SERO PREVALENCE OF BRUCELLOSIS IN PIGS IN 6 VDCs OF RUPANDEHI DISTRICT, NEPAL

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RECOMMENDATIONS

This is to recommend that the thesis entitled “Sero- prevalence of brucellosis in pigs in 6 VDCs of Rupandehi district, Nepal” has been carried out by Mr. Shree Ram Poudel for the partial Fulfilment of Master’s Degree of Science in Zoology with special paper Parasitology. This is his original work and has been carried out under our supervision. To the best of my knowledge, this thesis work has not been submitted for any other degree in any institution.

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CERTIFICATE OF ACCEPTANCE

This thesis work submitted by Mr. Shree Ram Poudel entitled "Sero- prevalence of brucellosis in pigs in 6 VDCs of Rupandehi district, Nepal" has been accepted as a partial fulfillment for the requirement of Master's Degree of Science in Zoology with special paper Parasitology.

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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).

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ABSTRACT

The objective of the present study is to determine the prevalence of brucellosis in pigs in 6 VDCs of Rupandehi district, Nepal. For this a cross- sectional study was conducted. Serum samples of 103 pigs were taken from 6 VDCs of Rupandehi district namely Devdaha, Dudhrakshya, Majuwa, Motipur, Parrohoa and Saljhandi. Fresh blood samples were collected from the ear vein of the farm pigs and were centrifuged to separate the serum from the blood. The separated serum samples by centrifuge were transported to NZFHRC laboratory for testing by maintaining proper cold chain condition. In the lab sthe test was done by qualitative slide agglutination test (SAT). Out of 103 samples sero-positive for brucellosis was found to be 13.59% (14/103). Among 53 samples of female pigs, 15.09% were found to be sero- positive for brucellosis where as out of 50 samples of the male pigs 12% were found to be sero-positive for *Brucella* antibody. Group-wise 5.26% from 38 samples of 0-3 months age group, 11.90 % from 42 samples of 3-6 months age group, 21.05% from 19 samples of 6-9 months age group and 75% from 4 samples of age above 9 months were found to be sero-positive for *Brucella* antibody. Similarly 15.38% from 52 samples of exotic breed group, 10 % from 10 samples of local breed group and 12.19% from 41 samples of crossbreed group were found to be sero-positive for *Brucella* antibody. The result from the analysis seems statistically significant in terms of age wise ($p=0.001$) whereas it seems statistically insignificant in terms of sex wise ($p=0.647$) and breed wise ($p=0.852$). This study shows an alarming situation not only in the Rupandehi district but also contribute in the public health sector of the country. By implementing strict and appropriate prevention and control strategy as adopted by many developed countries it can be possible to eradicate this disease from Nepal.

CONTENTS

Pages

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

AGID	Ager Gel Immuno- diffusion
AMRT	Abortus- Bang-Ring- Test
BDC	Brewer Diagnostic Card
BPAT	Buffered Plate Agglutination Test
cELISA	Competitive Enzyme- Linked Immunosorbent Assay
CFT	Complement Fixation Test
DDC	District Development Committee
FAO	Food and Agriculture Organization
FPA	Fluorescent Polarization Assay
MPCS	Milk Producers Cooperative Society
MRT	Milk- Ring Test
NZFHRC	National Zoonoses and Food Hygiene Research Center
OIE	International Office of Epizootics
PAT	Plate Agglutination Test
PCR	Polymerase Chain Reaction
RBPT	Rose Bengal Plate Test
RPT	Rivanol Plate Test
SAT	Serum/Slide Agglutination Test
SAT-EDTA	Serum Agglutination Test with Ethylenediaminotetracetic Acid
SPAT	Standard Plate Agglutination Test
STAT	Standard Tube Agglutination Test
SPSS	Statistical Package for Social Science
VDC	Village Development Committee
WHO	World Health Organization

1. INTRODUCTION

1.1 Brucellosis

Brucellosis is an infectious, contagious, and worldwide spread form of an important zoonotic disease caused by bacteria of the genus *Brucella*. *Brucella* belongs to family Brucellaceae and order Eubacteriales. Various *Brucella* species primarily affects cattle, sheep, goats, swine and dogs, and is characterized by abortion or infertility and also affects people and other animal species (Ray and Steele 1979). Human become infected by coming in contact with animal products that are contaminated with these bacteria. It is estimated that inhalation of only 10 to 100 bacteria is sufficient to cause the disease in man (Kaufmann et al. 1997). Human brucellosis is mainly an occupational disease affecting animal caretakers, livestock farmers, artificial inseminators, abattoir workers, meat inspectors and veterinarians due to frequent exposure to infected animals (Corbel 2006). Human brucellosis remains the most common zoonotic disease worldwide, with more than 500,000 new cases reported annually (Pappas et al. 2006). Globally this disease is woefully under-reported because of its vague clinical flu like symptoms, difficult in laboratory diagnosis and lack of familiarity by medical professionals (Corbel 2006). Published reports indicate that it is an occupational disease among livestock farmers, milkers, butcher and veterinary practitioners and caused economic losses due to abortion, loss of calf production, reduced milk yield and infertility (Rahman et al. 2012). From a recent survey in Mediterranean and Middle East countries, the annual incidence of human brucellosis varies from 1 to 78 cases per 100,000. Certain communities in South European countries reported up to 77 cases per 100,000 people (WHO 1986, Mousa and Elhag 1988).

Brucellosis is found worldwide, but it is well controlled in most developed countries. The disease occurs worldwide, except in those countries where bovine brucellosis (*B. abortus*) has been eradicated. The Mediterranean countries of Europe, northern and eastern Africa, Eastern countries, India, Bangladesh, Thailand, Central Asia, Central and South America are

still not brucellosis free (Robinson 2003). Brucellosis is more common in countries with poorly standardized animal and public health program (Capasso 2002). In camels, brucellosis has been reported from Arabian and African countries. 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, Myanmar, China, Thailand, and Indonesia and even in Nepal it has been reported.

1.2 Etiological agents

The etiological agents of brucellosis are *Brucella* species are facultative intracellular gram-negative cocco-bacilli, non-spore-forming and non-capsulated. Although *Brucella* spp. is described as non-motile, they carry all the genes except the chemotactic system (Fretin et al. 2005). Nine *Brucella* species are currently recognized, seven of them that affect terrestrial animals are: *B. abortus, B. melitensis, B. suis, B. ovis, B. canis, B. neotomae*, and *B. microti* (Scholz et al. 2008; Verger et al. 1987) and two that affect marine mammals are: *B. ceti* and *B. pinnipedialis* (Foster et al. 2007).

Brucella suis consists of five biovars, but the infection in pigs is caused by *B. suis* biovars 1, 2 and 3. The disease caused by biovars 1 and 3 is similar, while that caused by biovar 2 differs from 1 and 3 in its host range, its limited geographical distribution and its pathology. *Brucella melitensis, Brucella suis, Brucella abortus* and *Brucella canis* are the most frequently occurred *Brucella* species transmitted from animals to humans and are pathogenic.

1.3 Morphology and characteristics of *Brucella*

Brucella is small, non-motile, non-sporing, gram-negative coccobaccilli short rods. They grow rather slowly on ordinary nutrient media while their growth is improved by serum or blood. They are an aerobic; there is no growth under strictly anaerobic conditions. The *Brucella* species are intracellular parasites inside human-beings and animals and can usually be found in the reticuloendothelial and reproductive systems. Typically *Brucella* spp. occurs as small gram-negative cocco-baccilli, but coccal and bacillary forms also occur. The cells are short and slender; the axis is straight; the ends are rounded; the sides may be parallel or

convex outwards. In length they vary from about 0.5 - 0.7 μm, in breadth vary from 0.5 - 1.5 μm, occurring singly, in pairs or short chains (Leslie et al. 1998).

1.4 Reservoirs/sources of infection

Brucellosis is a zoonotic disease; hence the ultimate sources of infection are infected animals. The key species are the major food-producing animals: cattle, sheep, goats, pigs. Others, including bison, buffalo, camels, dogs, horses, reindeer and yaks are less important, but they can be very significant local sources of infection in some regions. Recently, the infection has also been identified in marine mammals, including dolphins, porpoises and seals, and these may present an emerging hazard to persons occupationally exposed to infected tissues from them.

1.5 Transmission

The main sources of infection in animals are fetuses, after birth and vaginal discharges containing a large number of *Brucella* organisms. They may lick the genital organs of infected animals, aborted fetuses or placental membranes or fluids like milk; or they may ingest grass and water contaminated with such materials or with urine or serum from infected animals. The sexual interaction between animals carrying with *Brucella* organisms also helps in the transmission of the disease. In human common routes of infection include direct inoculation through cuts and abrasions in the skin, inoculation via the conjunctival sac of the eyes, inhalation of infectious aerosols, and an ingestion of infectious unpasteurized milk or other dairy products. Blood transfusion, tissue transplantation and sexual transmission are possible but rare routes of infection.

1.6 Factors

The severity of the disease depends upon many factors such as previous vaccination, age, sex and management such as herd or flock size and density. Abortions are more prevalent in unvaccinated. Close contact with animals may occur when humans assist animals during parturition or abortion or handling of stillbirth. Environmental factors that affect the ability of

Brucella to survive outside the mammalian hosts are to be considered in the epidemiology of brucellosis. High humidity, low temperature and absence of direct sun light may favor survival of *Brucella* for several months in water, aborted fetuses, placental membranes, liquid manure, hay, buildings, equipment and clothes (Sammartino et al. 2005).

Another consideration that should be taken into account is the potential influence of ecological factors; particularly climate change (Keesing 2006). In the Greater Yellowstone Ecosystem, Wyoming, USA where the free-ranging elk (*Cervus elaphus*) is a maintenance host for *Brucella abortus,* a study assessed how the increase on the transmission of brucellosis in those animals, may be affected by climatic factors, such as snowpack (Cross et al. 2010).In a recent study from northern Alaska, USA the presence of specific antibodies to *Brucella* spp. in polar bears (*Ursus maritimus*) from southern Beaufort Sea during 2003-2006 was reported. One possible explanation suggested for the annual variability, ranging from 7% to 19% in the period, is climate change (Ohara et al. 2010).

1.7 Pathogencity

From epidemiological evidence, *Brucella* species has different host specificity. Among the *Brucella* species *B. abortus* is confined to cattle, buffaloes, bison, camel, yaks etc. whereas *B. melitensis* is associated with infection in sheep and goats. Similarly *B. canis* is associated with dogs. *Brucella suis* mainly infects pigs and is the main cause of porcine brucellosis. The remaining other members of the species have much greater host specificity. Typically, in all host species *Brucella* grows intracellulary, producing a variable bacteraemic phase followed by localization in the tissues of the genital tract and in the mammary gland. In particular, female animals that have reached sexual maturity are most susceptible to infection. It is usually detected in pregnant females through abortions (England et al. 2004). *Brucella* invade the body via the alimentary tract, conjunctival mucosa, respiratory tract, or skin and localization within regional lymph nodes, followed by ingestion either mononuclear or polymorphonuclear leucocytes. Virulent *Brucella* has the ability to survive in both polymorphonuclear and mononuclear phagocytes and also can depress chemotaxis and phagocytosis (Ocon and Reguera 1994).

After phagocytosis, *Brucella* probably multiplies in the lymph nodes as parasites and then enters the blood and produces the bacteraemia followed by the acute febrile phase of the disease. From the blood, the organisms are distributed throughout the reticuloendothelial system and become present in large numbers in the liver and spleen. They also localize in many other sites such as the joints, heart, kidneys, the central nervous system and genital tract (Baldwin 1994).

1.8 Sign and symptoms

In sexually mature animals the infection localizes in the reproductive system and typically produces placentitis followed by abortion in the pregnant female, birth of weak piglets, infertility and epididymitis and orchitis in case of male. The other clinical manifestations are spondylitis (inflammation in joints particularly of the lumbar and sacral regions), arthritis, paralysis of hind limbs, and lameness (movement disability) fever, depression, metritis occasionally with blood-stained discharge, mastitis, bursitis, stillbirth, weak calves in case of cattle, and abscesses or granulomas in various locations including subcutaneous tissue, reproductive organs, and mammary gland (Woldemeskel 2013). There is lowered milk production due to premature births. The udder is often permanently infected, especially in the case of cows and goats. In goats, cattle, swine and dogs similar complications may follow infection with *B. melitensis*, *B. abortus*, *B. suis* and *B. canis* respectively.

In case of human the common symptoms also include fever malaise, insomnia, anorexia, headache, arthralgia, constipation, sexual impotence, nervousness and depression, meningitis, spondylitis, arthritis, endocarditis, orchitis, and prostatitis (Acha et al. 2003). The clinical picture in human brucellosis can be mixed up with cases of gasterointestinal, respiratory, dermal, and neurologic manifestations predominantly, which are not uncommon (Shakir et al. 1987). Acute or subacute diseases follow after fever and bacteremia, and may vary from 1 week to 6 weeks or several months. Acute brucellosis is characterized by an intermittent fever ranged from 38 – 41 ºC. Death may occur from hyperpyrexia, severe toxaemia, endocarditis, and meningoencephalitis (Dalrymple-Champneys 1960). *Brucella* infection commonly causes mild hematologic abnormalities such as anemia and leukopenia. Chronic brucellosis may result from untreated patients when the acute or subacute disease has

persisted for 6 months or longer with or without complications. In that case, the disease is regarded as chronic. The symptoms are generally related to the state of hypersensitivity of the patient and then the illness may persist for years (Araj and Lulu 1986).

1.9 Diagnosis and treatment

In animals and human blood, lymph nodes, milk, placenta etc. are the main materials for diagnosis (Poiester et al. 2010). Furthermore, other materials rich in the organism include: stomach contents, spleen and lungs from aborted fetuses, vaginal swabs, semen and arthritis or hygroma fluids from adult animals. From animal carcasses, the preferred tissues for culture are the mammary gland, supramammary, lymph nodes & spleen (OIE 2009, Ahmed et al. 2010). The sample should be frozen until required for culture (OIE 2009).

Agglutination test: It includes different types of test.

Milk ring test (MRT): This test is also known as ABRT (Abortus-Bang-Ring-Test) which is done with milk or cream (Sutherland 1980).

Serum Agglutination Test: Serological tests can be used to screen for, or confirm brucellosis. It includesseveral types of tests (Kaltungo et al. 2014) like Standard plate agglutination test (SPAT), Serum agglutination test with ethylenediaminotetracetic acid (SAT-EDTA), Buffered plate agglutination test (BPAT), Rose Bengal plate test (RBPT), Rivanol plate test (RPT), Complement fixation test (CFT),The 2-mercaptoethanol test, Anti-globulin test**,** Heat inactivation test. Besides agglutination test there are other several tests which are used for diagnosis of the Brucellosis.

- Isolation of organism
- Laboratory animal inoculation
- Intra- Dermal test
- Molecular Method by using the polymerase chain reaction (PCR)
- Primary binding assays by using the competitive enzyme-linked immunosorbent assay (cELISA) and the fluorescent polarization assay (FPA) were developed to detect the antibodies (Gall and Nielsen 2004)

For the treatment the regimen of first choice is combination theraphy with doxycycline for 45 days and streptomycin for 14 days. Gentamicin or netilmicin for the first 7days may be substituted for streptomycin. The second choice of regimens consists of combination of doxycycline and rifampicin for 45 days or monotherapy with doxycycline for 45 days. Surgery should be considered for patients with endocarditis or cerebral abscess, spleen abscess or with other abscesses which are antibiotic resistant. Tetracycline are generally for pregnant patients and children <8 years old. Rifampicin 900mg once daily for 6 weeks is considered the drug of choice for treating brucellosis in pregnanat women. In children <8 years old the preferred regimen is rifampicin with cotrimaoxazole for 45 days. An alternative regimen consists of a combination of rifampicin for 45 days with gentamicin 5 to 6 mg/kg/day for the first 5 days (Solera et al. 1997).

1.10 Prevention and control

Brucellosis occurs due to direct or indirect exposure to infected animals or their products. Prevention must be based on elimination of such contact. But there is technical and social difficulties involved in eradicating the disease. In many situations there is little alternative that helps to minimize the impact of the disease and to reduce the risk of infection. The ways of prevention can be done by applying safety and effective rules on the points like occupational hygiene, personal hygiene, farm sanitation, laboratory precautions, hygienic precaution (meat, milk and milk products). Similarly public health aspects include the public health education and community participation program contributing in the prevention and control of the brucellosis.

1.11 Aims and objectives

1.12 General Objective

To determine the prevalence of brucellosis in pigs in 6 VDCs of Rupandehi district of Nepal.

Specific Objectives

To detect the antibody against *Brucella* in *suis* by serological test.

To determine brucellosis according to breed wise of *suis*.

To determine brucellosis according to sexr wise, age wise and VDCs wise of *suis*.

To determine the knowledge and practice of pig farmers in pig farming system.

1.13 Rationale of the study

Brucellosis is one of the world's major zoonoses that still are of veterinarian, public health and economic concern in many parts of the world. Brucellosis occurs worldwide but is much controlled in developed countries due to routine screening of domestic animals and animal vaccination program. But no routine screening is done regarding prevalence of brucellosis of pig in Nepal. It may cause considerable economic loss. In livestock, brucellosis results in reduced productivity, almost all domestic species can be affected. Epidemiological evidence shows that in Nepal brucellosis is present in different species of mammalian farm animals including cattle, goats, buffalo, yaks, camel, horses and pigs. Although the poultry, buffalo and goat are the major source of meat in our country the other source of meat are pig and sheep which are also consumed in high quantity. The country couldn't control the peoples demand on getting only the meat of poultry and goats in such cases like threat of "Bird Flu". In such case the demand of people is more comfortably provided by the meat of pigs and buffaloes. So now a day the farmers are motivating towards pig farming. Though nutritional, genetic, managerial and infectious diseases are the major problems in livestock husbandry but abortion losses are considered as one of the major constraints in livestock production system.

In Rupandehi district many cases of abortion and reproductive inefficiency in domestic animals are reported but their actual causes are remaining undiagnosed. Such cases of

abortion and reproductive inefficiency could be due to *Brucella* species. From Nepal though some research have been conducted on *Brucella* species in domestic animals like cattle, buffaloes, goats etc. but regarding brucellosis in pigs a very few article was found. The present study is fully specified to brucellosis in pigs. Study for diagnosis of Brucellosis in animals is of vital importance. This could be new in the region, which is of public health importance. So, identification of prevalence rate of brucellosis in different animals at different places of country has become important for making plan and polices regarding prevention, control and treatment and other zoonotic disease in the country. This study will be helpful for researchers doing work related to pig brucellosis in the country. Similarly, on establishing the epidemiology of the disease could be an important source of information for farmers, students, future researchers, control strategy planners and other concerned persons.

1.14 Limitation of the study

This study is based on the qualitative slide agglutination test. Using this test the false negative results may be obtained in the early phase of disease, prozone brucellosis (the range of antibody concentrations within which reaction is inhibited) and antibiotic treatment. Sera from low or non- immune responders can also produce false negative results. However using this test the false positive result may be obatained due to serological cross-reactions with some strains of *Vibrio cholera*, *Pasteurella*, *Proteus OX 19* and *Y. enterolitica* serotype 9. Sera from individuals without clinical signs of infection may show positive results with P-OX19 due to low titres of anti-Proteus antibodies, particularly in the slide agglutination (screening) test (Source: Humatex Febrile antigens produced by Human Gesellschaft fur Biochemica und Diagnoatica mbH, Wiesbaden- Germany). For accurate confirmation other tests like PCR, ELISA, CFT, blood culture for microscopic test etc are necessary. But these tests are very difficult to perform due to financial constraint. Other limiting factor includes small sampling size and limited time for study.

2. LITERATURE REVIEW

2.1 History of brucellosis

Among the all types of *Brucella* species the first recognized species was *Brucella melitensis*. Considering the history of *Brucella melitensis*, the member of the genus recognized, was isolated in 1887 from spleen of patients who died from Mediterranean fever or Bruce's septicemia, later called Malta fever by Sir David Bruce, a British army surgeon. However David Bruce isolated the organism that bears his name from the spleen of five patients with fatal cases on Malta. Bruce named the organism as *Micrococcus melitensis*. But an accurate description of the diseases in man was reported in 1860 and designated as Mediterranean or gastric intermittent fever. However, the organism was isolated in 1887 (Bruce 1887). After ten years later, in 1897, was isolation and identification of *Brucella abortus* from aborted bovine fetus and fetal membranes by the Danish veterinarian Frederick Bang; hence the infection in the cattle was also known as Bang's disease or Bang's abortion disease (Vegad et al. 2001). Traum (1914) identified the *Brucella suis* from the fetus of the sow in USA. Keefer (1924) first recognized case of undulant fever caused by *Brucella abortus*.

2.2 Brucellosis research in world

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. Nagal et al. (1992) determined the sero-prevalence of *Brucella abortus* infection in livestock of Kangra Valley in Himachal Pardesh, India. They reported the overall prevalence as 17.6%, however, in cattle of organized farm, field cattle, sheep and goats it was found to be 16.5%, 2.85%, 15.11% and 25.42% respectively on the basis of standard serum agglutination test. Chandra et al. (1993) studied bovine brucellosis in an endemic area. They collected bovine serum samples from zebu cattle and buffaloes in India. Out of the 138 serum samples tested 26 showed positive antibody level 18.84% by ELISA test. Brisibe et al.

(1996) studied brucellosis serologically in 210 sheep and 201 goats in Borno and Yobe Street in the Arid Zone of North Eastern Nigeria. Sera samples were tested by RBPT. The incidence of brucellosis was found to be positive for sheep and goats were as 4.8% (10/210) and 6.0% (12/201) respectively. Mrunalini and Ramasastry (1999) studied the serological survey on the occurrence of brucellosis in domestic animals and man in Andhra Pradesh, India. After analysis of 10 years of data, they revealed that the sero-positivity to brucellosis was found to be 7% in goats, 4.14% in buffaloes, 3.8% in cattle, 3.3% in sheep and 1.2% in pigs. However, the incidence of the disease in humans was found to be 15.86%. Thoppil (2000) observed 9.5% seroprevalence in 756 Pigs slaughter in Karnataka, India.

Amin et al. (2001) reported *Brucella* melitensis in bovine and ovine serum using the PCR assay in Saudi Arabia. A total of 120 serum samples were collected and examined by the PCR method. Out of 120 serum samples, 12 (10%) samples showed positive by PCR method, while direct culture detected on 7 (5.8%) in the same serum sample. A cross-sectional study was carried out to determine the seroprevalence and associated risk factors of bovine brucellosis in Guto-Gida district in East Wollega zone from November 2010 to March 2011 (Moti et al. 2012). A total of 406 blood samples were collected from cattle of above 6 months of age and sera were initially screened with Rose Bengal Plate Test (RBPT) and those samples found positive by RBPT were further tested by Complement Fixation Test (CFT) for confirmation. Out of 406 sera 12 (2.96%) were positive using RBPT and the overall seroprevalence of bovine brucellosis documented was 1.97% based on CFT result. The study showed no statistically significant difference ($P > 0.05$) in seroprevalence among the age groups and sexes considered. A serological survey of brucellosis in pigs was conducted in Makurdi, Benue state North Central Nigeria between October and November 2011. Blood-sera were collected from a total of 281 slaughtered pigs and their ages and sex were recorded. The sera were tested for brucellosis using the Rose Bengal Plate Test (RBPT). A total of 86 of the 281 (30.60%) pigs were serologically positive. The prevalence of positive pigs based on sex was 31.20% and 30.13% for male and female pigs, respectively. The age prevalence was 30.10% and 32.00% for young and adult pigs, respectively (Bala 2013)

2.3 *Brucella* as a potential biological weapon

Brucellosis is not only a major zoonotic problem but is also linked with bioterrorism (Anonymous 2001). The severity of this disease, lack of vaccines suitable for use in man and frequent failure of clinical laboratories to correctly identify isolates led to the investigation of *Brucella* as an agent for bioterrorism. Before 1954, when Britain was focusing on anthrax, brucellosis was the first microorganism chosen by the United States to develop as a weapon. Indeed, the American military weaponized *B. suis* in 1954, however, changing global politics resulted in abandonment of these efforts following the biological and toxic weapons convention in 1972. *Brucella* are not difficult to grow and disperse, and transmission to humans may result in prolonged illness and long-term sequelae (Yagupsky and Baron 2005). Aerosol or food contamination could be the sources of dispersion. It has been estimated that 10-100 organisms are sufficient to constitute an infectious aerosol dose for humans. The economic impact of a brucellosis bioterrorist attack would cost $ 477.7 million per 100,000 persons exposed (Kaufmann et al. 1997).

2.4 Brucellosis research in Nepal

Joshi et al. (1974a) conducted serological survey and collected 506 samples from cattle, buffaloes, sheep, goats, Yaks and, pigs, horses, mules, dogs and poultry from the different district of Nepal. Out of 59 cattle serum samples tested, 15 (24.42%) were found to be positive with plate tests and 6 (10.1%) with tube test in the diagnostic titre. Of the 37 buffaloes sera tested 4 (10.8%) were found positive with plate and tube tests. Similarly of the 51 sheep sera tested 5 were found positive with plate test and 18 (35.3%) with tube test in the diagnostic titre. Out of the 137 goats sera subjected to plate and tube tests 10 (7.3%) were positive. For horses and mules the percentage of sera found positive was 25 with plate test and 12.5 with tube test. None of the 26 pig sera was found positive but 21 samples were found positive out of 95-serum samples collected from poultry. Joshi et al. (1974b) collected seventy-seven blood samples from sheep, goats and pigs for investigation for brucellosis. Out of which 48 samples from sheep, 26 from goats and one from pig. All the sera samples were tested by plate and tube agglutination tests. The prevalence of brucellosis in sheep and goats was found to be 23%. Pig was free from the disease. Joshi (1976) conducted a serological

survey of animal brucellosis in Kathmandu. He collected sera samples of 79 goats, 47 horses and mules, 46 cattle, 24 pigs, 20 dogs, 3 yaks and 55 poultry were examined by tube plate and card test. The incidence of positive case was found 11.39% in goats, 30.43% in cattle, 23.4% in horses and mules, 15% in dogs and 14.45% in poultry by plate test. Similarly 8.86% in goats, 10.86% in cattle, 10.63% in horses and mules, 15% in dogs and 16.36% in poultry were shown by tube test. Card test showed positive cases only in goats (1.26%), cattle (8.69%), horses and mules (4.25%) and poultry (10.9%). However, sera of pigs and yaks were not found to be positive.

Pyakural and Mishara (1977) studied on sero-epidemiological evidence of animal beucellosis in Nepal. They collected animal sera from different geographical areas of mountains, plains and valleys from September 1975 to February1977. The sera consisted of 31(yak and nak) from Solukhumbu, 95 (yak, nak, chauri and cattle) from Jumla, 146 (cattle, pig and goat) from Kathamandu, 53 buffaloes from Pokhara, 65 (goat and sheep) from Lumle, 24 (cattle) from Chitwan and 72 (cattle) from Biratnagar. All these sera were tested by tube agglutination tests. The prevalence of disease was found to be highest in buffaloes from Pokhara (22.64%) followed by cattle from Kathmandu (17.47%) and the lowest to those coming from Biratnagar. Upadhyay (1998) studied on sero prevalence of human and animal brucellosis. Upadhyay collected 500 blood samples of out and indoor patients from Mahendra Adarsha Hospital, Chitwan. Out of 500 blood sample she reported 14% 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. Dahal (2003) done a sero epidemiological surveillance of brucellosis among human and animals of Dolkha district. He collected 200 human serum samples from patients with and without fever of private hospitals and clinics. He found 0.5% (1/200) positive in human sera samples by using Brucella card test and milk samples were tested by MRT but not a single positive case was identified. Rana (2005) studied sero surveillance of brucellosis in pigs in Kathmandu valley. Sera samples 190 slaughter pigs from different slaughter sites were examined. Incidence rate was found to be 21.58%. According to (Shrestha et al. 2008) total 376 blood samples were collected randomly from meat animals of Nepal.153 buffalo blood sample from Thankot slaughter house, 70 goats from IAAS and 153 pigs from Itahari. The incidence

rate 17.14% (12/70) was found to be positive in goat and 7.18% (11/153) in case of pig but all buffaloes showed up to be negative reactors. Shrestha (2012) carried out survey on animal brucellosis in raw milk samples in Kathmandu valley. 200 milk samples were collected from private dairy and livestock handlers and tested by MRT. Out of 200 samples examined, 49% (98/200) were found to be positive by MRT. Pandeya (2013) studied sero prevalence of brucellosis in cattle, buffalo and goat of Kailali district, Nepal. He collected 50 serum samples of cattle, 67 of buffaloes and 116 of goats. All of them were tested by plate agglutination test (PAT). Out of 50 cattle 32% (16/50), 13.43% (9/67) of buffalo, 2.59% (3/116) of were found to be positive. Over all 12.01% positive prevalence was found.

3. MATERIALS AND METHODS

3.1 Study area

Rupandehi district of Nepal was taken as site of study. Rupandehi is a district of Terai region in Lumbini zone of Nepal. The district covers an area of 1360 sq. km. and lies between 83°12' to 83°38' longitude and 27°20' to 27°47' latitude. The boarder of the district is touched with the district Nawalparasi in east, Kapilvastu in west, Palpa in north and the Utter Pardesh of India. The altitude range 100 m. from lowland to 1219 m. up to Churia hills from sea level. The temperature range is from 8.75℃ in winter and maximum increase upto 42.4℃ in summer. The average rainfall is 1391 mm. The climate of the district is sub-tropical (DDC Rupandehi). Samples were collected from Majuwa ward of Butwal Municipality, Devdha VDC, Dudhrkshya VDC, Motipur VDC, Parroha VDC and Saljhandi VDC. About the scenario of the VDCs, the Majuwa is located in the island made by the Butwal Tinau river of Butwal Municipality where almost all the householder are without any cultivated land. The land topography of this area is almost made up of large size stone to gravel and pebbles. No any sort of agricultural products were cultivated. Each pig farmer had 1 pig in average number. But other VDCs have similar type of land topography in having cultivated land. Most of the houses have their own land in which they farmed their pigs. A few pig farmers had middle size farm house having an average of 10 pigs. Other pig farmers had small size farm house having an average number of 2 pigs and other livestock.

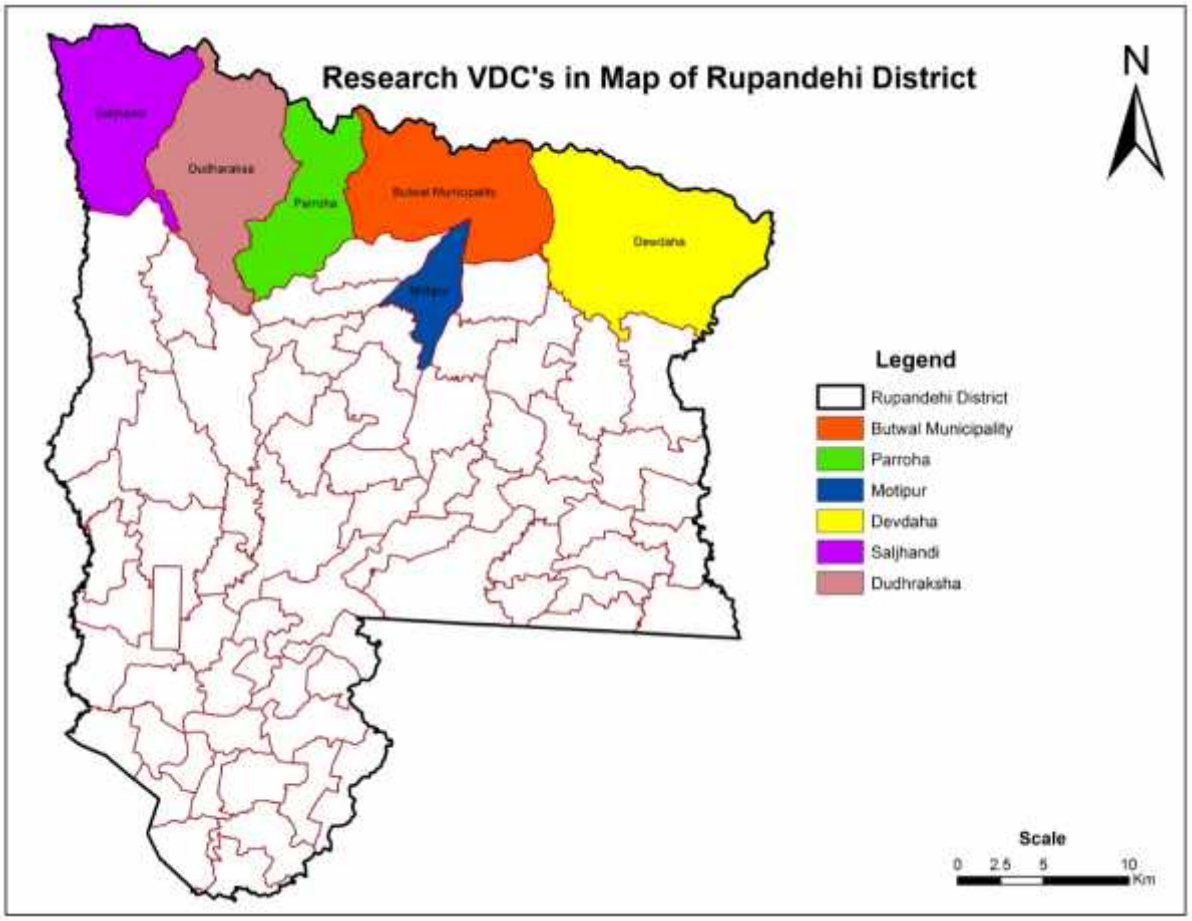

Fig. 1 Study area (VDCs) in the map of Rupandehi district, Nepal.

3.2 Study period

The study was conducted from May 2013 to September 2014.

3.3 Research design

A cross-sectional study was designed for research and sampling was done purposively.

3.4 Sample size determination

The following formula (Daniel 1999) was used for sample size determination.

$N= Z^2 P (1-P)/d^2$

Where N= sample size

Z= Z statistic for level of confidence

P= expected Prevalence or Proportion

d= precision

(In proportion of one; if 5%, d= 0.05)

Z statistic (Z): For the level of confidence of 95%, which is conventional, Z value is 1.96.

In the study the results were presented with 95% confidence intervals (CI).Taking the average of research done in Nepal by different researchers (Shrestha and Joshi 2008, Rana and Joshi 2005), expected prevalence of Brucellosis in pig was found to be 14.38 %. For P value the recent finding prevalence (expected prevalence) rate was divided by 100 which came 0.1438. Putting this P value (0.1438) in the Daniel formula the required sample size can be obtained.

So required sample size of pig is 189.

3.5 Sample collection, transportation and storage

103 blood samples of pig were collected. The blood samples were collected from the ear vein using individual needles and sterile vacuum tubes. These collected tubes of blood samples were maintained at cold chain by putting in ice box. Serum separation was done by centrifuging blood sample at 10,000 rpm for 12-15 minutes with the help of centrifuge machine. The separated serum was kept in deep freeze condition until transportation for test. These serum samples were taken to NZFHRC laboratory by maintaining cold chain and were kept under deep freeze until test.

3.6 Questionnaire survey

The questionnaires were prepared and pre-tested for pig farmers. The questionnaires were based on their introduction, purpose of pig farming, knowledge and practices of pig farming which is presented in Annex I.

3.7 Testing

With the help of pipette a drop (50µl) of Brucella test antigen (Humatex Febrile antigens produced by Human Gesellschaft fur Biochemica und Diagnoatica mbH, Wiesbaden-Germany) was put on clean and sterile slide. To this slide with help of pipette a drop of 50µl of serum was added and with the help of disposable stick the mixed drop was stirred and spread the fluid over the entire area of the particular cell and the slide was placed on an automated rotator at 100 r.p.m. At the end of rotation the results were observed under bright artificial light within 1 minute after rotation. In positive case agglutination (clumping) was formed in slide and negative lacked agglutination.

All serum samples were tested as per procedure described above and results were noted.

3.8 Statistical analysis

The data collected were analyzed by using MS-Excel 2007 and SPSS 19. The statistical parameter like prevalence was calculated by simple arithmetic procedure as below:

The prevalence rate of brucellosis in pig is as follow.

$$\text{Prevalence (P)} = \frac{\text{Total number of positive case observed}}{\text{Total number of animal tested}} \times 100$$

Descriptive statistics was used and data were considered significant at 5% level of significance. Prevalence was calculated by percentage of the positive cases considering all the subjects under study.

4. RESULTS

The study is based on lab finding for brucellosis and questionnaire survey.

4.1 Laboratory test: From the laboratory test the following results were obtained.

4.1.1 General prevalence of brucellosis in pigs

In the present study, from the 50 stakeholders/farmers, 103 pig's blood samples were collected and tested. Out of 103 pigs sera tested using qualitative slide agglutination test (SAT) 13.59% (14/103) were found to be sero-positive.

Table 1 General prevalence of Brucellosis in pigs

Total	Positive	Negative	Positive %
103	14	89	13.59

4.1.2 Breed wise prevalence of brucellosis in pigs

Altogether three pig breeds were studied. The highest prevalence of *Brucella* antibody was found in the exotic breed 15.38.07% (8/52), followed by 12.19% (5/41) in crossbreed and 10% (1/10) in local breed.

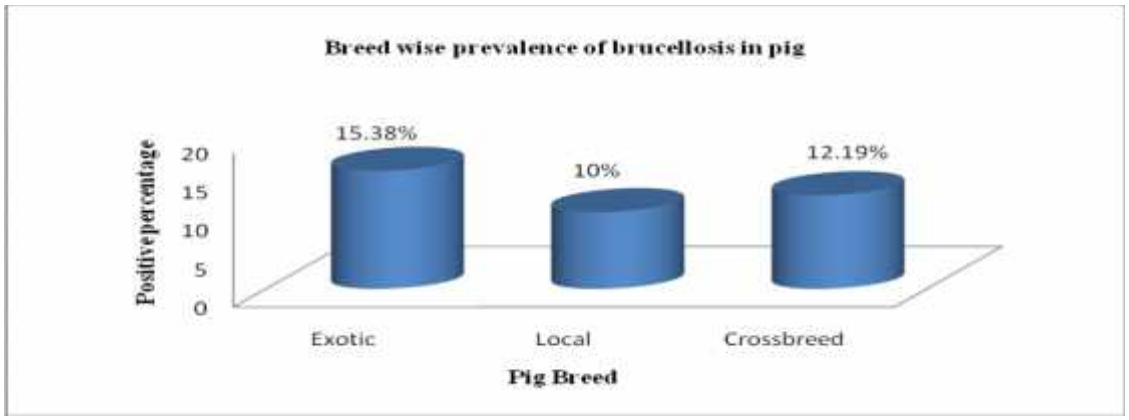

Fig. 2 Breed wise prevalence of brucellosis in pig

No statistically significant difference ($p>0.05$) was found in prevalence of brucellosis in different type of breed ($p= 0.852$, $\chi2= 0.320$, at $d.f= 2$).

4.1.3 Sex wise prevalence of brucellosis in pigs

Of the 103 sera samples of pig tested for *Brucella,* 50 were the sera of male pigs and 12% (6/50) of it was found to be sero-positive. Similarly, in 53 sera samples of female pigs 15.09% (8/53) were found to be sero-positive.

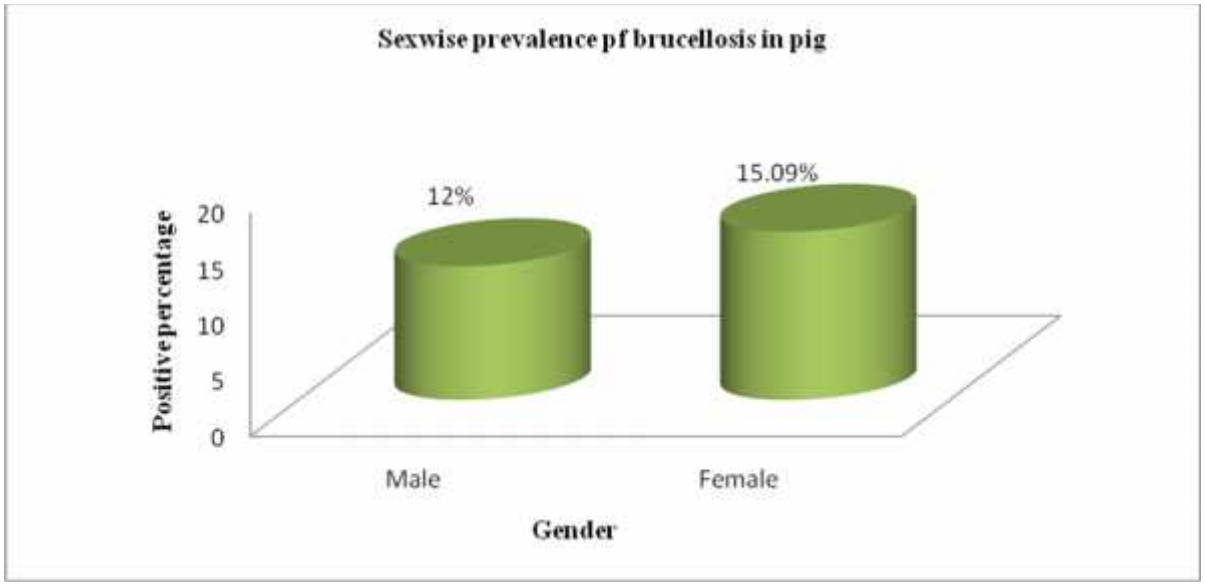

Fig. 3 Sex wise prevalence of brucellosis in pigs

The females showed the higher percentage of seropositivity than the males. But there was no statistically significant difference ($p > 0.05$) in sex wise prevalence of brucellosis in pig ($p = 0.647$, $\chi 2 = 0.210$ at d.f= 1)

4.1.4 Age wise prevalence of brucellosis in pigs

Prevalence of brucellosis was detected in different age groups of pig. Age wise four group were made. The groups were made as continuous interval. Age group above 9 months showed higher prevalence of positive cases of *Brucella* infection, followed by other age groups (6-9 months, 3-6 months and 0-3 months). The age group 0-3 months had 5.26% (2/38), 3-6 months had 11.9% (5/42), 6-9 months had 21.05% (4/19) and above 9 months had 75% (3/4) sero-positivity for brucellosis.

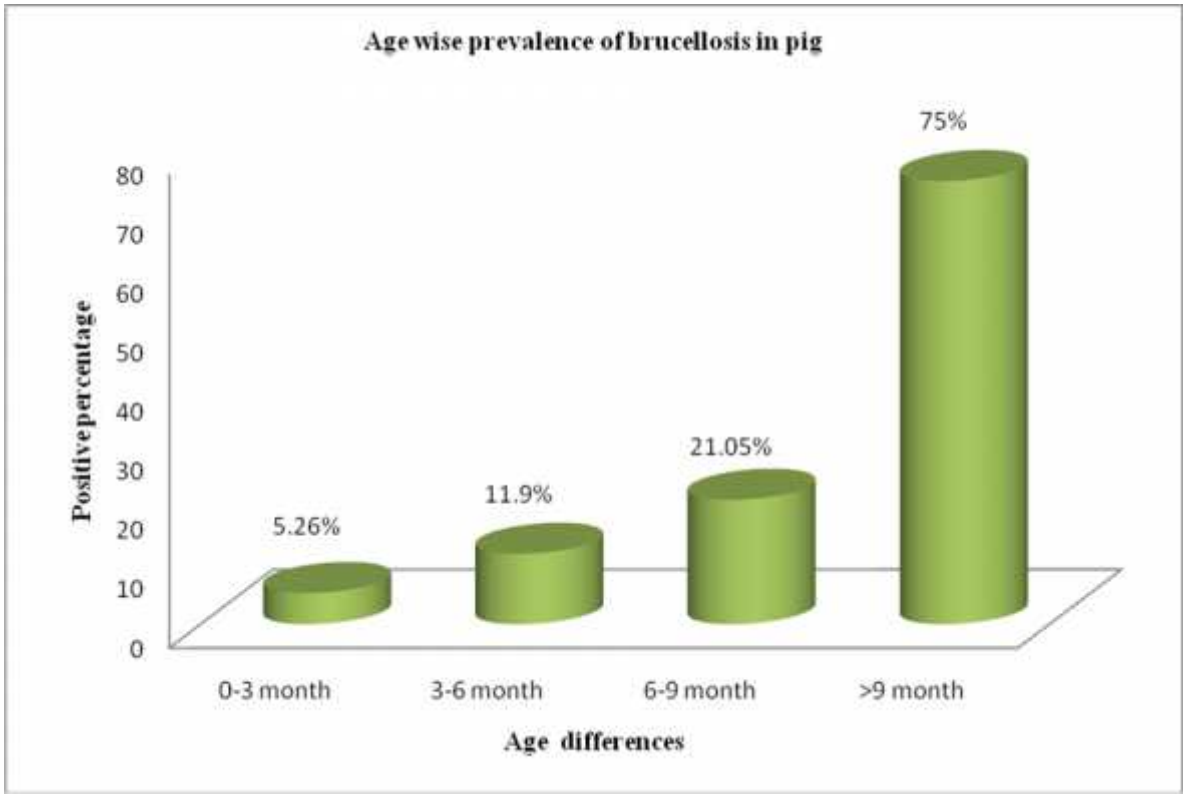

Fig. 4 Age- wise prevalence of brucellosis in pigs

Statistically significant difference ($p < 0.05$) was found in age wise prevalence of brucellosis in pigs, ($p = 0.001$, $\chi 2 = 16.090$ at d.f =3). This shows that the chances of *Brucella* infection can be increased with the increase in the age of the animal.

4.1.5 VDCs wise prevalence of brucellosis in pigs

The study was conducted in 6 VDCs of Rupandehi district. The VDCs were Devdaha, Dudhrakshya, Majuwa, Motipur, Parrohoa and Saljhandi. From each VDC random numbers of samples were collected. Out of 103 sera samples tested, 13.59% (14/103) were found to be positive. The positive case was 17.64% (3/17) from Devdaha VDC, 23.07% (3/13) from Dudhrakshya VDC, 22.22% (2/9) from Majuwa, 4.34% (1/23) from Motipur, 18.75% (3/16) from Parrohoa and 8% (2/25) from Saljhandi.

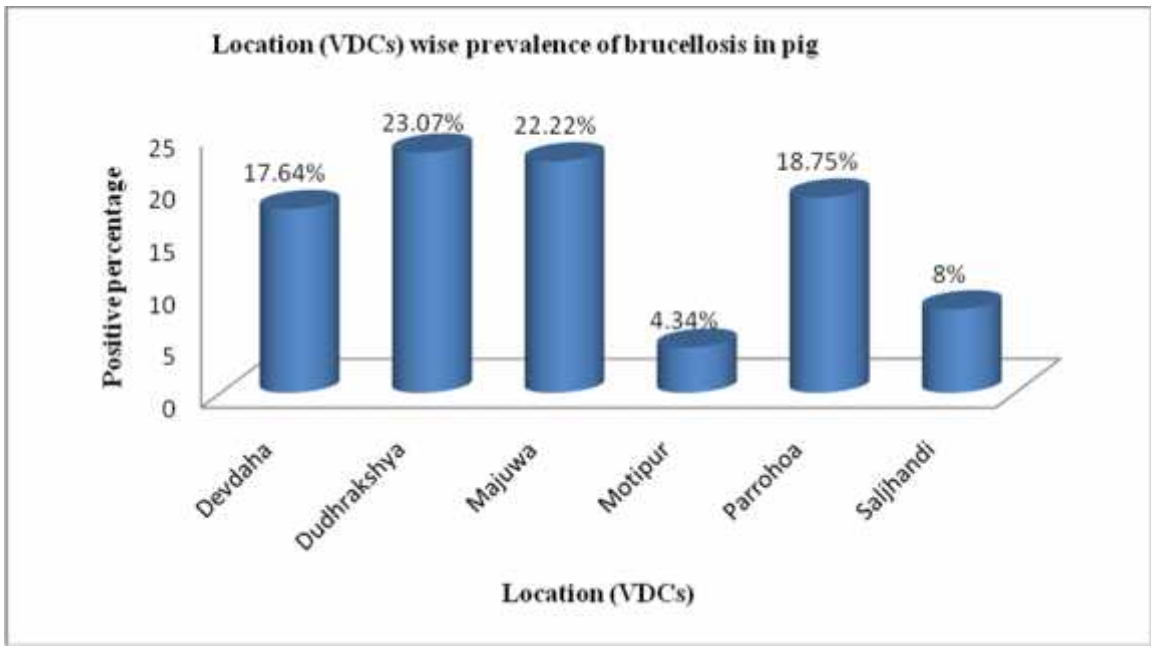

Fig. 5 Location wise prevalence of brucellosis in pigs

It was found that there was no significant difference ($p > 0.05$) in VDCs wise prevalence of brucellosis in pigs in Rupandehi district i.e ($p = 0.479$, $\chi 2 = 4.506$ at d.f= 5). But comparatively the more positive percentage of the disease was found in Dudhrakshya and Majuwa VDC as compared to other VDCs.

4.1.6 Sex wise prevalence of brucellosis in pigs of different breed

The prevalence of brucellosis in different breed of pig in relation to gender showed that the brucellosis prevalence in females was higher than that of males in any of the breed type.

Table 2 Sex wise prevalence of brucellosis in pigs of different breed

Pig Breed	Total	Gender	Negative	Positive	Positive %
Exotic	52	Male	23	3	5.76
		Female	21	5	9.61
Crossbreed	41	Male	18	2	4.87
		Female	18	3	7.31
Local	10	Male	3	1	10
		Female	6	0	0

It was found that there was no statistically significant difference between the types of breed with gender to the prevalence of brucellosis in pig. The significant value ($p=0.442$) of exotic breed in relation with gender was found to be higher than that of expected p value i.e. ($p> 0.05$) at ($\chi2= 0.591$, d.f= 1). Similarly significant value of local and crossbreed are also greater than expected p value i.e. ($p>0.05$) at ($p= 0.675$, $\chi2= 0.176$, d.f= 1) for crossbreed and ($p= 0.197$ $\chi2=1.66$, d.f= 1) for local.

4.1.7 Sex wise prevalence of brucellosis in different age groups of pigs

Table 3 Sex wise prevalence of brucellosis in different age group of pigs

Sex	Age	Total	Positive	Negative	Positive %
Female	0-3 month	17	1	16	5.88
	3-6 month	20	2	18	10
	6-9 month	14	3	11	21.42
	Total	53	8	45	15.09
Male	0-3 month	21	1	20	4.76
	3-6 month	22	3	19	13.63
	6-9 month	5	1	4	20
	>9 month	2	1	1	50
	Total	50	6	44	12

It was found that there was no significant difference in prevalence of brucellosis in gender with the different group of age. The p value of female i.e. ($p=0.004$, $\chi2=13.219$ at d.f= 3,) was smaller than the significant value i.e. ($p <0.05$). This shows that there was significant difference in pig with the prevalence of brucellosis in respect to age of female. Hence result shows that the chances of occurring of infection will be higher in adult age group i.e. above 9 months in case of female. Similarly, the p value of male is 0.347 at $\chi2= 4.136$, d.f = 3. Hence there was no statistically significant difference with the prevalence of brucellosis in respect to age of male.

4.2 Questionnaire survey

The questionnaire survey was based on the knowledge and practices of pig farming system. Of the 50 respondents/stakeholders, when asked about pig farming, according to them the purpose of pig farming were for selling piglet, breeding purpose as well as for meat and for religious purposes. Mostly the age required for selling piglet was within 1 month after birth and for the meat purpose the age of the pig must reach at least 5-6 months. Based on questionnaires the following results were obtained.

4.2.1 Knowledge about brucellosis in stakeholders/farmers

Although the education level of Rupandehi district is high, awareness regarding brucellosis was found to be weak in Rupandehi district. Only 12% of the stakeholders/farmers were known about the disease. This shows that there is scarce of knowledge about the disease brucellosis.

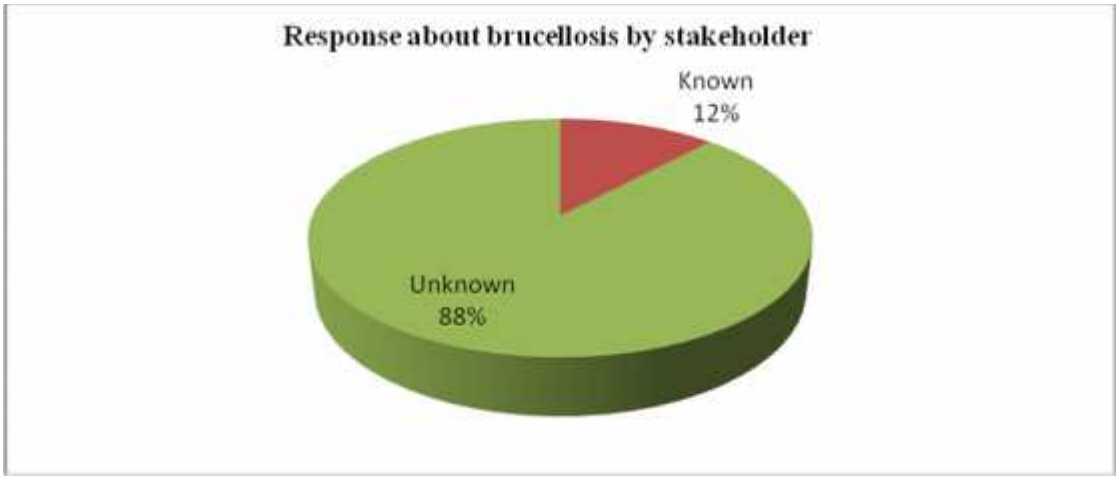

Fig. 6 Response about brucellosis in stakeholder/farmers

4.2.2 Knowledge and practices of pig farmers in pig farming system

Table 4 Knowledge and practices of pig farmers in pig farming system

Questionnaires context	No. of farmers	Knowledge	Practice
Vaccination	50	42%	20%
Clean farm		90%	80%
Use of disinfectants		48%	32%
Training of pig farming		40%	24%

50 farmers were interviewed based on the questionnaire about the knowledge and practices of pig farming. Among them 42% of farmers had knowledge about the vaccination program but only 20% of them practiced on it. The vaccination program on brucellosis hasn't been conducted till now in Nepal, therefore the vaccination program was applied only for the other disease like Japanese encephalitis. Similarly 90% of the farmers had knowledge about the cleanliness of the farm but 80% of them used to clean the farm at regular interval. Though 48% of the farmers had knowledge about the use of disinfectants only 32% of them had used the disinfecting materials. The rest of other farmers who didn't use the disinfectant materials was because of their low economic status which they could not afford to buy the disinfecting agents and in few cases due to carelessness they were not using. Similarly 40% of the pig farmers were trained from government training program but only 24% of them had practiced in their daily life. Those farmers who could not practice the trained way was due to of their low economic status which they could not able to collect/buy the infrastructure material for the systematic farming system. From the study it was found that there were both types of scavenging/open air and close pen/ confined pig farming system. The food materials used for feeding were kitchen leftover, hotel wastes and bone and meats of poultry in small size farm house but in case of middle size farm house the farmers used mostly the byproducts of rice in large quantity. 99 % of the farmers used tap water for drinking purposes for pigs. In case of any disorder in health of pigs moreover of the farmers consulted to the local trained veterinarians.

5. DISCUSSION

The main objective of the study was to determine the prevalence of brucellosis in pigs of Rupandehi district. This is an important study with regard to public health, and it may help to control this zoonotic disease efficiently in the commercial field of animal husbandry. Pig husbandry and pork production in Nepal is at an early stage of development compared to other livestock systems. The Nepalese economy is primarily agriculture based and livestock farming has always been popular among the farmers. Traditionally, pigs have been associated with low social groups. As the pig is only reared by certain groups of people its production does not combine well with the whole farming system, in the way that of other livestock does. Neplalese farmers are doing mixed type of farming i.e. crop cultivation and livestock farming simultaneously. In livestock farming Nepalese farmers keep all types of domestic animals like cow, buffalo and goat. However, pigs farming are being done by certain ethnic groups of Nepal such as the Rai, Limbu, Magar, Kami and Damai. In Nepal, two main types of indigenous pig are kept, these are the Chwanche, which are small in size, black in colour and mostly reared in the hills, and the Hurra which are rust brown or black in colour, are relatively large in size and are reared in the terai region. Over the years, government institutions and non-governmental agencies have imported some exotic breeds like the Hampshire, Landrace, Tamworth, Saddleback and Fauyen, with a view to upgrading native swine. These days the exotic breeds are also reared in large numbers in Nepalease society.

Brucellosis is a bacterial zoonotic disease. Different types of research has been been done regarding brucellosis outside the country. In Nepal, though the countable reports based on brucellosis has been reported in case of ruminants but still very few report on brucellosis in pigs has been reported. Being a zoonotic disease, these days it has become an issue of major concern on the public health as well as animal farming system. This study of brucellosis in case of pig was conducted first time in Rupandehi district of the country. This study shows an alarming ring bell in case of brucellosis and its public health importance.

In this study 6 VDCs of Rupandehi district were selected and from each VDCs random number of samples of pigs were collected. From 50 farmers's pigs total of 103 blood samples were collected and tested. A set of questionnaire were also pre-tested for the knowledge, attitude and practices on the farmers about the pig farming system. In the study 15 cases of abortion were recorded within last 3 year. But these abortions were due to either by brucellosis or by other reasons were not known exactly. Similarly in these VDCs some farmers had knowledge about pig farming but less percent of them were being as practicable. Most of them were unaware of the knowledge, attitude and practice of the pig farming system.

According to (Shrestha et al. 2008) serum samples of 153 pigs from Itahari were tested by using the Brewer Diagnostic Card (BDC), 11(i.e.7.18%) showed positive. Among them female showed high prevalence of 9.23% (6/65) than that of males 5.7% (5/88) which is less than the present finding. This dissimilarity result may be due to using different technique of testing.

Rana (2005) collected the serum samples of 190 slaughtered pigs in Koteshwor and Talchhikhel areas in the Kathmandu valley for the serological study of prevalence of brucellosis in swines from June to December 2005. Out of 190 serum samples of slaughtered pigs tested for brucellosis, 41 were found to be positive i.e. 21.58% of the total serum samples tested was found to be positive. The present study result (13.59%) shows that it is quite convincing to the study of Rana. This could be because of using same procedure of testing.

(Dhakal et al. 2005) found 5.36% (3/56) of prevalence rate of goat in Chitwan district which is comparatively lesser than prevalence rate 13.59% (14/103) obtained from this present study. Similarly prevalence rate of buffalo was found to be 2.86% (1/35) which is lesser than prevalence rate found in this study. This could be due to higher sample size as well as due to increment of disease in recent years.

The result of this study has been found to be even higher than that in the dogs 10% (10/100) in the Kathmandu valley as presented by Gurung (2003).

Bala (2013) conducted serological survey of brucellosis in pigs in Makurdi, Benue state North Central Nigeria between October and November 2011. Blood-sera were collected from a total of 281 slaughtered pigs and their age and sex were recorded. The sera were tested for brucellosis using the Rose Bengal Plate Test (RBPT). A total of 86 of the 281 (30.60%) pigs were serologically positive. The prevalence of positive pigs based on sex was 31.20% and 30.13% for male and female pigs, respectively. But the prevalence rate of this present study is 13.59% (14/103) which is quite less than the study of Bala 2013. The present study shows, that 12% (6/50) for male and 15.09% (8/53) for female were found to be positive. This could be due to difference in geographical area and could be due to smaller sample size of the present study.

The prevalence of brucellosis in pigs obtained from this study i.e. (13.59%) is found to be closer to the prevalence rate that was found by Van Der and Priadi (1988) of which 13.1% (22/175) prevalence of brucellosis in pigs slaughtered in Kapuk Jakarta,West Java and 15.09% (36/226) of pigs slaughtered in Surabaya, East Java. This could be due to similar sample size proportion and similar type of farming system.

A statistically significant difference ($p< 0.05$) was found considering the age wise determinants towards the prevalence of brucellosis in pig in 6 VDCs of Rupandehi district. But there was no significanct difference in context of sex wise and breed type ($p>0.05$). Despite of all other circumstances, the main aim of this study was to determine the prevalence of brucellosis in the study area. The study reveals that pigs are at risk of the disease. The present study showed that the numbers of females are affected slightly higher than those of male percentage. Hence investigations using reliable tools are needed in order to know the exact epidemiological distribution of brucellosis in Rupandehi district and to set plan for control and prevention of the disease accordingly. The public in general and high risk groups in particular should be made aware of the zoonotic diseases and economic importance of brucellosis through veterinary extension education and possible means like media.

This study was based on only one type of detecting method i.e qualitative slide agglutination test. So in case of brucellosis the prevalence cannot be confirmed as golden test by conducting only this test. Accidently the false positive result may be obtained. This is due to presence of high cholesterol in sample, as serum obtained from the infected animals may contain other strains of bacteria like Vibrio Cholerae, Pasteurella, Proteus OX19 and Y. enterolitica, serotype, 9. Sera from individuals without clinical signs of infection may show positive false results with P-OX19 due to low titers of anti-Proteus anti-bodies, particularly in the slide agglutination (screening test). Therefore other relative tests are to be applied for the confirmatory test. For accurate confirmation test like CFT, ELISA, AGID, blood culture isolation, molecular test through (PCR) etc are necessary but these tests prove to be out of reach with limited resources.

6. CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

This study represents that there is prevalence of brucellosis in pigs of Rupandehi district, a terai district of Western Devlopment region of Nepal. From the present study following points can be concluded.

- The prevalence rate of brucellosis in pigs is 13.59 % in 6 VDCs of Rupandehi district.
- The age group of above 9 months pigs were comparatively more affected. 75% of this age group were affected.
- Female pigs showed higher prevalence of sero-positive than male pigs. 15.09% of the female showed sero-positive where as 12% of the male showed sero-positive.
- Exotic breed were highly affected in comparison to crossbreed and local pigs. 15.38% of exotic breed showed sero-positive where as 12.19% and 10% sero-positive in crossbreed and local breed respectively.
- Knowledge about the brucellosis and practices of pig farming system were poor and less in the pig farmers of Rupandehi district.

Being a zoonotic disease it not only harms the animals but economically gives loss to the farmers and can also create a hazardous terrorism in health sector of public in a society as well as in the country. This study shows an alarming situation not only in the Rupandehi district but also contribute in the National public health sector of the country. By implementing strict and appropriate prevention and control strategy as adopted by many farmers of developed countries we could be successful in eradicating this disease from our country.

6.2 Recommendations

This study was done only in one district with limited sample size. Such type of research should be done in other districts also not only on pig but also in other domestic animals of the country. Based on the outcome results, the following recommendations have been made to reduce the risk of brucellosis.

- Regular sero-monitoring or screening must be conducted in animal raising areas.
- There must be the facilities for the diagnosis of brucellosis in veterinary hospitals, milk collection centers and medical hospitals.
- People involved in pig husbandry and pork handlers should be use gloves while handling if there is any suspicion of brucellosis in the animals.
- It is necessary to raise the program about the level of health knowledge and farm management practices among the public and the farmers.
- Strict quarantine measures should be brought into play.
- In order to control the transmission of brucellosis disease, strengthening of the National Animal Health and Development Law is necessary.

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APPENDICES

Appendix- 1: Structured Questionnaire

A. Background Information

a) ID……

b) Name…….

c) Age…….

d) District……..

e) VDC/MP……Ward No.

B. Questionnaire

1. Purpose of pig Farming?
   a. Selling piglet b. Breeding purpose c. Religious/Cultural purpose d. Food for own Family.
2. Total number of pig in the farm
3. What are the housing systems that are common in your farm?
   a. Indoor/Close pen/Confined system b. Scavenging/Open air system c. Combination
4. What are the management strategies for the disease problem?
   a. Consult veterinarian b. Traditional measures c. Nothing
5. When do you vaccinate your pigs?
   a. Regularly b. In outbreaks/epidemics c. Never
6. What are the common sources of the Foodstuffs?
   a. Kitchen leftovers b. Hotel wastes c. Animal Feeds d. Others
7. What are the sources of water?
   a. Tap water b. Well/pond water c. Others
8. How often do you clean your pen by watering?
   a. Everyday b. Twice a week c. Once a week d. over the week
9. Do you any disinfectants in your farm? If yes, what type of you use.
10. How often do you use the disinfectants in your farm?
    a. Regularly b. Sometimes c. Never

11. After how old they become matured and ready for sell for slaughter purpose?

    a) 1/2 yrs. b) 1 yrs. c) 1 and ½ yrs. d) 2 yrs.

12. What are the other pets you have?
13. Do you know any kind of disease that attacks your livestock frequently?
    If yes, what kind of disease is that?
14. Do you ever heard about brucellosis and its causes, symptoms?
15. Has there been any case of abortion among your pigs for the last 3 years?
16. Who does treatment of animals if they are sick?
17. Do you know anybody in your family eat raw meat?
18. Do you know any disease transmitted from raw or uncooked meat?
19. If your animals encountered the disease, which sex and age group did it frequent?
20. Do you wear protection gear while handling aborted materials?
21. Have you seen a pig or a person infected with the disease Brucellosis?

Appendix- 2 Materials used

- Disposable syringe and needles
- Pigs
- Cotton
- Blood collecting tubes
- Centrifuge machine
- Serum collecting vials
- Cold box
- Refrigerator
- Disposable sticks
- Fresh sterile slides
- Pipette
- Serum
- *Brucella abortus* Reagent
- Automated rotater

Appendix- 3 Survey Data

S.N	V.D.C	Sex	Age	Breed	Pevalence
1	Motipur	Female	0-3 month	Exotic	Negative
2	Motipur	Female	0-3 month	Exotic	Negative
3	Motipur	Female	3-6 month	Local	Negative
4	Motipur	Female	3-6 month	Local	Negative
5	Motipur	Male	0-3 month	Exotic	Negative
6	Motipur	Male	0-3 month	Exotic	Negative
7	Motipur	Male	0-3 month	Exotic	Negative
8	Motipur	Female	0-3 month	Exotic	Negative
9	Motipur	Female	0-3 month	Exotic	Negative
10	Motipur	Female	0-3 month	Exotic	Negative
11	Motipur	Female	0-3 month	Exotic	Negative
12	Motipur	Female	0-3 month	Exotic	Negative
13	Motipur	Female	0-3 month	Local	Negative
14	Motipur	Female	3-6 month	Crossbreed	Negative
15	Motipur	Female	0-3 month	Crossbreed	Negative
16	Motipur	Male	0-3 month	Crossbreed	Negative
17	Motipur	Female	6-9 month	Crossbreed	Negative
18	Motipur	Male	>9 month	Crossbreed	Positive
19	Motipur	Female	6-9 month	Crossbreed	Negative
20	Motipur	Female	6-9 month	Crossbreed	Negative
21	Motipur	Female	3-6 month	Crossbreed	Negative
22	Motipur	Female	3-6 month	Crossbreed	Negative
23	Motipur	Female	6-9 month	Crossbreed	Negative
24	Parrohoa	Female	6-9 month	Exotic	Negative
25	Parrohoa	Female	6-9 month	Exotic	Negative
26	Parrohoa	Female	6-9 month	Exotic	Positive
27	Parrohoa	Male	6-9 month	Exotic	Negative
28	Parrohoa	Male	6-9 month	Exotic	Negative
29	Parrohoa	Female	6-9 month	Exotic	Negative
30	Parrohoa	Female	6-9 month	Exotic	Negative
31	Parrohoa	Female	6-9 month	Exotic	Negative
32	Parrohoa	Female	6-9 month	Exotic	Positive
33	Dudhrakshya	Male	6-9 month	Exotic	Negative
34	Dudhrakshya	Male	3-6 month	Exotic	Negative
35	Dudhrakshya	Male	3-6 month	Exotic	Negative
36	Dudhrakshya	Male	3-6 month	Exotic	Negative

S.N	V.D.C	Sex	Age	Breed	Pevalence
37	Dudhrakshya	Male	3-6 month	Exotic	Positive
38	Dudhrakshya	Male	3-6 month	Exotic	Positive
39	Dudhrakshya	Female	3-6 month	Crossbreed	Negative
40	Dudhrakshya	Female	3-6 month	Crossbreed	Negative
41	Dudhrakshya	Male	3-6 month	Exotic	Negative
42	Dudhrakshya	Male	3-6 month	Exotic	Negative
43	Dudhrakshya	Female	3-6 month	Exotic	Positive
44	Dudhrakshya	Female	3-6 month	Exotic	Negative
45	Dudhrakshya	Female	0-3 month	Exotic	Negative
46	Parrohoa	Male	0-3 month	Exotic	Negative
47	Parrohoa	Male	0-3 month	Crossbreed	Negative
48	Parrohoa	Male	0-3 month	Crossbreed	Negative
49	Parrohoa	Male	3-6 month	Crossbreed	Negative
50	Parrohoa	Male	3-6 month	Crossbreed	Negative
51	Parrohoa	Female	6-9 month	Crossbreed	Negative
52	Parrohoa	Female	6-9 month	Crossbreed	Positive
53	Saljhandi	Male	0-3 month	Crossbreed	Negative
54	Saljhandi	Male	0-3 month	Crossbreed	Negative
55	Saljhandi	Female	0-3 month	Crossbreed	Negative
56	Saljhandi	Male	3-6 month	Local	Negative
57	Saljhandi	Female	3-6 month	Exotic	Positive
58	Saljhandi	Female	3-6 month	Exotic	Negative
59	Saljhandi	Male	3-6 month	Exotic	Negative
60	Saljhandi	Female	3-6 month	Exotic	Negative
61	Saljhandi	Female	3-6 month	Crossbreed	Negative
62	Saljhandi	Male	3-6 month	Crossbreed	Negative
63	Saljhandi	Female	3-6 month	Crossbreed	Negative
64	Saljhandi	Male	>9 month	Exotic	Negative
65	Saljhandi	Male	0-3 month	Exotic	Negative
66	Saljhandi	Male	3-6 month	Crossbreed	Negative
67	Saljhandi	Female	>9 month	Crossbreed	Positive
68	Saljhandi	Male	3-6 month	Crossbreed	Negative
69	Saljhandi	Male	3-6 month	Crossbreed	Negative
70	Saljhandi	Male	0-3 month	Crossbreed	Negative
71	Saljhandi	Male	0-3 month	Crossbreed	Negative
72	Saljhandi	Male	3-6 month	Crossbreed	Negative
73	Saljhandi	Female	3-6 month	Crossbreed	Negative

S.N	V.D.C	Sex	Age	Breed	Pevalence
74	Saljhandi	Male	6-9 month	Crossbreed	Negative
75	Saljhandi	Male	3-6 month	Exotic	Negative
76	Saljhandi	Female	3-6 month	Crossbreed	Negative
77	Saljhandi	Female	3-6 month	Crossbreed	Negative
78	Devdaha	Female	0-3 month	Exotic	Negative
79	Devdaha	Female	0-3 month	Exotic	Negative
80	Devdaha	Male	0-3 month	Exotic	Negative
81	Devdaha	Female	0-3 month	Exotic	Negative
82	Devdaha	Female	3-6 month	Exotic	Negative
83	Devdaha	Female	0-3 month	Crossbreed	Negative
84	Devdaha	Male	0-3 month	Exotic	Negative
85	Devdaha	Male	0-3 month	Exotic	Negative
86	Devdaha	Male	3-6 month	Exotic	Negative
87	Devdaha	Male	0-3 month	Crossbreed	Negative
88	Devdaha	Male	3-6 month	Exotic	Negative
89	Devdaha	Male	3-6 month	Crossbreed	Positive
90	Devdaha	Male	3-6 month	Crossbreed	Negative
91	Devdaha	Male	3-6 month	Crossbreed	Negative
92	Devdaha	Female	>9 month	Crossbreed	Positive
93	Devdaha	Female	6-9 month	Local	Negative
94	Devdaha	Male	6-9 month	Local	Positive
95	Majuwa	Female	0-3 month	Exotic	Negative
96	Majuwa	Male	0-3 month	Exotic	Negative
97	Majuwa	Male	0-3 month	Exotic	Positive
98	Majuwa	Female	3-6 month	Local	Negative
99	Majuwa	Female	3-6 month	Local	Negative
100	Majuwa	Male	0-3 month	Local	Negative
101	Majuwa	Male	0-3 month	Local	Negative
102	Majuwa	Male	0-3 month	Exotic	Negative
103	Majuwa	Female	0-3 month	Exotic	Positive

Appendix- 4

HUMATEX FEBRILE ANTIGENS

HUMATEX FEBRILE ANTIGENS are used to detect antibodies against the most common febrile antigens. They are intended for the detection of febrile infections such as Salmonellosis (typhoid fever), Brucellosis and certain Rickettsia diseases.

The antigen solutions consist of stained bacterial suspensions which are used either for screening purposes by rapid slide agglutination, or for confirmation by tube agglutination (Widal test). Usually, confirmatory tests are performed to verify positive results found by the slide method.

HUMATEX FEBRILE ANTIGENS can be used for the qualitative detection of antibodies, and also for monitoring of infection processes by determining changes in the respective antibody titer. HUMATEX FEBRILE ANTIGENS contain different bacterial, vitally stained inactivated with formaldehyde or phenol and standardized suspensions named hereafter antigen solution (AG).

Qualitative Slide Agglutination Test (Screening Test)

1. Pipette/ drop serum onto separate cells of the slide (1 drop = 50 µl):	Cell 1	Cell 2
2. Add (AG) next to all samples and controls	1 drop	1 drop
3. Mix with separate, disposable sticks and spread the fluid over the entire area of the particular cell.		
4. Tilt the slide back and forth for 1 minute so that the mixture rotates slowly inside the cells or place the slide on an automated rotator at 100 r.p.m.		
5. At the end of rotation read results under bright artificial light.		

Interpretation of Results

Qualitative Slide Agglutination Test (Screening Test): Examine macroscopically for the presence/ absence of district agglutination within 1 minute after rotation. Positive sera (visible clumping) may be titrated by the tube agglutination test. Negative sera should result in no visible clumping.

Question: Why do we offer only *B. abortus*, and no *B. melitensis* and no *B. suis* suspensions?

Answer: *Brucella abortus* reacts with antibodies to all three *Brucella* species that are pathogenic for humans. Therefore, it is not necessary to test separately for *B. melitensis or B. suis.*