SERO-PREVALENCE OF BRUCELLOSIS IN PREGNANT WOMEN VISITING GYNAECOLOGY DEPARTMENT OF KATHMANDU MODEL HOSPITAL OF NEPAL.

SEEMA THAPA
T.U. Registration No. : 5-2-48-2579-2007
T.U. Examination Roll. No. : 21696
Batch : 2068/69

A thesis submitted in partial fulfillment of the requirements for the award of the degree of Masters of Science in Zoology with special paper Parasitology

Submitted to
Central Department of Zoology
Institute of Science and Technology
Tribhuvan University
Kirtipur, Kathmandu
Nepal
March, 2016

DECLARATION

I hereby declare that the work presented in this thesis has been done by myself, and has not been submitted elsewhere for the award of any degree. All sources of information have been specifically acknowledged by reference to the author(s) or institution(s).

Date: **……..…** **……**

Seema Thapa

T.U. Registration No. : 5-2-48-2579-2007

T.U. Examination Roll No. : 21696

Batch : 2068/69

RECOMMENDATION

This is to recommend that the thesis entitled **"Sero-prevalence of brucellosis in pregnant women visiting gynaecology department of Kathmandu model hospital of Nepal."** has been carried out by Seema Thapa for the partial fulfillment of Master's Degree of Science in Zoology with special paper Parasitology. This is her original work and has been carried out under my supervision. To the best of my knowledge, this thesis work has not been submitted for any other degree in any institutions.

Date: **……..** **……**

Supervisor

Dr. Mahendra Maharjan

Associate Professor

Central Department of Zoology

Tribhuvan University

Kirtipur, Kathmandu, Nepal

LETTER OF APPROVAL

On the recommendation of supervisor **"Dr. Mahendra Maharjan"** this thesis submitted by Seema Thapa entitled **"Sero-prevalence of brucellosis in pregnant women visiting gynaecology department of Kathmandu model hospital of Nepal."** is approved for the examination and submitted to the Tribhuvan University in partial fulfillment of the requirements for Master's Degree of Science in Zoology with special paper Parasitology.

Date: ……. **…….**

Prof. Dr. Ranjana Gupta
Head of Department
Central Department of Zoology
Tribhuvan University
Kirtipur, Kathmandu, Nepal

CERTIFICATE OF ACCEPTANCE

This thesis work submitted by Seema Thapa entitled **"Sero-prevalence of brucellosis in pregnant women visiting gynaecology department of Kathmandu model hospital of Nepal."** has been approved as a partial fulfillment for the requirements of Master's Degree of Science in Zoology with special paper Parasitology.

EVALUATION COMMITTEE

…….	……
(Supervisor)	(**Head of Department)**
Dr. Mahendra Maharjan	**Prof. Dr. Ranjana Gupta**
Central Department of Zoology	Central Department of Zoology
Tribhuvan University	Tribhuvan University
Kirtipur, Kathmandu, Nepal	Kirtipur, Kathmandu, Nepal
……...	**…….....**
External Examiner	**Internal Examiner**

Date: ……

ACKNOWLEDGEMENTS

I would like to express my hearty gratitude to my supervisor Associate Prof. Dr. Mahendra Maharjan, Central Department of Zoology, T.U. for his supervision, guidance, tremendous support and invaluable suggestion throughout my study. Similarly I am thankful to Gynaecology Department of Kathmandu Model Hospital, Baghbazar, Kathmandu for providing the blood sample. I am thankful to our honorable Head of Department Prof. Dr. Ranjana Gupta, Central Department of Zoology, T.U. for providing me such an opportunity to carry out this dissertation work.

I am indebted to National Zoonoses and Food Hygiene Research Center (NZFHRC) for providing laboratory facilities for the preparation of my present dissertation. My gratefulness goes also to Mrs. Kabita Shahi staff of NZFHRC for her kind help during laboratory work. I am also very thankful to Mr. Dhan Kumar Pant, Dr. Mina Sharma and Mrs. Meena Dahal for their good suggestions and guidance. I would like to thank all the staffs of NZFHRC. I also acknowledge to all the teachers and staffs of Central Department of Zoology for their continuous aspiration and motivation.

I would like to express my deepest gratitude to my family and relatives for their support and inspiration in my whole academic career.

Last but not least I would like to acknowledge my friends for their kind support throughout my dissertation work and all those persons who helped me directly or indirectly to complete this work.

Seema Thapa
T. U Registration No: 5-2-48-2579-2007
Symbol No- 21696
Batch 2068/69

CONTENTS

LIST OF TABLES

LIST OF PHOTOGRAPHS

LIST OF ABBREVIATIONS

BPAT	Buffered plate agglutination test
CFT	Complement fixation test
CVL	Central Veterinary Laboratory
DoAH	Directorate of Animal Health
DoHS	Department of Health Services
EDCD	Epidemiology and Disease Control Division
ELISA	Enzyme-linked immunosorbent assay
FAO	Food and Agriculture Organization
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
NZFHRC	National Zoonoses and Food Hygiene Research Centre
OD	Optical Densities
OIE	World Organization for Animal Health
PAT	Plate Agglutination Test
PCR	Polymerase chain reaction
PUO	Pyrexia of unknown origin
RBPT	Rose Bengal Plate Test
RPT	Rivanol plate test
RT-PCR	Real time polymerase chain reaction
SAT-EDTA	Serum agglutination test with ethylenediaminotetracetic acid
SPAT	Standard Plate Agglutination Test
SPSS	Statistical Package for Social Science
STAT	Standard Tube Agglutination Test
WHO	World Health Organizations
ZDU	Zoonotic Disease Unit

ABSTRACT

Brucellosis is a highly contagious zoonotic disease caused by ingestion of unpasteurized milk or undercooked meat from infected animals or close contact with their secretions. Sero-prevalence of brucellosis in pregnant women was conducted for the first time in Kathmandu, the capital of Nepal. A total of 80 sera samples were collected from the pregnant women visiting Kathmandu Model Hospital. The patients were categorized on the basis of age, trimester and ethnic groups. The sera samples were tested by ELISA method. The sero-prevalence of brucellosis among pregnant women was found to be 11.25%. Madhesi ethnic group showed the highest (16.66%) seropositivity rates followed by janajati (11.53%) and the lowest was in Brahmin (8.33%) ethnic group. Similarly, the age group 31-35 years showed highest prevalence (29.41%) followed by the age group 26-30 years (13.33%). There is absence of seropositivity among the age group 16-20 years and 21-25 years. The highest sero-prevalence rate (12.76%) was found in the third trimester followed by first trimester (10%) and the lowest was in second trimester (8.69%). In a questionnaire survey of 200 pregnant women done to assess their knowledge, attitudes and practices regarding the brucellosis, knowledge about the disease was found very poor. About 3% of them consume raw milk directly from milking animals which is one of the risk factors of brucellosis in pregnant women.

The prevalence was found to be high in pregnant women and ELISA was a sensitive and specific test for the detection of IgG antibodies against *Brucella*.

1. INTRODUCTION

1.1 Background

Brucellosis is a chronic granulomatous infection caused by intracellular bacteria and requires combined, protracted antibiotic treatment (Pappas et al. 2005). It is an old disease with minimal mortality and remains the most common zoonotic infections globally (Pappas et al. 2005, Ariza et al. 2007). It is an infectious disease caused by *Brucella* species belonging to family Brucellaceace and order Eubacterials. It is an infection that mainly affects animals including goats, sheep, pigs, deer, cattle, dogs etc. Brucellosis is a bacterial zoonotic disease transmitted to humans by consumption of infected, unpasteurized animal milk or through direct contact with infected animals, particularly aborted fetuses (Dean et al. 2012). In human, brucellosis can be a serious, debilitating and chronic disease which may affect a variety of organs (Poester et al. 2014). It is estimated that inhalation of only 10 to 100 bacteria is sufficient to cause the disease in man (Kaufmann et al. 1997). The disease is commonly known as undulant fever, Mediterranean fever or Malta fever in human since the disease is characterized by irregular fever, headache constipation, dizziness etc. (WHO 2006). Brucellosis in pregnancy is highly associated with adverse obstetric outcomes including abortion (threatened and spontaneous) and fetal/maternal and neonatal death (Vilchez et al. 2015). *Brucella* bacteremia can result in abortion especially during the early trimesters (WHO 2006). The incidence of spontaneous abortion and intrauterine death among pregnant women with acute brucellosis is primarily due to *Brucella melitensis* (Khan et al. 2001). Although brucellosis in domestic animals has been controlled in most developed countries, it remains an important public and animal health problem in several parts of the world, including the Middle East (Tsolia et al. 2002). It is a common and endemic zoonotic disease in many regions of the world, particularly where livestock are a major source of food and income. Despite control programs, it remains endemic in most developing countries (Corbel 1997). Brucellosis is an occupational hazard of livestock farmers, dairy workers, veterinarians, slaughterhouse workers, and laboratory personnel (Rahman et al. 2012).

The disease occurs worldwide, except in those countries where bovine brucellosis (*B. abortus*) has been eradicated (Robinson 2003). Brucellosis is more common in countries

with poorly standardized animal and public health program (Capasso 2002). The countries with the highest incidence of human brucellosis include Saudi Arabia, Iran, Palestinian Authority, Syria, Jordan and Oman (Halling and Boyle 2002). Asian countries like India, Bangladesh, Pakistan, China etc. and even in Nepal it has been reported.

1.2 Brucellosis in human and animals

Brucellosis is caused by the various species of *Brucella*, a gram-negative, non-motile, non-spore forming, rod-shaped (coccobacilli) bacteria. Nine *Brucella* species are currently recognized, seven of them affect terrestrial animals. They are *Brucella abortus* in cattle, *B. melitensis* in sheep and goat, *B. suis* in pig, *B. canis* in dog, *B. ovis* in sheep, *B. neotomae* in desert wood rat, *B. microti* in common vole (Scholz et al. 2008; Verger et al. 1987) and two that affect marine mammals are: *B. ceti* and *B. pinnipedialis* (Foster et al. 2007). *B.abortus, B. melitensis, B. suis* and *B. canis* are usually transmitted between animals by contact with placenta, fetus, fetal fluids and vaginal discharges from an infected animal. Entry into the body occurs by ingestion and through the mucous membranes, broken skin and possibly intact skin (Hassanain and Ahmed, 2012). The species that are linked to human brucellosis are *B. abortus, B. melitensis, B.suis and* to minor extent *B.canis* which has been reported as zoonotic important species (Moreno 2014). More recently, *B. inopinata* (single isolate from a human) have been recognized (Kazmierczak 2012). *Brucella* species particularly *B. melitensis* and *B. abortus* have been reported as possible biological weapons (Santis et al. 2011). *B. melitensis* is the most invasive and pathogenic for human and is cause of Malta or Mediteranrean fever (Xavier et al. 2010). Few cases of brucellosis caused by *B. canis* have been reported and most of these infections have been mild. However, human infections with *B. canis* may be underdiagnosed (CFSPH 2012). *B. melitensis* accounts for the majority of cases of brucellosis in humans (Yumuk and Callaghan 2012, Pappas et al. 2006, Lucero et al. 2008). After *B. melitensis, B. abortus* is most frequent in humans (Yumuk and Callaghan 2012, Omer et al. 2010, Corbel 1997).

1.3 Epidemiological aspect of Brucellosis

The new foci of human brucellosis have emerged, particularly in central Asia, while the situation in certain countries like Syria is rapidly worsening (Pappas et al. 2006). The

disease is still present in varying trends both in European countries and in the USA (Pappas et al. 2006). The highest recorded incidence of human brucellosis occurs in Middle East and Central Asia and the disease causes substantial morbidity in human and animal populations (Rubach et al. 2013). OIE 2002 reported that the absence of brucellosis in Australia, Canada, Cyprus, Denmark, Finland, the Netherlands, New Zealand, Norway, Sweden and the United Kingdom. The incidence of the disease in Central Asian countries such as Kyrgyzstan and Azerbaijan is also high (Bonfon et al. 2012, Abdullayer et al. 2012). In Asian continent, the incidence of brucellosis ranges from 1-77%. For e.g. in india 1.6-25% (Mantur et al. 2004, Pathak et al. 2014, Din et al. 2013, Mangalgi et al. 2015, Yohannes and Gill 2011), Lebanon 65% (Tohme et al. 2001), Kuwait 77% (Lulu et al. 1988), Pakistan 33% (Shahid et al. 2014), Mongolia 53% (Erdenebaatar et al. 2004) etc. In Nepal it ranges from 1-20% according to the various reports (Joshi et al. 1998, Dahal 2003, Joshi et al.2009, Rana 2002). Similarly, in African continent, the disease incidence ranges from 0.5-47%. e.g. in Tanzania 0.6-20.5% (Assenga et al. 2015, Swai et al. 2009, James 2013, Shirima 2005), Kenya 16% (Osoro et al. 2015), Libya 47% (Ahmed et al. 2010) etc. In United States, its incidence is less than 0.5 cases per 100,000 people (CFSPH 2012). The incidence of disease is high in many countries of the European continent like Macedonia (Maninska et al. 2008), Bosnia and Herzogovina (Dautovic et al. 2010), Greece (Minas et al. 2007, Bikas et al. 2003), Serbia (Cekanac et al. 2010), Albania (Bego and Byku 2015) etc.

1.4 Diagnostic Aspects

A definite diagnostic technique is required for the isolation of *Brucella* from blood, bone marrow or other tissues (Dahouk et al. 2007). In animals and human blood, lymph nodes, milk, placenta etc. are the main sample taken for diagnosis. Various diagnostic techniques have been developed for brucellosis with high sensitivity and specificity. e.g. Bacteriological, serological and molecular methods (Poiester et al. 2010).

1.4.1 Bacteriological methods

In this method, isolation, identification, detection of smooth colonies and biotyping of *Brucella* (Amin et al. 2012), stained smears and culture (Poiester et al. 2010) are carried out. Direct smear microscopic examination and cultural isolation of *Brucella* organism are also used for diagnosis as bacteriological methods (Kaltungo et al. 2014).

1.4.2 Serological methods

The most widely used method of diagnosis is serology. Direct agglutination, Rose Bengal test, Coomb's test and ELISA are the most commonly used techniques for the serologic diagnosis of brucellosis.

Agglutination tests*:* This test is very sensitive and specific for the diagnosis of brucellosis (Mert et al. 2003). It is based on the reactivity of antibodies against the smooth lipopolysaccharide of *Brucella* (Franco et al. 2007). The traditional gold standard of diagnostic testing is the serum agglutination test (SAT), developed in 1897, which measures both IgG and IgM (Lim and Rickman 2004).The principle of the slow agglutination test (SAT) is that it detects agglutinin antibodies (mainly IgM, but also IgG) against *Brucella* spp. Large antigen-antibody complexes form when antibodies are present in the sample and precipitate at the bottom of the test tube or plate (Godfroid et al., 2010). Standard plate agglutinations test (SPAT), Serum agglutination test with ethylenediaminotetracetic acid (SAT-EDTA), Buffered plate agglutination test (BPAT), Rivanol plate test (RPT), Complement fixation test (CFT), the 2-mercaptoethanol test (Kaltungo et al. 2014) also the common diagnostic test based upon agglutination principles.

Rose Bengal test:

The Rose Bengal plate test can be used as a sensitive rapid screening test but the results should be confirmed by bacteriological and other serological tests (WHO 2006). The sensitivity of the Rose Bengal test is very high, however, and false-negative results are rarely observed (Serra and Vinas 2004). It is a simple spot agglutination test where drops of stained antigen and serum are mixed on a plate and any resulting agglutination signifies a positive reaction. The results are received in several minutes (Smirnova et al. 2013).

The antihuman globulin test or Coomb's test:

The Coombs test is an extension of SAT, used for the detection of incomplete, blocking or non-agglutinating IgG (Araj 2010). It determines IgG and IgA so it does not work in the earliest stages of acute illness when only IgM is present. It is considered together with ELISA IgG and ELISA IgA the best test for chronic brucellosis although it fails to detect many cases. The main problem with this test is that it is slow and labour intensive. It usually takes at least 3 days to complete the test whereas ELISA IgG and IgA can be done in a few hours (Baddour 2012).

***ELISA tests**:*

The ELISA technique is sensitive and specific for the detection of IgG antibodies against *Brucella.* It has become increasingly popular as a well standardized assay for brucellosis. ELISAs are divided into two categories, the indirect ELISA (iELISAs) and the competitive ELISA (cELISAs) (Godfroid et al. 2010). Basically, ELISA tests are used for either detection of circulating bacterial antigen or antibodies produced by the host against the specific bacterial antigen. ELISA tests for total (IgG+IgM+IgA), IgG and IgM anti-*brucella* antibodies, which utilized only commercially available reagents, were used to diagnose human brucellosis (Sippel et al. 1982). The basic principle of ELISA is based on Basic Immunology Response in the Lock and Key Concept in which the antigen (key) is the substance when introduced into the body produces antibodies and the antibody (lock) is the protein in the body that is used by immune system to identify and neutralize foreign targets (referred to as antigens).The key fits into the lock. Secondly the Enzyme conjugate substrates are used. The enzyme that converts colorless substrates to a colored product and bound to the antibody that is part of the antibody-antigen complex (Hsueh and Hegerfeld 2011). The ELISA method for brucellosis is based upon the reaction of antibodies in the sample tested with the antigen adsorbed on the polystyrene surface. Unbound immunoglobulins are washed off. An enzyme-labeled anti-human globulin binds the antigen-antibody complex in a second step. After a new washing step, bound conjugate is developed with the aid of a substrate solution to render a blue coloured soluble product which turns into yellow after adding stop solution.

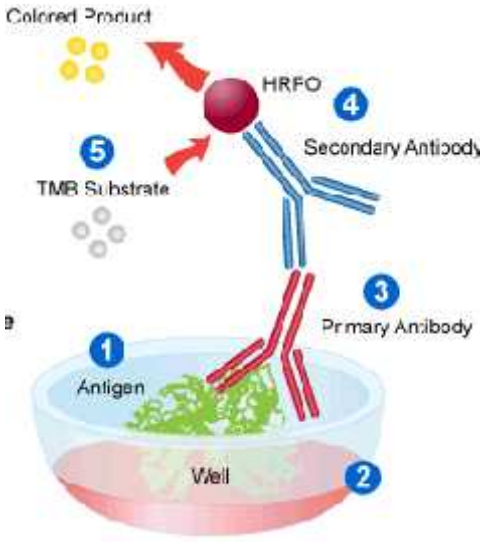

Fig: Ab-ELISA procedure

1.4.2 *Molecular methods*

PCR and RT-PCR falls under molecular methods. The polymerase chain reaction (PCR) is a recent and promising technique that allows for rapid and accurate diagnosis of brucellosis without the limitations of conventional methodology (Baddour, 2012). Advances in molecular-based technology have been utilised for the laboratory diagnosis of human brucellosis. In-house developed conventional polymerase chain reaction (PCR) and real-time PCR (RT-PCR) assays have been attempted for the direct detection of *Brucella* from clinical specimens, to monitor treatment response, and for the identification, speciation and differentiation of recovered *Brucella* spp. (Araj 2010).

1.5 Objectives

1.5.1 General Objective

To determine the sero-prevalence of brucellosis along with Knowledge, Attitude and Practices among the pregnant women visiting Gynaecology Department of Kathmandu Model Hospital, Nepal.

1.5.2 Specific Objectives

- To determine the sero-prevalence of brucellosis among the pregnant women.
- To assess the knowledge, attitude and practice regarding brucellosis among the pregnant women.

2. LITERATURE REVIEW

Brucellosis is a zoonotic disease of worldwide distribution (Doganay and Aygen 2003, Memish and Balkhy 2004). Despite being controlled in many developed countries, the disease remains endemic in many parts of the world including Latin America, the Middle East, Spain and many parts of Africa and western Asia (Memish and Balkhy 2004). Brucellosis occurs worldwide, but it is well controlled in most developed countries. The disease is rare in industrialized nations because of routine screening of domestic livestock and animal vaccination programs. Clinical disease is still common in the Middle East, Asia, Africa, South and Central America, the Mediterranean Basin, and the Caribbean (Lopes et al. 2010). The epidemiology of human brucellosis, the commonest zoonotic infection worldwide, has drastically changed over the past decade because of various sanitary, socioeconomic and political reasons, together with the evolution of international travel. Several areas traditionally considered to be endemic-e.g. France, Israel and most of Latin America have achieved control of the disease (Pappas et al. 2006). More than 500,000 people are affected by brucellosis each year worldwide (Saeed et al. 2013).

Scenario of Human Brucellosis in the Context of World

In Asian Continent:

Brucellosis is highly prevalent in Asia with the highest recorded incidence of human brucellosis in the Middle East and Central Asia (Zhang et al. 2010, Rubach et al. 2013). The disease is endemic with incidence estimates more than 100 cases per 100,000 populations in Iraq, Jordan and Saudi Arabia (Yacoub et al. 2006, Qasem and Shaqra 2000, Tawfiq and Abukhamsin 2009). The incidence of the disease in Central Asian countries such as Kyrgyzstan and Azerbaijan is also high (Bonfon et al. 2012, Abdullayev et al. 2012).

Brucellosis is endemic in India with numerous reports of cases from isolated areas (Thakur and Thapliyal 2002). The prevalence of brucellosis among PUO cases and occupationally exposed individuals in Goa has been reported to be 4-6% by various tests like RBPT, SAT, Indirect ELISA and IgG ELISA (Pathak et al. 2014). Brucellosis in India is an important but often neglected disease (Smits and Kadri 2005, Mantur and Amaranth 2008). The seroprevalence of brucellosis in children in Bijapur was found to be 1.6% (Mantur et al. 2004). The overall seroprevalence of brucellosis in humans of Bhimber, Azad Jammu and Kashmir districts has been reported to be 6-10% by RBPT,

SPAT and STAT (Din et al. 2013). Similarly in Ludhiana, India, 24.5% were found to be positive by RBPT (Yohannes and Gill 2011). The overall seroprevalence of human brucellosis between 1987 and 1996 has been reported to be 15.86% in Andra Pradesh (Mrunalini and Ramasatry 1999). The overall positivity rates by RBPT, SAT and 2-ME test were reported to be 10.50%, 7.32% and 5.88% among individuals residing in rural areas of India (Mangalgi et al. 2015).

Brucellosis is endemic in Turkey (Kaya et al. 2013, Yumuk and Collaghan 2012). The overall seroprevalence of brucellosis in people living in the rural area of Kayseri, Central Anatolia has been reported to be 3.4% in which the lowest prevalence (2.0%) was observed in the 25-34 age group and the highest prevalence (4.3%and 4.1%) were in the 35-44 and 15-24 age groups, respectively which shows that the seropositivity rate is low in Turkey (Centinkaya et al. 2004). Out of 1028 patients with brucellosis, the overall relapse rate was recorded 4.7% in a 10 year study period, so human brucellosis may lead to serious morbidity and it continues to be a major health problem in Turkey (Buzgan et al. 2010). A hospital based case report in Turkey showed that the central nervous system involvement is rarely seen in brucellosis patients, with an incidence of 0.5-2.5% (Saime et al. 2010). The seroprevalence of human brucellosis in rural area of Western Anatolia, Turkey has been reported to be 4.8% (Cetinkaya et al. 2005).

Brucellosis is endemic and a significant health problem in Iran (Sofian et al. 2007, Bokaie et al. 2008). According to an epidemiological survey of brucellosis in Azna County, Western Iran, 41 people were found to be brucellosis positive in laboratory tests and the incidence rate was calculated to be 56.65 in 100,000 populations (Kassiri et al. 2012). The prevalence of brucellosis among nomads in Khuzestan a province of Iran was found to be 6.3% which shows that the prevalence of brucellosis among nomads in Iran is high due to their lifestyle (Alavi et al. 2007). A cross-sectional survey of brucellosis serology in HIV-infected patients as well as in healthy controls of Iran indicates that out of 90 HIV-positive patients and 100 healthy age-matched controls, positive brucellosis serology was significantly higher in HIV-infected patients than in controls (Abdollahi et al. 2010). The prevalence rate of brucellosis during 2002-2006 in human of Birjand, Iran was reported to be 37 per 100,000 which were much lower than in the reports of other countries of the region (Bokaie et al. 2008). The annual incidence of human brucellosis in Bardsir district of Iran has been reported to be 141.6 cases per 100,000 inhabitants (Haghdoost et al. 2007). The incidence of human brucellosis in Shazand county and Markazi state, Iran has been reported to be 13.1 and 7.3 per 100,000 population respectively so the prevalence of

brucellosis in Shazand county is much higher than expected (Hosseini et al. 2008). The incidence of brucellosis was found to be 59.31 per 100,000 populations in Azna, western Iran and the most common age group was 15-24 (27.9%) and about 60.5% of the patients were between 15-44 years old (Kassiri et al. 2013). The seroprevalence of human brucellosis has been reported to be 6.4% in Kurdistan Province, western Iran (Esmaeili et al. 2014) while *Brucella melitensis* is highly prevalent in animals and humans of Ishafan province in which 1526 clinically suspected human cases, 476 showed laboratory evidence of brucellosis (Sabbaghian and Nadim, 1974). Similarly brucellosis is endemic in Anbar Governorate, west of Iraq in which 96 cases were presented with subacute brucellosis (Koubaisy and Lafi 2011).

Consistent outbreaks of brucellosis have been reported in Afghanistan (Saeed et al. 2013). Brucellosis is known to be endemic in ruminant population throughout Afghanistan and the overall prevalence of seropositivities in the human samples tested was found to be 5.2% (Akbarian et al. 2015).

Brucellosis is endemic in Saudi Arabia with the seroprevalence of 15% in humans (Sekait 1999). Among 115 children ranging in age between 6 months and 12 years, the bacteremia was observed in 45% and of the isolates speciated, 96% were *Brucella melitensis* (Shaalan et al. 2002). The incidence of brucellosis among pregnant women has been reported to be 12.2% according to the prospective study conducted by Taif, S.A and Masoura University Hospital from august 2005-december 2007 (Elshamy and Ahmed 2008). Among 133 children screened for brucellosis in Al-Khafji region, Saudi Arabia, 84 were found to be positive by rapid slide test (Afify et al. 2012). In Saudi Arabia the seroprevalence of human brucellosis was reported to be 19% (Alsubaie et al. 2005) and 34 per 100,000 populations in Tabuk province in 1997 (Elbeltagy 2001). Overall 43% of pregnant women with brucellosis had spontaneous abortions during the first and second trimester and 2% had intrauterine fetal death in the third trimester which shows the incidence of spontaneous abortion among pregnant women with brucellosis is high. The cumulative incidence of pregnancy and brucellosis was 1.3 cases per 1000 delivered obstetrical discharges (Khan et al. 2001).

Brucellosis is endemic in Lebanon and Bangladesh (Tohme et al. 2001, Rahman et al. 2011). In Lebanon maximum 65% acute cases of brucellosis has been reported (Tohme et al. 2001) while in Bangladesh, maximum livestock farmers(21.6%) and milkers (18.6%) were found infected with brucellosis compared to butcher and veterinarians (Islam et al. 2013). Similarly, a total of 7842 cases of human brucellosis have been registered till 1997

at the Ministry of Health in Jordan. The number of cases was found to be the lowest in children below 4 years and highest in the 5 -14 years age group (Shaqra 2000).

The prevalence of brucellosis is increasing in China over the past several years (Li et al. 2009). The incidence of human brucellosis increased especially from 1995 to 2010 in China (Deqiu 2002). The incidence of human brucellosis rose substantially in the provinces of Inner Mongolia, Shanxi3, Heilongjiang, Hebei, Jilin and Shanxi1 and it also increased gradually in some southern provinces such as Henan, Guangdong and Fijian. The annual incidence rate of disease varied from 1.41 to 2.7 per 100,000 populations during the study period in China (Zhong et al. 2013).

The incidence of human brucellosis in the Gaza strip of Palestine was reported to be 8/100,000 in which the cases were reported from all districts with a particularly high incidence in the mid-zone district and Gaza city (Awad 1998). Human brucellosis epidemic presents a clear upward trend in Ningbo and 1.08% seropositivity has been reported from 2002 to 2012 of which 50 cases were diagnosed as brucellosis and 63 cases were bacteria carriers (Ya-wei et al. 2013).

In Georgia the incidence of human brucellosis has been reported to be 2.8 cases per 100,000 persons (Pappas et al. 2006) while in Kuwait maximum 77% sub-acute cases of brucellosis has been reported (Lulu et al. 1988). Brucellosis is highly endemic in Kyrgyzstan. Kyrgyzstan reported 77.5 new cases of human brucellosis per 100,000 inhabitants in 2007, which is one of the highest incidences in the world. The overall apparent seroprevalences of brucellosis has been reported to be 8.8% in humans of Naryn, Chuy and Osh Blasts (Bonfoh et al. 2012).

Brucellosis is endemic in Pakistan (Munir et al. 2011). In Peshawar maximum farmers (32.90%) and livestock owners (32.67%) were found to be infected with brucellosis compared to employees and other patients over the period of 3 years. So, brucellosis is important public health problem in and around Peshawar district (Shahid et al. 2014).

The areas of central and eastern Inner Mongolia provide a long term suitable environment where brucellosis outbreaks have occurred and can be expected to persist (Jia and Joyner 2015). The overall seroprevalence of brucellosis in human has been reported to be 53.25% in Mongolia (Erdenebaatar et al. 2004) while 27.3% in Sukhbaatar and Zavkhan provinces which shows the high seroprevalence of human brucellosis in Mongolia (Baijinnyam et al. 2014). Similarly among the randomly selected rural people age 4 to 90 years, the seroprevalence of *Brucella* spp. has been reported to be 11.15 ranging

between 2.3% and 22.6% in the eight provinces which confirms that human brucellosis seroprevalence among rural people in Mongolia is high (Tsend et al. 2014).

A total of eleven cases were identified at the local outbreak; the cultures were positive in four cases and seven cases were serologically diagnosed which shows that the Republic of Korea is no longer free of human brucellosis (Park et al. 2005). Likewise a total of 7,983 cases of human brucellosis were reported during the 15-year study period from 1995 to 2009 in Azerbaijan (Abdullayev et al. 2012). Brucellosis is endemic in the Gulf Cooperation Council region (GCC). *Brucella melitensis* is the etiological agent of brucellosis in Qatar (Deshmukh et al. 2015).

In African continent:

Brucellosis is an important disease among livestock and people in Sub-Saharan Africa (Dermott and Arimi 2002). The disease is endemic in Northern Africa and remains a major public health problem in the Maghreb region (Algeria/Morocco/Tunisia) (Lounes et al. 2014).

The overall seroprevalence of antibodies to *Brucella abortus* was found to be 5.52% in Tanga, Tanzania (Swai et al. 2009). The overall seroprevalence of brucellosis in human in Tanzania has been reported as 20.5% in Morogorro region (James 2013), 28% in Arusha and Manyara regions (Shirima 2005), 7.7% in rural northern Tanzania (John et al. 2010) and 0.6% in the Katavi-Rukwa ecosystem (Assenga et al. 2015). Likewise the incidence of brucellosis averages 6.26% in humans of Egypt (Hassanain and Ahmed 2012) whereas the proportions of seropositive sera have been reported to be 0.0 and 1.7% among the inhabitants of an endemic area in Egypt (Sherbini et al. 2007).

The prevalence of brucellosis antibodies among the humans in Kiambu county, Kenya is low (ZDU 2012). The data on the prevalence of brucellosis among humans and animals is limited in Kenya. According to a cross-sectional survey conducted in 3 counties, the seroprevalence of human brucellosis was found to be 16% (Osoro et al. 2015).

The study of human brucellosis in Ethiopia is sparse with even less information on risk factors for human infection (Yohannes et al. 2013). The seroprevalence of human brucellosis in and around Adami Tulu, Ethiopia has been reported to be 2.15% by RBPT and CFT (Tibesso et al. 2014). Similarly in Libya, the overall prevalence of human brucella seropositivity has been reported to be 40% in the Yafran municipality, 47% in Jado and 46% in Yifrin (Ahmed et al. 2010). In Rwanda, 25% of pregnant women have been found to have a positive serology towards *Brucella* sp. (Rujeni et al. 2008).

Serological survey of human brucellosis as conducted by Adekolu-John et al. 1991 in Nigeria revealed 22.3% positive cases. Similarly the highest prevalence (20%) of brucellosis has been observed among cattle handlers followed in decreasing order of prevalence by goat rearers (10%), mixed sheep and cattle rearers (9%), mixed sheep and goat rearers (8%) and 4% among each of sheep rearers and non-rearers of animals in Nigeria (Baba et al. 2001). The seroprevalence of human brucellosis among abattoir workers in Abuja was found to be high (Aworh et al. 2013).

In Uganda, the seroprevalence of human brucellosis has been reported to be 652 in urban, peri-urban and rural areas in Kampala (Makita et al. 2008), 5.8% and 9% among exposed cattle keepers in Mbarara and consumers of unpasteurized milk in Kampala district respectively (Nasinyama et al. 2014) and 17% in Kiboga district (Tumwine et al. 2015). Likewise the prevalence of human brucellosis is high i.e. 24.4% in the Sahelian region of Gourma, Mali (Tasei et al. 1982).

Brucellosis is endemic in Algeria (Aggad and Boukraa 2006) while in eastern Sudan, only 1% of positive case has been reported among butchers, slaughter house workers, milkers and cow attendants (Ansary et al. 2001). Similarly, the seroprevalence of human brucellosis in nomadic communities of Chad was found to be 3.8% (Schelling et al. 2003).

In Australian Continent:

Brucellosis due to *Brucella abortus* is a disappearing disease in Australia as a result of effective eradication programmes in cattle. However, the disease is re-emerging in Queensland because of recreational and occupational exposure to feral pigs infected with *Brucella suis* (Robson et al. 1993).

In American Continent:

Human brucellosis cases are rare in U.S. however; this disease remains a common and serious problem in some parts of the world (CFSPH 2009). The incidence of brucellosis in United States is less than 0.5 cases per 100,000 people. Most cases have been reported from California, Florida, Texas and Virginia (CFSPH 2012). With the eradication of *Brucella abortus* in cattle from Florida, *Brucella suis* has been emerged as the primary cause of human and animal brucellosis in the state. *Brucella melitensis* is not present in Florida but is endemic in Mexico (Stanek et al. 2010). Brucella presents adverse obstetric outcomes including fetal and maternal/neonatal death. Among pregnant women with brucellosis 27.7% had a threatened abortion/preterm labor, 12.8% experienced spontaneous abortion, 13.9% preterm delivery, 8.1% fetal death and 1.1% congenital

malformations and after delivery neonatal death occurred in 8.1%, low birth weight in 14.5% and congenital brucellosis in 6.4% (Vilchez et al. 2015).

In European Continent:

Brucellosis is a rarely encountered infection in northern Europe (Eriksen et al. 2002).It has become an emerging zoonosis in Bosnia and Herzegovina. It has become a continuous infection and for the past ten years, it has become an endemic disease (Dautovic et al. 2010). Brucellosis has become a very important health problem since 2001 and in the period 2001-2008, there has been 1639 human brucellosis cases reported and the number of cases increased every year with the morbidity rate over the study period ranged from 3.8 to 33.4 per 100,000 inhabitants in Bosnia and Herzegovina (Obradovic and Velic 2010).

Human brucellosis is a serious problem in the Republic of Macedonia presenting with a high percentage of localized forms, relapses and therapeutic failures (Bosilkovski et al. 2007). Similarly brucellosis is endemic in Strumica, Macedonia. During the 15 years, 1992-2006, 975 patients with acute form of brucellosis have been registered and in the last six years the number of new cases per year is decreased and in 2006 were registered 18 patients (Maninska et al. 2008).

The incidence of human brucellosis in the area of Larissa in Central Greece from 2003 to 2005 has been reported to be 32.49 cases per 100,000 inhabitants (Minas et al. 2007) while the overall incidence is extremely high in the rural area of Achaia in Western Greece i.e.1110 per 100,000 population per year (Bikas et al. 2003). A total of 152 newly diagnosed brucellosis cases have been recorded during a two year study period in a defined region of North Western Greece (Avdikou et al. 2005).

The incidence of human brucellosis in Sicily increased from 5.5 cases/100,000 inhabitants in 1990 to 8.0 cases/100,000 inhabitants in 2002, with a peak of 18.8 cases/100,000 inhabitants in 1997 (Massis et al. 2005). Brucellosis has been a significant public health concern in Serbia with 1521 human brucellosis cases reported and the disease was most prevalent among people aged 30-49 years accounting for 81 of 177(46%) of the cases (Cekanac et al. 2010).

Brucellosis is a rare zoonotic disease in UK but there is a case of brucellosis in the extensive multi-ethnic area of East London which may be due to emigration or by vacationing in *Brucella* endemic regions (Javaid et al. 2013). In Spain 75 laboratory workers had suffered from laboratory acquired brucellosis (Bouza et al. 2005) and among

158 patients diagnosed with brucellosis, 27.8% had osteoarticular complication in the Lugo region of Northwestern Spain (Gonzalez et al. 1999).

Bulgaria had been free from brucellosis since 1958 but during 2005-2007 a re-emergence of human and animal disease has been recorded (Russo et al. 2009) while human brucellosis has become a rare disease in Germany since the eradication of bovine and ovine/caprine brucellosis but it has now emerged as a disease among Turkish immigrants (Dahouk et al. 2007). The seroprevalence of *Brucella* spp. has been reported to be 19% in Tirana, Albania and by age group the highest seroprevalence was found to be in 15-24 years at 26.9% with the lowest in the 1-4 years age group at 4.5% (Bego and Byku 2015).

Scenario of Brucellosis in the context of Nepal

Brucellosis, which is transmitted to people by drinking unpasteurized milk or through close contact with aborted reproductive fluids and tissues, is the most common bacterial zoonotic infection in the world and is endemic to Nepal (Specchio, 2014). Brucellosis has been identified as priorities zoonotic diseases in Nepal with epidemic potentials (DoHS 2013/2014). Brucellosis was an unconsidered entity till 1979 when it was first reported as a result of infection by *Brucella melitensis*, in shepherds of sheep rearing stations of Pokhara (Dickenson and Thaller 1979).

A recent case recorded by Epidemiology and Disease Control Division (EDCD) of the Department of Health Services (DoHS) found that brucellosis bacteria have infected seven people so far in Dhukharkha VDC of Kavre district (My Republica 2015).

The overall sero-prevalence of human brucellosis in Chitwan district has been reported to be 1.4% by brucellosis card test. By age group 10-19 years 1(1.92%), 20-29 years 1(0.54%), 30-39 years 2(2.53), and above 50 years 3(2.72%) had the prevalence of brucellosis which indicates that people above 50 years age group have more prevalence rate (Upadhyay 1998). Similarly, the overall sero-prevalence of human brucellosis in Surkhet district was found to be 20% with the highest prevalence rate in the age group of 20-29 (29%) while the least prevalence rate was found in the group of 50 and above (10%) and 14% seropositivity has been recorded from the patients visiting Bir Hospital (Rana 2002).

The hospital based study on brucellosis showed that 8.17% seropositivity in Siddhi Polyclinic, Charkhal, Teku Infectious Hospital, Bir Hospital and Bharatpur Hospital (Joshi et al. 2009), 0.4% in Kathmandu (Knox et al. 2000) and 2.7% in Bir hospital and Teku infectious diseases hospital (Joshi 1984).

The sero-prevalence of human brucellosis in Kathmandu has been reported to be 11.95% in which the age group 6-15 years showed highest prevalence 29.17% followed by age group 0-5 years with16.67 followed by age group 16-45 years with 11.85% and the least age group 46 years and above with 10% (Aryal 2007). Similarly the sero-prevalence rate of human brucellosis in Dolakha district was found to be 0.5% (Dahal 2003) and 6.08% in Kathmandu (Joshi 1983).

The seropositivity rates of human brucellosis has been reported as 5.26% in Solukhumbu, 5.36% in Langtang, 2.56% in Malunga, 1.49% in Pokhara, 2.32% in Biratnagar, 3.63% in Bhairahawa, 1.65% in Kathmandu by plate test and 5.26%, 7.14%, 3.84%, 2.23%, 2.79%, 4.54%, 2.47% by tube agglutination test respectively (Pyakural 1980).

DHS (2003) indicated that in Nepal around 2-3% of the cattle and buffaloes are seropositive. Similarly, 1.48% seropositivity has been recorded by CVL (2003-2013) among the serum of cattle, buffalo and goat suspected for Brucellosis through RBPT and only 16 samples of animals tested in CVL by ELISA and PAT were found to be positive (DoAH 2070/71). All the samples of swine and goat tested for *Brucella abortus* antibody in CVL by PAT method were found to be negative (DoAH 2067/068).

The seroprevalence of brucellosis in animals has been reported as 6% in Godawari villages of Lalitpur district (Heidmann et al.1998), 17.6% in Chandanbari and Kyanjin Gompa (NZFHRC 2002), 12% in Kailali district (Pandeya et al. 2013) and 3% in the Hills of Nepal (Joshi and Shrestha 2011).

The sero-prevalence of brucellosis in the slaughter swines in the Kathmandu valley was found to be 21.58% (Joshi and Rana 2005) while the seroprevalence in pigs of Rupandehi district has been reported to be 13.59% (Poudel 2014). Similarly goats showed 17.14% and pig showed 7.18% positive reactors among the samples collected randomly from buffaloes, goats and pigs from different parts of Nepal (Shrestha et al. 2008). In Bhaktapur district, the seropositivity of brucellosis in buffaloes has been reported to be 15% by RBPT (Mishra et al. 2009) while the yak population of Mustang and Myagdi districts are found to be free from brucellosis (Aryal and Paudel 2007).

Scenario of Knowledge, Attitude and Practices (KAPs) of Brucellosis

Brucellosis as a bacterial disease has been heard maximum by farmers and shepherds of Egypt (Holt et al. 2011, Hegazy et al. 2016), Ethiopia (Kuma et al. 2013), livestock owner of Jordan (Musallam et al. 2015), pastoralists of Kenya (Obonyo and Gufu 2015),

households of Tajikistan (Grahn 2013), people of Lebanon (Shaar et al. 2014), farmers of Turkey (Cakmur et al. 2015), agro-pastoral and pastoral communities of Uganda (Tumwine et al. 2015, Kansiime et al. 2014), patients of Saudi Arabia (Bilal et al. 1991), respondents of Ethiopia (Desta 2015), people of Iran (Ghomashlooyan et al. 2015), people residing in rural areas of India (Mangalgi et al. 2015) and respondents of Rupandehi district, Nepal (Poudel 2014).

Regarding the transmission of disease from animals to human maximum people of Egypt (Holt et al.2011), Ethiopia (Kuma et al. 2013), Jordan (Musallam et al. 2015), Kenya (Obonyo and Gufu 2015, Omemo et al. 2012), Tajikistan (Grahn 2013), Nigeria (Buhari et al. 2015), Turkey (Cakmur et al. 2015), Tanzania (Swai et al. 2015),Saudi Arabia (Bilal et al. 1991) and Kiambu county, Kenya (ZDU 2012) had knowledge that it can be transmitted from animals to human.

Brucellosis transmission has been found to be due to drinking unpasteurized milk and eating unpasteurized dairy products (Adesiji et al. 2005, Cetinkaya et al. 2005, Holt et al. 2011, ZDU 2012, Musallam et al. 2015).

Regarding practices, majority of the people from Ethiopia (Kuma et al. 2013, Desta 2015), Kenya (Obonyo and Gufu 2015), Tajikistan (Grahn 2013, Lindahl et al. 2015), Uganda (Tumwine et al. 2015, Nasinyama et al. 2015), Turkey (Centinkaya et al. 2005), Nigeria (Adesiji et al. 2005) and Surkhet, Nepal (Rana2002) consumed raw milk and milk products made from raw milk. Likewise people of Egypt (Holt et al. 2011), Tajikistan (Grahn 2013), Turkey (Cakmur et al. 2015), Afghanistan (Saeed et al. 2013) and Chitwan and Dolakha, Nepal (Upadhayay 1998, Dahal 2003) consumed boiled milk and milk products. Similarly people from Ethiopia (Kuma et al. 2013, Desta 2015), Turkey (Cakmur et al. 2015) and Nigeria (Adesiji et al. 2005) consumed raw meat.

Regarding attitudes towards treatment of the diseases, people of Iran has been reported to be familiar with herbal and traditional medicines for treatment (Ghomashlooyan et al. 2015) while people of Kenya would visit health facility, divine intervention, herbal medicines and local chemist for treatment (Obonyo and Gufu 2015). Similarly majority of the respondents practiced self-medication and relatively large number were using traditional healers in Tanzania (James 2013) and in Nepal majority of the people go to a faith healer when their livestock are ill (Dahal 2003).

3. MATERIALS AND METHODS

3.1 Study Area

This was a hospital based study conducted by visiting Gynaecology Department of Kathmandu Model Hospital, Bagbazar, Kathmandu. Kathmandu is the capital and largest municipality of Nepal which is situated in Central Development Region. The district covers an area of 395 sq. km with the population of 975,453 according to the census of 2011. The average altitude is about 1400 m above sea level. It is located at the longitude of 27'43"E and latitude of 85'21"N. The average summer temperature varies from 28-30 degree celsius and the average winter temperature is 101.1 degree celsius. Kathmandu Model Hospital was established in 1993 as an 18 bed community referral hospital. In accordance with the beliefs of phect-NEPAL, KMH firmly believes that health services should empower the sick to fight all types of suffering.

3.2 Study design

The study was divided into two phases to fulfill the objectives.

(a) General screening of patient visiting Gynaecology Department of Kathmandu Model Hospital to determine the seroprevalence of brucellosis.

(b) Questionnaire survey to assess the knowledge, Attitude and Preventive Practices (KAP) of the patients towards brucellosis.

3.3 Study Period

The study was conducted from 2014 to 2015.

3.4 Sample Size

A total of 80 blood samples were collected from the pregnant women visiting Gynaecology Department of Kathmandu Model Hospital for the regular checkup.

3.5 Sampling Procedures

3.5.1 Sample Collection and Transport

The blood samples (5ml) were collected in sterile, clean and leak-proof vials and labeled properly from the pregnant women visiting Gynaecology Department. Then serum

separation was done by centrifuging blood sample for 12-15 minutes with the help of centrifuge machine. The separated serum was pipette out in a sterile eppendorf tubes and were frozen at -20^{o}C till analysis. These serum samples were taken to the laboratory of National Zoonoses and Food Hygiene Research Centre (NZFHRC), Tahachal Kathmandu for test. The serum was tested by Enzyme Linked Immunosorbent Assay (ELISA) (Delta Biologicals S.r.l. INC.Via Nicaragua) method for the further diagnosis of brucellosis.

3.5.2 Serological study

ELISA was conducted at NZFHRC, Kathmandu for the detection of IgG antibodies against *Brucella.* It was performed in polystyrene 96- well microplates. All unknown serum samples and four positive control samples were tested in duplicate. The protocol of ELISA was followed according to manufacturer which is briefly described below.

3.5.2.1 Protocol of ELISA for the detection of IgG antibodies against Brucella

- First of all wash solution was prepared in advance by filling 50 ml of 20x wash concentrate up to 1 litre with distilled water and the solution was warmed to 37°C before diluting. After diluting it was stored at 2-8°C.
- All the reagents were brought to the room temperature before use (approximately 1 hour), without removing the plate from the bag.
- After shaking all the components, the plate was removed from the package. The number of wells employed with four wells for the controls, two for the cut off serum and one each for the negative and positive sera was determined.
- Now, 100µl of serum dilution solution was added to all the wells. Then, 5µl of each sample, 5µl of positive control, 5µl of cut off serum and 5µl of negative control was added into the corresponding wells with the help of pipette.
- The plate was covered with a sealing sheet and incubated for 45 minutes at 37°C.
- The seal was removed and the liquid was aspirated from all the wells and washed with 0.3ml of washing solution per well for five times. The remaining liquid was drained off.
- Immediately 100µl of IgG conjugate solution was added into each well.

- The plate was covered with a sealing sheet and incubated for 30 minutes at 37°C.
- The seal was removed and the liquid was aspirated from all the wells and washed with 0.3ml of washing solution per well for five times. The remaining liquid was drained off.
- Immediately 100µl of substrate solution was added into each well.
- The plate was covered with a sealing sheet and incubated for 20 minutes at room temperature protected from light.
- The seal was removed and immediately 50µl of stopping solution was added into all the wells.
- Then the plate was read by using ELISA reader within 1 hour of stopping.

3.5.2.2 Interpretation of Results

All positive and unknown samples were done in duplicate. The mean OD of the samples and cut off serum was calculated and the antibody index was calculated by using the following formula,

$$\text{Antibody index} = \frac{\text{mean OD of samples}}{\text{cut off serum mean OD}} \text{ X 10}$$

The samples with antibody index above 11 were considered as having IgG specific antibodies against *Brucella.*

3.6 Quality control

To obtain the reliable results strict quality control was maintained and the favourable condition was maintained throughout the lab work. The internal control of each test was done by a conjugate control, a substrate control, cut off, negative and positive controls. The conjugate react specifically to plate-bound sample analytes. The substrate reacts with enzyme portion of the conjugate to produce colour. The positive control is a solution that contains antibody or antigen. The negative control is a solution without antibody or antigen. The controls are used to validate the assay and to calculate sample results.

3.7 Questionnaire survey to assess KAP towards Brucellosis

The questionnaires were prepared and pre-tested and was modified to make it reliable for the respondents to answer. Then it was applied to a total of 200 pregnant women visiting Gynaecology Department during the study period.

The questions were based on knowledge, attitude and practices (KAP) towards Brucellosis and face to face interview was performed among the pregnant women. The questions include knowledge about zoonotic diseases, meat and milk borne diseases, miscarriage and brucellosis. Similarly their attitudes towards different forms of treatment were also included.

3.8 Statistical Analysis

The data collected were checked for the completeness. The data were analyzed by using SPSS version 21. The association between categorical variable was assessed by chi-square ($\chi 2$) test. The result was considered significant at 5% level of significance (P value < 0.05). The analyzed data were summarized in table and percentages.

Photo plates

Photo no.1 Questionnaire with pregnant women

Photo no.2 Materials used in ELISA test

Photo no 3. Pipetting the sample in the ELISA kit

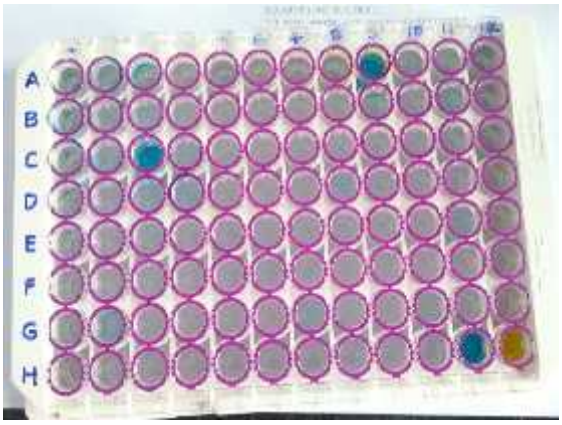

Photo no.4 Change in colour after adding substrate solution

Photo no.5 Adding stop solution

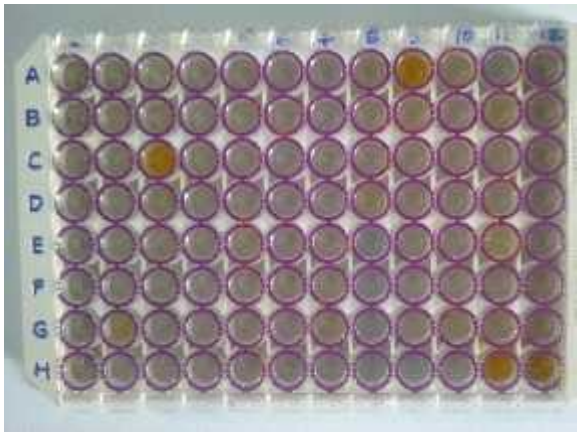

Photo no. 6 Interpretation of ELISA results

Photo no.7 ELISA reader reading OD value

4. RESULTS

4.1 Sero-prevalence of brucellosis among pregnant women

A total of 80 sera samples were collected from the pregnant women visiting Kathmandu Model Hospital. The individuals from which the sera sample was taken were categorized on the basis of ethnic group, age and trimester. Of the 80 samples tested, 9 (11.25%) were found to be brucellosis positive (Table1). The statistical analysis revealed that there were no significance differences ($p>0.05$) between seropositivity of brucellosis, ethnicity and trimester of the pregnant women but found significant differences between seropositivity of brucellosis and age of the pregnant women indicating that seropositivity of brucellosis is high among the age group >30 years.

4.1.1 Ethnic wise prevalence

Among 80 samples collected, ethnicity has been differentiated into four major groups such as brahmin, chhetri, janajati and madhesi on the basis of the surname of the respondents. The highest sero-prevalence rates of brucellosis was found among madhesi (16.66%) followed by janajati (11.53%) and the lowest was in brahmin (8.33%) even though the sample size were not equally divided (Table1). There is no significance difference between seropositivity of brucellosis and ethnicity of pregnant women.

4.1.2 Age wise prevalence

Of the total samples collected the lowest age is 18 and the highest age is 35 so the age group has been classified into four groups with the class interval of five. The highest sero-prevalence rate (29.411%) was found within the age group 31-35 years followed by the age group 26-30 years (13.33%) whereas there is absence of seropositivity among the age group 16-20 years and 21-25 years (Table1). The statistical analysis shows that there is significance difference between seropositivity of brucellosis and age of the pregnant women.

4.1.3 Trimester wise prevalence

Among 80 samples collected, trimester has been differentiated into three groups (first, second and third trimester) on the basis of the month of pregnancy. The highest sero-prevalence rate (12.76%) was found in the third trimester followed by first trimester (10%) and the lowest was in second trimester (8.69%) even though the sample sizes were not equally divided. The statistical analysis shows that there is no significance difference ($p>0.05$) between seropositivity of brucellosis and trimester of pregnant women (Table1).

Table1: Sero-positivity distribution of pregnant women by ethnicity, age and trimester by ELISA

Variables	Frequency n=80	Positive (%)	Value of $\chi 2$	d.f	P-value
Ethnicity			0.560	3	0.906
Brahmin	24	2 (8.33)			
Chhetri	18	2 (11.11)			
Janajati	26	3 (11.53)			
Madhesi	12	2 (16.66)			
Age			9.930	3	0.019
16-20	8	0 (0)			
21-25	25	0 (0)			
26-30	30	4 (13.33)			
31-35	17	5 (29.411)			
Trimester			0.274	2	0.872
First trimester (1-3 months)	10	1 (10)			
Second trimester (4-6 months)	23	2 (8.69)			
Third trimester (7-9 months)	47	6 (12.76)			

4.2 Assessment of knowledge, attitude and practices among the pregnant women

4.2.1 Socio demographic characteristics

A total of 200 pregnant women were interviewed with structured questionnaire to assess their knowledge, attitude and practices towards brucellosis. Most of the women were from the brahmin (37.5%) ethnic group followed by janajati (29%) and others. Of the total participants 38.5% fall under the age group 26-30 years likewise 29.5% fall under 21-25 age group. According to trimester wise distribution 48% of pregnant women falls under third trimester followed by second trimester and first (Table2).

Table 2: Socio demographic characteristics of study participants

Variables	**Characteristics**	**Frequency (n=200) (%)**
Ethnic group	Brahmin	75 (37.5)
	Chhetri	38 (19)
	Janajati	58 (29)
	Dalit	9 (4.5)
	Madhesi	20 (10)
Age group	16-20	24 (12)
	21-25	59 (29.5)
	26-30	77 (38.5)
	31-35	35 (17.5)
	36-40	5 (2.5)
Trimester	First trimester(1-3months)	31 (15.5)
	Second trimester(4-6months)	73 (36.5)
	Third trimester(7-9months)	96 (48)

4.2.2 Assessment on knowledge

Out of 200 respondents, all had the knowledge about zoonotic diseases. Most of them had heard about rabies and others on some of the common zoonotic diseases like bird flu, swine flu etc. Of the total respondents 83% have knowledge on meat borne diseases (bird flu and swine flu). Among the total respondents 52.5% have knowledge about diseases transmitted from milk and milk products (TB and fever). There is significant difference between ethnicity and knowledge about diseases transmitted from milk and milk products ($p<0.05$) indicating that the knowledge about disease transmitted from milk and milk products is high among brahmin ethnic group followed by chhetri and other ethnic groups. There is an also significant difference between age and knowledge about diseases transmitted from milk and milk products. Of the total respondents knowledge about diseases transmitted from milk and milk products is high on 26-30 years age group followed by 21-25 years age group. Of the total respondents 95% of women have knowledge about miscarriage (Table3). They heard about miscarriage cases from their family, relatives and in village/town. Of the total women who knows about miscarriage 78.4% know the reasons of miscarriage. There is significant difference between age and knowledge about reasons of miscarriage indicating that knowledge about reasons of miscarriage is high among respondents falling under 26-30 years age group. Most of them said that heavy work and long journey by bus is the major cause of miscarriage. Other said that problem in uterus, weak balanced diet, carelessness; physical injuries and diseases are also the causes of miscarriage. Only 4% of the total respondents have knowledge about brucellosis (Table3). They heard about brucellosis from social media and while visiting hospital but they don’t have clear idea about it.

Table 3: Knowledge assessment

Variables for knowledge	Frequency (%)	Ethnicity (p value)	Age (p value)	Trimester (p value)
Knowledge about zoonotic diseases	200 (100)			
Knowledge on meat borne diseases	166 (83)	0.136	0.329	0.574
Knowledge about diseases transmitted from milk	105 (52.5)	0.007	0.001	0.600
Knowledge about miscarriage	190 (95)	0.084	0.457	0.503
Knowledge on reasons of miscarriage	149 (78.4)	0.088	0.002	0.685
Knowledge about brucellosis	8 (4)	0.788	0.955	0.972

4.2.3 Assessment on attitude

Of the 200 respondents 80% go to the hospital and medical center for treatment when they feel unwell and 20% of them prefer both hospital and dhami/jhakri for treatment (Table4). There is significant difference between ethnicity and places from where respondents get treatment. Of the total respondents, women getting treatment from hospital is highest in brahmin ethnic group followed by janajati ethnic group and other ethnic groups. Likewise women getting treatment from both hospital and dhami/jhakri is highest in janajati ethnic group followed by other ethnic groups. Of the total respondents suffering from fever, most of them recover without taking medicine in two or three days and they even don't know the reasons of fever and others take medicine as they were suffering from typhoid.

Table 4: Attitude assessment

Variables for attitude	Frequency (%)	Ethnicity (p value)	Age (p value)	Trimester (p value)
Places from where respondents get treatment				
Hospital	160 (80%)	0.000	0.134	0.553
Hospital and dhami/jhakri	40 (20%)			

4.2.4 Assessment on practice

Generally most of the respondents consume meat of goat (64.4%). All the respondents consume well-cooked meat. There is a significant difference between ethnicity of people and food habit and types of meat consumed. Of the total respondents, women with non-vegetarian food habit are highest in brahmin ethnic group followed by janajati and other ethnic groups. Similarly respondents consuming meat of goat is highest in brahmin ethnic group followed by chhetri and other ethnic groups. There is an also significant difference between trimester of pregnant women and food habit. Of the total respondents, women with non-vegetarian food habit are highest in third trimester followed by second and first trimester. Out of 200 respondents 84.5% consumes milk. Most of them (58.6%) consume dairy milk. Only 3% of them consume raw milk. There is significant difference between ethnicity of people and milk consumed and consumption of raw milk. Of the total respondents, women consuming milk is highest in brahmin ethnic group followed by janajati and other ethnic groups. Similarly respondents consuming raw milk are highest in madhesi ethnic group followed by janajati ethnic group and other ethnic groups do not consume raw milk. There is also significant difference between trimester and types of milk consumed. Of the total respondents women's consuming dairy milk is highest in third trimester followed by second and first trimester. Out of the total respondents consuming milk 65.1% take ten minutes to boil the milk while 34.9% take five minutes to boil the milk. Generally 50.3% people don't prepare any items from milk. Those who prepare various items from milk, they all prepared items from well boiled milk.

Table 5: Practices of respondents towards brucellosis

Variables on practices	Frequency (%)	Ethnicity (p value)	Age (p value)	Trimester (p value)
Food habit Vegetarian Non vegetarian	 26 (13) 174 (87)	0.030	0.676	0.040
Types of meat consumed Goat Buffalo and goat Buffalo, goat and pig	 112 (64.4) 33 (19) 29 (16.7)	0.000	0.427	0.115
Method of meat preparation Well cooked	 174 (100)			
Milk consumed	169 (84.5)	0.001	0.508	0.478
Types of milk consumed Dairy Buffalo Cow Buffalo and cow Dairy, buffalo and cow	 99 (58.6) 33 (19.5) 22 (13) 10 (5.9) 5 (3)	0.077	0.860	0.022
Consumption of raw milk	5 (3)	0.003	0.786	0.119
Time taken to boil the milk 5mins 10mins	 59 (34.9) 110 (65.1)	0.233	0.94	0.323
Items prepared from milk Curd Curd, ghee and butter None of them	 31 (18.3) 53 (31.4) 85 (50.3)	0.099	0.500	0.432
Items prepared from well boiled milk	84 (42)			

5. DISCUSSION

Brucellosis is a worldwide zoonosis and a common cause of economic loss and ill health among animal and human populations (Baba et al. 2001). Human brucellosis can be a very debilitating disease, although the case fatality rate is generally low; it often becomes sub-clinical or chronic, especially if not recognized early and treated promptly. All ages are susceptible, and even congenital cases have been recorded. Few studies have attempted to measure infection in the general population, but a recent study in southern Saudi Arabia showed about 20% of the population had serological evidence of exposure (Robinson 2003). Although human brucellosis is the most common bacterial zoonotic infection worldwide it is still a regionally neglected disease. Human brucellosis is known to be highly endemic in the Mediterranean basin, Middle East, Western Asia, Africa, and South America (Pappas et al. 2006). Approximately 25.8 million human beings and around 18.84 million of the livestock are susceptible to the disease indicating potent economic looser disease. In man it cause Malta or Mediterranean fever regarding as important zoonosis rendering serious health hazard and easily transferred from feed material like raw cheese, meat and other contaminated materials (Joshi 2007). As brucellosis is transmitted from meat and milk products to humans, in Nepal buffaloes contribute about 64% of the meat consumed, followed by goat meat (20%), pork (7%), poultry (6%) and mutton (2%) (Joshi et al. 2003). Similarly about 88% of urban households consume fluid milk regularly and another 7% occasionally and for milk products, consumption is primarily concentrated on traditional products like ghee (45% of households) and yoghurt (33% of households) in Nepal (Joshi and K.C 2001).

Brucellosis is a bacterial zoonotic disease. Different types of research have been done regarding human brucellosis but globally only little research has been done on brucellosis in pregnant women. In Nepal, though the countable reports based on human brucellosis has been reported but still there is no report on brucellosis in pregnant women. Being a zoonotic disease, these days it has become an issue of major concern on the public health as well as animal husbandry. This study of brucellosis in case of pregnant women was conducted for the first time in the capital of the country. This study determined the seroprevalence of brucellosis in pregnant women visiting gynaecology department of KMH. A total of 80 sera samples were collected from the pregnant women visiting for regular checkup in Kathmandu Model Hospital and ELISA were performed to detect the presence of IgG antibodies against *Brucella*. Of the 80 samples tested, 9 were found to be

positive for brucellosis. The seroprevalence of brucellosis in pregnant women was found to be 11.25%. Previously, Aryal (2007) reported the seroprevalence of human brucellosis in Kathmandu to be 11.95% in which the sample size was 1006. Similarly, Rana (2002) reported the prevalence rate of human brucellosis to be 20% in Surkhet district and 14% in the patients visiting Bir hospital. Likewise other hospital based studies also showed similar results 8.17% from the serum samples collected from Siddhi Polyclinic, Teku infectious hospital, Bir hospital and Bharatpur Hospital (Joshi et al. 2009), 0.4% from the samples collected from hospitals in Kathmandu (Knox et al. 2000) and 2.7% from the samples collected from Bir hospital and Teku infectious hospital (Joshi 1984). The overall seroprevalence of human brucellosis in Chitwan district was 1.4% (Upadhyay 1998), in Dolakha district was 0.5% (Dahal 2003) and in Kathmandu was 6.08% (Joshi 1983).

About 4.96% prevalence of brucellosis has been reported among PUO and occupationally exposed individuals in Goa (Pathak et al. 2014). Similarly, the prevalence has been reported 24.5% in Ludhiana, India (Yohannes and Gill 2011). Globally several studies showed the similar seroprevalence such as 6.4% in Kurdistan, Iran (Esmaeili et al. 2014), 5.2% in Afghanistan (Akarbian et al. 2015), 3.4% in rural area of Kayseri, Central Anatolia (Centinkaya et al. 2004), 19% in Saudi Arabia (Alsubaie et al. 2005), 2.8 cases per 100,000 persons in Georgia (Pappas et al. 2006), 53.25% in Mongolia (Erdenebaatar et al. 2004), 20.5% in Tanzania (James 2013), 6.26% in Egypt (Hassanain and Ahmed 2012), 16% in Kenya (Osoro et al. 2015), 2.15% in Ethiopia (Tibesso et al. 2014), 24.1% prevalence in abbatoir workers of Abuja (Aworh et al. 2013), 17% in Uganda (Tumwine et al. 2015) and 3.8% in nomadic communities of Chad (Schelling et al. 2003). Also the prevalence of human brucellosis was 32.49 cases per 100,000 inhabitants in Larissa, Central Greece (Minas et al. 2007) and 75 had suffered from laboratory acquired brucellosis in Spain (Bouza et al. 2005). Likewise the seroprevalence of brucellosis was 19% in Albania (Bego and Byku 2015). The incidence of brucellosis among pregnant women was 12.2% in Egypt (Elshamy and Ahmed 2008).

In the present study, the statistical analysis showed that there is significance difference between seropositivity of brucellosis and age of the pregnant women in which the highest seroprevalence rate (29.41%) was found within the age group 31-35 years followed by the age group 26-30 years (13.33%). By age group the seroprevalence of brucellosis was high (2.72%) among the people above 50 years age group in Chitwan (Upadhyay 1998). The

prevalence rate was high in the age group of 20-29 years (29%) in Surkhet (Rana 2002) and in Kathmandu the highest prevalence 29.17% was seen in 6-15 years age group (Aryal 2007).

The highest prevalence (4.3% and 4.1%) was found in the 35-44 and 15-24 age groups and the lowest prevalence (2%) was observed in 25-34 age groups among the people living in rural area of Central Anatolia, Turkey (Centinkaya et al. 2004). Similarly, the most common age of human brucellosis in Azna, Western Iran was 15-24(27.9%) and about 60.5% of the patients were between 15-44 years old (Kassiri et al. 2013). Brucellosis was most prevalent among people aged 30-49 years accounting for 46% of the cases in Serbia (Cekanac et al. 2010) and the highest seroprevalence (26.9%) was found in 15-24 years in Albania (Bego and Byku 2015).

The present study showed the highest seroprevalence rate in the third trimester (12.76%) followed by first trimester (10%) and the lowest was in second trimester (8.69%). Similarly, the highest seroprevalence rates of brucellosis was found among madheshi (16.66%) followed by janajati (11.53%) and the lowest was in brahmin (8.33%).

Knowledge, Attitude and Practices towards Brucellosis

Among 200 respondents all had the knowledge about zoonotic diseases but only 4% of them have heard about brucellosis without any clear idea on it. The knowledge about brucellosis among the farmers of Chitwan was found to be nil (Upadhyay et al. 1998). Similarly 12% of farmers of Rupandehi district knew about brucellosis (Poudel 2014). In Egypt 83.2% of participants responsible for rearing animals were sure that they heard of a disease named brucellosis and 16.8% believed they had heard of the disease but were not sure (Holt et al. 2011). Similarly 22.1% of farmers of Jimma zone, Ethiopia have heard about brucellosis among them only 29.2% knew that it is zoonotic disease (Kuma et al. 2013). Likewise 43.8% of people of Iran revealed that they had a lot of information regarding brucellosis (Ghomashlooyan et al. 2015). The questionnaire survey conducted among the livestock owner (n=537) of Jordan revealed that 100% had heard about brucellosis (Musallam et al. 2015). Likewise 79% pastoralists (n=120) of Kenya had heard of brucellosis and among them 71% knew brucellosis as a zoonotic disease (Obonyo and Gufu 2015), 9% public health workers (n=110) of Nyanza province, Kenya knew brucellosis as a zoonotic disease (Omemo et al. 2012), 57% of the household owners knew about brucellosis in Tajikistan (Grahn 2013), 12.2% had heard of

brucellosis but were not fully knowledgeable on the disease in Tanzania (James 2013), 92.9% of the pastoralists in Nigeria were aware of brucellosis (Buhari et al. 2015), only 21.9% farmers of Kars Turkey knew about zoonotic diseases and 88.1% had heard about brucellosis (Cakmur et al. 2015), 41.7% agro-pastoral communities of Uganda had knowledge about brucellosis (Tumwine et al. 2015), only 7.7% respondents in Ethiopia had knowledge about brucellosis (Desta 2015) and 19.6% were aware of brucellosis and 6.6% knew that the disease is caused by a bacterial agent in Nigeria (Adesiji et al. 2005).

In the present study 83% have knowledge on meat borne diseases, most of them knew about bird flu and swine flu as meat borne diseases and 52.5% have knowledge on milk borne diseases (TB and fever). In Dolakha district, out of 100 respondents 96% of them did not know about milk-borne and meat borne diseases (Dahal 2003).

The present study showed that 84.5% consume milk and most of them (58.6%) consume dairy milk. Only 3% of them consume raw milk. Among the respondents consuming milk 65.1% take ten minutes to boil the milk while 34.9% take five minutes to boil the milk. Among the respondents consuming meat, all of them consumed well-cooked meat. Those who prepare various items from milk, they all prepared items from well boiled milk. All (n=30) the respondents in Chitwan consumed boiled milk (Upadhyay 1998). Likewise 30% of the respondents in Surkhet consumed raw milk and among them also children up to 5 years of age were found to be fed by their parents for nourishment (Rana 2002). All of the respondents (n=100) in Dolakha boiled milk for over 5 minutes before consumption (Dahal 2003).

All the participants of Menufiya Governorate of Egypt said they boiled milk before consumption but no participants boiled milk before processing it into other dairy products (Holt et al. 2011). Among 294 farmers of Mana and Limmukosa districts of Jimma zone, Ethiopia, 43.2% consumed raw milk and 27.2% consumed raw meat (Kuma et al. 2013). Similarly 96% of the respondents in Garrisa, Kenya consumed raw milk and 14% consumed milk products processed from raw milk (Obonyo and Gufu 2015), 18% of the interviewees in Tajikistan drank fresh milk and 58% boiled the milk before drinking it and made Smetana out of it (Grahn 2013), almost 30% of the households consumed unpasteurized dairy products on regular basis in Tajikistan (Lindahl et al. 2015), 57% of the respondents in Turkey consumed milk by boiling, 45% make cheese from boiled milk and 55% make cheese from raw milk and 23.8% consumed raw milk (Cakmur et al.

2015), 12.8% of people in Kiboga district of Uganda consume raw milk and 53.2% consumed milk products (Tumwine et al. 2015), 26.5% of people in Mbarara and Kampala districts, Uganda consumed raw milk (Nasinyama et al. 2014), 66% of people in Afghanistan consumed fresh cheese,40.6% consumed unsalted butter and 14.9% consumed cream made from raw milk (Cetinkaya et al. 2005), all(168) of the respondents in Ethiopia consumed raw milk and also liver is consumed raw (Desta 2015), 36% of the people in Nigeria have habits of eating raw meat and 13% drink unpasteurized milk (Adesiji et al. 2005).

In the present study 80% of the respondents go to the hospital and medical centre for treatment when they feel unwell and 20% of them prefer both hospital and dhami/jhakri for treatment. In Dolakha 59.94% go to a faith healer when their livestock are ill, 25% seek veterinary help and 18.6% use their domestic treatment methods (Dahal 2003). In Ishafan, Iran 6.8% were familiar with herbal medicines to cure brucellosis and 72.2% have been treated and 53 participants believed that herbal therapy was most effective and these patients (32.7%) turned to traditional medicine for treatment of brucellosis while 9.3% did not addressed the traditional physician (Ghomashlooyan et al. 2015). Similarly 42% of people in Garrisa, Kenya mentioned visiting health facility, 24% seeking divine intervention, 18% consuming herbal medicine and 15% would purchase medicine from a local chemist when they suffer from brucellosis (Obonyo and Gufu 2015). Majority of the respondents (49.3%) in Tanzania practiced self-medication whenever felt sick and 30.1% were using traditional healers to get health services (James 2013).

6. CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

Sero-prevalence of brucellosis among pregnant women visiting Gynaecology Department of Kathmandu Model Hospital, Kathmandu carried out by ELISA tests revealed that the seropositivity was 11.25%. The seropositivity was found highest among madhesi (16.66%) ethnic group followed by janajati and the lowest was in brahmin (8.33%). Similarly, the highest sero-prevalence rate (29.411%) was found within the age group 31-35 years followed by the age group 26-30 years whereas there is absence of seropositivity among the age group 16-20 years and 21-25 years. According to the trimester wise prevalence the highest seroprevalence rate (12.76%) was found in the third trimester followed by first trimester.

In a questionnaire survey of 200 pregnant women done to assess the knowledge, attitude and practices towards brucellosis, all had knowledge about zoonotic diseases but only few of them heard about brucellosis. Majority of the pregnant women had positive attitudes but the knowledge about the disease was very poor. So there is a need to strengthen the knowledge about the disease through mass media or any other means.

Being a zoonotic disease, human gets brucellosis through infected animal which may be due to direct contact with the infected animals or through consumption of milk or milk products and meat of infected animals. So in order to control brucellosis in humans, the first step is to control and eradicate the diseases from livestock.

6.2 Recommendations

- Sero-epidemiological surveillance of human brucellosis should be carried out regularly throughout the nation.
- There must be facilities for the diagnosis of brucellosis in every Medicals hospitals and clinics.
- Educate people and generate public awareness about the causative agent, route of transmission, symptoms, treatment, prevention and control of the disease through mass media like television, radio, as well as inclusion in text books etc.

7. REFERENCES

Abdollahi, A., Morteza, A., Omid, K. and Rasoulinejad, M. 2010. Brucellosis serology in HIV-infected patients. International Journal of Infectious Diseases **14**: e904-e906.

Abdullayev, R., Kracalik, I., Ismayilova, R., Ustun, N., Talibzade, A. and Blackburn, J.K. 2012. Analyzing the spatial and temporal distribution of human brucellosis in Azerbaijan (1995-2009) using spatial and temporal statistics. BMC Infectious Diseases **12**: 185.

Adekolu-John, F.O. 1991. A serological survey of human brucellosis in Kainji lake population of Nigeria. Tropical Veterinary **7**(3/4): 141-148.

Adesiji, Y.O., Adesiji, G.B. and Fagbami, A.H. 2005. Brucellosis: knowledge, attitude and practices among occupationally exposed individuals in Osun state. Science Focus **10**(1): 38-41.

Afify, M., Al-Zahrani, S. H., and El-Koumi, A.M. 2012. Brucellosis-induced Pancytopenia in children: a prospective study. Journal of American Science **8**(10): 112-116.

Aggad, H. and Boukraa, L. 2006. Prevalence of bovine and human brucellosis in western Algeria: Comparison of screening tests. Eastern Mediterranean Health Journal **12**(1/2): 119-128.

Ahmed, M.O., Elmeshri, S.E., Abuzweda, A.R., Blauo, M., Abouzeed, Y.M., Salem, H., et al. 2010. Seroprevalence of brucellosis in animals and human populations in the western mountains region in Libya, December 2006- January 2008. Eurosurveillance **15**(30):19625-19627.

Akbarian, Z., Ziay, G., Schauwers, W., Noormal, B., Saeed, I., Qanee, A.H., et al. 2015. Brucellosis and *Coxiella burnetii* infection in householders and their animals in secure villages in Herat Province, Afghanistan: A cross-sectional study. PLoS Neglected Tropical Diseases **9**(10): e0004112.

Alavi, M.S., Rafiei, A. and Nikkhooi, A. 2007. The effect of lifestyle on brucellosis among nomads in Khuzestan province of Iran. Pakistan Journal of Medical Sciences **23**(3): 358-360.

Alsubaie, S., Almuneef, M., Alshaalan, M., Balkhy, H., Albanyan, E., Alola, S., et al. 2005. Acute brucellosis in Saudi families: Relationship between *Brucella* serology and clinical symptoms. International Journal of Infectious Diseases **9**: 218-224.

Amin, M.M., Ahmed, S.A., Zaki, H.M., and Ismail, R.I. 2012. Serological and molecular studies on the Diagnosis of Bovine Brucellosis. Nature and Science **10**(11): 68-76.

Ansary, E.H, Mohammed, B.A., Hamad, A.R., and Karom, A.G. 2001. Brucellosis among animals and human contacts in eastern Sudan. Saudi Medical Journal **22**(7): 577-579.

Araj, G.F. 2010. Update on laboratory diagnosis of human brucellosis. International Journal of Antimicrobial Agents **36**: S12-S17.

Ariza, J., Bosilkovski, M., Cascio, A., Colmenero, J.D., Corbel, M.J., Falagas, M.E., et al. 2007. Prospectives for the treatment of Brucellosis in the 21st century: the loannina recommendations. PLoS Medicine **4**(12): e317.

Aryal, A. 2007. Seroprevalence of human brucellosis in Kathmandu. A report submitted to TU, IAAS, Rampur Chitwan, Nepal.

Aryal, S. and Poudel K.P. 2007. Reproductive disorders and seroprevalence of brucellosis in Yaks. Nepal Agriculture Research Journal **8**: 130-132.

Assenga, J.A., Matemba, L.E., Muller, S.K., Malakalinga. J.J. and Kazwala, R.R. 2015. Epidemiology of *Brucella* infection in the human, livestock and wildlife Interface in the Katavi-Rukwa ecosystem,Tanzania. BMC Veterinary Research **11**: 189.

Avdikou, I., Maipa, V. and Alamanos, Y. 2005. Epidemiology of human brucellosis in a defined area of Northwestern Greece. Epidemiology and Infection **133**(5): 905-910.

Awad R. 1998. Human brucellosis in the Gaza Strip, Palestine. Eastern Mediterranean Health Journal **4**(2): 225-233.

Aworh, M.K., Okolocha, E., Kwaga, J., Fasina, F., Lazarus, D., Suleman, I., et al. 2013. Human brucellosis: seroprevalence and associated exposure factors among abattoir workers in Abuja, Nigeria-2011. Pan African Medical Journal **16**: 103.

Baba, M.M., Sarkindared, S.E. and Brisibe, F. 2001. Serological evidence of brucellosis among predisposed patients with pyrexia of unknown origin in the north eastern Nigeria. Central European Journal of Public Health **9**(3): 158-161.

Baddour, M.M. 2012. Diagnosis of brucellosis in humans:a review. Journal of Veterinary Advances **2**(4): 149-156.

Baljinnyam, Z., Tsend, S., Tsegeen, N., Dorj, G., Dashzevge, E., Zinsstag J., et al. 2014. Representative seroprevalences of human and livestock brucellosis in two Mongolian Provinces. Ecohealth **11**(3): 356-371.

Bego, A. and Byku, B. 2015. Seroprevalence of brucellosis in Albania, 2004-2012. International Journal of Science and Research **4**(8): 1519-1521.

Bikas, C., Jelastopulu, E., Leotsinidis, M. and Kondakis, X. 2003. Epidemiology of human brucellosis in a rural area of north-western Peloponnese in Greece. European Journal of Epidemiology **18**: 267-274.

Bilal, N.E., Jamjoom, G.A., Bobo, R.A., Aly, O.F. and Nashar, N.M. 1991. A study of the knowledge, attitude and practice (KAP) of a Saudi Arabian community towards the problem of brucellosis. Journal of Egypt Public Health Association **66**(1-2): 227-38.

Bokaie, S., Sharifi, L. and Alizadeh, H. 2008. A seroepidemiologic survey of brucellosis in humans and animals in Birjand, east of Iran. International Journal of Infectious Diseases **12**(Suppl1): e448.

Bonfoh, B., Kasymbekov, J., Durr, S., Toktobaev, N., Doherr, M.G., Schueth, T., et al. 2012. Representative seroprevalences of brucellosis in humans and livestock in Kyrgyzstan. EcoHealth **9**: 132-138.

Bosilkovski, M., Krteva, L., Dimzova, M. and Kondova, I. 2007. Brucellosis in 418 patients from the Balkan Peninsula: exposure-related differences in clinical manifestation, laboratory test results and therapy outcome. International Journal of Infectious Diseases **11**: 342-347.

Bouza, E., Sanchez, C.C., Hernangomez, S. and Gonzalez, M.J. 2005. Laboratory acquired brucellosis: a Spanish national survey. Journal of Hospital Infections **61**(1): 80-83.

Buhari, H,U., Saidu, S.N.A., Mohammed, G. and Raji, M.A. 2015. Knowledge, attitude and practices of pastoralists on Bovine brucellosis in the north senatorial district of Kaduna state, Nigeria. Journal of Animal Health and Production **3**(2): 28-34.

Buzgan, T., Karahocagil, M.K., Irmark, H., Baran, A.I., Karsen, H., Evirgen, O., et al. 2010. Clinical manifestations and complications in 1028 cases of brucellosis:a retrospective evaluation and review of the literature. International Journal of Infectious Diseases **14**: e469-e478.

Cakmur, H., Akoglu, L., Kahraman, E. and Atasever, M. 2015. Evaluation of farmers knowledge-attitude-practice about zoonotic diseases in Kars, Turkey. Kafkas Journal of Medical Sciences **5**(3): 87-93.

Capasso, L. 2002. Bacteria in two-millennia-old cheese and related epizoonoses in Roman populations. Journal of Infections **45**: 122–127.

Cekanac, R., Mladenovic, J., Ristanovic, E. and Lazic, S. 2010. Epidemiological characteristics of brucellosis in Serbia, 1980-2008. Croatian Medical Journal **51**(4): 337-344.

Cetinkaya, F., Nacar, M., Nedretkoc, A., Gokahmetoglu, S. and Aydin, T. 2004. Prevalence of brucellosis in the rural area of Kayseri, Central Anatolia, Turkey. Turkish Journal of Medical Sciences **35**: 121-126.

Cetinkaya, Z., Aktepa, O.C., Ciftci, H.I. and Demirel, R. 2005. Seroprevalence of human brucellosis in a rural area of western anatolia,Turkey. Journal of Health Population and Nutrition **23**(2): 137-141.

CFSPH. 2009. Brucellosis. The Centre for Food Security and Public Health. Ames, Lowa.

CFSPH. 2012. Canine brucellosis: Brucella canis. The Centre for Food Security and Public Health. Ames, Lowa.

Corbell. 1997. Brucellosis: an overview. Emerging Infectious diseases **2**: 213-21.

Dahal, R. 2003. Sero-epidemiological surveillance of brucellosis among humans and animals in Dolakha districts, Nepal. M.Sc. Thesis. Central Department of Zoology, Tribhuvan University, Kathmandu, Nepal.

Dahouk, Al.S., Neubauer, H., Hensel, A., Schoneberg, I., Nockler, K., Alpers, K., et al. 2007. Changing epidemiology of human brucellosis, Germany, 1962-2005. Emerging Infectious Diseases **13**(12): 1895-1900.

Dautovic, S.K., Cengic, M.H., Mehanic, S., Ahmetagic, S., Ibrahimpasic, N., Hadzic, E., et al. 2010. Brucellosis-Emerging zoonosis in Bosnia and Herzegovina. International Journal of Infectious Diseases **14**(Suppl1): e161.

Dean, A.S., Crump, L., Greter, H., Schelling, E. and Zinsstag, J. 2012. Global burden of human Brucellosis: a systematic review of disease frequency. PLOS Neglected Tropical Diseases **6**(10): e1865.

Deqiu, S., Donglou, X. and Jiming, J. 2002. Epidemiology and control of brucellosis in China. Veterinary Microbiology **90**(1-4): 165-182.

Dermott, J. and Arimi, S.M. 2002. Brucellosis in sub-saharan Africa: epidemiology, control and impact. Veterinary Microbiology **90**(1-4): 111-134.

Deshmuk, A., Hagen, F., Alsharabasi, O., Abraham, M., Wilson, G., Doiphode, S., et al. 2015. In vitro antimicrobial susceptibility testing of human *Brucella melitensis* isolates from Qatar between 2014-2015. BMC Microbiology **15**: 121.

Desta, A.H. 2015. Public awareness and practices of pastoral and agro-pastoral community towards zoonotic *Brucella* infection in Afar regional state of north east Ethiopia. Euro Journal of Preventive Medicine **3**(5): 141-146.

DHS.2003.National recommended case definition and surveillance standards 2003.HMG, Ministry of health, Department of health services, Epidemiology and disease control divison. Kathmandu, Nepal.

Dickenson, J.G. and Thaller, V.T. 1979. Brucellosis in shepherds in Nepal. Journal of Nepalese Medical Association **17**(3): 33-38.

Din, A.M.U., Khan, S.A., Ahmad, I., Rind, R., Hussain, T., Shahid, M., et al. 2013. A study on the seroprevalence of brucellosis in human and goat populations of district Bhimber, Azad Jammu and Kashmir. The Journal of Animal and Plant Sciences **23**(1 Suppl.): 113-118.

DoAH. 2067/68. Annual technical report. Government of Nepal, Ministry of agricultural development, Department of livestock services, Tripureshwor, Kathmandu, Nepal.

DoAH. 2070/71. Annual technical report. Government of Nepal, Ministry of agricultural development, Department of livestock services, Tripureshwor, Kathmandu, Nepal.

Doganay, M. and Aygen, B. 2003. Human brucellosis: An overview. International Journal of Infectious Diseases **7**: 173-182.

Elbeltagy, K.E. 2001. An epidemiological profile of brucellosis in Tabuk Province, Saudi Arabia. Eastern Mediterrean Health Journal **7**(4/5): 791-798.

Elshamy, M. and Ahmed, A. 2008. The effects of maternal brucellosis on pregnancy outcome. Journal of Infections of Developing countries **2**(3): 230-234.

Erdenebaatar, J., Bayarsaikhan, B., Yondondorj, A., Watarai, M., Shirahata, T., Jargalsaikhan, E., et al. 2004. Epidemiological and serological survey of brucellosis in Mongolia by ELISA using Sarcosine extracts. Microbiology and Immunology **48**(8): 571-577.

Eriksen, N., Lemming, L., Hojlyng, N. and Bruun, B. 2002. Brucellosis in immigrants in Denmark. Scandinavian Journal of Infectious Diseases **34**(7): 540-2.

Esmaeili, S., Pourhossein, B., Gouya, M.M., Amiri, F.B. and Mostafavi E. 2014. Seroepidemiological survey of Q fever and brucellosis in Kurdistan province, western Iran. Vector borne and Zoonotic Diseases **14**(1): 41-45.

Foster, G., Osterman, B.S., Godfroid, J., Jacques, I. and Cloeckaert, A. 2007. *Brucella ceti* sp. nov. and *Brucella pinnipedialis* sp. nov. for *Brucella* strains with cetaceans and seals as their preferred hosts. International Journal of Systematic Evolution and Microbiology **57**: 2688–2693.

Franco, M.P., Mulder, M., Gilman, R.H .and Smits, H. L. 2007. Human brucellosis. The Lancet Infectious Diseases **7**: 775-86.

Ghomashlooyan, M., Azami, M., Mohaghegh, M.A., Hadipour, M., Jahani, S., Hejazi, S.H., et al. 2015. Evaluation of Ishafan inhabitant attitude about brucellosis and common its herbal treatment. International Journal of Infections **2**(3): e25061.

Godfroid, J., Nielsen, K. and Saegerman, C. 2010. Diagnosis of brucellosis in Livestock and wildlife. Croatian Medical Journal **51**(4): 296-305.

Gonzalez-Gay, M.A., Garcia, P.C., Ibanez, D. and Garcia, P.M.J. 1999. Osteoarticular complications of brucellosis in an Atlantic area of Spain. Journal Rheumatal **26**(1): 141-145.

Grahn, C. 2013. Brucellosis in small ruminants – an investigation of knowledge, attitude and practices in peri-urban farming around the region of Dushanbe, Tajikistan. Tajik Agrarian University, Tajikistan.

Haghdoost, A.A., Kawaguchu, L., Mirzazadeh, A., Rashidi, H., Sarafinejad, A., Baniasadi, A.R., et al. 2007. Using GIS in explaining spatial distribution of brucellosis in an endemic district in Iran. Iranian Journal of Public Health **36**(1): 27-34.

Halling, S.M. and Boyle, S.M. 2002. Incidence and control of brucellosis in the Near East region. Veterinary Microbiology **90**: 81-110.

Hassanain, N.A. and Ahmed, W.M. 2012. Efficacy of Serological Tests in Comparison with PCR for Diagnosis of Brucellosis. World Journal of Medical Sciences **7**(4): 243-247.

Hassanain, N.A. and Ahmed, W.M. 2012. Seroprevalence of brucellosis in Egypt with Emphasis on potential risk factors. World Journal of Medical Sciences **7**(2): 81-86.

Hegazy, Y., Elmonir, W., Abdel-Hamid, N.H. and Elbauomy, M. 2016. Seroprevalence and "Knowledge, attitudes and practices" (KAPs) survey of endemic ovine brucellosis in Egypt. Acta Vetenaria Scandinavica **58**: 1.

Heidmann, P., Joshi, D.D. and Sollod, A. 1998. Evidence of brucellosis in dairy animals in Lalitpur. Zoonoses and Food Hygiene News **4**(4): 87.

Holt, H.R., Eltholth, M.M., Hegazy, Y.M., El-Tras, W.F., Tayel, A.A. and Guitian, J. 2011. *Brucella* spp. infection in large ruminants in an endemic area of Egypt: cross-sectional study investigating seroprevalence, risk factors and livestock owner's knowledge, attitude and practices (KAPs). BMC Public Health **11**: 341.

Hosseini, M.R., Sadeghi, M., Khondabi, E., Bakhshali, H. and Omidi, A.J. 2008. Incidence of brucellosis in Shazand county Iran. International Journal of Infectiuos Diseases **5**: 1183.

Hsuen, H.Y. and Baker, J.H. 2011. Applied food safety education laboratory course III NFS 492/592.

Islam, A., Khatun, M., Were, R.S., Sriranganathan, N. and Boyle, M.S. 2013. A review of Brucella seroprevalence among humans and animals in Bangladesh with special emphasis on epidemiology, risk factors and control opportunities. Veterinary Microbiology **166**: 317-326.

James, L.W. 2013. Studies on human brucellosis in the Mikumi Selous ecosystem, Morogoro, Tanzania. M.Sc.Thesis. Senate of Sokoine University of Agriculture. Morogoro, Tanzania.

Javaia, M.R., Farrugia, M. and Ahmed, N.Y. 2013. Anything rare is possible here: Brucellosis in London in 2012. BMJ Case report.

Jia, P. and Joyner, A. 2015. Human brucellosis occurences in inner Mongolia,China: a spatio-temporal distribution and ecological niche modeling approach. BMC Infectious Diseases **15**:36.

John, K., Fitzpatrick, J., French, N., Kazwala, R., Kambarage, D., Mfinanga, G.S., et al. 2010. Quantifying Risk Factors for Human Brucellosis in Rural Northern Tanzania. PLoS One **5**(4): e9968.

Joshi, D.D. 1984. Incidence of human brucellosis in Kathmandu. Journal of Nepalese Medical Association **22**: 1-7.

Joshi, D.D. 2007. Evidence of brucellosis in human and animals in Nepal. Proceedings of the 12th international conference of the association of institutions for tropical veterinary medicine (ALTVM), Montpellier, France. Does control of animal infectious risks offer new international perspectives? 259-261.

Joshi, D.D. and K.C. T.B. 2001. An overview of smallholder dairy production and marketing in Nepal. Proceedings of a south-south workshop held at National dairy development board (NDDB), Anand, India.

Joshi, D.D. and Rana, S. 2005. Seroprevalence of brucellosis in pig of Kathmandu. Zoonoses and Food Hygiene News **11**(4): 282-285.

Joshi, D.D. and Shrestha, B.S. 2011. Studies on normal fertility indices and factors associated with bovine infertility in the hills of Nepal. Nepalese Veterinary Journal **30**: 86-102.

Joshi, D.D., Maharjan, M., Johansen, M.V., Willingham, A.L. and Sharma, M. 2003. Improving meat inspection and control in resource poor communities: the Nepal example. Acta Tropica **87**: 119-127.

Joshi, D.D., Shrestha, I.L. and Aryal, A. 2009. Seroprevalence of brucellosis in Nepal. Nepalese Veterinary Journal **29.**

Joshi, D.D.1983. Brucellosis a public health problem in Nepal. Bulletin of Veterinary Science and Animal Husbandry **15**: 16.

Kaltungo, B.Y., Saidu, S.N.A., Sackey, A.K.B. and Kazeem, H.M. 2014. A review of diagnostic techniques for brucellosis. African Journal of Biotechnology **13**(1): 1-10.

Kansiime, C., Mugisha, A., Makumbi, F., Mugisha, S., Rwego, I.B., Sempa, J., et al. 2014. Knowledge and perceptions of brucellosis in the pastoral communities adjacent to Lake Mburo national park, Uganda. BMC Public Health **14**: 242.

Kassiri, H., Amani, H. and Lotfi, M. 2013. Epidemiological, laboratory, diagnostic and public health aspects of human brucellosis in western Iran. Asian Pacific Journal of Tropical Biomedicine **3**(8): 589-594.

Kassiri, H., Amani, H., Lotfi, M. and Hosseini, S. 2012. An epidemiological survey of brucellosis in Azna county, Luristan Province, Western Iran. International Journal of Infectious Diseases **16**(Supp.1): e454.

Kaufmann, A.F, Meltzer, M.I. and Schmid, G. P. 1997. The economic impact of a bioterrorist attack. Are prevention and postattack intervention programmes justifiable? Emerging Infectious Diseases **3**(2): 83–94.

Kaya, S., Eskazan, A.E. and Elaldi, N. 2013. Brucellar pericarditis: a report of four cases and review of the literature. International Journal of Infectious Diseases **17**: e428-e432.

Kazmierczak, J. 2012. Public health implications of *Brucella canis* infections in humans. Summary. Findings and Recommendations of the Brucella canis workgroup.

Khan, Y. M., Mah, M.W. and Memish, Z.A. 2001. Brucellosis in pregnant women. Clinical Infectious Diseases **32**: 1172-7.

Knox, C., Gillies, L. and Joshi, D.D. 2000. Veterinary public health in the Nepal Himalaya. Canadian Veterinary Journal **41**(11): 879-881.

Koubaisy, H. and Lafi, A.S. 2011. Presentation of brucellosis in an endemic area; West of Iraq. Egyptian Academic Journal of Biology and Sciences **3**(1): 13-18.

Kuma, T., Deressa, B., Alem, F. and Tigre, W. 2013. Farmers awareness and practices on rabies, bovine tuberculosis, taeniasis, hydatidosis and brucellosis in Mana and Limmukosa districts of Jimma zone, south west Ethiopia. World Applied Science Journal **23**(6): 782-787.

Li, G., Gao, R., Zhang, R. and Yang, Y. 2009. Sika deer as a new source of human infection by brucella. International Journal of Infectious Diseases **13**(Suppl1): 554.

Lim, M.L. and Rickman, L.S. 2004. Brucellosis. Infectious Diseases in Clinical Practice **12**(1): 7-14.

Lindahl, E., Sattorov, N., Boquist, S. and Magnusson, U. 2015. A study of knowledge, attitudes and practices relating to brucellosis among small scale dairy farmers in an urban and peri-urban area of Tajikistan. Plos One **10**(2): e0117318.

Lopes, L.B., Nicolino, R. and Haddad, J. 2010. Brucellosis-risk factors and prevalence: A review. The Open Veterinary Science Journal **4**: 72-84.

Lounes, N., Cherfa, M.A., Carrou, G.L., Bouyoucef, A., Jay, M., Bastaji, B.G., et al. 2014. Human brucellosis in Maghreb: Existence of a lineage related to socio-historical connections with Europe. Plos One **9**(12): e115319.

Lucero, N.E., Ayala, S.M., Escobar, G.I. and Jacob, N.R. 2008. Brucella isolated in humans and animals in Latin America from 1968 to 2006. Epidemiology and Infections **136**: 6-503.

Lulu, A.R., Araj, G.F., Khateeb, M.I., Mustafa, M.Y., Yusuf, A.R. and Fenench, F.F. 1988. Human brucellosis in Kuwait; a prospective study of 400 cases. Quarterly Journal of Medicine Association **66**(249): 39-54.

Makita, K., Fevre, E.M., Waiswa, C., Kaboyo, W., Bronsvoort, B.M.C., Eisler, M.C., et al. 2008. Human brucellosis in urban and peri-urban areas of Kampala, Uganda. Annals of the New York Academy of Sciences **1149**: 309-311.

Mangalgi, S.S., Sajjan, A.G., Mohite, S.T. and Kakade, S.V. 2015. Serological, clinical and epidemiological profile of human brucellosis in rural India. Indian Journal of Community Medicine **40**(3): 163-167.

Maninska, L., Marangozova, K., Timova, T. and Petkoska, S. 2008. Human brucellosis in the region of Strumica, Macedonia. International Journal of Infectious Diseases **12**(Suppl1): e215-e216.

Mantur, B.G., Akki, A.S., Mangalgi, S.S., Patil, S.V., Gobbur, R.H. and Peerapur, B.V. 2004. Childhood Brucellosis-a microbiological, epidemiological and clinical study. Journal of Tropical Pediatric **50**(3): 153-157.

Mantur, G.B. and Amarnatha, K. S. 2008. Brucellosis in India - a review. Bioscience **33**(4): 539-547.

Massis, F.D., Girolamo, A.D., Petrini, A., Pizzigallo, E. and Giovannini, A. 2005. Correlation between animal and human brucellosis in Italy during the period 1997-2002. Clinical Microbiology and Infections **11**: 632-636.

Memish, Z.A. and Balkhy, H.H. 2004. Brucellosis and international travel. Journal of Travel Medicine **11**(1): 49-55.

Mert, A., Ozaros, R., Tabak, F., Billir, M., Yilmaz, M., Kurt, C., et al. 2003. The sensitivity and specificity of Brucella agglutination tests. Diagnostic Microbiology and Infectious Disease **46**(4): 241-243.

Minas, M., Minas, A., Gourgulianis, K. and Stournara, A. 2007. Epidemiological and clinical aspects of human brucellosis in central Greece. Japanese Journal of Infectious Diseases **60**: 362-366.

Mishra, Y.P., Joshi, D.D., Gautam, S.P., Aryal, A. and Shahi, K. 2009. Brucellosis in milking buffaloes of Bhaktapur milk shed areas. Zoonoses and Food Hygiene News **15**(1): 376-378.

Moreno, E. 2014. Retrospective and prospective perspectives on zoonotic brucellosis. Frontiers in Microbiology **5**: 213.

Mrunalini, N. and Ramasatry, P. 1999. Serological survey on the occurrence of brucellosis in domestic animals and man in Andhra Pradesh. Indian Veterinary Journal **76**(6): 483-484.

Munir, R., Farooq, U., Fatima, Z., Afzal, M., Anwar, Z. and Jahangir, M. 2011. Seroprevalence of brucellosis in Bovines at farms under different management conditions. British Journal of Dairy Sciences **2**(3): 35-39.

Musallam, I., Abo-Shehada, M.N. and Guitian, J. 2015. Knowledge, attitudes and practices associated with brucellosis in livestock owners in Jordan. The American Society of Tropical Medicine and Hygiene **93**(6): 1148-1155.

Nasinyama, G., Sekawojwa, E., Opuda, J., Grimaud, P., Etter, E. and Bellinguez, A. 2014. *Brucella* seroprevalence and modifiable risk factors among predisposed cattle keepers and consumers of unpasteurized milk in Mbarara and Kampala districts, Uganda. African Health Sciences **4**: 790-796.

NZFHRC. 2002. Prevalence of brucellosis in Yaks, Chauries, cattle and buffaloes in Rasuwa, Kathmandu and kavre districts. Zoonoses and Food Hygiene News **8**(2): 180-181.

Obonyo, M. and Gufu, W.B. 2015. Knowledge, attitude and practices towards brucellosis among pastoral community in Kenya, 2013. International Journal of Innovative Research and Development **4**(10): 375-384.

Obradovic, Z. and Velic, R. 2010. Epidemiological characteristics of brucellosis in federation of Bosnia and Herzegovina. Croatian Medical Journal **51**(4): 345-350.

Omemo, P., Ogola, E., Omondi, J.W. and Knobel, D. 2012. Knowledge, attitude and practice towards zoonoses among public health workers in Nyanza province, Kenya. Journal of Public Health in Africa **3**: e22.

Omer, M.M., Musa, M.T., Bakhiet, M.R. and Perrett, L. 2010. Brucellosis in camels, cattles and humans: associations and evaluation of serological tests used for diagnosis of

the disease in certain nomadic localities in Sudan. Scientific and Technical Review of the Office International Epizootics **29**(3): 663-669.

Osoro, E.M., Munyua, P., Omulo, S., Ogola, E., Ade, F., Mbatha, P. et al. 2015. Strong association between human and animal Brucella seropositivity in a linked study in Kenya 2012-2013. American Journal of Tropical Medicine and Hygiene **93**(2): 224-231.

Pandeya, Y.R., Joshi, D.D., Dhakal, S., Ghimire, L., Mahato, B.R., Chaulagain, S., et al. 2013. Seroprevalence of brucellosis in different animal species of Kailali district, Nepal. International Journal of Infectious Microbiology **2**(1): 22-25.

Pappas, G., Akritidis, N., Bosilkovski, M., and Tsianos, E. 2005. Brucellosis. The New England Journal of Medicine **352**: 2325-36.

Pappas, G., Papadimitriou, P., Akritidis, N., Christou, L. and Tsianos, E.V. 2006. The new global map of human brucellosis. Lancet Infectious Diseases **6**: 91–99.

Park, M.Y., Seoplee, C., Choi, Y.S., Park, S.J., Lee, J.S. and Lee, H.B. 2005. A sporadic outbreak of human brucellosis in Korea. Journal of Korean Medicine and Sciences **20**(6): 941-946.

Pathak, A.D., Dubal, Z.B., Doijad, S., Raorane, A., Rodrigues, S., Naik, R., et al. 2014. Human brucellosis among pyrexia of unknown origin cases and occupationally exposed individuals in Goa region,India. Emerging Health Threats Journal **7**: 23846.

Poester, F.P., Nielsen, K., Samartino, L.E., and Yu, W.L. 2010. Diagnosis of Brucellosis. The Open Veterinary Science Journal **4**: 46-60.

Poudel, S. 2014. Seroprevalence of brucellosis in pigs in 6 VDCs of Rupandehi district, Nepal. M.Sc. Thesis. Central Department of Zoology, Tribhuwan University, Kathmandu, Nepal.

Pyakural, S. 1980. Brucella agglutinins in human serum in Nepal. Bulletin of Veterinary Science and Animal Husbandry, Nepal **8**: 9-14.

Qasem, M. and Shaqra, A. 2000. Epidemiological aspects of brucellosis in Jordan. European Journal of Epidemiology **16**(6): 581-584.

Rahman, A.K., Dirk, B., Fretin, D., Saegerman, C., Ahmed, M.U., Muhammad, N. et al. 2012. Seroprevalence and risk factors for brucellosis in a high-risk group of individuals in Bangladesh. Foodborne Pathological Diseases **9**(3): 190–197.

Rahman, M.S., Faruk, M.O., Her, M., Kim, J.Y., Kang, S.I. and Jing, S.C. 2011. Prevalence of brucellosis in ruminants in Bangladesh. Veterinarni Medicina **56**(8): 379-385.

Rana, H. 2002. Seroepidemiological surveillance of human and animal brucellosis in Surkhet district, Mid-western region of Nepal. M.Sc.Thesis. Central Department of Zoology, Tribhuvan University, Kathmandu, Nepal.

Robinson, A. 2003. Guidelines for coordinated human and animal brucellosis surveillance. FAO animal production and health paper 156.

Robson, J.M., Harrison, M.W., Wood, R.N., Tilse, M.H., Mckay, A.B. and Brodribb, T.R. 1993. Brucellosis: re-emergence and changing epidemiology in Queensland. Medical Journal of Australia **159**(3): 153-158.

Rubacha, M., Halliday, E.B., Cleaveland, S. and Crump, J. 2013. Brucellosis in low-income and middle-income countries. Current Opinion in Infectious Diseases **26**(5): 404-412.

Rujeni, N., Gasogo, A. and Mbanzamihigo, L. 2008. Contribution to the study of the prevalence of brucellosis in Rwanda. Case study of Huye district. International Journal of Infectious Diseases **12**(Suppl 1): e128.

Russo, G., Pasquali, P., Nenova, R., Alexandrov, T., Ralcher, S., Vullo, V., et al. 2009. Reemergence of human and animal brucellosis, Bulgaria. Emerging Infectious Diseases **15**(2): 314-316.

Sabbaghian, H. and Nadim, A. 1974. Epidemiology of human brucellosis in Isfahan Iran. Journal of Hygiene **73**(2): 221-228.

Saeed, K.M.I., Ahadi, J., Sahak, M.N., Ghiasi, A.F. and Ashgar R.J. 2013. Concurrent brucellosis and Q fever infection: a case control study in Bamyan Province, Afghanistan. Central Asian Journal of Global Health **2**(2):58.

Saime, A., Tur, B.S. and Kutlay, S. 2010. Cerebral infarct due to meningovascular neurobrucellosis:a case report. International Journal of Infectious Diseases **14**S: e202-e204.

Santis, R.D., Ciammaruconi, A., Pomponi, A., Fillo, S. and Lista, F. 2011. Brucella. Molecular diagnostic techniques in response to bioterrorism threat. Journal of Bioterrorism and Biodefence **S2**: 004.

Schelling, E., Diguimbaye, C., Daoud, S., Nicolet, J., Boerlin, P., Tannei, M., et al. 2003. Brucellosis and Q fever seroprevalences of nomadic pastoralists and their livestock in Chad. Preventive Veterinary Medicine **16**(4): 279-293.

Scholz, H.C., Hubalek, Z., Sedlacek, I., Vergnaud, G., Tomaso, H., Al Dahouk, S., et al. 2008. *Brucella microti* sp. nov., isolated from the common vole *Microtus arvalis*. International Journal of Systematic Evolution and Microbiology **58**: 375–382.

Sekait, M.A.Al. 1999. Seroepidemiological survey of brucellosis antibodies in Saudi Arabia. Annals of Saudi Medicine **19**(3): 219-222.

Serra, J. and Vinas, M. 2004. Laboratory diagnosis of brucellosis in a rural endemic area in northeastern Spain. International Microbiology Journal **7**: 53-58.

Shaalan, M.A., Memish, A.Z., Mahmoud, A.S., Alomari, A., Khan, M.Y., Almuneef, M., et al. 2002. Brucellosis in children: clinical observations in 115 cases. International Journal of Infectious Diseases **6**: 182-186.

Shaar, L.Al., Chaaya, M., Ghosn, N.and Manfoud, Z. 2014. Brucellosis outbreak in Chouf district of Lebanon in 2009: a case control study. Eastern Mediterranean Health Journal **20**(4): 250-256.

Shahid, M., Basit, A. and Khan, M.A. 2014. Prevalence of brucellosis among the hospital patients of Peshawar, Khyber Pakhtunkhwa. Journal of Infection and Molecular Biology **2**(2): 19-21.

Shaqra, M.A.Q. 2000. Epidemiological aspects of brucellosis in Jordan. European Journal of Epidemiology **16**: 581-584.

Sherbini, A.El., Kabbash, I., Schelling, E., Shennawy, S.El., Shalapy, N., Hasab, G., et al. 2007. Seroprevalences and local variation of human and livestock brucellosis in two

villages in Gharbia Governorate, Egypt. Transactions of the Royal Society of Tropical Medicine and Hygiene **101**(9): 923-928.

Shirima, G.M. 2005. The epidemiology of brucellosis in animals and humans in Arusha and Manyara regions of Tanzania. PhD Thesis. Department of Veterinary clinical studies, University of Glasgow, Scotland, U.K.

Shrestha, B., Joshi, D.D., Aryal, A. and Shahi, K. 2008. Serological evidence of brucellosis in different species of meat animals of Nepal. Zoonoses and Food Hygiene News **14**(3): 361-363.

Sippel, J.E., Ayad, N.M. and Farid, Z. 1982. Diagnosis of human brucellosis with ELISA. The Lancet **2**(8288): 19-21.

Smirnova, E. A., Vasin, A. V., Sandybaev, N.T., Klotchenko, S.A., Plotnikova, M.A., Chervyakova, O.V., et al. 2013. Current methods of human and animal brucellosis diagnostics. Advances in Infectious Diseases **3**: 117-184.

Smits, H.L. and Kadri, S.M. 2005. Brucellosis in India: a deceptive infectious disease. Indian Journal of Medical Research **122**: 375-384.

Sofian, M., Aghakhani, A., Velayati, A.A., Banifazl, M., Eslamifar, A. and Ramezani, A. 2008. Risk factors for human brucellosis in Iran:a case-control study. International Journal of Infectious Diseases **12**: 157-161.

Specchio, S. 2014. Student's yak research may reduce infection in Nepal. Cornell Chronicle.

Stanek, D., Nicoletti, P., Harrell, D., Churchwell, G., Lee, P. and Blackmore, C. 2010. Epidemiologic comparison of Human *Brucella suis* and *Brucella melitensis* infections. International Journal of Infectious Diseases **14**(Suppl1): e212.

Swai, E.S. and Schoonman L. 2009. Human brucellosis: Seroprevalence and risk factors related to high risk occupation groups in Tanga municipality, Tanzania. Zoonotic and Public Health **56**(4): 183-187.

Swai, E.S., Schoonman, L. and Daborn, C.J. 2010. Knowledge and Attitude Towards Zoonoses among Animal Health Workers and Livestock Keepers in Arusha and Tanga, Tanzania. Tanzania Journal of Health Research **12**(4): 282-288.

Tasei, J.P., Rangue, P., Balique, H., Traore, A.M. and Quilici, M. 1982. Human brucellosis in Mali. Results of a seroepidemiological study. Acta Tropica **39**(3): 253-264.

Tawfiq, A.J. and Abukhamsin, A. 2009. A 24-year study of the epidemiology of human brucellosis in a health care system in Eastern Saudi Arabia. Journal of Infection and Public Health **2**(2): 81-85.

Thakur, S.D. and Thapliyal, D.C. 2002. Seroprevalence of brucellosis in man. The Journal of Comminicable Diseases **34**(2): 106-109.

Tibesso, G., Ibrahim, N. and Tolosa, T. 2014. Seroprevalence of Bovine and human brucellosis in Adami Tulu, Central Ethiopia. World Applied Sciences Journal **31**(5): 776-780.

Tohme, A., Hammoud, A., Rassi, B., Germanos, H.M. and Ghayad, E. 2001. Human brucellosis. Retrospective studies of 63 cases in Lebanon. Press Medical **30**(27): 1339-43.

Tsend, S., Baljinnyam, Z., Suuri, B., Dashbal, E., Oidov, B., Roth, F., et al. 2014. Seroprevalence of brucellosis among rural people in Mongolia. Western Pacific Surveillance Response Journal **5**(4): 13-20.

Tsolia, M., Drakonaki, S., Messaritaki, A., Farmakakis, T., Kostaki, M., Tsapra, H., et al. 2002. Clinical features, complications and treatment. Outcome of childhood brucellosis in Central Greece. Journal of Infection **44**(4): 257-262.

Tumwine, G., Matovu, E., Kabasa, J.D., Owiny, D.O. and Majalija, S. 2015. Human brucellosis: seroprevalence and associated risk factors in agro-pastoral communities of Kiboga district, central Uganda. BMC Public Health **15**: 900.

Upadhayay, M. 1998. Seroprevalence of human and animal brucellosis in Chitwan District, central region of Nepal. M.Sc. Thesis. Central Department of Zoology, Tribhuvan University, Kathmandu, Nepal.

Verger, J.M., Grimont, F., Grimont, P.A. and Grayon, M. 1987. Taxonomy of the genus *Brucella*. Annales de Institut Pasteur Microbiology **138**: 235–238.

Vilchez, G., Espinoza, M., Onadio, G., Saona , P., and Gotuzzo, E. 2015. Brucellosis in pregnancy: clinical aspects and obstetric outcomes. International Journal of Infectious Diseases **38**: 95-100.

WHO. 2006. Brucellosis in humans and animals. Food and agriculture organization of the United Nations, World Organization for Animal Health, World Health Organization, Geneva.

Xavier, M.N., Paixao, T.B., Hartigh, A.B., Tsolis, R.M., and Santos, R.L. 2010. Pathogenesis of *Brucella* spp. The Open Veterinary Science Journal **4**: 109-118.

Yacoub, A., Bakr, S., Hameed, A.M., Thamery, A. and Fartoci, M.J. 2006. Seroepidemiology of selected zoonotic infections in Basra region of Iraq. Eastern Mediterranean Health Journal **12**(1/2): 112-118.

Ya-wei, S., Jie-nan, Z., Xue-fei, Z., Man-hong, Y., and Ke-jie, C. 2013. Surveillance analysis of human brucellosis in Ningbo from 2002 to 2012. Chinese Journal of Health Laboratory Technology **05.**

Yohannes, M. and Gill, P.S. J. 2011. Seroepidemiological survey of human brucellosis in and around Ludhiana, India. Emerging Health Threats Journal **4**(10): 7361.

Yohannes, M., Degefu, H., Tolosa, T., Belihu, K., Cutler, R. and Cutler, S. 2013. Brucellosis in Ethiopia. African Journal of Microbiology Research **7**(14): 1150-1157.

Yumuk, Z. and Callaghan, D. 2012. Brucellosis in Turkey-an overview. International Journal of Infectious Diseases **16**: e228-e235.

ZDU. 2012. Brucellosis seroprevalences study Kiambu. Zoonotic Disease Unit, Kenya.

Zhong, W.Y., Guo, W.D., Sun, S.H., Jiang, J.F., Sun, H.L., Li, S.L., et al. 2010. Human brucellosis, Inner Mongolia, China. Emerging Infectious Diseases **16**(12): 2001-2003.

Zhong, Z., Yu, S., Wang, X., Dong, S., Xu, J., Wang, Y. et al. 2013. Human brucellosis in the people's Republic of China during 2005-2010. International Journal of Infectious Diseases **17**: e289-e292.

APPENDIX I

Questionnaire related to KAP

Name:

Age:

1. What type of food habit do you have?
   a) Vegetarian b) Non-vegetarian

2. If non-vegetarian what type of food do you consume?
   a) Buffalo b) Goat c) Pig d) Sheep d) All

3. How do you prepare meat you eat?
   a) Well-cooked b) Half cooked c) Raw preparation

4. What type of milk do you consume?
   a) Dairy b) Buffalo milk c) Cow milk

5. Do you consume raw milk?
   a) Yes b) No

6. How long do you boil the milk?
   a) 5mins b) 10mins

7. What are the various items prepared from milk in your home?
   a) Curd b) Ghee c) Ice-cream d) Butter e) None of them

8. Do you prepare all the milk items from well-boiled milk?
   a) Yes b) No

9. Do you know about zoonotic diseases?
   a) Yes b) No

10. Do you know of any diseases transmitted from raw/uncooked meat? If yes, what are the diseases?

11. Do you know of any diseases transmitted from milk and milk products? If yes, what are the diseases?

12. Have you suffer from prolonged fever?

a) Yes b) No

13. If yes, how frequent and how long you suffer?

14. If you fell unwell, where do you go for treatment?

a) Medical center b) Health post c) Hospital d) Ayurvedic e) Dhami/jhakri

15. Have you heard about abortion cases in women?

a) Yes b) No

16. If yes, in

a) Family b) Neighbours c) Relatives d) Village/town

17. If family, how frequent?

18. Do you know about the reasons of abortion?

19. Have you ever heard of a disease called brucellosis? If yes then what do you know about it?