THE ROLE OF BRUCELLOSIS IN SPONTANEOUS ABORTION

AT NAROK DISTRICT HOSPITAL

A research dissertation submitted to the University of Nairobi, Department of Obstetrics and Gynaecology as part fulfillment of the requirements for the award of the degree of Master of Medicine in Obstetrics and Gynaecology by:

Dr Onzere Norris Ijayo

H58/70851/2007

INVESTIGATOR: Dr Onzere Norris Ijayo

Registrar Department of Obstetrics and Gynaecology, University of Nairobi

SUPERVISORS: Dr. Samson Wanjala

Senior Lecturer Department of Obstetrics & Gynaecology, University of Nairobi

Consultant Obstetrician and Gynaecologist, Kenyatta National Hospital

Dr. Lubano Kizito

Lecturer Department of Obstetrics & Gynaecology, University of Nairobi

Consultant Obstetrician and Gynaecologist

Assistant Director Kenya Medical Research Institute

DECLARATION

This dissertation is my original work and has not been presented elsewhere to the best of my knowledge. Reference to work done by others has been clearly indicated.

Signature..... Date..... 07/07/11.

Dr. Onzere Norris Ijayo

Approval:

This dissertation has been submitted with our approval as University supervisors:

Signature..... Date....07/07/11

Dr Samson Wanjala

Signature..... Date.....7/7/2011

Dr. Lubano Kizito

CERTIFICATE OF AUTHENTICITY

This is to certify that this dissertation is the original work of Dr Norris Ijayo Onzere

Master of Medicine student in Department of obstetrics and Gynaecology,

Registration number H58/70851/2007 University of Nairobi (2007-2011). The research was carried out in the department of obstetrics and Gynaecology, School of Medicine, College of Health Sciences. It has not been presented in any other university for award of a degree.

Signature.....

Date.....12/5/11.....

Prof. Koigi Kamau,

Associate Professor of Obstetrics and Gynaecology

Consultant Obstetrician and Gynaecologist,

Chairman,

Department of Obstetrics and Gynaecology,

University of Nairobi

ACKNOWLEDGEMENT

To patients and staff of Narok District Hospital for allowing me to carry out this study in an enabling environment.

To my University of Nairobi supervisors, Dr.Samson Wanjala and Dr. Lubano Kizito for their invaluable guidance and support.

To the Department of Obstetrics and Gynaecology University of Nairobi

To Mr. Francis Njiri for statistical design, data management and analysis

THANK YOU

DEDICATION

To my wife and daughter

LIST OF ABBREVIATIONS

Ab.....Antibody

BD.....Twice Daily

DIC.....Disseminated Intravascular Coagulation

ELISA.....Enzyme Linked Immunosorbent Assay

HIV.....Human Immunodeficiency Virus

I/M.....Intramuscular

IUFD.....Intrauterine Fetal Death

KNH.....Kenyatta National Hospital

LNMP.....Last Normal Menstrual Period

OD.....Once Daily

OR.....Odds Ratio

P/O.....Per Oral

RR.....Relative Risk

SAb.....Spontaneous Abortion

SD.....Standard Deviation

SPSS.....Statistical Package for Social Sciences

VDRL.....Venereal Disease Research Laboratory

WHO.....World Health Organization

LIST OF FIGURES

LIST OF TABLES

TABLE OF CONTENTS

ABSTRACT

Background: Brucellosis is a zoonotic infection caused by bacteria of the genus brucella. It is endemic in the Middle East, the Mediterranean and Africa. Brucella is a major aetiological agent of spontaneous abortion in cattle but its role in humans remains controversial. This study was designed to explore the relationship between maternal brucellosis and spontaneous abortion in an endemic area in Kenya.

Design: Case control study

Setting: This study was based at Narok District Hospital in Narok County.

Objectives: 1) To evaluate the serum brucella antibody titres in women presenting with spontaneous abortion compared to women with live deliveries at term. 2) To determine the association between brucellosis and spontaneous abortion in humans.

Methods: A total of 324 women were enrolled into the study.82 women with spontaneous abortion were enrolled as cases while 242 with live deliveries after 37 completed weeks of gestation and who in addition had never had an abortion served as the control group. A standard tube agglutination test was performed for estimation of brucella antibody titers. Titers of $\geq$ 1:160 were considered positive for brucellosis.

Results: Out of the 82 cases of spontaneous abortion, 22 had brucella antibody titres $\geq$ 1:160 while 37 out of a total of the 242 of the women in the control group were positive. There was a strong association between brucella antibody titres $\geq$ 1:160 and spontaneous abortion OR=2.03; 95% C.I (1.114-3.705).

Conclusion

Women with brucella antibody titres ≥1:160 are twice at risk of having a spontaneous abortion as compared to those with titres <1:160.

Recommendations:

In endemic areas educating women of childbearing age on brucellosis prevention may help to prevent the disease and its complications in pregnancy. It is further recommended that testing for human brucellosis should be carried out in at risk women with spontaneous abortion as part of their management. Further studies should be carried out to determine the association between brucella seropositivity and adverse pregnancy outcomes after twenty weeks of gestation.

1.0 BACKGROUND AND LITERATURE REVIEW

Brucellosis is a zoonosis transmitted to humans primarily through the consumption of infected animals or their products(1).Worldwide, the reported incidence varies widely, from <0.01 to >200 per 100 000 population (2). Brucellosis remains a major public health problem in developing countries with prevalence rates of 19.2% being reported for Saudi Arabia (3), 7.6% for Nigeria(4) and 13.3% for Uganda(5). Maichomo et al (6) working in Narok district of Kenya found a prevalence of 12%. Brucellosis is a systemic disease that can involve any organ or system of the body. In humans, the clinical spectrum of disease can vary from asymptomatic to - severe forms. Disease is mostly asymptomatic, and is usually diagnosed by serological testing in the endemic areas and among the high-risk groups (7).Brucellosis is a well established cause of spontaneous abortion in animals but its role in humans is lower. This is principally due to the presence of erythritol, a sugar alcohol occurring in high concentration in placental tissue which is believed to play an important role in the localization of brucella (8). An additional reason for the less significant role of brucella infection in human abortion is the presence of anti-brucella activity in the human amniotic fluid (9). It is postulated that maternal bacteremia, toxaemia, acute febrile reaction and DIC are causes of spontaneous abortion and IUFD in brucellosis (10). Bone marrow cultures are considered the gold standard for the diagnosis of brucellosis (11) however, harvesting bone marrow for culture is an invasive procedure and results are not universally reproducible. The serum agglutination test remains the most popular diagnostic tool for brucellosis. Titers above 1:160 are considered diagnostic in conjunction with a compatible clinical presentation(12).Spontaneous abortion (SAb) which refers to the expulsion of an embryo or fetus of gestational age of 20 weeks or less without medical or mechanical means (13) is a common complication of early pregnancy (14). Up to 20% of clinically recognized pregnancies

less than 20 weeks of gestation undergo SAb (15-17).Approximately 60% of embryos and early fetuses that are spontaneously aborted contain chromosomal anomalies. Maternal factors such as anatomical uterine anomalies, infections, chronic illnesses, endocrine disorders, immunological factors, environmental factors and trauma contribute the remaining 40% (18).The role of infective causes in spontaneous abortion remains controversial. Toxoplasmosis, rubella, cytomegalovirus, herpes simplex, syphilis, brucellosis, listeriosis, mycoplasma, ureaplasma and HIV have all been associated with spontaneous abortion in humans (19). Investigators vary in the importance they place on Brucella as a cause of spontaneous abortion in humans. Several studies carried out in brucellosis endemic areas have shown that both symptomatic and asymptomatic brucellosis can cause spontaneous abortion. In a study conducted in Saudi Arabia, Maged et al (20) assessed the outcome of pregnancies complicated by Brucella infection and found a 27.27% incidence of abortion in pregnant women infected with brucellosis while Sarram et al (21) observed an 11.6 % incidence in Iran. Other studies have found a much higher incidence with Madkour et al and Lulu et al reporting incidences of 40% and 35% respectively (22,23).In his work, Khan et al (24) demonstrated a 43 % incidence and in addition showed that occurrence of abortion was not associated with either the magnitude of the serum agglutinin titre or the presence of Brucella bacteraemia.Mertihan et al (25) analysed the reproductive outcomes (spontaneous abortion, intrauterine fetal death, preterm delivery) of pregnant women with brucellosis and found that only the spontaneous abortion rate substantially exceeded that among the general population without an association with the clinical type of brucellosis.Although brucellosis can also result in human abortion, it has been debated whether it is any more frequent than with the other bacterial infections (26). In a case control study in Iran, Nassaj et al (27) showed that brucella seropositivity was no more common in women with spontaneous abortion

than in normal pregnancy while Seoud et al (28) in his works demonstrated a lesser role for Brucella infection in human abortion.Fernihough et al (29) carried out a study in Natal province of South Africa and concluded that brucella seropositivity was similar in women with spontaneous abortion as in those with a normal pregnancy outcome.

2.0 JUSTIFICATION, RESEARCH QUESTIONS, HYPOTHESIS AND OBJECTIVES

2.1 Justification

Human brucellosis remains a major public health problem in developing countries including Kenya due to the consumption of unpasteurized dairy products (30). In its annual health sector status report that covered the years 2005-2007, the Ministry of Health identified brucellosis as a reemerging infection especially in Rift Valley Province (31).With the increase in reported cases of human brucellosis comes a possible rise in the numerous sequelae associated with the disease (32-36). Controversy surrounds the relationship between brucellosis and spontaneous abortion in humans. Evidence from studies conducted in brucella endemic areas suggests that brucellosis causes a higher rate of abortion than other bacterial infections whereas other investigators working in similar settings have not been able to demonstrate the same. Investigating for all the known causes of spontaneous abortion in women presenting at health facilities is costly especially in resource constrained settings and thus a need for the use of tests whose utility is supported by scientific evidence. Despite the finding of a high prevalence in Narok (12%), no study to our knowledge had been conducted to determine the role of human brucellosis in spontaneous abortion. This study was therefore designed to carry out such an analysis.

2.2 Research Questions

a) What are the serum brucella antibody titres in women presenting with spontaneous abortion at Narok district hospital?

b) What are the serum brucella antibody titres in women with live births at term at Narok district hospital?

2.3 Hypothesis

Brucella seropositivity is more common among women with spontaneous abortion compared to women with live births at term.

2.4 Objectives

2.4.1 General Objective

To determine the association between high brucella titres (≥1:160) with spontaneous abortion.

2.4.2 Specific Objectives

1. To determine serum brucella antibody titres in women with spontaneous abortion at Narok district hospital.
2. To determine serum brucella antibody titres in women in women with live births at term at Narok district hospital
3. To compare brucella seropositivity in women with spontaneous abortion with that of women with live births at term at Narok district hospital.
4. To determine factors associated with brucella seropositivity in women with spontaneous abortion and women with live births at Narok district hospital.

3.0 MATERIALS AND METHODS

3.1 Study Design

This was an unmatched case control study. Women presenting with spontaneous abortion were enrolled as cases while those with a live birth after 37 completed weeks of gestation who had never had an abortion served as the control group. In both groups, there was no positive history of diabetes mellitus, thyroid disease or previous treatment for brucellosis and all had been residents of Narok district for more than one year.

3.2 Study Site: Narok District Hospital

Narok District lies in the southern part of the Rift Valley Province of Kenya covering approximately 15,000 square kilometres two thirds of which is semi-arid (37). The district has an estimated population of 431,363 most of whom practice a semi-nomadic lifestyle traditionally relying on meat, milk and blood from cattle for their dietary needs. This population is served by Narok district hospital which is also a referral facility for 19 health centers and 40 dispensaries. The maternity unit is manned by registered midwives who handle an average of 220 deliveries a month. A medical officer is on standby to assist in conducting difficult vaginal deliveries and carrying out emergency caesarean sections where indicated. After delivery, women are counseled on nutrition, lactation, danger signs in puerperium and care for the newborn .Upon discharge, they are reviewed at the maternal and child welfare clinic after a period of two weeks. The gynaecology unit offers post abortal care services to an estimated 50 patients every month. Uncomplicated cases are managed by clinical officers at the accident and emergency department as outpatients while those with various degrees of complications such as sepsis or anaemia are

admitted to the gynaecology ward where they are reviewed and managed by a medical officer in consultation with the resident gynaecologist. All patients with abortion receive emergency treatment for complications of spontaneous or induced abortion including but not limited to manual vacuum aspiration and counselled on contraceptive use. Those with recurrent abortions and sexually transmitted infections are referred to the gynaecology clinic where they are followed up by the resident obstetrician and gynaecologist. The hospital has a fully equipped laboratory manned by a medical laboratory technologist who routinely tests for human brucellosis by use of the standard-agglutination test. Non pregnant patients with brucella antibody titres $\geq$ 1:160 are treated with IM Streptomycin 1g OD for a period of three weeks in combination with P/O Doxycycline 100mg BD for a period of 6 weeks by clinical officers at the accident and emergency department while those who are pregnant receive P/O Rifampicin 600mg OD for a period of 6 weeks under the care of the resident obstetrician and gynaecologist at the antenatal clinic.

3.3 Study Population

Women presenting with spontaneous abortion and women with a live birth after 37 completed weeks of gestation who had never had an abortion.

3.4 Operational Definition of Cases and Controls

3.4.1 Definition of Cases

In accordance with the WHO 1987 protocol for distinguishing induced from spontaneous abortion , women presenting with expulsion of an embryo or fetus of gestational age of 20 weeks or less stating that the pregnancy was planned and not practicing contraception were considered as cases.

3.4.2 Definition of Controls

Women delivering a live infant vaginally or via caesarean section after 37 completed weeks of gestation and who in addition had never had an abortion.

3.5 Inclusion Criteria For Cases And Controls

3.5.1 Cases

Written informed consent

Woman stating the pregnancy was planned

Not practicing contraception

3.5.2 Controls

Woman delivering a live infant after 37 completed weeks of gestation

Woman who had never had an abortion

Written informed consent

3.6 Exclusion Criteria For Cases and Controls

Positive history of diabetes

Positive history of thyroid disease

Not a resident of Narok district for more than a year

Previous treatment for brucellosis

3.7 Sample Size Determination

Maichomo et al (6) working in Narok district of Kenya found a human brucellosis prevalence of 12%.Using OpenEpi software* the hypothetical proportion of cases with exposure was taken as 25% while that in the controls put at 12%. The ratio of cases to controls was set at 1:3 in order to achieve a 95% confidence interval and a power of 80.Using this method a total sample size of 324 was obtained comprising 82 cases and 242 controls.

*OpenEpi version 2.3 Copyright (c) 2003, 2008 Andrew G. Dean and Kevin M. Sullivan, Atlanta, GA, USA

3.8 Sampling Technique

3.8.1 Cases

All patients presenting with spontaneous abortion who met the inclusion criteria were sampled until the required sample size was attained.

3.8.2 Controls

All women delivering a live infant after 37 completed weeks of gestation who met the inclusion criteria were sampled until the required sample size was attained.

04.0 PROCEDURES

4.1 Recruitment

4.1.1 Cases

All women who presented with spontaneous abortion either at the acute gynaecology ward or the accident and emergency department were recruited by a research assistant conversant with the research protocol.

4.1.2 Controls

For every patient with spontaneous abortion recruited, 3 women who had delivered a live infant/s after 37 completed weeks of gestation either vaginally in the labour ward or via caesarean section and who had never aborted were recruited as controls.

4.1.3 Recruitment Protocol

4 i.3.1Cases

Recruitment of cases was done by the principal investigator and eight research assistants comprising of two clinical officers and six nurses who before commencement of the study, had undergone a one day training on the nature of the study, screening protocols, standard operating procedures of recruitment (appendix 1) and filling in of the questionnaire. Cases were recruited at either the acute gynaecology ward or the accident and emergency department. The nature of the study, its benefits and possible risks was explained to the participants as outlined in the consent explanation form in English (appendix 2) or Swahili (appendix 3). Women willing to participate signed a written consent in either English (appendix 4) or Swahili (appendix 5) before

recruitment and allocation of a study number. A standardized structured questionnaire (appendix 8) was then administered to obtain demographic and clinical data. 5 ml of blood was thereafter drawn and sent to Narok District Hospital laboratory microbiology unit for determination of brucella antibody titres according to the standard operating procedure for testing (appendix 9). Those with brucella antibody titres $\geq$ 1:160 were referred for appropriate care. Women with spontaneous abortion received post abortal care at Narok district hospital as per the standard National guidelines. All participants otherwise received treatment and management of any conditions noted during the study as per the standard National guidelines. Results of syphilis, HIV and any other serological tests if available were captured by use of the questionnaire. Patients who did not consent to participate and those found not eligible as per screening protocol were discharged from the study.

4.1.3.2Controls

Recruitment of controls was done by the principal investigator and eight research assistants comprising of two clinical officers and six nurses who before commencement of the study, had undergone a one day training on the nature of the study, screening protocols, standard operating procedures of recruitment (appendix 1) and filling in of the questionnaire. Controls were recruited at the labour ward of Narok district hospital. The nature of the study, its benefits and possible risks was explained to the participants as outlined in the study explanation form in either English or Swahili. Women willing to participate signed a consent form in either English or Swahili before recruitment and allocation of a study number. A standardized structured questionnaire (appendix 5) was then administered to obtain demographic and clinical data. 5 ml of blood was thereafter drawn and sent to the laboratory for determination of brucella antibody

titres according to the standard operating procedure for testing. Those with brucella antibody titres ≥ 1:160 were referred for appropriate care. Women with live births at term were referred for appropriate postnatal care. All participants otherwise received treatment and management of any conditions noted during the study as per the standard National guidelines. Results of syphilis, HIV and any other serological tests if available were captured by use of the questionnaire. Patients who did not consent to participate were discharged from the study.

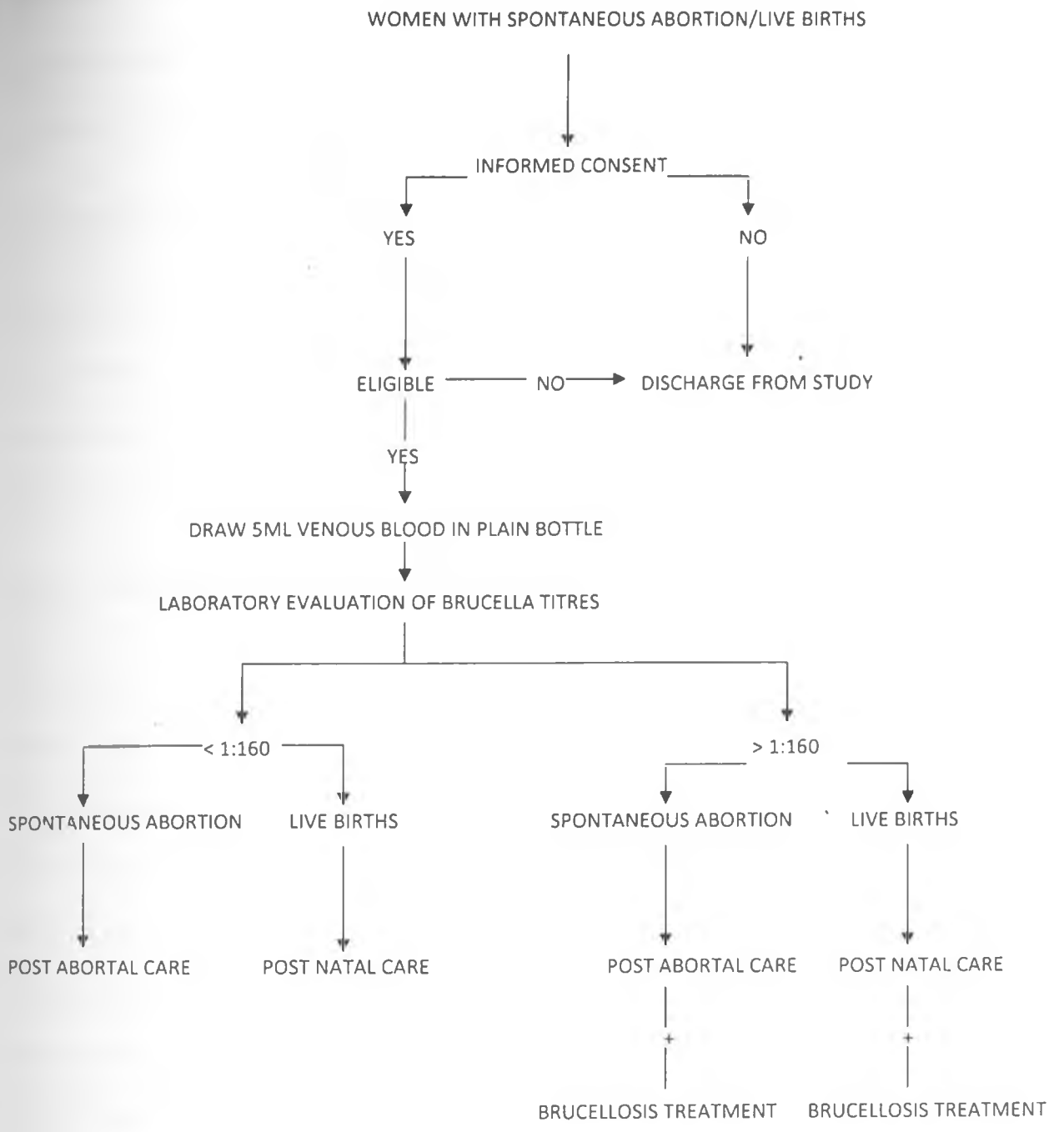

Figure 1: General recruitment and testing protocol

4.2 Sample Collection

5ml of venous blood was drawn from the antecubital vein into a plain vacutainer bottle and labeled with the appropriate study number.

4.3 Sample Storage and Transport

Samples collected between 8:00am and 6:00pm were immediately transferred to the microbiology unit of Narok District Hospital for analysis of brucella antibody titres. Those collected between 6:00pm and 8:00am were stored in a cooler box at between 2-8^{o} c before transportation to the laboratory.

4.4 Sample Processing

Samples underwent laboratory analysis of brucella antibody titres according to the procedure recommended by the manufacturers of Febrile antigen® (Fortress Diagnostics Limited U.K).Blood samples were kept at room temperature to clot then centrifuged at 3000rpm for 10 minutes to enable serum to separate.8 small round bottom tubes were then labeled 1 to 8. By adding 0.1 ml serum to 1.9 ml normal saline a 1/20 dilution was obtained. I ml of this was transferred to tube 2 and 1.0 ml normal saline added providing a 1/40 dilution. This process was repeated until a 1/1280 dilution obtained in tube 7.Tube 8 served as a control containing only saline. One drop of the brucella antigen suspension was then added to each tube and mixed well. The samples were incubated at 37°C in a water bath for 24 hours. Each tube was examined for signs of agglutination. The titre to be considered was the last tube to show agglutination. Titers of $\geq$ 1:160 were considered positive for brucellosis. The antigen suspension used did not differentiate between positivity for Brucella abortus and Brucella melitensis the two main species

that have been equally associated with spontaneous abortion in humans. It was however employed since clinically, identification to the genus level is adequate to initiate therapy.

4.5 Quality Control

The research assistants received a one day training that focused on the nature of the study, standard operating procedures for recruitment (appendix 1), screening protocols, and filling in of the standardised structured questionnaire. By having the questionnaire standardised and structured, the same set of questions were administered to the participants and in the same order. This ensured that the answers provided were within the same context as the questions asked, could be reliably analyzed and comparisons made between the cases and controls.

Pre-analytical quality control measures including use of suitable serum (non-contaminated, non-hemolysed or non-lipemic samples) and standard reagents were employed. Analysis of brucella antibody titres was done according to the procedure recommended by the manufacturers of Febrile antigen® (Fortress Diagnostics Limited U.K) to ensure analytical quality control. Post analytical quality control was achieved by having the brucella antibody titre interpreted as per the reagent manufacturers.

5.0 DATA MANAGEMENT

5.1 Data Collection

A standardized structured questionnaire (appendix 5) was administered to eligible patients.

5.2 Data Storage

Data collected during the study was manually entered into a Microsoft access database.

5.3 Data Cleaning

To reduce errors in data entry, manual verification by the data entry clerk was done.

5.4 Data Analysis

The data was analyzed using SPSS version 15.0. Univariate analysis was used initially in analyzing the data before being subjected to further bivariate and multivariate analysis. All the quantitative parameters were expressed as a mean with standard deviation in both groups. The student t-test was used to analyse for differences in the mean values between the cases and controls for quantitative parameters following a normal distribution while the Mann-Whitney test was applied to skewed data. The Chi-square test tested for differences in proportions between categorical variables. Statistical significance was taken as $p < 0.05$. To quantify the association between human brucellosis and spontaneous abortion a 95% confidence interval together with an odds ratio of 2.4 was employed. Because the cases and controls were unmatched, logistic regression analysis was performed to estimate the effect of suspected risk factors on spontaneous abortion.

6.0 ETHICAL CONSIDERATIONS

6.1 Consent Explanation Form

The principal investigator or a research assistant explained the purpose and nature of the study, its benefits and potential risks to the participants as contained in the consent explanation form in either English (appendix 2) or Swahili (appendix 3).

6.2 Consent Form (written consent)

Written consent was obtained in either English (appendix 4) or Swahili (appendix 5) from eligible participants before they took part in the study. Patients who consented but were unable to write were assisted by the PI or a research assistant to have a thumb print of either hand taken in place of their signature.

6.3 Privacy and Confidentiality

The principal investigator ensured auditory and visual confidentiality and privacy of participants during the study. After enrolment, participants were identified by their study numbers only. Paper records were secured in locked cabinets while those in electronic format were password protected. Patient study numbers and results were delinked from the demographic data. Only the principal investigator was able to link the data to enable communication of test results to the patient and forwarding to their clinician for appropriate treatment in cases of infection. Access to records was limited to researchers and data entry clerks involved in the study.

6.4 Ethical Review and Clearance

The study protocol was submitted to the Department Of Obstetrics and Gynaecology -University of Nairobi and ethical clearance obtained before further approval by the Kenyatta National Hospital Scientific and Ethical Review Committee. It was then presented to the Narok District Hospital management committee who reviewed and gave authorisation before commencement of the study.

6.5 Study Limitations

This study had the following limitations:-

1. Inability to completely rule out cases of medically and mechanically induced abortions.

2. Possible false positives due to cross-reactions of class M immunoglobulins with other gram negative bacilli sharing common antigens.

3. Serological tests for other infectious agents associated with spontaneous abortion such as toxoplasmosis, rubella, cytomegalovirus, herpes simplex and HIV were not conducted. In addition, tests for known genetic, anatomic, endocrine and immunologic causes of spontaneous abortions were not carried out.

3. Recall limitation and bias

4. Use of hospital controls which were not a true representation of the general population.

7.0 RESULTS

Table 1: Socio-demographic Variables		
	Cases	Controls
Variable	N=82 (Freq %)	N=242 (Freq %)
Marital status		
Single	19(23.2)	27(11.2)
Married	63(76.8)	215(88.8)
Residence		
Rural	53(64.6)	156(64.5)
Urban	29(35.4)	86(35.5)
Formal Education		
Primary	28(34.2)	75(31.0)
Secondary	32(39.0)	110(45.5)
Tertiary	20(24.4)	54(22.3)
None	2(2.4)	3(1.2)

As shown in table 1 above, a total of 324 subjects (82 cases, 242 controls) were enrolled into study. 23.2% of women in the case group were single as compared to 11.2 % of controls. Among the cases 64.6% resided in rural areas while those in the control group were 64.5%. Majority of the study participants had some formal education with only 2.4% of the cases and 1.2% of the controls having received no formal education.

Table 2: Sharing of House With Livestock

	Cases N=82	Controls N=242
Yes	42	147
No	40	95

As shown in table 2 above, a total of 42 cases lived in the same house with livestock as compared to 147 controls though the difference was not statistically significant (P=0.4623).

Table 3: Handling of Raw Animal Products

	Cases N=82	Controls N=242
Yes	20	33
No	62	209

OR= (20 x 209) ÷ (62 x 33) = 2.04

Handling of raw animal products such as meat, milk, blood and hides was noted among 20 cases and in 33 controls. The association with spontaneous abortion was found to be significant OR=2.04 (95% CI=1.095-3.811)

Table 4: Consumption of Raw Animal Products

		Control		Case		
		N=242	Freq (%)	N=82	Freq (%)	OR(95% CI)
Meat Consumer	No	201	83.1%	58	70.7%	2.03 (1.133-3.631)
	Yes	41	16.9%	24	29.3%	
	Total	242	100.0%	82	100.0%	
Milk Consumer	No	162	66.9%	54	65.9%	
	Yes	80	33.1%	28	34.1%	
	Total	242	100.0%	82	100.0%	
Blood Consumer	No	219	90.5%	72	87.8%	
	Yes	23	9.5%	10	12.2%	
	Total	242	100.0%	82	100.0%	

Since cases and controls were unmatched for history of raw meat, milk and blood consumption, data was analyzed by logistic regression method and a significant relationship only for raw meat consumption was found OR=2.03 (95% CI=1.133-3.631).

Table 5: HIV and VDRL Serostatus

		Case		Control		Total	
		N=82	Freq (%)	N=242	Freq (%)	N=324	Freq (%)
HIV	Negative	60	73.2%	221	91.3%	281	86.7%
	Positive	6	7.3%	9	3.7%	15	4.6%
	Unknown	16	19.5%	12	5.0%	28	8.6%
	Total	**82**	**100.0%**	**242**	**100.0%**	**324**	**100.0%**
VDRL	Negative	41	50.0%	176	72.7%	217	67.0%
	Positive	0	0%	2	0.8%	2	0.6%
	Unknown	41	50.0%	64	26.4%	105	32.4%
	Total	**82**	**100.0%**	**242**	**100.0%**	**324**	**100.0%**

Results of previous serological tests were not readily available especially among cases. As a result, the HIV serostatus remained unknown in 19.5% of cases while VDRL results were unavailable in 50%. Consequently, analysis for a possible association between spontaneous abortion and HIV and VDRL serostatus could not be done. However, it was noted that among controls, 3.7% were positive for HIV and 0.8% for VDRL.

Table 6: High Brucella Titres in Cases and Controls

	CASES N=82	CONTROLS N=242
>1:160	22	37
<1:160	60	205

OR= (22 x 205) ÷ (60 x 37) =2.03

As shown in table 6, there were 22 cases that tested positive for brucellosis as compared to 37 in the control group. This was statistically significant OR=2.03 (95% C.I=1.114-3.705)

Table 7: High Brucella Titres and Number of Spontaneous Abortions

	Number of Spontaneous Abortions N=82				
	1	2	3	>3	Total
≥1:160	11	5	2	4	22
<1:160	26	23	5	6	60

Of the total number of cases, 22 tested positive for brucellosis. A direct correlation between the brucella antibody titre level and the number of spontaneous abortions could not be established.

Table 8: High Brucella Titres and Number of Live Births

	Number of Live Births N=242				
	1	2	3	>3	Total
≥1:160	3	10	4	20	37
<1:160	25	57	58	65	205

As captured in table 8, a total of 37 women with live births tested positive for brucellosis. A direct correlation between the brucella antibody titre level and the number of live births could however not be established.

8.0 DISCUSSION

Spontaneous abortion (SAb) is a common complication of early pregnancy (14). Up to 20% of clinically recognized pregnancies less than 20 weeks of gestation undergo SAb (15-17).Approximately 60% of embryos and early fetuses that are spontaneously aborted contain chromosomal anomalies. Maternal factors such as anatomical uterine anomalies, infections, chronic illnesses, endocrine disorders, immunological factors, environmental factors and trauma contribute the remaining 40% (18).

The association of infection with abortion is a very controversial issue and has only been partly explored as a potential cause of abortion (19). Investigators vary in the importance they place on brucella as a cause of spontaneous abortion in humans. Several studies have shown that both symptomatic and asymptomatic brucellosis can cause spontaneous abortion.

It is postulated that maternal bacteremia, toxaemia, acute febrile reaction and DIC are causes of spontaneous abortion and IUFD in brucellosis (10). In our study, all subjects had similar sociodemographic characteristics, were in good general condition and had their vital signs (temperature, pulse, respiratory rate, blood pressure) within normal range.

In our study, out of the 82 cases of spontaneous abortion a total of 22 had brucella antibody titres $\geq$ 1:160 while 37 of the 242 of the women in the control group were positive. There was a strong association between brucella antibody titres $\geq$ 1:160 and spontaneous abortion OR=2.03; 95% C.I (1.114-3.705).

However, the study was not large enough to establish a correlation between brucella antibody titre levels and the total number of pregnancies lost. These findings are in agreement with the

study by Khan et al (24). Malone et al. (38), Makhseed et al. (39) and Maged et al (20); however, these results are contrary to the studies of Seoud et al. (9) and Nassaj et al (27) who stated that brucella infection has a lesser role in human abortion.

Brucellosis is transmitted to humans primarily through the consumption or contact with infected animals or their products (1).In our study, handling of raw animal products such as meat, milk, blood, or hides was noted among 20 cases and in 33 controls. The association with spontaneous abortion was found to be significant OR=2.04 (95% CI 1.095-3.811).
Raw meat consumption was noted among 24 cases and 41 controls. Its association with spontaneous abortion was also noted to significant OR=2.03 (95% CI 1.133-3.631).

Syphilis and HIV have, among other infectious agents, been associated with spontaneous abortion in humans (19).Screening for these two conditions also forms part of the routine antenatal care offered to pregnant women presenting at health facilities. Our study did not include serological testing for HIV and VDRL. However, results of these tests where available were captured from the patient's records by use of the questionnaire. The incidental finding was that in a majority of the cases, antenatal profile screening for these two conditions was not complete with the HIV serostatus being unknown in 19.5% of cases and VDRL results unavailable in 50%,a possible indicator of the quality of antenatal care among this population. However, HIV and VDRL screening was complete in all subjects among the controls. Among this group, 3.7% were positive for HIV and 0.8% for VDRL. Due to the antenatal profile screening not being complete, analysis for a possible association between spontaneous abortion and HIV/VDRL serostatus could not be done.

9.0 CONCLUSION

Women with brucella antibody titres ≥1:160 are twice at risk of having a spontaneous abortion than those with titres <1:160.

10.0 RECOMMENDATIONS

In endemic areas educating women of childbearing age on brucellosis prevention may help to prevent the disease and its complications in pregnancy. It is further recommended that testing for human brucellosis should be carried out in at risk women with spontaneous abortion as part of their management.

Further studies need to be carried out to determine the association between brucella seropositivity and adverse pregnancy outcomes after twenty weeks of gestation.

11.0 APPENDICES

Appendix 1: Standard Operating Procedure for Recruitment

- Patients presenting with spontaneous abortion and those with live births after 37 completed weeks of gestation who have never had an abortion will be presented with a consent explanation form to read before being asked to give a written informed consent. If they are unable to read, a research assistant will help explain the contents of the consent explanation form to the patient and guide them in signing the consent form or taking their thumb print if they give verbal consent to take part in the study.
- Participants will then be screened for eligibility according to the defined protocol.

Those found eligible will be assigned a study while those who do not qualify will be discharged from the study.

Appendix 2: Consent Explanation Form (English Version)

Introduction and Objectives of the study

You are invited to participate in a research study conducted by investigators from the University Of Nairobi School Of Medicine, to find out the role of brucellosis in spontaneous abortion in humans. Brucellosis is a zoonosis transmitted to humans primarily through the consumption of infected animals or their products. The disease has been associated with spontaneous abortion in animals but the role in humans remains unclear. The researchers would like to find out the association between infection with brucellosis and spontaneous abortion in humans.

Benefits and risks of the Study to you

Benefits

- Participating in the study at no cost
- Receive health education on the causes of human brucellosis, its mode of transmission, symptoms, complications, treatment options available and how to prevent infection if found negative and reinfection if you are found positive.
- Be referred for appropriate care if diagnosed with brucellosis.
- If you have had a spontaneous abortion, you will receive information regarding the known causes, current management and referral for specialized care and follow up in the gynaecology clinic at Narok District Hospital.

Risks

- 5 ml of blood will be drawn from your forearm. The prick will be painful.

If you agree to participate, you will:

- Sign a Consent form
- Be asked a few questions to assess your eligibility
- Answer a number of questions contained in the study questionnaire
- Have your blood pressure, pulse, temperature and respiratory rate taken
- Have 5ml of blood drawn from your forearm for laboratory estimation of your brucella antibody titers
- Be referred for appropriate care if found positive for human brucellosis
- Receive appropriate post abortal care if you have presented with spontaneous abortion
- Be referred for standard post natal care if you will have delivered a live infant after 37 completed weeks of gestation

Participation is voluntary and you can withdraw at any time. Any information given to us will remain confidential. You may ask questions regarding the study now or at any time during the study.

If you have any question relating to the study, kindly contact:

1. Dr Onzere Norris Ijayo Tel No. 0722665865

2. The Secretary to the Ethical Research Committee. KNH Tel No. 272260 Ext. 44102

Appendix 3: Consent Explanation Form (Swahili version)

Fomu ya maelezo kuhusu utafiti

Azma ya Utafiti

Umealikwa kushiriki katika utafiti unaofanywa na watafiti kutoka Chuo Kikuu cha Nairobi (bewa la tiba) ili kuchunguza uhusiano wa ugonjwa wa brucellosis na tatizo la mimba kuharibika kabla ya miezi mitano katika hospitali ya wilaya ya Narok.Ugonjwa huu huadhiri mifugo lakini huenezwa kwa wanadamu wanapozila nyama au bidhaa kutoka kwa wanyama walioadhirika. Ugonjwa huu umehusishwa na kuharibika kwa mimba kwenye mifugo lakini haijabainika wazi ikiwa husababisha tatizo hili kwa wanadamu.Watafiti wangependa kubahiri ikiwa kuna uhusiano kati ya kuambukizwa maradhi ya brucellosis na kuharibika kwa mimba katika wanadamu.

Manufaa na hatari za kushiriki

Manufaa

- Utaweza kushiriki bila kutozwa ada yoyoyote.
- Utaelekezwa utakapopata matibabu maaluum ikiwa utapatikana na ugonjwa wa brucellosis, virusi vinavyosababisha ugonjwa wa ukimwi au kaswende
- Utapokea maelezo kuhusu viini vinavyosababisha ugonjwa wa brucellosis,jinsi unavyoenezwa,dalili zake,makali yanayotokana na ugonjwa huo na matibabu yaliyopo. Ikiwa utapatikana na viini vya brucellosis utapokea ushauri jinsi ya kuepuka maambukizi mapya na usipokuwa na viini hivyo, namna ya kuepuka kuambukizwa.

- Ikiwa mimba yako imeharibika kabla ya miezi mitano,utapata maelezo kuhusu kinachosababisha mimba kuharibika,matibabu yaliyopo na baadaye kuelekezwa kwenye kiliniki ya mtaalamu wa afya ya akina mama katika hospitali ya wilaya ya Narok utakapopata huduma zaidi.

Hatari

Mililita tano za damu zitatolewa kuotoka kwenye mkono wako kwa kutumia sindano. Utahisi maumivu .

Ikiwa utakubali kushiriki:

- Utatakiwa kutia sahihi fomu ya ridhaa iliyotolewa kwa hiari.
- Utaulizwa maswali kubaini ikiwa waweza kushiriki
- Utajibu maswali kadhaa yaliyomo kwenye hojaji maalum.
- Utapimwa mwili wako kuona hali yako ya afya kiujumla.
- Mililita tano za damu zitachululiwa kutoka kwenye mkono wako na kupelekwa kwenya maabara ili kuchunguzwa kiwango cha bakteria za brucellosis.
- Utapokea matibabu unayostahili ikwa mimba yako imeharibika kulingana na mwongozo wa Wizara ya Afya.
- Waliojifungua watoto walio hai wataelekezwa kwenye kiliniki ya akina mama waliojifungua ili kupata ushauri na matibabu ikiwa ipasavyo.

Haulazimishwi kushiriki katika utafiti huu na waweza kujiondoa wakati wowote.Jambo hili halitakuzuia kupokea au kuadhiri kwa vyovyote vile huduma ambazo ungepaswa kupokea.Habari utakazozitoa kwetu zitachukuliwa kwa usiri.

Ikiwa una maswali kuhusu utafiti huu,wasiliana na wafuatao:

1. Daktari Onzere Norris ijayo Nambari ya simu: 0722665865
2. Katibu wa Kamati ya Utafiti Hospitali kuu ya Kenyatta.Nambari ya simu:020-272260

Appendix 4: Written Consent (English version)

I.....after carefully reading the consent explanation form and getting a detailed explanation on the nature of the study by ..... a research assistant involved in the study, do hereby give informed consent to participate in the study on The Role of Brucellosis in Spontaneous Abortion at Narok District Hospital. I am also aware that I can withdraw from the study at any time during its course and that such a decision will not in any way interfere with the medical care I am entitled to receive for my condition.

Signed..... OR Thumbprint.....Left/Right

Date .....

Research Assistant

Name .....

Signature .....

Date.....

Witness

Name.....

Signature.....

Date.....

Appendix 5: Written Consent (Swahili version)

Ridhaa iliyotolewa kwa ufahamu

Mimi .....baada ya kusoma kwa makini fomu ya maelezo kuhusu utafiti na kuelezwa kwa undani kuhusu utafiti huo na .....msaidizi mtafiti,natoa ridhaa kwa ufahamu kushiriki kwenye utafiti kuhusu Uhusiano wa Ugonjwa wa Brucellosis na tatizo la mimba kuharibika kabla ya miezi mitano katika hospitali ya wilaya ya Narok.Nafahamu ya kwamba naweza kujiondoa kwenye utafiti huu wakati wowote na uamuzi huu hautaadhiri huduma ambazo ningestahili kupokea.

Sahihi..... au Alama ya kidole cha gumba.....

Tarehe.....

Msaidizi mtafiti

Jina.....

Sahihi.....

Shahidi

Jina.....

Sahihi.....

Tarehe.....

Appendix 6: Screening protocol for Cases

	YES	NO
a) A resident of Narok District for more than 1year		
b) Never had a history of diabetes mellitus		
c) Never had a history of thyroid disease		
d) Never been treated for brucellosis before		
e) On a contraceptive method		
f) This was a planned pregnancy		
g) Gestation of 20 weeks or less at the time of abortion		

If answers to ALL questions are YES, proceed to Study Questionnaire (Appendix 5).

Appendix 7: Screening protocol for Controls

	YES	NO
a) A resident of Narok District for more than 1year		
b) Never had a history of diabetes mellitus		
c) Never had a history of thyroid disease		
d) Never been treated for brucellosis		
e) Never had an abortion		
f) Pregnancy greater than 37 weeks at the time of delivery		
g) Pregnancy outcome was a live infant		

If answers to ALL questions are YES, proceed to Study Questionnaire (Appendix 5).

Appendix 8: Study questionnaire

THE ROLE OF BRUCELLOSIS IN SPONTANEOUS ABORTION AT NAROK DISTRICT HOSPITAL

Study Number ☐☐☐ Case ☐ Control ☐

(Tick in appropriate box)

Surname First Name Middle Name

Year of Birth ☐☐☐☐ Age in Years ☐☐

Tick the correct response in the check box to the right of the option provided.

SOCIAL AND DEMOGRAPHIC DATA

1. What is your current relationship status?

   Single ☐ Married ☐ Separated ☐ Divorced ☐ Widowed ☐

2. What is your current residence?

   Rural ☐ Urban ☐

3. Do you have formal education?

   Yes ☐ No ☐

4. What is your level of formal education?

   Primary ☐ Secondary ☐ Tertiary ☐ N/A ☐

5. Do you share your living quarters with livestock?

   Yes ☐ No ☐

6. Are you involved in the handling of raw animal products such as meat, milk, blood, hides or disposal of dead livestock either as part of your occupation or daily chores?

Yes ☐ No ☐

7. Do you consume any of the following products raw?

Meat Yes ☐ No ☐

Milk Yes ☐ No ☐

Blood Yes ☐ No ☐

OBSTETRIC AND GYNAECOLOGIC HISTORY

8. When was your Last Normal Menstrual Period (LNMP)?

(dd/mm/yy) ☐☐☐

Gestation in weeks as per LNMP ☐☐

If unsure of LNMP, indicate the estimated gestation as per exact conception date if known, early pregnancy symptoms, first trimester ultrasound, fundal height at first antenatal visit or quickening.

Estimated Gestation in weeks ☐☐

9. How many times have you lost a pregnancy at a gestation equal to or less than 20 weeks gestation?

0 ☐ 1 ☐ 2 ☐ 3 ☐ >3 ☐

10. How many times have you delivered a live infant after 37 completed weeks of gestation?

0 ☐ 1 ☐ 2 ☐ 3 ☐ >3 ☐

PHYSICAL EXAMINATION

11. General condition

Good ☐ Sick looking ☐

12. Vital signs

Temperature ☐ ^{0}C

Blood pressure ☐ mm/Hg

Respiratory rate ☐ breaths/min

Pulse rate ☐ beats/min

13. Results of previous serological tests (if available)

HIV Positive ☐ Negative ☐

VDRL Positive ☐ Negative ☐

LABORATORY RESULTS

14. Patient's Brucella antibody titres

1:20 ☐

1:40 ☐

1:80 ☐

1:160 ☐

1:160 ☐

1:320 ☐

1:640 ☐

1:1280 ☐

Research Assistant .....

Signature .....

Date ... .

Appendix 9: Standard Operating Procedure for Testing

- 5ml of venous blood will draw from the antecubital vein into a plain vacutainer bottle using aseptic techniques.
- The blood sample will be transferred in a cooler box at 2-8 □c to the laboratory.
- A laboratory technician will keep the blood samples at room temperature to clot then centrifuge at 3000rpm for 10 minutes to enable serum to separate.
- 8 small round bottom tubes will be labeled 1 to 8. By adding 0.1 ml serum to 1.9 ml normal saline a 1/20 dilution will be obtained. I ml of this will be transferred to tube 2 with 1.0 ml normal saline added providing a 1/40 dilution. This process will be repeated until a 1/1280 dilution is obtained in tube 7.Tube 8 will serve as a control containing only saline.
- One drop of Febrile antigen® (Fortress Diagnostics Limited U.K) suspension will be added to each tube and mixed well.
- Samples will be incubated at 37°C in a water bath for 24 hours.
- Each tube will be examined for signs of agglutination.
- The titre to be considered will be the last tube to show agglutination.

Appendix 10: Budget

ITEM	QUANTITY	UNIT COST (KSH)	TOTAL COST (KSH)
PROPOSAL DEVELOPMENT			
Printing paper	3 Reams	360	1,080
Printing	240 Sheets	5	1,200
Binding- soft Cover	6 Copies	100	600
Sub-Total			**2,880**
PERSONNEL			
Clinical officers	1	7,500 per month	7,500
Nurses	2	5,000 per month	10,000
Data entry clerk	1	5,000 per month	10,000
Biostatistician	1	30,000	30,000
Sub-Total			**57,500**
TRAINING OF RESEARCH ASSISTANTS			
Note books	10	50	500
Ball Point pens	12	15	180
Pocket files	10	50	500
Stapler	1	200	200
Staples	1 Pack	200	200
Paper Punch	1	400	400
Permanent Marker pens	1 Pack	400	400
Sub-Total			**2,380**
PRE-TESTING OF TOOLS			
Printing Paper	1 Ream	360	360
Printing	500 Sheets	5	2,500
Pocket files	10	50	500
Brucella Antigen	20	30	600
Permanent Marker pens	4	100	400

5cc syringes	100pc pack	400	400
One-half inch 26 or 27 Gauge needles	100pc pack	120	120
Gloves	1 box	250	250
Cotton Wool	3 Rolls	200	600
Methylated Spirit	1 Liter	200	200
Sub-Total			**5,930**
DATA COLLECTION			
Printing Paper	10 Reams	360	3,600
Printing	40 sheets	5	200
Photocopying	4,000 Sheets	2	8,000
File folders	400	30	12,000
5 cc Syringes	400	4	1,600
One-half inch 26 or 27 Gauge needles	400	2	800
Gloves	10 Boxes	170	1,700
Methylated Spirit	5 liters	1000	1,000
Marker pens	10	100	1,000
Vacutainer tubes	400	8	3,200
Cooler box	1	5000	5,000
Brucella Antigen	400 Doses	30	12,000
Sub-Total			**50,100**
COMMUNICATION			
Mobile phone calling cards (for 2 months)	3000/=pm	6000	6,000
Sub-Total			**6,000**
FINAL REPORT			
Printing Paper	2 Reams	360	720
Printing	1000 Sheets	5	5,000
Binding- Hard Cover	6 Copies *	500	3,000
Sub-Total			**8,720**
Total			**133,510**
Contingencies(10% of total)			**13,351**
Grand Total			**146,861**

Appendix 11: References

1. Gotuzzo E and Carrilo C. Brucella. In: Gorbach SL, Bartelett JG, Blacklow NR. Infectious Diseases, 2nd ed. Philadelphia: Saunders; 1998; 1837-43.
2. Boschiroli ML, Foulongne V and O'Callaghan D. Brucellosis: A worldwide zoonosis. Curr Opin Microbiol 2001; 4:58-64.
3. Alballa SR. Epidemiology of human brucellosis in southern Saudi Arabia. J Trop Med Hyg. 1995 Jun;98(3);185-9.
4. Ofukwu AR, Yohanna CA and Abuh HA. Medicine on-line: Int. J. Med. 2008; http://www.priory.com/med/brucella.htm
5. Mutanda LN: Selected laboratory tests in febrile patients in Kampala, Uganda. East African Medical Journal 1998; 75(2):68-72.
6. Maichomo MW, McDermott JJ, Arimi SM and Gathura PB: Assessment of the Rose-Bengal plate test for the diagnosis of human brucellosis in health facilities in Narok district, Kenya. East African Medical Journal 1998; 75(4):219-222.
7. Salata RA, Brucellosis. In: Goldman L, Ausiello D, editors. Cecil Textbook of Medicine, 22th ed. Philadelphia: Saunders; 2004:1887-90.
8. Poole PM, Whitehouse DB and Gilchrist MM .A case of abortion consequent upon infection with Brucella abortus biotype 2. J Clin Pathol 1972; 25: 882-4.
9. Seoud M, Saade G, Awar G, Uwaydah M (1991) Brucellosis in pregnancy. J Reprods Med 1991; 36: 441-5.
10. Ketty S, Ebraheim N and Ismail N. Brucellosis in humans. Research Centre on Brucellosis Tehran, Iran. J Infect Developing Countries 2008; 2(3):230-234

11. Gotuzzo E, Carrillo C, Guerra J and Llosa L. An evaluation of diagnostic methods for brucellosis .The value of bone marrow culture. J Infect Dis 1986; 153:122-125

12. Salata RA. Brucellosis. In: Goldman L, Ausiello D. Cecil Textbook of Medicine, 22th ed. Philadelphia: Saunders; 2004:1887-90

13. Cunningham GF, Leveno KJ and Bloom SL. Williams Obstetrics 22nd ed.New York: Mc-Graw Hill; 2005:232

14. Regan L and Rai R.Epidemiology and the medical causes of miscarriage.Baillieres Best Pract Res Clin Obstet Gynaecol 2000; 14:839

15 Harlap S and Shiono PH.Alcohol, smoking and incidence of spontaneous abortions in The first and second trimester, Lancet 1980; 2:173

16. Wilcox AJ, Weinberg CR and O'Connor JF. Incidence of early loss of pregnancy. New Engl J Med 1988; 319:189

17. Wang X Chen and C Wang L. Conception, early pregnancy loss and time to clinical pregnancy: a population-based prospective study.Fertil Steril 2003; 79:577

18. Cunningham GF, Leveno KJ and Bloom SL. Williams Obstetrics 22nd ed.New York: Mc-Graw Hill, 2005:231-251

19. Schut DJ and Joseph H. Recurrent Pregnancy Loss in. Novak's Gynecology, 13th ed. Philadelphia: Lippincott Williams and Wilkins. 2002:1073- 5

20. Maged E and Amien A. The effects of maternal brucellosis on pregnancy outcome. Journal of infection in developing countries. 2008; 2(3):230-234.

21. Sarram M, Feiz J and Foruzanfarpour P. Intrauterine fetal infection with brucella melitensis as a possible cause of second-trimester abortion. Am J Obstet Gynacol. 1974; 119:657-60.

22. Madkour MM. Pregnancy and brucellosis. In: Madkour MM ed. Brucellosis. London: Butterworth. 1989:197 -204.

23. Lulu AR, Araj GF, Khateeb MI, Mustafa MY, Yusuf AR and French FF. Human brucellosis in Kuwait.A Prospective study of 400 cases .Q J Med 66: 39 -54

24. Khan MY, Mah MW and Memish ZA. Brucellosis in pregnant women. Clin infect Dis 2001: 32; 1172-7.

25. Mertihan Kurdoglu, Ertan Adali, Zehra Kurdoglu, Mustafa Kasim Karâhocagil, Ali Kolusari, Recep Yildizhan, Zehra Kucukaydin, Hanim Guler Sahin, Mansur Kamaci and Hayrettin Akdeniz. Brucellosis in pregnancy: a 6-year clinical analysis. Archives of Gynecology and Obstetrics. 2010: 281:2;201-206

26. Young EJ. Brucella species. In: Mandell GL, Bennett JE, Dolin R. Principles and Practice of Infectious Diseases. 6th ed. Philadelphia: Churchill Livingstone; 2005:2669-72.

27. Nassaj M, Rahbar N, Ghorbani and R, Lavaf S .The Role of Brucella Infection Among Women with Spontaneous Abortion in an Endemic Region. Journal of The Turkish German Gynecological Association 2008; 9(1):20-23

28. Seoud M, Saade G and Uwaydah M. Brucellosis in pregnancy. J Reprod Med 1991; 36:441-445

29. Fernihough TJ, Munoz WP and Mahadeyo I. The role of Brucella abortus in Spontaneous abortion among the black population. S Afr Med J 1985; 68(5):379-80

30. Muriuki SM, McDermott JJ, Arimi SM, Mugambi JT, Wamola IA. Criteria for better detection of brucellosis in the Narok District of Kenya. East Afr. Med. J 1997; 74: 317-320.

31. Republic of Kenya Ministry of Health Annual Health Sector Status Report 2005-2007: 38

32. Santini C, Baiocchi P, Berardelli A, Venditti M and Serra P. A case of brain abscess due to Brucella melitensis. Clin Infect Dis 1994; 19: 977-978.

33. Potasman I, Even L, Banai M, Cohen E, Angel D and, Jaffe M. Brucellosis: An unusual diagnosis for a seronegative patient with abscesses, osteomeylitis and ulcerative colitis. Rev Clin Dis 1991; 13: 1039-1042.

34. Shakir RA, Al-Din ASN, Araj GF, Lulu AR, Mousa AR and Saadah MA. Clinical diagnosis of neurobrucellosis. A report on 19 cases. Brain 1987; 110: 213-223.

35. Colmenero JD, Reguera JM, Martos F, Sanchez-De-Mora D, Delgado M, Causse M, MartÃn-Farman A and Juarez C. Complications associated with Brucella melitensis infection: a study of 530 cases. Medicine (Baltimore) 1996; 75: 195-211

36. Galanakis E, Bourantas KL, Leveidiotou S.

Lapatsanis PD. Childhood brucellosis in north-western

Greece. Euro J Pediatr 1996; 155: 1-6.

37. Narok District Vision and Mission 2005-2015:3

38. Malone FD, Athanassiou A, Nores LA, Dalton ME (1997) Poor perinatal outcome

associated with Maternal Brucella abortus infection. Obstet gynacol 90 (4PT2): 674-6.

39. Makhseed M, Harouny A, Araj G. Obstetric and Gynecologic

implication of brucellosis in Kuwait. J Perinatol 1998; 18(3):196-6.

KENYATTA NATIONAL HOSPITAL
Hospital Rd. along, Ngong Rd.
P.O. Box 20723, Nairobi.
Tel: 726300-9
Fax: 725272
Telegrams: MEDSUP", Nairobi.
Email: KNHplan@Ken.Healthnet.org

Ref: KNH-ERC/ A/627

11th November 2010

Dr. Onzere Norris Ijayo
Dept. of Obstetrics & Gynaecology
School of Medicine
University of Nairobi

Dear Ijayo

Research proposal: "The Role of Brucellosis in spontaneous abortion at Narok District Hospital" (P253/07/2010)

This is to inform you that the KNH/UON-Ethics & Research Committee has reviewed and **approved** your above revised research proposal for the period 11th November 2010 - 10th November 2011.

You will be required to request for a renewal of the approval if you intend to continue with the study beyond the deadline given. Clearance for export of biological specimens must also be obtained from KNH/UON-Ethics & Research Committee for each batch.

On behalf of the Committee, I wish you a fruitful research and look forward to receiving a summary of the research findings upon completion of the study.

This information will form part of the data base that will be consulted in future when processing related research study so as to minimize chances of study duplication.

Yours sincerely

PROF A N GUANTAI
SECRETARY, KNH/UON-ERC

c.c. The Deputy Director CS, KNH
The Dean, School of Medicine, UON
The HOD, Records, KNH
The Chairman, Dept. of Obs/Gynae, UON
Supervisors: Dr. Samson Wanjala, Dept.of Obs/Gynae, UON
Dr. Lubano Kizito, Dept.of Obs/Gynae, UON