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Comparative study on kits used for diagnosis of Brucellosis in humans

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Abstract

Brucellosis is an infectious zoonotic disease caused by bacteria of the genera *Brucella.* Brucellosis is mainly a disease of livestock infecting cattle, sheep, goats, pigs, dogs and marine mammals. In humans, the disease is acquired directly or indirectly from infected animals or animal products. *Brucella* species of public health concern are *B. abortus*, *B. melitensis, B. suis, B. canis, B. ovis and B. neotamae* which cause disease both in animals and human. Clinical symptoms include fever, headache, chills, depression, profound weakness, weight loss and muscle and joint pain. If untreated, the infection persists and progresses to a chronic illness that may present as endocarditis, epididymo-orchitis and infertility in males, spontaneous abortion in females, neurobrucellosis and may result in death. Diagnosis of human brucellosis is based on isolation of the bacteria from clinical specimens followed by microscopy. This method may miss some positive specimens especially when the bacteremia is low. Other methods include serology and molecular biology techniques. Serological test are not internationally standardized, thus the sensitivity and specificity of the tests may be unknown. Other limitations include cross reactivity of some tests with closely related bacteria and inability to distinguish between active infection and previous exposure. These may lead to false positive or false negative results, leading to poor clinical management of patients. Molecular methods, such as polymerase chain reactions (PCR) are very sensitive and specific but require expertise, are expensive and are unavailable in many clinical laboratories in Kenya. This study will compare the sensitivity and specificity of the serological kits used for *Brucella* diagnosis in health care facilities in Kenya with indirect enzyme immunosorbent assay (ELISA) and the Rose Bengal test, tests that have been shown to have high sensitivity and specificity. Polymerase chain reaction will be used to confirm the *Brucella*-positive samples and to identify the *Brucella* species affecting patients in the participating health facilities. The study will provide information on the specificity, sensitivity and cost of each diagnosis kit to the participating health facilities and to the Kenya Ministry of Health to help inform decisions on diagnosis of brucellosis in humans in Kenya.

Keywords: Baringo, *Brucella,* diagnosis

Résumé

La brucellose est une maladie zoonose infectieuse causée par des bactéries du genre *Brucella*. La brucellose est une maladie touchant principalement l'élevage infectant les bovins, les moutons, les chèvres, les cochons, les chiens et les mammifères marins. Chez l'être humain, la maladie est acquise directement ou indirectement à partir d'animaux infectés

ou des produits d'origine animale. Les espèces de *Brucella* qui interpellent la santé publique sont *B. abortus, B. melitensis, B. suis, B. canis, B. ovis et B. neotamae* qui causent la maladie chez les animaux et les êtres humains. Les symptômes cliniques sont la fièvre, des maux de tête, des frissons, la dépression, la faiblesse profonde, la perte de poids et des douleurs musculaires et articulaires. Si la maladie est non traitée, l'infection persiste et progresse vers une maladie chronique qui peut se présenter comme l'endocardite, orchiépididymite et l'infertilité chez les hommes, l'avortement spontané chez les femmes, la neurobrucellose et peut entraîner la mort. Le diagnostic de la brucellose humaine est basé sur l'isolement des bactéries à partir de prélèvements cliniques, suivie par microscopie. Cette méthode peut manquer quelques spécimens positifs surtout quand la bactériémie est faible. D'autres procédés comprennent la sérologie et des techniques de biologie moléculaire. Les tests sérologiques ne sont pas normalisés au plan international, ainsi la sensibilité et la spécificité des tests peuvent être inconnues. D'autres limitations incluent la réactivité croisée de certains tests avec des bactéries et l'incapacité à faire la distinction entre l'infection active et l'exposition précédente qui sont étroitement liés. Ceux-ci peuvent résulter à des résultats faux positifs ou faux négatifs, conduisant à une mauvaise prise en charge clinique des patients. Les méthodes moléculaires, tels que des réactions en chaîne de la polymérase (PCR) sont très sensibles et spécifiques, mais nécessitent une expertise, et sont coûteux et ne sont pas disponibles dans de nombreux laboratoires cliniques au Kenya. Cette étude permettra de comparer la sensibilité et la spécificité des trousses sérologiques utilisés pour le diagnostic de *Brucella* dans les établissements de soins de santé au Kenya avec l'enzyme indirect de dosage immunoenzymatique (ELISA) et le test au Rose Bengale, les tests qui ont démontré une haute sensibilité et spécificité. La réaction en chaîne de la polymérase sera utilisée pour confirmer les échantillons de *Brucella* positif et d'identifier les espèces de *Brucella* affectant des patients dans les établissements s'ocupant de la facilités de santé. L'étude fournira des informations sur la spécificité, la sensibilité et le coût de chaque kit de diagnostic pour les établissements de santé, au ministère kenyan de la Santé pour aider à prendre des décisions éclairées sur le diagnostic de la brucellose chez l'homme au Kenya.

Mots clés: Baringo, *Brucella,* le diagnostic

Introduction

Brucellosis is a zoonotic disease infecting cattle, sheep, goats, pigs and humans. It is caused by bacteria of the genera *Brucella. Brucella* species are small, facultative, intracellular gram negative, rod shaped (coccobacilli) bacteria. Six major *Brucella* species known to cause disease in human are *B. abortus*, *B.melitensis, B. suis, B. canis, B. ovis and B. neotamae* (Glynn *et al.,* 2008). *Brucella melitensis* is the most pathogenic to human (OIE, 2009). In some of the natural host like cattle, sheep, goats, pigs and dogs the infection usually establishes itself in the reproductive tract, resulting in placentitis followed by abortion, still birth, birth of weak offspring, retained placenta in females. In males *Brucella spp* cause epididymitis, orchitis and infertility (Corbel, 2006). Excretion in genital discharge and milk is common and is a major source of human infection.

In humans, the disease is acquired directly or indirectly from infected animals and animal products. It manifests itself as an acute febrile illness (AFI) presenting with undulant fever, headache, chills, depression, profound weakens, arthralgia, myalgia, weight loss and back pain. When not treated, the disease persists and progresses to a chronically incapacitating disease with severe complication. These include endocarditis, osteoarthritis, meningitis or meningo-encephalitis, epididymo-orchitis and pre-term termination of pregnancy.

Diagnosis of brucellosis in humans may be made by isolation of the microorganism in blood, bone marrow, cerebral spinal fluid, which provides absolute proof of the infection. Isolation of *Brucella* species requires high containment laboratory facilities (Biosafety Level 3), highly skilled personnel and has long turnaround time (Nielsen and Yu, 2010). Other methods used in diagnosis of human brucellosis include detection of *Brucella* antibodies or antigen in blood using serological tests such as Rose Bengal test, serum agglutination test (SAT), complement fixation test (CFT) and enzyme linked immunoabsorbent assay (ELISA). Other diagnostic methods include molecular tests such as the polymerase chain reaction methods (PCR) that detect bacterial genetic material in blood or other tissues.

Methods

Study area. The study area is Baringo County located in the Rift valley in Kenya. Baringo has an area of 8,655 square km and a population 555,561 people (2009 census). About 140.5 square km of land is covered by water surface. Temperatures range from 25°Cto 30°C, it has an altitude of approximately 1000M to 2600M above sea level and these contribute to the weather patterns. The main economic activities include pastoralism, crop farming, and sand harvesting. For the pastoralists livestock keeping is the main activity, it occupy central part in their cultural life as food and as currency for dowry.

Baringo was chosen because no similar study has been carried out in the region; the county has pastoralist and agro-pastoralist communities, with traditional practices that would enhance *Brucella* transmission in humans and thus there is a likelihood of a high prevalence of people in this area. The study is being carried out in Kabarnet, Marigat and Eldama Ravine district hospitals.

Study design. This is a laboratory based study in which samples are obtained from specimen left over after routine diagnosis of brucellosis in health facilities in study health facilities. Field site visits and key informant interviews were done prior to beginning the study to identify and categorize various health care facilities in the county, where sampling is being carried out. Diagnostic kits used for brucellosis diagnosis in the selected health facilities and those available in the markets have been identified and are being analyzed.

Sample size. The following formula was used to calculate the required sample size

$$n = \frac{Z\alpha^2 pq}{L^2}$$ (Martin *et al.,* 1987)

Where;

n = Sample size,
$Z\alpha$ = confidence level 95% (1.96)
P = assume the prevalence estimate (13.7%)
q = 1-p,
L = the precision error (5%)
Sample size = 181.67samples

Thus, a total of 182 blood specimens will be collected.

In additional, vaginal swabs will be collected from women who present with spontaneous abortion and from who brucellosis diagnosis is requested by attending physicians.

Inclusion criteria. All patients 18 years and older from who the clinician have requested for brucellosis testing and who consent to participate in the study.

Exclusion criteria. All patients less than 18 years old, prisoners, patients for who brucellosis test is not requested by the attending clinician and all patients 18 years and older with brucellosis test request but who do not consent to participating in the study.

Ethical approval. Ethical approval has been obtained from the Kenyatta National Hospital/ University of Nairobi-Ethic and Research committee (KNH/UON-ERC).

Data collection and management. Semi structured pre-tested questionnaire, will be used for data collection that will be analyzed for risk factors identification as well as challenges associated with brucellosis diagnosis in the health care facilities.

Samples collection, processing, storage and transportation. Specimen 10ml of blood sample is collected in a sterile vacutainer from each participant and routine diagnosis of *Brucella* and other assays required for patient management carried out. In addition the samples are tested using two rapid diagnosis kits, Fortress diagnostic kit and Plasmatec, that are commonly used in health facilities in the country for Brucellosis diagnosis. Serum and clot are separated from the remaining blood, stored in sterile -20^0c, and transported to Department of Biochemistry, University of Nairobi within five days for additional assays.

Laboratory analysis of samples. Serum samples are tested for brucellosis using Rose Bengal Test (RBT), Indirect Enzyme Linked Immunoabsorbent Assay (ELISA) and reverse transcription polymerase chain reaction (PCR).

Data analysis. Data analysis will be done using statistical package for social sciences (SPSS), Chi- square test and Analysis of variance (ANOVA). To calculate the sensitivity, specificity and predictive values negative, positive of each diagnostic kit in relation to ELISA and RBT.

Results

After data collection and analysis, sensitivity and specificity of each test will be reported. Predictive values negative and positive will also be reported. The best diagnostic kit in terms of sensitivity and specificity will be reported compared to ELISA. Risk factor associated with brucellosis infection will be reported. The report will be given to the health care facilities who participated in the study.

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