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BRUCELLOSIS IN TEREKEKA COUNTY, CENTRAL EQUATORIA STATE, SOUTHERN SUDAN

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ABSTRACT

Objectives: To identify factors associated with Brucellosis in patients attending Terekeka Health Facility, Terekeka County, Central Equatoria State, Southern Sudan and to evaluate the utility of the rapid test kit Euracil®.

Design: A facility based case-control study.

Setting: Terekeka Health Facility, Terekeka County, Central Equatoria State, Southern Sudan.

Subjects: Cases were patients presenting at the Terekeka Health Facility with clinical symptoms suggestive of Brucellosis and tested positive for Brucellosis by rapid antigen test while controls were selected from individuals attending Terekeka Health facility with health problems unrelated to brucellosis or febrile illness.

Results: A total of fifty eight cases with clinical symptoms suggestive of and tested positive for Brucellosis by rapid antigen test presented. A total of 116 consented controls were recruited into the study. Males accounted for 52% of the cases and 53% of the controls. The mean age was 31 years for both groups. Cases without formal education were 84% while 40% had no source of income, 20% of the cases and 14% of the controls were cattle keepers while 5% of the cases and 13% of the controls were students. In multivariate analysis there were many factors associated with Brucellosis like consumption of raw meat, living with animals at the same place, raising of goats, farm cleaning contact, eating of aborted and wild animals. Logistic regression revealed two factors associated with the disease; consumption of raw milk (OR=3.9, P-value 0.001, 95% CI 1.6666 - 9.0700) was a risk factor while drinking boiled milk was protective (OR= 0.09, p- value 0.000, 95% CI, 0.1 - 0.2).

Conclusions: The main age-groups affected were 20 – 30 years with males being affected more than females. Drinking of raw milk was significantly associated with Brucellosis while drinking boiled milk was protective. There should be active public health education on the benefits of boiling milk before consumption. Further studies to elucidate the extent and epidemiology of brucellosis in humans and animals in Southern Sudan are recommended.

INTRODUCTION

Brucellosis is a zoonotic disease with a global distribution but is more common in countries with poorly standardised animal and public health programs (Turatbek *et al.*, 2003). The true global prevalence of human brucellosis is unknown because of the imprecision of diagnosis, inadequacy of reporting and lack of surveillance systems in many countries. Brucellosis has been reported from

almost all countries in Africa (Refai, 2002). The prevalence of the disease is unknown in Southern Sudan due to lack of awareness by both veterinarians and health care staff. One of the major impediments to the treatment and control of brucellosis is the diagnosis of new cases. As a consequence brucellosis remains a largely neglected disease with little attention given to control and prevention. In addition, the effect of brucellosis on human health in Southern Sudan is unknown.

Most people in Terekeka County depend on livestock for their food and income. Livestock is also important for social status and marriage. However, this dependence on livestock makes people vulnerable to zoonotic diseases. Animal owners in Terekeka are at risk of exposure to brucellosis because they live in close contact with their animals in cattle camps and drink raw and fermented milk which may contain large numbers of *Brucella* and other pathogenic organisms. No serological and microscopic diagnosis of animal brucellosis has been made in the area.

The objective of this study was to determine factors associated with brucellosis in a rural health facility in Southern Sudan and also to evaluate the utility of the rapid test kit Euracil®.

MATERIALS AND METHODS

Study Design: Case control study.

Study Area: Terekeka Health Facility located in Terekeka Payam in Terekeka County which is one of Central Equatoria State Counties. The county is located approximately 75 Kilometers North of Juba, the capital of South Sudan. The county has a population of 194,847 (census report, 2008). The Health Facility is managed by medical assistants, community health workers (CHWs) and traditional birth attendants (TBAs).

Study Participants: The study participants were recruited from all patients presenting with a febrile illness at Terekeka Health facility during the study period of August to November, 2009. Patients who tested positive for brucellosis using the Rapid Brucella Antigen Test and showing at least two of the following clinical features: headache, recurrent or continuous fever, sweating, joint pain, joint swelling, general body malaise or backache, were defined as brucellosis cases. For every case two hospital based controls having a negative brucellosis result by Rapid Brucella Antigen Test were selected by systematic sampling; every person had a probability of being selected as a control. The study was approved by the ethical review board, Ministry of Health Government of South Sudan and the board of postgraduate studies, Jomo Kenyatta University of Agriculture and Technology.

Information on cases and controls was supplemented with data obtained by interview with study subjects or their parents/caregivers using questionnaires which covered demographic data (marital status, occupation, education level and family size), data on onset of symptoms, and questions on direct as well as indirect exposure to brucellosis.

Laboratory diagnosis: Blood samples (5 ml) were collected by qualified medical personnel from

cephalic vein into properly labeled sterile bottle and centrifuged at 150g for 5 minutes. Sera were collected and stored at -20°C until analyzed. All specimens were screened using rapid brucella antigen test for *B. abortus* and *B. melitensis* antigens according to the manufacturer's instructions (Euremid Equip Ltd, West Harrow, UK).

Briefly, a drop of serum was placed on clean glass slide and a drop of *Brucella* antigen (M) for *Brucella melitensis* was added. Onto a second slide a drop of serum was placed and then a drop of *Brucella abortus* antigen (A) was added. The mixture was rotated by moving the slides. Agglutination occurred within 10 to 15 minutes in the samples positive for *Brucella* antibodies. The negative samples showed no agglutination. Internal positive and negative controls were tested alongside the patient samples for quality assurance. All positive sera with rapid antigen test were subsequently tested using Standard Tube Agglutination Test, (BIOTECH, CHINA) and a titre of $\geq 1:80$ were considered as positive.

Fifty microlitres of serum was diluted with 950 μL normal saline (1:20) and 500 μL from the diluted serum was added in to another tube with 500 μL from brucella antigen (1000 μL) and 500 μL was taken and added into another 500 μL and incubated at 37 °C for 24 hours. The positive results showed agglutination and the negative results did not show any agglutination.

Data management and analysis: This was performed using Epi Info version 3.5 (CDC, Atlanta, USA). Conditional logistic regression was used to study the association between exposure variables and brucellosis. Potential risk factors were first assessed in the univariate analysis. All variables which were significant in univariate analysis were selected for multivariate analysis. The stepwise method of model building was used to arrive at the final statistical model. A p-value of ≤ 0.05 was defined as statistically significant.

RESULTS

Demographic characteristics of study participants: Out of a total of 126 subjects presenting with clinical symptoms of brucellosis, 58 tested positive and they were selected as cases. One hundred and sixteen consented controls were recruited into the study. The females were 48% of the cases and 47% of the controls. The mean age of the cases was 31 years (range 10 - 67 years) and for controls was 31 years (range 4 - 60 years). The disease was most prevalent in housewives (32%). Those without formal education accounted for 86% of the cases while 40% of the cases had no source of income (Table 1). Most of the cases (71%) were in Terekeka Payam.

Table 1
Demographic characteristics of patients presenting with clinical symptoms of brucellosis, Terekeka Health Facility, 2009

Variable	Case (%)	Controls(%)
Sex		
Male	52	53
Female	48	47
Occupation		
Cattle keeper	20.7	14.7
Informal employment	5.2	1.7
Government employed	20.7	28.4
Housewife	32	27.6
Student	5.2	13.8
Unemployed	15	13.8
Formal education		
None	86	56
Basic (primary)	7	22.4
Secondary	7	19
Tertiary	0.00	1.7
Monthly income (Sudanese pounds)		
Zero	40	46.6
100 – 200	24.1	7.0
201 –400	27.6	27.6
401 – 600	8.6	15
> 601	00	4.3

Table 2
Analysis of factors associated with brucellosis in Terekeka Health Facility

Variable	Case%	Controls %	OR 95%CI	P. value
Risk factors				
Consumption of raw meat	12.3	0.9	15.8	0.003
Living with animals	73.7	47.0	3.1	0.0015
Keeping goats	72.4	3.7	3.9	0.000
Farm cleaning	62	44	2.0	0.04
Contact with aborted animal	56.1	19.8	5.1	0.000
Eating aborted fetus	18.4	0.9	18.4	0.001
Eating wild animal	57.9	35.3	2.5	0.004
Milking of animals	60	40	5.6	0.000
Drinking goat's milk	46.6	9.5	8.3	0.0000
Drinking raw milk	70.7	24.1	7.5	0.0000
Protective factors				
Drinking boiled milk	19	79	0.03	0.001
Awareness about brucellosis	50.9	27.4	0.39	0.008
Knowledge of brucellosis transmission	19.3	80.7	0.38	0.01
None statistically significant factors				
Slaughter of animals	15	13	1.1	0.9
Drinking milk	91	81	2.3	0.15
Drinking of cattle's milk	82	81	1.1	0.9

Table 3
Unconditional Logistic Regression Final "Best Model"

Variable characteristics	Odd Ratio	95% CI	P-value
Drinking boiled milk	0.0992	0.041-0.23	0.0001
Drinking goats milk	6.4212	2.28-18.03	0.0004
Drinking raw milk (Yes/No)	3.8879	1.66 - 9.07	0.0017

Figure 1
Clinical symptoms of brucellosis cases in THF Terekeka County South Sudan, 2009

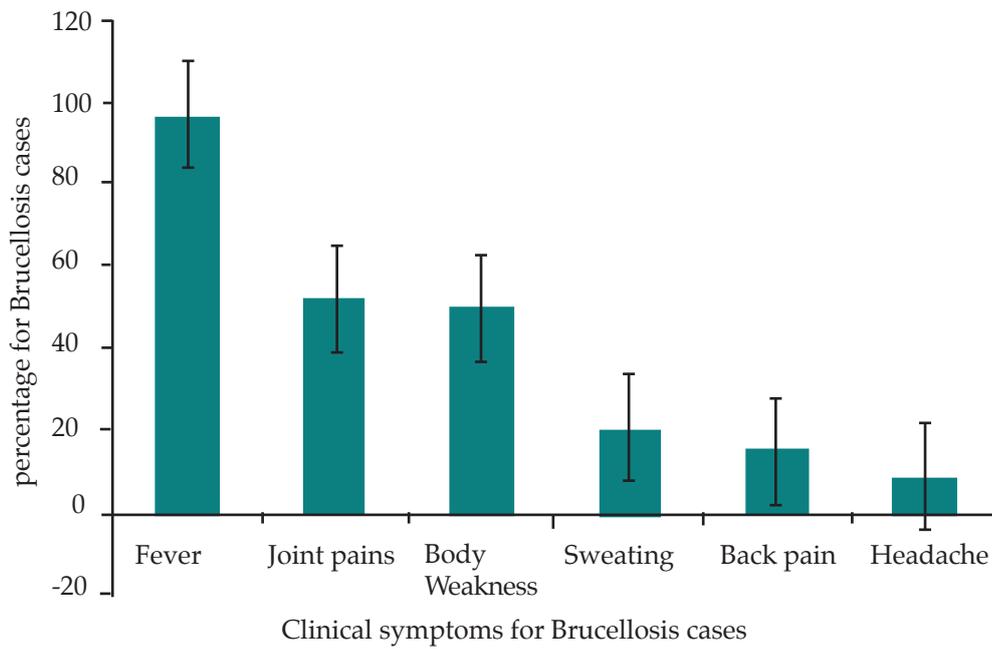
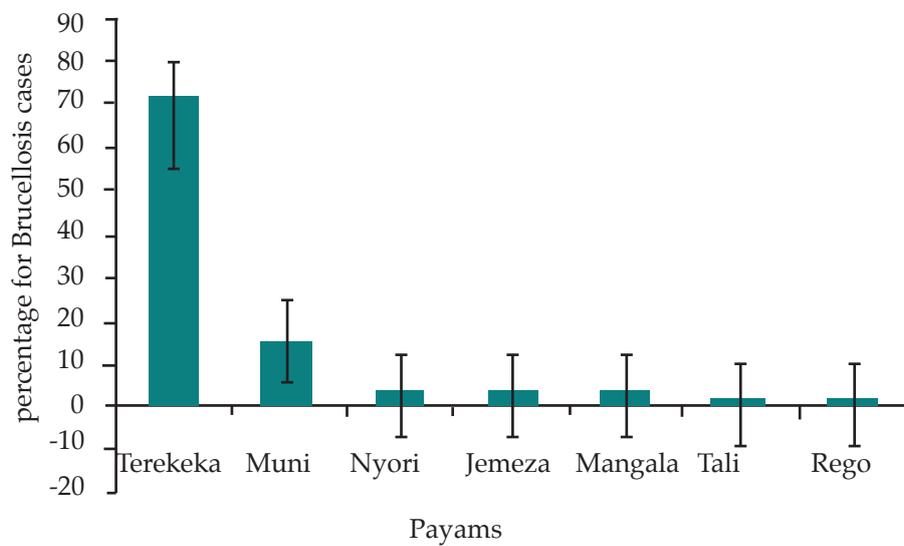


Figure 2
Distribution of brucellosis cases of by Payam THF South Sudan, 2009



Clinical characteristics: Fever was the most common symptom among the cases (97%) followed by joint-pain and generalized body weakness. About 28% of the cases and 34% of the controls had normal body mass index (BMI). However, slightly more than half of the cases (52%) had BMI below normal compared to controls (34%).

Associated factors: The main animal species kept by people of Terekeka were cattle, goats and sheep. Goats were kept by 72% of the cases and 39% of the controls (OR=3.9, $p<0.000$) while 29% of the cases and 21% of the controls were raising sheep (OR=1.5, $P=0.34$). Having animals in the household was a significant risk factor (OR=3, $p=0.001$) but when animal species was considered, the odds for presenting with brucellosis was significantly higher only for goats (OR=3.9, $p<0.000$). Cattle and sheep revealed no significant association with the risk of disease.

Consumption of raw meat was a significant risk factor (OR=15.8, $p=0.003$) while drinking boiled milk (OR=0.03, $p=0.00$) was a protective factor. Slaughtering of animals, drinking of sheep's and cow's milk revealed no significant association with brucellosis. In multivariate analysis there were many factors associated with brucellosis like contact with animals was a significant risk factor (OR=3, $P=0.001$), consumption of raw meat, living with animals at the same place, raising of goats, farm cleaning, eating of aborted animals (OR=18.4, $p=0.001$) and eating of wild animals. The significant factors for brucellosis were entered into a final best fit model where two factors remained significantly associated with brucellosis; drinking raw milk (OR=3.8, $p\text{-value}=0.0004$ was a significant risk factor and drinking boiled milk OR=0.09, $p\text{-value}=0.000$. was a significant protective factor.

Sensitivity and specificity of the rapid test: Detection rate of the rapid test kit was compared with the serum agglutination test kit as the gold standard. Fifty eight sera were positive with rapid test, while 62 were negative with Serum Agglutination Test (OR =0.003, $P\text{-value}<0.000$) (Table 4 and table 5).

Table 4
Comparison between Rapid Test and Serum Agglutination Test used in the brucellosis patients attending THF, S. Sudan 2009

	Rapid test	SAT
Positive	58	56
Negative	68	62

Table 5

Determination of Sensitivity of the Rapid Test kit used for the detection of brucella cases in patients attending THF, S. Sudan 2009

Test result	SAT (+)	SAT (-)	Total
Rapid (+)	56	2	58
Rapid (-)	6	62	68
Total	62	64	126

Sensitivity of the rapid test = $56/56+2 = 97\%$

Specificity of the rapid test = $62/62+6 = 91$

DISCUSSION

Brucellosis is one of the major zoonotic infections worldwide (Pappas *et al.*, 2006). The burden of disease is especially important in developing countries. This is especially so among animal keeping communities. However, due to insufficient reporting systems, the true burden of the problem is not known. The epidemiological data on the disease is frequently incomplete. This is partly explained by the lack of proper laboratory facilities in some remote areas as well as by poor cooperation and exchange of information between veterinary and health services (Sadrizadeh *et al.*, 1999). In this study conducted between August and November 2009, a total of 58 cases and 116 controls were recruited and tested for brucellosis. Human brucellosis is an occupational disease and it mainly affects persons whose occupation brings them into direct contact with animals. This observation was noted in this study where brucellosis was more common in males. This may be because all slaughterhouse workers and animal dealers in this area are males. Moreover, the most common occupation among the men in Terekeka County was cattle keeping.

Although brucellosis was observed in every age-group, this study established that the disease was most common in the 20 to 30-years age group. This was similar to a study done in Mexico by Lopez *et al.*, (1992) and Xue and Fu, (1998) who did a survey in healthy persons between one year old to 89 years old and they found that this age group is chiefly responsible for raising animals hence being at the greatest risk for brucellosis and other animal-borne infections.

In the community studied, housewives were involved with raising animals and this was reflected in the fact that 32% of case patients were housewives. This observation is similar to those made by Lopez *et al.*, (1992). The majority (86%) of the cases in this study had not had any education and was mostly of a low socioeconomic status. This would place the participants at great risk of getting brucellosis. A case-control study conducted in Yemen showed that dealing with animals was a significant risk factor for infection where socio-economic factors and education were independent risk factors (Al Shamahy *et al.*, 2000).

The main health facility in the current study is located in Terekeka Payam approximately a mile from Terekeka Payam Headquarters and this could explain why most cases (71%) were from Payam. Moreover, the health facility was accessible to people living around this area. Besides, some households were far from hospitals and the poor infrastructure made accessibility to healthcare facilities difficult.

The clinical symptoms of brucellosis are not specific. In this study the common clinical symptom was fever (97%) and this was similar to a study in Kuwait which reported that fever was common in brucellosis patients (Mariam *et al.*, 2008). Joint pains occurred in 51% of the cases and generalized body weakness (50%) being observed in the study.

People in contact with livestock and especially having animals in the house were more likely to contract brucellosis than the general population (OR=3, P= 0.001). These findings are similar to observations of a study in Yemen that defined contact with animals as milking, cleaning, shearing, and animal parturition (Al-Shamahy *et al.*, 2000). Although proper heat treatment of milk or milk products is important for the effective prevention of brucellosis in humans, only 15% of the cases were aware that brucellosis can be transmitted by drinking raw contaminated milk. Consumption of raw milk was a common practice in the study area because of the social beliefs that boiled milk is only drunk by persons with fever-like symptoms. This study found that consumption of boiled milk was a significant protective factor (OR= 0.03, P= 0.00). Meat from goats, sheep and cattle infected with brucellosis may also be a source of infection if not properly cooked. Consumption of raw meat was associated with brucellosis (OR=15, P= 0.003). This is similar to findings of a study in Saudi Arabia (Benjamin *et al.*, 1992) who found that consumption of raw liver was a significant risk factor. On the contrary, the consumption of raw meat revealed no association with risk of brucellosis in Yemen (Alshamahy *et al.*, 2000). That could be explained by the fact that raw liver is normally mixed with bile. The bile is thought to be able to reduce the pathogenesis of the bacteria. This mixture is a traditional meal in Sudan called marara. Knowing the mode of brucellosis transmission was protective in this study. This is in agreement with the findings of Turatbek *et al.*, 2003 in his study in Lylek and Kadamjay districts in Kyrgyzstan.

Most clinical laboratories prefer rapid slide agglutination test if the rapid test has been well standardized. When the rapid test was compared to Serum Agglutination Test the sensitivity was found to be 97% and the specificity was 91%. Tests with a high sensitivity are often used to screen for disease. Screening tests tend to cast a wide net in order to pick up all cases of a disease and not miss anyone. However, tests with a high specificity are used to confirm the results of sensitive, but less specific screening tests. People who come up positive on a very sensitive screening test might come up negative on a specific confirmatory test, which means that we can reassure them they do not actually have the disease.

Rapid brucella antigen test can be used in a place where the laboratory facility is adequately equipped or for screening test. However, serum agglutination test can be used as a confirmatory test. In terms of cost and availability the rapid antigen test is inexpensive. One kit contains two different antigen (*Brucella abortus* and *Brucella melitensis*) that can perform 100 samples and costs 25 dollars thus one test costs 25 cents. Conversely the Serum agglutination test is a confirmatory test and needs well developed laboratory to perform this test. The test costs \$5 per test and is only available in Khartoum, North Sudan.

In conclusion, this study confirms the presence of *Brucella* species among humans in Terekeka County, Central Equatoria State Southern Sudan. It is the first of its kind to be carried out in post-war Southern Sudan. The study reveals the public health implications of Brucellosis in agro-pastoral and pastoral communities. The main affected age-groups were 20 to 30 years old and the prevalence of the disease is higher in males than in females. Consumption of raw milk, contact with animals were the main risk factors for brucellosis while drinking boiled milk was protective for brucellosis. The rapid test is an inexpensive screening test for brucellosis.

RECOMMENDATIONS

There is a need for the implementation of an active surveillance and control program for human and animal brucellosis in this area in particular and in Southern Sudan in general. Further studies to elucidate the epidemiology of brucellosis in humans and animals in Southern Sudan are recommended.

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