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Seroprevalence of Brucellosis among Patients Attending a District Hospital in Rwanda

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Abstract. Studies on human brucellosis in Rwanda are scarce, and the incidence is likely to be higher than official estimates. In a recent study using Rose Bengal Test (RBT) on women who had aborted or had still births in Huye district, 25% were *Brucella* seroprevalent. Thus, purpose of the present study was to investigate the *Brucella* seroprevalence in patients presenting with the key signs and symptoms of brucellosis. Cross-sectional study was done in Nyagatare District in the Eastern Province of Rwanda. A total of 198 patients were recruited from Nyagatare District Hospital, blood samples were collected, and sera analyzed with RBT. A questionnaire was used to explore the risk factors. A total of 12 patients (6.1%; 95% confidence interval [CI] = 0.662–7.820) were *Brucella* seropositive. Infection was significantly associated with drinking unboiled milk (odds ratio [OR] = 8.3; 95% CI = 2.4–29.2) and having had recurrent fever (OR = 5.6; 95% CI = 1.5–21.3). Drinking unboiled milk is a risk factor for *Brucella* infection. Provision of adequate resources and trainings to staff in brucellosis diagnosis is needed to reduce recurrence of fevers probably because of misdiagnosis. Public awareness creation on transmission routes of brucellosis is to be intensified.

BACKGROUND

Human brucellosis is one of the most widespread zoonoses in the world and occurs annually with many cases going unreported.[1–4] Brucellosis in humans, while not usually fatal, causes chronic suffering resulting in considerable economic losses from inability to work well and due to prolonged treatment costs.[5–8] In livestock, the disease affects almost all animals, where it results in reduced or complete loss of milk production, abortions, and sterility, which contributes notably to poverty in the affected communities.[6,9]

The disease is caused by the bacterial species of the genus *Brucella*, namely *Brucella abortus*, *B. suis*, *B. melitensis*, and *B. canis*, but brucellosis in humans is mainly caused by a more severe *B. melitensis* from goats and sheep and which produces most severe symptoms, and the more mild *B. abortus* from cattle, and *B. suis* from pigs.[10,11] Human brucellosis does not have a safe vaccine in humans[12] and is largely characterized by undulating or persistent fever, weight loss, and night sweats, which makes it easily misdiagnosed as malaria in areas with limited laboratory facilities.[5,13] In countries of low economic status, the major transmission route of brucellosis is considered to be the consumption of raw milk or milk products, but direct contact with infected animal parts as well as by inhalation are also implicated.[7,10,11] It is conceded that to control brucellosis in humans effectively, the disease intervention measures need to be intensified in the livestock and other animals since there is no vaccine yet available for humans.[8,11]

The studies on brucellosis in livestock have widely been conducted; there is limited information about human brucellosis in most low-income countries including those in sub-Saharan African.[8] In Rwanda, a recent study conducted on groups of women who had aborted and those who had had stillbirth showed a 25% *Brucella* seroprevalence,[7] but the information on the disease distribution in the country remains scanty.

The Eastern Province of Rwanda is one of the most important areas for dairy production with more than 60% of cattle population in the country, and therefore having frequent contact with livestock and their raw products including drinking raw milk,[8,14,15] and thus at greater risk of contracting brucellosis. In a survey carried out in 2008 in one of the districts of the Eastern Province, namely, Nyagatare District, an overall 9.9% seroprevalence of brucellosis was reported in cattle, and thus raised the speculation that the diseases could have been incriminated as one of the possible causes of infertility of unidentified origin in the animals.[5] On the other hand, bovine seroprevalence of brucellosis in Kigali city has been found to be much lower at 2.3%.[16] Livestock rearing in the district involves keeping some of the livestock in a family house mainly for the security of their animals and other reasons as reported elsewhere, which increases the risk of animal to human diseases transmission.[17,18]

The purpose of this study was therefore to assess the seroprevalence of brucellosis among the patients as well as knowledge and practices related to the disease in the Nyagatare District.

METHODS

The cross-sectional study was conducted in Nyagatare District, Eastern Province of Rwanda at the Nyagatare District Hospital. All the patients who were at the hospital, between June 18 and July 7, 2014, and were diagnosed by the clinician to have any one of the following signs and symptoms willingly were included in the study: intermittent or persistent fever, headache, weakness, profuse sweating, chills, arthralgia, weight loss, and joint pain. Patients who presented with different signs and symptoms and/or were unwilling to participate were excluded from the study.

Study setting. The District of Nyagatare is one of the seven districts making up the Eastern Province. It spreads over an area of 1920.11 km^2, with Uganda at its North, Tanzania at its East, Gatsibo District at its South, and Gicumbi District at its West (District monograph 2012). It is the one of the largest districts in the country with the total population of 466,944 (NISR2012) population census results. The district hospital has 200-bed capacity. The study area was chosen because of the existence of large livestock animals as well as its accessibility as the hospital is built on the main road.

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Collection and processing of samples. The blood samples were collected from a total of 198 patients over the duration of the study and were processed on regular basis. About 5–10 mL of venous blood was taken from the arm by venipuncture method, and collected into silicon-coated plain tubes for isolation of sera. The blood was centrifuged at 3,000 RPM for 5 minutes to obtain the serum used for detection of antibody using *Brucella* antigens. The collected sera were checked for the presence of *Brucella* antibodies using Rose Bengal Test (RBT; TRANSAK, Leuven, Belgium)[19] as follows: 50 µL of test serum was mixed with an equal volume of colored *Brucella* antigen on a clean slide placed on a white paper. The mixture was agitated gently for 4 minutes at ambient temperature, and then observed for agglutination. Any visible reaction (agglutination) was considered to be positive and nonagglutination was negative.[19] A sample of serum from a negative individual was used as a negative control to be compared with positive samples. In addition, a questionnaire was developed, pretested, and corrected, and was used to record respondents' demographic data such as age, occupation, and knowledge of the *Brucellosis* transmission by mentioning of at least one example of route of transmission, recurrence of symptoms, whether it was the first time or otherwise, and possible exposure to animals.

Statistical analysis. SPSS software (IBM Corporation, Armonk, NY, 1994) was used for analysis to determine the seropositivity and its possible association with recorded categorical characteristics of the participants using Fisher's exact test at less than or equal to 0.05 level of significance. To identify risk factors to observed brucellosis seropositivity, a univariable logistic regression analysis was performed for each variable and all that had a P value of < 0.2 were considered for the multivariable model. The final model was developed using a manual backward stepwise elimination of factors, stopping when all remaining variables were significant at $P < 0.05$.

RESULTS

The results of this study are presented in Table 1 showing that 198 patients participated, of whom 52% were females and 48% were males. The most participants were aged 21–40 years at 53.5%, those who drank unboiled milk accounted for 22.2% (95% CI = 0.034–0.421), and livestock keepers were 79.3% of the total. Most (88.4%) did not know how brucellosis is transmitted, whereas 37.4% had recurring fever.

The RBT revealed that 6.1% (95% CI = 1.461–21.34) of the participants were seropositive for brucellosis.

Tests for relationship between seropositivity status and sex, age group, livestock keeping, or knowledge of brucellosis transmission indicated that there was no significant association between the infection statuses with these attributes. However, infection status was significantly associated with drinking unboiled milk (OR = 8.3; 95% CI = 0.034–0.421) and having recurrent fever (OR = 5.6; 95% CI = 1.461–21.34) (Tables 1 and 2).

DISCUSSION

The purpose of this study was to determine the seroprevalence of brucellosis in patients attending Nyagatare District Hospital and the possible risk factors for the infection with *Brucella* in the area. Results from our study show that 6.1% of the patients who had been diagnosed with signs and symptoms similar to those of brucellosis were seropositive for *Brucella* by the RBT method. However, the results may not necessarily mean that the patients had active infection due to the limited specificity of the diagnostic method used.

TABLE 1
Comparison of seropositivity among respondents groups (N = 198)

	RBT result			Fisher's exact test		
Characteristics	Positive *n* (%)	Negative *n* (%)	Total *n* (%)	cOR	95% CI	*P* value*
Gender						
Male	8 (8.4)	87(91.6)	95	2.28	0.662–7.820	0.237
Female	4 (3.9)	99 (96.1)	103			
Total	12 (6.1)	186 (93.9)	198			
Age-group						
≤ 40	9 (8.6)	96 (91.4)	105	2.81	0.738–10.72	0.143
≥ 40	3 (3.2)	90 (96.8)	93			
Total	12 (6.1)	186 (93.9)	198			
Drink boiled milk						
Yes	4 (2.6)	150 (97.4)	154	0.12	0.034–0.421	**0.001**
No	8 (18.2)	36 (81.8)	44			
Total	12 (6.1)	186 (93.9)	198			
Livestock keeping						
Yes	8 (5.1)	149 (94.9)	157 (79.3)	0.50	0.142–1.739	0.276
No	4 (9.8)	37 (90.2)	41 (20.7)			
Total	12 (6.1)	186 (93.9)	198 (100)			
Knowledge of brucellosis transmission						
Yes	3 (13.0)	20 (87.0)	23 (11.6)	2.77	0.691–11.07	0.150
No	9 (5.1)	166 (94.9)	175 (88.4)			
Total	12 (6.1)	186 (93.9)	198 (100)			
Recurrence of symptoms						
Yes	9 (12.2)	65 (87.8)	74 (37.4)	5.58	1.461–21.34	**0.01**
No	3 (2.4)	121 (97.6)	124 (62.6)			
Total	12 (6.1)	186 (93.9)	198 (100)			

CI = confidence interval; cOR = crude odds ratio; RBT = Rose Bengal Test. The bold values mean significant value where P value is lesser than 0.05.
* 95% confidence limit.

TABLE 2
Multivariable logistic regression analysis to show the odds ratios for seropositivity ($N = 198$)

	RBT			Multivariable logistic regression analysis		
Characteristics	Positive *n* (%)	Negative *n* (%)	cOR	aOR	95% CI	*P* value*
Gender						
Female	4 (3.9)	87 (91.6)	2.28	1		
Male	8 (8.4)	99 (96.1)		2.83	0.710–11.335	0.14
Drink boiled milk						
Yes	4 (2.6)	150 (97.4)	0.12	1		
No	8 (18.2)	36 (81.8)		9.638	2.401–38.681	**0.001**
Keep livestock						
No	4 (9.8)	149 (94.9)	0.5	1		
Yes	8 (5.1)	37 (90.2)		0.854	0.196–3.730	0.834
Recurrence of fever and other symptoms						
No	3 (2.4)	121 (97.6)	5.58	1		
Yes	9 (12.2)	**65 (87.8)**		6.129	1.414–26.563	**0.015**

aOR = adjusted odds ratio; CI = confidence interval; cOR = crude odds ratio; RBT = Rose Bengal Test. The bold values mean significant value where *P* value is lesser than 0.05.

In a similar study conducted in Kenya involving 488 patients with brucellosis-like symptoms, a little higher proportion of 13.0% were diagnosed with brucellosis using RBT, 6% were found with malaria by blood slide microscopy, 40% with typhoid by Widal test, 6% with streptococcal infections in anti-streptolysin O test, and 9% rheumatoid arthritis in rheumatoid factor test.[20–22] These laboratory results clearly show how dependence on clinical signs alone for diagnosis of such infections as malaria could have easily missed out a few other infections including brucellosis leading to wrong or no treatment of some patients and resulting in persistence or recurrence of the symptoms.[23] The significant association of reported recurrence of fever symptom with seropositivity status in the present study could be attributed to non-diagnosis of some causes of fever and calling the symptom pyrexia of unknown origin or just due to misdiagnosis.[22,24,25] The laboratory misdiagnosis is a challenge facing the effort to control brucellosis. Therefore, the present study presses the need to introduce the *Brucella* diagnostic kit in the routine laboratory screening in all health facilities in Rwanda.

In a 5-year (1996–2000) report on human cases of brucellosis, it was surprisingly noted that quite low numbers were reported in sub-Saharan African countries, indicating limited differential diagnostic capacity for brucellosis, and possible low prioritization of the disease sometimes due to high cost involved.[22] Other factors contributing to underreporting of human brucellosis include low numbers of those seeking for health care due to long distances to health facilities, unreliable supplies of diagnostic materials, limited diagnostic capacity, lack of awareness and limited knowledge of brucellosis among health providers and the community, and inadequate inter-sectoral collaboration.[22,23,26]

Lack of knowledge of the transmission and symptoms of brucellosis was found in 88.4% of the respondents in the present study, but it was not significantly associated with seropositivity. Related investigations have reported high lack of awareness and knowledge of brucellosis in Tajikistan (85%),[27] Nigeria (78.5%),[28] and Uganda (69.6%),[29] while in one study in Kenya up to 78% of the respondents did not know that brucellosis existed.[30] In some instances, limited awareness of brucellosis of the community has been attributed to the absence of its diagnosis and treatment at the health facilities and instead other febrile conditions such as malaria and typhoid being the most commonly mentioned and managed.[31] On the other hand, high proportions of people with knowledge of brucellosis symptoms were reported in Uganda, (75.5%), by Kansiime and others.[32] The drinking of raw milk was found to be a significant risk factor for acquiring *Brucella* infection (OR = 8.3; 95% CI = 2.4–29.2). This is not surprising given that the role of raw milk in the transmission of brucellosis has been reported in several studies.[22,24,29,33,34] Raw milk consumption has been on the increase not only in Rwanda but also in other countries in the region, owing to the increasing costs of milk processing, which has resulted in closure of some plants in some countries.[14,35] As a result, unpasteurized fermented milk sold by such outlets as restaurants, small shops, and street vendors expose consumers to milk-borne diseases including brucellosis.[35] Milk and other animal products, therefore, have come to be the focus for intervention activities against brucellosis in many parts of the world.[4,14,36] In Rwanda, the target for the 2009–2012 Strategic Plan for the Transformation of Agriculture in Rwanda–Phase II[37] was to reduce the incidence of Brucellosis from 2.6% to zero in livestock. The move toward improving the quality of milk value chain that is underway in Rwanda, therefore, is considered to be a step in the right direction toward tackling the problem of drinking unpasteurized milk to safeguard the consumers.[14]

In the present study, livestock keeping was not associated with brucellosis seropositivity. This is in agreement with the study conducted in Tanzania,[38] in which there was no observed significant difference between the proportion of brucellosis seropositive males and females. This could be attributed to the fact that in the East African region men and women get involved in the handling of cows with some distinct division of labor, of herding reserved for men and children of 6 or 7 years of age, and the milking and taking care of health of the livestock under the charge of women.[39] This involvement of most members of the household apart from very early age puts the whole population at risk and might also account for the observed lack of difference in the infection rate among the studied age groups.[4] In high-income countries, brucellosis is mainly an occupational disease affecting mainly men aged between 25 and 45 years.[4]

LIMITATIONS OF THE STUDY

The limitation of this study is that RBT, which is the screening test, was the only one used without further complementary test to take into account the variability in sensitivity and specificity of rapid serological tests and thus the results might not mean an active infection. Therefore, we recommend that more specific diagnostic tests such as ALISA and PCR molecular techniques are to be used in future studies. In addition, the study subjects were patients at a hospital, and thus the results cannot represent a community-wide seroprevalence. Furthermore, the sample size was very small because the study period was so short (less than a month). This is due to the fact that the authors wanted to have quicker data that could guide a bigger study we are planning to conduct in the near future.

CONCLUSION

This study has shown that human brucellosis exists in Nyagatare District. Consumption of raw milk was found to be a risk factor for *Brucella* infection. Recurrent fever was associated with the infection, whereas age, gender, and knowledge respondent for brucellosis transmission and symptoms were not associated with seropositivity, probably because of the involvement of the whole population in the management of the livestock and their products from a tender age. Awareness creation of the community in Nyagatare about the need to boil milk before it is drunk or processed into other products should be intensified. Provision of adequate resources and trainings to staff in brucellosis diagnosis is needed to reduce recurrence of fevers probably due to misdiagnosis. More research involving a larger sample size and community based is needed to shed more light on the status of brucellosis among livestock keepers.

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