnosis, but this must go hand-in-hand with implementation of control measures at source in the animal reservoir and capacity to treat human cases.

1. Introduction

Brucellosis is one of the most widespread zoonoses in the world caused by several species of the genus *Brucella* and accounting for 500,000 cases worldwide per year (Pappas et al., 2006). This paper focuses on the African context, where *B. melitensis* and *B. abortus* (whose host preference are small ruminants and cattle respectively) have the biggest impact on livestock productivity and human health (Ducrotoy et al., 2017; Godfroid et al., 2011). There is recognition that brucellosis, both in animals and humans, is hugely under-reported in Africa (Dean et al., 2012b; Ducrotoy et al., 2014; McDermott and Arimi, 2002; Rubach et al., 2013). Underestimation of disease burden hinders policy development and the implementation of control measures (Rubach et al., 2013). Human prevalence reflects the situation in the animal reservoir, as human cases are always a result of direct contact with infected animals or indirect transmission through the consumption of contaminated dairy products. Distribution of the disease in both humans and animals is predicted to be heterogeneous in Africa, hence accurate data on 'hot spots' of disease would enable more targeted (and cheaper) vaccination strategies to be implemented in livestock, thereby preventing human cases (ILRI, 2012).

Preventing disease at source through vaccination of livestock has been demonstrated to be a cost-effective approach for brucellosis; both in term of improvements in animal productivity and averted cost of treating human cases (Roth et al., 2003). Capacity to identify human brucellosis cases enables treatment of affected individuals. Brucellosis requires prolonged and combined antibiotic therapy (use of specific antibiotics vary depending on the form of brucellosis and patient profile), but appropriate treatment is curative for the disease (Ariza et al., 2007; Corbel, 2006; Pappas et al., 2005; Skalsky et al., 2008). Pasteurisation of milk is also important to reduce animal to human transmission. This is poorly regulated in poor countries where a large proportion of the population still consumes raw milk and dairy products and is in close contact with susceptible animals (Marcotty et al., 2009).

In livestock, brucellosis manifests as abortions in females and infertility in males, neither of which are pathognomonic of brucellosis (Cunningham, 1977; OIE, 2016a). Moreover, most infected animals do not show any clinical signs at least for a certain period of the infection course. Human brucellosis is a disease with protean clinical manifestations (Ariza, 1999; Dean et al., 2012a) and

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is often misdiagnosed for other fever-inducing conditions such as malaria (Pappas et al., 2006). Humans can present no clinical sign during the acute phase but could suffer decades later from chronic focalised forms of the disease (arthritis, hepatic, splenic or encephalic abscesses or valvulopathy, for instance).

The diagnosis of brucellosis is based on the use of direct or indirect tests. Diagnosis in humans identifies cases in order to treat the disease at the individual level. In animals diagnosis is implemented at the population level in the context of appropriate control or eradication strategies. These different contexts influence the choice of tests.

An in-depth comparison of the performance of available tests in animals and humans is beyond the scope of this paper. We argue, however, that the rose Bengal test stands out as efficient and cheap option for the resource poor context and justify this claim by comparing it with other tests for the different contexts of ruminant and human diagnosis here below. We propose that a social science or system's approach to explore the practicality of RBT distribution to high-risk brucellosis areas in rural locations in Africa may be a step towards solving the issue of under-diagnosis.

2. Direct tests

Direct tests detect viable *Brucella* or *Brucella* DNA from samples. Molecular and bacteriological diagnosis are rarely undertaken in the developing country context for routine diagnosis because of their prohibitive cost, requirement for high standards of laboratory infrastructure, and the fact that they are cumbersome and require a great deal of expertise. However, molecular epidemiology tools, like Multiple-Locus Variable number tandem repeat Analysis (MLVA, see below), are essential to investigate the epidemiology of the disease.

2.1. Direct testing in animals

Direct testing for brucellosis relies on culture and isolation of *Brucella* and is the gold standard against which performance of other tests is assessed. This has to be undertaken in laboratories with Biosafety Level 3 facilities due to the operator risk of acquiring this zoonotic infection. Definitive identification and biotyping to determine *Brucella* species and biovar relies on assessment of general morphological and metabolic characteristics (OIE, 2016a). Despite being 100% specific, bacteriological culture can lack sensitivity depending on the viability and concentration of Brucellae in the sample and on the kind and number of samples chosen and selective media used (de Miguel et al., 2011).

Molecular tests are run on bacteria isolated through bacteriology, and this limits their widespread use. Molecular tests exist for species, biovar and vaccine typing and can be applied to colonies on isolation plates avoiding dangerous manipulations and discrimination capacity depends on the molecular markers used (Yu and Nielsen, 2010). While not currently applied for routine diagnosis, they are valuable for epidemiological purposes. Whereas most identify the species, this is not always the case with the classical biovars. Part of the discrepancy stems from the reproducibility problems of the classical typing (Whatmore, 2009; Whatmore et al., 2007). Many strains grouped by classical typing do not always reflect an epidemiological situation or outbreak, and some molecular methods reveal these inconsistencies.

Methods like Bruce-ladder for species identification or MLVA for finer analyses will probably be used extensively in the future. A recent paper has described running MLVA directly on field samples, which can be useful for epidemiological purposes (Gopaul et al., 2014). PCR protocols have been optimized in laboratory experiments for analytical sensitivity and specificity, but there are few studies on diagnostic performance, Molecular methods such as Bruce-ladder allow typing at species and, in some cases, biovar level (Lopez-Goni et al., 2011, 2008). The variety of samples, DNA extraction and PCR protocols, limitations intrinsic to the type of samples, and the fact that reference bacteriological or serological procedures are not uniform, complicate diagnostic development.

2.2. Direct testing in humans

Cultures should be performed whenever possible and preferably in the pyretic phase, although this is rarely undertaken in Africa due to lack of capacity. Isolation can be attempted from articular, cerebrospinal and other fluids or some tissues in focal forms but blood culture under 10% CO_2 is the routine method. Since growth is not visually perceived and repeated subculturing on agar media to isolate the microorganism is highly risky, modern bacterial growth detecting systems or Ruiz-Castañeda's biphasic system are recommended. In either case, prolonged incubation (up to 21 and 45 days, respectively) is necessary before discarding a suspicious culture. Large (5–10 ml) samples in duplicate flasks and two or three independent blood samplings at adequate intervals are advisable. The leukocyte lysis-concentration procedure or the use of bone marrow may improve detection. Rates of isolation can be high (up to 86%) in the pyretic phase, less in apyretic intervals. Indeed, when antibiotic treatment is applied before culturing, the success is low. Unless infection by Rev. 1 or RB51 is suspected,[1] identification to genus level is enough for medical purposes and the species is not a factor in choosing the treatment (Díaz and Moriyón, 1989). PCR-based methods have been developed to detect *Brucella* DNA in human samples but the variety of protocols and reproducibility problems preclude any recommendation.

3. Indirect tests

Indirect tests detect antibody or cellular responses (the latter are not discussed in this paper due their limited practical use). A huge range of serological tests have been developed overtime (rose Bengal test [RBT], serum agglutination test [SAT], complement fixation test [CFT], lateral flow immunochromatophy assay [LFIA], fluorescent polarisation assay [FPA], competitive ELISA [cELISA], indirect ELISA [iELISA], double gel diffusion with Native Hapten, radial immunodiffusion, counter electrophoresis etc.) which emphasises that the perfect test (easy to use, robust, affordable and able to identify all infected individuals) is not yet available. The classical rose Bengal test, however, stands out as an efficient, practical and very cheap test, and we justify this claim here below.

3.1. Immunological diagnosis of ruminant brucellosis

The epidemiological context and target host species, influence the performance of tests in terms of diagnostic sensitivity (DSe) and specificity (DSp). The silent behaviour of the pathogen towards the immune system and its intracellular niche (Guerra, 2007; Roop et al., 2004) imposes limitations with regards to sensitivity, as there is a lag time between infection and development of antibodies; antibody responses may not be detected during early stages of infection in very old animals and in congenitally infected offspring (Beh and Lascelles, 1973). Two situations have to be considered with regards to specificity issues. The first is when Rev.1 or S19 vaccination is applied in adult animals or in young animals by the

[1] Rev. 1 is streptomycin (but not gentamycin)-resistant, and RB51 is rifampin-resistant. Therefore, these antibiotics (normally used in brucellosis treatment) cannot be used in these infections.