INTRODUCTION

1.1 STUDY AREA

Nepal is a landlocked agriculture based country located in South Asia. It is located in the Himalayan bounded by India to the South, East and West where as by China to the North with the latitude 26˚ 22’ to 32˚ 27’ North and longitude 80˚ 4’ to 88˚ 12’ East and elevation ranges from 90 to 8848 meters. Nepal is the world’s 93rd largest country by land mass with an area of 1, 47,181 square kilometers. It is of roughly trapezoidal shape, with 800 kilometers long and 200 kilometers wide.

The country of Sagarmatha having population of approximately 30 millions, it contains three parallel ecological zones described as the Mountains, Hills and Terai with five development regions.

Kathmandu is the capital and the largest metropolitan city of Nepal. It is the headquarter o of the central development region and is located in 27˚ 42’ N latitude and 85˚ 22’ E longitude of Bagmati Zone. It stands at an elevation of approximately 1400 meters in the bowl-shaped valley in central Nepal surrounded by four major hills Shivapuri, Phulchowki, Nagarjun and Chandragiri.

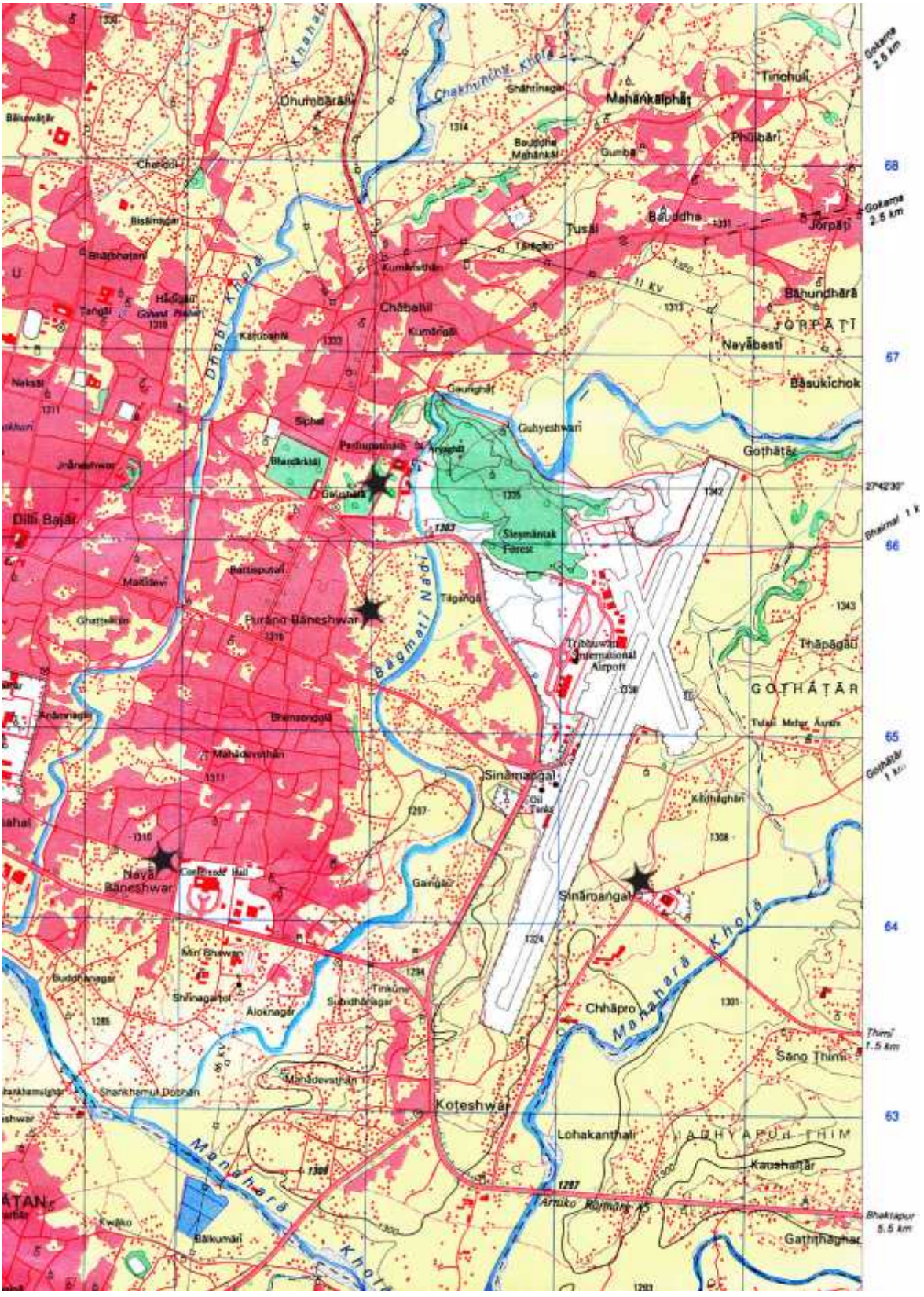
The total area of Kathmandu is 50.67 square kilometers. Kathmandu valley is part of three districts, Kathmandu District, Lalitpur District and Bhaktapur District. The population of Kathmandu valley is 5 million as estimated in 2011.

The average temperature in Kathmandu is 18.3˚ C during the month of January the minimum temperature goes upto1˚ C and in the month of May, maximum temperature goes up 30˚ C. The annual rainfall in Kathmandu generally exceeds 1300 mm.

Bovine Brucellosis study was conducted in different areas of Kathmandu Valley namely

Gausala, Old Baneshwor, New Baneshwor and Sinamangal.

Photograph I: MAP OF KATHMANDU VALLEY



(Star indicates the study sites)

1.2 BACKGROUND OF BRUCELLOSIS

All the living things found on the earth are inter-related to each other. They depend on each other for the survival. Animals and humans are close associates from the ancient period. Animals play vital role in the survival of human race. They are used in different aspects such as nutrition, as beast of burden, socio-economic development, entertainments, companionship etc. beyond their importance they are unfortunately knowingly and unknowingly main cause of transmission of zoonoses diseases in human either by intimate association or by the consumption of the products.

The diseases transmitted from animals to human are known as zoonoses. Zoonotic diseases are transmitted to human by the different sources. One of the main sources is milk. Milk is known as complete nutritious food. It is consumed by all the age group of people and of both sexes. Different products like cheese, ghee, curd, ice-cream, butter, sweets etc are prepared from milk. Milk contain significant amount of saturated fat, protein, calcium and vitamin 'C'. Milk provides all the nutrients necessary to human but it may also be the source of zoonotic diseases. Among them one of the sources of zoonotic disease transmitted through milk is "Brucellosis".

Brucellosis is a bacterial zoonotic disease affecting both public health as well as economic significance in the most of the countries. It is consider as the most wide spread zoonotic disease in the world by WHO, OIE and FAO. It is known as undulant fever, Mediterranean fever, Malta fever, Gibraltar fever, Nepolitan fever, infectious abortions, Bang's disease contagious abortions, epizootic abortion and Ram epididymitis in animals.

Brucellosis is essentially a disease of animals, especially domesticated livestock caused by the bacteria of the genus *Brucella*. The bacterium *Brucella* belongs to family Bucellaceae and order Eubacteriales. Various *Brucella* spp. affect many species of animals but especially of those that produce food (especially milk producing) sheep, goats, cattle, camels, yak, deer, dogs and several other animals. Human as an accidental host become infected by coming in contact with animals or animal products that are contaminated with

Brucella bacteria. It is estimated that inhalation of only 10 to 100 bacteria is sufficient to cause disease in human.

The disease Brucellosis contributes largely to infertility, retained placenta, low birth rate, stillbirth, and low milk production in case of animals resulting in heavy economic crisis. In case of human, Brucellosis cause fever, chills, profuse sweating, weakness, fatigue, malaise, weight loss, headache, diarrhoea, abdominal pain, vomiting, arthralgia and depression sometime become rarely fatal due to immune deficiency of infected person. Everyone is susceptible to this disease if exposed to contaminated environment, occupational exposure and food borne transmission. The people especially livestock handlers, butchers, the veterinarians, laboratory workers are in great risk to cause this disease.

In many developing countries, there has been great progress in controlling the disease but still remain regions where the infection persists in domestic animal and consequently transmission to the human population frequently occurs. There are only few countries in the world that are officially free of the disease although cases still occurs in people returning from endemic countries. Thus, the disease Brucellosis in term of human health hazard and economic crises remains a matter of major concern.

1.3 *BRUCELLA*

1.3.1 General Account

Brucella the bacterial micro-organisms which are non motile, non-sporulating, aerobic, slow growing, gram negative, short rod, non-capsular, coccobacilli with straight convex sides and rounded ends measuring 0.5 to 0.7 µm wide by 0.6 to 1.5 µm long. They are usually arranged singly or less commonly in pair, short chains or small clusters. They do not produce capsule but if they do the capsules are soon lost. They are sensitive to light and live for not more than five hours when exposed to direct sunlight. They are not acid fast but may resist decolorization by week acids or alkalis in the macchiarello or modified koster staining procedures. They can survive for five days when kept in an ordinary room. Thirty seven days, when dried slowly in soil, one hundred and twenty days in *Bovine* faeces dried very slowly in dark cupboard, four days in *Bovine* urine and 75 days in cool

weather (Rana, 2002). The age readily killed by common disinfectant and by standard pasteurization.

1.3.2 Etiological Agent

There are six main species of *Brucella* distinguished. *B. abortus*, *B. suis*, *B. melitensis*, *B. neotomae*, *B. ovis*, *B. canis* (Corbel, 2006). *B. abortus* is normally associate with cattle, *B. melitensis* with sheep and goats, *B. suis* with swine, *B. ovis* causes an infection specific for sheep and has not been conclusively implicated in human disease, *B. suis* biovars 5 has only been isolated on a few occasions from rodents and *B. canis* is usually associated with disease in dogs but occasionally cause human brucellosis. *B. neotomae* has been isolated on few occasions and has never been implicated in human disease.

1.3.3 Geographical Distribution

Brucella infections are worldwide in distribution. It is more common in countries that do not have good standardized and effective public health and domestic animal health programs. Areas currently listed as high risk are the Mediterranean Basin, South and Central America, Eastern Europe, Asia, Africa, the Caribbean and the Middle East.

The distribution of different species of *Brucella* and their biotypes varies according to geographical areas. *B. abortus* is the most wide spread. Infection by *B. melitensis* is largely confined to those regions where goats are grazed, especially Europe and North Africa.
B. suis is confined to hog raising regions (Merchant and Packer, 1967). *B. neotomae* is an infection with natural foci in the Western United States. The presence of *B. canis* has been bacteriologically confined in the United States, Brazil, Argentina, Mexico, Madagascar and India. *B. ovis* appears to be distributed in all major sheep raising countries.

1.3.4 Growth Characteristics

The *Brucellae* are aerobic and do not grow under anaerobic condition. Growth on artificial media is slow and colonies may not become visible for forty eight hours. When freshly isolated the colonies are smooth circular, convex, translucent but these organism readily undergo smooth to rough variation to yield a slightly rough, yellowish-brown colony,

when grown on solid media and particularly on potato the *Brucellae* produce a characteristic chocolate brown discoloration of the medium visible after some days incubation. Maximum growth is obtained on liver extract agar (Rana 2002)

Most *Brucella* strains require multiple amino acids thiamine, nicotineamide, niacin, biotin and patnthothenic acid for growth. Optimal growth temperature is 37° C, with a temperature range from 10 to 40° C (Dahal 2003). The optimum pH range is 6.6 to 7.4.

Brucella is adapted to an intercellular habitat and their nutritional requirements are complex. Some stains have been cultivated on defined media of 18 amino acid, vitamin, salts and glucose. *B. abortus* requires 5-10 % Co_2 for growth, where as the other species grow in presence of air.

Brucella species utilize carbohydrates but produce neither acid nor gas. They are moderately sensitive to heat and acidity. They are killed by pasteurization. Their metabolism is oxidative.

1.3.5 Variation

Three variants of *Brucella*, namely smooth(s), mucoid (M) and rough (R) are known. They can be recognized on the basis of their colonial appearance and virulence. The smooth forms produce round, translucent colonies with an entire edge and a smooth glistening surface, giving a slight bluish white opalescence in reflected light, although they are translucent pale yellow in transmitted light. This form is virulent. Rough or mucoid, which are non smooth form produce colonies and are often slightly larger than smooth colonies and have more granular surface. The colors of these forms vary from off white to brown in transmitted or reflected light. These forms are non virulent.

1.3.6 Phage

Isolation of new phage strains has considerably widened the host range of *Brucella* phages. It has made more convenient for us to divide the genus on the basis of phage type. They are useful for identification at both genus and species level as these phages don't lyse bacteria of other genera. *Brucella* phages have been classified into six stains on the basis of host range which are as follows:

- Tibilisi (Tb) stain
- Firenze (Fr) stain
- Weybridge (Wb) stain
- Berkeley (Bk0, Bk1 and Bk2)
- Izatnagar (Iz) and
- sixth stain include those phages lytic for non smooth Brucella culture derived from phage R.

1.3.7 Antigenic Structure

The present theory of basic antigenic structure of the three species, *B. abortus B. suis* and *B.melitensis* in their smooth antigenic phase is that there are two main antigenic determinants, A and M, which are present in different amount in the three species. Sera prepared against any *Brucella* species contain antibody to both A and M antigens, but can be rendered specific for either A or M by careful adsorption. Thus all anti-M antibodies can be removed from a *B. abortus* serum by adsorption with a *B. melitensis* suspension. The resulting mono-specific serum will agglutinate only organisms in which the homologous antigen is present as the major antigenic components. Thus the serum-containing antibody A only will agglutinate both *B. abortus* and *B. melitensis*. The A antigen is present in *B.melitensis* but apparently not in sufficient amount to render the organisms susceptible to agglutination by A anti-serum. Major antigenic changes occur when *Brucellae* undergoes the smooth-rough variation. These changes appear to be due to the loss of the specific S-antigen and may be accompanied by colonial alternation and reduction in virulence for lab animals. Organisms characteristically in the smooth phase in

nature are *B. abortus*, *B. melitensis*, and *B. suis*, whereas *B. ovis* and *B. canis* seems to occur naturally in non-smooth phase.

1.3.8 Source of Infection

Brucellosis is a zoonotic disease. The main sources of infection of Brucellosis disease is infected animals. The major food producing animals are the main sources to cause the infections of Brucellosis disease in human whereas, others are less importance. Recently the infection has also been identified in marine animals in some region may present an emerging hazard to person occupationally exposed to infected tissues from them (Corbel, 2006).

The risk of disease and its severity is determined by the type of *Brucella* to which an individual is exposed. This will be influenced by the species of host animal acting as source of infection.

B. melitensis in sheep and goats is the type most frequently reported as a cause of human disease and the most frequently isolated from cases. It is the most virulent type and associated with sever acute disease. It is recorded as endemic in several countries.

The transmission of *Brucella* infection to human and its prevalence in different areas of the world depends upon the food habit, social customs, type of animal husbandry, climatic condition, standard of personal and environmental hygiene, method of processing milk for cream, butter and cheese.

B. suis occur mostly in the areas where pigs are kept. Its prevalence is generally low except in part of South America and South East Asia. It occurs in people handling pig on farms, during slaughtering and processing including the hunting of feral swine.

B. abortus cause *Bovine* Brucellosis which is most wide spread cause of infection but has been eradicated from Canada, Northern Europe, Australia and Japan. Infection can be acquired by drinking unpasteurized milk and milk products by milk of infected cattle. Abattoir workers and veterinarians are in the great risk of infection.

B. canis is a widespread infection of dogs in many countries. It is infrequently associated with human disease. Reported cases have been mild. It especially occurs only in dog handlers.

Brucella infection occurs in many species of wild animals but these are rarely implicated as source of human disease.

1.3.9 Mode of Transmission

The possible mode of transmission of Brucellosis in human includes person to person transmission, infection from a contaminated environment, occupational exposure usually resulting from direct contact with infected animals and food borne transmission.

Rare instances of person to person transmission have been recorded either in circumstances implicating sexual contact (Goossens et al., 1983) or by the transfer of tissue including blood or bone marrow (Naparslek et al., 1982). Infection may occur via the gastrointestinal tract or by penetration of the mucous membrane of the throat. Contact with *Brucellae* in vaginal discharges, features, placentas urine, manure, carcasses and salvaged animal causes a large proportion of human cases. Water supplies such as cisterns and wells contaminated by infected animal excreta or carcasses are also the source of infection. Infection also occurs when man inhales infected dried materials of animal origin such as dusts from sheep wool, railway trucks and Lorries that have transported infected animals, abattoirs, infected farm premises and *Brucella* laboratories.

The main source of infection in cattle are features, afterbirth and vaginal discharged containing large number of *Brucella* organisms cattle become infected with brucellosis chiefly through the alimentary tract. They may lick the genital organs of infected cows, aborted features or placental membranes and fluids or they may ingest grass and water contaminated with such materials or with urine or serum from infected bulls. The agent may also enter through the skins, eyes, nose and in certain methods of artificial insemination of cow will be infected by serum from an infected bull (Abdou et al. 1948, Manthei et al. 1956)

1.3.10 Pathogenesis

The infection of *Brucella* species occur a wide range of animals including human beings. It especially affects the domesticated livestock. *Brucella* can enter into human being through break in the skin, mucous membrane, conjunctiva, respiratory and gastro intestinal tracks. Sexual transmission may be occasional. Ingestion of unpasteurized milk, milk products, meat may be the main source of infection in human beings. The common routes of infections are inhalation, conjunctiva exposure through eye splash and precutaneous needle stick exposure etc.

Brucella may cause sub-acute febrile illness which in the absence of specific treatment may persist for weeks or months. It may also cause of enlargement of the liver, spleen, lymph node which may progress to a chronic one in human beings in absence of treatment.

For cattle, infection is usually caused by *B.abortus*. However *B.melitenis* and rarely *B.suis* also causes infection. *B.abortus* has got affinity to invade uterus, mammary gland, testes, lymph nodes and joints. Organisms gain access through mucous membrane of the oropharynx, upper respiratory tract, conjuctival mucosa, abraded skin, cervix of genitalia (Berman, 1981).

The bacteria invade lymph nodes or other lymphoid tissues (spleen, iliac, lymph nodes) reach circulation, multiply and set up bacteremia. They sometime colonize in the gravid uterus and placenta, therefore, produce degenerative changes in the placenta.

Localized infection may persist in the udder and supra mammary lymph nodes. Thus there is constant or periodical shedding of bacteria through milk. Sexually immature calves are resistant to infection but calves born out the infected cows may acquire the infection via lymph nodes and pulmonary tissues. Similarly infected milk fed calves may contract the infection through gastro intestinal tract and shed the organisms through their faeces. A sizeable portion of calves born out of infected mother may remain latently infected. The clinical signs and serological responses may appear at the time of abortion or following abortion in the first pregnancy (Plommet, 1977 cited by Chakrabarty, 2007)

In male, the organisms multiply in large proportion and localize in epididymis, testes and other accessory sex organs. There is epididymitis and orchitis. The organisms are shed through semen. Thus males remain as cause of infertility in a herd.

1.4 DIAGNOSIS

The main aim of diagnosis of brucellosis is to identify the animals which are infected and shedding the organism and thus spreading the disease. Various serological methods have been used in evaluating the humoral response of *B abortus* and they are used to detect the disease in eradication programmer (Morgan, 1967 cited by Chakrabarty, 2001). IgM, IgG $_1$and IgG_2 antibody in the serum of cattle vaccinated with S_{19} will differ from that of *B.abortus* infected cattle.

The laboratory test used in diagnosis of Brucellosis in animals includes:

- Isolation of organisms
- Animal pathogenecity test
- Test for the presence of antibodies
- CFT (Compliment fixation test).

The milk ring test (MRT) is used as periodic test for Brucellosis free herds and identifying infected herds. There are other different methods for diagnosis of Brucellosis in animals i.e. Rose Bengal Plate Test (RBPT), Rivanol and Mercaptoethanol test, Rapid or plate agglutination test, Vaginal mucus test, Microplate agglutination Test, Elisa Test, Anti *Bovine* gamma globuline test.

In Nepal, incidence of Brucellosis infection has been increasing day by day. Adequate research has not been done. Most of the people are ignorant about the disease and hence raw milk and milk products such as curd, cheese, butter, whey, ice-cream etc are being consumed in both rural and urban areas (Joshi, 1973). An epidemiological surveillance has been carried out by National Zoonoses and Food Hygiene Research Centre (NZFHRC) emphasizing the different sector of the country including Kathmandu Metropolitan City. The present study is carried out in collaboration with NZFHRC, Tahachal, Kathmandu, Nepal.

1.5 HUSBANDRY SYSTEM

A research is performed on New Baneshwor, Sinamangal, Gausala and Old Baneshwor. Cattle are reared in these areas for milk production in order to gain economic benefit. It is found that mostly large populations of cows are reared in these areas than other animals. Generally cows are kept in separate small house (shed) due to lack of space in these areas with poor management system, moist floor and poor ventilation. Large numbers of cattle are kept in one place. No separate place for sick animals.

Being urban area there is no open grazing place for cattle so they are kept in shed and are raised in stall feeding system. Cattle get concentrate twice daily along with roughages like straw grasses, spoiled vegetables, flour etc.

1.6 OBJECTIVES OF RESEARCH

1.6.1 General Objective

- To determine the prevalence of Brucellosis in the raw milk samples of cattle.

1.6.2 Specific Objectives

- To determine prevalence of animal Brucellosis by milk ring test (MRT) in the sample taken directly by livestock handlers.
- To determine prevalence of animal Brucellosis in the sample taken from the small private dairy.
- To raise awareness about Brucellosis within the study community.
- To suggest the possible method for the prevention and control measures against animal Brucellosis

1.7 SIGNIFICANCE OF STUDY

Nepal is an agricultural country. Beside agriculture, livestock farming is also one of most popular occupation in Nepal. Most of people involve in livestock farming for the food purposes and economic benefit. Milk is known as nutritious food. All age group either child youngster or old people have milk as well as the product made form milk. It may be the source of infection of zoonotic diseases either in rural areas or urban areas. Zoonotic diseases are the major problem in livestock husbandry and human beings.

Nutritional genetic managerial and infectious diseases are the major problems in livestock husbandry. Abortion losses by infectious and non infectious causes are considered as one of the major constraints in livestock production, which is the major cause of the significant economic loss. Brucellosis is one of the most important zoonotic diseases, which cause serious health hazard and easily transferred from feed materials like milk, meat and other contaminated materials. It could be great threat in future due to the reason of lack of knowledge unawareness about zoonotic disease, lack of milk inspection authority, carelessness of handling livestock.

Hence, establishment of the epidemiological information could be an important tool in controlling and eradicating of this disease. It could also be an important source of information for farmers, students, control strategy planner, future researchers and other people as well.

1.8 LIMITATION OF STUDY

The study is based on the milk ring test. However using this test the false positive reactions are due to the colostral antibody in calves or cross reaction with some bacteria like *Bordetella* species Pseudomonas, *Moraxella* and *Salmonella*, *Yersinia entericolitica* etc (Mishra, 2008). False negative reaction may result during early incubation of disease or immediately after abortion. For accurate conformation test like CFT, ELISA, AGID, blood culture isolation etc are necessary. But these tests prove to be out of reach with limited time and resources. Other limiting factors include small sampling size, limited time and small sampling area and coast.

2. LITERATURE REVIEW

2.1 HISTORY OF BRUCELLOSIS

Description of this disease dates back to the days of Hippocrates. An accurate description of the disease in man was reported in 1860 and designated as Mediterranean or gastric intermittent fever. However, the organism was not isolated until 1887, when the British Army physician, David Bruce isolated the organism that bears his name from the spleens of five patients with fatal cases on Malta. Bruce named the organism as *Micrococcus melitensis*. The generic name *Brucella* was first published validly in 1920 by Meyer and Shaw.

In 1897, Wright and Smith reported *M. melitensis* in the serum of man and animals, and the disease in man was given the descriptive name undulant fever. Zammit in 1905 detected infections in Maltese goats and showed the goat to be its natural host. Man became infected by consuming raw milk or cheese. Bang, 1897 discovered *B. abortus* among cows in Denmark, and now the disease in known Bang's disease. The actual valid naming of the organism was done by Schmidt in 1901.

The third member of the group Traum in 1914 reported that abortion in swine is caused by *B. suis* and named by Huddelson in 1929. *B. ovis* was first observed in Australia and New Zealand by Buddle and Boyes in 1953 and identified as the cause of sexually transmitted epidiymitis inrams by Simmons and Hall in 1953.
B. neotomae was discovered from desert wood in Utah, USA by Stoenner and Lackman in 1957. *B. canis* was reported from Beagle dogs in USA by Carmichael and Burner (1968).

2.2 GLOBAL BRUCELLOSIS RESEARCH

Globally, a lot of research work on Brucellosis has been carried out. The major research carried out throughout the world and in the Indian sub-continent is as follows:

Kapoor et al. (1885) carried a sero survey of 174 goats and MRT of 58 milch goats in Rajasthan. In the milch goats, the prevalence was found to be 11.42% while is slaughtered goats, it was found to be 1.92%.

Nandgonkar and Rao (1971) collected 1,291 buffalo milk samples and 25 cow milk sample from Andhra Pradesh. The milk sample was examined by milk ring test (MRT) and 0.25% buffaloes and 4.0% cows were found to be positive. They also studied the incidence of positive case for Brucellosis in buffaloes and cows in the basis of sero diagnosis. They examined serum of 212 buffaloes and 91 cows and found 3.7% and 9.9% to be positive.

Shaw (1987) studied infectious fertility and abortion incidences of *Bovine* Brucellosis in Kashmir Valley (India) from 1979 to 1983. He conducted the test on the basis of MRT and SAT on 3,386 milk samples and 2,104 sera samples respectively. During this period, he found the overall incidence of Brucellosis as 1.7% & and 1.28% on the basis of MRT and SAT respectively. Similar studies conducted about two and half decades ago (1968-1971) revealed corresponding incidence of 2.3% and 2.6% respectively.

Vaid et al. (1992) studied sero prevalence of animal disease in Himachal Pradesh. They obtained the result, as 1.55% cattle and 1.09% sheep were positive for Brucellosis.

Shakya et al. (1995) studied sero epidemiological survey of *Bovine* Brucellosis in village of Madhya Pradesh, India, A total of 115 milk samples (pooled) were collected and were tested for Brucellosis using milk ring test (MRT). Out of 115 milk sample, 21 (18.26%) were found to be positive for Brucellosis by MRT. 139 sera samples were also screened for *Brucella* antibodies using Plate Agglutination Test (PAT) followed by Standard tube Agglutination Test (STAT). 39 (28.66%) samples were found positive for Brucellosis with PAT and STAT.

Mohan et al. (1996) surveyed Brucellosis and its control in Zimbabwe. MRT was employed to test the bulk-pooled milk sample once a month for 14 months. The test was recorded highly positive on all 14 occasions. Milk samples from 36 individual cows were similarly tested. Of these 21 (among 59%) were found to be reacting positively. 175 animals were marked for sero testing, of these 40 (25% approximately) showed quite high serum titres >1:360 in both the STT and RBT.

Acedo et al. (1997) investigated the incidence of *Brucella* in raw milk and regional white cheese in Mexico. 289 raw milk samples and 355 white soft cheese samples from Cajeme County were analyzed to isolate *Brucella*. The MRT was performed in the raw milk

samples and only 15% were found to be positive. Of the 335 cheese samples, 25 (75%) were found to be positive. The species identification in both samples were *Brucella abortus* 21 (66%) and *B. melitensis* 7 (22%) and 4 (12%) of atypical strains.

Gurturk et al. (1999) compared the dot-immunobinding assay (DIA) with serum agglutination test (SAT), RBPT and MRT for the diagnosis of *Bovine* Brucellosis in Trukey. For this purpose, they collected a total of 116 paired blood and milk samples at the same time from 56 aborted and 60 healthy dairy cows and examined by DIA, SAT, RBPT and MRT. Of the 116 paired blood and milk samples, 24 were found to be positive and 72 found to be negative by all tests used. Serum samples of 6 aborted cows were found to be positive by DIA, SAT and RBPT where as milk samples were showed negative by DIA and MRT. Serum and milk samples of 4 aborted cows gave positive reaction only by MRT and 2 of them negative by both RBPT and MRT. 4 sera samples of healthy cows were found to be positive only by SAT.

Folhadella et al. (2001) reported *Bovine* Brucellosis in cattle farms in the State of Rio de Janeiro, Brazil. In the period of February to August of 2000, a total of 1,229 *Bovine* sera samples were examined, originating from 135 rural properties, distributed in 59 municipal districts. The incidence of disease was observed only in females as 4.06% where as all the examined males were found to be negative.

Lefkomitz et al. (2003) conducted a study on Brucellosis in Yak Naks, churies and hilly cattle of Langtang Valley by MRT which revealed the prevalence to be 17.6%.

Shafee et al. (2011) investigated prevalence of *Bovine* Brucellosis in organized dairy farms. A total of 200 milk samples from cattle (n=86) and buffalo (n=114) were evaluated. The overall prevalence was found to be 3% and 8.5% in cattle and buffaloes using MRT and I-ELISA, respectively. The prevalence was higher in government dairy farm compared to privately owned dairy farm.

2.3 BRUCELLOSIS RESEARCH IN NEPAL

Joshi (1976) investigated the prevalence of Brucellosis among cows, buffaloes and goats. He examined milk samples from 4 cows, 40 buffaloes and 1 goat by MRT and whey plate tests. The incidence of positive case was found 25.0% in cows (1/4) and 100% (1/1) in goat. Milk samples of buffaloes did not show positive reaction.

Pyakurel et al. (1997) reported that the incidence of diseases was observed 22.64% in water buffaloes, 17.4% in cattle, and 1.54% in sheep.

Joshi et al. (1998) conducted the epidemiologically survey of human and animal Brucellosis in Chitwan district. They collected a total of 500 milk samples of cows and buffaloes (single, pooled or mixed) from six different MPCS of Bharatpur municipality, Chitwan. They also collected 500 human sera of outpatients and indoor patients of Mahendra Adarsha Hospital, Bharatpur. The milk samples and human sera were tested by MRT and *Brucella* card test respectively. Out of total 500 milk samples, 23.17% (70/302) from single, 30.06% (46/153) from pooled and 28.8% (13/45) from mixed milk samples were found MRT positive. Similarly, out of 500 human sera examined, the incidence of disease was found 1.4% (7/500) positive. They also reported that the age groups 10-19 years, 20-29 years, and 30-39 years and above 50 years had the prevalence of Brucellosis as 1.92%, 0.54%, 2.53% and 2.72% respectively. Regarding the prevalence of Brucellosis with sex, the disease was found 1.79% in male and 1.08% in female (P32).

Upadhyay (1998) studied on sero prevalence of human and animal Brucellosis. 500 blood samples of out and indoor patients from Mahendra Adarsha Hospital, Chitwan were collected. Out of 500 blood sample 14% were positive for human *Brucella*. For milk ring test, she collected 500 milk samples from six MPCS of Bharatpur. Out of 500 milk samples, 129 (25.8%) were found MRT positive result.

Joshi (2000) studied animal Brucellosis in KTM and around the valley. He collected total 660 milk samples (pooled) of buffaloes and cows from private DDC of KTM and tested. BRT the positive reaction was found to be 4.69% (31/660). Similarly he examined 4229 milk samples (single) of buffaloes and cows from DDC centers milk vendors and villages in KTM and around the Valley viz Panchkhalk, Bhaktapur and Banepa and observed

1.25% positive by MRT. He also collected 53 sera of cows and buffaloes that had aborted in advance pregnancy and tested by *Brucella* plate agglutination test. The incidence was found to be 3.8% (2/53). In a epidemiological surveillance conducted by Joshi (2000) of human and animal brucellosis in milk collection area of DDC 4.5% (25 out of 558) goat and 4.5% (25 out of 558) human sera collected from different hospital showed to be sero positive.

Rana (2002) Sero-epidemiological and animal Brucellosis in Surkhet district, mid western region of Nepal. Total sample 200 samples examined by *Brucella* card test the prevalence rate was found 20% positive and Bir Hospital by collecting 200 human serum samples examined by card test 14% found positive. Animal Brucellosis was studied among cow and buffalo by collecting total 400 milk samples. All Samples were analyzed by MRT. Out of 400 samples examined prevalence rate in buffalo 29% and cow 19%.

Dahal (2003) A sero-epidemiological surveillance of Brucellosis among human and animals in Dolkha district, Nepal out of 200 human sera samples were collected from patient. The sera sample was investigated using *Brucella* card test. The incidence of *Brucella* was found to be positive from human 0.5% and milk samples were tested by MRT and not a single positive case were identified.

Mishra (2008) studied to determine the prevalence of Brucellosis in buffaloes of six VDC of Bhaktapur district. Altogether 60 milk samples were collected for milk ring test. Out of 60 samples 23.33% showed positive result and the percentage of prevalence ranges from 13.75 to 36.30 at 95% confidence interval.

3. MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Equipment Used for Milk Ring Test

i) Test Tubes (Khan Tubes)
ii) Rubber Capped Sterile Vials
iii) Thermometer
iv) Incubator
v) Dropper
vi) Pipette (1 ml capacity) tip
vii) Test Tube stand
viii) Parafilm
ix) Gloves
x) Mask
xi) Glass

3.1.2 Chemical Requirement for Milk Ring Test

i) Milk Ring Test Antigen (*B.abortus* antigen)

3.2 METHODOLOGY

3.2.1 Sample Collection

A total of 200 samples were collected. 77 single milk samples (69 cows and 8 buffaloes), 75 pooled milk samples (either cows or buffaloes) and 48 mixed samples (both cows and buffaloes) were collected randomly for the study of survey on Animal Brucellosis in raw milk sample. Single milk samples and pooled milk samples were collected directly through milking animals by livestock handlers. Mixed samples were obtained from the small private dairy and livestock handlers. Mixed and pooled samples were differentiated by labelling directly. All the milk samples were collected in sterile rubber capped vials.

Table I: Number of Samples Collected at Site

S.No.	Type of Milk Samples	Number of Samples
1	Single (cows and buffaloes)	77
2	Pooled (either cows or buffaloes)	75
3	Mixed (both cows and buffaloes)	48
	Total	**200**

3.2.2 Questionnaire Survey

The questionnaire were prepared and pre-tested for livestock handlers and the owner of the small private dairy. The questionnaire for livestock handlers were basically focused on their introduction, amount of milk production, preparation of milk products, consumption of raw milk habit, awareness about disease, precaution of livestock sanitation, veterinary care whichever presented in Annex I.

The questionnaire for the small private owners were basically focused on introduction, amount of milk supply, amount of preparation of milk products, awareness about disease, precaution while preparing milk products, which were presented in Annex II

3.2.3 Data Collection and Analysis

The data was collected from the livestock handlers and the small private dairy owners with the help of questionnaires, collected samples and field observation.

Similarly milk samples were analyzed on the basis of ring formation by MRT. Milk samples which form ring after incubation for an hour at 37^{o}C with *Bovine* Brucellosis antigen were considered as positive and without ring were considered as negative. Thus the obtained data were analyzed to find the prevalence rate of Brucellosis disease infected in the raw milk of cattle.

3.3 LABORATORY PROCEDURES

3.3.1 Collection of Milk Samples

5ml milk samples were collected in sterile rubber capped vials without using preservation. Single milk samples were collected directly from livestock handlers by milking cows or buffalos. Pooled and mixed milk samples were collected from livestock handlers and from the small private dairy where milk are brought from villages in cans without pasteurization. Then these collected samples were kept under refrigeration until the tests were performed. After collection of milk samples labelling were done properly for the separation of single, pooled and mixed milk samples.

3.3.2 Milk Ring Test (MRT)

The milk ring test is also called *Brucella* Ring Test (BRT). The MRT is a simple diagnostic test for use on bulk milk supplies. It is the most practical and screening method for locating infected dairy herds and for surveillance of Brucellosis in free herd. This test was first described by Fleichhauer, (1973) in Germany in identifying infected herd. First of all collected milk samples in sterile vials were brought to the National Zoonoses and Food Hygiene Research Centre lab, Tahachal, Kathmandu for MRT test, the milk samples were thoroughly shaken to dispense the cream. 1 ml of milk sample was transferred from sterile vials to a narrow test tube known as khan tube. One drop (0.03ml) of BRT antigen was added to sample. Then the mouth of Khan Tube was covered by parafilm so that sample might not drop from Khan Tube. It was mixed thoroughly by gentle shaking and inverting the test tube several times. Then the mixed tube was allowed to stand for about 1 minute to make sure that the antigen is thoroughly mixed with milk samples before the samples were incubated at 37° C for one hour in an incubator.

After one hour the samples were taken out from the incubator for observation whether samples were positive or negative. A strongly positive reaction was indicated by the formation of a dark pink ring at the top of the tubes. The test was considered negative if the colour of the underlying milk exceeded that of the cream layer. The positive antigen *(B. abortus*) was provided by NZFHRC lab where the MRT test was done.

4. RESULTS

4.1 RESULT OF QUESTIONNAIRE SURVEY

The questionnaire survey was conducted randomly among livestock handlers and private dairy owners in the area of Kathmandu Valley viz. Gausala, New Baneshwor, Old Baneshwor and Sinamangal. During questionnaire survey, 30 livestock handlers of different areas were asked questions (given in annex I). According to survey it was found that the populations of cows were more than buffaloes. Most of livestock handlers had reared cows which give large quantity of milk per day. Mostly Jarsi, Holdstand, Swissbrand and their cross cows were found during survey. The number of cows in minimum four and in maximum up to two hundred where as the number of buffaloes in minimum two and in maximum four were reared by livestock handlers. That was because of lack of pasture areas. The buffaloes need large amount of food in comparison of cows which is quite impossible for livestock handlers in metropolitan city. Also there is scarce of grazing areas and cattle were raised in stall feeding which result low production of milk and might have affected in economic benefit as well.

Regarding the types of selling milk, it was known that most of livestock handlers were found to be selling mixed milk. According to survey, it was also known that home services were provided and some of them sold milk to private dairy or small hotels of related areas. During survey, it was known that due to scarce of enough place both healthy and sick animals were kept in same place. It was also known that most of livestock handlers were unknown to disease Brucellosis and its disasters.

Similarly, the questionnaire survey was done with private dairy owners. Altogether 25 dairy owners were asked questions (given in annex II). According to survey it was known that mostly mixed milk were found which were brought from Banepa, Bhaktapur and also taken from livestock handlers of related areas. During survey it was known that different dairy products like curd, ghee, butter, cheese, ice-cream, paneer, sweets etc were prepared from the milk which was sold to customers of related areas. It was also known that Brucellosis disease were new name for them.

4.2 Prevalence of Brucellosis in raw milk of cattle in KTM Valley

During the study of Brucellosis in raw milk of cattle in KTM Valley, a total of 200 milk samples were collected from different areas viz. Old Baneshwor, New Baneshwor, Sinamangal and Gausala. The milk samples were tested by milk ring test (MRT) which showed 98 (49%) positive result out of 200 samples examined.

Out of 200 samples, the maximum samples were collected from Old Baneshwor, which consist of 109 samples and among them 55 (50%) showed positive result to MRT. Similarly 65 samples from Sinamangal which showed 32 (49%) positive result and the least samples were collected from New Baneshwor and Gausala where 6 (46%) and 5 (38%) samples showed positive result respectively out of 13 samples examined.

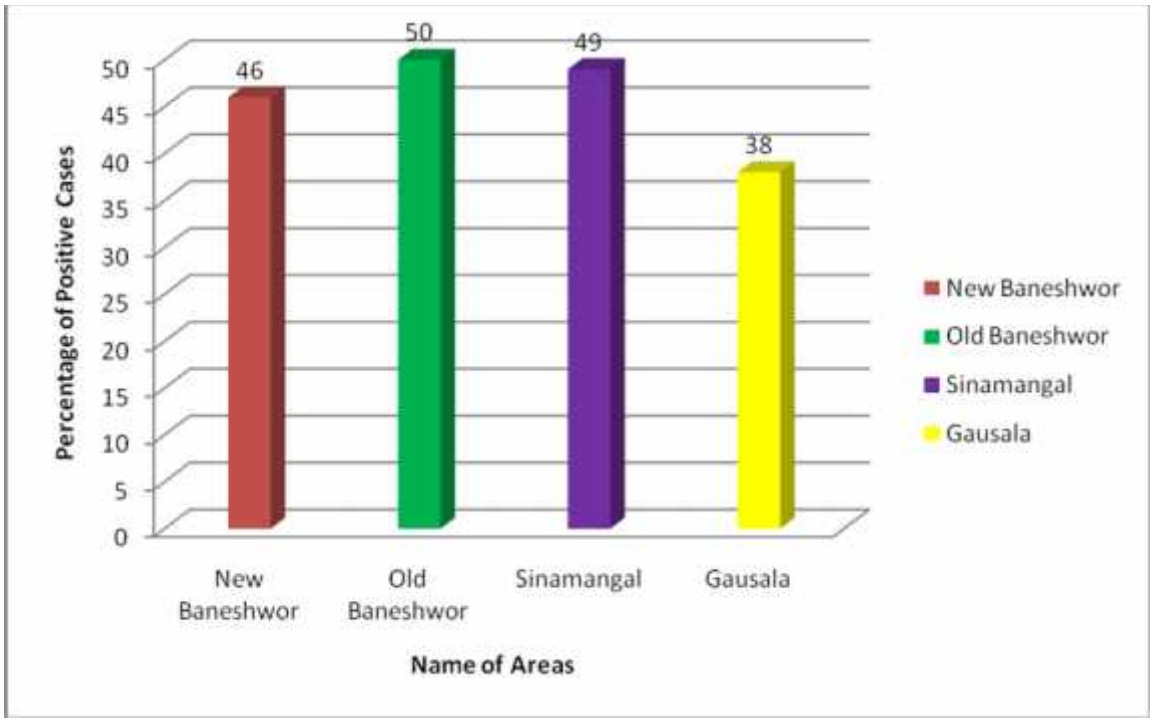


Figure I: Prevalence of Brucellosis in raw milk of cattle in KTM Valley

The diagram shows that the highest prevalence of Brucellosis was obtained from Old Baneshwor area (50%) while least rate was obtained from Gausala area (38%)

4.3 Comparative Brucellosis among single mixed and pooled milk samples

During, milk samples collection from livestock handlers and private dairy out of 200 milk samples 77 single milk samples, 75 pooled samples and 48 mixed milk samples were collected and analyzed to determine Brucellosis in raw milk of cattle. All milk samples were tested by milk ring test and the obtained positive result are given in the table II.

Table II: Comparative Brucellosis among single mixed and pooled milk samples

Name of area	Single			Pooled			Mixed			Total		
	Samples	Positive	%	Samples	Positive	%	Samples	Positive	%	Samples	Positive	%
New Baneshwor	5	1	20	5	3	60	3	2	66	13	6	46
Old Baneshwor	42	17	40	42	25	59	25	13	52	109	55	50
Sinamangal	25	9	36	24	13	54	16	10	62	65	32	49
Gausala	5	1	20	4	3	75	4	1	25	13	5	38
Total	**77**	**28**	**36**	**75**	**44**	**58**	**48**	**26**	**54**	**200**	**98**	**49**

The result shows that the highest prevalence rate of Brucellosis in raw milk was obtained from pooled samples (58%) followed by mixed milk samples (54%) and single milk samples (36%)

4.4 Prevalence of Brucellosis in raw milk of cattle collected from private dairy and livestock handlers

During the collection of milk samples from livestock handlers and private dairy, out of 48 mixed milk samples 25mixed milk samples were collected from private dairy and 23 mixed milk samples from livestock handlers. The milk samples were tested by milk ring test. Out of 25mixed milk samples obtained from private dairy showed 10 positive results to MRT whereas 23 samples obtained from livestock handlers showed 16 positive results to MRT.

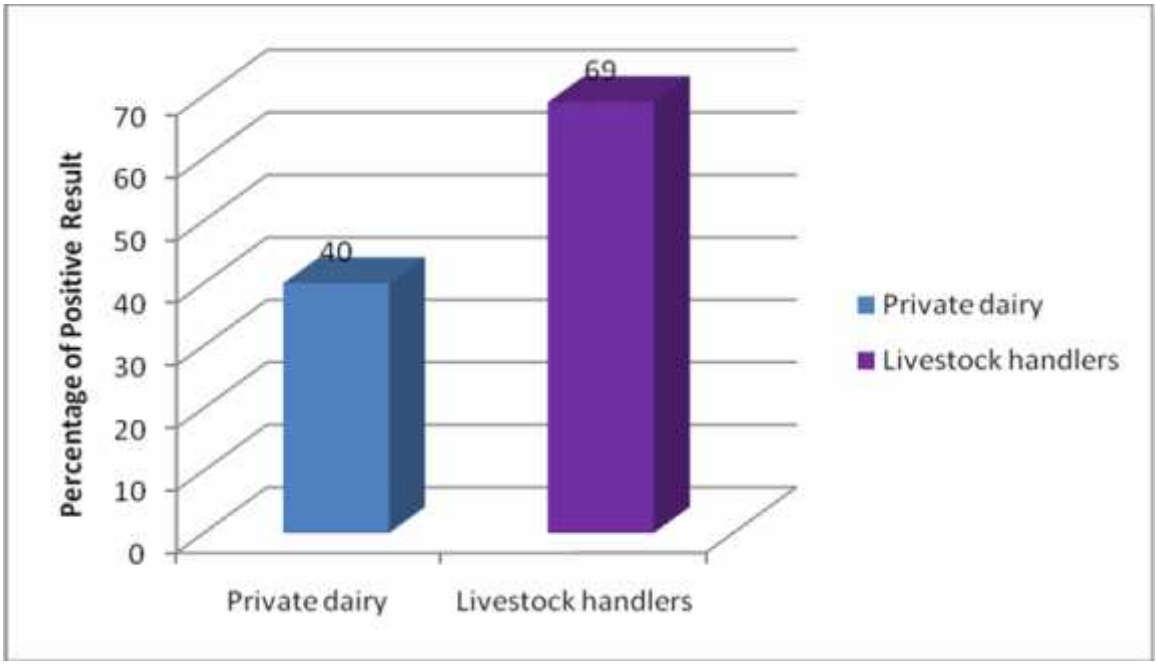


Figure II: Prevalence of Brucellosis in raw milk of cattle collected from private dairy and livestock handlers

The result shows that the higher prevalence rate of Brucellosis in mixed samples was obtained from livestock-handlers (69%) than from private dairy (40%).

4.5 Comparative Brucellosis infection in cows and buffaloes

The study of Brucellosis was carried out among cows and buffaloes. During the study period single milk samples were collected from 69 cows and 8 buffaloes directly through milking animals of livestock handlers. Out 69 milk samples of cows, 28 samples were detected as positive while out of 8 milk samples of buffaloes; all samples were found to be negative.

Table III: Comparative Brucellosis infection in cows and buffaloes

Animals	Samples	Positive	Percentage
Cows	69	28	40
Buffaloes	8	0	0
Total	77	28	36

Comparative Brucellosis infection in cows and buffaloes revealed that the prevalence rate was found in cows (40%) whereas prevalence rate in buffaloes was 0. The result showed that cows were highly infected by Brucellosis disease.

5. DISCUSSION

Milk is the nutritious food for the human beings. It is also called a complete food. It is consumed by all the age group people and of both sexes. Nowadays there is a large demand of milk and milk products. So, most of the people are interested in livestock farming business. Livestock farming has always been popular among the farmers but nowadays other peoples are also interested in livestock farming business.

Livestock farming has always been popular among the farmers but nowadays other peoples are also interested in livestock farming for the economic benefits. It has not only become popular in village areas but also has become popular even in metropolitan cities.

Dairy farming in Nepal is developing day by day which result rapid increase of milk collection centers, dairy industries and many private dairy are established throughout the countries. In spite of establishment of these recent advance dairy industries, the state veterinary and health service in the country is still rudimentary. The lack of awareness about sanitation, personal hygiene as well as lack of screening and monitoring facilities for diseased animals may be the increasing risk of zoonotic diseases.

Brucellosis is one of most important example of zoonotic discease which may affect both human beings and animals. Recent information clearly shows that the Brucellosis, a zoonotic disease in Nepal has become major concern in the view of impact on both public health and animal production development schemes. The basic aim of the present study was to investigate the prevalence of Brucellosis in raw milk of cattle in Kathmandu Valley. The survey was conducted among livestock-handlers and private dairy owners by collecting milk samples as well as questionnaire method. During the study period, 200 milk samples from different areas of Kathmandu Valley viz Old Baneshwor, New Baneshwor, Sinamangal and Gausala were collected. Out of 200 samples examined 49% (n=98) showed positive result to milk ring test (MRT) The highest prevalence rate was obtained from Old Baneshwor (50%) while the least prevalence rate was obtained from Gausala (38%). The highest prevalence rate was obtained from livestock handlers (69%) than from private dairy (40%) while\examined of mixed milk samples. The disease prevalence rate was highly found in cows (40%) whereas absent in buffaloes while examined of single milk samples.

The analysis carried out by Upadhayay,(1998) conducted epidemiological survey of human and animal Brucellosis in Chitwan district. 500 milk samples were collected and tested by MRT. She found 25.8% (n=129) to be MRT positive which is lesser than this findings. This contrast result may be due to animals outside the valley are less infected with this diseases and most of the livestock are local breed.

Rana (2002) conducted epidemiological survey of human and animal Brucellosis in Surkhet district collected 400 milk samples from Surkhet and used MRT to determine Brucellosis in single, pooled and mixed milk samples. He found the prevalence to be 26%, 32% and 29% respectively. The overall prevalence was 29% which is again lesser than this finding. The reason behind this may be due to less infection of this disease to animals outside the Valley.

Dhakal (2003) conducted epidemiological survey of human and animal Brucellosis in Dolkha district. He collected 350 milk samples from the study area in Dolkha. MRT did not reveal a single positive case of Brucellosis in either single or pooled or mixed milk samples which is totally different from this finding. The reason may be the livestock handlers are more aware about cleanliness and sanitation measures, may be the livestock are of local breed or may be high quality breed are not imported into the district from outside.

Mishra, (2008) carried out the study of prevalence of Brucellosis in buffaloes at different site of Bhaktapur district. He collected 60 milk samples from the study area of Bhaktapur. On milk ring test out of 60 samples, 14 samples (23.33%) showed positive which is lesser than this finding. This contrast result may be either due to less sample collection, or may be the buffaloes are less infected to this disease than the cows.

Joshi (2000) studied animal Brucellosis in Kathmandu and around the valley. He collected total 660 milk samples (pooled) of buffaloes and cows from DDC of Kathmandu and tested by MRT. The prevalence of positive reaction was found 4.89% (31/660). Similarly he examined 4229 samples (single) of buffaloes and cow from DDC centers milk vendors and village in Kathmandu Valley and around Valley viz Panchkhal, Bhaktapur and Banepa and observed 1.25% positive by MRT which is again lesser than this finding. The reason

may be cattle of DDC are vaccinated or due to less infected with this disease around valley or due to false negative reaction shown by MRT.

Although the result of this survey showed the higher percentage of Brucellosis in tested samples which might be due to false positive result of MRT. The prevalence cannot be detected by conducting only one test in case of Brucellosis. Both tests which determine sensitivity and specificity should be performed. The finding of this is not sufficient as other specific laboratory test has not conducted due to limited time and resources. The false positive result may be due to lack of serum test and presence of high cholesterol as well as bacteria in milk samples. For accurate conformation test like CFT, ELISA, AGID, blood culture isolation etc are necessary but these tests prove to be out of reach with limited time and resources. Hence result of study Control measures like screening and monitoring, segregation or even isolation of infected animals have not strictly been enforced by any agencies. Import of high quality breed from outside without veterinary examination should also be discouraged. Awareness program about the disease and its affect are not conducted in every part of country.

Thus, further research work should be conducted and possible control measures like educating people, training veterinaries, butchers and abattoir workers, monitoring vaccination campaign is vital. Import animals from outside after veterinary examination, awareness program about personnel hygiene and sanitation is necessary to reduce the intensity of this disease in Nepal.

6. CONCLUSION AND RECOMMENDATION

6.1 CONCLUSION

Brucellosis is a bacterial zoonatic disease caused by a small, gram-negative bacteria of the genus *Brucella* belonging to family Brucellaceae. It causes abortion, still birth sterility, low milk production and retention of placenta in animals.

It is also known as Undulant fever, Malta fever or Mediterranean fever in human being's. It is occupational diseases that have public as well as economic importance.

The study of **Survey of Brucellosis in raw milk of cattle in Kathmandu Valley** was carried out by collecting 200 milk samples from the different area like Gausala, Old Baneshwor, New Baneshwor and Sinamangal. These 200 milk samples were analyzed by MRT test. While collecting samples, questionnaire survey was also done among livestock handlers and private dairy owners. Out of 200 milk samples, 49% (n=98) were found positive by milk ring test method. The highest prevalence rate was obtained from Old Bneshwor (50%) while the least prevalence rate was obtained from Gausala (38%). Similarly, examination of single, pooled and mixed milk samples by MRT test showed that the highest prevalence rate of Brucellosis was obtained from pooled samples (58%) followed by mixed samples (54%) and the least prevalence rate from single milk samples (36%). Among livestock handlers and private diary, the prevalent rate was found more in livestock handlers (69%) than private dairy (40%). Also, the prevalent rate of disease was found highly in cows (40%) where as absent in buffaloes.

Considering the higher prevalence of Brucellosis in raw milk, suitable preventive and control measures including awareness programme for livestock handlers and private dairy owners, effective quarantine and legislative measure, test of quality of milk and milk products, examination of animals before import from outside have been recommended.

From the study, it was found that Brucellosis in raw milk of cattle is highly prevalent in Kathmandu Valley. So it reveals that a profound study of Brucellosis in raw milk of cattle is very urgent.

6.2 RECOMMENDATIONS

During the study of survey of Brucellosis in raw milk of cattle 49% of them were found positive which is quite high in such sampling in compared to other. This investigation indicates that there is the great risk of disease which should be checked properly and appropriate control measures should be effectively applied.

- The preventive measures against Brucellosis like proper sanitation, education, awareness, cleanliness program should be conducted in every part of country.
- There must be the facilities for the diagnosis of Brucellosis in veterinary hospitals, milk collection centers and medical hospitals.
- Government should make strict law for the inspection of the quality dairy products before introducing to consumers.
- Regular sero monitoring or screening and control program should be promoted in livestock raising areas. So that if the area is found to be endemic, vaccination, and campaign should be conducted against Brucellosis in these areas by government.
- The manufacturing place of dairy products should be hygienic and they should prepare only after inspection and effectively heating of milk.
- Free training is necessary in every part of the country for livestock workers, private dairy owners, dairy farmers about zoonotic disease
- Government should establish the proper laboratory facilities for detecting zoonotic disease species including *Brucella*, culture isolation and identification.
- Government should provide fund for research centre in order to eradicate the disease from country
- Precaution should be followed while handling of the suspected or infected animals.
- The surveillance activities of both public health and animal health sector should be fully conducted throughout the country. Administrative arrangement should be established between the two sectors.

REFERENCES

- Acedo, E., Diaz, M.E and Leon, A.B. 1997. Incidence of *Brucella* sp. in raw milk and regional white cheese. Alimentaria (Issn Span, with Span. And Engl. Summ) **35** (281): 57-60.
- Alton, G.G. 1981. The control of *Bovine* Brucellosis. World Animal Review, FAO, **39**: 17-24.
- Ansary, EI-E.H., Mohammed B.A., Hamad A.R and Karom A.G 2001. Brucellosis among animals and human contracts in eastern Sudan. Saudi Medicine Journal Jul. **22** – (7): 577-579.
- Avila, M.O., Ribas, J.A.S, Santos, M.D, Schein F.B., Camargo, L.M. & Brando, K.P. 2001. Presence of *Bovine* Brucellosis analyzed at the Microbiology Laboratory of the Veterinary Medical College, Cuiaba University, Mato Grosso state, Brazil. Revista Brasileria de Reproducao Animal **25** (2): 237-238.
- Barman, N.N. 1992. Therapeutic response of long-acting oxytetracycline combine with streptomycin in *Bovine* Brucellosis. Indian J. Animal Research **25** (2): 95-96.
- Benjamin, C.J. and Wiliamson C.C. 1989. A serological survey of *Bovine* Brucellosis in four district of Bophuthatswana (South Africa). J.S. Afr. Vet. Assoc. **60** (1): 50.
- Berman, D.T. 1981. Disease of Cattle in the Tropics. Edt, Ristic. M and Mclntyre, I. Martinus Nijhoff: 271.
- Chakrabarty, A.2001. A Text Book of Preventive Veterinary Medicine. 3rd ed. Kalyani Publishers, New Delhi: 312-322.
- Chakrabarty,A. 2007. A Text Book of Preventive Medicine. 7th ed. Kalyani Publishers, New Delhi: 317-324.
- Chandra M and Ramdas, C.P. P and Raghavan, N., 1993. Study on *Bovine* Brucellosis in an endemic area. India veterinary journal **69** (7): 581-583.
- Mohan. C., C.P, Ramdasa, P. And Raghavan, N. 1993. Studies on *Bovine* Brucellosis in an endemic area. Indian Vet. J. **69** (7): 581-583.
- Corbel, M.J. 2006. Brucellosis in humans and animals, World Health Organization in collaboration with Food and Agriculture Organization of the United Nation and World Organization for Animal Health.

- Dahal, R. 2003. Sero Epidemiological surveillance of Human and Animal Brucellosis in Dolkha district. Unpublished dissertation of M.Sc., submitted to the Central Department of Zoology, Tribhuvan University, Kirtipur.
- Folhadella, I.M., Jesus, V.L.T., Folhadella, D.S., Goulart, I. & Andrade C.M. 2001. Risk factors of *Bovine* Brucellosis in cattle farms in the state Rio de Janerio. Revista Brasileria de Repdoducao Animal. **25** (2): 239-240.
- Gurturk. K., Boynukara, B., Ilhan, Z., Hakki, I.E. & Gulhan, T. 1999. Comparison of the dot-immunobinding assay with the serum agglutination test, the Rose Bengal Plate Test and the Milk Ring Test for the detection of *Brucella* antibodies in *Bovine* sera and milk. Journal of Veterinary Medicine Serie B **46** (4): 279—285.
- Joshi, D.D. 2000. Epidemiological surveillance Human and Animal Brucellosis in milk collection Area of DDC Nepal. NZFHRC Tahachal: 35.
- Joshi, D.D. 1970. A serological survey of animal Brucellosis in KTM, Bull Vet, Sc & AH, Nepal, **5:** 32-37.
- Joshi, D.D. 1976. A serological survey of animal Brucellosis in KTM, Bull. Vet. Sc & AH, Nepal **5:** 32-37.
- Joshi,D.D. 1973. Public Health Importance of Brucellosis. Veterinary Public Health Hazards in Nepal: 47-52.
- Joshi, D.D., Upadhya , M. & Mishra, P.N. 1998. Brucellosis in Animal and Human of Chitwan. Workshop on status of Animal Health in Nepal, National Zoonosis and Food Hygiene and Research Center (NZFHRC): 37-48
- Joshi, D.D. 2000. Epidsemiological surveillance of Human and Animal Brucellosis in Milk collection Area of DDC Nepal. NZFHRC, Tahachal: 35.
- Khanal, D.R. and Jha V.C. 1996/97. Study on *Bovine* Abortion. Annual Report 1996/97 NARC, AHRD, Tripureshwor: 21-22.
- Khanal, D.R. and Jha V.C, 1997/98. Study on *Bovine* Abortion and Repeat Breeding due to Brucellosis in Chitwan and Eastern Terai. Annual Report 1997/98 NARC, AHRD, Tripureshwor: 22-23.
- Lefkowitz, N.A., Joshi, D.D., Herd, D., Chetri, B.K. and Sharma, M. 2003. Prevalence of Brucellosis in Yaks and other cattle of Langtang Valley. Nepalese Veterinary Journal, (27), Nepal Veterinary Association: 12 -17.

- Manandhar, P. 1999/00. Sero Study of Brucellosis in Cattle and Buffalo in the Western Development in Nepal Annual Technical Report, HMG, DLS, DAH, CVLADCS, Tripureshwor, Kathmandu: 52-54.
- Merchant, I.A. and R.A. Packer (1967) Veterinary Bacteriology and Virology CBS publisher and distributor 485 Jin Bhawan Bholanath Nagar Shahdra, Delhi 110032 (India) 7 th edition, 315-317.
- Mishra, Y. 2008. Prevalence of Brucellosis in Buffaloes at different site of Bhaktapur district. Unpublished mini thesis submitted to Purbanchal University facility of Science and Technology.
- Nandgoankar, D. & Rao, P.L.N. 1971. A Survey of the incidence of *Bovine* Brucellosis in the integrated milk project area in some of the dairy farms of Andhra Pradesh. The Indian Veterinary Journal **48** (1): 12-18.
- Pradhan, A. 1996. Sero prevalence of Brucellosis in cattle and Buffaloes in Chitwan. Proceedings of First Livestock and Fisheries Research Workshop: 227-231.
- Pykural., S. and Mishra, U. 1997. Sero-Epidemiological Evidence of Animal Brucellosis in Nepal, Bulletin of Veterinary Science & Animal Husbandry. Nepal **6**: 1-6.
- Rana, H. 2002. Sero – Epidemiological surveillance of Human and Animal Burcellosis in Surkhet district. Unpublished dissertation of M.Sc., submitted to the Central Department of Zoology, Tribhuvan University, Kritipur.
- Shafee M., Rabbani, M., Sheikh A.A., Ahmed M., and Razzaq, A. 2011. Prevalence of Bovine Brucellosis in organized dairy farms, using milk ELISA in Quetta City, Balochistan, Pakistan.
- Shakya, S., Joshi, R.K. & Ali, S.L. 1995. Sero-epidemiological survey of *Bovine* Brucellosis in a village of Madhaya Pradesh. The Indian Veterinary Journal **72:** 12.
- Shaw, A.A. 1987. Studies on infectious infertility and abortion incidences of *Bovine* Brucellosis in Kashmir (India). India Veterinary Medical Journal **10** (3): 137-140.
- Upadhaya, Maya. 1998. Sero-prevalence of Human and Animal Brucellosis in Chitwan District. Unpublished dissertation of M.Sc. Submitted to the Central Department of Zoology, Tribhuvan University Kirtipur.
- Vaid, L.J. and Kaushal, R.S. 1992. Seroprevalence of Animal Disease in Himachal Pradesh. Indian veterinary journal **68**(8):705-707.

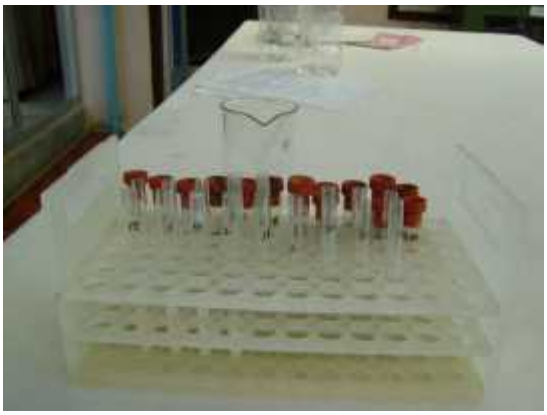
LIST OF PHOTOGRAPH

Photographs II & III: Questionnaire survey with livestock handler

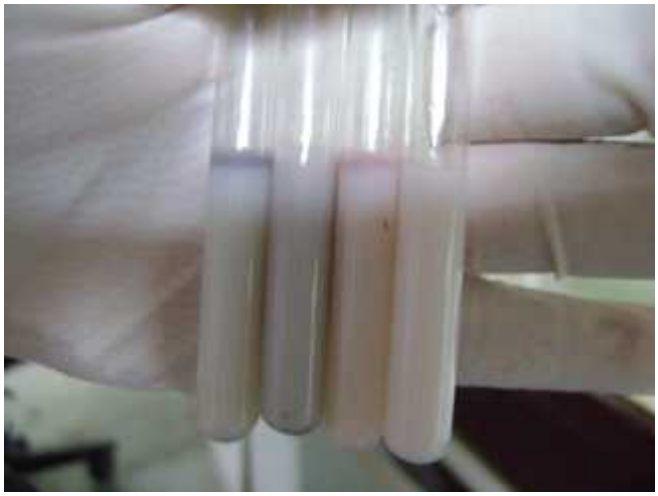
Photographs IV & V: Collection of single milk sample directly by milking cow

Photographs VI & VII: Questionnaire survey with private dairy owner


Photograph VIII & IX: Collection of mixed milk sample from private dairy




Photographs X & XI: Collection of milk samples in sterile rubber capped vials and Milk sample transferred from vial by pipette

Photograph XII & XIII : Observation of Milk Ring Test and Ring formation shows positive & without ring shows negative samples

Annex I: Questionnaires for Livestock handlers

Form No. :

Name:

Address:

Age/Sex:

Number of milk animals

Milk production – Lt/day

Where do you sell milk?

What all items do you prepare from milk?

Do you prepare items from well boiled milk?

Do any member in your family drinks raw milk?

Yes No

Do you know any disease transmitted from milk?

What precaution do you take with sick animals?

Who does the treatment if the animals are sick?

Have you heard the name of disease called Brucellosis?

Do you know about the disaster caused by Brucellosis?

Annex II: Questionnaire for Private Dairy Owner

Form No:

- Name of Dairy :
- When was it established?
- What types of milk products it prepares?
- What is the production capacity?
- To fulfill their requirement, from where all milk is being collected?
- What king of precaution is taken in the preparation of milk product?
- Do you know about milk borne diseases?

  Yes No
- Have you heard the name of disease called Brucellosis?
- Do you know about the disaster caused by Brucellosis?