Molecular Epidemiology, Clinical Aspects and Adverse Obstetric Outcomes of Emerging Zoonotic Diseases in Women of Khyber Pakhtunkhwa, Pakistan

BY

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DEDICATED TO MY PARENTS,

ALL FAMILY MEMBERS AND TEACHERS

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LIST OF ABBREVIATIONS

2-ME	2-Mercaptoethanol
Ab	Antibody
Ab-ELISA	Avidin-biotin enzyme linked immunosorbent assay
Ag	Antigen
ALT	Alanine aminotransferase
APOs	Adverse pregnancy outcomes
AST	Aspartate aminotransferase
BHI	Brain heart infusion
BHU	Basic health units
BOH	Bad obstetric history
Bp	Base pair
BSL-3	Bio-safety level-3
CDC	Centre for disease control
c-ELISA	Competitive enzyme linked immunosorbent assay
CNS	Central nervous system
CO_2	Carbon dioxide
CRL	Crown rump length
CRP	C-reactive protein
CSTE	Council of state and territorial epidemiologists
CT	Computed tomography
DAT	Direct Agglutination Test
DNA	Deoxyribonucleic acid
dNTP	Deoxy nucleoside triphosphates
EDTA	Ethylene diamine tetra acetic acid
ELISA	Enzyme linked immunosorbent assay
ESR	Erythrocyte sedimentation rate
F.L	Femur length
FAO	Food and agriculture organization
FDA	Federal drug authority
G.A	Gestational age
GDP	Gross domestic product
GIS	Geographical inflammation system
H_2S	Hydrogen sulphide
Hb	Hemoglobin
HCV	Hematocrit volume
ICT	Immunochromatographic techniques
I-ELISA	Indirect enzyme linked immunosorbent assay
IFA	Indirect fluorescent antibody
IFAT	Indirect fluorescent antibody test
Ig	Immunoglobulin
IgA	Immunoglobulin alpha heavy chain
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IUFD	Intra uterine fetal death
IUGR	Intra uterine growth retardation
kDa	Kilo Daltons
KPK	Khyber Pakhtunkhwa
LAT	Latex agglutination test
LBW	Low birth weight

LMR	Lymphocyte monocytes ratio
LPS	Lipopolysaccharide
MAT	Modified agglutination test
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
$MgCl_2$	Magnesium chloride
MPV	Mean platelet volume
MRI	Magnetic resonance imaging
MRT	Milk ring test
NaCl	Sodium chloride
NLR	Neutrophils lymphocyte ratio
OD	Optical density
OIE	Office international des epizooties (World organization for Animals)
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PCT	Platelet count
PCV	Packed cell volume
PDW	Platelets distribution width
pH	Power of hydrogen ions
PID	Pelvic inflammatory diseases
PLR	Platelet-lymphocyte ratio
PPM	Part per million
PUO	Pyrexia of unknown origin
RBC	Regional blood banks
RBCs	Red blood cells
RBPT	Rose bengal plate agglutination test
RCI	Red cell indices
RHC	Regional health centers
RIF	Rifampicin
R-LPS	Rough lipo-polysaccharides
rpm	Revolutions per-minutes
SAT	Serum agglutination test
S-LPS	Smooth lipo-polysaccharide
SOT	Solid organ transplantation
Spp	Species
SPSS	Statistical package for social sciences
Sq	Square
STAT	Serum tube agglutination test
TGF-β	Transforming growth factor
TMB	Tetra methyl benzidine
TMP-SMX	Trimethoprim-sulfamethoxazole
V.R.I	Veterinary research institute
W.H.O	World health organization
WAT	Wright agglutination test
WBCs	White blood cells

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All glory to Allah, the most merciful and munificent who gave me strength for successfully completion of this research work. Cordial thanks are also offered to our Holy Prophet Muhammad (Peace be upon him), who taught us spiritual depths and supreme standard in every field of life. On the completion of this research work, I want to express my cordial gratitude to all those people, who have contributed towards making this research possible.

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Abstract

Introduction: Zoonotic diseases are communicable diseases of vertebrate animals, caused by various pathogens, which can transmit to human through various routes and cause diseases. These diseases are naturally transmitted from vertebrate animals to humans. Among zoonotic diseases, Brucellosis and Toxoplasmosis are zoonotic diseases, which are transmitted from animals and cause various adverse obstetric outcomes in women.

Material and Method: The current study was conducted from 2017 to 2020 in Khyber Pakhtunkhwa province of Pakistan. 3586 women of reproductive age (17-45 years) from 7 divisions and 24 districts of KP province of Pakistan were clinically examined and screened for emerging zoonotic diseases (Brucellosis, Toxoplasmosis) using SPAT and RBPT for initial screening of Brucellosis and LAT and ICT assays for Toxoplasmosis, while ELISA and PCR was used as confirmatory assay for Brucellosis and STAT and ELISA for Toxoplasmosis. Hematological parameters like CBC, ESR were determined,

Results: Out of 3586 women, 1599 (44.59%) were found positive for zoonotic diseases. Toxoplasmosis was found in 881 (24.56%) and Brucellosis in 718 (20.02%) women. Brucellosis was found in acute, sub-acute and chronic cases in 428 (59.61%), 239 (33.28%) and 51 (7.11%) women with a significant $P<0.05$ difference.

A significant difference ($p<0.05$) was found in distribution frequency of zoonotic diseases (Brucellosis, Toxoplasmosis) among various district of the province. Prevalence of zoonotic diseases was significantly $P<0.05$ high in Karak (72.47%), Chitral (60.95%), Bannu (60.01%) and Kohat (57.81%) district. Assessment of zoonotic diseases was carried out in various seasons in the studied region. A significant difference $P<0.05$ was found in prevalence of zoonotic diseases in different seasons. Prevalence of zoonotic diseases was significantly $P<0.05$ high in summer 762 (50.76%) season as compared to spring, autumn and winter season

Annual frequency of zoonotic diseases (Brucellosis, Toxoplasmosis) was determined during three year study, but no significant $P>0.05$ difference was found in prevalence of these diseases (Brucellosis, Toxoplasmosis).

Among various socio-demographic characteristics, prevalence of zoonotic diseases (Brucellosis, Toxoplasmosis) was significantly $P<0.05$ high in (47.75%), (48.51%), (50.41%), (47.21%), (46.61%), (47.91%) and (45.71%) women, who were in age group of 27-36 year, resident of rural area, non-educated, house wives, low socio-economic statues, bad hygienic and who no knowledge about zoonotic diseases.

Among various epidemiological factors, a significant $P<0.05$ association was found in prevalence of diseases with various factors like animals in homes, exposure to animals, handling meat, involvement in processing of dairy products and storing animal dung in homes for agriculture purposes, Prevalence of

zoonotic diseases (Brucellosis, Toxoplasmosis) was significantly P<0.05 high in (50.67%), (48.09%), (47.05%), (51.23%) and (50.82%) women, who had animals at homes, exposed to animals, handling meat, involved in processing dairy products and store animals manure in homes, while rate of diseases was low in women, who had no history of these factors.

Various laboratory assays like SPAT, RBPT, IgM ELISA and IgG ELISA were evaluated for diagnosis of Brucellosis. SPAT and RBPT significantly P<0.05 detected Brucellosis, while ELISA and PCR significantly P<0.05 confirmed Brucellosis. SPAT was positive in 21.05% cases with sensitivity of 94.29%, specificity 97.28%, PPV 89.67%, NPV 98.55%, while RBPT was positive in 20.21% cases, with a sensitivity of 92.48%, specificity 97.87%, PPV 91.58% and NPV 98.12%. Similarly ELISA was positive in 70.36% cases with sensitivity of 100%, specificity 98.07%, PPV 99.17% and NPV 100%. IgM ELISA was positive in 23.51% cases with sensitivity of 33.15, specificity 98.72, PPV 98.35% and NPV 39.01%. IgG ELISA was positive in 11.66% cases with a sensitivity of 16.44, specificity 99.36%, PPV 98.34%, NPV 33.99%, while ELISA for IgM and IgG was collectively found positive in 35.18% cases with a sensitivity of 50.42, specificity 100%, PPV 100% and NPV 46.63% respectively.

Quantitative assessment of SPAT assay was carried out. A highest degree of agglutination 75% was shown by 35.63% women, while lowest agglutination P<25% in 56.9% cases.

IgM and IgG antibodies titer was quantitatively estimated by Brucella ELISA assay. A significant difference P<0.05 was found in positivity of IgM and IgG antibodies. IgM was positive in 23.52% women, IgG in 11.66% women, while IgM, IgG were collectively positive in 35.18% women. Brucella specific IgM and IgG were significantly P<0.05 different in acute, sub-acute and chronic cases of Brucellosis

Various laboratory assays like LAT, ICT and ELISA were also evaluated for diagnosis of Toxoplasmosis. LAT and ICT assays significantly P<0.05 detected Toxoplasmosis, while no significant difference P>0.05 was found in positivity between these two assays. According to positive cutoff point, LAT assay was found positive in 25.46% cases with a sensitivity of 97.27%, specificity 97.92%, PPV 93.86% and NPV 99.11%. Similarly ICT assay was collectively found positive in 25.51% women with a sensitivity of 96.25%, specificity 97.52%, PPV 92.68% and NPV 98.76%,

ICT and ELISA assay was compared for positivity of *T. gondii* specific IgM and IgG, which was based on qualitative and quantitative detection of these antibodies. A significant P<0.05 difference was found in positivity of IgM and IgG antibodies by these two assays.

STAT was used for quantification of *T. gondii* antibodies. Among 915 women, 3.72%, 68.52% and 27.76% women showed agglutination at 1:32, 1:128 and 1:2048 dilation, which indicate absence of specific antibodies, evolving immunity and recent immunity.

Clinical features of Brucellosis were determined in infected women. Fever, artheralgia, back pain, fatigue, malaise, anorexia, sweating, myalgia, headache, chills, nausea, mild chest pain, splenomegaly and hepatomegaly were significantly P<0.05 high in women with Brucellosis as compared to non-brucellosis cases A significant difference (<0.05) was also observed in frequency of these clinical symptoms in acute, sub-acute and chronic cases of Brucellosis.

Clinical features of Toxoplasmosis were also determined in infected women. Frequency of fatigue, headache, myalgia and lymphadenopathy were significantly P<0.05 high in 42.45%, 29.17%, 23.61% and 19.63% women with Toxoplasmosis as compared to non-toxoplasmosis.

Various hematological and biochemical parameters were determined in women with zoonotic diseases (Brucellosis, Toxoplasmosis). Among 718 women with Brucellosis, Hb level was significantly low (<0.05) in 45.12% women, which indicated anemia. High TLC count was significantly P<0.05 found in 24.51% cases. Neutropenia P<0.05, lymphopenia P>0.05, monocytopenia P>0.05, eosinopenia P>0.05 and thrombocytopenia P<0.05 was observed in 37.18%, 3.21%, 4.03%, 4.17% and 24.93% infected women. A mild form of pancytopenia was observed P>0.05 in 1.11% cases. Similarly neutrophilia P>0.05, lymphocytosis P<0.05, monocytosis P<0.05, eosinophilia P>0.05, basophilia P>0.05 and thrombocytosis P>0.05 was observed in 7.11%, 26.32%, 24.65%, 3.34%, 5.57% and 1.39% cases of Brucellosis. These hematological abnormalities were observed with a significant P<0.05 difference in acute, sub-acute and chronic clinical stages of Brucellosis.

Hematological parameters were also determined in 881 cases of Toxoplasmosis. Low Hb level P<11.5 g/dL was significantly P<0.05 found in 22.81% women, which indicated anemia, Low TLC count was found P>0.05 in 4.54% infected cases, while high TLC count was significantly P<0.05 found in 39.95% infected women. Neutropenia was found P>0.05) in 7.15% cases, while neutrophilia was significantly (<0.05) observed in 35.41% cases. Lymphopenia was found P>0.05 in 5.33% cases, while lymphocytosis was significantly P<0.05 found in 23.72% infected cases. Monocytopenia was found P>0.05 in 3.06% cases, while monocytosis was significantly P<0.05 observed in 14.98% infected cases. Eosinopenia was found P>0.05 in 2.61% women, while eosinophilia was significantly P<0.05 observed in 23.16% cases. Basophilia was found P>0.05 in 9.42% cases. Similarly thrombocytopenia was significantly P<0.05 found in 10.66% women, while thrombocytosis was observed P>0.05 in 2.49% infected cases.

Clinical inflammatory biomarkers like ESR, CRP and liver profiles like AST and ALT were determined in women with Brucellosis. Elevated ESR and CRP was significantly P<0.05 found in 64.62% and 61.55% cases of Brucellosis. Similarly elevated AST and ALT was found P>0.05 in 30.22% and 33.28% cases of Brucellosis.

ESR, CRP, AST and ALT were also determined in women with Toxoplasmosis**.** Elevated ESR, CRP, AST and ALT was found P>0.05 in 30.31%, 33.25%, 24.85% and 27.92% cases of Toxoplasmosis

Various therapeutic regimen and associated clinical outcomes were observed in Brucellosis patients. Among 718 women, who used various antibacterial regimen for management of Brucellosis, 83.43% were symptomatically improved, while therapeutic failure occurred in 16.57% cases. A significant $P<0.05$ difference was observed in symptomatic improvement in infected women, who used mono-therapeutic, dual therapeutic and triple therapeutic antibacterial regimen. Among 57.66%, 32.45% and 9.89% patients, who used single-drug, dual-drug and triple-drug regimen, frequency of symptomatic improvement significantly $P<0.05$ increased from 78.11% to 88.84% and 97.18% patients, while rate of therapeutic failure was significantly $P<0.05$ decreased from 21.98% to 11.16% and 2.81% cases of Brucellosis.

Various factors such as type of drug regimens, disease stage, Hb level, pregnancy status, ages and gravidity of women were evaluated for therapeutic failure in 718 cases of Brucellosis. A significant $P<0.05$ association was found in rate of symptomatic improvement and therapeutic failure in various type of drug regimens, stages of diseases and Hb level, while no significant $P<0.05$ association was found between therapeutic failure and obstetric statues, age and gravidity of patients.

Various parameters like gravidity, obstetric and gestation statues were evaluated for prevalence of emerging zoonotic diseases. A significant $P<0.05$ difference was found in prevalence of zoonotic diseases among women, who were primary gravida and multigravida, pregnant and non-pregnant and among various gestational stages.

Frequency of zoonotic diseases was determined in women, who were positive for various types of adverse obstetric outcomes and those, who had no history of adverse obstetric outcomes. A high rate of zoonotic diseases was found in 45.35%, 45.59%, 44.67%, 45.69%, 45.74%, 45.31% and 45.14% women, who were not positive for history of previous abortion multiple abortions, recent abortion, clinical induced abortion, preterm birth, stillbirth and congenital anomalies in children, while low rate of zoonotic diseases was found in 38.92%, 23.12%, 43.96%, 31.69%, 22.78%, 21.49% and 22.73% women, who were positive for these adverse obstetric outcomes.

Similarly prevalence of zoonotic diseases was significantly $P<0.0P$ high 54.04% in those women, who had pelvic inflammatory diseases, while rate of diseases was low 43.12% in those women, who had no PID. Among zoonotic diseases, prevalence of Brucellosis was significantly $P<0.05$ high 30.45% in women with PID, while rate of infection was low 18.41% in those women, who had no PID.

CONCLUSION: The current study showed an alarming situation about these diseases among women community and co-existence of social-cultural and animal husbandry activities promote transmission of these infections in women community in the region. The animal owners still adopted old methods of animal husbandry practices, which facilitate spread of zoonotic diseases in the region. Due to poverty and lack of resources, most of the families in rural areas keep livestock animals in sleeping rooms in their home for protection from rains and cool weather during rainy and winter season. The diseases are

spreading due to living in close contact with animals and consuming infected unpasteurized milk, other dairy products and handling meat of infected animals in homes for cocking. These zoonotic diseases are becoming a serious public health problem in women of reproductive age group, as these infection cause serious clinical complications such as anemia, various other hematological and biochemical abnormalities and adverse obstetric outcomes such as early or late abortion, intrauterine fetal death (IUFD), preterm birth, still birth, congenital anomalies like microcephales, macrocephales, mental retardation, blindness, ocular abnormalities, deepness and many other postnatal problems. Due to unauthorized medical practices, non-specific clinical symptoms and medications, lack of proper awareness and diagnostic facilities, these emerging zoonotic infections usually misdiagnosed and lead to chronicity in infected individuals, which cause various clinical, diagnostic and therapeutic challenges for clinician. Various cheap serological test kits are used for diagnosis of Brucellosis and Toxoplasmosis in local diagnostic laboratories in our province, which is not 100% sensitive and specific and cannot differentiate to diagnose false negative and positive cases of these emerging infections. ELISA and PCR are confirmatory assays for diagnosis of these infections, which is still not frequently available in local diagnostic centers in Pakistan. These assays are available in some private expensive diagnostic laboratories in Pakistan, where majority patient cannot access due to unawareness, expensive rates, poverty and living in rural areas, which compels clinician for relay on single serological assay. Advanced diagnostic assays are important for clinical differentiation and management of acute, sub-acute and chronic cases of Brucellosis and Toxoplasmosis infection. The study also revealed a comprehensive clinical knowledge related to therapeutic modalities and failure of various antibacterial regimens frequently recommended for clinical management of Brucellosis infection. Monotherapy was not so effective, while dual and triple drug regimen has shown good clinical response. The data provide a comprehensive clinical knowledge related to in vivo efficacy of various antibiotics, which will help to select effective antimicrobial therapeutic strategies to improve patient's health. Various mono-therapeutic regimens are still effective in many patients, but therapeutic failure also occurred in some cases. Due to intracellular replicating nature, those antibiotics should be given which can maintain maximum antibacterial activity. Moreover antibiotic susceptibility assay should be guided before prescribing a particular course of therapeutic regimen. Assessment of patients for disease stage, anemia, gravidity, pregnancy status, ages and body weight should be top priority of clinician before prescribing a particular course of antibiotic. Persistent clinical symptoms of Brucellosis after using a particular recommended therapeutic regimen for at least 2-3 week should be followed and confirmed through clinical and laboratory assays like SPAT, STAT, RBPT, ELISA or PCR assays for other possible and effective alternative therapeutic regimens. Human infection can be controlled by controlling disease in livestock animals through joint efforts of animal and public health authorities, technical and financial cooperation of various regional and international organizations.

There is no screening program for surveillance, understanding epidemiology, awareness and control of such neglected zoonotic infections in Pakistan, which is a potential threat for pregnant women. Therefore we strongly recommend that WHO and other health authorities may launch awareness and screening program for proper control of these emerging zoonotic infections and associated adverse obstetric outcomes in Pakistan and other endemic countries of the world.

INTRODUCTION

1.1 Zoonotic Diseases: Zoonotic diseases or zoonoses are communicable diseases of vertebrate animals, caused by various pathogens, which can transmit to human through various routes and cause diseases (Barbara *et al.,* 2016). These diseases are naturally transmitted from vertebrate animals to humans (Shanko *et al.,* 2015). Emerging zoonotic diseases are either newly evolved or previously occurred in an area, but shows an increases incidence and expansion in a particular geographical region. Various pathogenic agents such as fungi, parasite, bacteria, viruses and prions can cause zoonotic diseases in human (Wang and Crameri, 2014) and both wild and domestic animals are acting as reservoirs for these pathogens. Zoonosis involves an interaction between a pathogen and two hosts, an animals and human. Both domestic and wild animals play an important role in diseases transmission (Shanko *et al.,* 2015). Emerging zoonotic diseases represent a significant burden and public health threat in low and middle income countries (Chatterjee *et al.,* 2017). The impact of zoonotic diseases on human health and economy is more severe on low income families in developing countries, where most people live in rural areas and largely depend on animals for their food, farm work and transportation, while urban population are also not safe and usually affected. Most of zoonotic diseases are under diagnosed in low income families due to limited resources and weak health service coverage that create many challenges for proper diagnosis, treatment and transmission of these infections (WHO, 2019). Lack of education and control strategies also play a key role in transmission of these infections and a high incidence rate in developing countries (Sanyaolu *et al.,* 2016). Different potential factors such as biological, environmental, ecological, socioeconomic factors, interface of wild and domestic animals and human factors are influencing zoonotic diseases (Shanko *et al.,* 2015). The socioeconomic impact of zoonosis is currently increasing (Omar and Al-Tayib, 2019).

1.1.1 Impact of Zoonotic Diseases on Public Health: Zoonotic infections represent a major issue of public health all over the world (Sekamatte *et al.,* 2018), because about 60% pathogens of human have zoonotic origin. Various pathogens are either zoonotic or originated as important zoonosis before adapting to humans, due to which human is continuously being exposed to various novel animal pathogens (Smits *et al.,* 2015). In the present modern era, epidemiological safety has been threatened with many other new emerging zoonotic infections. Various studies on risk assessment have recently estimated that about 75% of emerging pathogens are zoonotic in origin. The rise of these emerging pathogens might be due to an increase in human population, expansion of urbanization, destruction of natural habitats, use of modernized methods in agricultural practices and changes in climate (Wang *et al.,* 2014). Rapid transmission with a high morbidity and mortality rates are some of the main features of various zoonotic diseases, which lead to great personal and economic losses within a short time period (Sri Raghava *et al.,*

2017). Zoonotic diseases are transmitted either by ingestion of contaminated food and water, exposure to pathogens during processing and preparation of various dairy products or by direct contact with infected animals. Various studies have shown that approximately 75% human emerging infectious disease originates from different animals. Many factors like human, animal demography, agricultural practices, religious, cultural, social, and life-style habits have led to emergence of various zoonotic infections since 1940 (Sanyaolu *et al.,*2016).

1.2 Brucellosis: Among zoonotic diseases, Brucellosis is a leading cause of worldwide zoonosis (Abdullah *et al.,* 2017) and stands first in list of bacterial zoonotic diseases. About 500,000 new cases of human infection are annually reported from disease-endemic regions (Von *et al.,* 2012; Olsen *et al.,* 2014; Byndloss *et al.,* 2016; Johansen, *et al.,* 2017), while approximately 2.4 billion people are at risk (Franc *et al.,* 2018). The disease is globally considered as a common contagious zoonotic infection (Khan and Zahoor, 2018). Brucellosis is caused by several species of genus Brucella (Ducrotoy *et al.,* 2017), which is worldwide in distribution (Welburn *et al.,* 2015).

1.2.1 History of Brucellosis: Brucellosis is an ancient zoonotic disease. The Genome of *B. melitensis* has been discovered from a 700 year-old skeleton during exploration of ruins of a Medieval Italian village using shotgun metagenomics (Kay *et al.,* 2014). Brucellosis was discovered in Malta during Crimean war in 1859. Many British troops had suffered from high fever and the British Royal medical staff had called British medical officer named David Bruce, who investigated what he called as Malta or Mediterranean fever. In 1887, he successfully cultured and isolated the bacterium that was responsible for the disease. Later on, Themistocles Zammit found that those people, who reared goats, lived in animals farms and drank milk from these animals showed similarity in symptoms with patients of Mediterranean fever. Using these findings, Zammit reproduced infection in healthy goats and also succeeded to isolate the bacterium from milk and blood. He deduced that British troops had contracted infection through consumption of milk from infected animals in local area in Malta. In 1906, the British army decided to bane consumption of goats' milk to control infection in British troops, but Malta fever was still not controlled in the region and other suspicions arose regarding consumption of contaminated dairy products such as cheese, ice-cream and fudge (Wyatt, 2013). Due to its global expansion, Brucellosis gained many other names such as 'Malta or Maltese fever (Buttigieg *et al.,* 2018), undulant fever (Adetunji *et al.,* 2019), Gastric fever, Bang's disease (OIE, 2014), Mediterranean fever (Manual on Communicable Diseases, 2017), Gibraltar fever, intermittent fever, contagious abortion, rock fever and Crimean fever (Godfroid *et al.,*2014).

1.2.2 Genus Brucella: Bacteria in the genus Brucella are pathogenic and extremely well adapted to their hosts (Moreno, 2014). The genus contains small, facultative, non-sporeing, non-motile, non-encapsulated,

Gram-negative, intracellular, aerobic bacteria, cocco-bacilli in shape, lack flagella, capsules and is slow growing organisms (Al-Dahouk *et al.,* 2017). Brucella belong to family Brucellaceae, order Rhizobiales, class alpha Proteo-bacteria (Von Bargen *et al.,* 2012; Ruiz-Ranwez *et al.,* 2013; Kämpfer *et al.,* 2014; Olsen and Palmer, 2014). The Genomic analyses revealed higher similarities between members of genus Brucella (Atluri *et al.,* 2011; Godfroid *et al.,* 2014). The genus contains eight species; *Brucella abortus* (cow, buffaloes), *Brucella melitensis* (sheep and goats), *Brucella canis* (canine), *Brucella suis* (swine), *Brucella neotomae* (desert rats), *Brucella ovis* (sheep), *Brucella ceti* (cetaceans) and *Brucella pinnipedialis* (pinnipeds) (CDC, 2017). However, *B. abortus* and *B. melitensis* are most common and virulent species infecting human and cause severe disease (Ducrotoy *et al.,* 2015 and Franc *et al.,* 2018), while *B. melitensis* is most invasive and pathogenic among all species of Brucella (Aydın, *et al.,* 2013).

1.2.3 Epidemiology of Brucellosis: Brucellosis is a neglected zoonotic disease and many factors may contribute to epidemiology of Brucellosis infection (Norman *et al.,* 2016). According to CDC, (2017), the disease is considered as a serious problem due to its low infectious doses (10–100 bacteria), ability to survive and persist for long time in environment, easily transmission to humans through aerosols (OIE., 2014) and difficult to treat by common antibiotics (El-Sayed and Awad, 2018). There is a little variation in risk factors of Brucellosis infection in different countries, but most identified risk factors are similar (Bamaiyi *et al.,* 2016). The possible risk factors of human infection are occupational exposure, feeding behavior and contact with infected animals, their products and discharges (Addis, 2015). Brucellosis is endemic in various regions of the world, including Africa, Middle East, South America, Eastern Europe central Asian, China, India and many regions of Mediterranean basin (Liu, *et al.,* 2015). In many developed countries of the world, where Brucellosis was eradicated, there seem to be a re-emergence of infection as zoonotic disease appears to claim more territory (Bamaiyi *et al.,* 2016). Human infection is strongly associated with management of domesticated livestock animals (Moreno, 2014) and consuming their contaminated food products (Felipe *et al.,* 2017). Brucella is sensitive to direct sun-light exposure and heat for a long period, but resistant to drying, freezing and can also easily survive for two months in cheese made from milk of infected goat and sheep (Franc *et al.,* 2018). Infection is entirely dependent on infected animal reservoirs (Plumb *et al.,* 2013).Various animals are main reservoir for human infection. Goats, sheep and camels are reservoirs for *B. melitensis*, cows for *B. abortus*, dogs for *B. canis*, swine for *B. suis* and sheep for *B. ovis* that is considered non-pathogenic for human (Franc *et al.,* 2018). Human is infected by direct or indirect contact with an infected animals (Abdullah *et al.,* 2017), inhalation of dust contaminated with feces of infected animals and consumption of animals products (Tuon *et al.,* 2017a). Animal blood, meat, aborted fetus, placenta or uterine secretions and consumption of raw animal products such as unpasteurized milk are other major sources of human infection (Saadat *et al.,*2017; Unuvar *et*

*al.,*2019). Eating of undercooked meat is a less common source of disease transmission. Human infections may also occur during working in laboratory through inhaling bacteria or needle prick (CDC, 2012). Human infection may also occur through skin cuts or abrasion during unprotected contact with blood, tissues, vaginal discharges, aborted fetuses, placenta and urine from infected animals (Roushan and Ebrahimpour, 2015). Fresh and raw vegetables grown in soil containing manure of farms animals is also rich sources of human infection. Airborne infection may occur during working in heavily infected animal sheds through inhalation. The bacteria may easily inhale through aerosol from cowsheds, slaughter houses and diagnostic laboratories (Manual on Communicable Diseases, 2017). It was previously thought that humans infected with Brucellosis are dead end of infection, but human to human transmission have recently been documented (Tuon *et al.,* 2017) through sexual intercourse (Al-Anazi and Al-Jasser, 2016), breast milk (Peker *et al.,* 2011), blood transfusion, bone marrow and organs transplantation, trans-placental or perinatal exposure (Tuon *et al.,* 2017a).Travel to endemic regions is also a risk factor for transmission of Brucellosis infection (Liu *et al.,* 2015).

1.2.4 Clinical features of Brucellosis: Brucella enters into host through respiratory and gastrointestinal mucosal epithelium (De-Figueiredo *et al.,* 2015; López-Santiago *et al.,* 2019). Once bacteria enter in human body through mucosal surfaces of the body, they are phagocytized by polymorphonuclear WBCs and carried to localized lymph nodes by lymphatic vessels and spread in various body organs through blood circulation (Aydın *et al.,* 2013). Brucella preferentially invades and replicates in macrophages, polymorphonuclear, dendritic cells, lymphoid and other preferred host tissues (Baldi and Giambartolomei, 2013; Scian *et al.,* 2013). Being intracellular in nature, Brucella has ability to survive and replicate inside host cells and tissues by expressing various virulence factors and using different strategies to avoid immune response of hosts, which enables them to survive during strong immune responses of hosts and replicate within an intracellular niches, which leads progression of disease from acute to chronic phase of Brucellosis infection (Gopalakrishnan *et al.,* 2016; Omolbanin Amjadi *et al.,* 2019). The lipopolysaccharide (LPS) of cell is a major virulence factor of Brucella. The LPS exhibits a low toxicity and its uncharacteristic structure was postulated to delay immune response of host, which favor establishment of chronic infection in infected individual (Yun *et al.,* 2017). The Incubation period of brucellosis is highly variable, usually (5 days–6 months), while the average onset is 2-4 weeks (CDC, 2017). These organisms induce a broad range of clinical manifestations that generate clinical challenges for prompt diagnosis (Traxler *et al.,* 2013). Brucella manages to avoid immune response of host and establish chronic disease (Ahmed *et al.,*2016), due to which human infection become serious, often long lasting and results in chronic disease (Seyyed-Gholizadeh, 2013), leading to diverse clinical, reproductive and pathological sequelae (Ayoola *et al.,* 2017) and every body organ can be involved (Moslemi *et al.,*

2018) which mimic many other infectious and non-infectious diseases (Ahmed *et al.,*2016). Human infection usually presents an acute febrile illness with a flu-like symptoms and uncharacteristic undulant fever (López-Santiago *et al.,* 2019) with profuse sweating, chills, headache, artheralgia, myalgia, fatigue, anorexia, weakness, weight loss and depression. Undulating fever is a commonly observed clinical sign (Addis, 2015). Lymphadenopathy (10–20%) and splenomegaly (20–30%) are most common clinical signs, which usually occur in sub-clinical infection. Meningitis, arthritis/spondylitis or focal organ involvement such as orchitis, epididymitis, endocarditis, hepatomegaly and splenomegaly may also occur in infected individuals (CDC, 2017). Brucellosis is a systemic infection that involves any body organ or tissue and may persist for weeks or months in absence of appropriate antibacterial treatment. The chronicity and relapses usually cause high morbidities (Whatmore, 2009; Atluri *et al.,* 2011) osteoarticular involvement and osteoarthritis (Dean *et al.,* 2012; Unuvar *et al.,* 2019; López-Santiago *et al.,* 2019).

1.2.5 Adverse pregnancy outcomes: During pregnancy, brucellosis may cause mischarges, congenital or neonatal brucellosis, IUFD, premature deliveries or normal full-term deliveries (Al-Tawfiq and Memish, 2013). Spontaneous abortion usually occurred during first and second trimesters of pregnancy. Intrauterine fetal transmission may also occur (Young, 1983). Spontaneous abortion and IUFD may occur in 31-46% infected cases. Fetal loss usually occurred in first and second trimester (Al-Tawfiq and Memish, 2013). Spontaneous abortions are usually associated with isolation of Brucella from aborted fetuses and placenta (Seoud *et al.,* 1991; Young 1983). Brucellosis causes less spontaneous abortions in humans as compared to animals, because animal placenta contain erythritol, a chemical constituent of ungulate fetal and placental tissue that facilitate growth of Brucella, while human placenta and fetus lack erythritol (Poole *et al.,*1972). Erythritol is considered as a growth factor and excellent medium for Brucella in animal placenta. The amniotic fluids of human contain anti-Brucella activity, which reduce abortion rate in human (Seoud *et al.,* 1991).

1.2.6 Diagnosis of Brucellosis: Brucellosis is usually diagnosed through a clinical history along with positive serological tests or culture of blood or synovial fluids (Unuvar *et al.,* 2019). Serum agglutination test (SAT), 2-ME test, complement fixation test (CFT) and enzyme linked immune-sorbent assay (ELISA) are some serological assays, which detect Brucella specific antibodies in sera of infected individuals (Lounes *et al.,* 2014; Al-Anazi and Al-Jasser 2016). SAT assay measures amount of immunoglobulin (IgM) and (IgG) antibodies. During infection, IgM first appears in blood, followed by IgG antibody, which are detected in agglutination and other serological tests. Standard agglutination test detect IgM, IgG, while 2-ME test detect only IgG antibody, which is used to follow improvement after initiation of therapy. A titer ≥1:160 for SAT and ≥1:80 for 2-ME test show positivity of brucellosis

infection (Bao *et al.,* 2017; Unuvar *et al.,* 2019). These serological tests are quick, safe, more sensitive and can easily applied for routine diagnosis. The common serological method is serum agglutination test (SAT), which is sensitive from 84.6% to 91.7%. However, other agglutination tests such as slide, plate or card agglutination assays are also used. Rose Bengal test (RBT) is a card agglutination test, which is recommended by WHO with a sensitivity of over 99%. Complement fixation test (CFT) is other serological assay, but it is not suggested for routine use in small diagnostic laboratories due to its technical complexity (Al-Dahouk *et al.,* 2013). Bacteriology is considered as a gold standard for isolation and identification of pathogen from clinical samples (Mirnejad *et al.,* 2013), but it is time consuming, require BSL-3 for biosafety (Ali *et al.,* 2014) and required skilled technician (Yu and Nielsen, 2010). Although, automated cultural techniques have significantly improved yield rates and biosafety issues, but it is still not used in developing countries (Al-Anazi and Al-Jasser, 2016). Molecular tools such as PCR accurately detect Brucella DNA in patient samples, Recently, real-time PCR based assay have also introduced to identify and quantify Brucella DNA in sera of infected individuals (Lounes *et al.,* 2014), but these are still not widely used for diagnosis in developing countries like Pakistan (Ali *et al.,* 2013). Immuno-histochemistry is an alternative technique, which is used for direct detection and diagnosis of Brucella in different histological samples (Geresu and Kassa, 2016). Other indirect diagnostic assays such C-reactive protein, erythrocyte sedimentation rate (ESR) and leukocyte count are additional diagnostic methods, which are beneficial to follow up therapeutic response in infected individuals (Balın *et al.,* 2018). Radiological assessments, such as x-rays, computed tomography (CT) and magnetic resonance imaging (MRI) are also helpful to diagnose complication associated with brucellosis and can determine affected sites (Bao *et al.,* 2017 and Unuvar *et al.,* 2019).

1.2.7 Therapeutic strategy for Brucellosis: Due to intracellular nature of Brucella, only limited and selected antibiotics groups are effective against these pathogens. Most common antibiotics, which are used for management of human brucellosis, include ciprofloxacin, gentamicin, doxycycline, streptomycin, tetracycline, amino-glycosides, trimethoprim-sulphamethoxazole, quinolones, chloramphenicol and rifampicin. Due to high incidence of relapse rate in single-therapeutic, these antibiotics are commonly used in various therapeutic combination (Al-Tawfiq, 2008).Therapeutic failure occurs in 5-15% cases and usually occurred in chronic Brucellosis infection, which is characterized by severe complications of cardiac, nervous and musculo-skeletal system (Franco *et al.,* 2007). Recommended regimens used for treatment of Brucellosis associated complication involve combination of two or three antibiotics and physicians do not prescribe any specific and standard treatment drugs regimens for a particular condition of disease. They only recommend combination of two or more drugs and prolong course of antibiotic therapy to prevent relapses (Unuvar *et al.,*2019).The relapses rate

decrease with therapeutic combination of two different antibiotics (Ulu-Kilic *et al.,* 2014). Brucella isolated from a relapsed patient maintains the same antibiotic-susceptibility. Therefore, most of relapsed patients respond to a repeated course of antimicrobial therapy (Zinsstag *et al.,* 2011; Corbel, 2006). Tetracycline should not be recommended to pregnant women (Zheng *et al.,* 2018). Therapy with TMP-SMX and rifampicin are still effective and appear to be safe during pregnancy (Al-Tawfiq and Memish, 2013).

1.2.8 Prevention of Brucellosis: Due to limited resources and poor animal management system, intra and inter-specific infection rates has significantly increased during the recent years in various developing countries including Pakistan (Khan and Zahoor, 2018).WHO focused on control of neglected zoonotic infections as a target for economic development (WHO, 2014). Education is important strategy for prevention along with a comprehensive knowledge on antibacterial characteristics, clinical features of disease, proper diagnosis, treatment, prophylaxis and protective measures of diseases (Bao *et al.,* 2017). The disease could be minimized by educating high risk individuals of a particular community in endemic regions (Rahman *et al.,* 2012a). Vaccination is an effective strategy for protection, but there is still no effective and safe licensed vaccine for prevention of human brucellosis (Bao *et al.,* 2017). Live Brucella vaccines are available for diseases control in animals, but it is not safe for human and can certainly cause infection. Killed or subunit Brucella vaccines are also not effective as these vaccines do not provoke long term protection (Harms *et al.,* 2009). In case of accidental infected animal or laboratory exposures to pathogen or animal Brucella vaccines, rifampicin 600 mg, plus doxycycline 200 mg should be used on daily basis for prophylaxis (Bao *et al.,* 2017). After long efforts, many developed countries have eradicated brucellosis, but it still remains a major neglected zoonotic disease in developing nations that impact on human health and livestock industries (McDermott *et al.,* 2013).The recent advancement in genetic, genomic and immunology has explored pathogenesis of Brucella, which will be helpful in development of safe vaccine, therapeutic agent for effective treatment and prophylaxis of disease (Al-Anazi and Al-Jasser, 2016). The efficient way to reduce and control infection of brucellosis in human is to eliminate infection in domestic animals, but wild animals are also suggested a possible source for transmission of infection to domestic animals, human and permanent and inaccessible reservoirs (Anka *et al.,* 2013). Therefore, one health approach is a successful method for controlling zoonotic Brucellosis, which involves human health, animal health and environmental health (Bamaiyi *et al.,* 2016), but to achieve this goal, a huge amount of financial resources would be required (Moreno, 2014).

1.2.9 Toxoplasmosis: Toxoplasmosis is another zoonotic disease caused by *Toxoplasma gondii*, a cyst forming obligate, coccidian parasite (Aguirre *et al.,* 2019), unicellular (Zhang *et al.,* 2016), intracellular in nature and infect both animals, humans (Maçin *et al.,* 2018) and is globally a common parasitic zoonosis

(Subauste *et al.,* 2011). The parasite is one of a common zoonotic pathogen and widespread in distribution (Shwaba *et al.,* 2018) and cause Toxoplasmosis in humans (Nardoni *et al.,* 2019). About one third of human population in the world is infected by *T. gondii* (Torgerson *et al.,* 2015) and is globally an important zoonotic issue with medical and veterinary importance (Liu *et al.,* 2015). The parasite belong to kingdom Protista, phylum Apicomplexa, class Sporozoasida, order Eucoccidiorida, family Sarcocystidae, genus Toxoplasma and species gondii (Maçin *et al.,* 2018).

1.2.10 Hosts of *T. gondii*: The parasite infect all warm-blooded animals (Flegr *et al.,* 2014), but domestic cats are main hosts (CDC, 2015).

1.2.11 Definitive Host of *T. gondii*: All felids cats eat infected intermediate hosts containing cysts in tissues. The parasite sexually reproduces in digestive tract of cats and shed oocysts in feces (Tidy *et al.,* 2017 and Aguirre *et al.,* 2019).

1.2.12 Intermediate Host of *T. gondii*: All mammals, including birds and humans are susceptible to infection through consuming of oocysts, felids, and other infected intermediate hosts (Aguirre *et al.,* 2019).

1.2.13 Incidental Host of *T. gondii*: Marine mammals like sea otters and monk seals exposed via consuming other infected marine organisms or direct ingestion of oocysts from sea water. Human may infected by consuming infected marine mammals (Aguirre *et al.,* 2019).

1.2.14 Life cycle of *T. gondii*: Sexual cycle of parasite occurs in enteroepithelial cells of feline host, which results in production of oocysts. After primary infection in cat, these oocysts may shed in cat feces for many days. Sporulation of oocysts occurs within 1-5 days in environment and become infective, which is resistant to environment and remains infective for many years. These sporulated oocysts contain four sporozoites in each of two sporocysts (Dubey and Beattie, 1988). The sporozoites are released from sporulated oocysts, whenever any susceptible animal ingests these sporulated oocysts and penetrate in intestinal lining of host, where convert to tachyzoites form and establish infection in host (OIE, 2017).

1.2.15 Epidemiology of Toxoplasmosis: *T. gondii* has capability to infect human and several other genera of warm-blooded animals (Flegr *et al.,* 2014; OIE, 2017) that is highly prevalent and globally widespread in distribution (Wyrosdick and Schaefer, 2015; Lüder and Rahman, 2017; Saberi *et al.,*2018). Globally, about 20-90% of adult populations of human in many regions have been reported to expose with *T. gondii* parasite (Zemene *et al.,* 2012). Various emerging infections in human are transmitted from animals and human behavior can influence exposure to zoonotic pathogens (Shwaba *et al.,* 2018). Human is infected by infective stages of *T. gondii*. sporulated oocysts present in environment contaminated by felines stools or consumption of viable bradyzoites in tissue cysts and finally through tachyzoites form, responsible for congenital infection (Rougier *et al.,* 2017). Routes of infection may vary according to

habits of people in a particular endemic region (Samudio *et al.,* 2015). Transmission may be horizontal or vertical (Dubey, 2010; Lopes *et al.,* 2013). Horizontal transmission occurred through ingestion of unwashed vegetables, fruits, raw milk and cheese (Dubey *et al.,* 2014) or consuming undercooked meat containing parasite cysts (Samudio, *et al.,* 2015; Wang *et al.,*2015; Wang *et al.,*2016; Yang *et al.,*2017b). Meat in raw and undercooked form containing viable tissue cysts is an important source for human infection (Tonouhewa *et al.,* 2017 and Belluco *et al.,* 2018). Human and animals also become infected through drinking water contaminated with oocysts (Smit *et al.,* 2017; Sroka *et al.,* 2018). The cysts may also swallow through hand to-mouth contact after handling meat. The handling of knives, utensils or cutting boards used in contaminated raw animal meat also transmit infection. Ingestion of oocysts dropped with cat feces, through hand-to-mouth contact during gardening, cleaning a cat's litter box, contact with children's sandpits or touching any thing that came into direct contact with infected cat feces are other sources of disease transmission (AL-Agroudi *et al.,* 2017). Exposure to soil, suspended dusts in homes and contaminated animal shelters are also source of parasite transmission (Baquero-Artigao, 2013) and Liu *et al.,* 2017). Infection can also occur through blood transfusion and organ transplant (Robert-Gangneux and Dardé, 2012; AL-Agroudi *et al.,* 2017; Alvarado-Esquivel *et al.,* 2018). Trans-placental or vertical transmission of *T. gondii* may also occur, when a pregnant woman is primary infected, which leads to congenital infection (Singh, 2016; AL-Agroudi *et al.,* 2017; Piao *et al.,* 2018).

1.2.16 Clinical Features of Toxoplasmosis: Globally, parasite infects more than 30% human population, but, most infected persons remain asymptomatic and disease remains unrecognized (Villard *et al.,* 2016). The infection could also cause variable clinical signs and symptoms in some infected individual, which depend on immunological status of infected individual (Maçin *et al.,* 2018). Pathogenicity of parasite may also vary by strains and host susceptibility, which is based on host genetic traits (Ngo *et al.,* 2017). Genotypes in French Guiana cause a significant damages and even death in some infected adults, who were not known to be immune-compromised (Carme *et al.,* 2009). Some infected individual show a mild, flulike symptoms (Paquet and Yudin, 2013). Primary infection with *T. gondii* is usually sub-clinical, but ocular disease and cervical lymphadenopathy may present in some infected individuals. Active infection of parasite is characterized by tachyzoites, while persistent tissue cysts characterize latent infection. Infection in fetus and immune-compromised persons can cause devastating disease (Dunay *et al.,* 2018). Most common symptomatic features, which are classically associate with Toxoplasmosis include lymphadenopathy (Pinto *et al.,* 2017), fever, asthenia. The patient will have initially a slight fever for few days or weeks that will spontaneously disappear (Garweg, 2016). Fever of an unknown origin may occur in some infected individual during early stages of Toxoplasmosis (Abhilash *et al.,* 2013) along with malaise and lymphadenopathy (Pradhan, 2015). The parasite can also cause decrees in vision or even

blindness (Samudio, *et al.,* 2015). The disease is also benign in most healthy immune-competent individual and spontaneously recovered (Garweg, 2016), while in immune-compromised individual, parasite can lead to life-threatening clinical manifestations in undiagnosed and untreated cases (Montoya and Gomez, 2016). Immune-compromised individuals are at high risk from severe disease in primary and reactivated infection (Khan and Khan, 2018). Chronic infection that was previously considered benign is a source of increased concern nowadays, because it has been associated with cerebral decline in older patients (Gajewski *et al.,* 2014). Reactivated infection may also occur in immunocompromised patients and may damage CNS that lead to life threatening condition (Machala *et al.,* 2015). Recently, Toxoplasmosis has also been linked to schizophrenia and many other neuropsychiatric disorders (Sutterland *et al.,* 2015; Alvarado-Esquivel *et al.,* 2011), like depression, suicide attempts (Flegr *et al.,* 2014) and anxiety. The basic mechanisms behind these disorders are still unknown, but may relate to host immune response to persistent cyst in tissue and bradyzoite within the brain of infected individuals (Xiao *et al.,* 2016). Acquired infection in some immune-competent persons may also cause ocular abnormality with uveitis and retinochoroiditis in different percentage that varies with circulating strains. Highest rate of ocular lesions is observed in various parts of South America (Garweg, 2016).

1.2.17 Adverse Pregnancy Outcomes: Toxoplasmosis has public and socioeconomic impact due to its pregnancy associated complication and cost of caring for patients (Zhou *et al.,* 2017). Primary infection, when occur in pregnant woman or within 3 months before conception can cause congenital infection, which has devastating consequences for unborn fetus that include stillbirth, neurological disorders and severe ocular disease (Maldonado and Read, 2017). Primary infection in pregnant women is either asymptomatic or has some mild, neglected symptoms. About 70-90% of infants born with congenital infection also remain asymptomatic at birth. However, infection may also show various adverse obstetric problems such as spontaneous abortions, premature births, stillbirths or serious fetal damage (Villard *et al.,* 2016). Many prenatally infected children remain asymptomatic at birth, but usually develop retinochorioiditis and many other serious outcomes in later life (Moncada and Montoya, 2012). In immune-competent pregnant women, primary infection cause intrauterine infection, which is also transmitted to fetus (Fallahi *et al.,* 2018). Globally, congenital Toxoplasmosis is diagnosed with appearance of symptoms at birth or ultrasonographic findings before birth (Maldonado and Read 2017). Infection is subclinical in immuno-competent and non-pregnant women (Wang *et al.,* 2017), but induce abortion or other serious abnormalities during fetal development (Villard *et al.,* 2016; Maldonado and Read, 2017), whenever parasite is vertically transmitted from infected mothers (Shah *et al.,* 2017). Therefore, congenital transmission is considered as a serious complication of Toxoplasma infection in pregnant women (Antczak *et al.,* 2016). The diseases lead to early or late miscarriage (Drapała *et al.,*

2014), intrauterine fetal death, stillbirth, neurologic, genetic defects, chorio-retinitis and various disability (WHO, 2015) or fetal abnormalities before birth (Dhombres *et al.,* 2017 and Piao *et al.,* 2018). Risk of congenital associated clinical manifestations and its severity are influenced by various strains of *T gondii*, gestational age during maternal infection and pattern of antenatal treatment (Pomares and Montoya, 2016). Infection in first trimester of pregnancy causes abortion, while late infection cause premature birth and many other adverse complications like hepatomegaly, splenomegaly, inflammation in retina or other parts of eye, seizures, postnatal jaundice, mental retardation and even fetal death (Moncada and Montoya, 2012). The parasite also causes high rates of severe hydrocephalus and intracranial calcifications before birth (Olariu *et al.,* 2011). However, Toxoplasmosis associated morbidity and mortality is treatable and preventable (Peyron *et al.,* 2015). Early maternal screening is recommended to detect and prevent fetal abnormalities such as hydrocephaly, anatomic damages of CNS, restricted fetal growth (Pradhan, 2015) for proper diagnosis and treatment (Fallahi *et al.,* 2018).

1.2.18 Diagnosis of Toxoplasmosis: Diagnosis of Toxoplasma infection during pregnancy has great significance due to risk of parasite transmission to unborn fetus and newborn babies (Ahmad and Qayyum, 2014). Early diagnosis and treatment during gestational period reduce vertical transmission and adverse pregnancy outcomes. Diagnosis is routinely based on serological assays, which are used to determine *T. gondii* specific antibodies in patients' sera (Zhang *et al.,* 2016). Accurate laboratory methods should be used for early detection of parasite in infected women and infants with congenital infection. Diagnostic approaches should include detection of specific IgM, IgG and IgA antibodies along with maternal history, gestational age and treatment (Pomares and Montoya, 2016). Various tests like ELISA, IFAT, DAT, MAT, PCR and histo-pathological techniques are used for surveillance, prevalence and confirmation of clinical suspected cases. Dye test is a serological test and consider as a 'gold standard, in which live, virulent tachyzoites are used as complement-like 'accessory-factor' in test serum, but the assay carries potential risk for human infection and require technical expertise. Indirect fluorescent antibody (IFA) can be used for proper differentiation of IgM and IgG antibodies. Direct agglutination and latex agglutination assays are commonly used, which are rapid and easily conducted (OIE, 2017). ELISA is currently used for detection of Toxoplasma specific IgG and IgM antibodies in sera of patients (Murata *et al.,* 2017). Avidity test is also used for detection of *T. gondii* specific IgG antibodies and to discriminate between recent and past infection during pregnancy (Barros *et al.,* 2017). Western blot (Sroka *et al.,* 2016) and indirect immune fluorescence techniques (Naghili *et al.,* 2017) can also perfumed for confirmation of *T. gondii* specific antibodies (Khammari *et al.,* 2014). PCR-based molecular techniques are also useful for detection of *T. gondii* DNA in clinical samples (Liu *et al.,* 2015), but PCR and histopathological techniques are expensive, laborious and cannot be used in large-scale surveys.

1.2.19 Therapeutic strategies for Toxoplasmosis: Toxoplasmosis associated morbidity and mortality is treatable and preventable (Peyron *et al.,* 2015), but unfortunately, treatment failure rates remain significantly high (Dunay *et al.,* 2018). Early and effective treatment in gestational period during pregnancy significantly decrease transmission of parasite from infected mother to child and improve clinical outcomes (Wallon *et al.,* 2013; Prusa *et al.,* 2015). A combination of pyrimethamine and sulfadiazine, which target active stage of *T. gondii* is a gold standard combination for treatment of Toxoplasmosis, but rate of therapeutic failure is significantly high. Other drug regimens include pyrimethamine in combination with atovaquone, clindamycin, clarithromycin or azithromycin or mono-therapy with TMP-SMX or atovaquone, but none have been found to be superior to pyrimethamine and sulfadiazine drug and no regimen is active against latent stage of parasite. The efficacy of these drug regimens in ocular disease is still uncertain. Spiramycin is effective for acute maternal infection and pyrimethamine, sulfadiazine combination for established fetal infection and prevention of vertical transmission of parasite. These drugs also reduce severity of congenital infection and showed a significant progress in treatment of infection. There is still a strong need for development of novel therapeutics agents for management of acute and latent infection of *T. gondii* during pregnancy (Dunay *et al.,* 2018).

1.2.20 Prevention of Toxoplasmosis: Majority of human emerging infections are zoonotic. For prevention of zoonotic infections, it is important to understand factors, which are associated with emergence and transmission of zoonotic infections (Shwaba *et al.,* 2018). As *T. gondii* has ability to infect domestic, free ranging animals and human being, therefore proper control is based on one health approach. The disease also impacts on ecosystems and is a common and continues threat to those families, who depends on animal resources in endemic regions (Jenkins *et al.,* 2015; Aguirre *et al.,* 2019). Integrating disease data on human, domestic and wild animals could better assess risk factors and develop methods of disease control. Development of these approaches for disease control would need to acquire an epidemiological knowledge of zoonotic infections in endemic regions (Thompson, 2013).

1.3 Objectives: Majority of human emerging diseases are zoonotic, which are transmitted from infected animal to human. Being an agriculture country, people depend on livestock animals for their earning in rural regions in Pakistan. Epidemiological, clinical, diagnostic and therapeutic information and information related to adverse obstetric outcomes due to emerging zoonotic diseases (Brucellosis, Toxoplasmosis) is very scarce in published literature in Pakistan. Therefore, the current study was designed to achieve the following objectives:

1. Assessment of geographical, annual and seasonal prevalence of zoonotic diseases (Brucellosis, Toxoplasmosis) in women of Khyber Pakhtunkhwa, Pakistan.

2. Assessments of socio-demographic and epidemiological factors associated with transmission of zoonotic diseases in Khyber Pakhtunkhwa, Pakistan.
3. To compare qualitative and quantitative assays used for diagnosis of Brucellosis, Toxoplasmosis and to determine statistical validity of these assays.
4. Assessment of clinical manifestation of zoonotic diseases for prediction of focal involvement and therapeutic failure.
5. Assessment of various hematological (CBC, ESR) and biochemical parameters (CRP, AST, ALT) for prediction of associated complication in women with these zoonotic diseases.
6. Assessment for clinical management of Brucellosis and associated risk factors of therapeutic failure.
7. Assessment of various adverse obstetric outcomes and zoonotic diseases (Brucellosis, Toxoplasmosis) in women.

REVIEW OF LITERATURE

2.1 Brucellosis

2.1.1 Global Prevalence of Brucellosis: Globally, serious health challenges have arisen from various diseases, including emergence and reemergence of old and new infections, which are accelerated due to fast human development like frequent changes in demographics, human, animal population and surrounding environment. These changing parameters have also led to many zoonoses in a changing ecosystem of humans and animals, which are stuck by growing globalized society, where most pathogens do not recognize geopolitical borders (Mackey *et al.,* 2014). Frequency of various zoonotic infections seems to be increased, although overall frequency of various zoonoses in hospital and general environment is not known, but risk of many zoonotic pandemics have been recognized as a global warning to human health (Morse *et al.,* 2012). The selected zoonoses included in the current study focused upon infection of Brucellosis and Toxoplasmosis in women patients. Epidemiology of zoonotic Brucellosis in many developing countries is challengeable to control and prevent due to complex ecology of pathogen (Gomo *et al.,* 2012; Treanor, 2013) with many known animal reservoir hosts (Dean *et al.,* 2012a). Brucellosis has a significant epidemiological, global, economic and health impact, mainly not only for animal population, but also to human in various developing countries that depend on cooperative animal farming and agricultural practices (Haque *et al.,* 2011; Moreno, 2014) like Pakistan, where 30-40% people in rural regions depends on livestock for their income and 30-35 million individuals are engaged to raise livestock. Pakistan dairy sector play important role in national economy, which is more than wheat and cotton sectors. During 2014-2015, about 52.6 million liter was an estimated annual milk production, due to which Pakistan was one of top milk producer (Pakistan Economic Survey 2014-2015). Brucellosis has been reported in Pakistan for many years and its increasing prevalence need to stress on regular animal screening at abattoirs and livestock markets (Abubakar *et al.,* 2010).

2.1.2 Epidemiology and Geographical Distribution of Brucellosis: Brucellosis has been reported from more than 86 countries (Tadesse, 2016), while global burden of human infection was considered more than 500,000 cases per year (Godfroid *et al.,* 2013), but annual incidence exceeds 800.000 cases (Kirk *et al.,* 2015). The increased incidence of human infection may be due to a fact that many livestock animals species are hosting the pathogens and infected animals do not show any prominent clinical symptoms, while chain for milk distributions encourage supply of unprocessed milk in developing countries (Wareth *et al.,* 2014), which spread diseases from animal to human (Racloz *et al.,* 2013). About 50% of annually estimated cases of human infection are due to use of contaminated food (Havelaar *et al.,* 2015). The disease is neglected in developing countries (Wareth *et al.,* 2014), which cause significant morbidity in human and animal population (Bosilkovski *et al.,* 2015). Accurate data on zoonotic Brucellosis is still lacking from many countries, which leads to underestimation of disease burden (Dean *et al.,* 2012).

2.1.3 Global Incidence of Brucellosis: Epidemiological statistics show that incidence of human Brucellosis significantly varies in different times and counters (Bosilkovski, 2016). The incidence fluctuates widely, not only between various countries, but also within different region in a particular country due to various factors. According to Garba *et al.,* (2017), human occupation and socioeconomic status are closely associated with rate of infection in Iraq and Egypt. These variations have also been reported to associate with other factors like standards of individual, environmental hygiene, methods of animal husbandry practices, methods of local food processing and existence of Brucella species in a particular endemic region (Pal *et al.,* 2017). The disease is rare in some countries, while greatly affects human community in some regions. The disease is generally rare in industrialized countries, while common in developing countries. During 2002, human incidence was 100 cases in USA, while it was 40 cases in Australia (Right Diagnosis from Health grades. 2014). About 40% human populations in Latin Americans live in Brucellosis endemic regions (Ron-Roman *et al.,* 2014). It is considered an emerging zoonotic disease in various Southeast Asia (Jama'ayah *et al.,* 2015), particularly reported from Indonesia (Berger, 2010), Malaysia (Tyagita *et al.,* 2014). The exact burden of Brucellosis is still not well-documented due to lack of strong effective surveillance programs and many institutional challenges, facing by veterinary and public health agencies in various developing countries, that make disease surveillance system difficult, as a result Brucellosis has become underreported in animals and humans (McDermott *et al.,* 2013 and Ducrotoy *et al.,* 2014). Brucellosis is endemic in some developed and many developing countries and cause significant morbidity in human and animal. Globally major endemic regions are Persian Gulf, Mediterranean Basin, Indian subcontinent, Mexico, South and Central America (Bosilkovski, 2016). The disease is also endemic in Sub-Saharan African countries and seroprevalence have been reported 3.3% in Central African Republic, 7.7% and 24.1% in Tanzania (Kunda *et al.,* 2010 and Aworh *et al.,* 2013), 31.82% in Nigeria (Cadmus *et al.,* 2006), 17% in Uganda (Tumwine *et al.,* 2015), in Peru 8%, Azerbaijan 10%, Saudi Arabia 19% and Iran 20% (Sanodze *et al.,* 2015). Brucellosis is also widespread in many Asian countries, including Pakistan, India, Sri Lanka and China (Norman *et al.,* 2016; Chen *et al.,* 2016) and Bangladesh (Islam *et al.,* 2013).

Pakistan: Brucellosis in animals has been associated with disease propagation in human due to handling of animals and consumption of animal products (Hegazy *et al.,* 2016). Infection in human is largely dependent on animal reservoirs (Hasanjani *et al.,* 2015) and infection is reported in Animals in Pakistan (Ali *et al.,* 2013; Shahzad *et al.,* 2014; Ali *et al.,* 2016; Ali *et al.,* 2017). Gwida *et al.,* (2011) stated that human Brucellosis and its associated risk factors exist in Pakistan. Al-Majali *et al.,* (2009) and Shahid *et al.,* (2014) reported Brucellosis in human with a prevalence of 6.9% and 30.5% in two different regions of Pakistan. Hussain *et al.,* (2008) reported human Brucellosis with a frequency of 14% and 11% based on RBPT and ELISA methods. Mukhtar and Kokab (2008) also detected and reported human infection in

high risk communities in Lahore, Pakistan. They reported 21.7% prevalence by IgG ELISA. Ali *et al.,* (2013) used RBPT and SAT test and reported infection of Brucellosis with a prevalence rate of 6.9% among different occupational groups in Potohar plateau of northeastern Pakistan.

INDIA: Prakash *et al.,* (2012) screened out 570 different categories of high risk individuals including PUO in western Rajasthan of India. All individuals were tested by stained febrile antigen. Positivity for anti-Brucella antibodies was found in 72%, 26.66%, 37.14% and 6.00% in PUO patients, meat handlers, milk handler and healthy individuals. Smita *et al.,* (2015) cultured blood samples of 169 patients for isolation of Brucella among 593 cases, who were clinically and serologically suspected for infection of Brucellosis. Brucella was isolated from 77 blood samples. The isolation rate of Brucella was 45.5%. Among 77 isolates, 75 were identified *B. melitensis* and 2 isolates were *B. abortus*. They observed that *B. melitensis* was a common specie in various regions of Indian.

Bangladesh: Brucellosis has been reported with different prevalence from Bangladesh. Rahman *et al.,* (2012a) reported infection in high risk exposed communities in Bangladesh. They used serological assays like RBPT, STAT, iELISA and detected infection with a rate of 2.6%, 18.6%, 2.5% and 5.3% in livestock farmers, milkers, butchers and animal handlers. Prevalence of infection was higher 11.4% in people, who had a history of consumption of raw milk as compared to 3.9% cases in those people, who had no history of consuming raw milk. Rate of infection was high 16.2% in those individuals, who had history of animal contact. Similarly Islam *et al.,* (2013) conducted a review study on human brucellosis, which was based on different serological techniques like SAT, RBPT, 2-MET, STAT, FPA, iELISA and cELISA assays in Bangladesh. They stated that sensitivity and specificity of these serological techniques influence infection rate in endemic regions. They reported Brucellosis infection with a frequency of 2.6-21.6%, 18.6%, 2.5% and 5.3-11.1% in animal owners, milkers, meat and animal handlers. They observed a prevalence variation of 2.5-18.6%, which was based on occupations of people.

China: Republic of China is a neighbor country of Pakistan, where Brucellosis has been reported. Shengjie *et al.,* (2017) analyzed data on incidence and distribution of Brucellosis in human using surveillance aggregated data and data of individual cases. They recorded 513,034 cases of human brucellosis, 99.3% were reported from northern China. Similarly Zolzaya *et al.,* (2014) reported Brucellosis in human with a rate of 27.3% in Mongolia.

Kyrgyzstan: Kyrgyzstan is also considered an endemic region for Brucellosis. Dürr *et al.,* (2016) reported 77.5% new cases of human Brucellosis per 100,000 inhabitants in the year 2007, which is consider highest incidences in the world. RBPT and Huddleson test are commonly used as diagnostic tests in ruminants and humans in Kyrgyzstan.

Malaysia: Malaysia is also an endemic country for brucellosis, where pathogen infects not only dairy animals, but also human population (Hartady *et al.,* 2014). According to Tay *et al.,* (2015), human

seropositive cases usually occurred in animal handlers and farmers, who had close animal contact in Malaysia. They stated that prevalence of infection was more common in people, who were in age group of 20-45 years. According to Bamaiyi (2016), risk factors for Brucellosis are age, occupation and habits of drinking unpasteurized milk In Malaysia.

Central America: Brucellosis is also widespread throughout Central America and infection is mostly caused by *B. abortus* and *B. suis* (McDermott *et al.,* 2013). Winkle-Preston and Drew (2014) reported a human case from USA, who was diagnosed with Brucella infection during an acute febrile illness. Infection was acquired due to consuming unpasteurized dairy products like soft goat cheeses, most commonly consumed in some traditional diet of cultures in many immigrants in USA.

North America: Human Brucellosis has been reported from different countries of North America due to consumption of unpasteurized milk and many other dairy products. Annually, 839 new cases are reported with 55 cases of hospitalization and 1 case of death (Scharff *et al.,* 2012). Beside North America, infection of Brucellosis also persists in South American countries. Agrarias *et al.,* (2013) claimed that 13% poor populations were positive for *B. abortus* and 4.6% for *B. canis* in urban areas.

European countries: Brucellosis is also found in many European countries, which is thought to associate with travelers and immigrants, who come from Middle East to Europe or due to importing huge amount of dairy products from endemic regions (Garofolo *et al.,* 2016; Georgi *et al.,* 2017). Mancini *et al.,* (2014) designed an epidemiological model to correct under reporting cases of Brucellosis. They found that number of positive cases should have ranged from 41,821 to 155,324 in Italy, which indicates a more severe picture of human Brucellosis than predicted by surveillance system. Wen-Chien Ko (2014) reported 53 cases of human Brucellosis from Northern Ireland, 52 (98%) cases were occupational risk factors, 37 (70%) cattle farmers, 11 (21%) abattoir workers and 4 (8%) cases were veterinarians.

Australia: Brucella Infection also occurred in Australian continent. Ridoutt *et al,*. (2014) stated that during 1991 to 2012, an average of 34 cases were annually occurred in Australia. Although the country is not endemic for human brucellosis, but persistence sporadic reports of human infection indicates that it is either endemic in animals or occurred due to frequent movement of people from endemic regions to Australia or consuming infected products.

Saudi Arabia: Saudi Arabia is also an endemic zone for zoonotic infection of Brucellosis. Persistence of *B- abortus* and *B. melitensis* have been conformed and reported from many Middle Eastern countries (Musallam *et al.,* 2016 and Garofolo *et al.,* 2016). Aloufi *et al.,* (2016) reported 19,130 cases of human infection from Saudi Arabia. Most human cases occurred during spring and summer season. Highest incidence was found 25% in Al-Qassim, Aseer and Hail regions. Al-Tawfiqa and Abu-Khamsin (2009) analyzed data on human infection of Brucellosis from 1983 to 2007 in a health-care center in eastern Saudi Arabia. They found 913 cases of human infection. Frequency of infected cases was highest from

April to June 361 (39.5%), while lowest cases were reported in the month of January. Among these patients, 219 patients had animals in their homes, 125 (57.1%) camels, 49 (22.4%) sheep, 24 (11%) goats and 21 (9.6%) cows. They found no association between patient's age and rate of positive blood culture. Serological titer of STAT test was 1:320 in (34.3%), 1:640 in (31%), 1:1280 in (24.7%) and ≥1:2560 in (10%) infected cases.

Qatar: Qatar is another region in Middle East, where cases of human Brucellosis frequently occurred. Garcell *et al.,* (2016) studied clinical, epidemiological and laboratory aspect of an outbreak of Brucellosis infection in 14 family members, who were living in a rural region in Qatar. They studied 14 patients with confirmed infection and 12 non-confirmed family members from hospital record. They observed mixed infection of *B. abortus* and *B. melitensis* identified through serological and bacteriological study. The identified source of outbreak was consumption of milk from infected camel.

Yemen: Yemen is another endemic country for brucellosis. Al-Arnoot *et al.,* (2017) conducted a review study and reported seroprevalence of human infection from 0.3 to 32.3% cases in various regions of Yemen.

Egypt: Egypt is also considered an endemic region for Brucellosis. Abdelbaset *et al.,* (2018) examined epidemiological aspects of human Brucellosis in 53 cases in Egypt, who had sheep in their homes and confirmed Brucellosis infection in 5 (9.44%) cases through RBPT test, They farther confirmed through *B. abortus* and *B. melitensis* specific antigen test that 80% RBPT-positive cases had antibodies for *B. abortus* and 20% cases had antibodies for both species of Brucella. No statistical association was found with infection and other factors like age, education and profession.

Turkey: Turkey is also endemic region for brucellosis. Buzgan *et al.,* (2010) retrospectively evaluated 1028 hospitalized patients of Brucellosis for history, clinical, laboratory, therapeutic features and complications. Among 1028 infected cases, 435 (42.3%) had a history for raising livestock, 55.2% infected cases were no occupational risk, while 654 (63.6%) cases had a history of consuming raw milk and dairy products

Israel: Brucellosis also occurred in various regions of Israel. Fuchs *et al.,* (2016) reported Brucellosis in Bedouin tribes, who were living in close proximity to their herds in southern Israel. They consume unpasteurized milk and dairy products, while Ethiopian Jews neither raise animals nor consume dairy products, but they still suffer from brucellosis, which might be due to traditional ritual slaughter of animals, commonly practiced among Ethiopian Jews in Israel. They slaughtered animal, skinned, remove guts, crushed meat and thus expose to zoonotic infectious pathogen.

Iran: Brucellosis also occurs in Iran. Nassaji *et al.,* (2015) reported 52.1% cases of Brucellosis in hospitalized patients and stated that source of infection was consuming unpasteurized fresh cheese and occupational exposure of animals. Nematollahia *et al.,* (2017) conducted epidemiological study and

reported 7318 cases of human brucellosis, in which 472 (6.45%) cases were recurrent infection. They reported that rate of recurrent Brucellosis was associated with increased age, history of consuming unpasteurized dairy products and winter season. Dastjerdi *et al.,* (2012) conducted a retrospective study to determine epidemiological features of Brucellosis in endemic region in central Iran. They reported 1996 cases of human brucellosis, 68% cases were from rural areas, while rate of animal contact was 81% in rural and 61% in urban cases. Raw milk was consumed by 37% infected cases.

African continents: African continent is the most common endemic region for Brucellosis infection (Musallam *et al.,* 2016; Garofolo *et al.,* 2016). Infection is widespread and prevalence varies from one region to other region in various countries in Africa (Ducrotoy *et al.,* 2015). Kardjadj *et al.,* (2015) conducted a review study to describe epidemiological aspects of human Brucellosis in Algeria. They observed that number of new cases of human infection followed a similar trend in animals, which highlight impact of animal reservoir on human health. They stated that transmission of disease depends on animal reservoirs along with many other factors like food habits, milk processing methods, type of dairy products, social customs, regional climatic, socioeconomic status, animal husbandry practices and individual hygiene in Algeria. About 85% human infection occurred due to consumption of raw milk and dairy products in Algeria. Uganda is another African country, where cases of human Brucellosis have been reported. Tumwine *et al.,* (2015) screened 235 participant by SAT and RBPT tests to detect anti-brucella antibodies and associated risk factors in agro-pastoral communities in Kiboga region of Central Uganda, where Brucellosis was found with a frequency of 17.0 %. Residence in rural areas, consuming locally processed milk products and unmarried individuals were found with increased risk of Brucellosis. Aworh *et al.,* (2011) reported 24.1% cases of human Brucellosis in Abuja, Nigeria and detected individual occupation and eating of raw meat as a major risk factors. Similarly Rujeni and Mbanzamihigo (2014) reported 25% infected women from Huye, Rwanda, who had positive history of adverse pregnancy outcomes. Oueslati *et al.,* (2016) reported 13 cases of neuro Brucellosis in hospitalized patients from Tunis. Awah-Ndukum *et al.,* (2018) conducted a cross sectional study to determine seroprevalence and risk factors, which are associated with human Brucellosis in 107 abattoir worker and 709 pregnant women in Cameroon. They used RBPT and ELISA assays for detection of Brucellosis specific antibodies. Infection was found in 5.6% cases by RBPT and 12.15% cases by Brucella IgG ELISA among abattoir workers and 0.28% in pregnant women by RBPT and ELISA. The global prevalence of Brucella infection in different countries indicate that Brucellosis is a major animal and public health issues in underdeveloped countries and even in human communities in developed countries, where people have poor socioeconomic status. Due to contagious nature of Brucella, risk of infection might further extend to Brucellosis free regions in near future (Leiser *et al.,* 2013).

2.1.4 Clinical and Laboratory Aspects of Brucellosis: Brucellosis is a systemic infection, in which many organ and body system can be involved. The most common affected body systems are gastrointestinal, locomotors genitourinary, cardiovascular, respiratory, central nervous and hematological systems (Pınar Korkmaz and Elif-Doyuk-Kartal, 2016). The Disease` show various clinical symptoms, due to which most patients are usually misdiagnosed as malaria, arthritis or many other diseases (Dean *et al.,* 2012b; Wang *et al.*, 2012). Incubation periods of infection can be long (up to 6 months) and clinical symptoms in infected individuals may persist for years in untreated cases (Godfroid *et al.,* 2013). Clinical, laboratory and therapeutic aspects of Brucellosis infection in human are reviewed as under:

- Garcell *et al.,* (2016) investigated clinical and laboratory aspects in 14 patients, who had confirmed Brucellosis and 12 cases of non-confirmed family members. Fever was reported for 14 days, associated with artheralgia, weakness, headache, diarrhea and abdominal discomfort. Elevated level of transaminase was observed in these patients. Serology and blood culture reveled and identified *B. abortus* and *B. melitensis* as disease causative agents.
- Demir *et al.,* (2012) reported fatigue, malaise, fever, artheralgia, loss of appetite, night sweats, headache, myalgia, back pain, weight loss and abdominal pain with a prevalence of 42 (88%), 36 (75%), 34 (71%), 31 (65%), 29 (60%), 21 (44%), 19 (40%), 16 (34%), 13 (27%), 6 (12%), while clinical signs like splenomegaly, hepatomegaly, hepato-splenomegaly and lymphadenopathy were observed in 24 (50%), 20 (42%), 9 (19%) and 12 (25%) cases of Brucellosis patients.
- Buzgan *et al.,* (2010) retrospectively evaluated clinical and laboratory findings, complications and therapeutic features in 1028 patients of Brucellosis. They reported artheralgia in 73.7%, fever (72.2%) and hepatomegaly in 20.6% patients. C-reactive protein was high in 58.4% cases. STA and Coombs test was positive in 1016 (98.8%) cases. Focal involvement was observed in 371 (36.1%) cases, in which osteo-articular involvement was observed in 260 (25.3%) infected cases. Relapse was found in 4.7% cases, which was highest 8.5% in osteo-articular patient. Doxycycline and streptomycin with and without rifampin was found more effective than other drug in osteo-articular patients.
- Oueslati *et al.,* (2016) reported 13 cases of neuro-brucellosis in hospitalized patients, who were positive for Brucella specific antibodies. Antibiotic therapy was started in various combinations. Among these infected cases, 9 patients were treated with rifampicin and co-trimoxazole, 4 with doxycycline for an average of eight months and 2 patients were also given steroids. Among these cases, 10 patients were successfully recovered.
- Masallat *et al.,* (2013) evaluated PCR and various serological techniques for diagnosis of Brucellosis in 90 pregnant women, who were clinically suspected for Brucellosis infection. These

patients were screened by blood culture, serological methods like STAT and ELISA. PCR was also used for amplification of 223-bp region within a gene, which code for 31kD Brucella antigen. Blood culture was positive in 20 (22.22%) patients, while positivity of STAT, IgM and IgG ELISA among 90 patients were 27 (30%), 23 (25.55) and 20 (22.22) respectively. PCR detected DNA of Brucella antigen in 26 (28.88) patients. STAT assay showed best diagnostic results at a titer of (≥320) in serological tests, while PCR diagnosed 6 positive women, who were negative by blood culture method.

- Wen-Chien Ko (2014) studied clinical features of Brucellosis in 53 cases of occupational cattle exposure in Northern Ireland. He observed lethargy, sweating, artheralgia, headache and fever, arthritis and endocarditis with a frequency of 52 (98%), 46 (87%), 42 (79%), 37 (70%), 26 (49%), 1 (2%) and 1 (2%). Endocarditis was detected through echocardiography (ECG) with isolation of *B- abortus* by blood culture. Among 53 patients, 46 were treated by rifampicin and doxycycline, while 4 cases were treated with rifampicin and doxycycline. Those infected individuals, who were diagnosed with endocarditis due to brucellosis, were treated by intravenous gentamicin, rifampicin and doxycycline. Among 53 infected cases, clinical symptoms were completely resolved in 33 (62%) patients with antimicrobial therapy, while 20 (38%) patients showed persistent symptoms due to therapeutic failure.
- Zamani *et al.,* (2011) conducted retrospective study and reported 96 patients, who were diagnosed with Brucellosis. Arthritis was found in 24 (25%), fever 21 (87.5%) and fatigue in 18 (75%) infected cases. Relapse was not seen in 24 patients, who were treated with co-trimoxazole and gentamicin for 6 weeks and 5 days respectively.
- Zribi *et al.,* (2009) studied 31 cases of acute and 14 cases of sub-acute infection of Brucella. Fever, sweating, artheralgia and splenomegaly were found in 93%, 82%, 78% and 51% patients. Elevated ESR was found in 80% cases, while leucopenia and anemia was found in 49% and 37% infected cases. *B. abortus* and *B. melitensis* were isolated by blood culture and 93% patients were successfully treated with therapeutic combination of rifampicin and doxycycline.
- Sowjanya Dasari *et al.,* (2013) retrospectively investigated 32 patients of Brucellosis. Chronic fever, productive cough, weight loss, undulant fever and night sweating were observed in 21 (65.63%), 13 (40.63%), 8 (25.00%), 11 (34.38%) and 5 (15.63%) cases. Similarly, prominent clinical signs like lymphadenopathy, hepatomegaly, splenomegaly and hepato-splenomegaly were observed in 7 (21.88%), 15 (46.88%), 12 (37.50%) and 8 (25.00%) patients. ESR elevated than 50 mm/hr and 100 mm/hr was found in 8 (25.00%) and 5 (15.63%) infected cases.
- Mugahi *et al.,* (2014) reviewed clinical and laboratory features in 81 hospitalized patients of Brucellosis. They reported fever, joint pain, chills, and sweating in 85.2%, 72.9%, 42% and 37%

patients, while arthritis, tachycardia, tenderness in lumbar spine were clinical signs, which were observed in 9.9%, 6.2% and 4.9% infected cases. Normal count of WBC was observed in 94.82%, anemia 75.3% and elevated ESR in 75.3% infected individuals.

- Ding *et al.,* (2017) observed clinical and treatment outcomes in 10 cases of Brucella associated endocarditis. Among these 350 patients of brucellosis, 10 (2.86%) were diagnosed with Brucella endocarditis, who had chest tightness and shortness in breath. Other associated clinical symptoms were fever and fatigue, sweating and weight loss, chills, cardiac insufficiency and joint pain in 9 (90%), 6 (60%), 5 (50%), 5 (50%) and in 4 (40%) cases respectively. They were successfully treated with doxycycline 100 mg, rifampicin 600 mg and levofloxacin 500 mg for 6 weeks.
- Teker *et al.,* (2014) reported a clinical case of brucellosis, who was allergic to TMP-SMX, but he was successfully treated with therapeutic combination of rifampicin, gentamicin and ciprofloxacin.
- Sen *et al.,* (2019) retrospectively evaluated predictive value of inflammatory markers in 187 patients of Brucellosis. The most common complications, associated with Brucellosis were osteoarticular, hematological, neurological, genitourinary, gastrointestinal, cardiovascular and ocular involvements. Neutrophil-lymphocyte (NLR), lymphocyte-monocyte (LMR) and platelet-lymphocyte ratio (PLR) were evaluated to predict complications and involvement of specific organ. Complications were observed in 125 infected cases. PLR and ESR was high in complicated cases. LMR, NLR were not significant in terms of predicting complications. They also evaluated involvement of specific organ as a complication due to recurrent hematological abnormalities in brucellosis. ESR, MPV, NLR, LMR and PLR were significantly changed in patients, who had specific organ involvement.
- Bukharie, (2009) clinically examined 84 patients of brucellosis. History of fever was found in 95%, artheralgia (64%), splenomegaly 13 (16%) and hepatomegaly in 9 (11%) infected cases, while 2 patients had fever of unknown origin, 2 cases with recurrent fever with menstruation, 1 had jaundice and spontaneous peritonitis, 11 (13%) had generalized lymphadenopathy. Meningitis and endocarditis were cardiac and neurological complications, found in 2 cases of Brucellosis. ESR was found with a range of 4-110 mm/hour. Anemia, leucocytosis, lymphocytosis and thrombocytopenia was found in 30 (36%), 5 (5.95%), 50 (60%) and 3 (4%) cases of Brucellosis. Elevated level of transaminase and γ-glutamyl transpeptidase (GGT) was found in 24 (29%) and 31 (37%) cases, while STAT assay was positive in 84 patients with an antibodies titer of ≥ 1:160.
- Demir *et al.,* (2012) investigated hematologic abnormalities through bone marrow biopsy examination in 48 patients of brucellosis, who had anemia 39 (81%), leukopenia 28 (58%),

thrombocytopenia 22 (46%) and pancytopenia 10 (21%). Examination of bone marrow reveled hypercellularity in 35 (73%), increased megacariocytic, erythroid and granulocytic series in 28 (58%), 15 (31%) and 5 (10%) patients. Moreover hemophagocytosis, granuloma and increased count of eosinophils and plasma cells was observed in 15 (31%), 12 (25%) and 9 (19%) infected cases. They claimed that hemophagocytosis, microgranuloma formation and hypersplenism are associated with hematologic complications, which occurred during infection of Brucellosis. Blood and bone marrow was cultured for isolation of Brucella. *B. melitensis* was isolated from blood samples of 11 (23%) cases and 25 (52%) samples of bone marrow culture. Elevated ESR (>20 mm/h) and CRP (>5 mg/dl) was found in 30 (62%) and 43 (90%) infected individuals.

- Fanni *et al.,* (2013) carried out a retrospective study and reviewed data associated with clinical manifestations, laboratory findings and therapeutic regimen in 34 cases of hospitalized patients of Brucellosis in Iran. Clinical findings at admission in hospital were fever, arthritis, hepatomegaly, splenomegaly, lymphadenopathy and macula-papular skin rashes. Anemia, leucopenia, leucocytosis and thrombocytopenic were observed in 53%, 33%, 2.95% and 12% patients, while no patients had pancytopenia. Blood culture was positive in 5 (23%), wright test 33 (97%), coombs test 23 (96%) and 2-Mercaptoethanoin test in 16 (72.7%) cases of Brucellosis. They concluded that prolonged fever with arthralgia and organomegaly increase possibility of Brucellosis in suspected individuals.
- Asaad and Alqahtani, (2012) compared ELISA and PCR with conventional diagnostic methods in 340 suspected patients of brucellosis. Blood samples of all patients were subjected to culture, SAT, IgM, IgG ELISA and PCR. Diagnosis was confirmed in 54 (15.9%) cases. Blood culture, SAT, ELISA for igM IgG and PCR diagnosed 14 (25.9%), 50 (92.6%), 46 (85.18%), 52 (96.29%) and 38 (70.37%) cases of brucellosis. The sensitivity of IgM and IgG ELISA was 85.2% and 96.3%, while specificity was 100% for both IgM and IgG. The sensitivity and specificity for PCR was 70.4% and 100% respectively. They claimed that ELISA offers a significant advantage over serological methods for proper assessment of brucellosis, while PCR was helpful in diagnosis of those patients, who have clinical signs and symptoms.

2.1.5 Adverse Obstetric Outcomes Associate with Brucellosis: Brucellosis has adverse effects on pregnancy. Infection may induce spontaneous abortion, which is more common in animals as compared to women, because human placenta and developing fetus lack erythritol, which is considered as growth factor of Brucella. Moreover, anti-brucella activities of amniotic fluid in infected women also decrease probability of abortion. In human, it is still controversial to associate abortion and stillbirth with infection of Brucellosis (Callaghan, 2013).

- Khan *et al.,* (2001) and Al-Tawfiq and Memish, (2013) stated that Brucellosis can induce spontaneous abortions in pregnant women. They reported spontaneous abortion from 2.5 to 53% cases of Brucellosis in pregnant women. They claimed that 43% spontaneous abortions occurred in first and second trimesters of pregnancy, while IUFD was 2% in pregnant women in Saudi Arabia.
- Rujeni and Mbanzamihigo, (2014) reported abortion and still birth in 25% women, who were positive for Brucellosis by RBPT assay in Huye, Rwanda. They linked many cases of abortion, stillbirth and other reproductive problems with Brucellosis associated pathology.
- Baud *et al.,* (2009) stated that Brucellosis is associated with miscarriage in women. They found *B. abortus* antibodies in two patients, who had miscarriages in London. Both women were immigrants from Sudan.
- Abdullah *et al.,* (2017) carried out a descriptive cross-sectional hospitals based study in order to assess Brucella specific IgM and IgG antibodies among 304 pregnant women by ELISA. IgM and IgG was found positive in 42 (13.82%) and 17 (5.95%) pregnant women. Headache and undulant fever was found in IgM and IgG positive women, but they did not find any significant association with patient's reproductive characteristics and infection of Brucellosis.
- Abo-shehada and Abu-Halaweh, (2011) used RBPT and CFT assay for detection of Brucellosis infection in women, who had miscarriages in Jordan. They found Brucellosis infection in 1.8% women, who had miscarriages, while 1% infected women had no miscarriages.
- Elshamy *et al.,* (2008) reported incidence of abortions, intra uterine fetal death and preterm labor with a frequency of 27.27%, 12.72% and 10.90% among those pregnant women, who had active infection of brucellosis. They further stated that frequency of fetal loss was high in infected patients.
- Alsaifa *et al.,* (2018) reviewed incidence of Brucellosis associated consequences during pregnancy and reported spontaneous abortions in 20 (31%), IUFDs 2 (3%) and congenital Brucellosis in 11 (17%) cases. During reviewing another study, they found 181 (27%) cases of spontaneous abortion in 679 pregnancies, IUFDs 19 (4%) in 458 pregnancies, preterm infants 44 (12%) and 6 (2%) infants with congenital Brucellosis in 362 pregnancies.
- Vilchez *et al.,* (2015) reviewed Brucellosis associated clinical and obstetric outcomes in hospitalized cases of pregnant women, who were diagnosed with active infection of Brucellosis. They observed that infection of Brucella cause severe obstetric complications in women patients, who had positive agglutination, blood or bone marrow culture test. They claimed evidences of various adverse obstetric outcomes like spontaneous abortion, fetal maternal and neonatal death.

- Peker *et al.,* (2011) presented a case of Brucella infection in 19 year old pregnant woman, who had 19-20 weeks gestation. Hematuria and nausea were clinical presentation at hospitalization. Blood culture became positive for Brucella and she was spontaneously aborted during treatment. They concluded that Brucella infection cause abortion, premature birth and IUFD, but there was no association between infection and congenital anomalies.
- Madkour (1989) and Seoud *et al.,* (1991) reported 40% spontaneous abortion and 46% IUFD in pregnant women, who were suffering from acute infection caused by *B. melitensis.*
- Ghanem-Zoubi *et al.,* (2018) investigate an association between incidence of Brucellosis and adverse pregnancy outcomes. They compared frequency of adverse pregnancy outcomes with high and low-incidence regions. IUFD was a primary outcome, followed by premature birth (less than 37 weeks) and poor fetal growth. They observed that APOs occurred more frequently in regions, where incidence of Brucellosis was high. They observed an absolute increase of 3.6 cases of IUFD, preterm births 4.8-18.3, 6.6 cases of threatened labor (2.2-10.9) and 7 cases of poor fetal growth per 10,000 live or dead births. They observed that annual frequency of Brucellosis and IUFD was high in Arab Bedouins, who were living in southern localities. They associated high frequency of Brucellosis with adverse obstetric outcomes.
- Kurdoglu *et al.,* (2010) conducted a case-control study to determined frequency of miscarriage in 342 pregnant women with Brucellosis as compared to 33936 women, who were negative for Brucellosis in the same hospital. Frequency of miscarriage was high 24.14% as compared to 7.59% cases in controls group, but these results could be influenced by statistical power, because infected cases were 100 times smaller than control group. They suggested that Brucellosis was a risk factor for miscarriages in endemic regions.
- Masallat *et al.,* (2013) evaluated PCR and serological techniques for accurate diagnosis of Brucellosis in 90 pregnant women. They followed up 20 culture positive cases during pregnancy and compared these infected cases to 20 negative cases by various tests to determine various adverse effects of infection on pregnancy. Frequency of spontaneous abortion was high 20% in Brucellosis positive women as compared to 15% in negative cases. Preterm deliveries were significantly observed in 9 (45%) infected women as compared to 2 cases in uninfected women. No IUFD was observed in infected and control group. They claimed that Brucellosis is a risk factor for preterm delivery during pregnancy.

2.2 Toxoplasmosis

2.2.1 Global Prevalence of Toxoplasmosis: Globally, Toxoplasmosis has been worldwide reported by serological detection of antibodies and through molecular techniques for presence of *T. gondii* DNA in blood and various other body tissues in infected host (Dubey *et al.,* 2015).

There is a large variation in seroprevalence and incidence of Toxoplasmosis infection in various geographical regions and within human population in a particular region, which depends on various factors like eating habits and general health of infected individuals (Subauste *et al.,* 2011). Countries, where frequency of infection is low, include USA and northern countries of Europe's like Norway, south-east Asian and Sahel Zone (Robert-Gangneux and Dardé, 2012). Globally about 30-50% human population has been exposed to parasite, while huge fractions may be chronically infected. A lowest seroprevalence was found 1% in some countries of Far East, while highest was 90% in many parts of South American and European countries (Flegr *et al.,* 2014). When focusing on regions of Southeast Asia, different prevalence rates was observed in adults with high in some equatorial zones, up to 70% sero-positively in human population living in Jakarta regions of Indonesia and 59.7% in inhabitants of Malaysia (Ahmad *et al*., 2014). A high frequency of infection has been reported among pregnant and women of childbearing age from various regions in Latin America, some parts of Central and Eastern Europe, Middle East and different parts of south-east Asia and Africa (Al-Tantawi *et al.,* 2014). Sero-prevalence of infection is highest in moist tropical regions of South America and Africa. Estimated range of infection was reported from 30-90% in South and Central America and European continent (Minbaeva *et al.,* 2013; Wilking *et al.,* 2016). Fonseca *et al.,* (2012) and Débora de Almeida Aloise, *et al.,* (2017) reported 49.5% and 66.2% cases from Brazil, Alvarado-Esquivel *et al.,* (2018) 3.6% and 12.5% cases from Hermosillo City, Mexico, while Fond *et al.,* (2013) reported 43% cases from France. According to Dubey, (2010), infection rates in Chinese is 8.2% (95%CI, 8.06-8.39%, 8,502/103,383), which is comparatively lower than India (24.0%), United States (38.0%), France (61.0%) and Brazil (84.5%). Many studies related to epidemiological aspects of Toxoplasmosis have been conducted in various countries and frequency of infection was different in different regions. Sagel *et al.,* (2011) reported 31% cases from Austria, Berghold *et al.,* (2016) reported 43.3% and 31.5% cases from Styria, which is an Austrian federal state, Smit *et al.,* (2019) reported 4.5% and 5.8% cases from Northern Vietnam, Pan *et al.,* (2017) and Dong *et al.,* (2018), 8.2% and 8.2% cases from China, Andiappan *et al.,* (2014) reported 42.47% and 30.70% cases from Malaysian and Myanmar. Similarly, Davami *et al.,* (2014), Ghasemi *et al.,* (2015), Borna *et al.,* (2013), Daryani *et al.,* (2014) and Ebrahim-Zadeh *et al.,* (2014) reported 13%, 2%, 15%, 25.5%, 26.4%, 39.9%, 40% and 58.3% infected cases from various regions of Iran, Kamal *et al.,* (2015) and Bassiony *et al.,* (2016) with 50.8% and 57.9% cases in Egypt, Mousa *et al.,* (2011) 44.8% and 8.4% cases from Libya, Awoke *et al.,* (2015) and Negeroa *et al.,* (2017) with 18.5% and 75.7% cases from northwest and southwestern Ethiopia, Frimpong *et al.,* (2017) 5.87% cases from Lusaka, Zambia, Ali *et al.,* (2017), Naglaa *et al.,* (2016) and Alghamdi *et al.,*

(2016) with 12%, 21.3%, and 32.5%, 6.4% cases from Saudi Arabia, MadhaviVyas and Rahul Acharya, (2018), Maçin *et al.,* (2018), Pandey, (2018), Samudio *et al.,* (2015) reported infection with a rate of 27.33%, 2.44% and 29.53%, 43% and 84% from many other region of the world. Besides other countries, some studies related to prevalence and epidemiological aspects of Toxoplasmosis have also been conducted and reported from Pakistan. Shah *et al.,* (2017), Shah *et al.,* (2015), Majid *et al.,* (2016), Faisal *et al.,* (2014), Shah *et al.,* (2016), Aleem *et al.,* (2018) and Khan *et al.,* (2014) reported 12%, 20%, 18.41%, 19.25%, 24.7%, 47.2%, and 65.71% cases from various regions of Khyber Pakhtunkhwa province of Pakistan, while Ahmad *et al.,* (2012), Latif *et al.,* (2017) and Tasawar *et al.,* (2012) reported 11.33%, 22%, and 29.45% infected cases from different regions in Punjab province of Pakistan.

2.2.2 Epidemiologic Aspects of Toxoplasmosis: There is epidemiological variation about Toxoplasmosis during pregnancy in various countries. In most European countries *T. gondii* specific IgM and IgG antibodies in pregnant women vary from 9% to 67% (Ramos *et al.,* 2011; Uysal *et al.,* 2013), while in Asia, a sero-prevalence of 0.8% to 63.9% were reported (Song *et al.,* 2005). Frequency of infection has been reported from 18.5% to 92.5% in Africa (Ayi *et al.,* 2009; Kefale *et al.,* 2015), while infection in pregnant women varies from 18.5 to 88.6% in Ethiopian regions (Endalew *et al.,* 2012; Mengistu *et al.,* 2014; Kefale *et al.,* 2015; Woyneshet *et al.,* 2015).

- Fond *et al.,* (2013) conducted a study in France. They observed that above 43% human population were infected by *T. gondii* due to their eating habits, lifestyle and frequent exposure to domestic cats.
- Hassanain *et al.,* (2018) conducted a cross-sectional study to investigate prevalence and associated risk factors of Toxoplasmosis among pregnant women in maternity centers in Egypt. They observed a high positivity rate of IgM rather than IgG antibodies, while low IgG avidity was observed, which indicated acute infection with a possibility of transmission risk to embryo and new-borne child. They reported high frequency of infection among pregnant women, who had history of using immunosuppressive drugs, abortion, cats, and other animals in homes and who had first and second trimester's pregnancy.
- Fonseca *et al.,* (2012) studied epidemiological aspects of Toxoplasmosis in pregnant women in Brazil. They found 49.5% women, who were sero-positive for IgG and 3.6% for IgM with an irregular distribution in frequency of infection in different locations. They observed that 93% pregnant women had no knowledge about Toxoplasmosis, while 24% showed positive serology, but had no clinical manifestations. They found a significant association between positivity of IgG antibodies in pregnant women with presence of pet animals in homes, which suggested that disease might occur in domestic environment.

- Alsammani (2016) conducted a review study to investigate prevalence and associated risk factors of Toxoplasmosis among pregnant women in African and Arab countries. He reported high sero-positivity in both regions, but more in African as compared to Arab countries, which was due to variation in risk factors. He stated that infection of Toxoplasmosis during pregnancy is an underestimated health problem of women in African and Arab countries. The identified risk factors were eating raw meat, proximity with domestic cats, undercooked food and increased maternal age.
- Alvarado-Esquivel *et al.,* (2018) conducted a cross-sectional survey to determine seroprevalence of *T. gondii* infection in women of reproductive age in Hermosillo City, Mexico. They screened 445 women, 16 (3.6%) were found positive for IgG and only 2 (12.5%) were positive for IgM antibodies. The level of IgG antibodies was higher than 150 IU/mL in 37.5% women, followed by 100-150 IU/mL in 25% and 9-99 IU/mL in 37.5% women. Socio-demographic, behavioral variables and consuming boar meat were associated with acquiring of infection.
- Davami *et al.,* (2014) conducted a systematic review on Toxoplasmosis and reported that overall prevalence was 40% among general Iranian population. They claimed that prevalence of infection was increased with age, direct contact with cat, consuming uncooked meat, raw vegetables, fruits, working in animal farms, housewife, low education and residence in rural areas. Among some Iranian women, who were screened for detection of *T. gondii* specific IgM and IgG antibodies by ELISA before marriage, positivity rate of IgM and IgG was 2% and 13%. The sero-positivity for IgG was different in different age groups, while high rate of positive IgM antibodies were found in women, who were <20 years old.
- Pan *et al.,* (2017) reported Toxoplasmosis with a rate of 8.6% in pregnant women, who had gynecological diseases and 16.8 in women, who were cancer patients. They claimed that women, who belong to various groups like animal keeper, butchers, animal takers, low educational level and who used to eat undercooked or raw meat were significantly associated with Toxoplasmosis. Human infection increased from west to east in China, which overlaps with incidence of *T. gondii* in food animals.
- Débora de Almeida Aloise, *et al.,* (2017) investigated seroprevalence and associated risk factors of human Toxoplasmosis from northeastern Brazil. They detected *T. gondii* specific IgG antibodies in 66.2% cases and claimed that age ≥45 years, illiteracy, presence of cats in home, no water tank and consumption of raw and unpasteurized milk were risk factors, which were associated with infection of Toxoplasmosis.

- Ali *et al.,* (2017) conducted a cross sectional study on prevalence of Toxoplasmosis in women in Rafha City of Saudi Arabia. They reported positive of IgG in 12% cases, which was higher in women of ≥30 years old as compared to <30 years old. The positivity of IgG was significantly high (13%) in pregnant as compared to (%%) cases in non-pregnant women and those, who had direct contact to soil (14%) as compared (5%) cases, who had no contact with soil.
- Maçin *et al.,* (2018) retrospectively evaluated suspected patients for sero-positivity of *T. gondii* specific IgM and IgG antibodies. *T. gondii* specific IgM was positive in 2.44% cases and IgG in 29.53% cases, while avidity test was positive in 171 (78.36%) infected cases.
- Smit *et al.,* (2019) studied sero-epidemiological aspects of *T. gondii* infection in pregnant women from Northern Vietnam. They found positivity of IgG with a rate of 4.5% and 5.8% in Hanoi and Thai Binh hospital. They claimed that only 2.0% pregnant women of Hanoi and 3.3% in Thai Binh hospital were aware about infection of Toxoplasmosis.
- Frimpong *et al.,* (2017) studied epidemiological parameters of Toxoplasmosis among pregnant women, who were attending antenatal clinics and Hospital in Lusaka, Zambia. They reported positivity of IgG in 5.87% cases, while no IgM positive case was observed. Among positive cases, 7.81% pregnant women had history of contact with cat and 15.5% were associated with farming profession. They found a weak association of infection with socio-economic status in pregnant women.
- Alghamdi *et al.,* (2016) conducted a cross sectional study to investigate prevalence and genotypes of *T. gondii* among 203 pregnant women in Saudi Arabia. They found IgG and IgM antibodies in 32.5% and 6.4% women. Genotype II was found in 29 (80.6%), while genotype III in 7 (19.4%) infected women.
- Bassiony *et al.,* (2016) carried out cross sectional study to investigate sero-prevalence and associated risk factors of *T. gondii* infection in 382 pregnant women, who had 8-40 weeks gestation and attended antenatal care clinics in Alexandria region in Egypt. ELISA and ICA assay was used for detection of infection. They found prevalence of infection with a rate of (11.3%) in women by RDT, which was significantly increased to 57.9% by ELISA. The sensitivity, specificity, PPV. NPV and diagnostic efficiency of RDT was found 15.8%, 95%, 81.4%, 45.1%, and 49.2%. Age of pregnant women was significantly associated with infection. The diagnostic efficacy of RDT test was less than ELISA in pregnant women.
- Awoke *et al.,* (2015) determined prevalence of Toxoplasmosis and associated risk factor among pregnant women, who attended antenatal care centers in northwest Ethiopia. Prevalence of infection was found in 18.5% pregnant women. Only IgG antibodies were found positive, which

were significantly associated with presence of cats at home, consumption of raw, undercooked meat and abortion history, while no significant association was found between infection and socio-demographic characters, gravidity, gestational age, consuming raw vegetable and history of blood transfusion in infected women.

- Negeroa *et al.,* (2017) conducted a cross sectional study to determine epidemiological aspects of Toxoplasmosis in pregnant women, who visited antenatal care centers in southwestern region of Ethiopia. They found 75.7% pregnant women, who were infected with *T. gondii* and observed Toxoplasmosis with a high frequency in those women, who were 36–44 year old, multigravidae, a history of eating raw meat, vegetables, abortion, drink water from river, streams and who had no history of handling raw meat.
- Faisal *et al.,* (2014) studied Toxoplasmosis in pregnant women in District Swabi, Khyber Pakhtunkhwa province of Pakistan and detected *T. gondii* specific antibodies in 19.25% women. Frequency of infection was high in those women, who were in age group 25-34 years as compared to >34 years old. Prevalence of infection was different in various regions of the same districts. High numbers of infected cases (26%) were recorded in Sodher and Yaqoobi and lower (10%) in Gohati and Dagi region. Prevalence of infection was high in women of young age group as compared to women of old age group.
- Majid *et al.,* (2015) investigated chronic Toxoplasmosis infection and associated risk factors in pregnant women in Khyber Pakhtunkhwa province of Pakistan. Overall infection was found in 18.41% cases of pregnant women. Anti-Toxoplasma IgG antibodies were found in 7.17% cases in first trimester, while highest 31.28% was found in third trimester of pregnancy. Infection was significantly increased with increased of age in infected women. Infection was found with different frequency in the same district and highest rate was found 33.03% cases in region of Upper Dir. History of eating undercooked meat was observed in 45.22% infected cases. Infection was significantly increased with low education level, consuming raw eggs, vegetables, contact with soil, cats, cattle, water sources and use of unpasteurized milk in the studied region.
- Aleem *et al.,* (2018) screened 360 pregnant women for Toxoplasmosis in Matta, Upper Swat region of Khyber Pakhtunkhwa province of Pakistan. They detected antibodies of *T. gondii* in 170 (47.2%) pregnant women. Frequency of infection was high in first trimester (61.7%) as compared to (58.4%) and (27.7%) cases in second and third trimester of pregnancy. Similarly rate of infection was also high (54.7%) in women, who were in age group 18-25 years as compared to (38.8%) cases in women, who were in age group 26-33 year old and uneducated (66.01%) as compared to (22.07%) cases in educated women.

- Shah *et al.,* (2016) studied epidemiological aspects of Toxoplasmosis among women in District Chitral regions of Khyber Pakhtunkhwa province in Pakistan. Lateral flow chromatographic immunoassay technique was employed for detection of *T. gondii* specific IgM and IgG antibodies. Frequency of infection was found in 24.7% women. They observed high infection rate (28.6%) in those women, who were in the age of 25 years, residents of rural areas (33.1%) and (27.2%) cases in those women, who raised cattle, goats, sheep and cattle in their homes.
- Khan *et al.,* (2014) investigated Toxoplasmosis and associated risk factors in women of Malakand Agency, Khyber Pakhtunkhwa province of Pakistan. They screened sera of 420 women by ICT, ELISA and LAT methods and detected Toxoplasmosis in 65.71% women, which was quite alarming. Frequency of infection was high (41.31%) in those women, who were in age group 21-30 years, pregnant than non-pregnant and who had a positive history of various type of abortions. They claimed that IgM ELISA was the most sensitive, reliable and accurate diagnostic method for diagnosis of Toxoplasmosis as compared to ICT and LAT methods.
- Ahmad *et al.,* (2012) investigated human Toxoplasmosis in various regions of Lahore, Pakistan. They used latex agglutination test (LAT) and detected antibodies in 11.33% cases. They found a significant difference of Toxoplasma infection in women of various age groups. Frequency of infection was significantly high (28%) in younger, while low (17.33%) in those, who were 40 years old.
- Latif *et al.,* (2017) studied Toxoplasmosis among pregnant women in Lahore region of Pakistan. They detected IgG antibodies in 22% patients and found high rate (29%) of infection in women, who were in age group 29-39 years as compared to (18%) cases in those women, who were 18-28 years old. Frequency of infection was high (26%) in third trimester as compared to (22%) and (15%) cases in first and second trimester of pregnancy. Frequency of infection was 31% in those women, who had a positive history of miscarriages, low socioeconomic statues and uneducated.
- Shah *et al.,* (2017) examined sera of maternity patient for anti-Toxo IgG and IgM antibodies and reported infection in 12% women. Toxoplasma specific IgG antibodies were found in 5 and IgM and IgG in 7 maternity patients. Frequency of infection was high 25% in women, who were 31-36 years old, while 20% infected women were at third trimester of pregnancy. Pregnant women, who had first pregnancy, low socioeconomic status, consumed undercooked meat, food and a positive history of contact with contaminated soil, cats were found positive with a rate of 3.96%, 36.36%, 30% and 27% respectively.
- Sadiqui *et al.,* (2019) studied distribution of *T. gondii* specific IgM and IgG antibodies among 500 pregnant women, who were in different age groups and gestational periods. They found

antibodies in 24.8% cases with acute infection in 8% women. Among positive women, 54.34% cases of acute infection were reported in first trimester of pregnancy. Among 8% cases of acute infection, 30 pregnant women had a recent history of contact with dogs and 40 with domestic animals.

- Tasawar *et al.,* (2012) investigated Toxoplasmosis in women from southern region of Punjab province in Pakistan. They reported 25.9% women, who were infected with Toxoplasmosis. Frequency of infection was high (57.14%) in women of lower age group and lowest (29.41%) in upper age group. They claimed that frequency of infection decreases with increased in age of women.

2.2.3 Clinical Manifestations of Toxoplasmosis: Infection of Toxoplasmosis has a wide spectrum of clinical diseases, asymptomatic infection in majority of immune-competent person to life-threatening condition in many immune-deficient patients. The disease is classified as asymptomatic, ocular infection, pregnancy associated infection, acquired and reactivated infection in patient with weak immunity and congenital infection (Alavi and Alavi, 2016). Toxoplasmosis associated clinical manifestation in immune-competent infected individuals include regional lymphadenopathy (Alavi and Alavi, 2010), neuro-psychologic disorders (Hamidinejat *et al.,* 2010), ocular and congenital abnormalities, while encephalitis or disseminated disease may also occurred in many immuno-suppressed patients. The classical and common clinical manifestation of recent infection during pregnancy is regional lymphadenopathy (Montoya *et al.,* 2010). Some infected pregnant women showed unusual clinical features such as FUO, tonsillitis, pneumonia and polyneuritis (Abhilash *et al.,* 2013; Nunura *et al.,* 2010; Cuomo *et al.,* 2013). Transmission of parasite to fetus during pregnancy may cause visual problems, hearing abnormalities, seizures mental retardation, hematological disorders and even fetal death (Montoya and Kovacs, 2010; Elahian-Firouz *et al.,* 2014). Congenital infection of Toxoplasmosis is complicated with hydrocephaly, microcephaly, encephalitis, hepatitis, lymphadenopathy and IUFD. Persistent parasite in placenta of infected women is usually associated with congenital infection (Asgari *et al.,* 2013).

- Chiang *et al.,* (2014) compared 30 cases of laboratory confirmed acute infection of Toxoplasmosis with healthy controls for assessment of clinical manifestations. Among infected cases, 20 patients showed flu-like symptoms, 4 had abnormalities in central nervous system, ocular problems in 3, miscarriages in 2, and 1 patient showed congenital infection.
- Carmo *et al.,* (2010) reported an outbreak of human Toxoplasmosis, which occurred in District of Pará State of Brazil, in which 186 infected individuals were epidemiologically, clinically and serologically evaluated. Among these cases, 40 (21%) infected cases showed a

serological profile of acute infection, 41 (22%) cases had suggestive clinical symptoms of acute infection like persistent fever, lymphadenopathy, hepato-splenomegaly, exanthema, while 12 (6.45%) infected cases had nonspecific clinical symptoms like nausea, migraine and respiratory symptoms, while remaining infected individuals were asymptomatic.

- Khurana and Batra (2016) mentioned that lymphadenopathy and chorioretinitis frequently occurred in Toxoplasmosis. Singh, (2016) stated that primary or acute infections in pregnant women causes miscarriages, stillbirths and congenital disease with involvement of eyes and CNS. Moncada and Montoya, (2012) stated that clinical manifestations of congenital disease in children slowly appear in life after birth.
- Abo-Hashim and Attya, (2015) evaluated various diagnostic assays such as rapid ICT, IgM ELISA, immune-blotting and PCR for detection of acute Toxoplasmosis infection in 30 pregnant women. They found infection with a frequency of 3 (10%) by rapid Toxo IgM method, 5 (16.67%) by IgM ELISA, 9 (30%) cases by immune-blotting and 6 (20%) cases by PCR. They claimed that sensitivity of IgM immune-blotting was high for detection of Toxoplasmosis in suspected pregnant women.
- Samudio *et al.,* (2015) studied clinical and epidemiological aspects of Toxoplasmosis infection in patients, who had problems in vision. They found infection in 67 (84%) cases, while ocular toxoplasmosis was detected in 67 (8.9%) sero-positive individuals. They found association of acquiring Toxoplasmosis infection with eating habit of unwashed vegetables and meat of wild animals in infected individuals.

2.2.4 Adverse Pregnancy Outcomes of Toxoplasmosis: Globally, Pakistan is considered a country where ratio of death during pregnancy (260 deaths in 100,000 live births) is high. The world ratio of neonate's mortality is 40% and in these deaths, 36% occurred due to various infections (Darmstadt *et al.,* 2005). Among global neonatal deaths, 7% occurred in Pakistan, where 41 to 70 neonates die per 1,000 in a year (World Health Statistics 2014). According to Mousa *et al.,* (2011), adverse pregnancy outcomes (APO) include two or more than two consecutive spontaneous abortions, IUFD, intrauterine growth retardation, low birth weight (LBW), stillbirths, early neonatal death and congenital anomalies after child deliveries. Maternal infections play a critical role in adverse pregnancy outcome. Many studies have been conducted to establish a relationship between infection of Toxoplasmosis and adverse pregnancy outcomes and rate of vertical transmission in infected pregnant women, but different parameters and methods were used (Abdoli *et al.,* 2014; Abdoli and Dalimi, 2014).

- Shah *et al.,* (2015) studied an association of TORCH group of pathogens with spontaneous abortion in 55 women patients, who had adverse obstetric history. Among these women, 11 (20%) were positive for Toxoplasma specific IgG antibodies, while IgM antibodies were not detected in any case. They claimed a significant association of Toxoplasmosis infection with bad obstetric history in infected women.
- Naglaa *et al.,* (2016) investigated Toxoplasmosis infection among pregnant women in Saudi Arabia. They reported 32 (21.3%) women, who were positive for *T. gondii* specific IgG antibodies, while no woman was positive for IgM antibodies. They observed a significant association between sero-positivity of Toxoplasmosis with maternal age, history of abortion and probability of a child birth with congenital anomalies in infected women.
- Mousa *et al.,* (2011) stated that maternal Toxoplasmosis infection in pregnant women play an important role in adverse pregnancy outcomes, which include two or more than two consecutive spontaneous miscarriages, IUFD, intrauterine growth retardation, babies of low birth weight, stillbirths, early death of neonate and congenital anomalies in children. They evaluated infection of *T. gondii* infection in those pregnant women, who had a previous history of adverse pregnancy outcome in Benghazi region of Libya. They included 143 obstetric patients, who had adverse pregnancy outcome and who had attended gynecology and obstetrics centers. They detected Toxoplasmosis infection in 64 (44.8%) women. *T. gondii* specific IgM was positive in 8.4% cases, while all women, who had a history of habitual abortions, were positive for *T. gondii* specific IgG and IgM antibodies.
- Ghasemi *et al.,* (2015) reported Toxoplasmosis associated abortion and stillbirth from Iran. Based on molecular (PCR) and serological (ELISA) techniques, they evaluated role of *T. gondii* in abortion and stillbirth in infected women. They enrolled 110 pregnant women, who had history of abortion and stillbirth as a case group and 110 pregnant women with normal delivery as control group. They detected positive IgG in 25.5% women of case group (26.8% in abortion and 21.4% in stillbirth) and 26.4% women in control group. The positivity of *T. gondii* specific IgM was detected in 2.7% women of case group (3.6% in abortion and 0% in stillbirth) and 0.9% in control group. DNA of *T. gondii* was detected in 6.4% women in case group (7.3% in abortion and 3.6% in stillbirth) and 1.8% in control group. Habits of eating undercooked meat was reported as a major risk factor for congenital infection They concluded that positivity of PCR for detection of *T. gondii* DNA was 3.7 times higher in women, who were positive for history of abortion and stillbirth as compared to those women, who had normal deliveries. They suggested that *T. gondii* was involved in etiology of abortion and stillbirth in infected pregnant women.

- Pandey, (2018) investigated seroprevalence of *T. gondii* in antenatal patients, who had history of adverse pregnancy outcome. He compared 174 women with BOH (cases; Group-1) with 106 antenatal women, who had normal obstetric history (controls; Group-2). He found *T. gondii* specific IgG antibodies in 43% and IgM in 25% cases, while IgG and IgM was simultaneously found positive in 14.4% cases. He reported adverse pregnancy outcomes like abortions, preterm delivery, IUFD, congenitally malformed babies and neonatal death in 0.7%, 50%, 4.2%, 9.7%, 3.5% and 0.7% infected women, while 31.3% women had term deliveries. He observed that *T. gondii* specific IgG and IgM antibodies were significantly higher in those women, who had positive history of adverse obstetric and infection in ongoing pregnancy.
- Kamal *et al.,* (2015) estimate sero-positivity of Toxoplasmosis by ELISA and its associated risk factors among women of high risk pregnancy and low risk antenatal care clinic in Egypt. They reported Toxoplasmosis in 50.8% cases of high-risk pregnancy group, which was significantly different from normal pregnancy group. They observed that abortion was a common adverse obstetric outcome, which was observed in 56.5% cases of infected women, while post-delivery adverse outcomes were detected in 80.3% cases of high-risk pregnancy group as compared to 20% in women of normal pregnancy group. Sero-positivity of Toxoplasmosis infection was significantly associated with residence in rural area, low socioeconomic statues and consumption of undercooked meat.
- Madhavi Vyas and Rahul Acharya, (2018) demonstrated a strong association between TORCH group of pathogens with recurrent miscarriages, intrauterine growth retardation, IUFD, preterm deliveries, early fetal death after delivery and congenital abnormality. They claimed that during current pregnancy, a history of previous pregnancy wastages with a positive serological test would be helpful in clinical management of Toxoplasmosis in order to reduce adverse pregnancy outcome in infected women.
- Li *et al.,* (2014) analyzed data on Toxoplasmosis associated adverse pregnancy outcomes. They quantified risks of congenital infection and abnormal pregnancy outcomes with acute maternal infection of Toxoplasmosis. The incidence of abnormal pregnancy outcomes (APO) in infected group was significantly higher than uninfected group of pregnant women. Prevalence of infection was significantly higher in women with abnormal-pregnancy outcome as compared to normal pregnancy group, while collective rate of vertical transmission of parasite was 20% in maternal infection. The incidence of vertical transmission of parasite in those women, who were infected in first, second and third trimester of pregnancy was increased from 5%, 13% and 32% respectively. Vertical transmission of parasite was observed in 13%, 13% and 24% women, who were treated

with spiramycin, PSF (pyrimethamine + sulfadiazine + folinic acid) or PS (pyrimethamine + sulfadiazine) with combination of spiramycin or other untypical treatments. They claimed that Toxoplasmosis can cause adverse obstetric outcomes in infected pregnant women.

- Umesh *et al.,* (2015) investigated Toxoplasmosis infection in 37 women, who had history of adverse obstetric. Among these women, 3 (8.10%) were infected with *T. gondii*. Abortions was observed in 2 (5.40) and IUFD in 1 (2.70%) infected cases.
- Pradhan, (2015) studied 109 patient from regions of eastern Nepal, who were in age group of 20-40 years. Among these patients, 28 (25.6%) were confirmed with recent infection of *T. gondii.*
- Dhruba *et al.,* (2014) conducted a descriptive case control serological study for assessment of TORCH group of infections and its association with spontaneous abortions. They investigated 43% cases of incomplete abortions and 26% cases of complete abortions. They detected 1.3% cases, who were positive for antibodies (IgM, IgG) of TORCH group of pathogens. Among these infected cases, frequency of Toxoplasmosis was 77.9%, while there was no significant difference in frequency of infection in women of various age group and type of abortion
- Aljumaily *et al.,* (2013) conducted a study to investigate infection of Toxoplasmosis in 2566 Iraqi women, who had confirmed history of various types of adverse obstetric outcomes. Toxoplasmosis was found in chronic form in 19.68% cases, while acute infection was detected in 1.05% cases.
- Liua *et al.,* (2018) investigate effect of *T. gondii* PRU strain infection on bias of decidual macrophage polarization and its involvement in adverse pregnancy outcomes. They established adverse pregnancy outcome in a mouse model by infection with *T. gondii* PRU strain. They measured and studied expression levels of various functional molecules in decidual macrophages of infected animal, which showed that *T. gondii* caused serious adverse pregnancy outcome in infected animal. They found congestion and infiltration of inflammatory cell in placentae of infected animal. They found a decrease in M2 markers (CD206, MHC-II and arginase-1) in decidual macrophages after infection, while expression of M1 markers (CD80, CD86, iNOS, cytokines TNF-α, IL-12) was increased. A positive expression of iNOS was also observed in decidua basalis of infected mice. They concluded that *T. gondii* was responsible for bias of M2 decidual macrophages toward M1, which caused alteration in immunosuppressive microenvironment at maternal-fetal boundary and contributes to adverse pregnancy outcomes.

Materials and Methods

3.1 Study Design

The present study was carried out from March 2017 to May 2020 in different districts of Khyber Pakhtunkhwa province of Pakistan. Those women patients, who were visiting government, private medical, healthcare and maternity centers for clinical examination were randomly included and consented. A simple questionnaire was designed for collection of epidemiological, socio-demographic, clinical and obstetric data (Annexture-1). The questioner was based on short clinical history of patient in Yes/No type questions. All enrolled patients were requested to provide data on designed questioner along with 10 ml blood sample in a clean disposable syringe for laboratory examination. All patients were enrolled on voluntary basis.

3.2 Sample Size

During the current study, (n=3586) patients were clinically examined and screened for emerging zoonotic diseases (Brucellosis, Toxoplasmosis) from 7 divisions (24 districts) of Khyber Pakhtunkhwa province of Pakistan (figure: 3.1). Among 3586 women, 247 women were screened from district Peshawar, 148 Nowshera, 189 Charsadha, 239 Mardan, 141 Swabi, 223 Swat, 193 Malakand, 107 Shangla, 140 Lower Dir, 172 Upper Dir, 134 Buner, 105 Chitral, 192 Kohat, 128 Hangu, 109 Karak, 120 Bannu, 103 Lakki Marwat, 188 D.I Khan, 99 Tank, 170 Abbottabad, 105 Haripur, 139 Mansehra, 110 Battagram and 85 women from district Kohistan respectively.

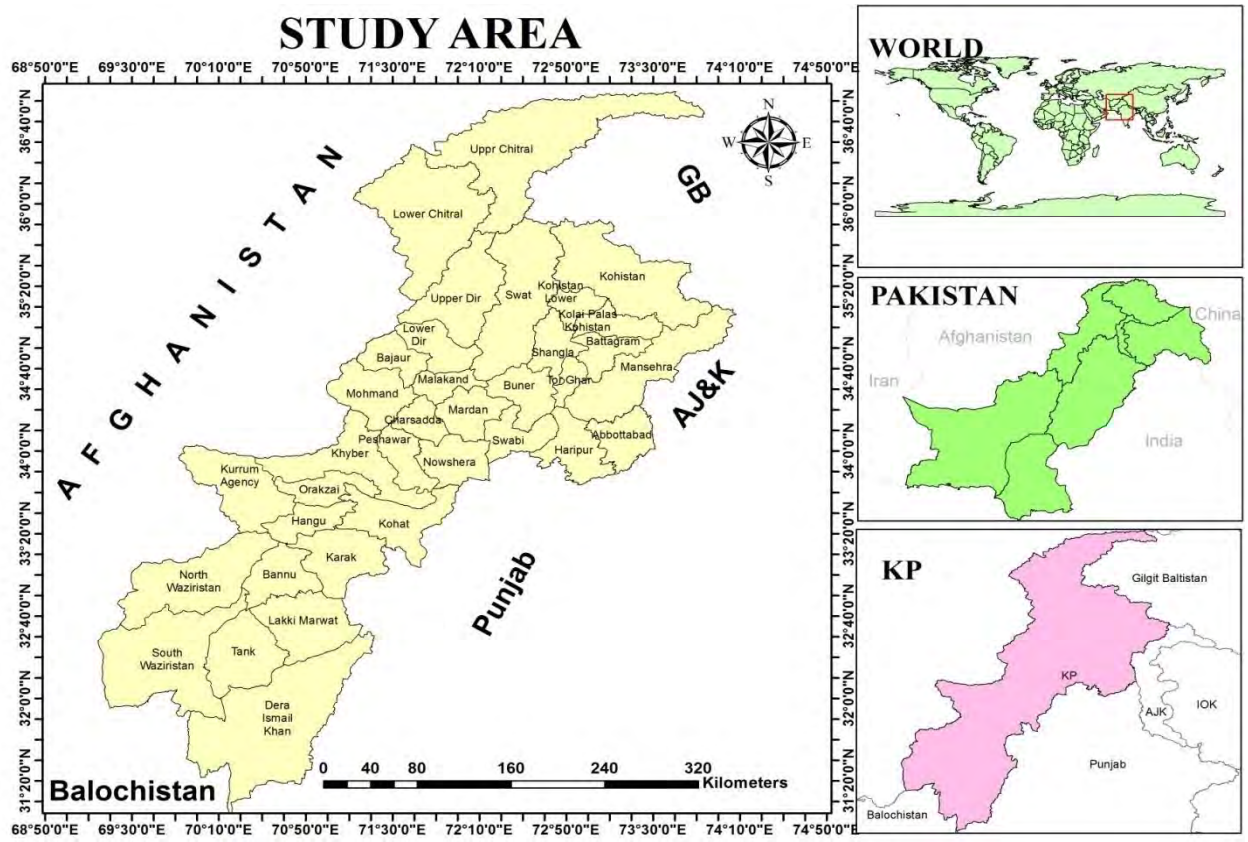

Figure 3.1: GIS map of study region, which show sampling regions (24 Districts) in Khyber Pakhtunkhwa province, Pakistan.

3.3 Collection of Venous Blood

Venous blood was collected as per recommended protocol mentioned by Cheesbrough, (2006). A Sterile and dry, 10 ml capacity disposable plastic syringes with 19 SWG disposable needles was used for collection of 10 ml venous blood from all enrolled women patient. A soft tubing tourniquet was applied to upper arm of patient to enable blood veins to be seen and felt for 2-3 minutes. The patient was asked to make a tight fist, which made blood veins more prominent. Before puncturing vein, blood collecting site was cleaned with cotton wool socked in 70% Isopropyl alcohol and allowed to dry. After collecting sufficient blood, tourniquet was released and patients were instructed to open their fists. The needle was slowly removed and puncture site was pressed with a piece of clean dry cotton wool and were instructed to continually press on puncture site until bleeding was stopped. The vein was checked for bleeding and was covered with a small sterile sticky dressing paper. The blood was immediately dispensed into two separated tubes, a gel tube for serological assays of brucellosis (SPAT, RBPT, ELISA), Toxoplasmosis (ICT, LAT, ELISA for quantitative detection of immunoglobulin (IgM, IgG) and biochemical analysis for

CRP, AST, ALT tests and into EDTA tubes for PCR test and hematological assays like ESR and CBC. These blood containing tubes were properly labeled by permanent marker with patient codes for proper identification. These blood tubes were kept in cooler containing ice and transferred to Microbiology Section, Veterinary Research Institute (VRI) Peshawar for serological tests, while hematological and biochemical tests of positive blood samples were performed in pathology Department, Government Post Graduate Lady Reading Hospital (LRH) Peshawar.

3.4 Collection of Data

Data was collected by two methods i.e questioner and laboratory methods. Data generated from questioner was patient's socio-demography, risk factor of disease transmission, obstetrical profiles, clinical signs, symptoms and duration of disease. Laboratory generated data was based on results of PCR and serological tests for brucellosis (SPAT, RBPT, IgM and IgG ELISA), Toxoplasmosis (ICT Lateral flow chromatography immunoassay, Latex agglutination test, Indirect ELISA for IgM and IgG, hematological tests such as CBC, ESR and biochemical tests like CRP, AST and ALT.

3.4.1 Inclusion criteria

All women patients were included in the current study, who were married and in reproductive (17-45 years) age group, pregnant, non-pregnant, permanent resident of any districts in Khyber Pakhtunkhwa province, who visited to obstetrician, either for obstetrical problems or other clinical problems and to whom, concerned clinician or obstetrician had recommended Brucellosis or Toxoplasmosis specific screening assays and who were also willingly accepted to participate in current study.

3.4.2 Exclusion criteria

All women patients were excluded from the current study, who were unmarried, <17 year or >45 year old, women at menopausal stage, clinically declared infertile or who had other diagnosed chronic diseases or infection or who were not willingly participate in the current study or to whom, the concerned clinician had not advised screening assays for brucellosis or Toxoplasmosis.

3.5 Diagnosis of Zoonotic Diseases (Brucellosis, Toxoplasmosis)

Recommended clinical and laboratory criteria was followed for diagnosis and confirmation of Brucellosis and Toxoplasmosis infection. For brucellosis, clinical criteria were followed as mentioned by Alavi and Alavi, (2011), which was based on major or minor criteria. Major clinical criteria were history of close animal contact along with fever and joint involvement, while minor clinical criteria was based only on selected clinical symptoms such as sweating, headache, weight loss, chills and malaise. These clinical manifestations were further confirmed by Brucella specific serological laboratory techniques such as SPAT, RBPT and IgM and IgG ELISA assays. The cutoff titer for Brucella specific SPAT assay was considered positive at 1:160 titers as recommended by Alton *et al.,* (1988) and Young *et al.,* (2005). The

positivity of these diagnostic assays were further confirmed by quantitative detection of Brucella specific immunoglobulin (IgM and IgG) on ELISA assay. Patients of Brucellosis were further classified as acute (≤8 weeks), sub-acute (8-52 weeks) and chronic (≥1 year) cases on clinical ground and according to duration of disease symptoms (CDC, 2020).

3.6 Serological Assays and PCR for Assessment of Brucellosis Infection:

3.6.1 Serological Assays

3.6.1.1 Method A: Rapid Slide Test

The test was perfumed according to recommended protocol of manufacturer (Laboratory Diagnostics Co. Inc. USA) as under:

i. A clean and transparent glass sheet was divide into 1 ½ inch squares with a diamond tipped pencil.
ii. Unheated and clear serum was dispensed from left to right in consecutive squares on glass sheet with an amount of 0.08ml: 0.04ml: 0.02ml: 0.01ml and 0.005ml. The procedure was repeated for positive and negative control sera.
iii. Vials containing *B. abortus* and *B. melitensis* febrile antigen was gently shaken to ensure a uniform suspension of antigen.
iv. One drop febrile antigen suspension was separately dispensed just below to each quantity of serum sample. Patient's sera and Brucella antigens was mixed well using a piece of wood applicator stick. Separate sticks were used for mixing each quantity of serum. Each mixture had covered an area of approximately ½ inch by 1 inch.
v. Glass sheet was rotated by a mechanical shaker at 150 rpm for 2-3 minutes.
vi. The test was observed for agglutination using a good indirect light source against a dark background.
vii. Positive serum of known titer and negative was run parallel as control with each batch of testing sera. The serum samples which showed agglutination were considered Brucella positive.

The results were interpreted as:

4+ 100% organisms were agglutinated
3+ 75% organisms were agglutinated
2+ 50% organisms were agglutinated
1+ 25% organisms were agglutinated
± Less than 25% organisms were agglutinated
_ Negative no agglutination was observed

3.6.1.2 Method B: Test Tube Titration Test

1. Ten 12×75mm transparent glass test tubes were placed in a rack.
2. 1.9 ml of 0.9% sodium chloride solution was dispensed into first tube.
3. 10ml of 0.9% sodium chloride solution was dispensed into remaining tubes.
4. 0.1ml of patient serum was dispensed into first tube and was mixed well. 1.0 ml diluted serum was transferred from first tube to second tube. The procedure was repeated until all ten tubes achieved serial two-fold serum dilutions from 1:20 to 1:10,240.

   1.0ml diluted serum was discarded from tube No-10. Tube No. 1 was considered as 1:20 dilution. The procedure was repeated in separated tubes for positive and negative control sera.
5. Vials containing *B. abortus* and *B. melitensis* febrile antigen suspension was gently shaken and one drop from these vials was dispensed into each tube.
6. The rack was shaken well to mix antigen and serum and was placed in water bath. Recommended temperature and incubation time was 37 Ċ for 48 hours.
7. After incubation, rack of test tubes was carefully removed from water bath and observed for agglutination with the help of indirect light source against a black background, which provided optimum condition for reading test tubes.
8. The results were interpreted as follows:

   4+ All organisms were clumped in bottom of test tubes and supernatant fluid was clear.

   3+ Approximately 75% organisms were clumped and supernatant fluid was slightly cloudy.

   2+ Approximately 50% organisms were clumped and supernatant fluid was moderately cloudy.

   1+ Approximately 25% organisms were clumped and supernatant fluid was cloudy.

   0 No agglutination was observed and suspension was completely cloudy.
9. Patient's sera, which gave 2+ reactions, were considered positive for Brucella infection.
10. Sera, which showed positive agglutination at last dilution was considered as serum titer.

3.6.1.3 Rose Bengal Plate Test (RBPT)

The patients' sera were also subjected to commercially available RBPT test antigen (Lillidale diagnostics, UK) prepared from strain 99 of *B. abortus* and was stained with Rose Bengal dye, suspended in acidic buffer (pH 3.65). The acidic pH of antigen suspension prevents non-specific bacterial agglutination, which increased specificity of test. The RBPT is a fast, simple and sensitive assay and detects IgM, IgG and IgA antibodies against *B. abortus*, *B. melitensis* and *B. suis* in animals and human sera. The test was carried out according to manufacturer instruction (Lillidale diagnostics, UK) as under:

1. Before starting RBPT assay, antigen and patients sera were brought to room temperature.
2. 30 µl of each serum sample was dispensed on a clean agglutination plate.
3. RBPT antigen vial was gently shaken before use and 30 µl was placed next to serum sample on agglutination plate. Antigen and serum was mixed well.
4. Agglutination plate was shaken for 5 minutes and results were interpreted as;

a. No Agglutination indicated absence of anti-Brucella antibodies in sera.
b. Agglutination indicated presence of anti-Brucella antibodies in sera.
c. A slight agglutination was also considered as a positive result.

5. For validation of RBPT antigen test, a positive serum of known titer was run parallel as a positive control with each batch of testing sera.

3.7 ELISA for Brucella specific Immunoglobulin IgM and IgG:

The patients were also screened for detection of Brucella specific immunoglobulin (IgM, IgG) by commercial available quantitative i-ELISA (Vircell microbiologist, Spain) and the assay was perfumed according to manufacturer protocol.

3.7.1ELISA Assay for Quantitative Detection of Brucella Specific Immunoglobulin (IgM)

Assay Procedure:

Before stating Brucella specific IgM and IgG quantitative ELISA assay:

1. Incubator was set at 37±1ºC.
2. All reagents with ELISA kit were brought to room temperature without removing ELISA plates from plastic bag.
3. All components were carefully shaken before use.
4. 96-wells ELISA plate coated with LPS antigen of *Brucella abortus*, strain S-99 was used. Four wells were used for control, two for cut off serum, one for negative and one for IgM positive sera. Separated ELISA kit for quantitative detection of IgM and IgG was used.
5. Except wells for controls, 25 µl human IgG sorbent has been dispensed into each required wells. 5 µl patient serum samples were further added into each well, which was followed by adding 75 µl serum diluents (blue color phosphate buffer containing protein stabilizers). The control wells were prepared by adding first 100 µl serum diluents solution to each well and then 5 µl IgM positive control serum, 5 µl IgM cut off control serum (in duplicate) and 5 µl IgM negative control serum to corresponding wells. The plate was shaken in plate shaker for 2 minutes for homogenous mixing of reagents.

6. The plate was covered with a sealing sheet and incubated at 37± 1ºC for 45 minutes.
7. The sealing sheet was removed after 45 minutes and liquid from all wells were removed. The plate was washed five times with 0.3 ml of 20x washing solution (a phosphate buffer containing Tween R-20 and Proclin) per well. All liquid was drained off from wells.
8. 100 µl IgM conjugate solution (anti-human IgM peroxidase conjugate dilution in orange-colored) was immediately dispensed into each well.
9. The plate was again covered with a sealing sheet and incubated at 37±1ºC for 30 minutes.
10. The sealing sheet was removed and liquid was aspirated from all wells and again washed five times with 0.3 ml washing solution.
11. 100 µl substrate solutions (containing tetra methyl benzidine, TMB) were immediately dispensed into all wells.
12. ELISA plate was protected from light in aluminum foil and incubated at room temperature for 20 minutes.
13. 50 µl stopping solution (0.5 M sulphuric acid) was immediately dispensed into all wells.
14. ELISA plate was read with a spectrophotometer at 450/620 nm within 1 hour after adding stopping solution.

Interpretation of Results

The mean O.D was calculated for cut off serum and antibodies index was measured by recommended formula of ELISA kit as mentioned below:

Antibody index: (sample O.D / cut off serum mean O.D) x10

Patients sera with antibody index <9 was reported negative, 9-11 equivocal and >11 positive for brucellosis. Sera with equivocal results (9-11) were retested on ELISA and were also confirmed by genus specific PCR for Brucella.

3.7.2 ELISA Assay for Quantitative Detection of Brucella Specific Immunoglobulin G (IgG)

Assay procedure:

1. 96-wells ELISA plate coated with LPS antigen of *B. abortus,* strain S-99 was used. Four wells were used for control, two for cut off serum, one for negative and one for positive sera.
2. 100µl serum diluents solution (blue color phosphate buffer containing protein stabilizers) was dispensed into all wells, which was followed by adding 5µl testing serum sample, 5µl IgG positive control serum, 5µl IgG cut off control serum (in duplicate) and 5µl IgG negative control

into corresponding wells. The plate was shaken in a plate shaker for 2 minutes in order to achieve homogenous mixture of reagents.

3. The plate was covered with aplastic sealing sheet and incubated at 37±1ºC for 45 minutes.
4. After incubation, sealing sheet was removed and liquid was drained off from all wells. The plate was washed five times with 0.3 ml 20x washing solution (a phosphate buffer containing Tween R-20 and Proclin) per well.
5. 100µl IgG conjugate solution (anti-human IgG peroxidase conjugate dilution in orange-colored containing buffer) was immediately dispensed into all wells of plate.
6. The plate was covered with sealing sheet and incubated at 37±1ºC for 30 minutes.
7. The sealing sheet was removed from ELISA plate and liquid was drained off from all wells and plate was washed five times with 0.3 ml 20x washing solution (a phosphate buffer containing Tween R-20 and Proclin) per well.
8. 100µl substrate solution containing tetra methyl benzidine (TMB) was immediately dispensed into all wells.
9. ELISA plate was protected in aluminum foil from light and kept at room temperature for 20 minutes.
10. 50 µl stopping solution (0.5M sulphuric acid) was immediately dispensed into all wells.
11. ELISA plate was read with a spectrophotometer at 450/620 nm within 1 hour after adding stopping solution.
12. The mean O.D was calculated for cut off serum and antibodies index was measured by recommended formula of ELISA kit as mentioned above in ELISA IgM procedure.
13. Positive, negative and cut off controls were run with each batch to check validation of IgM and IgG ELISA kit assay. The optical densities (O.D) were observed and matched with specific values mentioned in kit protocol. The test was considered invalid and repeated when values of positive control >0.9, negative control <0.5 and cut off control (>0.55, <1.5) were not observed within recommended ranges.

3.8 Molecular Techniques for Diagnosis of Brucellosis

3.8.1 Extraction of DNA from Blood

Procedure:

i. Bacterial DNA was isolated from patient blood with a DNA purification kit. (Bio-Basic Inc. kit, USA).

ii. 500 µl whole blood sample of women patient was taken into an Eppendorf. The tube was centrifuged at 3000 rpm for 3 minutes at 4 C. The supernatant was discarded.
iii. 0.8 µl TBP buffer was added into Eppendorf tube and gently vertex for a while and re-centrifuged at 4000 rpm for 3 minutes. Blood pellet became colorless and supernatant was again discarded from Eppendorf tube.
iv. The sample was again centrifuged for 3 minutes at 4 C.
v. 0.5 µl TBM buffer was added into Eppendorf tube. The tube was vigorously vertex and 3ml proteinase K enzyme was added in Eppendorf tube. The Eppendorf tube was incubated at 55C for 30 minutes
vi. Due to visibility of insoluble material in some cases, the tubes were re-centrifuged for 2 minutes at 5,000 rpm. The supernatant was transferred in 0.2 ml Eppendorf tube and 260µl absolute ethanol was added in the tube.
vii. The mixture was subjected to EZ-10 column, which is in 2.0ml collection tube. The tube was centrifuge at 10,000 rpm for 2 minutes. Discarded flow-through in collection tube.
viii. 500 µl washing solution was added and the tube was centrifuged at 10,000 rpm for 1 minute.
ix. The washing step was repeated.
x. The column was placed into 1.5 ml clean microfuge tube and 30-50ul elution buffer solution was added in center part of membrane in the column. The tube was incubated at 37 or 50 ºC for 2 minutes.
xi. To remove bacterial DNA from column, the tube was spin at 10,000 rpm for 1 minute.
xii. An aliquot of purified bacterial genomic DNA was stored at -20 ºC.

3.8.2 Amplification of DNA by PCR

Procedure

Purified genomic DNA of Brucella, which was isolated from blood samples of women patients were subjected to genus specific PCR for amplification of 223-bp DNA fragment of a gene in Brucella, which code synthesis of an immunogenic protein, which has a molecular mass of 31 kDa and located on external surface of cell membrane of *B-abortus* (BCSP31) as mentioned by Kamal *et al.,* (2013). BCSP 31 is a protein, which is present in all biovars of Brucella, while specific to genus of Brucella. DNA amplification was performed by PCR machine with general primers (Oligonucleotide) sequence BG-F, 5'-GCTCGGTTGCCAATATCAATGC-3' and BG-R 5'-GGGTAAAGCGTCGCCAGAAG-3'. PCR was conducted as per protocol mentioned by Khosravi *et al.,* (2006). The reaction was conducted in a final volume of 50 µl reaction mixture for each reaction of sample, which contained 5 µl of 10X PCR buffer; 5

µl of $MgCl_2$; 5ul of 2.5 mMdNTPs; 0.5ul of AmpliTaq DNA polymerase enzymes; 2ul of forward primer; 2ul of reverse primer; 5ul of DNA sample and 25.5ul of distilled water. PCR reaction mixture tubes were transferred to a thermal cycler for amplification of Brucella DNA. The cyclic parameters for PCR reaction were adjusted as: 95 ºC for 3 minutes, which was followed by 35 cycles of denaturation at 95 ºC for 2 minutes, annealing at a temperature of 55ºC for 2 minutes and extension at 72 ºC for 2 minutes and a final extension at 72 ºC for 4 minutes. PCR based amplified DNA product of Brucella was stained with 0.5 mg/ml ethidium bromide dye. The DNA bands of 223 base pair in a size of 31kD were analyzed by an Agarose gel (2% w/v) electrophoresis and were visualized by standardized equipment using ultra-violet (UV) trans illuminator (Bio-Rad Imager Gel Doc XR System, 2008).

3.8.3 Agarose Gel Electrophoresis for PCR Product

After amplification of bacterial DNA from patient blood, the samples of DNA were subjected to gel electrophoresis for confirmation of Brucella DNA in the sample as per protocol described by Garshasbi *et al*., 2014) with some modification.

i. A clean gel caster was placed on a horizontal smooth surface and the comb was fixed into gel caster.
ii. 1.2g Agarose (electrophoresis grade) was added into a clean flask, containing 100 ml of 1X TAE buffer (0.4 M Tris acetate, pH: 7.5, 20 mM EDTA) and was boiled, so that agar dissolve. The flask was cooled to 55 ºC.
iii. The liquefy Agarose was poured into gel caster from the flask and 0.5-1.0 mm space was kept between bottom of teeth and base of gel caster, so that sample wells were completely sealed.
iv. After complete solidification of gel, the comb was carefully removed from solidified gel and gel was placed in electrophoresis tank, which contained 1X TAE running buffer.
v. In one well, 100b of DNA ladder was loaded, while in remaining wells, 10 µl of PCR amplified DNA product and 5 µl DNA loading dye (50% glycerol, 6X TAE, 1% bromophenol blue) was carefully loaded by using micropipettes.
vi. The gel tank was connected to an electric power supply for DNA electrophoresis. Electric voltage was adjusted at 120 volts. The gel tank was kept in running position for 60 minutes.
vii. To visualized PCR product (DNA), the gel was carefully stained with a florescent tag (ethidium bromide, 1ug/ml) for 15 minutes.
viii. To remove extra stain, the gel was washed in water and kept in gel documentation system (Bio-Rad Imager Gel Doc XR System, 2008) for visualization of PCR amplified DNA product under UV trans-illuminator.

3.9 Serological Assays for Assessment of Toxoplasmosis Infection:

3.9.1 Toxoplasmosis Latex Agglutination Tests (LAT)

The test was performed according to manufacturer (Fortress Diagnostics Limited UK) protocols as under.

i. All reagents and samples were brought to room temperature.
ii. The serum samples were diluted by 1/16 in physiological saline.
iii. One drop diluted serum was placed on black circle of slide.
iv. The latex reagent was mixed well and one drop was added over each drop of testing serum.
v. Latex reagent and serum was mixed well with a micropipette on slide.
vi. The presence or absence of agglutination was observed within a period of 3 minutes.
vii. A clear evidence of agglutination was considered positive test.
viii. Each batch of tests was validated with a positive and negative control.

3.9.2 Toxo IgG/IgM Rapid Test Cassette Method: (Lateral Flow Chromatographic Immunoassay)

Toxo IgG/IgM Rapid Test Cassette was a lateral flow chromatographic immunoassay, which is used for qualitative detection of Toxoplasma specific IgG and IgM antibodies in human serum and provides an aid in rapid diagnosis of Toxoplasma infection. The test was performed according to method of Chinese pharmacopeia, China Biological Products Procedures as under:

Assay procedure:

i. The test cassette was removed from sealed pouch and kept at horizontal position.
ii. About 10µl patient sera was transferred from gel tube to "S" well of test cassette, followed by mixing of 2 drops buffer (about 70µl) with patients sera in test cassette and timer was started.
iii. The test cassette was left at room temperature for 15 minutes until colored lines appeared in test cassette. The result was interpreted within 15 minutes as:

a. Positive: Control line and at least one test line (IgM, IgG), which appeared on kit membrane was considered as a positive test.
b. Appearance of IgG test line was considered presence of *T. gondii* specific IgG antibodies, while appearance of IgM test line was considered presence of IgM antibodies. The appearance of both IgM and IgG were also considered as a positive test.
c. The test was considered negative, when only one colored line appeared in control negative region (C) and no colored line was observed in IgM or IgG test line region.
d. The test was considered invalid and repeated with a new test kit, when no line was observed in region of control line.

3.9.3 Toxoplasma ELISA IgM Capture method

i. 96-wells ELISA plate coated with anti-IgM antibodies (µ-specific) was used. Four wells were used for control, two for cut off serum and one for negative and positive sera.
ii. 100 µl serum dilution solution (blue color phosphate buffer containing protein stabilizers) was dispensed into all wells except four wells assigned for controls, which was followed by adding 5 µl patients sera into all testing wells, while 100 µl IgM positive control, 100 µl IgM cut off control (cut off in duplicate) and 100 µl IgM negative controls were also dispensed into corresponding wells.
iii. The plate was covered with a plastic sealing sheet and incubated at 37±1ºC for 60 minutes.
iv. The sealing sheet was removed from ELISA plate and liquid was aspirated from all wells. The plate was washed five times with 0.3 ml of 20x washing solution (a phosphate buffer containing Tween R-20 and Proclin) per well. Remaining liquid was drained off from all wells.
v. Conjugate solution was prepared by adding 3 ml Toxoplasma reconstitution solution (a buffered solution for reconstitution of lyophilized conjugate) to one vial of lyophilized Toxoplasma conjugate (*T. gondii* antigen labeled with peroxidase, lyophilized). The vial was kept at standing position for one minute to allowed rehydration and was mixed carefully by vortexing.
vi. 100 µl reconstituted conjugate was immediately dispensed into all wells.
vii. The plate was again covered with plastic sealing sheet and incubated at 37±1 ºC for 60 minutes.
viii. The seal was removed and liquid from all wells was again drained off. The plate was washed five times with 0.3 ml of 20x washing solution per well. All remaining liquid in wells was drained off.
ix. 100 µl substrate solutions containing tetramethyl benzidine (TMB) was immediately dispensed into all wells.
x. The plate was protected from light into aluminum foil and Incubate at room temperature for 20 minutes.
xi. 50 µl stopping solution (0.5 M sulphuric acid) was immediately dispensed into all wells.
xii. The plate was read with spectrophotometer at 450/620 nm within 1 hour after adding stopping reagents.
xiii. Positive, negative and cut off controls were run with each batch of testing sera, which validated performance of kit. The test was considered valid, when optical densities (O.D) of positive, negative and cut off control were >0.9, <0.5and (>0.55. <1.5), otherwise tested sera were retested on new kit.

xiv. **Result Interpretation:** Mean O.D was calculated for cut off serum and antibodies index was measured by recommended formula of ELISA kit.

Formula: Antibody index: (sample O.D / cut off serum mean O.D) x10

Antibody index < 9 was reported negative, 9-11 equivocal and >11 positive for Toxoplasma specific IgM. Sera with equivocal results (9-11) were retested on ELISA for confirmation.

3.9.4 Toxoplasma ELISA for Immunoglobulin G (IgG)

1. 96-wells ELISA plate coated with *T. gondii* antigen strain RH (ATCC 50174) were used. Four wells were used for control, two for cut off serum and one for negative and positive control sera.
2. 100 µl serum diluents (blue color phosphate buffer containing protein stabilizers) was dispensed into all wells, which was followed by adding 5µl testing sera into respective wells, 5 µl IgG positive control serum (IgG anti-Toxoplasma), 5µl IgG cut off control serum (IgG anti-Toxoplasma) in duplicate and 5µl IgG negative control serum into corresponding wells. The plate was shaken in shaker for 2 minutes for homogenous mixing of reagents.
3. ELISA plate was covered with aplastic sealing sheet and incubated at 37±1ºC for 45 minutes.
4. The sheet was removed and liquid was drained off from all wells and plate was washed for five times with 0.3 ml washing solution (phosphate buffer containing Tween R-20 and Proclin) per well. Remaining liquids in plate wells were properly drained off.
5. 100µl IgG conjugate solution (anti-human IgG peroxidase conjugate dilution in orange-color buffer) was dispensed into all wells.
6. The plate was again covered with plastic sealing sheet and incubated at 37±1ºC for 30 minutes.
7. Sealing sheet was removed and liquid was drained off from all wells. The plate was washed five times with 0.3 ml of 20x washing solution (a phosphate buffer containing Tween R-20 and Proclin) per well.
8. 100 µl substrate solution containing tetramethyl benzidine (TMB) was dispensed into all wells.
9. ELISA plate was protected from light in aluminum foil and Incubated at room temperature for 20 minutes.
10. 50 µl stopping reagent (0.5 M sulphuric acid) was immediately dispensed into all wells.
11. ELISA plate was read with spectrophotometer at 450/620 nm within 1 hour of stopping reactions.
12. The mean O.D was calculated for cut off serum and antibodies index was measured by recommended formula of ELISA kit as mentioned in procedure of ELISA IgM.

3.10 Hematological and Serum Biochemical Assays

3.10.1 Hematology

Hematological parameters of women patients, who were positive for Brucellosis and Toxoplasmosis, were determined from EDTA containing blood by automatic hematology analyzer machine. Only selected parameters such as hemoglobin (Hb), WBC, neutrophils, lymphocyte, monocytes, eosinophilis, basophiles and platelet counts were considered and recorded. According to Cheesbrough, (2006), hemoglobin values <11.5 g/dl, leukocyte count 4.00-12.0 x10^9/L and platelet count <150 x 10^9/L were considered and recorded as anemia, leucopenia and thrombocytopenia.

3.11 Analysis of Selected Inflammatory Markers (ESR, CRP)

3.11.1 Erythrocyte Sedimentation Rate (ESR):

ESR was measured by Westergreen ESR determination method (Jou *et al.,* 2011). A Westergreen tube with 200 mm in length was used. Exact 2ml fresh blood was dispensed into Westergreen tube which contained 0.5 ml of 3.8% sodium citrate and was mixed well. The Westergreen tube was filled to mark zero and kept in ESR stand in straight position and timer was switch on. The reading of blood distance from above to below in ESR tube was observed after 1 hour. The value of ESR was recorded as mm/hour (Kratz *et al.,* 2017). ESR value up to 20 mm/hour in women was considered as normal value (Cheesbrough, 2006).

3.11.2 C-Reactive Protein (CRP)

CRP was measured from fresh blood serum by automation in a CRP machine (i-CHROMA Bodi Tech Med. Inc**)** and values were displayed as mg/L. Detection limit and working range of CRP machine was 2.5-300 mg/L The result was displayed on machine LCD screen and recorded for analysis. Value of CRP >6mg/L was considered positive. CRP was measured only in positive cases of brucellosis and Toxoplasmosis.

3.12 Serum Biochemistry of Liver

3.12.1 AST (SGOT) and ALT (SGPT)

The level of alanine aminotransferase (ALT) and enzymes aspartate transaminase (AST) was measured from fresh serum by Mindray semi auto chemistry analyzer machine as per manufacturer protocols. The values for AST and ALT were displayed and recorded as units per liter (U/L) of serum. Reference ranges for AST and ALT assay were considered >40 U/L (Cheesbrough, 2006). Separate reagents were used for detection and measurement of AST and ALT on automation.

3.13 Development of Area Map

Maps of the region were developed by using ArcGIS 10.5. Before working in GIS environment, data was sorted and ensured that each polygon had the same values as enumerated by the time of the data

tabulation. Later one, spatial analyst tools were applied by using Kriging/Co-kriging techniques, followed by inverse distance weight (IDW) technique. Variogram and Semi-variogram properties were also incorporated in the form of ordinary methods with spherical Semi-variogram model, where minimum root mean square (RMS) error was ensured and 6-12 points were allocated as radius of cell size. Moreover, near neighborhood algorithm was used for IDW to depict raster output of map. Shapefiles of the study region were prepared by digitizing map as published by Survey of Pakistan, where world geographic system (WGS) 1984 as Datum and world geographic coordinated system 1984 were used. Generic symbology was used to adjust the maps with suitable color scheme ramps.

3.13 Ethical aspects

The study and questionnaire designed for collection of epidemiological, demographic and clinical parameters was approved by ethical committee of Quaid-I-Azam University Islamabad; Pakistan through approval number BEC-FBS-QAU2019-145. All those women patients, who willingly participated in the current study, were orally informed in urdu and local pashto languages about benefits of study. The included participants were agreed to participate in study on voluntary basis.

3.14 Statistical Analysis of Data

The Data generated from questionnaires (Annexure-A) and laboratory assays were stored in Microsoft Excel 2007 and were statistically analyzed for mean, mode, standard deviation and standard errors. Statistical tests like SPSS and R was used for analytic comparison of data, while prevalence, sensitivity, specificity, positive predictive and negative predictive values of various diagnostic assay were determined by the following recommended formula.

$$Prevalence\ (\%)\ = \frac{numberofpeoplewithdisease}{numberofpeopleinthepopulation} Xx\ 100\%$$

$$Sensitivity = \frac{Numberwhotestpositivewithdisease\ (a)}{Numberwithdisease\ (a\ +\ c)}$$

$$Specificity\ = \frac{Numberwhotestnegativewithoutdisease\ (d)}{Numberwith\ outdisease\ (b\ +\ d)}$$

$$Positivepredictivevalue = \frac{Numberwhotestpositivewithdisease\ (a)}{Numberwhotestpositive\ (a\ +\ b)}$$

$$Negativepredictivevalue\ = \frac{Numberwhotestnegativewith\ outdisease\ (d)}{Numberwhotestnegative\ (c\ +\ d)}$$

RESULTS

4.1 Geographical distributions, annual and seasonal prevalence of emerging zoonotic diseases (Brucellosis, Toxoplasmosis) in women in Khyber Pakhtunkhwa province of Pakistan.

During the current study, 3586 women patients of reproductive age (17-45 years) from 7 divisions (24 districts) of Khyber Pakhtunkhwa province of Pakistan (Figure, 4.1) were clinically examined and screened for detection of common emerging zoonotic diseases (Brucellosis, Toxoplasmosis) using SPAT and RBPT as initial screening test for Brucellosis and latex agglutination assay and ICT for Toxoplasmosis, while i-ELISA (IgM, IgG) and PCR was used as confirmatory assay for Brucellosis and i-ELISA for Toxoplasmosis.

Result of overall prevalence of common emerging zoonotic diseases (Brucellosis, Toxoplasmosis) in women of Khyber Pakhtunkhwa province of Pakistan is shown in Table (4.1) and graphically by figure (4.1).

Table: 4.1 Result of overall prevalence of common emerging zoonotic diseases (Brucellosis, Toxoplasmosis) in women in Khyber Pakhtunkhwa province, Pakistan.

Type of Disease	Prevalence	(%)	95% CI
Brucellosis	718	20.02	0.17-0.22
Toxoplasmosis	881	24.56	0.21-0.27
n= 3586	1599	44.59	

Out of (n=3586) women patients, 1599 were found positive for zoonotic diseases (Brucellosis, Toxoplasmosis), making an overall infection rate of 44.59%. Toxoplasmosis was found in 881 (24.56%) cases, while Brucellosis was confirmed in 718 (20.02%) women patients.

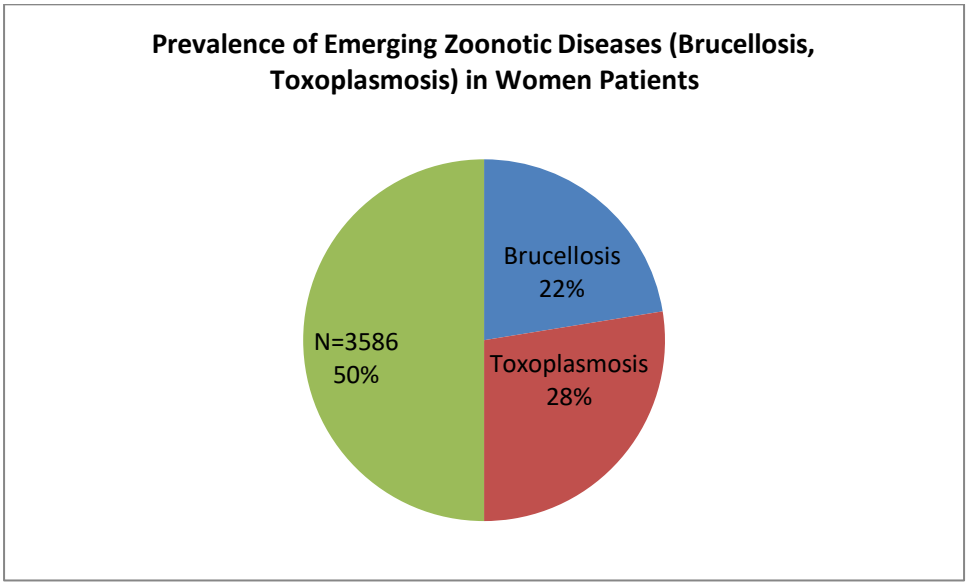

Figure 4.1: Result of overall prevalence of common emerging zoonotic diseases (Brucellosis, Toxoplasmosis) in women of Khyber Pakhtunkhwa province, Pakistan

Table: 4.2 Geographical distributions (Division-wise) of emerging zoonotic diseases (Brucellosis, Toxoplasmosis) in women in Khyber Pakhtunkhwa province, Pakistan.

Division	Patients	Zoonotic Diseases			Brucellosis			Toxoplasmosis		
	n=3586	1599	44.59%	p-value	718	20.02%	p-value	881	24.56%	p-value
Malakand	1074	434	40.41	1.00	147	13.68	1.00	287	26.72	1.00
Peshawar	584	245	41.95	0.99	165	28.25	1.00	80	13.69	1.00
Hazara	609	241	39.57	0.00	64	10.51	1.00	177	29.06	1.00
Kohat	429	241	56.17	0.00	112	26.11	1.00	129	30.06	1.00
Mardan	380	191	50.26	0.45	110	28.94	1.00	81	21.31	1.00
D.I Khan	287	138	48.08	0.74	58	20.21	1.00	80	27.87	1.00
Bannu	223	109	48.87	0.63	62	27.81	1.00	47	21.07	1.00
p-value	----	----	----	0.00*	----	----	0.00*	----	----	0.00*

1-sample proportions test
Division vs disease type. The Chi-square test for impendence of the two categorical variables (Division and Disease type) was used
*Pearson chi-square
*Likelihood ratio

Table (4.2) represents geographical distribution of zoonotic diseases (Brucellosis, Toxoplasmosis) in women patients in various divisions of Khyber Pakhtunkhwa province of Pakistan, which is graphically shown in Figure (4.2).

4.1.1 Assessment of zoonotic diseases (Brucellosis, Toxoplasmosis) in various divisions of Khyber Pakhtunkhwa province of Pakistan.

Zoonotic Diseases: A significant difference (<0.05) was found in distribution frequency of zoonotic diseases among various divisions in the study region. Prevalence of zoonotic diseases was high 241 (56.17%) in Kohat Division, followed by Mardan Division 191 (50.26%), Bannu Division 109 (48.87%), D.I. Khan Division 138 (48.08%), Peshawar Division 245 (41.95%), Malakand Division 434 (40.41%) and Hazara Division 241 (39.57%). Prevalence of zoonotic diseases was significantly (<0.05) high in Kohat and Hazara Divisions as compared to other division.

Brucellosis: A significant (<0.05) difference was found in prevalence of Brucellosis in various divisions. Prevalence of Brucellosis was high in Mardan Division 110 (28.94), followed by Peshawar Division 165 (28.25%), Bannu Division 62 (27.81%), Kohat Division 112 (26.11%), D.I. Khan Division 58 (20.21%), Malakand Division 147 (13.68%) and Hazara Division 64 (10.51%).

Toxoplasmosis: A significant difference (<0.05) was also found in prevalence of Toxoplasmosis infection in various divisions of the province. Prevalence of infection was high in Kohat Division 129 (30.06%), followed by Hazara Division 177 (29.06%), D.I Khan Division 80 (27.87%), Malakand Division 287 (26.72%), Mardan Division 81 (21.31%), Bannu Division 47 (21.07%) and Peshawar Division 80 (13.69%).

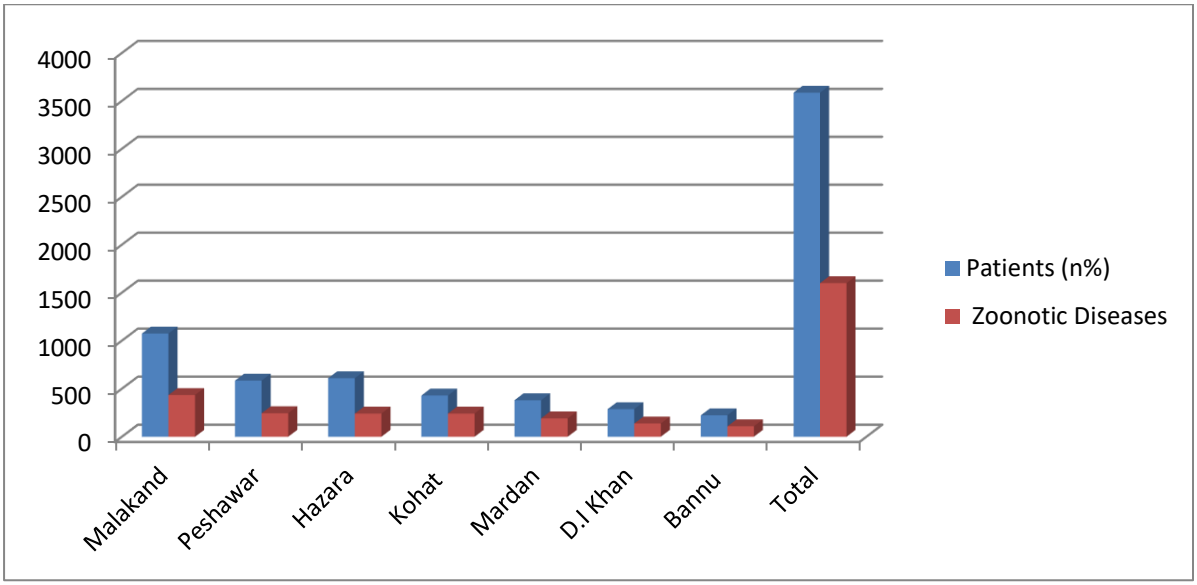

Figure 4.2: Geographical distributions (Division-wise) of emerging zoonotic diseases (Brucellosis, Toxoplasmosis) in women of Khyber Pakhtunkhwa province, Pakistan.

Table: 4.3 Geographical distributions (Divisional and District-wise) of emerging zoonotic diseases (Brucellosis, Toxoplasmosis) in women of Khyber Pakhtunkhwa province, Pakistan.

Division/District	Patients		Zoonotic Diseases			Brucellosis			Toxoplasmosis		
	n=3586	%	Positive	%	p-value	718	20.02%	p-value	881	24.56%	p-value
Malakand Division	1074	29.95	434	40.41	1.000	147	13.68	1.00	287	26.72	1.00
Swat	223	6.22	106	47.53	0.76	16	7.17	1.00	90	40.36	0.99
Malakand	193	5.38	84	43.52	0.96	29	15.02	1.00	55	28.49	1.00
Chitral	105	2.93	64	60.95	0.01	17	16.19	1.00	47	44.76	0.85
Buner	134	3.74	59	44.03	0.91	36	26.86	1.00	23	17.16	1.00
Upper Dir	172	4.79	52	30.23	1.00	21	12.21	1.00	31	18.02	1.00
Lower Dir	140	3.91	43	30.71	1.00	19	13.57	1.00	24	17.14	1.00
Shangla	107	2.98	26	24.29	1.00	9	8.41	1.00	17	15.88	1.00
p-value	----	----	----	----	0.00*	0.00*	0.00**	----	0.00*	0.00**	----
Peshawar Division	584	16.28	245	41.95	0.99	165	28.25	1.00	80	13.71	1.00
Peshawar	247	6.89	103	41.71	0.99	73	29.55	1.00	30	12.14	1.00
Charsadda	189	5.27	87	46.03	0.86	53	28.04	1.00	34	17.98	1.00
Nowshera	148	4.13	55	37.16	0.99	39	26.35	1.00	16	10.81	1.00
p-value	----	----	----	----	0.25* 0.26**	0.78*	0.78**	----	0.10*	0.11**	----
Kohat Division	429	11.97	241	56.17	0.00	112	26.11	1.00	129	30.07	1.00
Kohat	192	5.35	111	57.81	0.01	61	31.77	1.00	50	26.04	1.00
Karak	109	3.04	79	72.47	0.00	30	27.52	1.00	49	44.95	0.85
Hangu	128	3.57	51	39.84	0.98	21	16.41	1.00	30	23.43	1.00
p-value	----	----	----	----	0.00*	0.00*	0.00**	----	0.00*	0.00**	----
Hazara Division	609	16.98	241	39.57	1.00	64	10.51	1.00	177	29.06	1.00
Mansehra	139	3.87	68	48.92	0.60	17	12.23	1.00	51	36.69	0.99
Abbottabad	170	4.74	66	38.82	0.99	20	11.76	1.00	46	27.05	1.00
Battagram	110	3.07	42	38.18	0.99	6	5.45	1.00	36	32.72	0.99
Kohistan	85	2.37	38	44.71	0.83	12	14.12	1.00	26	30.58	0.99
Haripur	105	2.93	27	25.71	1.00	9	10.58	1.00	18	17.14	1.00
p-value	----	----	----	----	0.01* 0.00**	0.25*	0.21**	----	0.01*	0.01**	----
Mardan Division	380	10.61	191	50.26	0.45	110	28.94	1.00	81	21.21	1.00
Mardan	239	6.66	121	50.62	0.42	69	28.87	1.00	52	21.75	1.00
Swabi	141	3.93	70	49.64	0.53	41	29.07	1.00	29	20.56	1.00
p-value	----	----	----	----	0.96*	0.96*	0.96**	1.00***	0.78*	0.78**	0.89***
D.I Khan Division	287	8.01	138	48.08	0.74	58	20.21	1.00	80	27.87	1.000
Dera Ismail Khan	188	5.24	91	48.41	0.66	27	14.36	1.00	64	34.04	1.00

Tank	99	2.76	47	47.47	0.69	31	31.32	0.99	16	16.15	1.00
p-value	----	----	----	----	0.00*	0.00*	0.00**	0.00***	0.00*	0.00**	0.00***
Bannu Division	223	6.22	109	48.87	0.63	62	27.81	1.00	47	21.07	1.00
Bannu	120	3.35	72	60.01	0.01	39	32.51	0.99	33	27.51	1.00
Lakki Marwat	103	2.87	37	35.92	0.99	23	22.33	1.00	14	13.59	1.00
p-value	----	----	----	----	0.00*	0.09*	0.08**	0.10***	0.01*	0.01**	0.01***

Chi-square test for impendence of the two categorical variables districts and disease type was used.
*Pearson chi-square
**Likelihood ratio
***Fisher's exact test
1-sample proportions test

Table (4.3) represents geographical distribution of zoonotic diseases (Brucellosis, Toxoplasmosis) in women in 24 districts of 7 divisions in Khyber Pakhtunkhwa province of Pakistan, which is graphically shown by figure (4.3, 4.4, 4.5).

4.1.2 Prevalence of zoonotic diseases (Brucellosis, Toxoplasmosis) in various districts of Malakand division

A significant difference (<0.05) was found in prevalence of zoonotic diseases among 7 districts of Malakand division. Prevalence of zoonotic diseases was significantly (<0.05) high in district Chitral 64 (60.65%), followed by district Swat 106 (47.53%), district Malakand 84 (43.52%), district Lower Dir 43 (30.71%), district Upper Dir 52 (30.23%) and district Shangla 26 (24.29%) respectively.

Brucellosis: A significant difference (<0.05) was found in distribution frequency of Brucellosis among 7 districts of Malakand division. Prevalence of Brucellosis was high in district Buner 36 (26.86%), followed by district Chitral 17 (16.19%), district Malakand 29 (15.02%), district Lower Dir 19 (13.57%), district Upper Dir 21 (12.21%), district Shangla 9 (8.41%) and district Swat 16 (7.17%) respectively.

Toxoplasmosis: A significant difference (<0.05) was found in distribution of Toxoplasmosis among 7 districts of Malakand division. Prevalence of Toxoplasmosis was high 47 (44.76%) in district Chitral, followed by district Swat 90 (40.36%), district Malakand 55 (28.49%), district Upper Dir 31 (18.02%), district Buner 23 (17.16%), district Lower Dir 24 (17.14%) and district Shangla 17 (15.88%) respectively.

4.1.3 Prevalence of zoonotic diseases (Brucellosis, Toxoplasmosis) in various districts of Peshawar division

There was no significant difference (>0.05) in distribution frequency of zoonotic diseases (Brucellosis, Toxoplasmosis) among 3 districts of Peshawar division. Prevalence of zoonotic diseases was high in district Charsadda 87 (46.03%), followed by district Peshawar 103 (41.71%) and district Nowshera 55 (37.16%).

Brucellosis: There was no significant difference (>0.05) in distribution frequency of Brucellosis infection among 3 districts of Peshawar division. Prevalence of Brucellosis was high in district Peshawar 73 (29.55%), followed by district Charsadda 53 (28.04%) and district Nowshera 39 (26.35%).

Toxoplasmosis: There was no significant difference (>0.05) in distribution frequency of Toxoplasmosis among 3 districts of Peshawar division. Prevalence of Toxoplasmosis was high in district Charsadda 34 (17.98%), followed by district Peshawar 30 (12.14%) and district Nowshera 16 (10.81%).

4.1.4 Prevalence of zoonotic diseases (Brucellosis, Toxoplasmosis) in various districts of Kohat division

There was significant difference (<0.05) in distribution frequency of zoonotic diseases (Brucellosis, Toxoplasmosis) among 3 districts of Kohat division. Prevalence of zoonotic diseases was significantly (<0.05) high in district Karak 79 (72.47%), followed by district Kohat 111 (57.81%) and district Hangu 51 (39.84%) respectively.

Brucellosis: There was significant difference (<0.05) in distribution frequency of Brucellosis among 3 districts of Kohat division. Prevalence of Brucellosis was high in district Kohat 61 (31.77%), followed by district Karak 30 (27.52%) and district Hangu 21 (16.41%) respectively.

Toxoplasmosis: There was significant difference (<0.05) in distribution frequency of Toxoplasmosis among 3 districts of Kohat division. Prevalence of Toxoplasmosis was high in district Karak 49 (44.45%), followed by district Kohat 50 (26.04%) and district Hangu 30 (23.43%) respectively.

4.1.5 Prevalence of zoonotic diseases (Brucellosis, Toxoplasmosis) in various districts of Hazara division

There was significant difference (<0.05) in distribution frequency of zoonotic diseases (Brucellosis, Toxoplasmosis) among 5 districts of Hazara division. Prevalence of zoonotic diseases was high in district Mansehra 68 (48.92%), followed by district Kohistan 38 (44.71%), district Abbottabad 66 (38.82%), district Battagram 42 (38.18%) and district Haripur 27 (25.71%) respectively.

Brucellosis: There was no significant difference (>0.05) in distribution frequency of Brucellosis among 5 districts of Hazara division. Prevalence of Brucellosis was high in district Kohistan 12 (14.12%), followed by district Mansehra 17 (12.23%), district Abbottabad 20 (11.76%), district Haripur 9 (10.58%) and district Battagram 6 (5.45%) respectively.

Toxoplasmosis: There was significant difference (<0.05) in distribution frequency of Toxoplasmosis among 5 districts of Hazara Division. Prevalence of Toxoplasmosis was high in district Mansehra 51 (36.69%), followed by district Battagram 36 (32.72%), district Kohistan 26 (30.58%), district Abbottabad 46 (27.05%) and district Haripur 18 (17.14%) respectively.

4.1.6 Prevalence of zoonotic diseases (Brucellosis, Toxoplasmosis) in various districts of Mardan division

There was no significant difference (>0.05) in distribution frequency of zoonotic diseases (Brucellosis, Toxoplasmosis) in 2 districts of Mardan division. Prevalence of zoonotic diseases was high in district Mardan 121 (50.62%), followed by district Swabi 70 (49.64%).

Brucellosis: There was no significant difference (>0.05) in distribution frequency of Brucellosis infection in 2 districts of Mardan division. Prevalence of Brucellosis was high in district Swabi 41 (29.07%), followed by district Mardan 69 (28.87%).

Toxoplasmosis: There was no significant difference (>0.05) in distribution frequency of Toxoplasmosis in 2 districts of Mardan division. Prevalence of Toxoplasmosis was high in district Mardan 52 (21.75%), followed by district Swabi 29 (20.56%).

4.1.7 Prevalence of zoonotic diseases (Brucellosis, Toxoplasmosis) in various districts of D.I Khan division

There was significant difference (<0.05) in distribution frequency of zoonotic diseases (Brucellosis, Toxoplasmosis) in 2 districts of D.I Khan Division. Prevalence of zoonotic diseases was high in district Dera Ismail Khan 91 (48.41%), which was followed by district Tank 47 (47.47%).

Brucellosis: There was significant difference (<0.05) in distribution frequency of Brucellosis in 2 districts of D.I Khan Division. Prevalence of Brucellosis was high in district Tank 31 (31.32%), which was followed by district Dera Ismail Khan 27 (14.36%).

Toxoplasmosis: There was significant difference (<0.05) in distribution frequency of Toxoplasmosis in 2 districts of D.I Khan Division. Prevalence of Toxoplasmosis was high in district Dera Ismail Khan64 (34.04%), which was followed by district Tank 16 (16.15%).

4.1.8 Prevalence of zoonotic diseases (Brucellosis, Toxoplasmosis) in various districts of Bannu division

There was significant difference (<0.05) in distribution frequency of zoonotic diseases (Brucellosis, Toxoplasmosis) in 2 districts of Bannu division. Prevalence of zoonotic diseases was significantly (<0.05) high in district Bannu 72 (60.01%), which was followed by district Lakki Marwat 37 (35.92%).

Brucellosis: There was no significant difference (>0.05) in distribution frequency of Brucellosis in 2 districts of Bannu division. Prevalence of Brucellosis was high in district Bannu 39 (32.51%), which was followed by district Lakki Marwat 23 (22.33%).

Toxoplasmosis: There was significant difference (<0.05) in distribution frequency of Toxoplasmosis in 2 districts of Bannu division. Prevalence of Toxoplasmosis was high in district Bannu 33 (27.51%), which was followed by district Lakki Marwat 14 (13.59%).

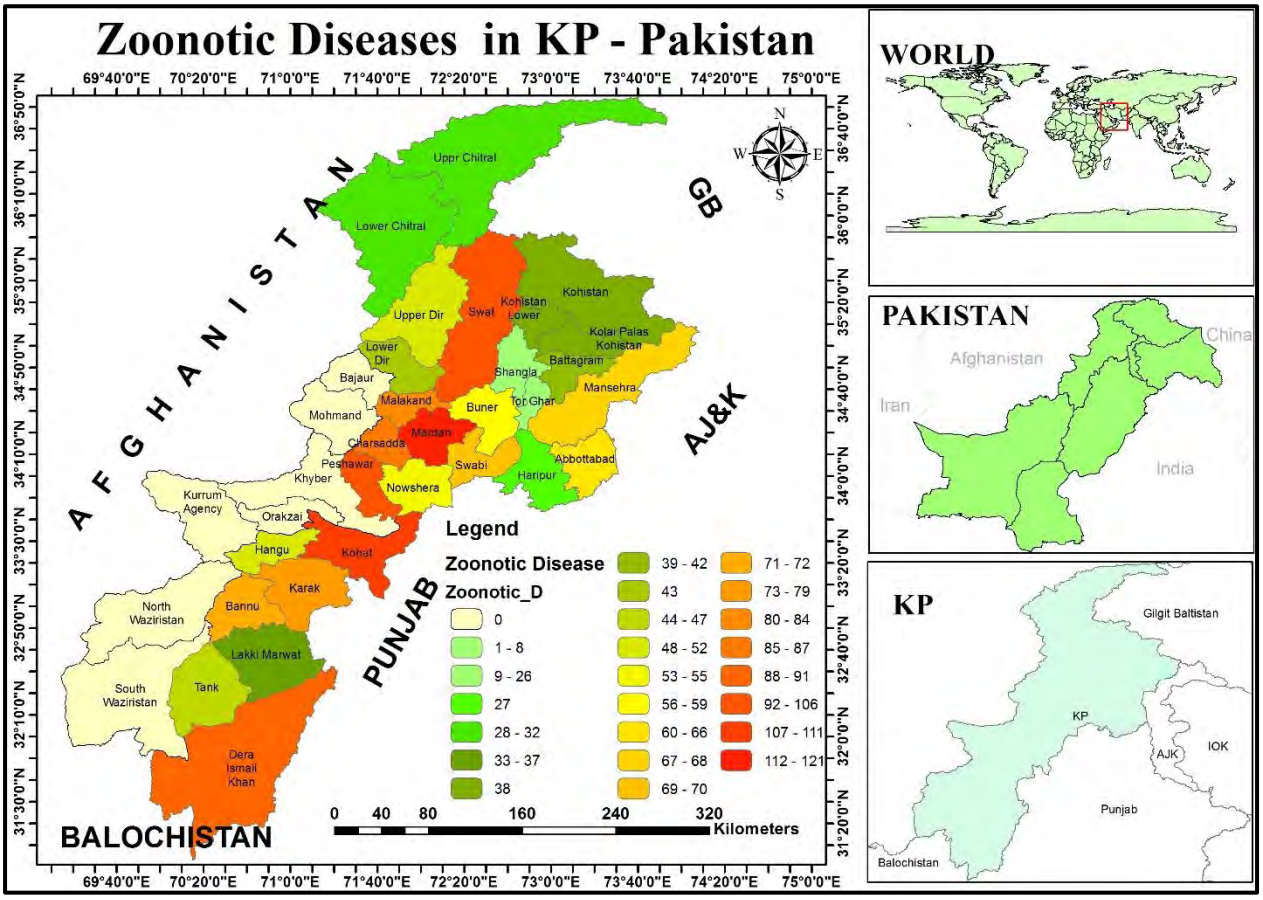

Figure 4.3: Maps of the studied regions, developed by geographical information (GIS) system, which show geographical distribution of emerging zoonotic diseases (Brucellosis, Toxoplasmosis) in women in various regions (Districts) of Khyber Pakhtunkhwa province, Pakistan.

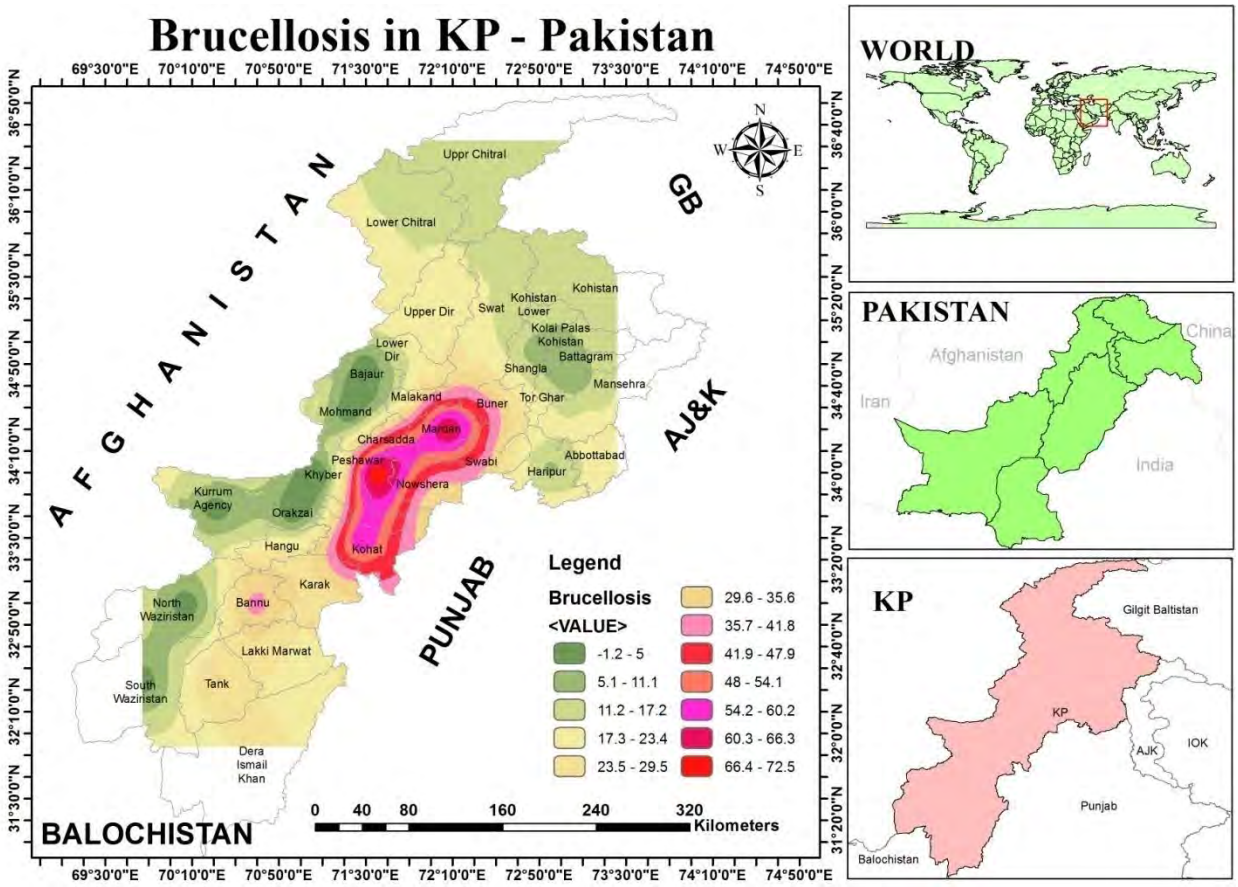

Figure 4.4: GIS maps showing geographical distribution of emerging zoonotic Brucellosis infection in women in various regions (Districts) of Khyber Pakhtunkhwa province, Pakistan

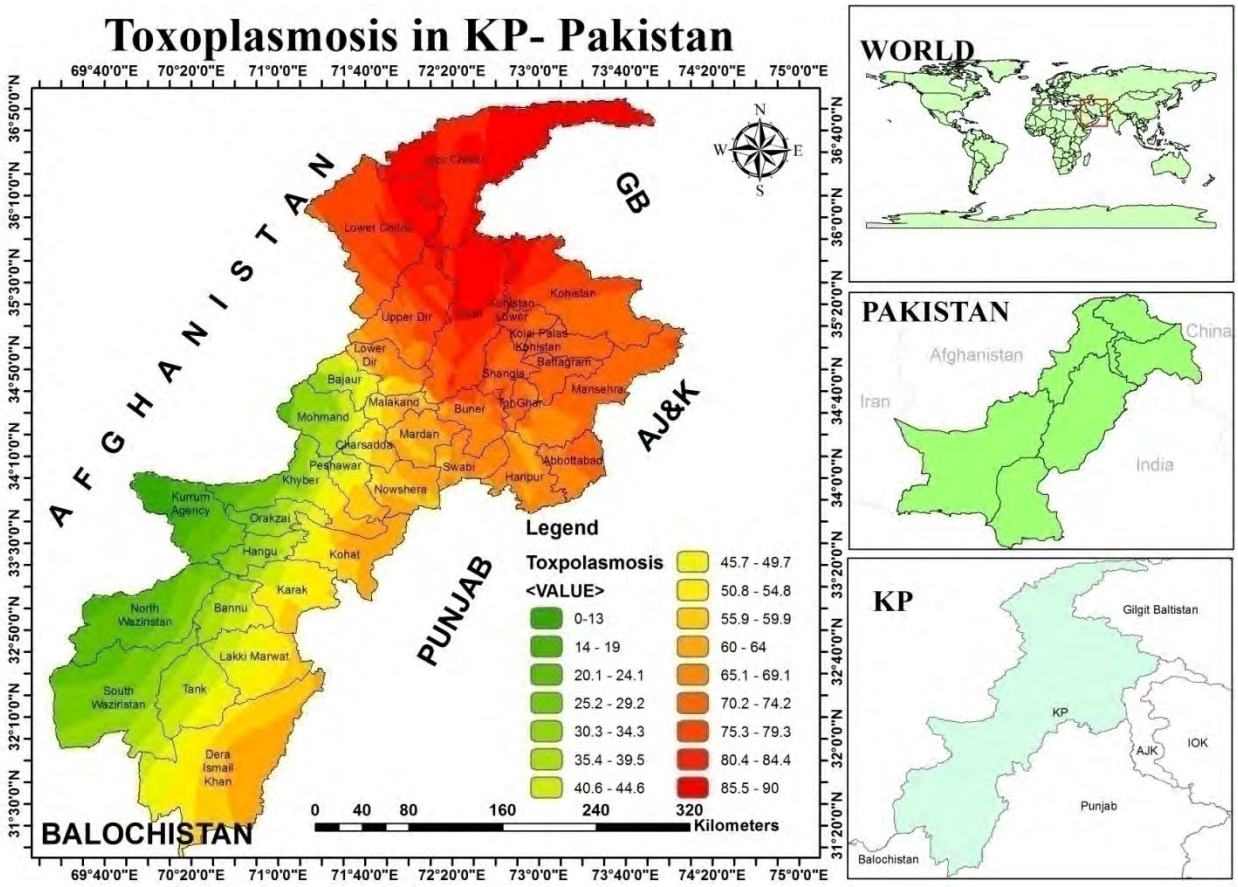

Figure 4.5: GIS maps, which show geographical distribution of emerging zoonotic Toxoplasmosis infection in women in various regions (Districts) of Khyber Pakhtunkhwa province, Pakistan.

Table 4.4 Prevalence of emerging zoonotic diseases (Brucellosis, Toxoplasmosis) in different months and seasons in women in Khyber Pakhtunkhwa province, Pakistan

Month and Season			Zoonotic Diseases			Brucellosis			Toxoplasmosis		
Spring	n=3586	(%)	1599 (44.59%)		p-value	718 (20.02%)		p-value	881 (24.56%)		p-value
	600	16.73	238	39.66	0.00 1.00	111	18.51	1.00	127	21.16	1.00
March	310	8.64	97	31.29	1.00	27	8.71	0.00 1.00	70	22.58	0.00 1.00
April	290	8.08	141	48.62	0.68	84	28.96	0.36 1.00	57	19.65	0.00 1.00
Summer	1501	41.85	762	50.76	0.00 0.27	382	25.45	1.00	380	25.31	1.00
May	373	10.41	196	52.54	0.16	87	23.32	0.78 1.00	109	29.22	0.38 1.00
June	251	7.11	105	41.83	0.99	51	20.31	0.22 1.00	54	21.51	0.00 1.00
July	329	9.17	159	48.32	0.72	119	36.17	0.04 1.00	40	12.15	0.00 1.00
August	307	8.56	171	55.71	0.02	74	24.11	0.47 1.00	97	31.59	0.92 1.00
September	241	6.72	131	54.35	0.08	51	21.16	1.00	80	33.19	1.00
Autumn October	232	6.46	112	48.27	0.87 0.70	54	23.27	0.89 1.00	58	25.01	0.06 1.00
Winter	1253	34.94	487	38.86	0.00 1.00	171	13.64	1.00	316	25.21	1.00
November	309	8.62	110	35.59	1.00	18	5.82	0.00 1.00	92	29.77	0.01 1.00
December	323	9.01	136	42.11	0.99	89	27.55	0.90 1.00	47	14.55	0.00 1.00
January	231	6.44	84	36.37	1.00	14	6.06	0.00 1.00	70	30.31	0.04 1.00
February	390	10.87	157	40.25	0.99	50	12.82	0.00 1.00	107	27.43	0.01 1.00

1-sample proportions test without continuity correction
The reference category is: OD. by SPSS

Table: 4.5 Prevalence of emerging zoonotic diseases (Brucellosis, Toxoplasmosis) in different seasons in women in Khyber Pakhtunkhwa province, Pakistan

Seasons	Patients	Zoonotic Diseases			Brucellosis			Toxoplasmosis		
	n=3586	1599	44.59%	p-value	718	20.02%	p-value	881	24.56%	p-value
Spring March-April	----	----	----	0.00	----	----	----	----	----	----
	600	238	39.66	1.00	111	18.51	1.00	127	21.16	1.00
Summer May-September	----	----	----	0.00	----	----	----	----	----	----
	1501	762	50.76	0.27	382	25.45	1.00	380	25.31	1.00
Autumn October	----	----	----	0.87	----	----	----	----	----	----
	232	112	48.27	0.70	54	23.27	1.00	58	25.01	1.00
Winter November-February)	----	----	----	0.00	----	----	----	----	----	----
	1253	487	38.86	1.00	171	13.64	1.00	316	25.22	1.00
p-value	----	----	----	----	----	----	0.00* 0.00**	881	24.56	0.21* 0.20**

1-sample proportions test
*Pearson Chi-Square (SPSS)
**Likelihood Ratio (SPSS)

Table: (4.4) and (4.5) represent assessment of emerging zoonotic diseases (Brucellosis, Toxoplasmosis) in different months and seasons in women in the studied region, which are graphically represented by figure (4.9).

4.1.9 Prevalence of zoonotic diseases (Brucellosis, Toxoplasmosis) in different months and seasons

Assessment of zoonotic diseases in different seasons was carried out in women patients. A significant difference (<0.05) was found in prevalence of zoonotic diseases (Brucellosis, Toxoplasmosis) in different seasons. Prevalence of zoonotic diseases was significantly (<0.05) high 762 (50.77%) in summer (May-September) season, which was followed by autumn (October) 112 (48.28%), spring (March-April) 238 (39.67%) and 487 (38.86%) cases in winter (November-February) season during three year study.

Brucellosis: There was significant difference (<0.05) in Prevalence of Brucellosis in different months and seasons. Prevalence of Brucellosis was high 382 (25.45%) in summer (May -September) season, which was followed by autumn (October) 54 (23.28%), spring (March-April) 111 (18.51%) and winter (November-February) 171 (13.65%) season.

Toxoplasmosis: There was no significant difference (>0.05) in prevalence of Toxoplasmosis in different seasons. Prevalence of infection was high 380 (25.32%) in summer (May-September) season, followed by winter (November-February) 316 (25.22%), autumn (October) 58 (25.00%) and spring (March-April) 127 (21.17%) season.

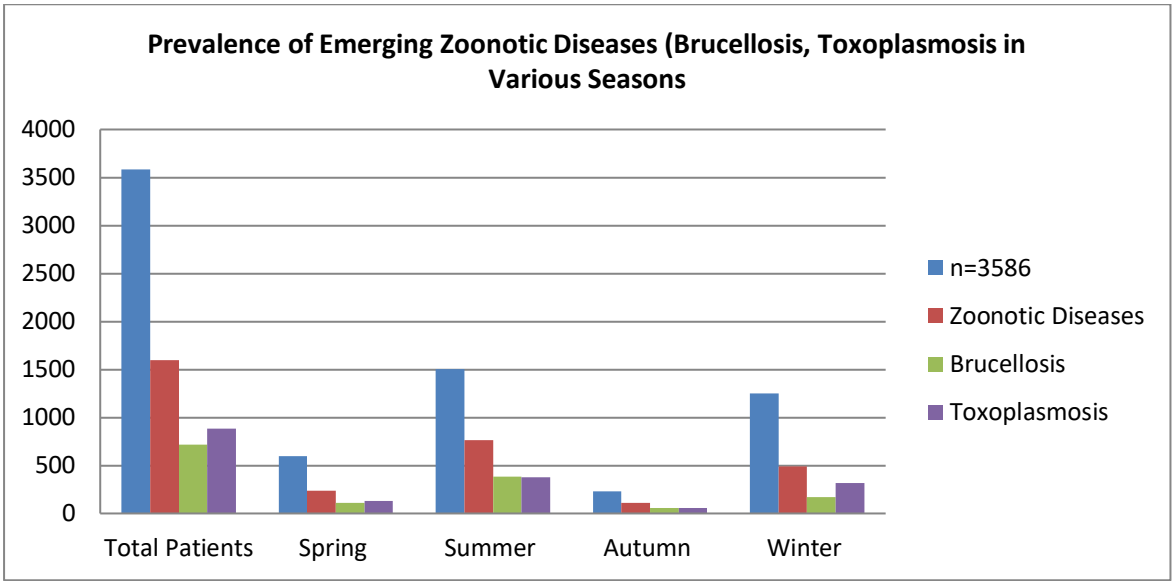

Figure 4.6: Prevalence of emerging zoonotic diseases (Brucellosis, Toxoplasmosis) in different seasons in women in Khyber Pakhtunkhwa province, Pakistan.

Table 4.6 Results of annual prevalence of emerging zoonotic diseases (Brucellosis, Toxoplasmosis) in women in Khyber Pakhtunkhwa province, Pakistan.

Year	Patients		Zoonotic Diseases			Brucellosis			Toxoplasmosis		
	n=3586	%	1599	44.59%	p-value	718	20.02%	p-value	881	24.56%	p-value
2017	1179	32.87	524	44.44	0.99	233	19.76	1.00	291	24.68	1.00
2018	1348	37.59	601	44.58	1.00	274	20.32	1.00	327	24.25	1.00
2019	1059	29.53	474	44.75	0.99	211	19.92	1.00	263	24.83	1.00
p-value	----	----	----	----	0.99*	----	----	0.93* 0.91*	----	----	0.94*

1-sample proportions test
*Pearson Chi-Square (SPSS)
*Likelihood Ratio (SPSS)
*Linear-by-Linear Association (SPSS)

Table 4.6 indicates results of annual prevalence of emerging zoonotic diseases (Brucellosis, Toxoplasmosis) in women in the studied region, which is graphically shown in figure 4.6.

4.1.10 Annual frequency of zoonotic diseases (Brucellosis, Toxoplasmosis) in women

Assessment for annual prevalence of zoonotic diseases was carried out in women patients in the province, but no significant (>0.05) annual difference was found in prevalence of zoonotic diseases during three years study. Prevalence of zoonotic diseases was high 474 (44.75%) in the year 2019, followed by 601 (44.58%) and 524 (44.45) cases in the year 2018 and 2017 respectively.

Brucellosis: No significant difference (>0.05) was observed in prevalence of Brucellosis infection during three years study. Prevalence of Brucellosis was high 274 (20.32%) in the year 2018, followed by 211 (19.92%) and 233 (19.76%) cases in the year 2019 and 2017 respectively.

Toxoplasmosis: No significant difference (>0.05) was observed in prevalence of Toxoplasmosis infection in three years. Prevalence of Toxoplasmosis was high 263 (24.83%) in the year 2019, followed by 291 (24.68%) and 327 (24.25%) cases in the year 2017 and 2018 respectively.

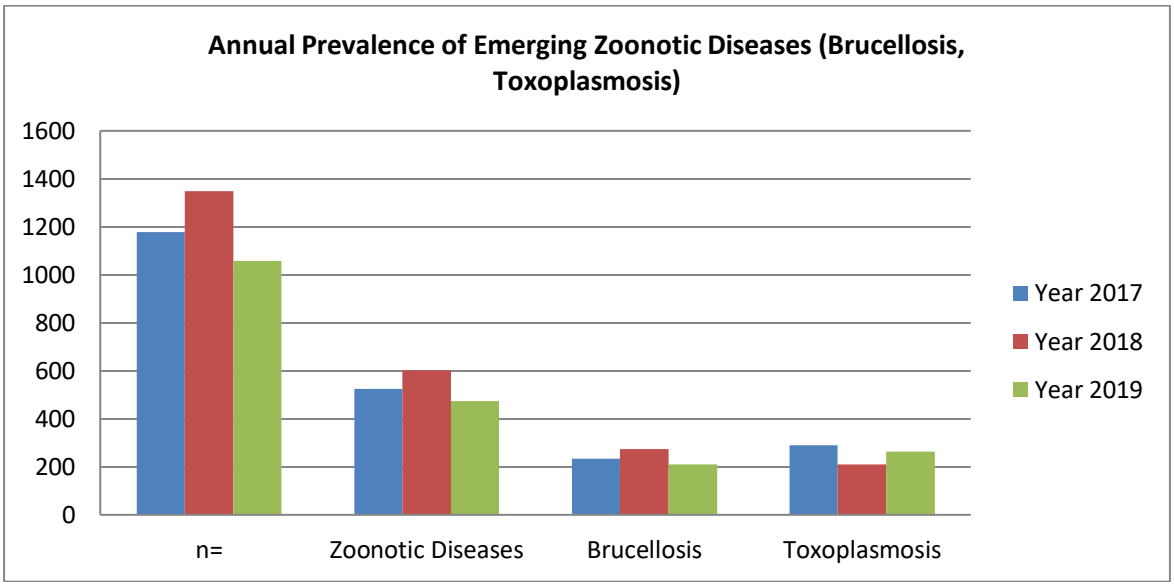

Figure 4.7: Results of annual prevalence of emerging zoonotic diseases (Brucellosis, Toxoplasmosis) in women of Khyber Pakhtunkhwa province, Pakistan.

4.2 Socio-demographic characteristics of women and sero-prevalence of emerging zoonotic diseases (Brucellosis, Toxoplasmosis) in Khyber Pakhtunkhwa province, Pakistan

Table 4.7 Results of descriptive socio-demographic characteristics and sero-prevalence of emerging zoonotic diseases (Brucellosis, Toxoplasmosis) in women in Khyber Pakhtunkhwa province, Pakistan

Demographic characteristics	Women		Zoonotic Diseases			Brucellosis			Toxoplasmosis		
Maternal Age	N=3586	%	1599	44.59%	p-value	718	20.02%	p-value	881	24.56%	p-value
17-26	1767	49.27	803	45.44	0.00	477	26.99	0.00	326	18.45	0.00
27-36	1248	34.81	596	47.75		193	15.46		403	32.29	
37-45	571	15.92	200	35.02		48	8.41		152	26.62	
28.19±0.11		----	----	----	----	----	----	----	----	----	----
Residential status of Patients											
Rural	2589	72.19	1256	48.51	0.00	581	22.44	0.00	675	26.07	0.00
Urban	997	27.81	343	34.41		137	13.74		206	20.66	
Educational status of Patients											
Illiterate	2386	66.53	1203	50.41	0.00	489	20.51	0.00	714	29.92	0.00
Primary school	834	23.25	293	35.13		177	21.22		116	13.91	
Secondary school	229	6.38	73	31.87		44	19.21		29	12.66	
Graduation	137	3.82	30	21.89		8	5.84		22	16.05	
Occupation status											
House-wife	3173	88.48	1498	47.21	0.00	683	21.52	0.00	815	25.68	0.00
Laborer	203	5.66	64	31.52		22	10.83		42	20.69	
Teacher	149	4.15	27	18.12		10	6.71		17	11.41	
Doctor/LHV/Nurse	61	1.71	10	16.39		3	4.92		7	11.47	
Socio-economic states of Patients											
Low/middle	3212	89.57	1497	46.61	0.00	680	21.17	0.00	817	25.43	0.00
High	374	10.43	102	27.27		38	10.16		64	17.11	
Patients Hygienic groups											
Bad	2563	71.47	1228	47.91	0.00	552	21.53	0.00	676	26.37	0.00
Good	1023	28.52	371	36.26		166	16.22		205	20.04	
Knowledge about Zoonotic Infections (Brucellosis, Toxoplasmosis)											
Yes	109	3.04	10	9.17	0.00	3	2.75	0.00	7	6.42	0.00
No	3477	96.96	1589	45.71		715	20.56		874	25.13	

3-sample test for equality of proportions without continuity correction
4-sample test for equality of proportions without continuity correction

Table 4.7 indicates results of selected descriptive socio-demographic characteristics and sero-prevalence of emerging zoonotic diseases (Brucellosis, Toxoplasmosis) in women patients in Khyber Pakhtunkhwa province of Pakistan, which is graphically shown by figure 4.7. Various socio-demographic characteristics like patient's age, residential, educational, occupational, socio-economic, hygienic status and knowledge level were separately

evaluated as epidemiological parameter for assessment of emerging zoonotic diseases in women, who were living in various regions of Pakhtunkhwa province of Pakistan.

4.2.1 Prevalence of zoonotic diseases in women of various age groups

Assessment of zoonotic diseases (Brucellosis, Toxoplasmosis) was carried out in women of various age groups. A significant difference (<0.05) was observed in rate of zoonotic diseases in women patients, who were of various age groups. Prevalence of zoonotic diseases was high 596 (47.75%) in women, who were 27-36 year old, followed by 803 (45.44%) and 200 (35.02%) positive cases in those women, who were in age group 17-26 and 37-45 year old respectively.

Brucellosis: A significant difference (<0.05) was also found in prevalence of Brucellosis infection in women patients of various age group. Prevalence of Brucella infection was high 477 (26.99%) in women, who were 17-26 year old, followed by 193 (15.46%) and 48 (8.41%) cases in women, who were in age group 27-36 and 37-45 year old.

Toxoplasmosis: A significant difference (<0.05) was also found in prevalence of Toxoplasmosis in women of various age group. Prevalence of Toxoplasmosis was high 403 (32.29%) in women, who were 27-36 year old, followed by 152 (26.62%) and 326 (18.45%) cases in women of age group 37-45 and 17-26 year old.

4.2.2 Prevalence of zoonotic diseases in women of different residential groups

Assessment of zoonotic diseases (Brucellosis, Toxoplasmosis) was carried out in women, who were living in various types of residential status. A significant difference (<0.05) was found in prevalence of zoonotic diseases in women, who were living in rural and urban region. Prevalence of zoonotic diseases was high 1256 (48.51%) in women, who were living in rural area, while rate of zoonotic diseases was low 343 (34.41%) in those women, who were living in urban region of the province.

Brucellosis: A significant difference was found in prevalence of Brucellosis infection in women of different residential status. Prevalence of Brucellosis was high 581 (22.44%) in women of rural region, while, rate of infection was low 137 (13.74%) in those women, who were living in urban region.

Toxoplasmosis: A significant difference was found in rate of Toxoplasmosis in women, who were living in different residential status. Prevalence of Toxoplasmosis was high 675 (26.07%) in women of rural region, while rate of infection was low 206 (20.66%) in women, who were living in urban region.

4.2.3 Prevalence of zoonotic diseases in women of different educational groups

Assessment of zoonotic diseases (Brucellosis, Toxoplasmosis) was carried out in women, who belonged to different educational level. A significant difference (<0.05) was found in rate of zoonotic diseases in women of various educational group. Prevalence of zoonotic diseases was high 1203 (50.41%) in women, who were non-educated, which decreased to 293 (35.13%), 73 (31.87%) and 30 (21.89%) cases in women, who belonged to primary education, secondary education and graduated level.

Brucellosis: A significant difference (<0.05) was found in rate of Brucellosis infection in women patients of different educational group. Prevalence of Brucellosis was high 177 (21.22%) in women, who had primary education, followed by 489 (20.51%), 44 (19.21%) and 8 (5.84%) cases in women, who were non-educated, secondary educated and graduated.

Toxoplasmosis: A significant difference (<0.05) was found in prevalence of Toxoplasmosis infection in women patients of different educational group. Prevalence of Toxoplasmosis was high 714 (29.92%) in women, who were non-educated, followed by 22 (16.05%), 116 (13.91%) and 29 (12.66%) cases in women, who were graduated, primary educated and secondary educated.

4.2.4 Prevalence of zoonotic diseases in women of different occupational groups

Occupational status of patients was evaluated for burden of zoonotic infection. A significant difference (<0.05) was found in prevalence of zoonotic diseases (Brucellosis, Toxoplasmosis) in women patients, who belonged to various occupational group. Prevalence of zoonotic diseases was high 1498 (47.21%) in house wives, which decreased to 64 (31.52%), 27 (18.12%) and 10 (16.39%) cases in those women, who were laborer, teacher and health professional.

Brucellosis: A significant difference (<0.05) was observed in rate of Brucellosis infection in women patients, who belong to various occupational groups. Prevalence of Brucellosis was high 683 (21.52%) in house wives, which decreased to 22 (10.83%), 10 (6.71%) and 3 (4.92%) cases in women, who belonged to laborer, teacher and health care professional.

Toxoplasmosis: A significant difference (<0.05) was observed in prevalence of Toxoplasmosis in women, who belong to various occupational groups. Prevalence of Toxoplasmosis was high 815 (25.68%) in women, who were house wives, which decreased to 42 (20.69%, 17 (11.41%) and 7 (11.47%) positive cases in those women, who were laborer, teacher and health care workers.

4.2.5 Prevalence of zoonotic diseases in women of different socio-economic groups

Socio-economic status of patients were evaluated for prevalence of zoonotic diseases. A significant difference (<0.05) was found in rate of zoonotic diseases (Brucellosis, Toxoplasmosis) in women, who belonged to low and high socio-economic status. Prevalence of zoonotic diseases was high 1497 (46.61%) in women, who belonged to low or middle socio-economic status, while rate of zoonotic diseases was low 102 (27.27%) in those women, who belonged to high socio-economic status.

Brucellosis: A significant difference (<0.05) was observed in prevalence of Brucellosis infection in women, who belonged to various socio-economic status. Prevalence of Brucellosis was high 680 (21.17%) in women of low socio-economic status, while rate of infection was low 38 (10.16%) in those women, who belonged to high socio-economic status.

Toxoplasmosis: A significant difference (<0.05) was observed in rate of Toxoplasmosis in women, who belonged to various socio-economic groups. Prevalence of Toxoplasmosis was high 817 (25.43%) in those women, who belonged to low or middle socio-economic status, while rate of infection was low 64 (17.11%) in those women, who had high socio-economic status.

4.2.6 Prevalence of zoonotic diseases in women of different hygienic groups

Hygienic status of patient's were also evaluated for prevalence of zoonotic diseases. A significant difference (<0.05) was found in rate of zoonotic disease in women of bad and good hygienic status. Prevalence of zoonotic diseases was high 1228 (47.91%) in women, who had bad hygienic status, while rate of zoonotic diseases was low 371 (36.26%) in those women, who had good hygienic status.

Brucellosis: A significant difference (<0.05) was observed in prevalence of Brucellosis infection in women, who belonged to various group of hygienic status. Prevalence of zoonotic diseases was high 552 (21.53%) in those women, who had bad hygienic status, while rate of zoonotic diseases was low 166 (16.22%) in those women, who had good hygienic status.

Toxoplasmosis: A significant difference (<0.05) was observed in rate of Toxoplasmosis in women of different hygienic status. Prevalence of Toxoplasmosis was high 676 (26.37%) in women, who had bad hygienic status, while rate of infection was low 205 (20.04%) in women, who belonged to good hygienic group.

4.2.7 Prevalence of zoonotic diseases in women of different knowledge level

Prevalence of zoonotic diseases was determined in women patients, who belonged to different group of knowledge level. A significant difference (<0.05) was found in rate of zoonotic diseases in women, who belonged to different groups of knowledge level. Prevalence of diseases was high 1589 (45.71%) in women, who had no knowledge about zoonotic diseases, while rate of zoonotic diseases was low10 (9.17%) in those women, who had knowledge about zoonotic diseases.

Brucellosis: A significant difference (<0.05) was observed in prevalence of Brucellosis infection in women patients, who belonged to different group of knowledge level. Prevalence of Brucellosis was high 715 (20.56%) in those women, who had no knowledge about zoonotic diseases, while rate of infection was low 3 (2.75%) in those women, who had proper knowledge about zoonotic diseases.

Toxoplasmosis: A significant difference (<0.05) was observed in prevalence of Toxoplasmosis infection in women patients, who had awareness about zoonotic diseases and those, who had no awareness. Prevalence of Toxoplasmosis was high 874 (25.13%) in women, who had no knowledge about zoonotic diseases, while rate of infection was low 7 (6.42%) in those women, who had proper awareness about zoonotic diseases.

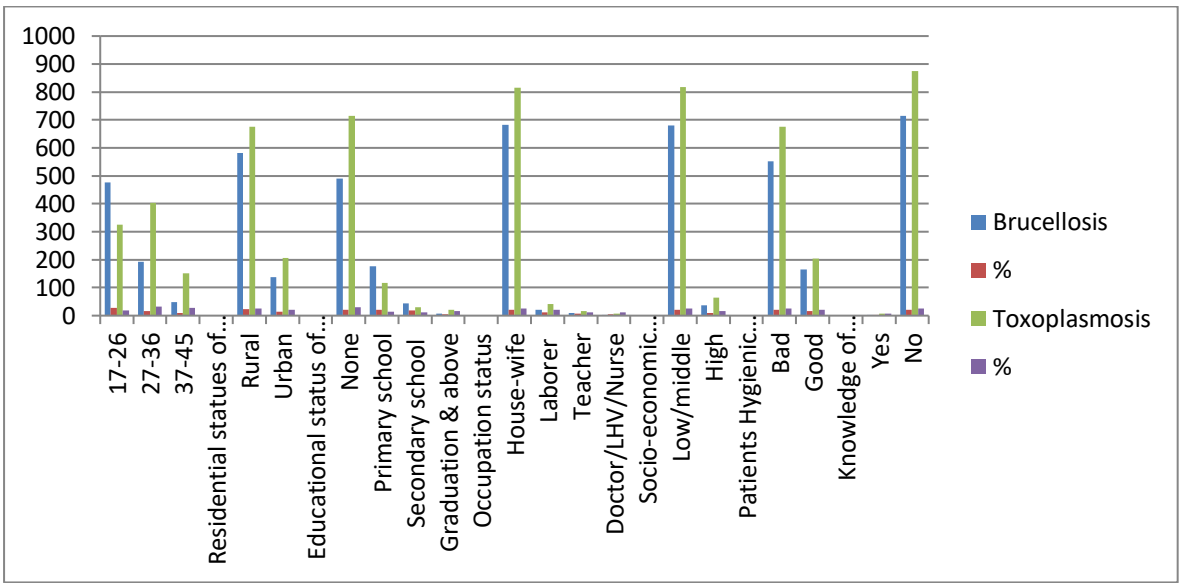

Figure 4.8: Socio-demographic characteristics and sero-prevalence of emerging zoonotic diseases (Brucellosis, Toxoplasmosis) in women of Khyber Pakhtunkhwa province, Pakistan

4.3 Epidemiological factors associated with zoonotic diseases (Brucellosis, Toxoplasmosis) in Khyber Pakhtunkhwa province, Pakistan.

Table 4.8 Assessments of various potential epidemiological risk factors, associated with zoonotic diseases (Brucellosis, Toxoplasmosis) in women of Khyber Pakhtunkhwa province, Pakistan.

Epidemiological Characteristics	No of Participants		Zoonotic Diseases			Brucellosis			Toxoplasmosis (%)	%	p-value
Variables	n=3586	%	1599	44.59%	p-value	718	20.02%	p-value	881	24.56%	
Animals at homes											
Yes	2157	60.15	1093	50.67	0.00	496	22.99	0.00	597	27.67	0.00
No	1429	39.85	506	35.41		222	15.53		284	19.87	
Shearing shelter with animals during winter											
Yes	296	8.25	72	24.32	1.00	55	18.58	0.74	17	5.74	1.00
No	3290	91.74	1527	46.41		663	20.15		864	26.26	
Exposure to animals/cleaning animal waste/shelters											
Yes	1867	52.06	898	48.09	0.00	401	21.47	0.01	497	26.62	0.00
No	1719	47.93	701	40.77		317	18.44		384	22.33	
Assisted animal births/abortion/stillbirth											
Yes	938	26.15	272	28.99	1.00	167	17.81	0.97	105	11.19	1.00
No	2648	73.84	1327	50.11		551	20.81		776	29.31	
Cutting/ handling animals meat at home for cocking											
Yes	3009	83.91	1416	47.05	0.00	649	21.57	0.00	767	25.49	0.00
No	577	16.09	183	31.71		69	11.95		114	19.75	
Regular hand washing habit after cutting meat/milking and caring animals/other works											
Yes	1282	35.75	433	33.77	1.00	200	15.61	1.00	233	18.17	1.00
No/Some time	2304	64.25	1166	50.61		518	22.48		648	28.12	
Previous or recent history/habit of drinking/raw/unpasteurized milk/dairy products											
Yes	503	14.02	129	25.64	1.00	98	19.48	0.62	31	6.16	1.00
No	3083	85.97	1470	47.68		620	20.11		850	27.57	
Processing animal milk/dairy products at homes											
Yes	1702	47.46	872	51.23	0.00	391	22.97	0.00	481	28.26	0.00
No	1884	52.54	727	38.58		327	17.35		400	21.23	
Recent/past Family history of zoonotic infections (Brucellosis, Toxoplasmosis)											

Yes	239	6.67	72	30.12	1.00	49	20.51	0.42	23	9.62	1.00
No	3347	93.33	1527	45.62		669	19.98		858	25.63	
History of blood transfusion											
Yes	219	6.11	27	12.32	1.00	19	8.67	1.00	8	3.65	1.00
No	3367	93.89	1572	46.68		699	20.76		873	25.92	
Habits of eating roasted/under cocked meat/beef											
Yes	419	11.68	37	8.83	1.00	10	2.38	1.00	27	6.44	1.00
No	3167	88.31	1562	49.32		708	22.35		854	26.96	
Habits of eating raw vegetables/salad/unwashed fruits											
Yes	732	20.41	237	32.37	1.00	32	4.37	1.00	205	28.01	0.00
No	2854	79.58	1362	47.72		686	24.03		676	23.68	
Drinking filter/boiled water at homes											
Yes	108	3.01	19	17.59	1.00	16	14.81	0.91	3	2.78	1.00
No	3478	96.98	1580	45.43		702	20.18		878	25.24	
Store animals waste/manure in homes/near homes for agriculture purposes											
Yes	1218	33.96	619	50.82	0.00	279	22.91	0.00	320	26.27	0.04
No	2368	66.03	980	41.38		439	18.53		561	23.69	

2-sample test for equality of proportions without continuity correction

Table 4.8 shows various potential epidemiological variables, which are associated with transmission of emerging zoonotic diseases (Brucellosis, Toxoplasmosis) in women in various regions of Khyber Pakhtunkhwa province of Pakistan, which is graphically shown by figure 4.8.

4.3.1 Livestock animals in homes and prevalence of zoonotic diseases in women

Assessment of zoonotic diseases (Brucellosis, Toxoplasmosis) was carried out in women patients, who were keeping different species of animals in homes and those, who had no animals in homes. A significant difference (<0.05) was found in rate of zoonotic diseases in women of these two groups. Prevalence of zoonotic diseases was significantly (<0.05) high 1093 (50.67%) in women, who had animals in homes, while rate of zoonotic diseases was low 506 (35.41%) in those women, who had no animal in homes.

Brucellosis: Prevalence of Brucellosis infection was significantly different (<0.05) in women of these two groups. Prevalence of infection was high 496 (22.99%) in those women, who were keeping animals in homes, while rate of infection was low 222 (15.53%) in those women, who had no animals in homes.

Toxoplasmosis: A significant difference (<0.05) was found in prevalence of Toxoplasmosis in women of these two groups. Prevalence of Toxoplasmosis was high 597 (27.67%) in women, who were keeping animals in homes, while rate of infection was low 284 (19.87%) in those patients, who had no animal in homes.

4.3.2 Shearing of shelter with animal during winter and prevalence of zoonotic diseases in women

Assessment of zoonotic diseases (Brucellosis, Toxoplasmosis) was carried out in women, who were shearing shelter with their animal during winter season and those, who were not involved in such type of activates. No significant difference (>0.05) was found in prevalence of zoonotic diseases between these two group of women. Prevalence of zoonotic diseases was high 1527 (46.41%) in those women, who had no history of shearing shelter with animals, while rate of zoonotic diseases was low 72 (24.32%) in women, who were involved in these activates.

Brucellosis: There was no significant difference (>0.05) in prevalence of Brucellosis infection between these two group of women. Prevalence of Brucellosis was high 663 (20.15%) in women, who were not involved in shearing shelter with animals, while rate of infection was low 55 (18.58%) in those women, who had positive history of shearing shelter with animals during winter season.

Toxoplasmosis: There was no significant difference (>0.05) in rate of Toxoplasmosis infection between these two groups of women. Prevalence of Toxoplasmosis was high 864 (26.26%) in women, who had no history of shearing shelter with animals, while rate of infection was comparatively low 17 (5.74%) in those women, who had positive history of shearing shelter with animals.

4.3.3 Animal exposure and prevalence of zoonotic diseases in women

Assessment of zoonotic diseases (Brucellosis, Toxoplasmosis) was carried out in women, who were exposed to animals during various types of activates like cleaning of animals, their wastes and shelters and those women, who were not involved in such types of activates. A significant difference (<0.05) was found in prevalence of

zoonotic diseases between these two group of patients. Prevalence of zoonotic diseases was high 898 (48.09%) in women, who were exposed to animals, while rate of zoonotic diseases was low 701 (40.77%) in those women, who had no history of animal exposures.

Brucellosis: A significant difference (<0.05) was found in rate of Brucellosis infection in women, who were exposed to animals and those, who had no history of animal exposure. Prevalence of Brucellosis was high 401 (21.47%) in women, who had positive history of animal exposure, while rate of infection was low 317 (18.44%) in those women, who had no history of animals exposure.

Toxoplasmosis: A significant difference (<0.05) was also found in rate of Toxoplasma infection in women of these two groups. Prevalence of Toxoplasmosis was high 497 (26.62%) in women, who had a positive history of animal exposure, while rate of infection was low 384 (22.33%) in those women, who had no history of animals exposure.

4.3.4 Exposure to animal deliveries and prevalence of zoonotic diseases in women

Assessment of zoonotic diseases was carried out in women, who had assisted animal births, abortion or stillbirth and those women, who were not involved in these activates. No significant difference (>0.05) was found in prevalence of zoonotic diseases between these two group of women. Prevalence of zoonotic diseases was high 1327 (50.11%) in women, who had no history of such type of activates, while rate of zoonotic diseases was low 272 (28.99%) in those women, who had assisted animal birth, abortion or still birth.

Brucellosis: There was no significant difference (>0.05) in rate of Brucellosis infection between these two groups of women patients. Prevalence of Brucellosis was high 551 (20.81%) in women, who had no history of dealing animal birth or abortions, while rate of infection was low 167 (17.81%) in those women, who had positive history of these activities.

Toxoplasmosis: There was no significant (>0.05) difference in rate of Toxoplasmosis between these two groups of women. Prevalence of infection was high 776 (29.31%) in women, who were not involved in assistance of animal birth or abortion, while rate of Toxoplasmosis was low 105 (11.19%) in those patients, who had positive history of these activities.

4.3.5 Handling of animals meat at home for cocking and prevalence of zoonotic diseases in women

Assessment of zoonotic diseases was carried out in women patients, who were involved in cutting and handling animal meat at home for cocking and in those women, who were not involved in such type of activates. A significant difference (<0.05) was found in prevalence of zoonotic diseases between these two group of women. Prevalence of zoonotic diseases was high 1416 (47.05%) in women, who were involved in cutting and handling animal meat in homes for cocking purposes, while rate of zoonotic diseases was low 183 (31.71%) in those women, who were not involved in these activities in homes.

Brucellosis: A significant difference (<0.05) was found in rate of Brucellosis infection between these two group of women. Prevalence of Brucellosis was high 649 (21.57%) in women, who were involved in handling animal

meat in homes, while rate of infection was low 69 (11.95%) in those women, who were not dealing animal meat for cocking in homes.

Toxoplasmosis: A significant difference (<0.05) was found in rate of Toxoplasmosis in women of these two groups. Prevalence of Toxoplasmosis was high 767 (25.49%) in women, who were involved in dealing animal meat for cocking, while rate of infection was low 114 (19.75%) in those women, who were not involved in such type of activities in their homes.

4.3.6 Habit of hand washing and prevalence of zoonotic diseases in women

Assessment of zoonotic diseases (Brucellosis, Toxoplasmosis) was carried out in those women, who had habits of regular hand wash after cutting meat, milking and caring of animal and in those women, who had no hand washing habit after performing such activities in homes. No significant difference (<0.05) was found in rate of zoonotic diseases between these two groups of women. Prevalence of zoonotic diseases was high 1166 (50.61%) in women, who had no habit of regular hand wash after dealing animal related activities in homes, while rate of zoonotic diseases was low 433 (33.77%) in those women, who had habits of regular hand wash after cutting meat, milking animals and caring of animals.

Brucellosis: No significant difference (>0.05) was found in rate of Brucellosis in women of these two groups. Prevalence of Brucellosis was high 518 (22.48%) in those women, who had no habit of regular hand wash, while rate of infection was low 200 (15.61%) in those women, who had habit of regular hand wash.

Toxoplasmosis: There was no significant difference (>0.05) in prevalence of Toxoplasma infection in women of these two groups. Prevalence of Toxoplasmosis was high 648 (28.12%) in women, who had no hand washing habit, while rate of infection was low 233 (18.17%) in those women, who had regular hand washing habit.

4.3.7 Consumption of unpasteurized dairy products and prevalence of zoonotic diseases in women

Assessment of zoonotic diseases was carried out in women, who had history of drinking raw, unpasteurized milk and other dairy products and in those women, who had no history of consuming unpasteurized dairy products. No significant difference (>0.05) was found in rate of zoonotic diseases in women of these two groups. Prevalence of zoonotic diseases was high 1470 (47.68%) in those women, who had no history of consuming unpasteurized dairy products, while rate of zoonotic diseases was low 129 (25.64%) in those women, who were positive for history of consuming unpasteurized dairy products.

Brucellosis: There was no significant difference (<0.05) in rate of Brucellosis infection in women of these two groups. Prevalence of Brucellosis was high 620 (20.11%) in women, who had no history of consuming unpasteurized dairy products, while rate of infection was low 98 (19.48%) in those women, who were positive for a history of consuming unpasteurized dairy products.

Toxoplasmosis: There was no significant difference (>0.05) in rate of Toxoplasmosis infection in women of these two groups. Prevalence of Toxoplasmosis was high 850 (27.57%) in women, who had no history of

consuming unpasteurized dairy products, while rate of infection was low 31 (6.16%) in those women, who had positive history of consuming various dairy products.

4.3.8 Processing of dairy products at homes and prevalence of zoonotic diseases in women

Assessment of zoonotic diseases was carried out in women group, who were involved in processing animal milk and various other dairy products at their homes and in those women, who were not involved in such type of activities. A significant difference (<0.05) was found in rate of zoonotic diseases between these two groups of women. Prevalence of zoonotic diseases was high 872 (51.23%) in women group, who were involved in processing animal milk and other dairy products at their homes, while rate of zoonotic diseases was low 727 (38.58%) in women group, who were not involved in such type of activities in their homes.

Brucellosis: There was significant difference (<0.05) in rate of Brucellosis in women of these two group. Prevalence of Brucellosis was high 391 (22.97%) in women, who were involved in processing dairy products in homes, while rate of infection was low 327 (17.35%) in those women, who were not involved in such activities in their homes.

Toxoplasmosis: There was significant difference (<0.05) in rate of Toxoplasmosis infection in women of these two group. Prevalence of Toxoplasmosis was high 481 (28.26%) in women, who were involved in processing dairy products at homes, while rate of infection was low 400 (21.23%) in those women, who were not engaged in such type of activities at their homes.

4.3.9 Family history of zoonotic diseases and prevalence of zoonotic infection in women

Assessment of zoonotic diseases was carried out in women groups, who had recent or past family history of zoonotic diseases and those women group, who had no positive history of zoonotic diseases. No significant difference (>0.05) was found in rate of zoonotic diseases in women of these two group. Prevalence of zoonotic diseases was high 1527 (45.62%) in women group, who had no family history of zoonotic diseases, while rate of diseases was low 72 (30.12%) in women group, who had positive family history of zoonotic diseases.

Brucellosis: There was no significant difference (>0.05) in rate of Brucellosis in women of two groups. Prevalence of Brucellosis was high 49 (20.51%) in those women, who had positive family history of Brucellosis, while rate of infection was low 669 (19.98%) in those women, who had no positive family history of Brucellosis.

Toxoplasmosis: There was no significant difference (>0.05) in rate of Toxoplasmosis in women of these two groups. Prevalence of Toxoplasmosis was high 858 (25.63%) in women, who had no family history of Toxoplasmosis, while rate of infection was low 23 (9.62%) in those women, who had positive family history of Toxoplasma infection.

4.3.10 History of blood transfusion and prevalence of zoonotic diseases in women

Assessment of zoonotic diseases (Brucellosis, Toxoplasmosis) was carried out in women, who had history of blood transfusion and in those women, who had no history of blood transfusion, No significant difference (>0.05) was found in rate of zoonotic diseases in women of these two groups. Prevalence of zoonotic diseases

was high 1572 (46.68%) in those women, who had no history of blood transfusion, while rate of zoonotic diseases was low 27 (12.32%) in those women, who had previous history of blood transfusion.

Brucellosis: There was no significant difference (>0.05) in rate of Brucellosis infection in women of these two groups. Prevalence of Brucellosis was high 699 (20.76%) in women, who had no history of blood transfusion, while rate of infection was low 19 (8.67%) in those women, who had previous history of blood transfusion.

Toxoplasmosis: There was no significant difference (>0.05) in rate of Toxoplasma infection in women of these two groups. Prevalence of Toxoplasmosis was high 873 (25.92%) in those women, who had no history of blood transfusion, while rate of infection was low 8 (3.65%) in those women, who had previous history of blood transfusion.

4.3.11 Habits of eating roasted or under cocked meat or beef and prevalence of zoonotic diseases in women

Assessment of zoonotic diseases was carried out in women, who had habits of eating roasted or under cocked meat, beef and in those women, who had no habit of eating under cocked meat. No significant difference (>0.05) was found in rate of zoonotic diseases in women of these two groups. Prevalence of zoonotic diseases was high 1562 (49.32%) in women group, who had no habit of eating under cocked meat, while rate of zoonotic diseases was low 37 (8.83%) in those women, who had habit of eating roasted or under cocked meat or beef.

Brucellosis: There was no significant difference (>0.05) in rate of Brucellosis infection in women of these two groups. Prevalence of Brucellosis was high 708 (22.35%) in those women, who had no habits of eating under cocked meat, while rate of infection was low 10 (2.38%) in those women, who had habits of eating roasted or under cocked meat or beef.

Toxoplasmosis: There was no significant difference (>0.05) in rate of Toxoplasma infection in women of these two groups. Prevalence of Toxoplasmosis was high 854 (26.96%) in women, who had no habits of eating under cocked meat, while infection rate was low 27 (6.44%) in those women, who had habits of eating roasted or under cocked meat or beef.

4.3.12 Habits of eating raw vegetables or unwashed fruits and prevalence of zoonotic diseases in women

Assessment of zoonotic diseases was carried out in women, who were eating raw vegetable or unwashed fruits and in those women, who were not eating raw vegetable or unwashed fruits. No significant difference (>0.05) was found in rate of zoonotic diseases in women of these two group. Prevalence of zoonotic diseases was high 1362 (47.72%) in those women, who had no habit of eating raw and unwashed vegetable, while rate of diseases was low 237 (32.37%) in those women, who had habits of eating raw vegetable, unwashed salad or fruits.

Brucellosis: There was no significant difference (>0.05) in rate of Brucellosis infection in women of these two group. Prevalence of Brucellosis infection was high 686 (24.03%) in those women, who had no habits of eating unwashed fruits or salad, while rate of infection was low 32 (4.37%) in those women, who were eating unwashed fruits or salad.

Toxoplasmosis: A significant difference (<0.05) was found in rate of Toxoplasma infection in women of these two groups. Prevalence of Toxoplasmosis was high 205 (28.01%) in women, who had habits of eating raw vegetable or unwashed salad and fruits, while rate of Toxoplasma infection was low 676 (23.68%) in those women, who had no habits of eating raw vegetable, unwashed salad or fruits.

4.3.13 Drinking unfiltered water at homes and prevalence of zoonotic diseases in women

Assessment of zoonotic diseases was carried out in women, who used to drink filter or boiled water at homes and in those women, who were not using filter or boil water. No significant difference (>0.05) was found in rate of zoonotic diseases in women of these two groups. Prevalence of zoonotic diseases was high 1580 (45.43%) in those women, who were not used to drink filter or boil water in homes, while rate of zoonotic diseases was low in those women, who used to drink filtered water in homes.

Brucellosis: No significant difference (>0.05) was observed in rate of Brucellosis infection in women of these two groups. Prevalence of Brucellosis was high 702 (20.18%) in those women, who were not drinking filter or boil water at homes, while rate of zoonotic diseases was low in those women, who used to drink filter or boil water in their homes.

Toxoplasmosis: No significant difference (>0.05) was found in rate of Toxoplasmosis infection in women of these two groups. Prevalence of Toxoplasma infection was high 878 (25.24%) in those women, who were not used to drink filter or boil water in homes, while rate of infection was low3 (2.78%) in those women, who used to drink filtered water in their homes.

4.3.14 Storage of animals manure in homes for agriculture purposes and prevalence of zoonotic diseases in women

Assessment of zoonotic diseases was carried out in those women, who were storing animal manure in homes or near homes for agriculture, economic and burning purposes and in those women, who were not involved in these activities. A significant difference (<0.05) was found in rate of zoonotic diseases between these two group of women. Prevalence of zoonotic diseases was high 619 (50.82%) in those women, who were storing animals manure in homes or near homes for making dung cake and agriculture purposes, while rate of zoonotic diseases was low 980 (41.38%) in those women, who were not engage in such type of activates.

Brucellosis: A significant difference (<0.05) was found in rate of Brucellosis infection between these two groups of women. Prevalence of Brucellosis was high 279 (22.91%) in those women, who used to store animal manure in homes for preparation of dung cake and agriculture purposes, while rate of infection was low 439 (18.53%) in those women, who were avoiding these unhygienic activities.

Toxoplasmosis: A significant difference (<0.05) was found in rate of Toxoplasmosis infection in women of these two groups. Prevalence of Toxoplasmosis was high 320 (26.27%) in women, who were storing animal dung in homes for various economic purposes, while rate of infection was low561 (23.69%) in those women, who were not involved in such risky and unhygienic activities in homes.

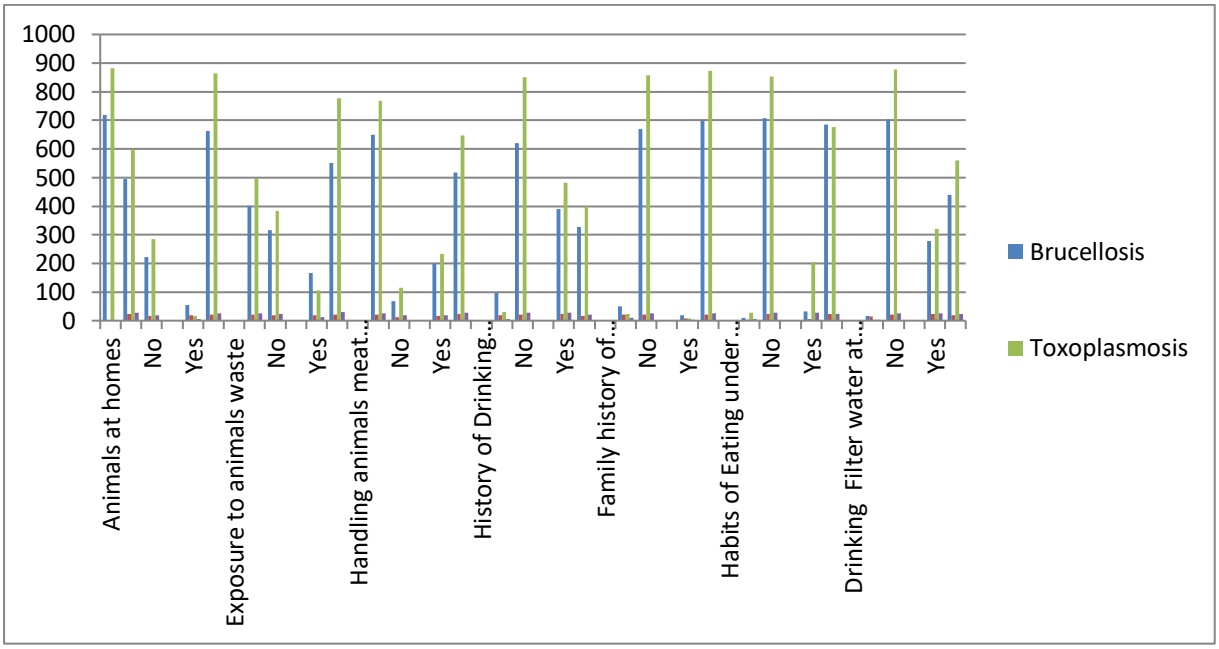

Figure 4.9: Assessments of various potential risk factors associated with emerging zoonotic diseases (Brucellosis, Toxoplasmosis) in women in Khyber Pakhtunkhwa province, Pakistan.

4.4 Laboratory assays used for diagnosis of zoonotic diseases (Brucellosis, Toxoplasmosis) in women

4.4.1 Laboratory assays used for diagnosis of Brucellosis

Table: 4.9 Comparative results, validity (sensitivity, specificity, positive, negative predictive value) of Brucella specific various serological assays (SPAT, RBPT ELISA) and PCR for diagnosis and confirmation of Brucellosis infection in women in Khyber Pakhtunkhwa province, Pakistan

Brucella specific serological assays and PCR Tests								
Method	n	Positive	%	Sensitivity	Specificity	PPV	NPV	p-value
	3586	718	20.02	----	----	----	----	----
SPAT	3586	755	21.05	94.29	97.28	89.67	98.55	0.00*
RBPT	3586	725	20.21	92.48	97.87	91.58	98.12	0.00*
ELISA	1029	724	70.36	100	98.07	99.17	100	0.00*
IgM	1029	242	23.51	33.15	98.72	98.35	39.01	----
IgG	1029	120	11.66	16.44	99.36	98.34	33.99	0.00**
IgM, IgG	1029	362	35.18	50.42	100	100	46.63	0.00**
PCR	269	61	22.67	----	----	----	----	1.00

*Pearson chi-square
*Likelihood ratio
*Fisher's exact test
*Linear-by-Linear association (SPSS values)
**Pearson's Chi-squared test
**Paired sample t test for the difference of IgG and IgM (SPSS)
PPV=Positive predictive value
NPV=Negative predictive value

Table: 4.9 show comparative results, validity (sensitivity, specificity, positive, negative predictive value) of Brucella specific serological assays like SPAT, RBPT ELISA and PCR for diagnosis and confirmation of Brucellosis infection in women, which is graphically shown by figure 4.9, 4.10, 4.11. SPAT and RBPT test was used as initial diagnostic procedures for Brucellosis, while IgM, IgG ELISA and PCR were used as confirmatory diagnostic assays. SPAT and RBPT assays were performed on sera of all women patients (n=3586), while IgM and IgG ELISA assay was conducted only on SPAT and RBPT positive sera and SPAT and RBPT negative, but clinically suspected patients (n= 1029). PCR was performed on patient's blood (n=269), which show equivocal results on IgM, IgG ELISA or show negative results on SPAT or RBPT and ELISA, but were suspected for Brucellosis infection on clinical criteria. SPAT, RBPT and ELISA assays significantly (<0.05) detected Brucellosis infection in women patients. According to positive cutoff point assigned for each diagnostic assay, 755 (21.05%) women were found positive (<0.05) for Brucellosis by SPAT assay, 725 (20.21%) by RBPT, 724 (70.36%) by ELISA assay and 61 (22.67%) cases were confirmed by Brucella genus specific PCR. A significant difference (<0.05) was found in positivity of Brucella specific IgM, IgG and IgM, IgG antibodies. IgM was positive in 242 (23.51%) cases, IgG in 120 (11.66%) and IgM, IgG in 362 (35.18%) infected women. Sensitivity, specificity, positive predictive value (PPV) and negative predictive values (NPV) were separately determined for each diagnostic assay with test positivity and WHO recommended clinical criteria. With reference to ELISA assay, sensitivity of SPAT assay was found 94.29%, specificity 97.28%, PPV 89.67%, NPV 98.55%, while RBPT assay showed a sensitivity of 92.48%, specificity 97.87%, PPV 91.58% and NPV 98.12%. Similarly ELISA assay as a whole showed a sensitivity of 100%, specificity 98.07%, PPV 99.17%, NPV 100% with reference to positivity of SPAT, RBPT, PCR assay and WHO recommended clinical criteria for diagnosis of Brucellosis. IgM ELISA showed a sensitivity of 33.15, specificity 98.72, PPV 98.35%, NPV 39.01% and IgG ELISA showed sensitivity of 16.44, specificity 99.36%, PPV 98.34%, NPV 33.99%, while ELISA for IgM and IgG was collectively found with a sensitivity of 50.42, specificity 100%, PPV 100% and NPV 46.63% respectively.

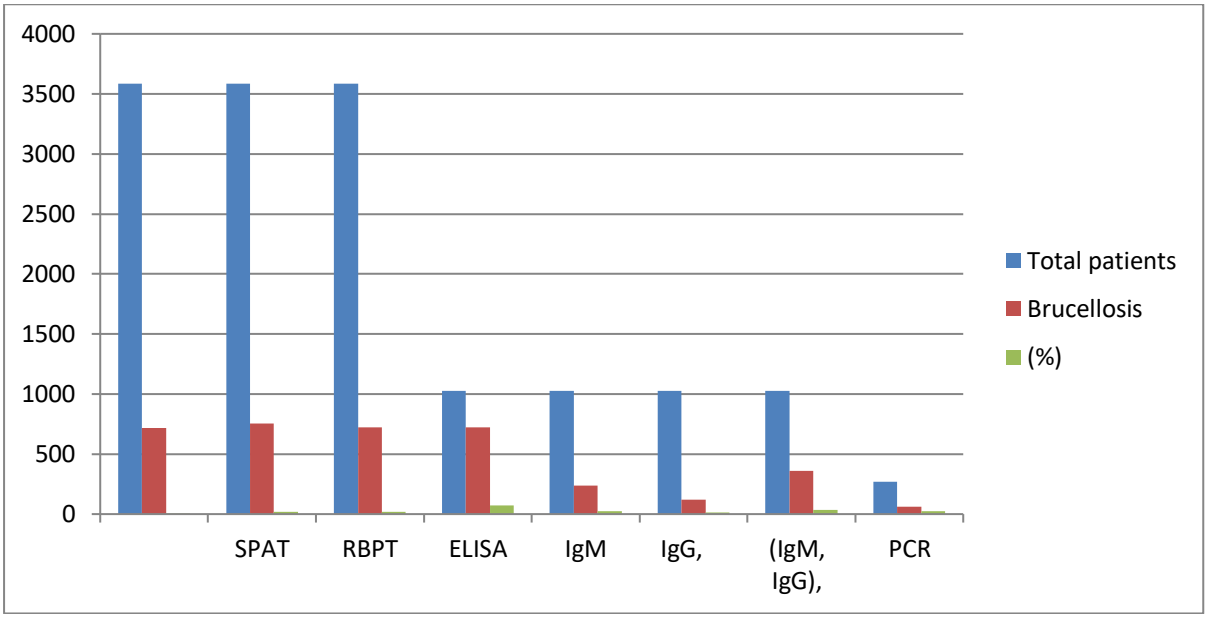

Figure 4.10: Comparative results of Brucella specific screening assay (SPAT, RBPT, ELISA) and PCR used for diagnosis and confirmation of Brucellosis infection in women in Khyber Pakhtunkhwa province, Pakistan.

Figure 4.11: Agarose gel electrophoresis, which show DNA product of PCR amplification (223 base pair) using general primer (Oligonucleotide) sequence, which is most commonly used for identification of DNA of genus Brucella through conventional PCR. A DNA ladder of 1000 bp was run with blood sample of patient for proper identification and confirmation of Brucella DNA.

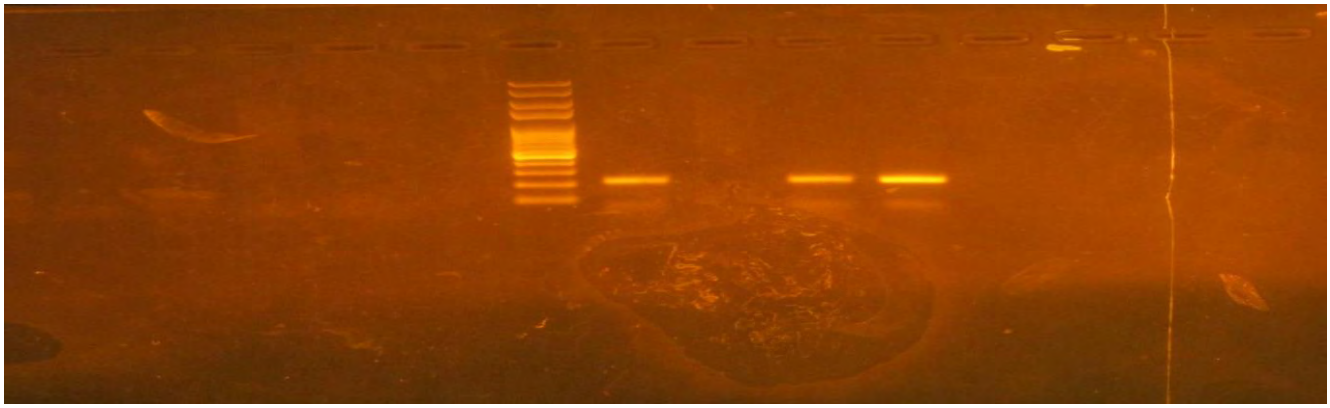

Figure 4.12: Agarose gel Electrophoresis showing product of PCR amplification of 223-bp region within a gene, which code for 31kD Brucella antigen using general primer (Oligonucleotide) sequence, which is commonly used for identification of genus Brucella through conventional PCR. A 1000 bp DNA ladder was run with patient blood sample for identification and confirmation of Brucella DNA.

Table: 4.10 Result of quantitative estimation of *Brucella abortus* and *Brucella melitensis* specific antibodies titer (direct SPAT method) in SPAT positive women in Khyber Pakhtunkhwa province, Pakistan.

Degree of Agglutination			*Br-abortus*		*Br-melitensis*		*Br-abortus& Br-melitensis*	
n= 3586	n=755	21.05%	240	31.78%	174	23.04%	341	45.16%
Assay Titer								
4+ (100%)	191	25.29	63	8.34	41	5.43	87	11,52
3+ (75%)	269	35.63	86	31.97	71	9.41	112	14.83
2+ (50%)	195	25.82	62	8.21	47	6.22	86	11.39
1+ (25%)	57	7.55	19	2.51	11	1.46	27	3.58
± (<25%)	43	5.69	10	1.32	4	0.53	29	3.84

Table 4.10 represents quantitative assessment of SPAT assay using febrile antigens for detection of *B. abortus* and *B. melitensis* specific antibodies titer (through direct SPAT method) in 755 women patients, who were positive for Brucellosis by SPAT assay, which is graphically shown by figure 4.12 and 4.13. Among 755 (21.05%) women, who were positive for Brucellosis by SPAT assay, 240 (31.78%) showed antibodies for *Br. abortus* 174 (23.04%) for *Br. melitensis* and 341 (45.16%) for mixed antibodies (*Br. abortus* and *Br. melitensis*). Among SPAT positive (n=755) women, a high degree of antigen-antibodies agglutination (100% and 75%) was shown by sera of 191 (25.29%) and 269 (35.63%) women, while low degree of agglutination (50%, 25% and <25%) was shown by sera of 195 (25.82%), 57 (7.55%) and 43 (5.69%) women.

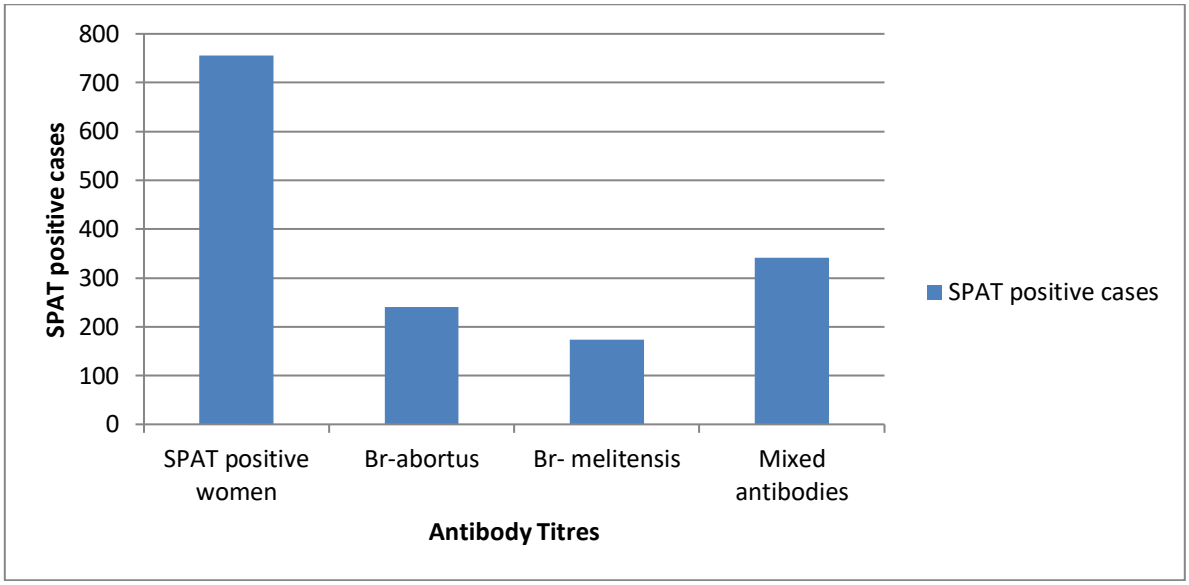

Figure 4.13: Result of quantitative estimation of *Brucella abortus* and *Brucella melitensis* specific antibodies titer in women of Khyber Pakhtunkhwa province, Pakistan.

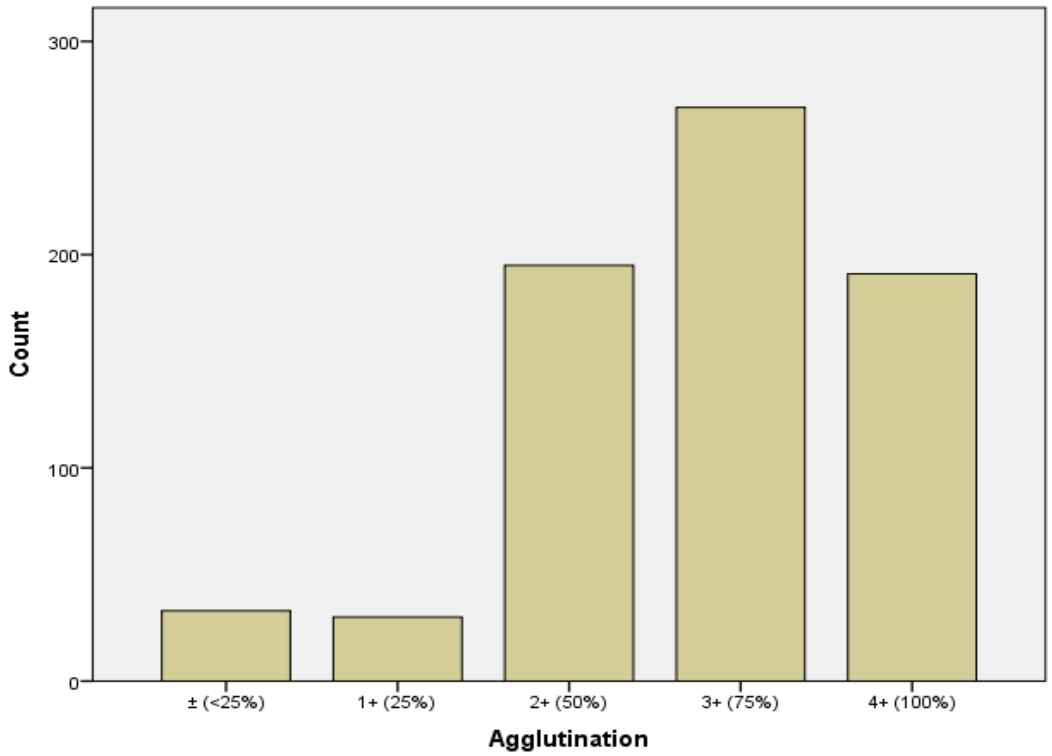

Figure 4.14: Result of quantitative estimation of Brucella specific antibodies titer (direct SPAT method) in women, who were positive for Brucellosis by SPAT assay in Khyber Pakhtunkhwa province, Pakistan.

Table: 4.11 Result of quantitative estimation of Brucella specific immunoglobulin (IgM, IgG) titer estimated by ELISA method in women of Khyber Pakhtunkhwa province, Pakistan

Variables	Positive	%	R-Range	n=1029	%	Mean ± S.E	SD	p-value
IgM	242	23.52	----	1029	----	16.35±0.26	8.1501	
			<9	186	18.08	6.39±0.13	1.6858	
			9-11	239	23.22	10.56±0.05	0.7665	
			>11	604	58.69	21.71±0.26	6.2833	
IgG	120	11.66	----	1029	----	14.52±0.24	7.4453	0.00*
			<9	204	19.82	6.41±0.14	1.9261	
			9-11	343	33.33	10.61±0.05	0.7601	
			>11	482	46.84	20.74±0.29	6.2349	
IgM, IgG	362	35.18	----	----	----	21.71±0.26	6.2833	0.00**
Total	724	70.36	----	----	----	----	----	----

P-value 7.171e-08= <0.05
*2-sample test for equality of proportions without continuity correction
**Paired sample t test for the difference of IgG and IgM (SPSS)

Table: 4.11 indicate Brucella specific immunoglobulin (IgM) and (IgG) titer, which was quantitatively estimated by Brucella specific ELISA method in 1029 women, who were suspected for Brucellosis. A significant difference (<0.05) was found in positivity of Brucella specific IgM and IgG antibodies. IgM was found positive in 242 (23.52%) women with a mean raised immunoglobulin (IgM) value of 21.71±0.26, while immunoglobulin (IgG) was found positive in 120 (11.66%) infected women with a mean raised titer value of 20.74±0.29, while IgM and IgG were collectively positive in 362 (35.18%) infected women.

Table 4.12 Results of Brucella specific IgM and IgG detected in different clinical stages of Brucellosis (acute, sub-acute, chronic) in infected women in Khyber Pakhtunkhwa province, Pakistan.

Clinical Stages of Brucellosis Infection								
Variables	Patients	Acute		Sub-acute		Chronic		
ELISA	718(20.02)	428(59.61%)	M±S.E	239(33.28%)	M±S.E	51(7.11%)	M±S.E	p-value
IgM >12	238(33.14)	207(48.36)	21.79±0.46	21(8.78)	21.65±1.29	10(19.61)	23.85±1.39	0.00
IgG>12	118(16.43)	21(4.91)	19.21±1.09	68(28.45)	20.81±0.86	29(56.86)	21.78±0.99	0.00
IgM & IgG>12	362(50.41)	200(46.73)	21.39±0.47	150(62.76)	21.75±0.55	12(23.53)	22.09±1.46	0.00

Pearson's Chi-squared test

Table 4.12 show assessment of Brucella specific immunoglobulin IgM and IgG in different clinical stages (acute, sub-acute and chronic) cases of Brucellosis infection in 718 women patients. A significant difference (<0.05) was found in positivity of (IgM), (IgG) and (IgM, IgG) during various clinical stages of Brucellosis infection., Among 718 patients, 362 (50.41%) women were found positive for Brucella specific (IgM, IgG), followed by 238 (33.14) for IgM and 118 (16.43%) for IgG. Immunoglobulin's (IgM and IgG) were detected in 200 (46.73%) cases of acute infection with a mean titer value of 21.39±0.47, 150 (62.76%) cases of sub-acute infection with a mean titer value of 21.75±0.55 and 12 (23.53%) cases of chronic Brucellosis infection with a

mean titer value of 22.09±1.46, while IgM was detected in 207 (48.36%) cases of acute infection with mean titer value of 21.79±0.46, 21 (8.78%) cases of sub-acute infection with a mean titer value of 21.65±1.29 and 10 (19.61%) cases of chronic infection with a mean titer value of 23.85±1.39. Similarly IgG was detected in 21 (4.91%) cases of acute infection with a mean titer value of 19.21±1.09, 68 (28.45%) cases of sub-acute infection with a mean titer value of 20.81±0.86 and 29 (56.86%) cases of chronic infection with a mean titer value of 21.78±0.99.

4.4.2 Laboratory assays used for diagnosis of Toxoplasmosis infection

Table 4.13 Comparative results and validity (sensitivity, specificity, positive and negative predictive values) of various serological assays used for diagnosis of Toxoplasmosis in women of Khyber Pakhtunkhwa province, Pakistan.

Method	Patients	Positive	(%)	Sensitivity	Specificity	PPV	NPV	p-value
n=3586	n=3586	881	24.56	----	----	----	----	----
Latex Agglutination Test								0.95**
LAT	----	913	25.46	97.27	97.92	93.86	99.11	0.00*
ICT Lateral Flow Chromatography Immunoassay								
	----	915	25.51	96.25	97.52	92.68	98.76	0.00*
IgM	----	112	3.12	78.13	98.93	66.96	99.41	----
IgG	----	694	19.35	98.82	99.31	97.11	99.72	----
IgM & IgG	----	109	3.04	96.12	99.71	90.82	99.88	----
ELISA	n=1195	881	73.72	----	----	----	----	0.00*
IgM	----	96	8.03	----	----	----	----	----
IgG	----	682	57.07	----	----	----	----	0.00***
IgM & IgG	----	103	8.62	----	----	----	----	----

*Pearson Chi-Square
*Continuity Correction[b]
*Likelihood Ratio
*Fisher's Exact Test
*Linear-by-Linear Association (By SPSS)
**Testing the difference of proportions between LAT and ICT test
**2-sample test for equality of proportions without continuity correction
***3-sample test for equality of proportions without continuity correction

Table 4.13 and figure 4.14 represent comparative results and validity (sensitivity, specificity, positive and negative predictive values) of various serological assays (latex agglutination, immunoblot ICT and ELISA), which were used for diagnosis and confirmation of Toxoplasmosis infection in women patients. Latex agglutination assay and lateral flow chromatography immunoassay (ICT) were used as initial screening procedures for diagnosis of Toxoplasmosis in 3586 women, while IgM and IgG ELISA was used as confirmatory diagnostic assay in 1195 women. Latex agglutination assay (LAT) and lateral flow chromatography immunoassay (ICT) significantly (<0.05) detected Toxoplasmosis in women and no significant difference (>0.05) was found in assessment of infection between these two assays. According to positive cutoff point recommended for each diagnostic assay, 913 (25.46%) women were significantly (<0.05) found positive

for Toxoplasmosis by latex agglutination assay, 915 (25.51%) by lateral flow chromatography immunoassay (ICT), while IgM and IgG ELISA significantly (<0.05) confirmed 881 (73.72%) women for infection of Toxoplasmosis. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of latex agglutination assay and lateral flow chromatography immunoassay (ICT) were separately determined for each diagnostic assay with comparison of i-ELISA. Sensitivity of latex agglutination assay was found 97.27%, specificity 97.92%, PPV 93.86% and NPV 99.11%. Similarly lateral flow chromatographic immunoassay (ICT) was found with a sensitivity of 96.25%, specificity 97.52%, PPV 92.68%, NPV 98.76%, while ICT IgM showed a sensitivity of 78.13, specificity 98.93, PPV 66.96%, NPV 99.41% and ICT IgG was found with a sensitivity of 98.82, specificity 99.31%, PPV 97.11%, NPV 99.72%, while ICT IgM, IgG was found with a sensitivity of 96.12, specificity 99.71%, PPV 90.82% and NPV 99.88%.

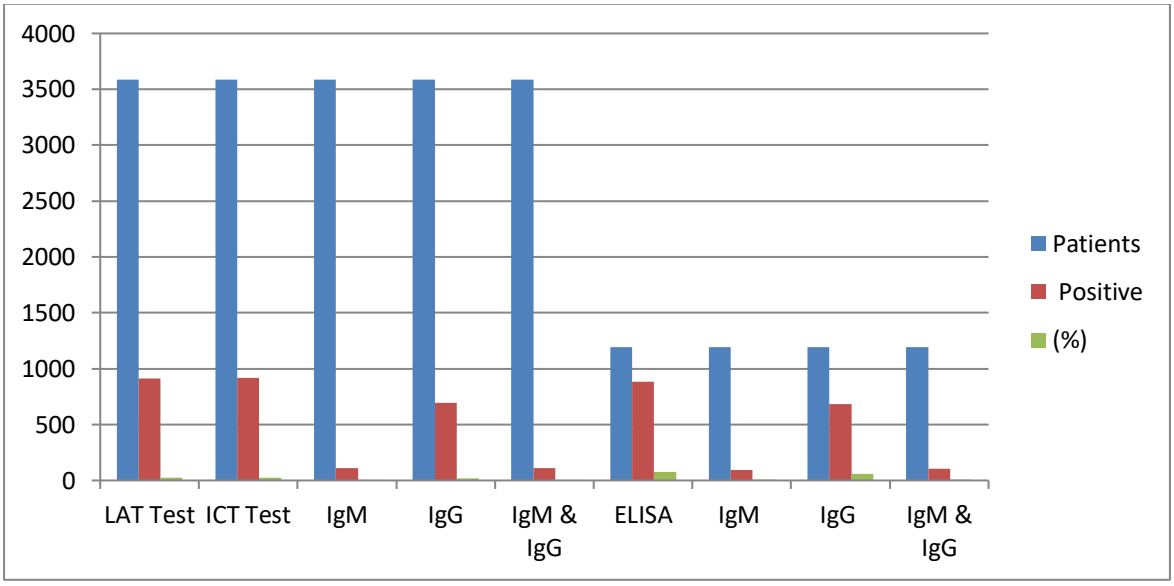

Figure 4.15: Comparative results of Toxoplasma specific serological assays (LAT, ICT, IgM and IgG ELISA) used for diagnosis of Toxoplasmosis infection in women in Khyber Pakhtunkhwa province, Pakistan.

Table: 4.14 Results of comparative study of Toxoplasmosis specific immunoglobulin (IgM, IgG) through qualitative ICT immuno-blot and quantitative ELISA assays in women of Khyber Pakhtunkhwa province, Pakistan

Method	n=3586		Method	n=1195		
ICT	Positive	%	ELISA	Positive	%	p-value
IgM	112	3.12	IgM	96	8.03	0.00
IgG	694	19.35	IgG	682	57.07	0.00
IgM & IgG	109	3.04	IgM & IgG	103	8.62	0.00
Total	915	25.51	Total	881	73.72	0.00

2-sample test for equality of proportions without continuity correction

Table: 4.14 represent results of comparative study of immunoglobulin (IgM) and (IgG), which were qualitatively detected through ICT immunoblot method and quantitatively by ELISA assays in sera of women. A significant (<0.05) difference was found in qualitative and quantitative detection of Toxoplasma specific (IgM), (IgG) and (IgM, IgG) antibodies by these two assays. According to positive cutoff point recommended for ICT and ELISA assay, Toxoplasma specific (IgM) was qualitatively detected in 112 (3.12%), (IgG) in 694 (19.35%) and (IgM, IgG) in 109 (3.04%) sera by ICT method, while Toxoplasma specific (IgM), (IgG) and (IgM, IgG) were quantitatively detected in 96 (8.03%), 682 (57.07%) and 103 (8.62%) women by quantitative ELISA method.

Table: 4.15 Results of quantitative estimation of Toxoplasma specific immunoglobulin (IgM, IgG) by ELISA assays in women of Khyber Pakhtunkhwa province, Pakistan.

ELISA	Positive Patients (n)			Total Patients n=1195				
Variables	n=881	73.72%	R-Range	n=1195	33.32	Mean± SE	SD	p-value
IgM	96	8.03	<9	750	62.76	6.09±0.07	1.8211	
			9-11	246	20.58	10.03±0.06	0.8483	
			>11	199	16.65	20.93±0.46	6.4757	
IgG	682	57.07	<9	262	21.92	6.71±0.11	1.7155	0.00
			9-11	148	12.38	10.35±0.08	0.8586	
			>11	785	65.69	24.31±0.28	7.6899	
IgM, IgG	103	8.62		1195	33.32	9.42± 0.18 18.72±0.29	6.2345 10.022	----

2-sample test for equality of proportions without continuity correction

Table: 4.15 represent quantitative estimation of Toxoplasma specific immunoglobulin (IgM) and (IgG) ELISA assays in women patient. A significant (<0.05) variation was found in positivity of IgM and IgG antibodies in infected women. According to positive cutoff point recommended for quantitative ELISA assay, immunoglobulin (IgM) was detected in 203 (16.98%) women with a mean positive IgM index value of 20.93±0.46, while IgG was detected in 785 (65.69%) women with a mean positive IgG index value of

24.31±0.28. Mean index value of IgM and IgG as a whole (n=1195) was found 9.42±0.18 and 18.72±0.29 respectively.

Table: 4.16 Result of Toxoplasma specific antibody titers (IgM, IgG) estimated by standard tube agglutination test (STAT) in ICT positive women of Khyber Pakhtunkhwa, Pakistan

Patient Group	Antibodies Titers	Patients n=915	%	p-value
Absence of specific antibodies	1:16	11	1.21	1.00
Residual or non-specific antibodies	1:32	23	2.51	1.00
	----	34	3.72	1.00
Acquired or evolving immunity	1:64	240	26.23	1.00
	1:128	387	42.29	1.00
	----	627	68.52	0.00
Recent infection	1:256	102	11.15	1.00
	1:512	86	9.41	1.00
	1:1024	47	5.14	1.00
	1:2048	19	2.07	1.00
	p-value	254	27.76	1.00

1-sample proportions test without continuity correction
1-sample proportions test with continuity correction

Table: 4.16 and figure 4.15 represent result of Toxoplasma specific antibody agglutination titers (IgM, IgG), which was estimated by standard tube agglutination test (STAT) in 915 patients, who were positive for Toxoplasma specific IgM and IgG antibodies by lateral flow chromatography immunoassay (ICT) method. The sera of 915 ICT positive women were serially cheeked at 1:16, 1:32, 1:64, 1:128, 1:256, 1:512, 1:1024 and1:1024 dilutions by standard tube agglutination (STAT) procedure, but no significant (>0.05) increased was observed in rate of agglutination at a particular dilution, while a significant (<0.05) increased was observed with collective dilution of 1:64 and 1:128. The sera of 11 (1.21%) women showed antibody titer at1:16 dilution, which indicated absence of Toxoplasma specific antibodies and false positivity of ICT assay, while 23 (2.51%) women showed antibodies titer at 1:32 dilution, which indicated residual or non-specific immunity and false positivity of ICT assay. Similarly, sera of 240 (26.23%) and 387 (42.29%) patients showed antibodies titer at 1:64 and 1:128 dilution, which indicated acquired or evolving immunity and true positivity of ICT assay, while sera of 102 (11.15%), 86 (9.41%), 47 (5.14%) and 19 (2.07%) women showed antibodies titer at 1:256, 1:512, 1:1024 and 1:2048 dilutions, which is suggested as a possible recent contact with risk factor of *Toxoplasma gondii* and true positivity of ICT assay.

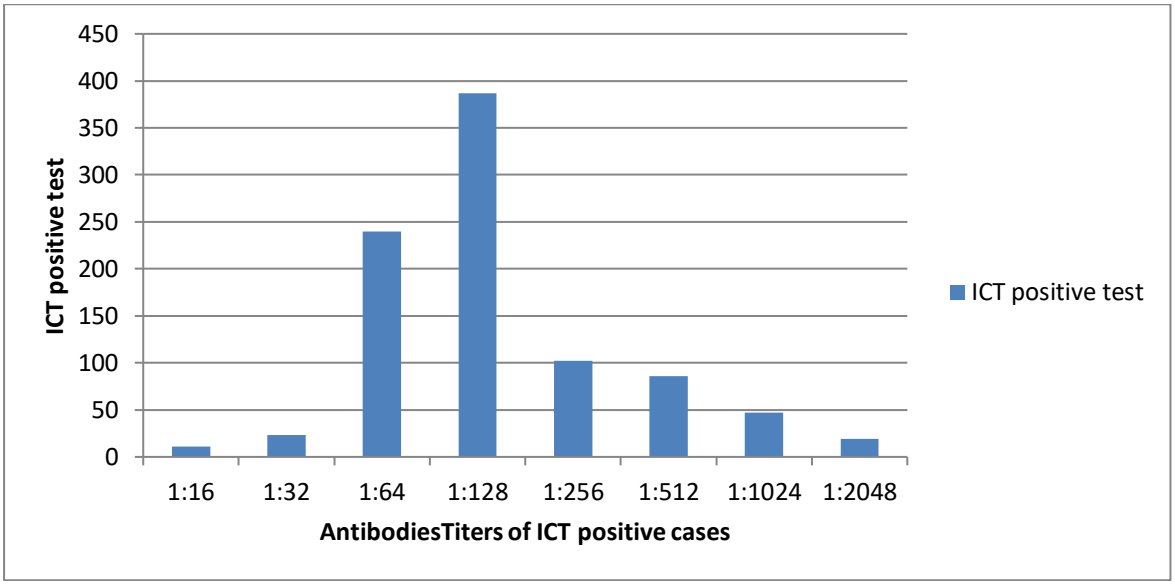

Figure 4.16: Result of Toxoplasma specific antibody titers (IgM, IgG) estimated by standard tube agglutination test (STAT) in women, who were positive by ICT assay in Khyber Pakhtunkhwa province, Pakistan.

4.5 Clinical stages of Brucellosis in infected women

Table: 4.17 Clinical pattern of Brucellosis infection observed in infected women in Khyber Pakhtunkhwa province, Pakistan.

Disease Stage	Duration of Disease	N=718 Prevalence (%)		Mean ±SE	SD	p-value
Acute	1-2 weeks)	25	3.48	1.92±0.06	0.277	0.00*
(≤8 weeks)	2-4 weeks)	149	20.75	3.47±0.05	0.611	
	1-2 months	254	35.37	5.95±0.06	0.931	
	----	428	59.61	4.86±0.08	1.587	
Sub-Acute	3-4 months	97	13.51	14.76±0.17	1.653	0.00
(8–52 weeks)	5-6 months	65	9.05	22.84±0.19	1.496	
	7-8 months	37	5.15	31.76±0.27	1.589	
	9-10months	26	3.62	39.85±0.31	1.489	
	11-12 months	14	1.94	48.79±0.39	1.424	
	----	239	33.28	24.31±0.68	10.511	
Chronic	≥1 year	51	7.11	68.36±1.89	13.452	----
	----	----	(weeks)	15.84±0.69	18.521	
Acute, Sub-acute and Chronic						0.00

Chi square test for given probability
3-sample test for equality of proportions without continuity correction

Table 4.18 shows various clinical stages of Brucellosis infection in 718 women, which is graphically shown in figure 4.16. On the basis of duration of clinical symptoms, Brucellosis was observed in acute, sub-acute and chronic infection in 718 patients. A significant difference (<0.05) was observed in rate of acute, sub-acute and chronic cases of Brucellosis. A significant difference (<0.05) was also found in durations of acute stage and sub-acute stage of Brucellosis. On the basis of duration of clinical symptoms, acute infection was significantly (<0.05) found in 428 (59.61%) patients with mean duration of 4.86±0.08 weeks, sub-acute infection was found in 239 (33.28%) cases with mean duration of 24.31±0.68 weeks and chronic infection was found in 51 (7.11%) women with mean duration of clinical symptoms 68.36±1.89 weeks. A significant (<0.05) difference was found in rate of acute 428 (59.61%), sub-actue 239 (33.28%) and chronic 51 (7.11%) cases of Brucellosis. Prevalence of acute and sub-acute infection was significantly (<0.05) high in infected women.

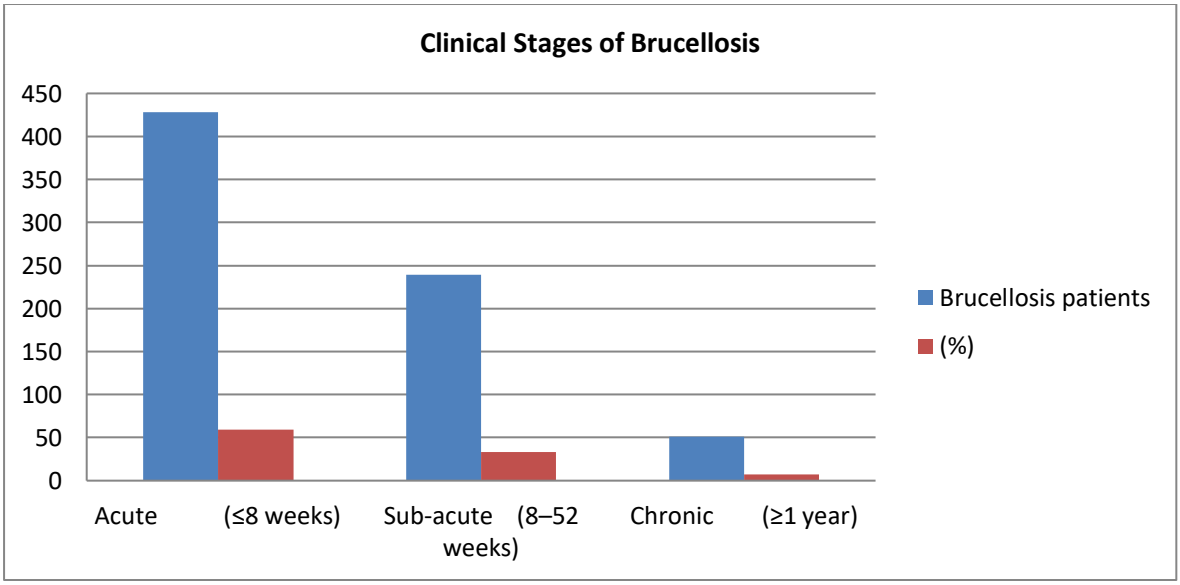

Figure 4.17: Clinical pattern of Brucellosis infection observed in infected women of Khyber Pakhtunkhwa province, Pakistan.

4.6 Clinical features of zoonotic diseases (Brucellosis, Toxoplasmosis) in women

4.6.1 Clinical features of Brucellosis infection

Table: 4.18 Results of comparative study of clinical features of Brucellosis with non-Brucellosis patients in Khyber Pakhtunkhwa province, Pakistan.

Clinical Symptom/ Signs	Brucellosis		Non-Brucellosis		
	n=718	20.02%	n=2868	79.98%	p-value
Fever	641	89.27	821	28.62	0.00
Arthralgia/Joint pain	587	81.75	657	22.91	0.00
Back Pain	518	72.14	1103	38.45	0.00
Fatigue,	478	66.57	628	21.89	0.00
Malaise	443	61.69	523	18.23	0.00
Anorexia	350	48.74	631	22.01	0.00
Sweating	312	43.45	283	9.86	0.00
Myalgia	270	37.61	438	15.27	0.00
Headache	224	31.19	424	14.78	0.00
Chills	164	22.84	205	7.14	0.00
Nausea	139	19.35	132	4.61	0.00
Cough and chest pain	101	14.06	123	4.28	0.00
Weight loss	87	12.11	442	15.41	0.98
Clinical signs					
Splenomegaly	269	37.46	373	13.01	0.00
Hepatomegaly	132	18.38	246	8.57	0.00
Splenomegaly &Hepatomegaly	27	3.76	95	3.31	0.27
Flue	23	3.21	266	9.27	1.00
Lymphadenopathy	17	2.36	320	11.15	1.00

2-sample test for equality of proportions without continuity correction

Table 4.18 and figure 4.17 show comparative results of major clinical, physical and abdominal ultrasonographic findings in 718 confirmed cases of Brucellosis with 2868 cases of non-Brucellosis patients. Fever, arthralgia, back pain, fatigue, malaise, anorexia, sweating, myalgia, headache, chills, nausea and dry cough were main clinical features, which were significantly (<0.05) high in Brucellosis patients as compared to non-brucellosis patients, while rate of weight loss was comparatively high in non-brucellosis patients. Fever was a prominent and significant (<0.05) clinical symptom observed in 641 (89.27%) cases of Brucellosis, which was followed by arthralgia 587 (81.75%), back pain 518 (72.14%), fatigue 478 (66.57%), malaise 443 (61.69%), anorexia 350 (48.74%), sweating 312 (43.45%), myalgia 270 (37.61%), headache 224 (31.19%), chills 164 (22.84%), nausea139 (19.35%), cough, 101 (14.06%) and weight loss in 87 (12.11%) cases of Brucellosis. Frequency of splenomegaly 269 (37.46%) and hepatomegaly 132 (18.38%) figure (4.18) were significantly (<0.05) high in

Brucellosis patients as compared to non-brucellosis patients, while no significant (>0.05) difference was found in spleeno-hepatomegaly, flue and lymphadenopathy in Brucellosis and non-brucellosis patients.

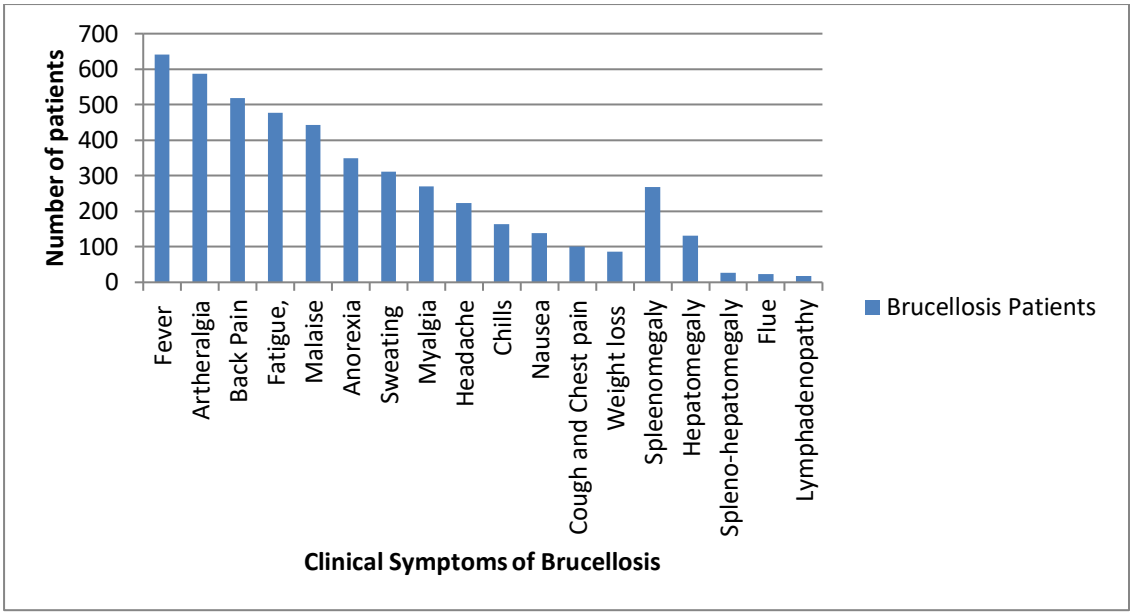

Figure 4.18: Results of clinical features of Brucellosis in infected women of Khyber Pakhtunkhwa province, Pakistan.

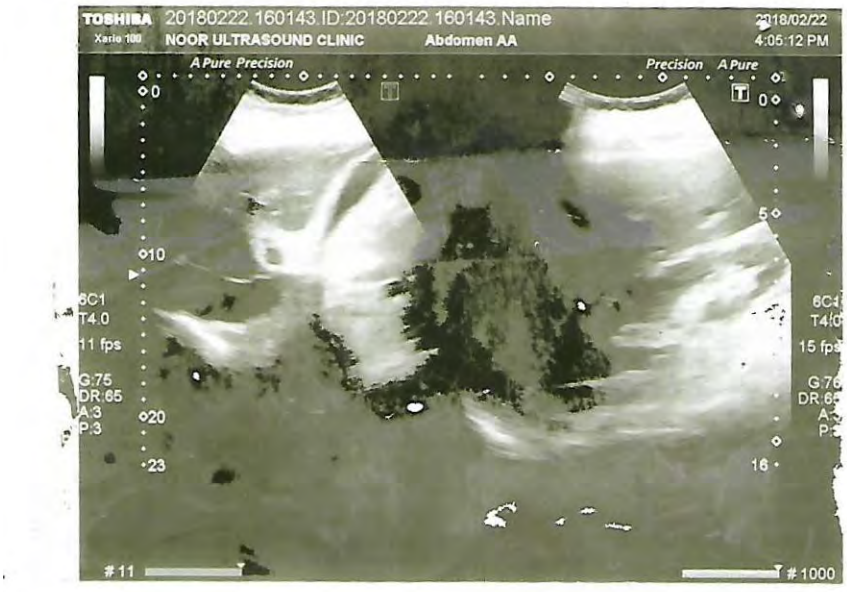

Figure 4.19: Abdominal ultrasonographic reports of 27 years primary gravida woman, who was positive for Brucellosis infection by STAT, RBPT, IgM, IgG ELISA and Brucella genus specific PCR.

Abdominal ultrasonographic examination showed mildly enlarged liver, which was measured 20 cm. Portal vein was normal in caliber. Gall bladder was normal in size, shape with normal thickness of walls. Pancreas was masked due to excessive shadows of gases in gut. Spleen was normal in size and had normal echo-texture. Para-aortic lymph nodes were not observed. There was no ascites.

Impression: Hepatomegaly

Table 4.19 Results of clinical features of Brucellosis in different clinical stages (acute, sub-acute, chronic) in infected women of Khyber Pakhtunkhwa province, Pakistan

Clinical Symptom/ Signs	Acute		Sub-Acute		Chronic		
	(≤8 weeks)		(8–52 weeks)		(≥52 weeks)		p-value
	428	59.61%	239	33.28%	51	7.11%	0.00
Fever	419	97.89	213	89.12	9	17.64	0.00
Arthralgia/Joint pain	332	77.57	209	87.45	46	90.19	0.00
Back Pain	297	69.39	179	74.89	42	82.35	0.07
Fatigue,	301	70.32	148	61.92	29	56.86	0.02
Malaise	270	63.08	145	60.67	28	54.91	0.48
Anorexia	249	58.17	84	35.14	17	33.34	0.00
Sweating	216	50.46	83	34.73	13	25.49	0.00
Myalgia	188	43.92	68	28.45	14	27.45	0.00
Headache	162	37.85	53	22.17	9	17.64	0.00
Chills	110	25.71	47	19.66	7	13.72	0.05
Nausea	95	22.19	39	16.31	5	9.81	0.03
Chest pain/cough	65	15.18	30	12.55	6	11.76	0.57
Weight loss	58	13.55	26	10.88	3	5.88	0.21
Clinical signs							
Splenomegaly	184	42.99	66	27.61	19	37.25	0.00
Hepatomegaly	80	18.69	42	17.57	10	19.61	0.91
Splenomegaly &Hepatomegaly	18	4.21	7	2.93	2	3.92	0.70
Flue	10	2.34	7	2.93	6	11.76	0.00
Lymphadenopathy	9	2.11	5	2.09	3	5.88	0.23

3-sample test for equality of proportions without continuity correction

Table 4.19 show comparative results of clinical features of Brucellosis in different clinical stages (acute, sub-acute, chronic) in 718 women, who were positive for Brucellosis. A significant difference (<0.05) was observed in frequency of fever, arthralgia, back pain, fatigue, anorexia, sweating, myalgia, headache, chills, nausea, splenomegaly and flue in acute, sub-acute and chronic cases of Brucellosis. Fever was significantly (<0.05) high in acute 419 (97.89%) and sub-acute 213 (89.12%) cases as compared to 9 (17.64%) cases of chronic infection. Frequency of arthralgia was significantly (<0.05) increased from acute 332 (77.57%) to sub-acute 209 (87.45%) and chronic 46 (90.19%) cases of Brucellosis. Similarly, rate of back pain was increased (>0.05)from acute 297

(69.39%) to sub-acute 179 (74.89%) and chronic 42 (82.35%) cases of infection. Frequency of fatigue was significantly (<0.05) decreased from 301 (70.32%) to 148 (61.92%) and 29 (56.86%) cases in acute to sub-acute and chronic infection, anorexia from acute 249 (58.17%) to sub-acute 84 (35.14%) and chronic 17 (33.34%) infection, sweating from acute 216 (50.46%) to sub-acute 83 (34.73%) and chronic 13 (25.49%) stage, myalgia from acute 188 (43.92%) to sub-acute 68 (28.45%) and chronic 14 (27.45%) infection, headache from acute 162 (37.85%) to sub-acute 53 (22.17%) and chronic 9 (17.64%) cases, chills from acute 110 (25.71%) to sub-acute 47 (19.66%) and chronic 7 (13.72%) cases, nausea from acute 95 (22.19%) to sub-acute 39 (16.31%) and chronic 5 (9.81%) cases of Brucellosis. Frequency of other symptoms like malaise was decreased (>0.05) from acute 270 (63.08%) to sub-acute 145 (60.67%) and chronic 28 (54.91%) infection, cough from acute 65 (15.18%) to sub-acute 30 (12.55%) and chronic 6 (11.76%) stage, weight loss from acute 58 (13.55%) to sub-acute 26 (10.88%) and chronic 3 (5.88%) cases of infection. Similarly frequency of other clinical signs like splenomegaly and flue were significantly (<0.05) different in acute, sub-acute and chronic infection, while no significant difference (>0.05) was found in frequency of hepatomegaly, hepato-splenomegaly and lymphadenopathy in acute, sub-acute and chronic stage of infection. Splenomegaly was more prominently observed in acute 184 (42.99%) and chronic 19 (37.25%) stage as compared to sub-acute 66 (27.61%) stage of Brucellosis, while rate of hepatomegaly was high in chronic 10 (19.61%) and acute 80 (18.69%) cases as compared to sub-acute 42 (17.57%) cases. Hepato-splenomegaly was observed in 18 (4.21%) cases of acute, 7 (2.93%) cases of sub-acute and 2 (3.92%) cases of chronic infection. Other clinical signs like flue and lymphadenopathy were observed more prominently in chronic cases as compared to acute and sub-acute cases of Brucellosis.

4.6.2 Clinical features of Toxoplasmosis infection in women

Table: 4.20 Results of comparative study of clinical findings in Toxoplasmosis with non-Toxoplasmosis patients in Khyber Pakhtunkhwa province, Pakistan.

Clinical Symptoms/ Signs	Toxoplasmosis Patients		Non-Toxoplasmosis Patients		
	n=881	24.56%	n=2705	75.43%	p-value
Back Pain	387	43.92	1234	45.62	0.80
Fatigue,	374	42.45	732	27.06	0.00
Arthralgia/Joint pain	309	35.07	935	34.56	0.39
Headache	257	29.17	575	21.25	0.00
Myalgia	208	23.61	500	18.48	0.00
Anorexia	184	20.88	797	29.46	1.00
Malaise	120	13.62	846	31.27	1.00
Nausea	117	13.28	412	15.23	0.92
Sweating	96	10.89	499	18.44	1.00
Fever	53	6.01	1409	52.08	1.00
Cough and Chest pain	13	1.47	211	7.81	1.00
Chills	11	1.24	358	13.23	1.00
Weight loss	4	0.45	267	9.87	1.00
Clinical signs					
Lymphadenopathy	173	19.63	164	6.06	0.00
Flue	66	7.49	223	8.24	0.76
Splenomegaly	60	6.81	582	21.51	1.00
Hepatomegaly	23	2.61	355	13.12	1.00
Splenomegaly &Hepatomegaly	4	0.45	118	4.36	1.00

Table 4.20 and figure 4.19 show comparative results of clinical, physical and abdominal ultrasonographic findings in 881 (24.56%) women, who were positive for Toxoplasmosis as compared to 2705 women, who were negative for Toxoplasmosis. Frequency of fatigue, headache, myalgia and lymphadenopathy was significantly (<0.05) high in women, who were positive for Toxoplasmosis as compared to those women, who were negative for Toxoplasmosis. Fatigue was a significant (<0.05) clinical symptom, which was observed in 374 (42.45%) women infected with Toxoplasma, which was followed by headache 257 (29.17%) and myalgia 208 (23.61%). Other clinical symptoms were back pain 387 (43.92%), arthralgia 309 (35.07%), anorexia 184 (20.88%), malaise120 (13.62%), nausea 117 (13.28%), sweating 96 (10.89%), fever 53 (6.01%), cough 13 (1.47%), chills 11(1.25%) and weight loss 4 (0.45%). Lymphadenopathy was a common and significant (<0.05) clinical sign, which was observed in 173 (19.63%) women, who were positive for Toxoplasmosis, while flue, splenomegaly,

hepatomegaly (figure; 4.20) and hepato-splenomegaly was found (>0.05) in 66 (7.49%), 60 (6.81%), 23 (2.61%) and 4 (0.45%) patients, who were positive for Toxoplasmosis.

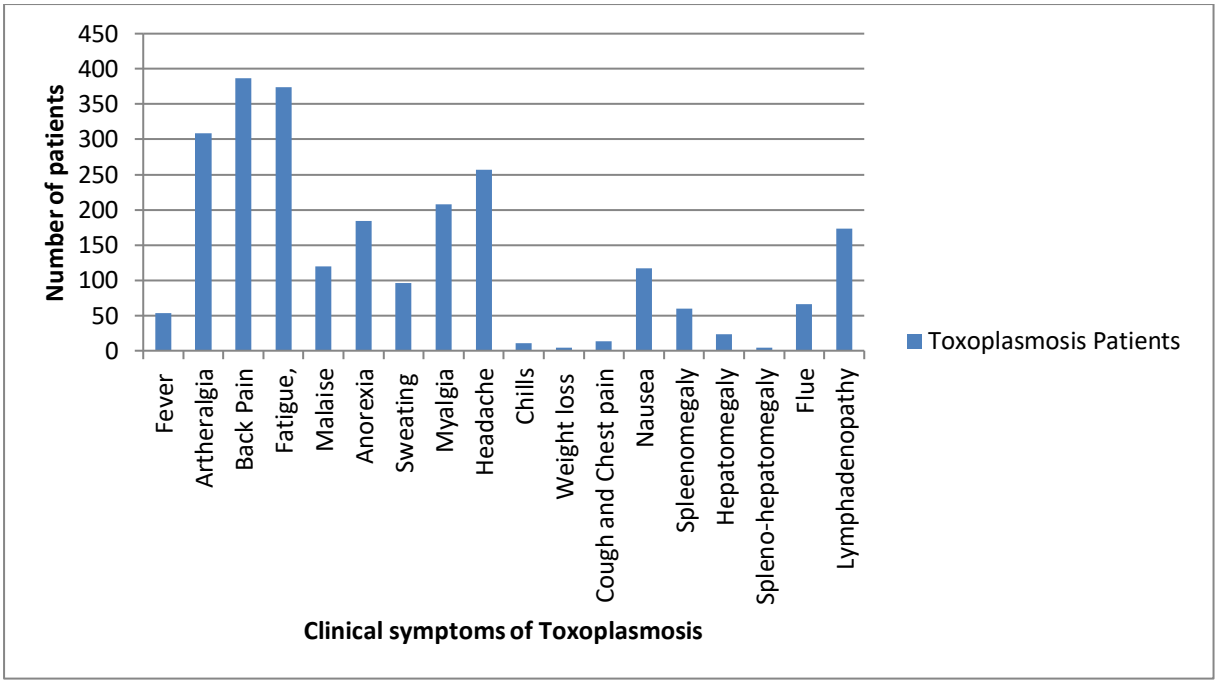

Figure 4.20: Results of clinical symptoms of Toxoplasmosis in infected women of Khyber Pakhtunkhwa province, Pakistan.

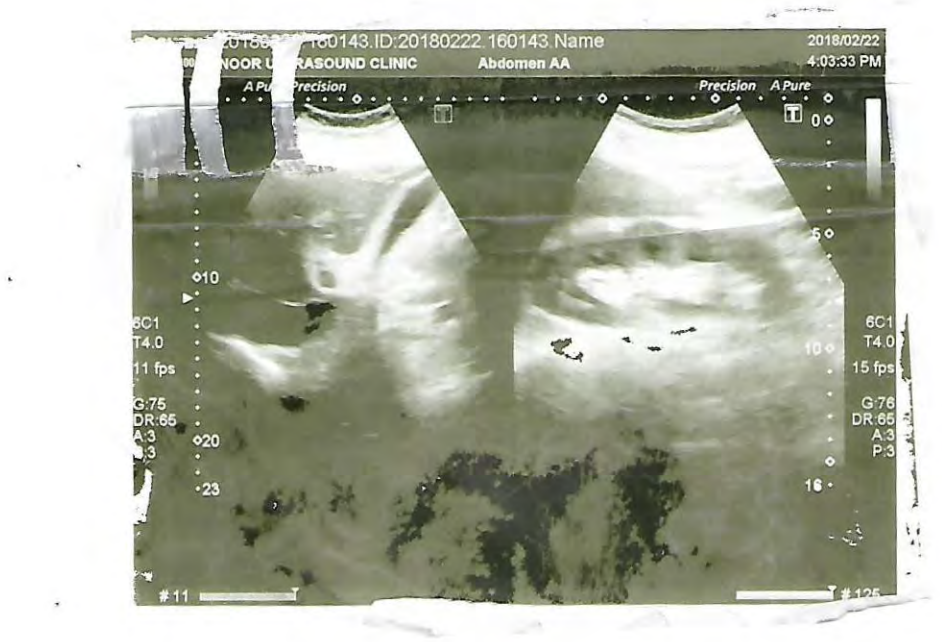

Figure 4.21: Abdominal ultrasonographic reports of 27 years primary gravida woman, who was positive for Brucellosis infection by STAT, RBPT, IgM, IgG ELISA and Brucella genus specific PCR. The scan showed enlarged liver (hepatomegaly), which was measured 20 cm, while spleen was normal in size with normal echo-texture.

4.7 Hematological and Biochemical parameters observed in women with zoonotic diseases (Brucellosis, Toxoplasmosis)

4.7.1 Clinical hematological laboratory parameters of Brucellosis in infected women

Table: 4.21 Results of various hematological parameters observed in women with Brucellosis in Khyber Pakhtunkhwa province, Pakistan.

Brucellosis Patients					
Hematological Variables	n=718	20.02%	Mean± SE	SD	p-value
Hemoglobin (Hb) g/dL					
<11.5	324	45.12	9.86±0.09	1.4982	0.00
11.5-17.5	386	53.76	13.37±0.07	1.3442	
>17.5	8	1.11	18.04±0.09	0.2822	1.00
			11.84±0.09	2.3329	
WBC 4.00-12.0 x10^9/L					
<4.00	117	16.29	3.39±0.05	0.5065	0.37
04-12	425	59.19	9.28±0.02	2.1463	
>12	176	24.51	14.68±0.13	1.6233	0.00
			9.64±0.15	4.0121	
Neutrophils (%)					
<40	267	37.18	37.27±0.14	2.1568	0.00
40-70	400	55.71	56.55±0.43	8.5835	
>70	51	7.11	75.91±0.52	3.6887	1.00
			50.76±0.51	13.236	
Lymphocyte (%)					
<15	23	3.21	11.28±0.55	2.6373	1.00
15-40	506	70.47	30.23±0.31	6.8206	
>40	189	26.32	46.73±0.35	4.7084	0.00
			33.99±0.39	10.462	
Monocytes					
<2	29	4.03	0.53±0.07	0.3842	1.00
2—10	512	71.31	6.13±0.09	2.0515	
>10	177	24.65	13.77±0.18	2.4136	0.00
			7.79±0.16	4.1671	
Eosinophilis (%)					
<1.0	30	4.17	0.65±0.04	0.2365	0.96

1-6	664	92.47	4.04±0.05	1.2028	
>6	24	3.34	8.14±0.29	1.4619	0.99
			4.03±0.06	1.5657	
Basophiles (%)					
0-1.7	678	94.42	0.87±0.02	0.4714	
>1.7	40	5.57	2.55±0.12	0.7404	1.00
			0.95±0.03	0.6244	
Platelet count <150 x 10^9/L					
<150 x 10^9/L	179	24.93	131.93±1.03	13.6523	0.00
150-450	529	73.67	243.54±2.69	61.7566	
>150 x 10^9/L	10	1.39	522.95±15.05	47.5899	0.99
			219.61±3.01	80.6767	

1-sample proportions t-test without continuity correction

Table 4.21 and figure 4.21 represent results of various abnormalities, observed in different hematological parameters in 718 women patients, who were positive for Brucellosis infection. Hemoglobin, WBC, DLC, neutrophils, lymphocyte, monocytes, eosinophils, basophiles and platelet counts were determined in 718 women, who had Brucellosis infection. Hemoglobin (Hb) level was significantly low (<0.05) in 324 (45.12%) women with a mean low Hb level of 9.86±0.09 g/dL, which indicated anemia in infected patients. Low (>0.05) and high (<0.05) TLC count was found in 117 (16.29%) and 176 (24.51%) infected cases with a mean low WBC count of 3.39±0.05/L and high 14.68±0.13/L respectively. Neutropenia (<0.05), lymphopenia (>0.05), monocytopenia (>0.05), eosinopenia (>0.05) and thrombocytopenia (<0.05) was observed in 267 (37.18%), 23 (3.21%), 29 (4.03%), 30 (4.17%) and 179 (24.93%) infected women with a mean low count of neutrophils 37.27±0.14, lymphocyte 11.28±0.55, monocytes 0.53±0.07, eosinophilis 0.65±0.04 and platelet 131.93±1.03/L respectively. A mild form of pancytopenia was observed (>0.05) in 8 (1.11%) infected cases of Brucellosis. Similarly neutrophilia (>0.05), lymphocytosis (<0.05), monocytosis (<0.05), eosinophilia (>0.05), basophilia (>0.05) and thrombocytosis (>0.05) was observed in 51 (7.11%), 189 (26.32%), 177 (24.65%), 24 (3.34%), 40 (5.57%) and 10 (1.39%) cases of Brucellosis with a mean high count of neutrophils 75.91±0.52, lymphocyte 46.73±0.35, monocytes 13.77±0.18, eosinophils 8.14±0.29, basophiles 2.55±0.12 and thrombocytes 522.95±15.05/L respectively.

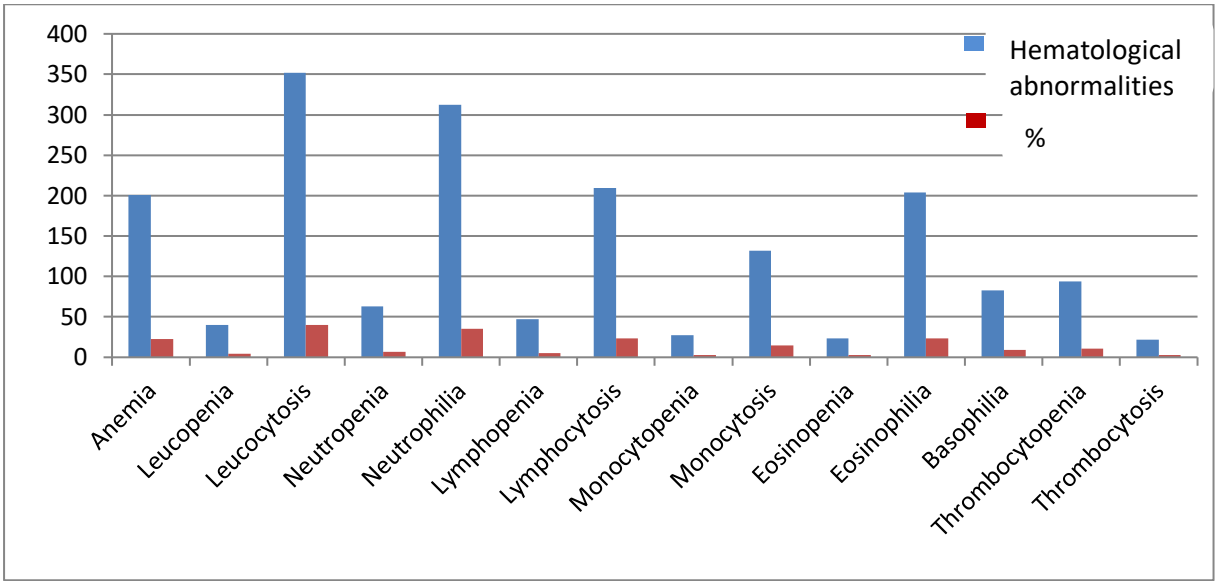

Figure 4.22: Results of various hematological abnormalities observed in women with Brucellosis infection in Khyber Pakhtunkhwa province, Pakistan.

Table: 4.22 Results of selected hematological parameters observed in different clinical stages of Brucellosis (Acute, Sub-Acute, Chronic) in infected women of Khyber Pakhtunkhwa province, Pakistan.

Clinical Stages of Brucellosis Infection							
Variables	Acute		Sub-Acute		Chronic		p-value
	n=428(59.61%)		n=239 (33.28%)		n=51(7.11%)		0.00*
Hemoglobin (Hb) g/dL		Mean ± S.E		Mean± S.E		Mean± S.E	0.02
<11.5	213 (49.76)	9.19±0.09	96 (40.16)	11.08±0.03	15(29.41)	11.39±0.01	0.00
>17.5	4 (0.93)	18.04±0.18	3 (1.25)	18.08±0.17	1 (1.96)	17.91±0	0.77
WBC 4.00-12.0 x10^9/L							0.00
<4.00	82 (19.15)	3.42±0.06	32 (13.38)	3.41±0.09	3 (5.88)	3.57±0.29	0.01
>12	120 (28.03)	14.62±0.15	49 (20.51)	14.71±0.24	7 (13.72)	15.37±0.49	0.01
Neutrophils (%)							0.00
<40	174 (40.65)	37.28±0.17	84 (35.14)	37.12±0.25	9 (17.64)	38.34±0.35	0.00
>70	22 (5.14)	76.44±0.77	25 (10.46)	75.71±0.77	4 (7.84)	75.15±1.41	0.03
Lymphocyte (%)							0.03
<15	15 (3.51)	11.47±0.78	7 (2.92)	10.45±0.49	1 (1.96)	14.03±0.00	0.80
>40	129 (30.14)	46.73±0.39	53 (22.17)	47.44±0.78	7 (13.72)	43.76±0.93	0.00
Monocytes %							0.15
<2	18 (4.21)	0.53±0.09	9 (3.76)	0.48±0.13	2 (3.92)	0.71±0.33	0.96
>10	119 (27.81)	13.92±0.23	50 (20.92)	13.17±0.27	8 (15.68)	15.25±1.46	0.04
Eosinophils (%)							0.23
<1.0	14 (3.27)	0.63±0.06	11 (4.61)	0.72±0.06	5 (9.81)	0.56±0.1622	0.08

>6	16 (3.73)	8.62±0.34	7 (2.92)	8.11±0.59	1 (1.96)	6.97±0.00	0.72
Basophiles (%)							0.19
>1.7	29 (6.77)	2.64±0.15	10 (4.18)	2.21±0.09	1 (1.96)	3.55±0.00	0.19
Platelet counts 150-450 x 10^9/L							0.04
<150	94 (21.96)	131.71±1.37	65 (27.19)	133.24±1.67	20(39.21)	128.61±3.55	0.01
>450	8 (1.86)	526.51±16.48	2 (0.83)	508.71±48.37	0 (0.00)	0.00±0.00	0.37

* Chi-squared test for given probabilities
Pearson's Chi-squared test

Table 4.22 represents results of various abnormalities, which were observed in different hematological parameters during various clinical stages (acute, sub-acute, chronic) of Brucellosis in 718 patients. A significant difference (<0.05) was found in low and high hemoglobin level, while a significant difference (<0.05) was also found in hemoglobin level in various clinical stages of Brucellosis. Anemia was found in 213 (49.76%) cases of acute infection with a mean low Hb value of 9.19±0.09 g/dL, which decreased to 96 (40.16%) and 15 (29.41%) cases in sub-acute and chronic infection with a mean low Hb value of 11.08±0.03 and 11.39±0.01 g/dL respectively. A significant difference (<0.05) was found in high and low TLC count, while a significant difference (<0.05) was also found in low and high TLC count in various clinical stages of Brucellosis. Low TLC count was observed in 82 (19.15%) cases of acute infection with a mean low TLC count of 3.42±0.06/L, which decreased to 32 (13.38%) and 3 (5.88%) infected cases in sub-acute and chronic infection with a mean low TLC count of 3.41±0.09 and 3.57±0.29/L respectively. High TLC counts was observed in 120 (28.03%) cases of acute infection with a mean high TLC count of 14.62±0.15/L, which decreased to 49(20.51%) and 7(13.72%) infected cases in sub-acute and chronic clinical stage with a mean high TLC count of 14.71±0.24 and 15.37±0.49 /L respectively. A significant difference (<0.05) was found in high and low neutrophils count, while a significant difference (<0.05) was also found in low and high neutrophils count in various clinical stages of Brucellosis. Neutropenia was observed in 174 (40.65%) cases of acute infection with a mean low neutrophils count of 37.28±0.17, which decreased to 84 (35.14%) and 9 (17.64%) infected cases in sub-acute and chronic Brucellosis with a mean low neutrophils count of 37.12±0.25 and 38.34±0.35 respectively, while neutrophilia was high 25 (10.46%) in sub-acute cases with a mean high neutrophils count of 75.71±0.77 as compared to acute 22 (5.14%) and chronic 4 (7.84%) cases with a mean high neutrophils count of 76.44±0.77 and 75.15±1.41 respectively. A significant difference (<0.05) was found in high and low lymphocyte count, while a significant difference (<0.05) was also found in high lymphocyte count in various clinical stages of Brucellosis. Lymphopenia was observed in 15 (3.51%) cases of acute, 7 (2.92%) sub-acute and 1 (1.96%) case of chronic infection with a mean low lymphocyte count of 11.47±0.78,10.45±0.49 and 14.03±0.00 respectively, while lymphocytosis was observed in 129 (30.14%) cases of acute infection, with a mean high lymphocyte count of 46.73±0.39, which decreased to 53 (22.17%) and 7 (13.72%) cases in sub-acute and chronic infection with a mean high lymphocytes count of 47.44±0.78 and 43.76±0.93 respectively. No significant difference (>0.05) was found in high and low monocytes count, while a significant difference (<0.05) was found in high monocytes count in

various clinical stages of Brucellosis. Monocytopenia was observed in 18 (4.21%) cases of acute, 9 (3.76%) sub-acute and 2 (3.92%) cases of chronic infection with a mean low monocytes count of 0.53±0.09, 0.48±0.13 and 0.71±0.33, while monocytosis was observed in 119 (27.81%), 50 (20.92%) and 8 (15.68%) cases of acute, sub-acute and chronic Brucellosis with a mean high monocytes count of 13.92±0.23, 13.17±0.27 and 15.25±1.46 respectively. There was no significant difference (>0.05) in low and high eosinophilic counts, while there was also no significant difference (>0.05) in eosinophilic counts in various clinical stages of Brucellosis. Eosinopenia was observed in 14 (3.27%) cases of acute, 11 (4.61%) sub-acute and 5 (9.81%) cases of chronic infection with a mean low eosinophils count of 0.63±0.06, 0.72±0.06 and 0.56±0.1622, while eosinophilia was observed in 16 (3.73%) cases of acute, 7 (2.92%) sub-acute and 1 (1.96%) case of chronic infection with a mean high eosinophils count of 8.62±0.34, 8.11±0.59 and 6.97±0.00 respectively. There was no significant difference (>0.05) in basophiles counts in various clinical stages of Brucellosis. Basophilia was observed in 29 (6.77%) cases of acute, 10 (4.18%) sub-acute and 1 (1.96%) case of chronic infection with a mean high basophiles count of 2.64±0.15, 2.21±0.09 and 3.55±0.00 respectively. There was significant difference (<0.05) in low and high thrombocytes counts, while there was also significant difference (<0.05) in low thrombocytes counts in various clinical stages of Brucellosis. Low thrombocytes count was observed in 94 (21.96%) cases of acute infection with a mean low count of 131.71±1.37/L, which increased to 65 (27.19%) and 20 (39.21%) cases in sub-acute and chronic infection of Brucellosis with a mean low thrombocytes count of 133.24±1.67 and 128.61±3.55/L respectively, while there was no significant (>0.05) difference in thrombocytosis in various clinical stages of Brucellosis. High count of thrombocytes was observed in 8 (1.86%) cases of acute, 2 (0.83%) cases of sub-acute and 0 (0.00%) cases in chronic infection with a mean high thrombocytes count of 526.51±16.48, 508.71±48.37 and 0.00±0.00 respectively.

4.7.2 Clinical hematological laboratory parameters observed in women with Toxoplasmosis infection

Table: 4.23 Results of various hematological parameters observed in women with Toxoplasmosis in Khyber Pakhtunkhwa province, Pakistan.

Toxoplasmosis Patients					
Hematological Variables	n=881	24.56%	Mean ± SE	SD	p-value
Hemoglobin (Hb) g/dL					
<11.5	201	22.81	10.49±0.06	0.8335	0.00
11.5-17.5	680	77.18	13.01±0.04	1.0739	
>17.5	0	0	0	0	1.00
			12.43±0.05	1.4712	
WBC 4.00-12.0 x10^9/L					
<4.00	40	4.54	3.85±0.02	0.1134	1.00
04-12	489	55.51	7.69±0.09	2.1914	
>12	352	39.95	13.35±0.05	0.8871	0.00
			9.78±0.12	3.4739	
Neutrophils (%)					
<40	63	7.15	38.61±0.14	1.1039	0.99
40-70	506	57.43	54.65±0.39	8.8845	
>70	312	35.41	72.51±0.08	1.3689	0.00
			64.36±5.21	154.554	
Lymphocyte (%)					
<15	47	5.33	13.57±0.13	0.8487	1.00
15-40	625	70.94	26.91±0.28	6.8283	
>40	209	23.72	42.26±0.09	1.3023	0.00
			29.84±0.33	9.5058	
Monocytes					
<2	27	3.06	1.25±0.09	0.4708	1.00
2-10	722	81.95	5.53±0.07	1.7672	
>10	132	14.98	21.88±8.98	103.07	0.00
			7.85±1.36	40.239	
Eosinophils (%)					
<1.0	23	2.61	0.57±0.05	0.2319	1.00
1-6	654	74.23	4.19±0.04	0.9635	
>6	204	23.16	7.74±0.07	0.8848	0.00
			4.91±0.06	1.89545	
Basophiles (%)					
0-1.7	798	90.57	0.98±0.02	0.4461	1.00
>1.7	83	9.42	2.71±0.08	0.70425	
			1.15±0.03	0.6906	
Platelet count <150 x 10^9/L					

<150 x 10^9/L	94	10.66	146.04±0.45	4.3971	0.00
150-450	765	86.83	260.05±2.51	69.107	
>150 x 10^9/L	22	2.49	454.13±0.85	3.9658	0.83
			252.73±2.71	80.156	

Table 4.23 and figure 4.22 represents results of various abnormalities, observed in different hematological parameters in 881 women, who were positive for Toxoplasmosis. Low hemoglobin level <11.5 g/dL was significantly (<0.05) found in 201 (22.81%) infected women with a mean low Hb value of 10.49±0.06 g/dL, which indicated anemia, while 680 (77.18%) infected women were found with normal hemoglobin level with a mean normal Hb value of 13.01±0.04 g/dL. Low TLC count was found (>0.05) in 40 (4.54%) infected cases with a mean low TLC count of 3.85±0.02/L, while high TLC count was significantly (<0.05) found in 352 (39.95%) infected cases with a mean high TLC count of 13.35±0.05/L. Neutropenia was found (>0.05) in 63 (7.15%) infected cases with a mean low neutrophils count of 38.61±0.14, while neutrophilia was significantly (<0.05) observed in 312 (35.41%) infected cases with a mean high neutrophils count of 72.51±0.08. Lymphopenia was found (>0.05) in 47 (5.33%) infected cases with a mean low lymphocyte counts of 13.57±0.13, while lymphocytosis was significantly (<0.05) found in 209 (23.72%) infected women with a mean high lymphocyte counts of 42.26±0.09. Monocytopenia was found (>0.05) in 27 (3.06%) infected cases with a mean low monocytes counts of 1.25±0.09, while monocytosis was significantly (<0.05) observed in 132 (14.98%) infected cases with a mean high monocytes counts of 21.88±8.98. Eosinopenia was found (>0.05) in 23 (2.61%) infected cases with a mean low eosinophils count of 0.57±0.05, while eosinophilia was significantly (<0.05) observed in 204 (23.16%) infected cases with a mean high eosinophils count of 7.74±0.07. Basophilia was found (>0.05) in 83 (9.42%) infected cases with a mean low basophile counts of 0.98±0.02. Similarly thrombocytopenia was significantly (<0.05) found in 94 (10.66%) women with a mean low thrombocytes count of 146.04±0.45, while thrombocytosis was observed (>0.05) in 22 (2.49%) infected cases with a mean high thrombocytes count of 454.13±0.85/L.

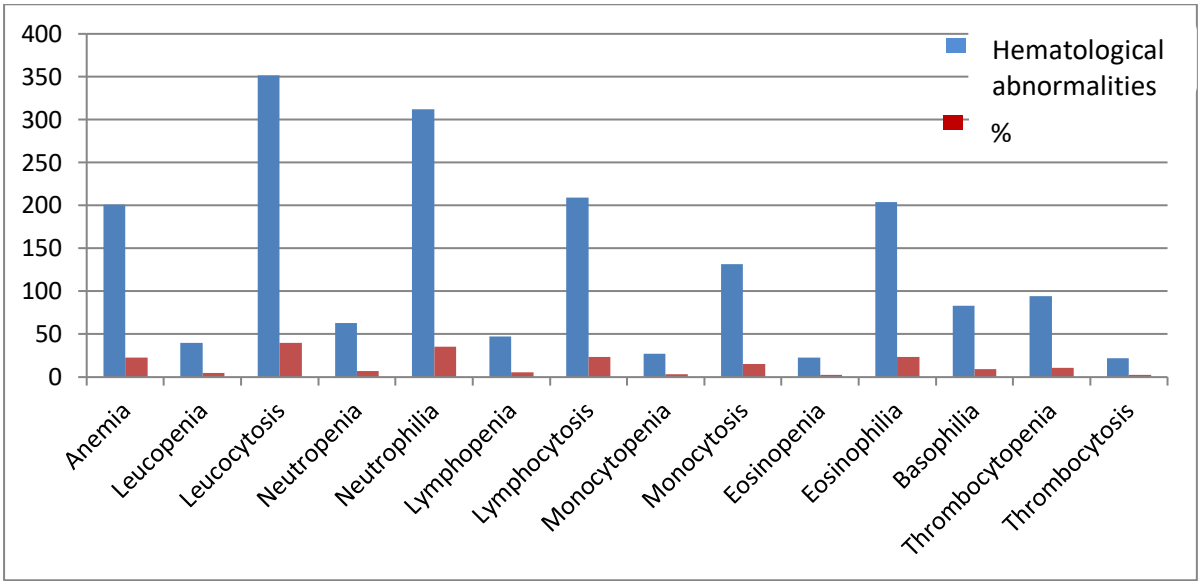

Figure 4.23: Results of various hematological abnormalities observed in women with Toxoplasmosis infection in Khyber Pakhtunkhwa province, Pakistan.

4.7.3 Clinical inflammatory biomarkers and liver profiles of women with Brucellosis infection

Table 4.24 Results of selected inflammatory biomarkers and liver profiles in women with Brucellosis infection in Khyber Pakhtunkhwa province, Pakistan.

Variables	Range (Units)	n=718	(%)	Mean±SE	SD	p-value
ESR	(mm/h)			52.22±1.48	39.459	0.00
	>20 mm/h	464	64.62	72.43±1.58	33.963	
	≤20 mm/h	254	35.37	14.93±0.25	3.8727	
CRP	(mg/L)			17.84±0.52	13.801	0.00
	>6mg/L	442	61.55	26.35±0.53	10.959	
	≤6mg/L	276	38.44	4.21±0.07	1.1341	
AST	(U/L)			46.87±1.23	32.732	1.00
	>42 U/L	217	30.22	90.21±1.73	25.458	
	≤42 U/L	501	69.77	28.09±0.42	9.3912	
ALT	(U/L)			50.67±1.52	40.6685	1.00
	>40 U/L	239	33.28	101.59±1.95	30.129	
	≤40 U/L	479	66.71	25.26±0.43	9.2472	

1-sample proportions test without continuity correction

Table 4.24 and figure 4.23 represents results of selected inflammatory biomarkers and liver profiles of 718 women patients, who were positive for Brucellosis infection. Elevated ESR was significantly (<0.05) found in 464 (64.62%) cases of Brucellosis with a mean elevated ESR value of 72.43±1.58 mm/h, while ESR of 254 (35.37%) patients was found normal with a mean normal ESR values of 14.93±0.25 mm/h. CRP was significantly (<0.05) found elevated in 442 (61.55%)infected women with a mean elevated CRP value of

26.35±0.53 mg/L, while CRP of 276 (38.44%) patients was normal with a mean normal CRP value of 4.21±0.07 mg/L. Similarly elevated AST was found (>0.05) in 217 (30.22%) infected cases with a mean elevated AST value of 90.21±1.73U/L, while AST of 501 (69.77%) patients was normal with a mean normal AST value of 28.09±0.42 U/L. ALT was found (>0.05) elevated in 239 (33.28%) infected cases of Brucellosis with a mean elevated ALT value of 101.59±1.95 U/L, while ALT of 479 (66.71%) infected women was normal with a mean normal ALT value of 25.26±0.43 U/L.

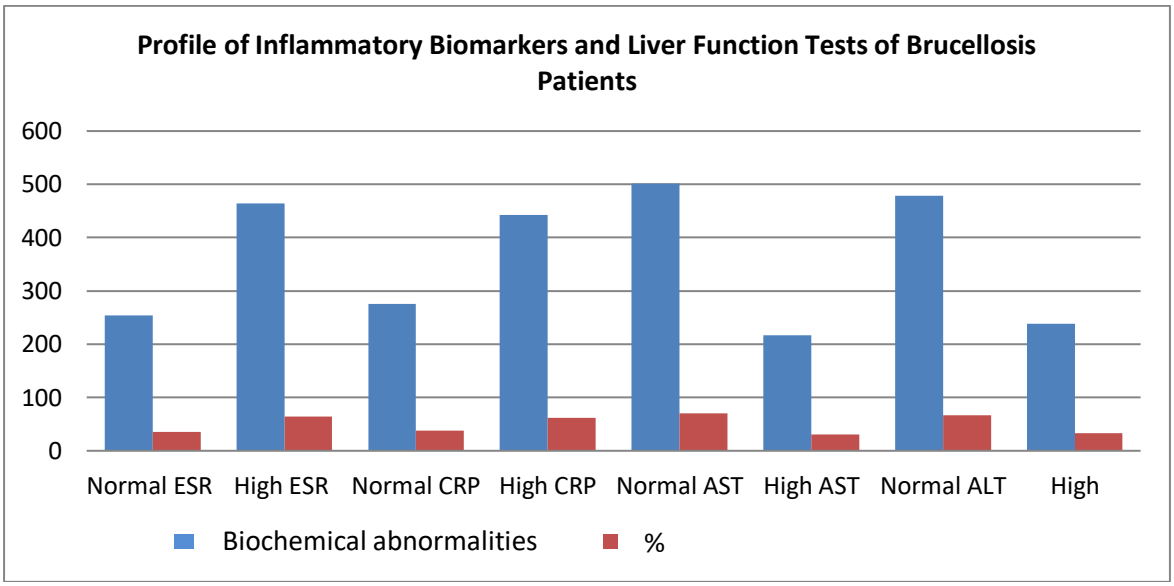

Figure 4.24: Results of selected inflammatory biomarkers and liver profiles in women with Brucellosis infection in Khyber Pakhtunkhwa province, Pakistan.

Table 4.25 Results of inflammatory biomarkers and liver profiles in various clinical stages (acute, sub-acute, chronic) of Brucellosis in women of Khyber Pakhtunkhwa province, Pakistan.

Variables	n=718	Acute (%)		Sub-acute (%)		Chronic (%)		
Assays	Range (unit)	428 (59.61)	Mean+ SE	239 (33.28)	Mean+ SE	51 (7.11)	Mean+ SE	p-value
ESR	>20 mm/h	309 (72.19)	74.07± 1.98	132 (55.23)	69.54± 2.82	23 (45.09)	67.09± 6.47	0.00
CRP	>6 mg/L	292 (68.22)	26.64± 0.66	139 (58.15)	26.05± 0.91	11 (21.56)	22.41± 3.19	0.00
AST	>42 U/L	149 (34.81)	90.53± 2.05	59 (24.68)	86.73± 3.44	9 (17.64)	94.23± 5.68	0.00
ALT	>40 U/L	169 (39.48)	98.83± 2.29	60 (25.11)	109.8± 4.02	10 (19.61)	109.8± 4.02	0.00

Pearson's Chi-squared test

Table 4.25 represent results of selected inflammatory markers (ESR, CRP) and liver profile (AST, ALT)during various clinical stages (acute, sub-acute, chronic) of Brucellosis in 718 patients. A significant difference (<0.05) was found in elevated ESR and CRP during various clinical stages of Brucella infection. Frequency of elevated

ESR was significantly (<0.05) high 309 (72.19%) in acute cases of infection, which decreased to 132 (55.23%) and 23 (45.09%) cases in sub-acute and chronic stages of Brucellosis with a mean ESR values of 74.07±1.98, 69.54±2.82 and 67.09±6.47 mm/h respectively. Similarly frequency of elevated CRP was significantly (<0.05) high 292 (68.22%) in acute stage of Brucellosis with a mean elevated CRP values of 26.64±0.66, which decreased to 139 (58.15%) and 11 (21.56%) cases in sub-acute and chronic clinical stages of infection with a mean values of 26.05±0.91 and 22.41±3.19 mg/L. Similarly, a significant difference (<0.05) was also found in elevated level of liver enzymes AST and ALT in various clinical stages of Brucellosis. Frequency of AST was high 149 (34.81%) in acute cases of infection with a mean elevated value of 90.53±2.05, which decreased to 59 (24.68%) and 9 (17.64%) cases in sub-acute and chronic clinical stage of infection with a mean elevated values of 86.73±3.44 and 94.23±5.68 U/L. Frequency of elevated ALT was high 169 (39.48%) in acute clinical stage of infection with a mean elevated values of 98.83±2.29 U/L, which decreased to 60 (25.11%) and 10 (19.61%) cases in sub-acute and chronic clinical stages of infection with a mean elevated value of 109.8±4.02 and 109.8±4.02 U/L respectively.

4.7.4 Clinical inflammatory biomarkers and liver profiles of women with Toxoplasmosis

Table 4.26 Results of selected inflammatory biomarkers and liver profiles in women with Toxoplasmosis infection in Khyber Pakhtunkhwa province, Pakistan

		Toxoplasmosis				
Variables	Range (Units)	n=881	(%)	Mean±SE	SD	p-value
ESR	(mm/h)			24.38±0.63	18.585	1.00
	>20 mm/h	267	30.31	47.07±1.18	19.199	
	≤20 mm/h	614	69.69	14.55±0.16	3.7721	
CRP	(mg/L)			9.29±0.27	7.9977	1.00
	>6mg/L	293	33.25	19.18±0.39	6.6189	
	≤6mg/L	588	66.74	4.37±0.04	0.9878	
AST	(U/L)			42.29±1.03	30.599	1.00
	>42 U/L	219	24.85	86.73±2.01	29.655	
	≤42 U/L	662	75.14	27.59±0.36	9.2629	
ALT	(U/L)			40.61±0.91	26.994	1.00
	>40 U/L	246	27.92	77.21±1.49	23.408	
	≤40 U/L	635	72.07	26.43±0.35	8.8585	

1-sample proportions test without continuity correction

Table 4.26 represent results of selected inflammatory markers (ESR, CRP) and liver profiles (AST, ALT) in 881 women patients, who were positive for Toxoplasmosis, which is graphically shown in figure 4.24. Elevated ESR was found (>0.05) in 267 (30.31%) infected cases with a mean increased ESR value of 47.07±1.18 mm/h, while 614 (69.69%) infected women were found with normal level of ESR with a mean value of 14.55±0.16 mm/h, CRP was increased (>0.05) in 293 (33.25%) infected cases with a mean increased CRP value of 19.18±0.39

mg/L, while CRP of 588 (66.74%) infected women was normal with a mean value of 4.37±0.04 mg/L. Liver profiles (AST, ALT) was evaluated in women with Toxoplasmosis. AST level was increased (>0.05) in 219 (24.85%) infected women with a mean increased AST value of 86.73±2.01 U/L, while AST level was normal in 662 (75.14%) infected cases with a mean values of 27.59±0.36 U/L. Similarly ALT level was increased (>0.05) in 246 (27.92%) infected cases with a mean increased ALT value of 77.21±1.49 U/L, while ALT level was normal in 635 (72.07%) infected cases with a mean normal value of 26.43±0.35 U/L.

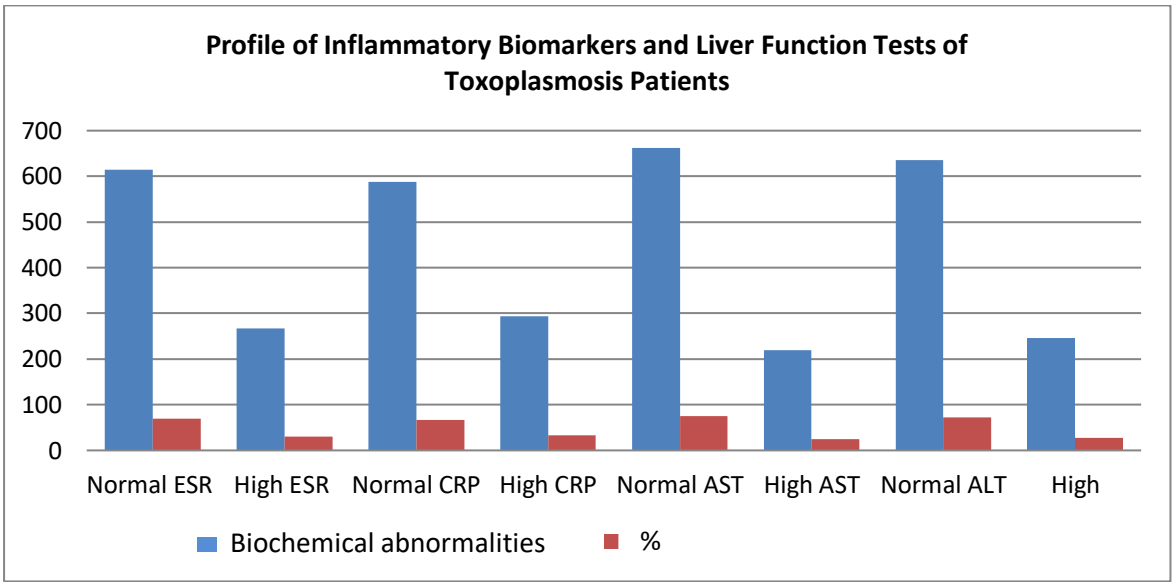

Figure 4.25: Results of selected inflammatory biomarkers and liver profiles in women with Toxoplasmosis infection in Khyber Pakhtunkhwa province, Pakistan

4.8 Clinical management of Brucellosis and associated therapeutic outcomes in infected women

Table: 4.27 Result of overall treatment modalities for management of Brucellosis infection and associated clinical outcomes in infected women of Khyber Pakhtunkhwa province, Pakistan.

Treatment Modalities	Patients		Symptomatic improvement			Therapeutic Failure		
Drug Regimens	n=718	20.02%	599	83.43%	p-value	119	16.57%	p-value
Single-Drug Therapy	414	57.66	323	78.11	0.00	91	21.98	0.00
Co-amoxiclav	5	0.69	2	40	0.81*	3	60	0.50
Azithromycin	12	1.67	5	41.66	0.71	7	58.33	0.61
Ciprofloxacin	17	2.37	11	64.71	0.11	6	35.29	0.16
Amoxicillin	66	9.19	44	66.66	0.00	22	33.34	0.00
Clarithromycin	40	5.57	27	67.5	0.01	13	32.5	0.01
Levofloxacin	38	5.29	26	68.42	0.01	12	31.58	0.01
Ampicillin	90	12.53	75	83.34	0.00	15	16.66	0.00
Cefixime	84	11.71	76	90.47	0.00	8	9.52	0.00
Tetracycline	62	8.63	57	91.93	0.00	5	8.06	0.00
Dual-Drug Therapy	233	32.45	207	88.84	0.00	26	11.16	0.00
Streptomycin+ Erythromycin	4	0.56	2	50	0.68*	2	50	0.50
Doxycycline +Streptomycin	65	9.05	55	84.61	0.00	10	15.39	0.00
Streptomycin+Tetracycline	13	1.81	11	84.61	0.00	2	15.38	0.01
Doxycycline+ Rifampicin	24	3.34	21	87.5	0.00	3	12.51	0.00
Doxycycline+Levofloxacin	44	6.13	40	90.91	0.00	4	9.09	0.00
Doxycycline + Gentamycin	46	6.41	43	93.48	0.00	3	6.52	0.00
Doxycycline+Ciprofloxacin	37	5.15	35	94.59	0.00	2	5.41	0.00
Triple-Drug Therapy	71	9.89	69	97.18	0.00	2	2.81	0.00
Doxycycline + Streptomycin+ Ciprofloxacin	26	3.62	24	92.31	0.00	2	7.69	0.00
Doxycycline + Rifampin + Streptomycin	10	1.41	10	100	0.00*	0	0	0.00
Doxycycline + Rifampicin + Gentamycin	13	1.81	13	100	0.00	0	0	0.00
Gentamicin+ Rifampicin+Ciprofloxacin	22	3.06	22	100	0.00	0	0	0.00

1-sample proportions test without continuity correction
*Exact binomial test
1-sample proportions test with continuity correction

Table 4.27 represents result of overall treatment modalities of Brucellosis management, symptomatic improvements and therapeutic failure in 718 patients, which is shown in figure 4.25, 4.26, 4.27 and 4.28. Among 718 (20.02%) infected women, 599 (83.43%) were symptomatically improved after using various therapeutic regimens for three weeks (≥21 days), while 119 (16.57%) were not symptomatically improved, which indicated therapeutic failure. A significant difference (<0.05) was observed in symptomatic improvement in women

patients, who used mono-therapeutic, dual therapeutic and triple therapeutic regimen. Among 414 (57.66%), 233 (32.45%) and 71 (9.89%) patients, who used single-drug, dual-drug and triple-drug regimen, symptomatic improvement significantly (<0.05) increased from 323 (78.11%) to 207 (88.84%) and 69 (97.18%) patients, while therapeutic failure significantly (<0.05) decreased from 91 (21.98%) to 26 (11.16%) and 2 (2.81%) patients of Brucellosis.

4.8.1 Single-drug therapy and associated outcomes

Among 414 (57.66%) women, who used single-therapeutic regimen, co-amoxiclav showed (>0.05) low rate 2 (40%) of symptomatic improvement and (>0.05) high rate 3 (60.0%) of therapeutic failure, azithromycin showed (>0.05) low rate 5 (41.66%) of symptomatic improvement and (>0.05) high rate 7 (58.33%) of therapeutic failure, ciprofloxacin showed (>0.05) high rate 11 (64.71%) of symptomatic improvement and (>0.05) low rate 6 (35.29%) of therapeutic failure, amoxicillin showed (<0.05) high rate 44 (66.66%) of symptomatic improvement and (<0.05) low rate 22 (33.34%) of therapeutic failure, clarithromycin showed (<0.05) high rate 27 (67.5%) of symptomatic improvement and (<0.05) low rate 13 (32.5%) of therapeutic failure, Levofloxacin showed (<0.05) high rate 26 (68.42%) of symptomatic improvement and (<0.05) low rate 12 (31.58%) of therapeutic failure, ampicillin showed high (<0.05) rate 75 (83.34%) of symptomatic improvement and (<0.05) low rate 15 (16.66%) of therapeutic failure, cefixime showed (<0.05) high rate 76 (90.47%) of symptomatic improvement and (<0.05) low rate 8 (9.52%) of therapeutic failure, tetracycline showed (<0.05) high rate 57 (91.93%) of symptomatic improvement and (<0.05) low rate 5 (8.06%) of therapeutic failure.

4.8.2 Dual-drug therapy and associated outcomes

Among 233 (32.45%) women, who used dual-drug regimen, streptomycin+ erythromycin showed (>0.05) an equal rate 2 (50%) of symptomatic improvement and therapeutic failure 2 (50%), doxycycline +streptomycin showed (<0.05) high rate 55 (84.61%) of symptomatic improvement and (<0.05) low rate 10 (15.39%) of therapeutic failure, streptomycin + tetracycline showed (<0.05) high rate 11 (84.61%) of symptomatic improvement and (<0.05) low rate 2 (15.38%) of therapeutic failure, doxycycline +rifampicin showed (<0.05) high rate 21 (87.5%) of symptomatic improvement and (<0.05) low rate 3 (12.51%) of therapeutic failure, doxycycline + levofloxacin showed (<0.05) high rate 40 (90.91%) of symptomatic improvement and (<0.05) low rate 4 (9.09%) of therapeutic failure, doxycycline + gentamycin showed (<0.05) high rate 43 (93.48%) of symptomatic improvement and (<0.05) low rate 3 (6.62%) of therapeutic failure, doxycycline + ciprofloxacin showed (<0.05) high rate 35 (94.59%) of symptomatic improvement and (<0.05) low rate 2 (5.48%) of therapeutic failure.

4.8.3 Triple-drug therapy and associated outcomes

Among 71 (9.89%) women, who used triple-therapeutic regimen, doxycycline + streptomycin + ciprofloxacin showed (<0.05) high rate 24 (92.31%) of symptomatic improvement and (<0.05) low rate 2 (7.69%) of therapeutic failure, doxycycline + rifampin + streptomycin showed (<0.05) high rate 10 (100%) of symptomatic

improvement and (<0.05) low rate 0 (0.00%) of therapeutic failure, doxycycline + rifampicin + gentamycin showed high (<0.05) rate 13 (100%) of symptomatic improvement and (<0.05) low rate 0 (0.00%) of therapeutic failure, gentamicin+ rifampicin + ciprofloxacin showed (<0.05) high rate 22 (100%) of symptomatic improvement and (<0.05) low rate 0 (0.00%) of therapeutic failure.

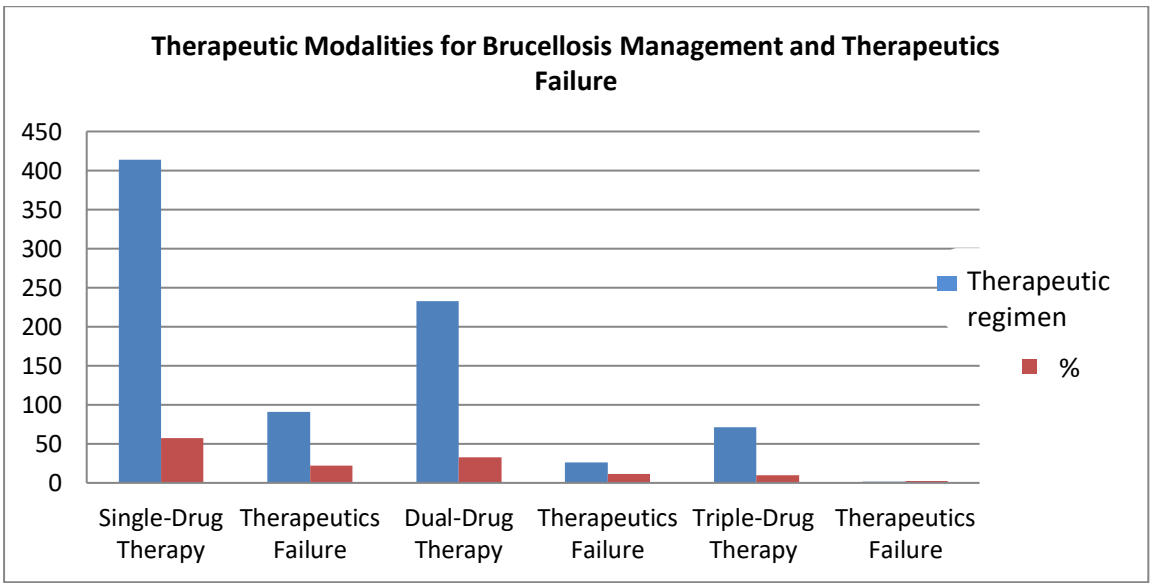

Figure 4.26: Result of various therapeutic modalities for clinical management of Brucellosis and associated clinical outcomes in infected women of Khyber Pakhtunkhwa province, Pakistan.

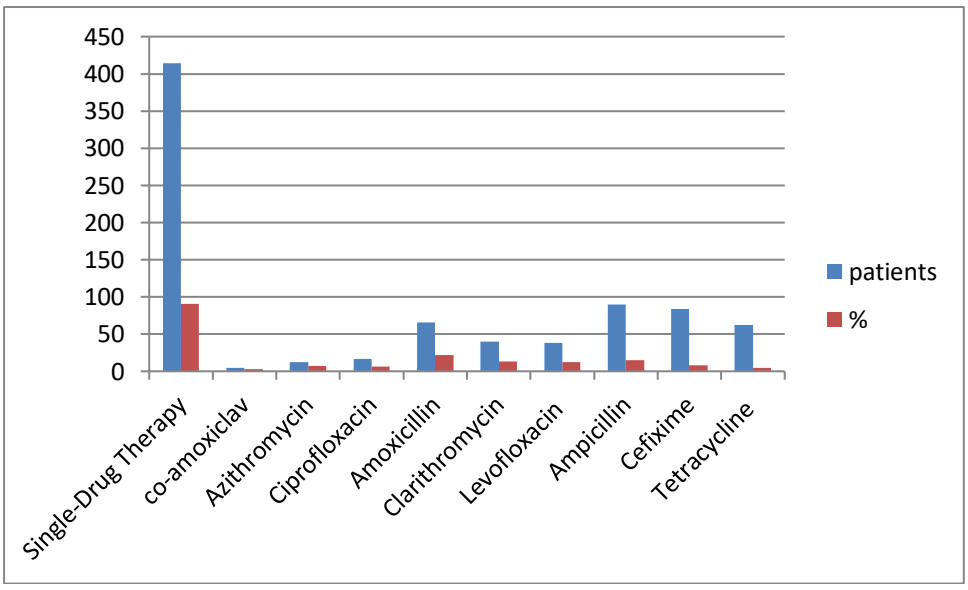

Figure 4.27: Result of single drug treatment modalities for clinical management of Brucellosis and associated clinical outcomes in infected women of Khyber Pakhtunkhwa province, Pakistan.

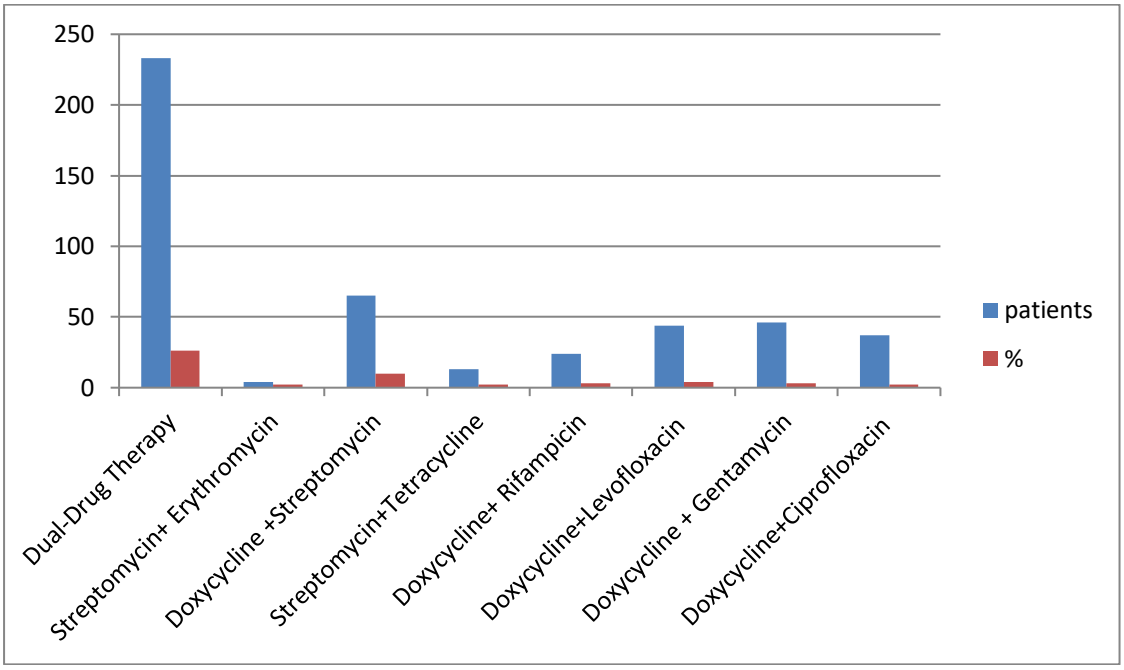

Figure 4.28: Result of dual drug treatment modalities for clinical management of Brucellosis and associated clinical outcomes in infected women of Khyber Pakhtunkhwa province, Pakistan.

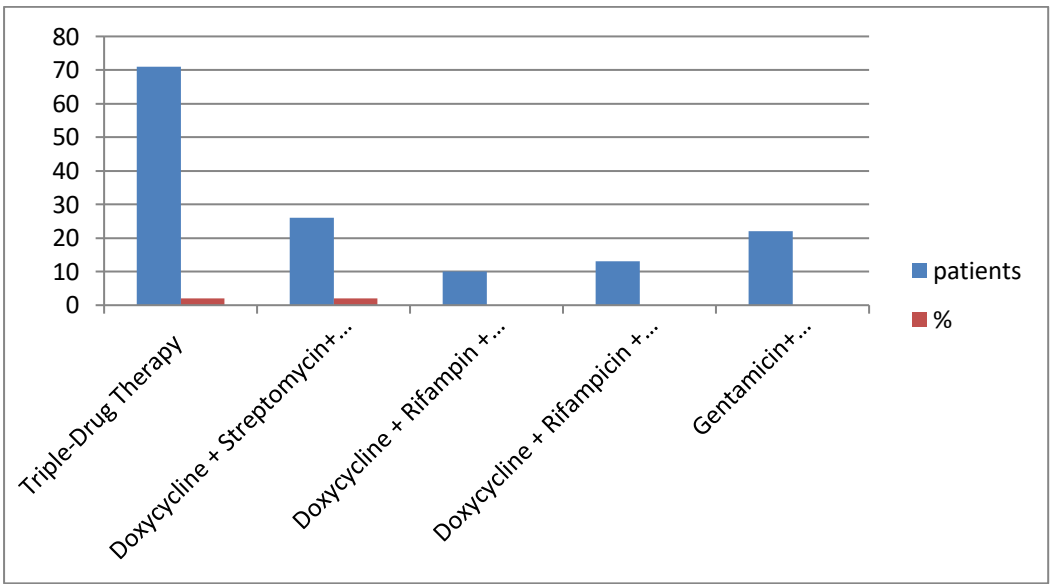

Figure 4.29: Result of triple drug treatment modalities for clinical management of Brucellosis infection and associated clinical outcomes in infected women of Khyber Pakhtunkhwa province, Pakistan.

4.8.4 Clinical management of Brucellosis and associated risk factors of therapeutic failure in women

Table 4.28 Result of various potential risk factors evaluated for therapeutic failure in women with Brucellosis in Khyber Pakhtunkhwa province, Pakistan.

Variables	Patients		Symptomatic Improvement			Therapeutic failure/Relapsed		
	n=718	%	599	83.43%	p-value	119	16.57%	p-value
Drug Regimen								0.00*
Monotherapy	414	57.66	323	78.02	0.00	91	21.98	0.00
Dual-Drug Therapy	233	32.45	207	88.84	0.00	26	11.16	0.00
Triple-Drug Therapy	71	9.89	69	97.18	0.00	2	2.81	0.00
Disease Staging								0.00*
Acute	428	59.61	368	85.98	0.00	60	14.02	0.00
Sub-Acute	239	33.28	203	84.94	0.00	36	15.06	0.00
Chronic	51	7.11	28	54.91	0.24	23	45.09	0.28
Mean Duration	15.84±0.69 (weeks)							
Hb status								0.00**
Anemia	324	45.12	249	76.85	0.00	75	23.15	0.00
Non-anemic	394	54.87	350	88.83	0.00	44	11.16	0.00
Mean Hb Level	11.83±0.09							
Pregnancy status								0.39*
Pregnant	106	14.76	87	82.07	0.00	19	17.92	0.00
Non-pregnant	612	85.24	512	83.66	0.00	100	16.34	0.00
Patients Ages								0.29*
<30 Year	507	70.61	420	82.84	0.00	87	17.16	0.00
>30 Year	211	29.38	179	84.83	0.00	32	15.17	0.00
Mean Age	26.72±0.19							
Gravida/Gravidity								0.13*
Prima-gravida	65	9.05	59	90.77	0.00	6	9.23	0.00
Second-gravida	653	90.95	540	82.71	0.00	113	17.31	0.00

1-sample proportions test with continuity correction
*Pearson's Chi-squared test
**2-sample test for equality of proportions with continuity correction

Table 4.28 indicate various factors such as drug regimens, disease stage, hemoglobin level, pregnancy status, ages and gravidity of patients, which were separately evaluated for rate of symptomatic improvement and therapeutic failure in 718 patients of Brucellosis. Type of drug regimens was evaluated for symptomatic improvement and therapeutic failure. A significant difference (<0.05) was found in rate of symptomatic improvement and therapeutic failure in various drug regimens. Frequency of symptomatic improvement was significantly (<0.05) high 69 (97.18%) in those patients, who used triple-drug regimens, which decreased to 207 (88.84%) and 323 (78.02%) cases in dual-drug and single-drug regimen, while frequency of therapeutic failure

as significantly (<0.05) high in single-drug regimen 91 (21.98%), which decreased to 26 (11.16%) and 2 (2.81%) cases in dual-drug and triple drug regimens. Disease stage was another parameter, which was evaluated for symptomatic improvement and therapeutic failure. A significant (<0.05) difference was found in rate of symptomatic improvement and therapeutic failure in acute, sub-acute and chronic cases of Brucellosis. Frequency of symptomatic improvement was significantly (<0.05) high 368 (85.98%) in acute stage of Brucellosis, which decreased to 203 (84.94%) and 28 (54.91%) cases in sub-acute and chronic stage of Brucellosis, while frequency of therapeutic failure was significantly (<0.05) high 23 (45.09%) in chronic stage, which decreased to 36 (15.06%) and 60 (14.02%) cases in sub-acute and acute stage of Brucellosis. Hemoglobin level was another parameter, which was evaluated for symptomatic improvement and therapeutic failure. A significant (<0.05) difference was found in rate of symptomatic improvement and therapeutic failure in anemic and non-anemic patients of Brucellosis. Frequency of symptomatic improvement was significantly (<0.05) high 350 (88.83%) in non-anemic patients, which decreased to 249 (76.85%) cases in anemic patients, while frequency of therapeutic failure was significantly (<0.05) high 75 (23.15%) in anemic patients, which decreased to 44 (11.16%) cases in non-anemic patients. Pregnancy status was another parameter, which was evaluated for symptomatic improvement and therapeutic failure. No significant (>0.05) difference was found in rate of symptomatic improvement in pregnant and non-pregnant patients. Frequency of symptomatic improvement was significantly (<0.05) high 512 (83.66%) in non-pregnant women, which decreased to 87 (82.07%) cases in pregnant patients, while frequency of therapeutic failure was significantly (<0.05) high 19 (17.92%) in pregnant women, which decreased to 100 (16.34%) cases in non-pregnant women. Patient's age was another parameter, which was evaluated for frequency of symptomatic improvement and therapeutic failure. There was no significant (>0.05) difference in rate of symptomatic improvement and therapeutic failure in women of age group <30 years and >30 years old. Frequency of symptomatic improvement was high 179 (84.83%) in women, who were >30 years old, which decreased to 420 (82.84%) cases in women, who were <30 year old. Frequency of therapeutic failure was high 87 (17.16%) in those women, who were <30 years old, which decreased to 32 (15.17%) cases in women, who were >30 years old. Gravidity of patients was also evaluated for symptomatic improvement and therapeutic failure. No significant (<0.05) difference was found in rate of symptomatic improvement and therapeutic failure in primary-gravida and multi-gravida women. Frequency of symptomatic improvement was significantly (<0.05) high 59 (90.77%) in women, who were primary-gravida, which decreased to 540 (82.71%) cases in women, who were multi- gravida. Frequency of therapeutic failure was significantly (<0.05) high 113 (17.31%) in women, who were multi- gravida, which decreased to 6 (9.23%) cases in those women, who were primary-gravida.

4.8.5 Clinical features of Brucellosis in infected women with therapeutic failure

Table: 4.29 Results of clinical features of Brucellosis in women with therapeutic failure in Khyber Pakhtunkhwa province, Pakistan.

Clinical Symptoms	n=119	16.57%	p-value
Fever	119	100	0.00
Arthralgia/Joint pain	103	86.55	0.00
Fatigue	77	64.71	0.00
Back Pain	63	52.94	0.26
Headache	30	25.21	1.00
Myalgia	25	21.01	1.00
Sweating	13	10.92	1.00

1-sample proportions t-test

Table: 4.29 represent results of various clinical symptoms, which were observed in 119 (16.57%) women patients, who were not symptomatically improved with recommended therapeutic regimens and categorized as therapeutic failure patients. Fever, arthralgia and fatigue were most prominent and significant (<0.05) clinical feature, which were found in patients of therapeutic failure. Among 119 (16.57%) cases of therapeutic failure, fever was significantly (<0.05) found in 119 (100%) patients, which was followed by arthralgia 103 (86.55%), fatigue 77 (64.71%), back pain 63 (52.94%), headache 30 (25.21%), myalgia 25 (21.01%) and sweating in 13 (10.92%) cases.

4.8.6 Clinical laboratory features of Brucellosis infection in patients of therapeutic failure

Table 4.30 Results of various clinical laboratory features of Brucellosis in women with therapeutic failure in Khyber Pakhtunkhwa province, Pakistan.

Laboratory Parameters	R-Range (Units)	n=119	16.57%	Mean ± S.E	SD	p-value
SPAT	≥ 1:160	112	94.12	----	----	0.00
SPAT	< 1:160	7	5.88	----	----	
RBPT	Positive	67	56.31	----	----	0.08
RBPT	Negative	52	43.69	----	----	
ELISA		119	16.57	19.29±0.69	7.6124	
IgM	<9	11	9.24	7.33±0.29	0.9489	
	9—11	11	9.24	10.51±0.23	0.7345	
	>11	97	81.51	21.64±0.65	6.3479	0.00
ELISA		119	16.57	19.57±0.54	5.8984	
IgG	<9	3	2.52	7.65±0.69	1.1852	
	9—11	11	9.24	10.46±0.14	0.4473	
	>11	105	88.23	20.87±0.49	4.9842	0.00
Hemoglobin (Hb) g/dL		119	16.57	10.85±0.21	2.2618	
Hb	≥11.5	41	34.45	13.31±0.25	1.5816	
	<11.5	78	65.54	9.56±0.15	1.2825	0.00
ESR	mm/h	119	16.57	58.63±3.18	34.742	
	≤20	20	16.81	17.75±0.55	2.4468	
	>20	99	83.19	66.89±3.25	32.284	0.00
CRP	mg/L	119	16.57	24.68±1.81	19.749	
	≤6	36	30.25	5.42±0.06	0.3372	
	>6mg	83	69.74	33.03±1.99	18.1023	0.00

1-sample proportions test

Table: 4.30 represent results of various abnormalities in clinical laboratory parameters, which were observed in 119 (16.57%) women, who were not symptomatically improved after using various therapeutic regimens and categorized cases of therapeutic failure. Among 119 (16.57%) cases of therapeutic failure, SPAT was significantly (<0.05) found positive in 112 (94.12%) cases, while RBPT was found (>0.05) positive in 67 (56.31%) cases of therapeutic failure. Similarly IgM ELISA was significantly (<0.05) found positive in 97 (81.51%) cases with mean positive immunoglobulin (IgM) index value of 21.64±0.65, while IgG ELISA was significantly (<0.05) found positive in 105 (88.23%) cases with mean positive immunoglobulin (IgG) index value of 20.87±0.49. Anemia was significantly (<0.05) found in 78 (65.54%) cases with a mean low hemoglobin (Hb) value of 9.56±0.15 g/dL. ESR was significantly (<0.05) found elevated in 99 (83.19%) cases with a mean

elevated level of 66.89±3.25 mm/h, while CRP was significantly (<0.05) found high in 83 (69.74%) cases with a mean increased value of 33.03±1.99 mg/L.

4.9 Obstetric profile and zoonotic diseases (Brucellosis, Toxoplasmosis) in women

Table 4.31 Result of obstetric profile of women evaluated for prevalence of emerging zoonotic diseases (Brucellosis, Toxoplasmosis) in Khyber Pakhtunkhwa province, Pakistan.

Obstetric Profile/information			Zoonotic Diseases			Brucellosis			Toxoplasmosis		
Gravidity	n=3586	%	1599	44.59%	p-value	718	20.02%	p-value	881	24.56%	p-value
Prim-gravida	539	15.03	170	31.53	0.00	65	12.06	0.00	105	19.48	0.00
Multi-gravida	3047	84.97	1429	46.89		653	21.43		776	25.46	
Patient recent obstetric statues											
Pregnant	1068	29.78	352	32.95	0.00	106	9.92	0.00	246	23.03	0.08
Non-Pregnant	2518	70.22	1247	49.52		612	24.31		635	25.21	
Recent gestational stage of fetus in 1068 pregnant women											
1st trimester (<14weeks)	493	13.75	175	35.49	0.00*	37	7.51	0.00	138	27.99	0.00*
2nd trimester (14–28weeks)	327	9.12	127	38.83		50	15.29		77	23.54	
3rd trimester (<28 weeks)	248	6.91	50	20.16		19	7.66		31	12.51	

*2-sample test for equality of proportions with continuity correction
**Chi-squared test for given probabilities

Table 4.31 and figure 29 indicates result of various maternal factors such as patient's gravidity, obstetric and gestational stages of pregnancy, which was evaluated for prevalence of common emerging zoonotic infection (Brucellosis, Toxoplasmosis).

4.9.1 Gravidity and zoonotic diseases in women

Patient's gravidity was evaluated for prevalence of zoonotic diseases. Frequency of zoonotic diseases (Brucellosis, Toxoplasmosis) was significantly (<0.05) different in primary-gravida and multi-gravida women. Prevalence of zoonotic diseases was significantly (<0.05) high 1429 (46.89%) in those women, who were multi-gravida, while rate of zoonotic diseases was low 170 (31.53%) in those women, who were primary gravida.

Brucellosis: Frequency of Brucellosis was significantly different (<0.05) in primary-gravida and multi-gravida women. Prevalence of Brucellosis was significantly (<0.05) high 653 (21.43%) in multi-gravida women, while rate of infection was low 65 (12.06%) in primary gravida.

Toxoplasmosis: Frequency of Toxoplasmosis was significantly different (<0.05) in primary-gravida and multi-gravida women. Prevalence of Toxoplasmosis was significantly (<0.05) high 776 (25.46%) in multi-gravida women, while rate of infection was low 105 (19.48%) in those women, who were primary gravida.

4.9.2 Obstetric statues and zoonotic diseases in women

Patient's obstetric statue was evaluated for prevalence of zoonotic diseases. Frequency of zoonotic diseases (Brucellosis, Toxoplasmosis) was significantly different (<0.05) in pregnant and non-pregnant women. Prevalence of zoonotic diseases was significantly (<0.05) high 1247 (49.52%) in non-pregnant women, while rate of zoonotic diseases was low 352 (32.95%) in pregnant women (Figure: 4.36, 4.37, 4.38, 4.39, 4.40, 4.41)

Brucellosis: Frequency of Brucellosis was significantly different (<0.05) in pregnant and non-pregnant women. Prevalence of Brucellosis infection was significantly (<0.05) high 612 (24.31%) in non-pregnant women, while rate of infection was low 106 (9.92%) in those women, who were pregnant.

Toxoplasmosis: There was no significant (>0.05) difference in frequency of Toxoplasmosis in pregnant and non-pregnant women. Prevalence of Toxoplasmosis was high 635 (25.21%) in non-pregnant women, while rate of infection was low 246 (23.03%) in pregnant women.

4.9.3 Gestational stages and zoonotic diseases in pregnant women

Assessment of zoonotic diseases (Brucellosis, Toxoplasmosis) was carried out in various gestational stages in 1068 pregnant women. There was significant difference (<0.05) in frequency of zoonotic diseases (Brucellosis, Toxoplasmosis) among 1st trimester, 2nd trimester and third trimester of pregnancy.

Prevalence of zoonotic diseases was significantly (<0.05) high 127 (38.83) in 2nd trimester of pregnancy, which decreased to 175 (35.49%) and 50 (20.16%) cases in 1st trimester and 3rd trimester of pregnancy.

Brucellosis: Prevalence of Brucellosis was significantly different (<0.05) in various gestational stages in pregnant women. Prevalence of infection was significantly (<0.05) high 50 (15.29%) in 2nd trimester of pregnancy, which decreased to 19 (7.66%) and 37 (7.51%) cases in 3rd and 1st trimester of pregnancy.

Toxoplasmosis: Prevalence of Toxoplasma infection was significantly different (<0.05) in various gestational stages of pregnancy. Prevalence of infection was significantly (<0.05) high 138 (27.99%) in 1st trimester of pregnancy, which decreased to 77 (23.54%) and 31 (12.51%) cases in 2nd and 3rdtrimester of pregnancy.

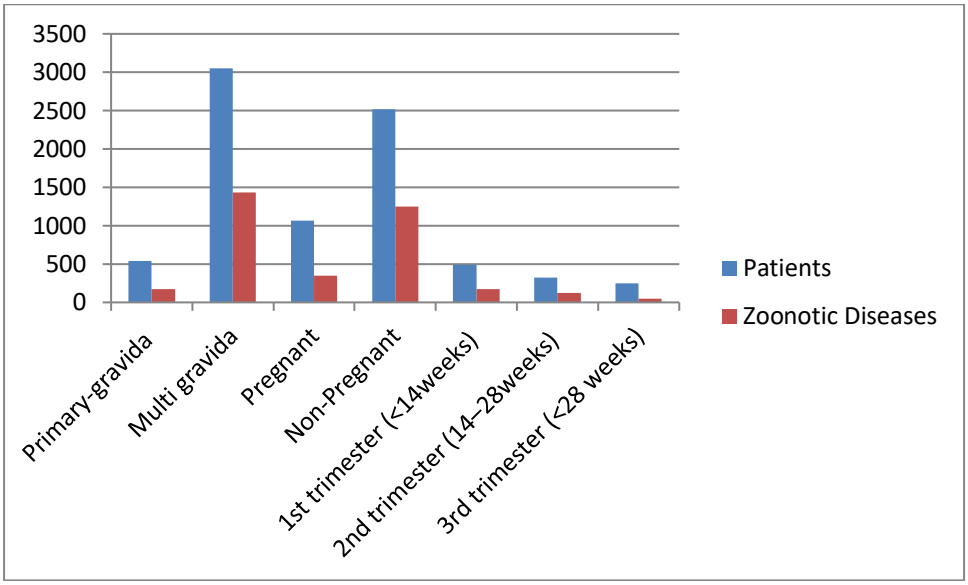

Figure 4.30: Result of obstetric profile of women and prevalence of emerging zoonotic diseases (Brucellosis, Toxoplasmosis) in Khyber Pakhtunkhwa province, Pakistan.

4.10 Various types of adverse obstetric outcomes and zoonotic diseases (Brucellosis, Toxoplasmosis) in women

Table 4.32 Results of various adverse obstetric outcomes in women evaluated for emerging zoonotic diseases (Brucellosis, Toxoplasmosis) in Khyber Pakhtunkhwa province, Pakistan.

Characteristic		Zoonotic Diseases				Brucellosis			Toxoplasmosis		
n=3586		%	1599	44.59%	p-value	718	20.02%	p-value	881	24.56%	p-value
History of previous spontaneous miscarriage/abortion											
Yes	429	11.96	167	38.92	0.99	43	10.02	1.00	124	28.91	0.01
No	3157	88.03	1432	45.35		675	21.38		757	23.97	
History of multiple spontaneous miscarriage/abortion											
Yes	160	4.46	37	23.12	1.00	7	4.37	1.00	30	18.75	0.95
No	3426	95.53	1562	45.59		711	20.75		851	24.84	
History of recent spontaneous miscarriage/abortion											
Yes	414	11.54	182	43.96	0.60	67	16.18	0.98	115	27.78	0.05
No	3172	88.45	1417	44.67		651	20.52		766	24.15	
Clinician advised medical induced abortion due to intrauterine fetal death(IUFD)/hydrocephalus											
Yes	284	7.91	90	31.69	1.00	23	8.09	1.00	67	23.59	0.65
No	3302	92.08	1509	45.69		695	21.05		814	24.65	
History of preterm birth *											
Yes	180	5.02	41	22.78	1.00	31	17.23	0.83	10	5.56	1.00
No	3406	94.98	1558	45.74		687	20.17		871	25.57	
History of stillbirth											

Yes	107	2.98	23	21.49	1.00	14	13.08	0.96	9	8.42	1.00
No	3479	97.01	1576	45.31		704	20.23		872	25.06	
History of congenital anomalies in children											
Yes	88	2.45	20	22.73	1.00	4	4.55	0.99	16	18.19	0.92
No	3498	97.54	1579	45.14		714	20.41		865	24.73	
Pelvic inflammatory diseases (PID) diagnosed through ultrasonographic examination											
Yes	483	13.46	261	54.04	0.00	147	30.45	0.00	114	23.61	0.70
No	3103	86.53	1338	43.12		571	18.41		767	24.72	

*2-sample test for equality

Table 4.32 indicates result of various parameters of adverse obstetric outcomes such as previous spontaneous abortion, recent spontaneous abortion (figure: 4.31), multiple spontaneous abortion, medical induced abortion due to IUFD (figure: 4.32), fetal abnormalities like hydrocephalus (figure:4.35), pre-term birth, stillbirth, congenital anomalies in children and pelvic inflammatory diseases (PID) figure:4.34, which were evaluated for prevalence of common emerging zoonotic diseases (Brucellosis, Toxoplasmosis) in women in the studied region.

4.10.1 History of previous spontaneous abortion and zoonotic diseases in women

Assessment of zoonotic diseases (Brucellosis, Toxoplasmosis) was carried out in women, who were positive for previous history of abortion and those women, who had no previous history of abortion. No significant difference (>0.05) was found in prevalence of zoonotic diseases in women of these two groups. Prevalence of zoonotic diseases was high 1432 (45.35%) in women, who had no previous history of abortion, while rate of zoonotic diseases was low 167 (38.92%) in those women, who were positive for previous history of abortion.

Brucellosis: There was no significant difference (>0.05) in prevalence of Brucellosis in women of these two groups. Prevalence of Brucellosis was high 675 (21.38%) in women, who had no previous history of abortion, while rate of infection was low 43 (10.02%) in women, who were positive for previous history of abortion.

Toxoplasmosis: There was significant difference (<0.05) in prevalence of Toxoplasmosis in women of these two groups. Prevalence of Toxoplasmosis was high 124 (28.91%) in women, who were positive for previous history of abortions (figure 4.33), while rate of infection was low 757 (23.97%) in women, who had no previous history of abortion.

4.10.2 History of recent spontaneous abortion and zoonotic diseases in women

Assessment of zoonotic diseases (Brucellosis, Toxoplasmosis) was carried out in women, who came to clinician with a recent history of abortion and those women, who had no history of recent abortion. No significant difference (>0.05) was found in prevalence of zoonotic diseases in women of these two groups. Prevalence of zoonotic diseases was high 1417 (44.67%) in those women, who had no recent clinical history of abortion, while rate of zoonotic diseases was low 182 (43.96%) in those women, who visited clinician for a recent history abortion.

Brucellosis: There was no significant difference (>0.05) in prevalence of Brucellosis in women of these two groups. Prevalence of Brucellosis was high 651 (20.52%) in women, who had no history of recent abortion, while rate of infection was low 67 (16.18%) in those women, who had recent clinical history of abortion.

Toxoplasmosis: There was significant difference (≤0.05) in prevalence of Toxoplasmosis in women of these two groups. Prevalence of Toxoplasmosis was high 115 (27.78%) in those women, who had positive history of recent abortion, while rate of infection was low 766 (24.15%) in those women, who had no recent clinical history of abortion.

4.10.3 History of multiple spontaneous abortion and zoonotic diseases in women

Assessment of zoonotic diseases (Brucellosis, Toxoplasmosis) was carried out in women patients, who came to clinician with a history of multiple abortions and those women, who had not presented such type of history. No significant difference (>0.05) was found in prevalence of zoonotic diseases in women of these two groups. Prevalence of zoonotic diseases was high 1562 (45.59%) in those women, who had no history of multiple abortions, while rate of zoonotic diseases was low 37 (23.12%) in women, who presented with a clinical history of multiple abortions.

Brucellosis: There was no significant difference (>0.05) in rate of Brucellosis in women of these two groups. Prevalence of Brucellosis was high 711 (20.75%) in women, who had no clinical history of multiple abortions, while rate of Brucellosis was low 7 (4.37%) in those women, who had history of multiple abortions.

Toxoplasmosis: There was no significant difference (>0.05) in prevalence of Toxoplasmosis in women of these two groups. Prevalence of Toxoplasmosis was high 851 (24.84%) in those women, who had no clinical history of multiple abortions, while rate of Toxoplasma infection was low 30 (18.75%)in those women, who were positive for history of multiple abortions.

4.10.4 Medical induced abortions in women and zoonotic diseases in women

Assessment of zoonotic diseases (Brucellosis, Toxoplasmosis) was carried out in those women, who were clinically advised abortions due to various fetal abnormalities like intrauterine fetal death (IUFD), hydrocephalus etc after proper ultrasonographic examination and those women, who were not advised clinically induce abortions. No significant difference (>0.05) was found in prevalence of zoonotic diseases in women of these two groups. Prevalence of zoonotic diseases was high 1509 (45.69%) in women, who were not advised clinical induce abortion, while rate of zoonotic diseases was low 90 (31.69%) in women, who were recommended clinically induce abortion.

Brucellosis: There was no significant difference (>0.05) in rate of Brucellosis in women of these two groups. Prevalence of Brucellosis was high 695 (21.05%) in women, who were not advised clinical induce abortion after clinical examination, while rate of Brucellosis was low 23 (8.09%) in those women, who were clinically advised abortions after proper ultrasonographic examination.

Toxoplasmosis: There was no significant difference (>0.05) in prevalence of Toxoplasmosis in women of these two groups. Prevalence of Toxoplasmosis was high 814 (24.65%) in women, who were not advised clinical induce abortion after clinical examination, while rate of infection was low 67 (23.59%) in women, who were advised abortions after ultrasonographic examination.

4.10.5 History of preterm birth and zoonotic diseases in women

Assessment of zoonotic diseases (Brucellosis, Toxoplasmosis) was carried out in women, who had history of preterm birth and those women, who had no clinical history of preterm birth. No significant difference (>0.05) was found in prevalence of zoonotic diseases in women of these two groups. Prevalence of zoonotic diseases was high 1558 (45.74%) in women, who had no history of preterm birth, while rate of zoonotic diseases was low 41 (22.78%) in women, who were positive for clinical history of preterm birth.

Brucellosis: There was no significant difference (>0.05) in rate of Brucellosis in women of these two groups. Prevalence of Brucellosis was high 687 (20.17%) in women, who had no history of preterm birth, while rate of infection was low 31 (17.23%) in those patients, who were positive for clinical history of preterm birth.

Toxoplasmosis: There was no significant difference (>0.05) in rate of Toxoplasmosis in women of these two groups. Prevalence of Toxoplasmosis was high 871 (25.57%) in women, who had no clinical history of preterm birth, while rate of Toxoplasma infection was low 10 (5.56%) in women, who were positive for clinical history of preterm birth.

4.10.6 History of stillbirth and zoonotic diseases in women

Assessment of zoonotic diseases (Brucellosis, Toxoplasmosis) was carried out in women, who had confirmed history of still birth and in those women, who had no clinical history of still birth. There was no significant difference (>0.05) in prevalence of zoonotic diseases in women of these two groups. Prevalence of zoonotic diseases was high 1576 (45.31%) in women, who had no history of still birth, while rate of zoonotic diseases was low 23 (21.49%) in those women, who had clinical history of still birth.

Brucellosis: There was no significant difference (>0.05) in rate of Brucellosis in women of these two groups. Prevalence of Brucellosis was high 704 (20.23%) in women, who had no history of still birth, while rate of Brucellosis was low 14 (13.08%) in women, who had clinical history of still birth.

Toxoplasmosis: No significant difference (>0.05) was found in rate of Toxoplasmosis in women of these two groups. Prevalence of Toxoplasmosis was high 872 (25.06%) in women, who had no history of still birth, while rate of Toxoplasmosis was low 9 (8.42%) in women, who were positive for clinical history of still birth.

4.10.7 History of congenital anomalies in children and zoonotic diseases in women

Assessment of zoonotic diseases (Brucellosis, Toxoplasmosis) was carried out in women, who were positive for history of various congenital anomalies (deafness, blindness, microcephalus, macrocephaly, mental retardation, any disability) in children and in those, who had no history of such congenital anomalies in children. No significant difference (>0.05) was found in rate of zoonotic diseases in women of these two groups. Prevalence

of zoonotic diseases was high 1579 (45.14%) in women, who had no history of congenital anomalies in children, while rate of zoonotic diseases was low 20 (22.73%) in women, who had congenital anomalies in children.

Brucellosis: No significant difference (>0.05) was found in rate of Brucellosis in women of these two groups. Prevalence of Brucellosis was high 714 (20.41%) in women, who had no congenital anomalies in children, while rate of Brucellosis was low 4 (4.55%) in women, who had congenital anomalies in children.

Toxoplasmosis: No significant difference (>0.05) was found in rate of Toxoplasmosis in women of these two groups. Prevalence of Toxoplasmosis was high 865 (24.73%) in women, who had no congenital anomalies in children, while rate of Toxoplasmosis was low 16 (18.19%) in those women, who had congenital anomalies in children.

4.10.8 Pelvic inflammatory diseases (PID) and zoonotic diseases in women

Assessment of zoonotic diseases (Brucellosis, Toxoplasmosis) was carried out in women, who were positive for pelvic inflammatory diseases (PID) and those women, who were negative for pelvic inflammatory disease after ultrasonographic examination (figure 36). A significant difference (<0.05) was found in prevalence of zoonotic diseases in women of these two groups. Prevalence of zoonotic diseases was high 261 (54.04%) in women, who were positive for pelvic inflammatory diseases, while rate of zoonotic diseases was low 1338 (43.12%) in women, who had not pelvic inflammatory disease.

Brucellosis: A significant difference (<0.05) was found in rate of Brucellosis infection in women of these two groups. Prevalence of Brucellosis was high 147 (30.45%) in women, who had pelvic inflammatory disease (figure 4.32), while rate of Brucellosis was low 571 (18.41%) in those women, who had no pelvic inflammatory disease.

Toxoplasmosis: No significant difference (>0.05) was found in rate of Toxoplasmosis in women of these two groups. Prevalence of Toxoplasmosis was high 767 (24.72%) in women, who had no pelvic inflammatory disease, while rate of Toxoplasmosis was low 114 (23.61%) in those women, who had pelvic inflammatory disease.

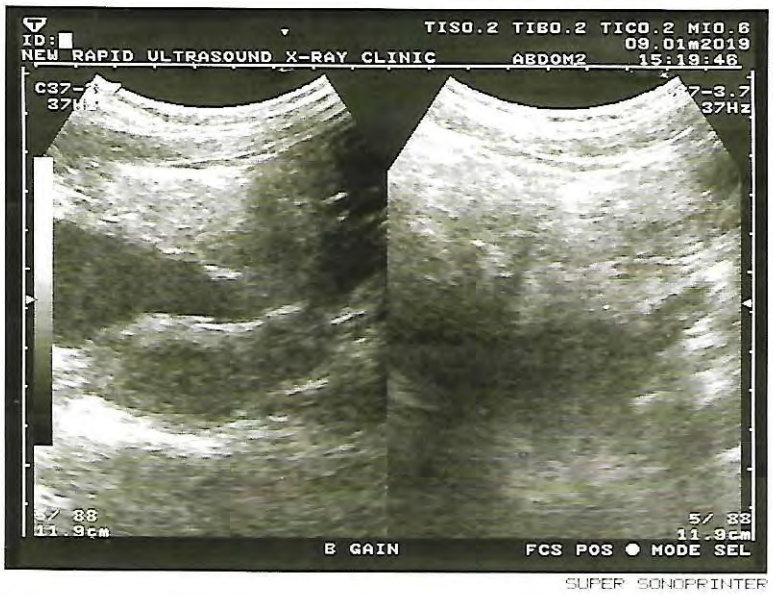

Figure 4.31: The pelvic ultrasonographic report of a 23 year primary gravida woman, who was positive for Brucellosis infection by Brucella genus, specific PCR.

The uterus of patient was normal in size, shape and echo-pattern. No focal mass or products of conception were seen in it. Both ovaries were normal in size and shape. The Endometrial thickness was normal. No adnexal mass was seen. The Follicular activity was normal. No amount of free fluid was seen in the cul-de-sac. The urinary bladder was normal.

Impression: Normal pelvic ultrasonographic study of woman with chronic Brucellosis infection.

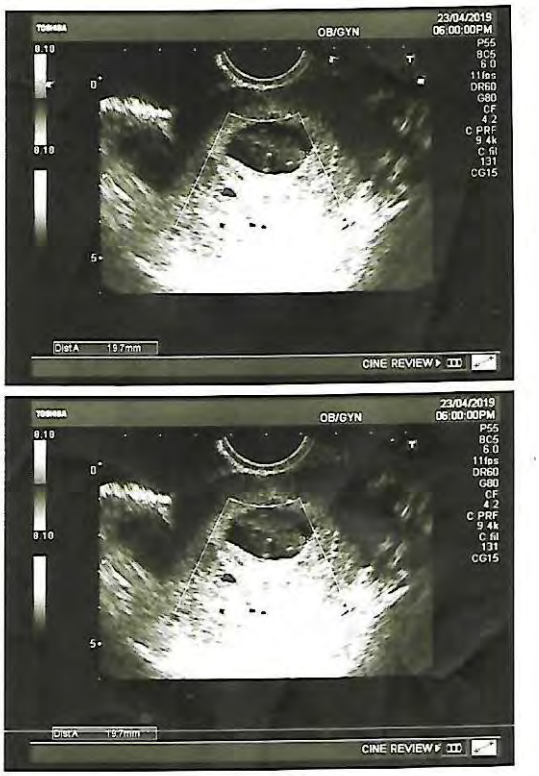

Figure 4.32: The pelvic ultrasonographic reports of a 24 year prima-gravida woman, who had missed abortion. She was positive for Toxoplasmosis by latex agglutination, ICT and Toxoplasma specific IgM and IgG ELISA test.

The scan shows uterus enlarged in size containing a large irregular gestational sac with fetal echoes in it. The fetal cardiac activity was not appreciated. A note was made of collapsed yolk sac. CRL was 1.9mm. The gestational age was 08 weeks ±5 days. No adnexal mass was seen. No free fluid was noted in the cul-de-sac. Normal urinary bladder was observed.

Impression:

1. Enlarged uterus containing a large irregular gestational sac with fetal echoes corresponding to the gestational age of 08 weeks ± 05 days.
2. Fetal cardiac activity was not seen with collapsed yolk sac suggestive of missed abortion.

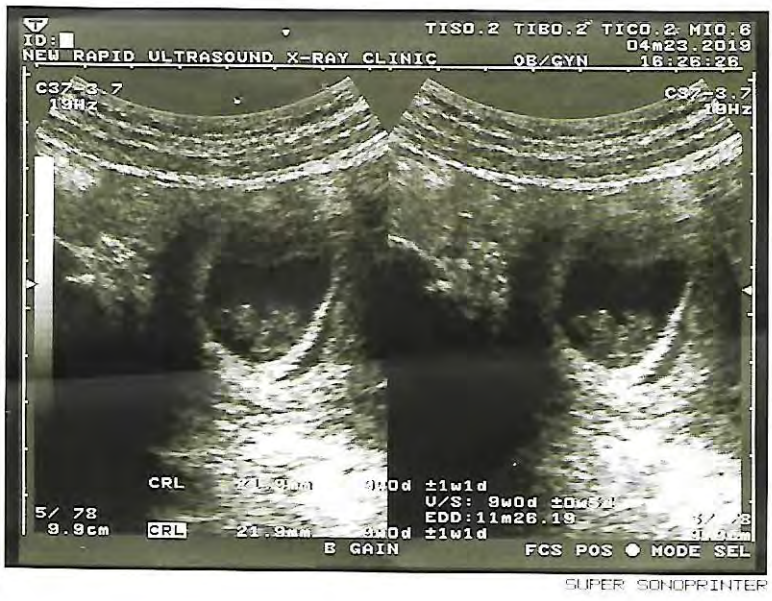

Figure 4.33: The pelvic ultrasonographic report of 23 year primary gravidia woman with dead intrauterine fetus of 9 weeks, who was positive for Brucellosis by SPAT, RBPT, IgM and IgG ELISA test.

The uterus of patient was enlarged in size and containing a gestational sac with a single non-active fetus in it. Fetal cardiac motions were not detected at the time of scanning. Crown rump length (C.R.L) was 21.9 mm, gestational age (G.A) 9 weeks and 0 day. No adnexal abnormality was detected. No free fluid was seen in the cul-de-sac. No lesion was observed in urinary bladder of the patient.

Impression: Dead intrauterine fetus of 9 weeks.

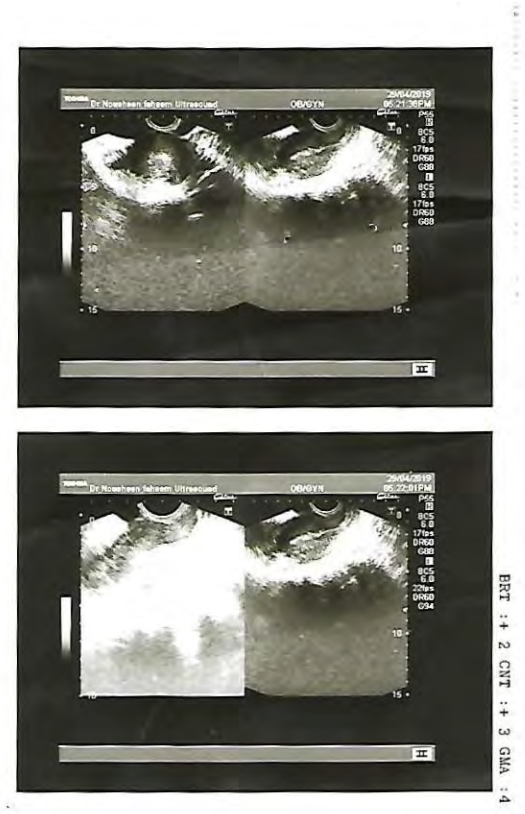

Figure 4.34: The pelvis ultrasonographic report of 26 years old primary gravidia woman, who was positive for Toxoplasmosis by LAT, ICT, IgM and IgG ELISA assay. She had enlarged uterus containing clotted blood and mild RPOCs.

The ultrasonographic scan showed mildly enlarged uterus with open endometrial cavity containing clotted blood and mild RPOCs measuring 13×14 mm. Both ovaries of patient were normal in size, shape and echo-pattern. No adnexal mass was seen. No free fluid was observed in the cul-de-sac. The urinary bladder was normal.

Impression: Mildly enlarged uterus with open endometrial cavity containing clotted blood and mild RPOCs

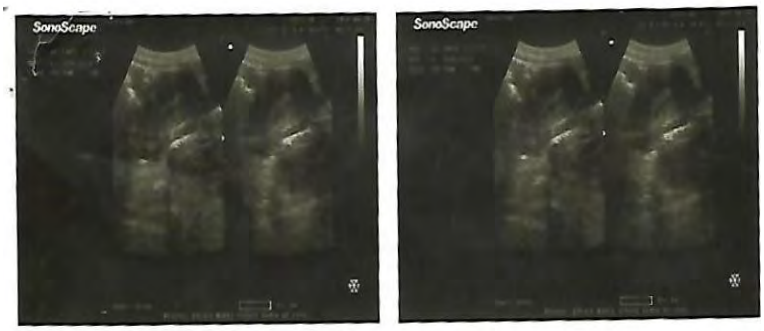

Figure 4.35: The pelvic ultrasound report of a 30 years multigravida woman, who had pelvic inflammatory disease (PID) and positive for Brucellosis by SPAT, RBPT, IgM and IgG ELISA.

The uterus appeared normal in size, shape and echo texture. No focal lesion was seen in it. Endometrial thickness was within normal limit. No adnexal pathology was seen. Small amount of free fluid was seen in the cul-de-sac. Both ovaries of patient were normal. The configuration of urinary bladder appeared normal.

Impression: The patient was diagnosed with pelvic inflammatory disease (PID).

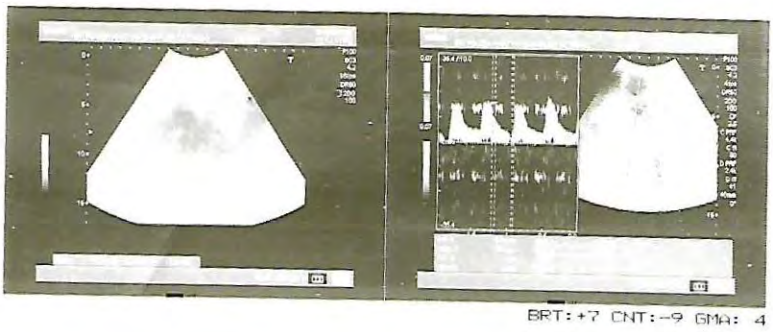

Figure 4.36: The pelvis ultrasonographic report of 32 years multi gravidia pregnant woman, who was positive for Toxoplasmosis infection by LAT, ICT and IgM, IgG ELISA assay. She had single active fetus of 37± 1 week with cephalic presentation. Scan shows fluid in the fetus head (hydrocephalus).

Obstetrical ultrasound: Real time trans-abdominal ultrasound study reveals single active fetus with following features:

Lie: longitudinal	Presentation- Cephalic
BPD: 11.2cm	more than 42 weeks 00 days ± 03 weeks
FL: 7.74 cm	39 weeks 05 days ± 03 weeks
FAC: 34.7cm	38 weeks 05 days ± 03 weeks

Estimated gestational age: 39 weeks 02 days ± 02 weeks (according to FAC & FL)

EDD: 09-08-2019 (according to FAC & FL)

EFBW: 3665 g ± 550 gm

There was marked enlargement of fetal head with evidence of mono-ventricular with rudimentary occipital horns and resultant marked compression of paper thin surrounding brain tissues. Posterior brain tissue was present. Thalami appeared normal. Partial flax present posteriorly. No corpus callosum or septum pellucidum was seen. Third and fourth ventricles were not identified. No spina bifida seen. No intracranial mass was detected. Semi lobar holoprosencephaly or marked hydrocephalus

Fetal stomach bubble, both kidney and urinary bladder were appreciated.

Amniotic fluid was adequate. Amniotic fluid index was 9.6 CM

Placenta: Fundal anterior

No umbilical cord loop coiling was seen around the fetal neck

BIOPHYSICAL PROFILE:

Variables	Score
Amniotic fluid	02
Cardiac re-activity	02
Respiration	02
Tone	02
Body movement	02
Total score	10/10

UMBILICAL ARTERY DOPPLER

Spectral Doppler tracing obtained from umbilical artery in the free cord segment reveals wave form with decreased diastolic flow and elevated S/D ratio for the gestation, suggesting resistance to the feto placental circulation at present scan (recommended S/D ratio 5^{th} -95^{th} percentile values for this gestational is 1.86 ----3.11). The velocity measurements of the umbilical artery were as follows:

Peak systolic velocity =15.0 cm/second Systolic/Diastolic ratio =4.86

End diastolic velocity =3.3 cm/second Resistance Index =0.80

Impression: As mentioned in above comments

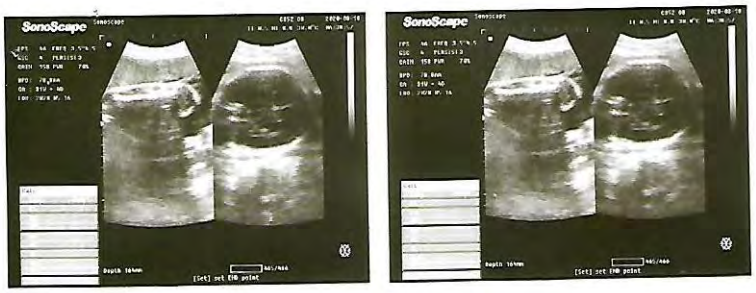

Figure 4.37: Obstetrical ultrasonographic report of 25 years pregnant woman, who was positive for Brucellosis by Brucella genus specific PCR and had threatened abortion.

Number of fetus was single. Presentation was cephalic. Fetal spine was regular. Amount of liquor was adequate Placental localization anterior. Fetal heart beat was regular. Fetal movement was seen. BPD was 6.6 cm and F.L 4.5 cm. Fetal gestation was 26 weeks ±5days

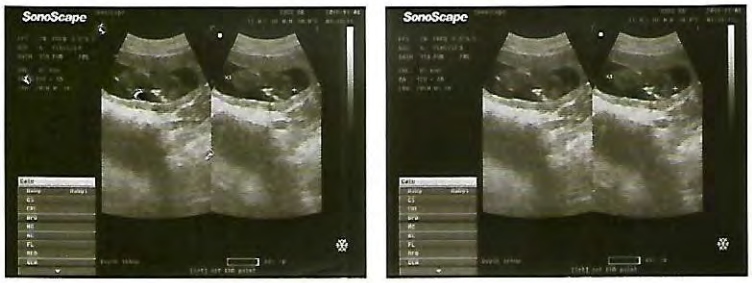

Figure 4.37: The obstetrical ultrasonographic reports of 34 years multi-gravida woman, who had pregnancy of 12 week gestational age. She was positive for Toxoplasmosis infection by IgM and IgG ELISA assay and had threatened abortion.

Number of fetuses was single and viable. Fetal spine was regular. Amount of liquor was adequate. Placental localization was Fundal. Fetal heart beats was regular. Fetal movement was seen. CRL was 6.5 cm. Fetal gestation age was 12 weeks +2 days.

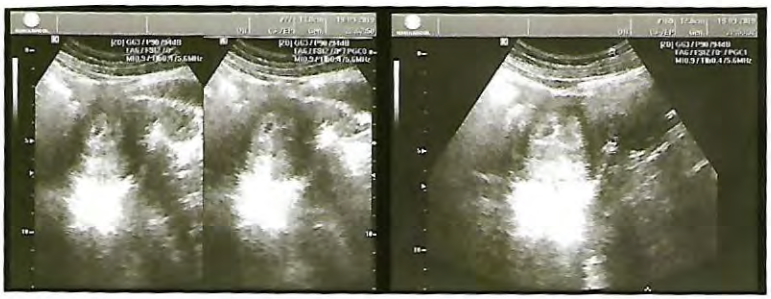

Figure 4.38: Obstetric ultrasonographic reports of 21 years gravida woman, who had early twins pregnancy. She was positive for Brucellosis infection by SPAT, IgM, IgG ELISA and Brucella genus specific PCR. She had at risk of early abortion.

The ultrasonographic scan showed gravid uterus having two small gestational sacs. G.S: 6mm. Fetal echoes and yolk sac were not seen. No adnexal mass was seen. No free fluid was noted in the cul-de-sac Impression: Early twins pregnancy.

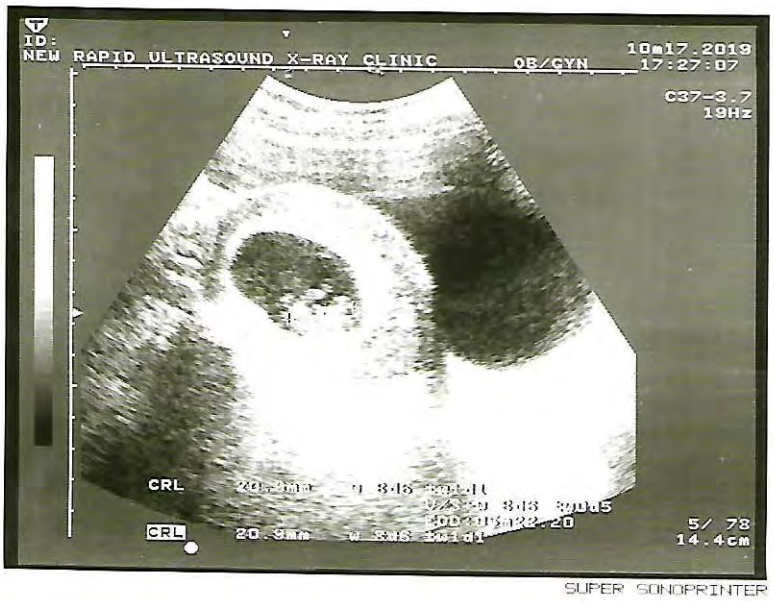

Figure 4.39: Obstetric ultrasonographic reports of 31 years multi gravida woman, who had normal intra uterine pregnancy of 08 weeks 06 days gestation. She was positive for Toxoplasmosis infection by LAT, ICT, IgM and IgG ELISA assays and was at risk of early abortion.

The pelvic ultrasonographic report showed single intra-uterine fetus. Fetal cardiac motion was detected by ultrasonographic examination. Crown rump length (C.R.L) was measured 20.9mm. Gestational age of fetus was 8 weeks 06 days. Internal cervical os was closed. Ultrasonically, no adnexal abnormality was detected

Impression: Normal intra uterine pregnancy of 08 weeks 06 days gestation.

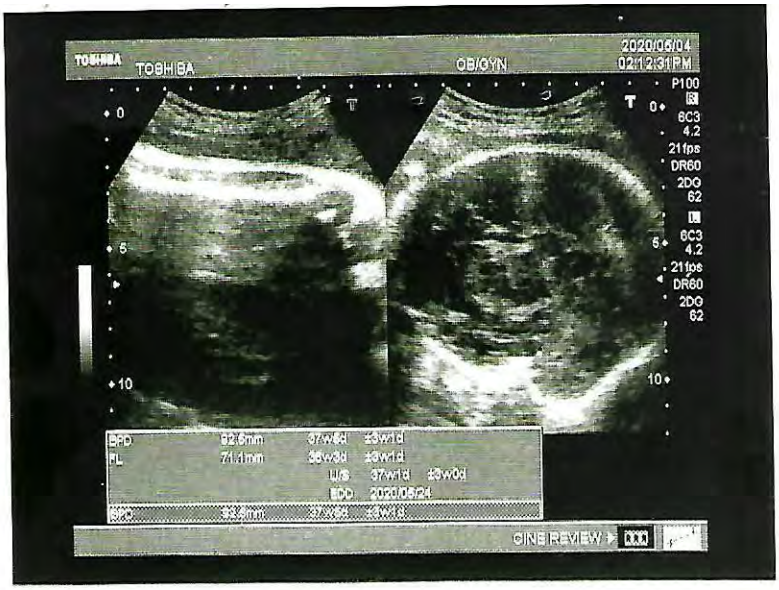

Figure 4.40: Obstetric ultrasonographic reports of 23 years primary gravida pregnant woman, who had single, alive, intrauterine fetus of 37 weeks 01 days and positive for Brucellosis infection by SPAT. IgM and IgG ELISA assay, who was at risk of abortion.

Obstetrical ultrasonographic finding showed a single alive intra uterine fetus .Presentation was cephalic (at present). BPD: 9.2cm. FL: 7.1 cm. Gestational age: 37 weeks 01 day. Placenta: Anterior in upper uterine segment. Amniotic fluid was mildly increased with internal echoes.

BIOPHYSICAL PROFILE

Criteria Score

Movements: 02

Respiration: 02

Heart rate: 02

Tone: 02

Amniotic fluid: 02

Total: 10/10

Impression: Single, alive, intrauterine fetus of 37 weeks 01 days with cephalic presentation and mild polyhydramnios. Biophysical profile score was 10/10

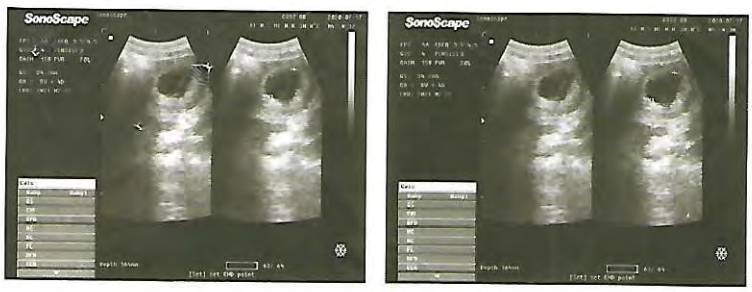

Figure 4.41: Obstetric ultrasonographic reports of 29 years multi gravida pregnant woman, who had pregnancy of a single, alive, intrauterine fetus of 36 weeks 04 days and was positive for Toxoplasmosis by LAT, ICT, IgM and IgG ELISA assay and at risk of abortion.

The ultrasonographic finding showed single alive fetus with cephalic presentation and placenta anterior with grade 1 calcification. Liquor was adequate with suspended echogenic particles. BPD was measured 9cm, FL =7.1 cm and femur length F.L 2.2mm. POG was 36 weeks 4 days.

Comments: No evidence of cord around neck at time of scan

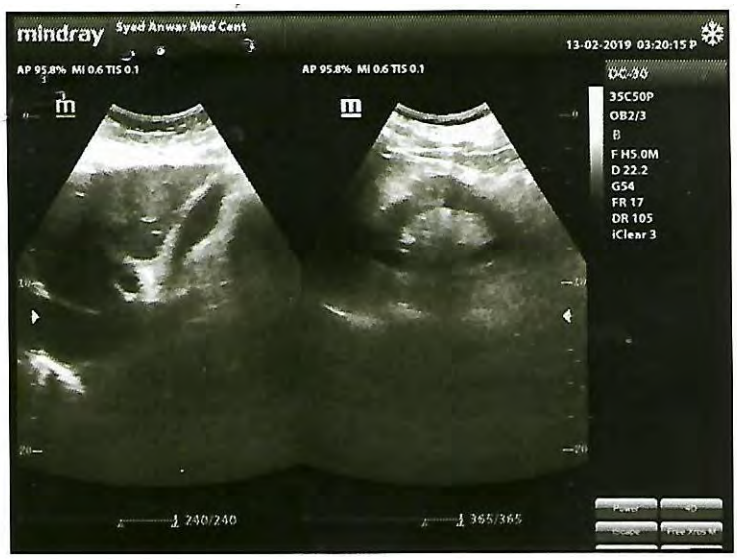

Figure 4.42: Abdominal and pelvis ultrasonographic reports of 34 years multi gravida woman, who was positive for Brucellosis infection by STAT, RBPT, IgM and IgG ELISA.

The liver was mildly enlarged in size measured 17cm, with normal shape and echo-texture. An about 11×9mm echogenic area was seen in lefty lobe of the liver having no flow on color Doppler,

"hamangioma". Portal vein was normal in caliber. Gall bladder was normal in size and shape with normal wall thickness. CBD was normal caliber. Pancreas was obscured due to excessive gut gases shadows. Spleen was normal in size with normal echo-texture. Both kidneys were normal in size, shape and echo-texture with intact CMD. No evidence of calculus, cyst and hydronephrosis was seen. Urinary Bladder was empty with catheter bulb in situ. Para-aortic lymph nodes were obscured. No ascites was found. Pelvis ultrasonographic examination showed postnatal bulky uterus with slightly gapping cavity. Note was made of few gas filled dilated gut loop especially in left abdomen with thickened wall and decreased peristalsis at places. X-ray correlation is advised to rule out intestinal obstruction

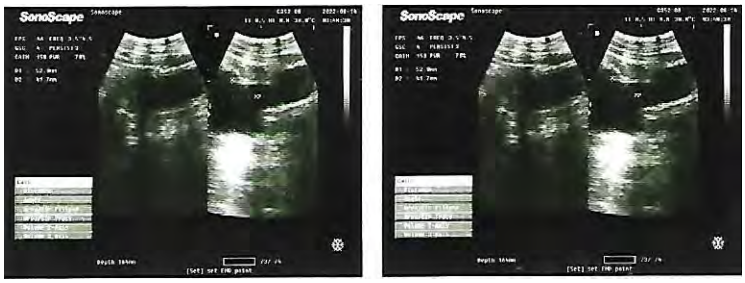

Figure 4.43: The pelvic ultrasonographic examination report of a 24 years primary gravida woman, who was not conceiving. She was positive for Toxoplasmosis by LAT, ICT and IgG ELISA assay.

The uterus was normal in size, shape and echo-pattern. No product of conception was seen in it. Both ovaries were normal in size and shape. The Endometrial thickness was normal. A fluid filled cyst was seen in uterine cavity, which was measured 4.21x 5.1cm

DISCUSSION

5.1 Geographical, seasonal and annual assessment of emerging zoonotic Brucellosis in women using geographical information system (GIS) in various geographical regions of Khyber Pakhtunkhwa province of Pakistan.

Brucellosis is a common infectious zoonotic disease, which was globally eradicated from many developed countries, but it is still a common zoonotic problem in various developing countries, where annually more than 500,000 new cases of human infection are reported (El-Sayed and Awad, 2018). The disease has been reported in 86 countries of the world. It is a serious threat for both livestock and human, cause economic losses and is associated with high morbidity in animal and human (Tadesse, 2016). It is a serious issue of those people, who live in low income countries, where poor families depend on livestock animals for their earning. (Mc Dermott *et al.,* 2013). Brucellosis is widespread in animals and human in Asian, South Asian countries like China, Sri Lanka, India and Pakistan (Norman *et al.,* 2016; Chen *et al.,* 2016). The current study adds important, updated and comprehensive information related to prevalence, geographical distribution, seasonal and annual assessment of zoonotic Brucellosis infection in women in various divisions (Table.4.2) and districts (Table.4.3) in Khyber Pakhtunkhwa province of Pakistan (Figure. 3.1). The results of current infected cases of women clearly indicate that zoonotic infection of Brucellosis persist in various species of livestock animals in different regions of the country, which clearly indicate that Pakistan is not only an endemic zone for animal Brucellosis, but risk factors also persist for transmission of infection to human community in the region. Gwida *et al.,* (2011) also claimed that risk factors exist for transmission of zoonotic Brucellosis to human in Pakistan, which support results of our current study. Bamaiyi *et al.,* (2015) stated that regional variations may occur in risk factors, but most of these risk factors are similar. Direct contact with infected animals, their products, consuming unpasteurized dairy products play important role in transmission of disease from infected animals to humans. Infected livestock animals are main reservoir and risk factor for human Brucellosis, while infection in various livestock animals have been previously reported by different researcher like Hussain *et al.,* (2008) in cattle, buffalo and human, Hamidullah *et al.,* (2009) in animals in district Kohat, Wadood *et al.,* (2009) in horses from Faisalabad regions, Akhtar *et al.,* (2010) in bovine hosts, Abubakar *et al.,* (2010) from Punjab province, Shafee *et al.,* (2011) in organized dairy farms from Quetta, Baluchistan, Ali *et al.,* (2013) from Rawalpindi and Attock regions of Punjab province, Bakhtullah *et al.,* (2014) in cattle from southern regions of Khyber Pakhtunkhwa and Safirullah *et al.,* (2014) in horses and donkeys from various regions in district Peshawar, Pakistan. These available published literatures on Brucellosis in various species of livestock animals indicate that persistent infection in animal in various regions of Pakistan is main source for zoonotic infection. Risk of human zoonotic infection increase, because

infected animals do not show any prominent clinical symptoms like human, due to which it is easily transmitted to human either by direct animal contact during milking, caring or consuming unpasteurized dairy product. Improper cooked meat, handling meat, direct contact with infected animal, working in contaminated environment and dust are other sources of infection (Christopher *et al.,* 2010), which are commonly seen in our rural community. During the current study, prevalence of zoonotic Brucellosis was different in different geographical division and district of Khyber Pakhtunkhwa province (Table.4.3), which could be due to difference in lifestyle, eating habits, cultural, socio-economic, climatic condition, environmental hygiene, socio-demographics, patient occupation and duration of occupational exposure. Other possible factor could be regional variations like agricultural, semi-agricultural and animal raising regions, while variation in prevalence of animal Brucellosis in different livestock animals could also be a possible factor for variation in human infection in different regions of Khyber Pakhtunkhwa province of Pakistan. Franc *et al.,* (2018) stated that prevalence of zoonotic diseases depends upon various factors like regional geography, method of food cooking and animal husbandry practices, while Gwida *et al.,* (2010) and Dean *et al.,* (2012), stated that number of human infection is directly correlated with number of infected animals within a particular geographical region in endemic countries. Unfortunately, there is no official and published data on incidence and prevalence of Brucellosis in various livestock animal in different geographical regions of Pakistan (Naeem *et al.,* 1990). Abubakar *et al.,* (2010) claimed that incidence of animal Brucellosis is increasing in various dairy farms of Government and private sector in different provinces and districts of Pakistan. Roushan and Ebrahimpour, (2015) stated that infection of Brucellosis is transmitted within animal herd by ingestion of contaminated material like feed and water. Mukhtar and Kokab (2008) reported human Brucellosis from various regions in Lahore and claimed that it is a serious public health problem in Pakistan. According to FAO, (2003), geographical distribution of Brucellosis is strongly correlated with those regions, where human community depends on livestock animals for food and source of revenue. Like other countries, human community reared livestock in the form of small and large ruminants in Pakistan, which contribute to their livelihoods. About 8 million families are involved in rearing of livestock animals and derived above 35% revenue from activates of livestock production in Pakistan (GoP, 2016). A large number of households are involved in rearing of livestock animals in various districts of Khyber Pakhtunkhwa province of Pakistan, which makes a significant contribution to their livelihood (Khan *et al.,* 2009; FAO, 2015). Those people, who live in various districts depend on various animal farming systems. They keep animals for food, use their manure as fertilizer for agriculture and live with animals in close proximity. Hasanjani *et al.,* (2015) claimed that contact with infected animals is a common source of zoonotic Brucellosis. Deshmukh *et al.,* (2015) stated that human infection is usually acquired by respiratory, oral and conjuctival routes during handling of

infected animals in occupational group of people. Mukhtar and Kokab, (2008) claimed that duration of occupational exposure is an important factor in transmission of zoonotic Brucellosis in those persons, who belong to high risk occupational group and sero-positivity increased with increased duration of animal exposure. Aworh *et al.,* (2013) reported a high positivity in those people, who had worked for above 5 years in high risk regions in Nigeria. Dean *et al.,* (2012) stated that frequency of human Brucellosis varies in various countries and within a particular region in endemic country due to different occupational, demographic and socioeconomic factors, while study biases could be also not ruled out. Ayoola *et al.,* (2017) stated that lack of education and proper awareness could also be a key factor, which play important role in difference of disease prevalence among high risk individuals in endemic regions. Limited literature is available on actual prevalence, incidence and geographical distribution of Brucellosis in Pakistan, which cause various epidemiological challenges in prevention of zoonotic infection. According to Scacchia *et al.,* (2013), comprehensive knowledge about prevalence and incidence of Brucellosis is importance in order to design strategy for effective control and eradication of disease. Unfortunately, there is no well-established animal and public health system in Pakistan, which investigates and annually monitors Brucellosis in livestock animals and divide various geographical regions into endemic, sporadic, epidemic, severe epidemic and disease free regions that would prevent chances of zoonotic infection. No geographical units have been designed by GIS and remote senescing technology in Pakistan, which regularly report and monitor these communicable diseases. No official data exist on human and animal Brucellosis at regional and national level due to weak and nonfunctional animal and public health system in Pakistan, which underestimate actual burden of zoonotic Brucellosis in various geographical regions of Pakistan. According to Dean *et al.,* (2012), aggregated data at regional and national levels do not capture complexities of disease dynamics. Ducrotoy *et al.,* (2015) stated that some species of Brucella are bound to specific host, but their pathogen and host relationship is still not exclusive. The growing population of human leads to intensive animal breeding of mixed livestock farming strategy that facilitates cross species infection. Hegazy *et al.,* (2016) stated that infection propagate to human due to handling animals and consuming bovine products. According to Plumb *et al.,* (2013), human infection is mostly dependent on infected animal reservoir and burden of disease appears more among pastoral communities. Norman *et al.,* (2016) stated that it is important to control infection in livestock for prevention of zoonotic infection, because Brucellosis is strongly linked to management of livestock and ingestion of animal products. During the current study, assessment of seasonal variations of Brucellosis was carried out and a significant difference (<0.05) was found in frequency of zoonotic infection during various seasons in the studied province of Khyber Pakhtunkhwa (Table.4.4 and 4.5). Prevalence of Brucellosis was significantly high in summer season, which was followed by autumn,

spring and winter seasons (Table: 4.4). A significantly (<0.05) high rate of zoonotic infection of Brucellosis in women in summer and autumn could be due to more animal exposure and involvement of occupational women in summer and autumn as compared to other seasons. Summer season start from the month of May to September in Pakistan, which is a lengthy period as compared to winter season, which start from the month of November to February, while spring season remain from the month of March to April. Due to hot weather in summer season in the region, people in rural communities use more dairy products in the form of soft drink, while people of urban regions frequently travel from hot metropolitan regions to some cool hilly regions, where they stay in hotels and usually exposed to various local dairy products. Moreover, there is no check on milk quality in urban regions. Milkman sale unpasteurized pool milk to restaurant and food shops. In published literature, there are controversial views about seasonal variation of Brucellosis infection in various countries. According to Shang *et al.,* (2002), Brucellosis may occur in any season of a year, but epidemic peak commonly occurred from the month of July and is strongly related to those months, which are associated with deliveries or abortion in livestock animals. Salari *et al.,* (2003) also reported a high rate (39.5%) of human Brucellosis in summer season in Iran. Memish *et al.,* (2000) stated that highest incidence of Brucellosis associated bacteremia occurs in spring and summer seasons due to consumption of more dairy products and frequently travel to animal raising regions for spending summer vocation, while lowest number of positive cases occurs in winter season due to change of food habit in cool weather. Sasan *et al.,* (2012) stated that 54% cases of Brucellosis occurred from the month of March to June in Greece, 71% from the month of June to September in Israel, while from April to August in Iran. Bosilkovski *et al.,* (2015) stated that human acquired Brucellosis through direct animal contact from the month of December to May in Macedonia, which is an intensive time for involvement in various animals activates. Boukary *et al.,*(2014) stated that risk of acquiring zoonotic infection from animals depend on various local factors like change in climatic condition, social behavior, eating habits and animal husbandry practices. Gul and Khan, (2007) stated that infection of Brucellosis reach at peak between April and June in Italy, which was associated with production and consumption of fresh cheese. During the current study, annual prevalence of zoonotic Brucellosis was monitored during three years (2017-2019) study (Table 4.6), but no significant difference (>0.05) was observed during annual frequency of Brucellosis, which might be due to sampling bias, highly variable (5 days to 6 months) incubation period of Brucellosis (CDC, 2017) and different clinical stages of disease like acute (≤8 weeks), sub-acute (8-52 weeks) and chronic (≥1 year) cases (Table: 4.17) with non-specific clinical symptoms, due to which Brucellosis infection may mimic with many other diseases and usually escape during diagnoses, which lead to chronicity of infection (Dossey, 2010). According to Holt *et al.,* (2011), annual incidence of human Brucellosis is more than 500,000 cases in the world, while regional incidence

is >200 per 100,000 cases. Franc *et al.,* (2018) stated that numbers of infected cases may be higher than annually reported cases in various developing countries in the world. Due to neglected nature of Brucella infection, about 2.4 billion people are at risk of infection in developing countries in the world. Due to intracellular nature, nonspecific symptoms, variable incubation period, various clinical stages, nonspecific use of antibiotic and non-specific agglutination of bacteria, Brucellosis usually become a diagnostic puzzle and progress to chronic infection, which generate many challenges for prompt diagnosis and treatment (Traxler *et al.,* 2013). Keeping in views these real facts, Brucella specific serological diagnostic assays like SPAT, RBPT, ELISA and genus specific PCR was used for detection, accurate diagnosis and confirmation of zoonotic infection of Brucellosis in women (Table:4.9). SPAT and RBPT assays significantly (<0.05) detected Brucellosis in clinically suspected women, while ELISA and PCR significantly (<0.05) confirmed Brucellosis in infected women (Figure 4.11, 4.12). A significant (<0.05) difference was found in positivity of Brucella specific IgM and IgG antibodies in infected women (Table: 4.11). Due to limited resources, SPAT and RBPT was used as initial screening assays, while ELISA and PCR were used as confirmatory tests for proper diagnosis and confirmation of Brucellosis (Figure: 4.10). During the current study, sensitivity, specificity, negative and positive predictive values of SPAT, RBPT and ELISA was determined, which was significantly (<0.05) reliable in diagnosis of Brucellosis infection (Table: 4.9). Febrile antigens of *B. abortus* and *B. melitensis* was used for detection of Brucella specific antibodies and its titer, in which 240 (31.78%) sera showed antibodies for *Br. abortus,* 174 (23.04%) for *Br. melitensis* and 341 (45.16%) for mixed antibodies i.e *Br. abortus* and *Br. melitensis* (Table: 4.10). A high degree of antigen-antibodies agglutination (100% and 75%) was shown by sera of 191 (25.29%) and 269 (35.63%) women, while low degree of agglutination (50%, 25% and <25%) was shown by sera of 195 (25.82%), 57 (7.55%) and 43 (5.69%) women (Figure: 4.14). PCR is a sensitive and specific method for diagnosis of Brucellosis. Brucella genus specific PCR was used in 269 clinical samples, which were positive on SPAT, RBPT, but either negative or showed equivocal results on IgM or IgG ELISA. PCR detected DNA of genus Brucella in 61 (22.67%) patients (Figure; 4.11 and 4.12). Unfortunately, we were unable to further screened and confirm all ELISA positive samples by PCR due to limited available resources. Bacteriological, serological and molecular methods are usually used for diagnosis of Brucellosis, while bacteriological method is a gold standard diagnostic method (Sathyanarayanan *et al.,* 2011), but we preferred serological methods like SPAT, RBPT, STAT and ELISA for diagnosis, because bacteriological method is not only time consuming, but also required BSL-3 protective laboratory for isolation and identification of Brucella, which is only available in some reference diagnostic laboratories in Pakistan. Serological assays like ELISA measures total quantity of IgM and IgG antibodies, while titer

of SAT ≥1:160 show positivity of SAT assay and used as a reference range for diagnosis of human Brucellosis (Unuvar *et al.,* 2019).

5.2 Geographical, seasonal and annual assessment of emerging zoonotic Toxoplasmosis in women using geographical information system (GIS) in various geographical regions of Khyber Pakhtunkhwa province of Pakistan.

Many emerging human infections are zoonotic. Toxoplasmosis is one of a zoonotic parasitic infection, caused by a protozoan parasite *Toxoplasma gondii*, which is geographically distributed in various regions of the world (Saberi *et al.,* 2018). The recent patterns of human-driven changes in environment and globalization of trade and travels has enhanced spillover and spillback of *T. gondii* parasites into human community and facilitating its further propagation to local, regional and global communities of human (Gortazar *et al*. 2014; Suzan *et al.* 2015). In Pakistan, large number of human population belong to low socioeconomic status, due to which women are usually more exposed to many infections (Mujtaba *et al.,* 2016). The current study is the first one, which was conducted on geographical distribution of zoonotic Toxoplasmosis infection in those women, who used to visit clinician in hospitals and different health care centers located in twenty four districts of Khyber Pakhtunkhwa province of Pakistan (Figure:3.1), which highlighted important and comprehensive information related to prevalence, annual and seasonal prevalence of zoonotic infection of Toxoplasmosis in women of Khyber Pakhtunkhwa province of Pakistan (Table: 4.3). The current results clearly indicated that zoonotic *T. gondii* parasite persist in different pet and food animal in these regions, because infected animals are main reservoir and risk for human infection. The disease in different livestock and pet animal species have been previously reported by several researcher in Pakistan in published literature like Ahmad and Tasawar, (2015), Perveen and Shah, (2015), Ahmad *et al.,* (2015), Ahmed *et al.,* (2016), Ahmad, and Tasawar, (2016), Khan *et al.,* (2018), while Ajmal *et al.,* (2013) detected *T. gondii* in different environmental materials like soil, drinking water, vegetable and local fruit. Similarly Mahmood *et al.,* (2014) reported high prevalence of *T. gondii* in uncaged (20.70%) chicken and low prevalence in caged (5.90 %) chickens, commonly intended for food from district Mardan region of Khyber Pakhtunkhwa province of Pakistan. They claimed that high prevalence of *T. gondii* in free ranged chickens indicates environmental and soil contamination with oocysts of *T. gondii*, because free ranged chickens are infected by feeding from ground or soil contaminated with oocysts of *T. gondii*. Ayaz *et al.,* (2011) and Khan *et al.,* (2013) detected *T. gondii* parasite in different sources of drinking water in many districts of Khyber Pakhtunkhwa province. Anvari *et al.,* (2018) reported a high prevalence of *T. gondii* in cattle meat, which was imported from Pakistan, while a low prevalence of infection in indigenous livestock meat in South East of Iran. Beside animals, Toxoplasmosis has also been reported by Faisal *et al.,* (2014), Majid *et al.,* (2016), Jan *et al.,* (2018), Zeb

et al., (2018), Aleem *et al.,* (2018) and Sadiqui *et al.,* (2018) in women from various other regions in Khyber Pakhtunkhwa province of Pakistan, which not only support results of our current study (Table:3), but also indicate that Pakistan is not only an endemic zone for zoonotic infection of Toxoplasmosis, but risk factors also persist for transmission of parasite to human. Jones *et al.,* (2001) and Xiao *et al.,* (2010) stated that prevalence of Toxoplasmosis in animals and human is used as indicator for widespread prevalence of a pathogen in a particular geographical region in the world. Majid *et al.,* (2016) reported various risk factors associated with disease transmission like close contact with pet dogs, cats, other livestock animals, consumption of unpasteurized milk, dairy products, undercooked meat, raw eggs, vegetables, water sources and residence in rural area, which were significantly (<0.05) associated with human infection in various districts of Malakand division in Pakistan, which support our current results (Table: 4.3). Chaudhary *et al.,* (2006) stated that *T. gondii* create a significant public health problems in Pakistan due to close contact between humans and various livestock animals. During the current study, an overall prevalence of Toxoplasmosis was recorded 24.56% in women of the studied regions (Table:4.1), while a significant variation (<0.05) was found in frequency of infection in women, who belonged to various divisions and districts of Khyber Pakhtunkhwa province (Table:4.3), which could be due to variations in various epidemiological parameters such as prevalence of disease in food animals in different geographical regions, sample size and convenient method of sample collection, socioeconomic status, level of education, knowledge level regarding Toxoplasmosis, eating habits, consumption of raw meat, unwashed fruits, vegetables or farming as an occupation, general and reproductive health of patients, immunological status, obstetric statues, age and residential statues (urban, rural) of patients. In rural area, people are usually living in houses, which are made of local mud, where sanitation system is very poor and people are also not educated and aware about zoonotic diseases. Moreover, people depend on various species of livestock animals for their revenue and frequently remain in close contact with animals. They usually drink unpasteurized animal milk and various other dairy products, which may contain tachyzoite stage of parasite. They drink un-boiled and non-filter water and eat half fried chicken eggs of infected chicken. Mahmood, *et al.,* (2014) stated that about 30-35 million people in rural regions are engaged in livestock farming in Pakistan. The current result on variations in prevalence of Toxoplasmosis in women in various geographical regions (Table; 4.3) is also supported by studies of many local researcher of Pakistan. Shah *et al.,* (2015), Faisal *et al.,* (2014), Majid *et al.,* (2016), Jan *et al.,* (2018), Zeb *et al.,* (2018), Aleem *et al.,* (2018) and Sadiqui *et al.,* (2018) also reported Toxoplasmosis with a prevalence of 28.44%, 19.25%, 65.71%, 21%, 2.5%, 47.2% and 24.8% cases from district Mardan, district Swabi, Malakand division, district Charsadda, district Peshawar, district Swat and Abbottabad, Mansehra district, while Shah *et al.,* (2015) reported 20% positive cases of human infection from other

selected regions of Khyber Pakhtunkhwa province of Pakistan, which strongly support results of our current study (Table: 4.3). Faisal *et al.,* (2014) also reported intra-regional variations in prevalence of Toxoplasmosis with a rate of 26% and 10% in Gohati and Dagi regions of district Swabi regions of the studied province (Figure: 3.1) and claimed that prevalence of Toxoplasmosis was high in those women, who were of young age group. Majid *et al.,* (2016) reported a high prevalence 33.03% of chronic infection of Toxoplasmosis from district upper Dir, while low prevalence in district Lower Dir and Swat in Malakand division of Khyber Pakhtunkhwa, which strongly support results of our current study (Table:4.3). Similarly Sadiqui *et al.,* (2018) also reported significant (<0.05) variations in prevalence of zoonotic Toxoplasmosis in women from Abbottabad and Mansehra district of Hazara division, which strongly support our current results (Figure; 4.5). Khan *et al.,* (2011) claimed that regional variations occur in seroprevalence of Toxoplasmosis among pregnant women with a range of 63% positive cases from Punjab, followed by 48.00%, 19.25% and 14.4% cases from Azad Kashmir, district Sawabi and district Kohat in Khyber Pakhtunkhwa province of Pakistan. Ali, (2016) stated that environment play important role in cross species transmission and regional variations in seroprevalence of *T-gondii* in Pakistan. The presence of large number of pet cats in homes in various regions could not be ignored, as these pet animals are important sources and reservoir for zoonotic Toxoplasmosis infection due to close relationship of these animals with human communities. Ahmad *et al.,* (2014) reported Toxoplasmosis with a prevalence of 26.43% and 28.43% in cats and dogs brought to different veterinary hospitals and private pet clinics in northern sub-tropical arid region of Pakistan. They observed that cats and dogs in rural regions were more infected with *T. gondii* as compared to urban regions. Ahmad and Qayyum, (2014) found that presence of cats in vicinity, poor hygienic conditions and extensive animal management systems is responsible for widespread prevalence of Toxoplasmosis infection in animal and human in Pakistan. They further stated that cattle and buffaloes are important source for milk and meat in the country and risk of infection is high due to lack of modern animal farming system in Pakistan. The current finding on regional variations in seroprevalence of Toxoplasmosis (Table: 4.3) is also supported by studies of many investigators from other geographical regions of the world. Subauste *et al.,* (2011), Zemene *et al.,* (2012), Flegr *et al.,* (2014) Tonouhewa *et al.,* (2017) and Maçin *et al.,* (2018) claimed that due to asymptomatic nature of *T. gondii* infection, it is difficult to determine exact source of infection, while human hygiene, nutritional habits, geographical regions, climate and cultural changes cause a significant (<0.05) variation in seroprevalence of Toxoplasmosis in various countries, geographical regions and even within a particular area in a region. During the current study, assessment for seasonal (Table: 4.5) and annual variations (Table: 4.6) was carried out in prevalence of Toxoplasmosis in women, but no significant (>0.05) difference was found in seasonal and annual variations in prevalence of

Toxoplasmosis (Table: 4.5 and 4.6). The reason could be due to sampling errors or persistent asymptomatic chronic infection of *T. gondii* in women in the regions. The clinician could not differentiate early, active, latent, chronic, congenital or reactivated infection of *T gondii* from serological assays like LAT, ICT and ELISA, which are based on qualitative and quantitative detection of various immunoglobulin's like (IgM) and (IgG), which cannot accurately differentiate in acute, chronic and reactivated infection in an individuals. Contreras, (2018) and Thangarajah *et al.,* (2019) also support our current views and stated that it always remained a diagnostic challenge for clinicians to differentiate acute infection from chronically infected cases by serological assessment of infected patients. Liu *et al.,* (2012) and Grzybowski, *et al.,* (2015) suggested that there is an urgent need to develop specific molecular markers for differentiation of acute stage of clinical infection from chronic stages of Toxoplasmosis. The asymptomatic and chronic nature of *T. gondii* infection causes various diagnostic, therapeutic and preventive challenges for health authorities in all over the world. During the current study, Toxoplasma specific LAT and ICT assays were used for initial screening, while STAT, IgM and IgG ELISA was used for confirmation of diagnosis in suspected women (Table:4.13), which was based on quantitative detection of *T. gondii* specific IgM and IgG antibodies in sera of suspected women. LAT, ICT and ELISA assays significantly (<0.05) detected *T. gondii* specific antibodies in suspected women (Table: 4.13). A significant (<0.05) difference was found between ICT and ELISA assays (Table: 4.14). ICT assay significantly (<0.05) detected *T. gondii* specific IgM and IgG antibodies in 915 (25.51%) women, while STAT and ELISA assays significantly (<0.05) confirmed true infection in 881 (24.56%) women (Table.4.15 and 4.16). No significant (>0.05) difference was found between diagnostic efficacy of LAT and ICT assay (Table: 4.13). The sensitivity, specificity, PPV and NPV of LAT and ICT assay was not significantly (>0.05) different. These assays were significantly (<0.05) effective for initial diagnosis of *T. gondii* infection in women, while ELISA significantly (<0.05) detected and confirmed true positive cases (Table: 4.13). A significant (<0.05) difference was observed in IgM and IgG antibodies. Toxoplasma specific IgM and IgG antibodies were detected in 96 (8.03) and 682 (57.07%) infected women, while IgM and IgG were detected in 103 (8.62%) infected cases (Table: 4.15). LAT and ICT are economical serological assays, which are commonly available and affordable in developing countries like Pakistan and frequently used as initial diagnostic assay for *T. gondii* infection in clinically suspected cases. During the current study, ELISA was used for confirmation of infection only, which significantly (<0.05) confirmed *T. gondii* infection in clinically suspected women (Table: 4.15). Tekkesin *et al,* (2011), Zhang *et al.,* (2016) and Hajissa *et al.,* (2017) also claimed that due to asymptomatic nature, various serological assays are primary, cheap and easy approach for achieving satisfactory diagnostic outcomes, which play

important role in management of human infection, but due to high sensitivities and specificities, clinicians usually relay and accept results of ELISA assay.

5.3 Molecular epidemiology and adverse obstetric outcomes of emerging zoonotic Brucellosis in women, attending maternity and health care centers in various geographical regions of Khyber Pakhtunkhwa province of Pakistan.

Brucellosis is one of the most common zoonotic disease, which is worldwide in distribution. More than 50,000 cases of human infection are annually reported from various geographical regions of the world (Ashenafi *et al.,* 2016), while, approximately 2.4 billion people are at risk for acquiring infection (Franc *et al.,* 2018). Brucellosis is usually transmitted to humans either by direct contacts with infected animals or through consumption of unpasteurized dairy products of infected animals (Ashenafi *et al.,* 2016). Globally, major endemic regions are Mediterranean countries, Middle East, Sub-Saharan Africa and Central Asia, China and India, while prevalence of infection depends upon various factors, which include regional geography, techniques of food cooking and animal husbandry practices (Franc *et al.,* 2018). During the current study, 718 (20.02%) women were found with Brucellosis (Table: 1). Due to zoonotic nature of Brucella, human infection is mainly dependent on infection in animal reservoir. According to Hegazy *et al.,* (2016), animal's infection is directly responsible for propagation of disease in human, but infected animals do not show any prominent clinical symptoms, which increase burden of zoonotic Brucellosis in women community, who live in close proximity with their livestock animals in various geographical endemic regions of the world. Brucellosis in various livestock animals have been reported by Nasir *et al.,* (2004), Hussain *et al.,* (2008), Wadood *et al.,* (2009), Hamidullah *et al.,* (2009), Abubakar *et al.,* (2010), Akhtar *et al.,* (2010), Shafee *et al.,* (2011), Ali *et al.,* (2013), Bakhtullah *et al.,* (2014), Safirullah *et al.,* (2014), Ali *et al.,* (2015), Ali *et al.,* (2017) and Yousaf *et al.,*(2017), while Hussain *et al*., (2008), Mukhtar and Kokab (2008), Ali *et al.,* (2013), Din *et al.,* (2013), Shahid *et al.,* (2014), Ali *et al.,* (2016) and Ali *et al.,* (2018) reported Brucellosis infection in human with a prevalence of 14%, 21.7%, 6.9%, 9.33%, 30.5%, 5.82% and 16% cases from various regions of Pakistan, which strongly support our current results (Table:1) and also indicate that Pakistan is not only an endemic zone for Brucellosis, but risk factors also exist for human infection. Gwida *et al.,* (2011) also stated that human Brucellosis and associated risk factors exist in Pakistan, while Mukhtar and Kokab (2008) reported that Brucellosis is a serious public health problem in Pakistan. Various socio-demographic (Table: 4.7) and potential epidemiological parameters (Table: 4.8) were evaluated for prevalence of emerging Brucellosis in women. Among socio-demographic characteristics, prevalence of Brucellosis was significantly (<0.05) high in 26.99%, 22.44%, 21.22%, 20.51%, 21.52%, 21.17%, 21.53% and 20.56% women, who were in the age group of 17-26 years, resident of rural area, lower educated, illiterate, house wife, low socio-

economic status, bad hygienic and who had no knowledge about zoonotic Brucellosis (Figure: 4.11). Among potential epidemiological variables, prevalence of zoonotic Brucellosis was significantly (<0.05) high in 22.99%, 21.47%, 21.57%, 22.48%, 22.97%, 20.51%, 20.18% and 22.91% women, who had animal at homes, history of animal exposure, history of handling animals, no hand washing habit, history of processing dairy products, positive family history of Brucellosis, drinking non-filtered water and those women, who used to stored animals' manure in homes for agriculture purposes (Table:4.8). The current results revealed that many socio-demographic characteristics (Table: 4.7) are significantly (<0.05) associated with transmission of zoonotic Brucellosis in the region, which should be parallel investigated with potential epidemiological parameters (Table: 4.8) for proper assessment of disease transmission in infected women. According to Njeru *et al.,* (2016), risk for recurrence and transmission of Brucella infection is evident due to co-existence of animal husbandry and social-cultural activities in a particular geographical endemic region, which promote transmission of zoonotic infections. Dean *et al.,* (2012) also support our current results (Table: 4.8) and stated that demographical, occupational and socioeconomic factors play important role in seroprevalence variations and transmission of zoonotic diseases within a particular endemic region. Hasanjani and Ebrahimpour, (2015) stated that beside animal contact and consumption of dairy products, environmental hygiene, climatic situation and socio-economic conditions are main factors, which play important role in transmission of zoonotic Brucellosis in endemic region. Prevalence of Brucellosis was significantly (<0.05) high in those women, who were 17-26 years old, resident of rural areas, illiterate, low educated, house-wife, low socio-economic statues, bad hygienic, lack of knowledge, animals keeper and handlers, handling animals meat, milk and dairy products at home, positive family history and those women, who used to store animals manure in homes for agriculture purposes (Table: 4.7 and 4.8)**.** Brucellosis affect women of all age group, but during the current study, prevalence of infection was significantly (<0.05) high in those women, who were 17-26 years old, which could be due to more involvement of young women in animal care and management as compared to other age groups, because they are main labor force in family and society and have more chances of access to livestock animals. According to Mirnejad *et al.,* (2013), Brucellosis affects men and women of all age groups. Zeinalian *et al.,* (2012) also stated that Brucellosis is prevalent in all age groups, which depend upon chance of contact with infected animals. Most families in rural communities of Khyber Pakhtunkhwa (Figure:3.1) belong to low socio-economic status and do not educate women due to various factors like cultural restriction, early marriages in young age, poverty, lack of educational and health facilities. Women are restricted to homes, where they work as house-wives and directly engaged in various activates in homes including rearing of livestock animals in their congested homes, due to which they are at risk of acquiring various zoonotic diseases. Assenga *et al.,* (2016) stated that non-educated

individuals are usually at higher risk for acquiring zoonotic Brucellosis than educated individuals and need prompt health education. Lack of knowledge about zoonotic Brucellosis was found a significant (<0.05) risk factor of acquiring infection (Table: 4.7), which indicates that burden of zoonotic diseases can reduced through creation of proper awareness about zoonotic diseases in human community in a particular region. Arif *et al.,* (2017) determined level of knowledge and understanding about zoonotic Brucellosis in smallholder dairy farmers in Pakistan. They found that 97% farmers were not aware about transmission of Brucellosis, 66% farmer's families were consuming raw milk and other dairy products, 49% were living in sheared houses with animals and 74% had not covered wounds on hands during contact with animals. They found poor understanding, presence of multiple risk factors in farm or household and observed incorrect perception about Brucellosis in smallholder dairy farmers. According to Arif *et al.,* (2017), majority of animal keepers are not aware about risk of zoonotic diseases, which are transmitted from domestic livestock animals. Those people, who have awareness about Brucellosis in animals, did not think that they could get the same infection from their animals. Arif *et al.,* (2017) further stated that poor knowledge about zoonotic diseases and poor animal practices poses a high risk of disease transmission in rural communities of Pakistan, where pasteurization of milk is not a common practice at smallholder level. They stressed proper health education for an increased awareness about Brucellosis and other zoonotic diseases, which is feasible and economical preventive measure of zoonotic infections in low income countries like Pakistan, where it is not economically possible to test animals before slaughter. They suggested education of "one health" approach in rural communities in order to ensure prevention of Brucellosis and various other zoonotic diseases in Pakistan Njuguna *et al.,* (2017) stated that it is important to determine knowledge level of human population on zoonotic Brucellosis in endemic regions, which will guide public and animal health authorities for designing and implementation of control measures for zoonotic diseases. According to Al-Ameen *et al.,* (2016), it is important to raise awareness about zoonotic disease in public to reduce and eradicate zoonotic infections in various endemic regions. Ayoola *et al.,* (2017) also reported that lack of awareness is a key factor of acquiring zoonotic Brucellosis. Scacchia *et al.,* (2013) stated that knowledge about prevalence of zoonotic Brucellosis in an endemic country is more importance in order to plain strategies for control and eradication of disease. Adesokan *et al.,* (2013) also stated that poor knowledge about mode of transmission of zoonotic Brucellosis among humans in rural regions appeared to be an important risk factor that facilitates infection in human. According to Kant *et al.,* (2018), spread of zoonotic diseases can be reduced by changing animal practices and adopting different safety measures. Al-Arnoot *et al.,* (2017) suggested that it is important to launch an education program for proper awareness about zoonotic diseases and different associated risk factors, which may also contain brochure regarding awareness about infections in human

community in various endemic regions of the world. Adesokan *et al.,* (2013) stated that poor knowledge about transmission of zoonotic Brucellosis among individuals, who belong to rural areas, is a major risk factor that facilitates zoonotic disease in human community in various endemic countries. Habtamu *et al.,* (2015) also support our results and stated that zoonotic Brucellosis is common in rural areas, because people live in close proximity with their livestock animals and frequently consume fresh and unpasteurized dairy products. According to Felipe *et al.,* (2017) and Tuon *et al.,* (2017), major source of zoonotic infection is direct contact with infected animals and indirect source is consumption of unpasteurized dairy products. Pandit and Pandit, (2013) stated that prevalence of zoonotic disease is directly proportion to infection in livestock animal and animal handlers are particularly more prone to these infections due to their occupation. Naz and Khan, (2018) stated that livestock animals are important source for economy of rural communities in Khyber Pakhtunkhwa province of Pakistan, while women play important role in rearing of these animals in homes for their livelihood. They remain directly engaged in animal care, feeding and milking. They also remain directly engage in milk processing for preparing different dairy products (Khan *et al.,* 2009; FAO, 2015). Most of these women have bad hygienic conditions, because they store animal manure in homes for agricultural purposes and also make dung cakes through pressing fresh animal manure by bare hand, dry dung cakes in sunlight and burn these dung cakes as fuel for cooking meal in homes. They also sold out these dung cakes in local markets and generate revenue for poor families. Dusting of animal's shelters, surrounding areas and preparation of dung cakes are significant (<0.05) risk factors of Brucellosis and other zoonotic infection in the region of Khyber Pakhtunkhwa (Figure: 3.1). According to Christopher *et al.,* (2010), zoonotic diseases are transmitted from infected animals by direct contact with contaminated area or through inhalation of animal feces, which dry off and become dust in environment. Bacteria of genus Brucella are resistant to different environmental condition and usually remain active for a long period of time. Infection in occupational individuals are usually acquired through respiratory, oral, and conjuctival routes due to handling animals, aborted fetuses or placenta of infected animals (Deshmukh *et al.,* 2015). According to Wang *et al.,* (2015), Bacteria in genus Brucella can remain alive in environment for several months, which depend on various environmental conditions such as pH, proper temperature and humidity. Roux, (1991) stated that Brucella survive in various environmental conditions outside the host body. Brucella survive in moist soil and animal dung for 70-80 days, while in air dust, it vary from 15-40 days, which depend on ambient humidity of air. Till, (2014) stated that Brucella organisms have ability to survive for about 11 weeks in aborted fetal materials and 3-4 weeks in animal milk or ice cream, while the organisms survive for several months in fresh cheese. Gül and Erdem, (2015) stated that Brucella is sensitive to heat, ionized radiation and disinfectants, while boiling and pasteurization of milk kill the organisms. Abubakar

et al., (2012) stated that Brucella is usually excreted in uterine discharges, animal manure, urine and milk of infected animals, which are rich sources of pathogen and a significant impact on human health and socio-economic impacts in rural communities of Pakistan, where rural income largely depend on livestock and dairy products. They claimed that incidence of zoonotic Brucellosis is increasing mainly in large dairy herds in private and government dairy farms located in various districts and provinces of Pakistan. Nasir *et al.,* (2004), Hussain *et al.,* (2008) and Shafee *et al.,* (2011) reported Brucellosis from private and government dairy farms located in various regions of Pakistan, which increase chance of human infection, because milk and dairy products of infected animals is a rich source for human infection. Beside rural community, people of urban regions are also at risk of acquiring Brucellosis (Table:4.7), because milk and other dairy food products of infected animals are sold at doors by unauthorized milkman on low price, which is a rich source of zoonotic infection for those people, who do not have any direct contact with infected livestock animals (Table:4.8). Wareth *et al.,* (2014) stated that milk distributing chains promote supply of unpasteurized pool milk and spread various type of zoonotic diseases in developing countries. Ali *et al.,* (2013) reported milk and other dairy food products contaminated with Brucella from various regions, which clearly indicated that people of rural and urban regions are at high risk for zoonotic infection of Brucellosis in Pakistan, because bacteria of genus Brucella survives in milk and dairy products and souring of milk does not destroy these pathogen as they are preserved in fat of milk. Although, formation of lactic acid has an inhibitory effect on some other pathogenic bacteria, but this cannot provide a safe and Brucella free milk products (Ducrotoy *et al.,* 2015). Due to contagious nature of bacteria in genus Brucella, a positive family history is important risk factor for Brucellosis, because it indicates that source of infection is same in infected family, which will draw attention of physician to screen other family member and identify unrecognized positive cases. Ciftdogan and Aslan, (2017) proposed that it is important to screen all family member, whenever a member in a family is found positive for zoonotic Brucellosis in endemic regions, because they shear same source of infection and associated risk factors, which will earlier identify other unrecognized positive family members, who will receive prompt treatment. Guler *et al.,* (2014) detected 22% cases of Brucellosis in those patients, who had positive family history of Brucellosis. They suggested that due to contagious nature of Brucellosis, other family members of infected patients must be investigated for Brucellosis. Blood transfusion is another possible risk factor of acquiring Brucellosis infection in endemic regions (Table: 4.8). During the current study, 8.67% women were found positive (>0.05) for zoonotic infection of Brucellosis, who had recent history of blood transfusion (Table: 4.8). Unfortunately, there is no screening program for Brucellosis in blood downer in hospital blood banks and regional blood banks (RBC) in Pakistan, while Brucellosis is endemic in the studied regions of Khyber Pakhtunkhwa province of Pakistan (Figure: 3.1),

which is a major risk factor for hospitalized and maternity patients, who need transfusion of blood. There is no published data on blood transfusion associated Brucellosis and other zoonotic infections in Khyber Pakhtunkhwa province (Figure: 3.1) and other regions of Pakistan. Rabbani *et al.,* (2008) reported Brucella specific antibodies in 0.07% blood donors in blood banks from our neighbor country Iran. They suggested that proper screening of blood is important for detection of Brucella antigen or antibodies in all blood banks and in those patients, who are regular recipient of blood due to various diseases. Wang *et al.,* (2015) screened 3896 blood donors and detected Brucella in 15 (0.39%) blood donors, who were positive for Brucella by PCR and DNA sequencing in China. They suggested that blood should be regularly screen for Brucella antibodies or antigen in hospital blood banks in endemic regions. Aydın *et al.,* (2013) stated that Brucellosis rarely occur through blood transfusion in human. Felipe *et al.,* (2017) also claimed that a low number of zoonotic Brucellosis cases occurred due to blood transfusion, which strongly support our current findings (Table: 4.8). Yavuz *et al.,* (2012) claimed that Brucellosis can transmit through blood transfusion in hospitals. They suggested that those blood donors, who come for blood donation from rural areas, should be regularly interviewed and screened for detection of Brucellosis in endemic regions. Gül and Erdem, (2015) stated that due to reported cases of zoonotic Brucellosis, which are related to transfusion of blood, it is important to ask about clinical symptoms of Brucellosis from blood donors and screen blood of donors for detection of Brucella antigen or antibodies. Various maternal factors such as patient's gravidity, obstetric statues and gestational stages of pregnancy were evaluated for prevalence of zoonotic Brucellosis (Table: 4.31). A significant (<0.05) difference was found in prevalence of Brucellosis infection in women of various maternal group. Prevalence of infection was significantly (<0.05) high in those women, who were multigravida (21.43%) and non-pregnant (24.31%) as compared to those women, who were primary gravid 12.06% and pregnant 9.92% (Table: 4.31). A significant (<0.05) difference was also found in prevalence of Brucellosis infection in pregnant women, who had various gestational stages. Among pregnant women, prevalence of zoonotic Brucellosis was significantly (<0.05) high 15.29% in those women, who had 2nd trimester of pregnancy as compared to 1st and 3rd trimester of pregnancy (Table: 4.31). During the current study, assessment of zoonotic Brucellosis was also carried out in those women, who were positive for history of various adverse obstetric outcomes (Table: 4.32). Brucellosis was found in women, who had history of previous spontaneous abortion, recent spontaneous abortion, multiple spontaneous abortion, medical induced abortion, preterm birth, stillbirth, congenital anomalies and those women, who were positive for pelvic inflammatory disease (PID) (Table:4.32). Zoonotic infection of Brucellosis has not only harmful effects on general health of women, but infection may also adversely effects reproductive life of women and cause many complications related to various aspects of reproductive well-being of women. Infection causes various obstetric complications

in pregnant women, but due to histological, biochemical and immunological variation between human and animal placenta, there are controversial reports about Brucellosis and its association with various adverse obstetric outcomes in women. Arenas-Gamboa *et al.,* (2016) stated that Brucellosis was previously recognized as animal reproductive disease and a flue like illness in women and had little role in maternal and newborn health, but recent findings and new evidences suggests that maternal infection of Brucellosis has a significant risk factor for various types of adverse obstetric outcomes such as increased risk of miscarriages during first or second trimester of gestation, premature deliveries and vertical transmission of pathogen to fetus. They farther stated that these adverse obstetric outcomes were clinically not associated with any specific symptoms. Al-Tawfiq and Memish, (2013) stated that zoonotic Brucellosis may induce abortions during pregnancy, but ratio of abortions in women is lower than animals due to absence of erythritol in placenta of women and fetus and the occurrence of various anti-brucella activates in amniotic fluids of women, which have protective role against many infection. According to Peker *et al.,* (2011), erythritol is a chemical substance, which is produced in placenta of animal and Bacteria of genus Brucella utilize erythritol for its growth, which is considered as a growth factor and a rich medium for growth of bacteria in genus Brucella. According to Barbier *et al.,* (2017), erythritol is a preferential carbon source for bacteria of genus Brucella, which is abundantly present in genital organs of ruminants, while human placenta lacks erythritol. They established infection of *B-abortus* in various animal models and observed that erythritol was frequently available, but not essential for multiplication of *B. abortus* in bovine trophoblasts cells. According to Karcaaltincaba *et al.,* (2010), an actual incidence of zoonotic Brucellosis infection in pregnant women is not clearly known in developing countries, due to which, there are controversial reports about zoonotic Brucellosis and its association with various types of adverse obstetric outcomes during pregnancy. Kurdoglu *et al.,* (2010) observed miscarriages with a prevalence of 24.14% cases in 342 pregnant women, who had Brucella infection. Ghanem-Zoubi *et al.,* (2018) reported existence of a strong and significant (<0.05) epidemiological association between incidence of Brucellosis and its association with major adverse obstetric outcomes in pregnant women. Vilchez *et al.,* (2015) stated that infection of zoonotic Brucellosis in pregnant women is a source of general and obstetric complications, while early diagnosis and management through specific therapeutic regimens reduce and prevent adverse effects on maternal and fetal life. Kurdoglu *et al.,* (2015) stated that Brucellosis is a risk factor for many obstetric complications in pregnant women. They claimed a high rate of spontaneous abortions as compared to preterm deliveries and intrauterine fetal death (IUFD) in infected pregnant women. They further stated that abortions were not associated with clinical stage and magnitude of agglutination titers. Carillo and Pappas, (2011) stated that incidence of spontaneous abortions varies from 7% to 45.6% cases in infected pregnant women.

Elshamy *et al.,* (2008) reported abortion, intrauterine fetal death and preterm labor in 27.27%, 12.72% and 10.90% cases of infected pregnant women. They stated that frequency of fetal loss was high in infected women. Duval *et al.,* (2014) reported spontaneous abortion and intra uterine fetal death (IUFD) during first trimesters of pregnancy among infected pregnant women. Peker *et al.,* (2011) presented a case of pregnant woman, who was 19 year old and she had 19-20 weeks gestation. Her blood culture was positive for Brucella and she spontaneously aborted during treatment of Brucellosis. Ghanem-Zoubi *et al.,* (2018) conducted a study on incidence of Brucellosis and its association with adverse obstetric outcomes in infected women in Israel. They observed intrauterine fetal demise (IUFD) as primary outcomes, while premature (less than 37 weeks) child birth, early or threatened labor and poor fetal growth were secondary adverse obstetric outcomes. APOs were expressed as events per 1,000 live or dead child births. They claimed that all adverse pregnancy outcomes (APOs) occurred more frequently and with a significant (<0.05) rate in high-incidence localities of Brucellosis. Elshamy *et al.,* (2008) reported incidence of abortion in 27.27%, IUFD in 12.72% and preterm labor in 10.90% women, who had active infection of Brucellosis. Vilchez *et al.,* (2015) stated that Brucella can cause adverse obstetric outcomes in the form of fetal and maternal/neonatal death. Rujeni and Mbanzamihigo, (2014) reported abortion and stillbirth in 25% women with Brucellosis. Criscuolo and Di Carlo, (1954) conducted a large series study on Brucellosis and its correlation with abortion in women. They reported abortion with a rate of 10% in 200 pregnant women, who were infected with *B. melitensis*. Baud *et al.,* (2009) found *B. abortus* antibodies in two women, who had history of miscarriages in London. Alsaifa *et al.,* (2018) reviewed Brucellosis associated consequences during pregnancy. They observed 20 (31%) cases of spontaneous abortions, 2 (3%) cases of IUFDs and 11 (17%) cases of congenital Brucella infection. Khan *et al.,* (2001) and Al-Tawfiq and Memish (2013) reported spontaneous abortions from 2.5-53% cases in pregnant women, who had Brucellosis. The incidence of spontaneous abortion was 43% in first and second trimesters, while IUFD was 2% in pregnant women in Saudi Arabia. Masallat *et al.,* (2013) reported spontaneous abortion in 20% and preterm deliveries in 9 (45%) women, who had Brucellosis. During the current study, a significant (<0.05) difference was found in positivity of Brucella specific IgMs and IgG immunoglobulin (Table: 4.11). Brucella specific immunoglobulin M (IgM) was prominent antibodies, which were detected in 23.52% infected women, while IgG were detected in 11.66% and IgM, IgG in 35.18% infected women (Table: 4.11). Abdullah *et al.,* (2017) also detected Brucella specific IgM in 13.82% and IgG in 5.95% pregnant women, who were positive for Brucellosis. Gül and Erdem, (2015) stated that cell mediated immunity has essential and primary role for controlling disease, while specific antibodies have a limited role in patient immune response, but these antibodies play important role in clinical diagnosis of infection. During first week of infection, IgM increase, which follow an increase in IgG antibodies in

second week. After 4th weeks, level of both IgM and IgG immunoglobulin rapidly decreases with a successful treatment, while level of IgG antibodies decrease faster than IgM with specific and effective treatment. After recovery from active infection, immunoglobulin M (IgM) can remain positive in low titers in serum for months or even years in infected individuals without any specific clinical symptom. A high level of IgA and IgG antibodies for longer than 6 months is a clinical sign of chronic infection or relapse of disease (Gül and Erdem, 2015). According to Solı ´s Garcı ´a del Pozo *et al.,* (2014), IgM antibodies are indicative of acute infection, but detection of IgM antibody in absence of IgGs create diagnostic challenges during interpretation of Brucella serology.

5.4 Epidemiology and adverse obstetric outcomes of emerging zoonotic Toxoplasmosis in women, attending hospitals, maternity and health care centers in various geographical regions of Khyber Pakhtunkhwa province of Pakistan.

Being a developing country, a large number of human population belong to low socioeconomic status in Pakistan, where women are mostly exposed to various zoonotic infection like Toxoplasmosis, while there is no proper screening programs for disease surveillance, understanding epidemiology, awareness and control of these infections. The current study was the first one, which was conducted on Toxoplasmosis in women patients, who were visiting maternity and health care center located in 7 division and 24 districts of Khyber Pakhtunkhwa province of Pakistan (Table:4.3). Prevalence of Toxoplasmosis was found in 24.56% cases in the form of acute, active or latent infection (Table:1), which clearly indicate presence of *T. gondii* in food animals and associated risk factors of parasite transmission to human in Pakistan. Infected food animals are reservoir and main risk factors for human infection. Pan *et al.,* (2017) stated that human infection increased with increased in incidence of *T. gondii* infection in food animals in endemic regions. Toxoplasmosis has been reported by Khan *et al.,* (2014), Faisal *et al.,* (2014), Shah *et al.,* (2015), Shah *et al.,* (2017), Majid *et al.,* (2016), Shah *et al.,* (2016) and Aleem *et al.,* (2018) with a prevalence of 65.71%, 19.25%, 20%, 12%, 18.41%, 24.7% and 47.2%, cases from various specific and limited regions located in Khyber Pakhtunkhwa province of Pakistan, while Tasawar *et al.,* (2012), Ahmad *et al.,* (2012) and Latif *et al.,* (2017) reported Toxoplasmosis with a prevalence of 29.45% 11.33% and 22% cases from various regions in Punjab province of Pakistan, which strongly support our current results (Figure: 4.5). Variation in seroprevalence of zoonotic Toxoplasmosis within human community in various geographical regions of Pakistan could be due to various socio-demographic, epidemiological factors and general health of infected individual. Various socio-demographic characteristics were evaluated for prevalence of emerging zoonotic Toxoplasmosis in women in various regions of Khyber Pakhtunkhwa province of Pakistan (Table; 4.7). Prevalence of infection was significantly (<0.05) high in those women, who were in age group of 27-36 year, resident of rural area,

non-educated, house-wife, low socio-economic statues, bad hygienic and in those women, who had no knowledge about zoonotic infection of Toxoplasmosis (Table: 4.7). Faisal *et al.,* (2014), Majid *et al.,* (2015) and Shah *et al.,* (2017) observed that these socio-demographic parameters are responsible for zoonotic infection of Toxoplasmosis in Pakistan. Mujtaba *et al.,* (2016) stated that a large number of human communities belong to low socioeconomic status in Pakistan, due to which, they are usually more exposed to infection. Shah *et al.,* (2016) reported high infection rate (28.6%) in those women, who were living in rural areas and (27.2%) positive cases in those women, who were raising livestock animals in homes in different regions in district Chitral region of Pakistan. Beside socio-demographic characteristics, various epidemiological factors were evaluated for zoonotic infection of Toxoplasmosis (Table: 4.8). A significantly (<0.05) high infection rate was found in those women, who had animals at homes, engaged in cleaning animal waste and shelters, handling animals meat at home for cocking, engaged in processing dairy products at homes, habits of eating raw vegetables, salad, unwashed fruits and in those women, who used to store animals manure in homes for agriculture purpose (Table: 4.8). Due to cultural restriction, most families in rural areas of our province do not educate women, who are restricted to homes. They work as house-wives and remained engaged in cooking meals, washing clothes and cleaning their homes. Due to poverty, a huge number of poor families are living in rural areas, as they cannot afford residence in urban regions. According to Naz and Khan, (2018), livestock animals are important for economy of people, who live in rural region and women play important role in rearing of these animals in rural communities of the province. They extensively reared various livestock animal species such as sheep, goat, cattle, buffaloes, donkey and poultries in their homes, which play a vital contribution to their livelihoods. These animals are usually reared by women and they remain engaged in their care, feeding, watering, cleaning, and milking. They also process milk at their homes for preparation of various local homemade dairy products along with other domestic chores (Khan *et al.,* 2009; FAO, 2015). According to FAO, (2015), livestock animals are main source of livelihoods, income and social protection of these women in rural community. Many women usually remained engaged in making dung cakes through pressing animal manure by bare hands. They dry animal dung cakes in open environment in sunlight and burn it as a fuel for cooking meal for families. They also sold out some proportion of dung cakes in local market to generate cash money for other needs in homes. Beside these activates, women store extra animal manure in homes on daily basis and use it as fertilizer for growing crops and vegetables. They also reared poultry in homes for eggs and food, but these birds are usually infected during feeding from ground or soil contaminated with oocysts of *T. gondii* and become a new source of human infection. Mahmood *et al.,* (2014) reported that prevalence of *T. gondii* was high (20.70%) in un-caged chicken, while rate of infection was low (5.90 %) in caged chickens, intended for food in local restaurants in

various region of Khyber Pakhtunkhwa province of Pakistan. Infected animals are major source of zoonotic infection, because there is no awareness, mass screening, disease surveillance and vaccination programs for control of infection in livestock, which cause serious issues of public health, socioeconomic, psychological and reproductions in women community of Pakistan. Pan *et al.,* (2017) stated that eating raw or undercooked meat, keeping animal, butchers, animal attendants illiteracy or and low education are various risk factors, which are associated with zoonotic Toxoplasmosis in China. Davami *et al.,* (2014) reported 40% cases of Toxoplasmosis in general Iranian population and observed an increased trend and association of human infection with age, consumption of uncooked meat, eating raw vegetables, fruits, working with animal in farms, housewife, low education and residence in rural areas. Similarly Kamal *et al.,* (2015) found a significant (<0.05) association between Toxoplasmosis and consumption of undercooked meat, low socioeconomic level and residence in rural area. Various maternal factors were evaluated for prevalence of zoonotic Toxoplasmosis (Table: 4.30). Prevalence of infection was significantly (<0.05) high in multi gravid and no-pregnant women. Similarly infection was significantly (<0.05) high during 1st trimester of pregnancy as compared to 2nd and 3rd trimester in pregnant women (Table: 4.30), Variation exists in seroprevalence of Toxoplasmosis in different obstetric conditions and in various adverse obstetric outcomes in infected women in published clinical literature. Aleem *et al.,* (2018) also detected *T. gondii* in 47.2% pregnant women with a high infection rate in 1st trimester 61.7% of pregnancy as compared to 58.4% cases in 2nd and 27.7% cases in 3rd trimester of pregnancy in Pakistan, while Khan *et al.,* (2014) reported Toxoplasmosis in women with a seroprevalence of 65.71% and claimed that infection was high 41.31% in pregnant women, who were 21-30 years old. Al-Tantawi *et al.,* (2014) reported high prevalence of Toxoplasmosis in those women, who were pregnant and in childbearing age from various regions in Latin America, Central and Eastern Europe, Middle East and various regions of south-east Asia and Africa. Negeroa *et al.,* (2017) found and reported a high 75.7% prevalence of Toxoplasmosis in pregnant and multigravidae women and in those women, who had confirmed history of abortions from south-western Ethiopia, which strongly support our current findings (Table:4.31). During the current study, Toxoplasmosis was also detected in a large number of women, who were positive for history of various types of adverse obstetric outcomes such as history of different types of abortions, preterm birth, still birth, congenital anomalies, pelvic inflammatory diseases (PID), intrauterine fetal death (IUFD), stillbirths, early death of neonate and congenital anomalies (Table:4.32). Mousa *et al.,* (2011) also stated that during pregnancy, maternal infections play an important and critical role in various forms of adverse obstetric outcomes (APO), which include two or more successive spontaneous miscarriages, intrauterine growth retardation (IUGR), low birth weight (LBW), intrauterine fetal death (IUFD), stillbirths and early death of neonate or congenital anomalies. They screened 143

obstetric patients, who had various types' adverse obstetric outcomes and detected Toxoplasma infection in 44.8% maternity patients. IgM was positive in 8.4%, while IgM and IgG were positive in those women, who had history of habitual abortions. They screened 143 obstetric patients, who had positive history of various adverse obstetric outcomes. Toxoplasmosis was detected in 44.8% maternity patients. IgM was positive in 8.4% infected cases, while IgM and IgG were positive in those women, who had positive history of habitual abortions. During pregnancy, parasite transmit to developing fetus and may cause visual, hearing abnormalities, mental retardation, seizures, hematological disorders and fetal death (Montoya and Kovacs, 2010; Elahian-Firouz *et al.,* 2014). According to Asgari *et al.,* (2013), persistent *T. gondii* parasite in women placenta is usually associated with congenital infection, which creates various fetal complications like microcephaly, hydrocephaly, encephalitis, severe hepatitis, moderate to severe lymphadenopathy and IUFD. Shah *et al.,* (2015) and Latif *et al.,* (2017) detected *T. gondii* specific IgG antibodies in 20% and 31% women, who had positive history of bad obstetric outcomes. They found a significant (<0.05) association of Toxoplasmosis with BOH. Ghasemi *et al.,* (2015) detected IgG antibody in 25.5% women (26.8% abortion, 21.4% stillbirth) and 26.4% women in control group. *T. gondii* specific IgM antibody was detected in 2.7% women of case group (3.6% abortion, 0% stillbirth) and 0.9% in control group. They claimed that infection of Toxoplasmosis play important role in etiology of abortions and stillbirths during pregnancy. Kamal *et al.,* (2015) reported zoonotic Toxoplasmosis in 50.8% cases of high-risk pregnant women. Abortion was a common adverse obstetric outcome in 56.5% infected women, while post-delivery adverse outcomes were detected in 80.3% cases of high-risk pregnancy group as compared to 20% normal pregnancy group. Pandey, (2018) reported a significantly (<0.05) higher antibodies level of *T. gondii* in those women, who had history of adverse obstetric outcomes and infection in ongoing pregnancy. He found IgG antibodies in 43% and IgM, IgG in 14.4% cases, while abortions 50%, preterm delivery 4.2%, IUFD 9.7%, congenitally malformed babies 3.5% and neonatal death 0.7% were various form of adverse obstetric outcomes. Singh, (2016) stated that primary or acute and active infection of *T. gondii* causes spontaneous abortion, stillbirths and various congenital diseases with involvement of eyes and CNS in pregnant women. All these published literature support our current results (Table: 4.32). Li *et al.,* (2014) observed a significantly (<0.05) high incidence of adverse pregnancy outcomes in infected pregnant women as compared to non-infected pregnant women. They observed that *T. gondii* infection was significantly higher in those women, who had abnormal pregnancy outcomes as compared to those women, who had normal pregnancy, while during maternal infection, collective rate of vertical parasite transmission was 20% and incidence of infection increased from 5%, 13% to 32% in first, second and third trimester of pregnancy. According to them, vertical transmission of parasite was found in 13%, 13% and 24% women, who were treated with combination of spiramycin and

PSF (pyrimethamine + sulfadiazine + folinic acid) or PS (pyrimethamine +sulfadiazine) with combination of spiramycin, or other untypical treatments. Moncada and Montoya, (2012) stated that clinical manifestations of Toxoplasma associated congenital diseases usually appear slow in life after child birth. Proper mechanism of *T. gondii* associated adverse obstetric outcome is still not clear in published literature. Liua *et al.,* (2018) investigate various adverse effects of PRU strain of *T. gondii* infection on bias of decidual macrophage polarization and its contribution in adverse obstetric outcomes in a mouse model. They measured expression levels of various functional molecules in decidual macrophages of infected mice and revealed that *T. gondii* parasite caused various adverse obstetric outcomes in mice. Congestion and infiltration of inflammatory cells were observed in placenta of infected mice. After infection, level of M2 markers (CD206, MHC-II, and arginase-1) was decreased, but expression of M1 markers (CD80, CD86, iNOS, cytokines TNF-α, IL-12) was increased in decidual macrophages. They reported that *T. gondii* parasite was responsible for bias of M2 decidual macrophages toward M1, which cause alterations in immunosuppressive microenvironment at maternal-fetal border and contributes in different form of adverse obstetric outcomes in infected host.

For diagnosis of zoonotic Toxoplasmosis, lateral flow chromatographic immunoassay (ICT) and latex agglutination test (LAT) were initially used, which significantly (<0.05) detected *T. gondii* specific antibodies, while standard tube agglutination (STAT) test (Table: 4.16) and ELISA were used for confirmation of infection, which significantly (<0.05) confirmed Toxoplasma infection in 24.56 infected women (Table: 4.13). The sera of 915 women, who were positive for Toxoplasma by ICT assay were serially cheeked at 1:16, 1:32, 1:64, 1:128, 1:256, 1:512, 1:1024 and 1:1024 dilutions by standard tube agglutination (STAT) procedure, but no significant (>0.05) increased was observed in rate of agglutination at a particular dilution, while a significant (<0.05) increased was observed in rate of agglutination at a collective dilution of 1:64 and 1:128, which indicated acquired or evolving immunity in infected women (Table: 4.16). The sera of 1.21% women showed antibody titer at 1:16 dilution, which indicated absence of *T. gondii* specific antibodies, while 2.51% women showed antibodies titer at 1:32 dilution, which indicated residual or non-specific immunity (Figure: 4.16). Positivity of STAT at 1:16 and 1:32 dilution indicated false positivity of ICT assay in 34 women. Similarly, 26.23% and 42.29% sera of patients showed antibodies titer at 1:64 and 1:128 dilution, which indicated acquired or evolving immunity, while sera of 11.15%, 9.41%, 5.14% and 2.07% women showed antibodies titer at 1:256, 1:512, 1:1024 and 1:2048 dilutions, which is suggested as a possible recent contact with risk factor of *Toxoplasma gondii*. ELISA assay significantly confirmed Toxoplasmosis in 881 women (Table: 4.15). *T. gondii* specific IgG was the most prominent antibody, which were detected in 57.07% women, followed by IgM 8.03% and IgM, IgG in 8.62% women (Table: 4.15). Fonseca *et al.,* (2012), Davami *et al.,*

(2014), Alghamdi *et al.,* (2016), Frimpong *et al.,* (2017), Maçin *et al.,* (2018) and Alvarado-Esquivel *et al.,* (2018) also reported Toxoplasma specific IgM in 3.6%, 2%, 6.4%, 0%, 2.44%, 12.5% and IgG in 49.5%, 13%, 32.5%, 5.87%, 29.53% and 3.6% women from various other countries of the world, which strongly support our current results (Table:4.15). Abo-Hashim and Attya, (2015) found IgM in 10%, 16.67% and 30% cases by rapid Toxo IgM method, ELISA and immunoblotting method. We used ELISA as a confirmatory assay for diagnosis of infection, which is a sensitive and reliable method and which significantly (<0.05) confirmed positive cases of *T. gondii* infection in women. Khan *et al.,* (2014) investigated Toxoplasmosis in women from various regions of Malakand Agency in Khyber Pakhtunkhwa province of Pakistan. They also stated that ELISA was the most sensitive, reliable and accurate method as compared to other methods, which were used for detection of Toxoplasmosis.

5.5 Clinical manifestations, ultrasonographic findings and laboratory aspects of 718 women with zoonotic Brucellosis infection in Khyber Pakhtunkhwa province of Pakistan.

There are variations in clinical features of Brucellosis (Jia *et al.,* 2017) and infection is gradually becoming a major health problem all over the world (Shi *et al.,* 2018). There is no published data on clinical and laboratory aspect of Brucellosis infection in women from Pakistan. The current data would deliver a comprehensive clinical knowledge, which is related to frequency of Brucellosis, clinical and laboratory features, stages of infection and associated complications in infected women. During the current study, variations were observed in clinical and laboratory parameters in infected women, which usually misguide clinician during diagnosis and prescription of medicines. According to Jiang *et al.,* (2019), clinicians have limited understanding about Brucellosis, while variation in clinical manifestation usually increases chances of misdiagnosis, which lead to chronicity of infection. Based on duration of clinical symptoms, Brucellosis was found in acute, (59.61%), sub-acute (33.28%) and chronic (7.11%) cases (Table: 4.17). During the current study, a significant (<0.05) difference was found in duration of clinical symptoms and stages of Brucellosis infection in infected women (Figure: 4.17), which is strongly supported by other investigators like Buzgan *et al.,* (2010), Roushan *et al.,* (2016), Shi *et al.,* (2018) and Jiang *et al.,* (2019), who reported Brucellosis in acute 61.6%, 73.8%, 74%, 91.9%, sub-acute 21.6%, 22.6%, 22% and chronic 13.6%, 3.7%, 4%, 8.1% cases from various other countries, but Eini *et al.,* (2012) reported 70.8% cases of sub-acute, 24% cases of acute and 5.2% cases of chronic infection, while Jia *et al.,* (2017) reported 53.6% cases of acute and 21.5% cases of chronic infection of Brucellosis. During the current study, a significantly (<0.05) high number 59.61% of acute cases of Brucellosis was observed (Table:4.17), which indicate that new cases of human infection is increasing due various persistent epidemiological risk factors including unawareness, infected animal in the region, close animal contact and frequent use of unpasteurized dairy products, while low rate of sub-acute 33.28% and chronic

7.11% cases could be due to previously undiagnosed or misdiagnosed cases, who were treated with nonspecific therapeutic regimens by unauthorized medical practitioner in rural regions of Pakistan, who are involved in illegal clinical practices in various regions of Khyber Pakhtunkhwa province of Pakistan (Figure;3.1). These non-specific therapeutic medications temporarily subsides severity of clinical symptom of Brucellosis and provide short term relief and confidence of recovery to infected individuals, which shift disease from acute to sub-acute and chronic stage. *Brucella abortus* and *B. melitensis* specific febrile antigen based standard agglutination test (SAT) is commonly used in local diagnostic laboratories in Khyber Pakhtunkhwa and other provinces of Pakistan (Table: 4.10) which is not 100% sensitive and specific and cannot differentiate to diagnose false negative, positive and chronic cases of Brucellosis. Jia *et al.,* (2017) reported 98.5% positivity in agglutination test. Brucellosis specific confirmatory assays like, IgM, IgG ELISA and PCR assays (Table: 4.9) are still not frequently available in local diagnostic centers in Pakistan. These assays are available in some private and expensive diagnostic laboratories in Pakistan, where majority patient cannot access due to unawareness, expensive test rates, poverty and living in rural areas, due to which, most of clinicians still relay on SAT assay and they start treatment of infected individuals on the basis of positivity of SAT assay. Advanced diagnostic assays are most important for clinical differentiation and management of sub-acute and chronic cases of Brucellosis infection (Shi *et al.,* 2018). During the current study, SPAT and RBPT were used for initial diagnosis of Brucellosis, while IgM, IgG ELISA and PCR were used for confirmation of disease in women (Table: 4.9). According to Shi *et al.,* (2018), age and chronicity of Brucellosis infection increased poor diagnostic rate, while chronicity of disease reduce positivity of standard agglutination test (SAT) and blood culture. Araj and Kaufmann, (1989) stated that classical serological assays are still important indicator, which diagnose culture negative patients, but chronic infection reduces host immune response, which increased false negativity in agglutination. They demonstrated a significant (<0.05) increase in antibodies formation in acute infection as compared to chronic cases of Brucellosis. Being intracellular in nature, Brucella affect any body organ in infected individuals, due to which no specific clinical sign and symptoms are usually associated, but complaint of fever, sweating, malaise, arthralgia headache, anorexia and backache usually occurred in various clinical stages (Shi *et al.,* 2018). During the current study, fever was a prominent and significant (<0.05) clinical manifestation, which was observed in 89.27% patient (Table: 4.18), which is in close agreement with study findings of Ulug *et al.,* (2011), Sac *et al.,* (2013), Roushan *et al.,* (2016), Jia *et al.,* (2017) and Shi *et al.,* (2018), who also reported that fever was a prominent clinical finding in Brucellosis patients. Jia *et al.,* (2017) observed blood culture in 86.9% cases of fever in Brucellosis, but they found only 34% cases of fever, who were positive for blood culture, which indicated that bacteremia was not exact cause of fever in Brucellosis infection. They observed that fever was disappeared in 2-14

days with treatment of doxycycline, rifampicin combination and 85.2% patients become normal within one week. Beside fever, arthralgia, was another clinical symptom, which was significantly (<0.05) observed in our Brucellosis patients (Table: 4.18). Bosilkovski *et al.,* (2009), Akhvlediani *et al.,* (2010), Bosilkovski *et al.,* (2010) and Shi *et al.,* (2018) also reported arthralgia in Brucellosis patients, which strongly support our current finding (Table:4.18). Fanni *et al.,* (2013) and Jiang *et al.,* (2019) also claimed that persistent fever with involvement of bones and organomegaly usually indicate probability of Brucellosis infection. Various other clinical symptoms such as back pain, fatigue, malaise, anorexia, sweating, myalgia, headache, chills, nausea, and cough were clinical symptoms, which were significantly (<0.05) found in Brucellosis patients (Figure: 4.18). Akhvlediani *et al.,* (2010), Buzgan *et al.,* (2010), Behnaz *et al.,* (2011), Olt *et al.,* (2015)**,** Roushan *et al.,* (2016), Jia *et al.,* (2017), Shi *et al.,* (2018) and Jiang *et al.,* (2019) strongly support our current clinical findings (Table: 4.18), who also reported these clinical symptoms in Brucellosis patients with different frequency from other endemic regions of the world. A mild chest pain with cough was observed in 14.06% infected women, that show involvement of respiratory system, which is not commonly occur in Brucellosis. Behnaz *et al.,* (2011), Erdem *et al.,* (2014) and Shi *et al.,* (2018) support our current finding, who reported respiratory involvement in various clinical stage of Brucellosis. Kerem *et al.,* (1994), Behnaz *et al.,* (2011), Erdem *et al.,* (2014) and Shi *et al.,* (2018) reported various form of pulmonary involvement in 16%, 7.6%, 30.8% and 10% cases of Brucellosis, which support our current findings (Table: 4.18). Being a major reticulo-endothelial organ, hepatomegaly and splenomegaly were significantly (<0.05) observed in infected women (Figure: 4.19). According to Buzgan *et al.,* (2010), liver is usually affected during Brucellosis infection. Globally, different investigator like Buzgan *et al.,* (2010), Behnaz *et al.,* (2011), Dean *et al.,* (2012), Jia *et al.,* (2017), Shi *et al.,* (2018) and Jiang *et al.,* (2019) also reported splenomegaly in 14.5%, 31.7%, 21-31%, 26.1%, 42%, 34.0% and hepatomegaly in 20.6%, 42%, 19-27%, 29.8%, 24% and 8.6% cases of Brucellosis, which strongly support our current clinical findings (Table: 4.18). Fanni *et al.,* (2013) claimed that splenomegaly and hepatomegaly occurred in one-third cases of Brucellosis, while Starakis *et al.,* (2010) stated that every body organ might be involved in Brucellosis. Lymphadenopathy was observed in 17 (2.36%) infected women (Table:4.18), which was also reported by Buzgan *et al.,* (2010), Behnaz *et al.,* (2011) and Shi *et al.,*(2018) in 2.4%, 8.4% and 2.0% cases of Brucellosis, which supports our current clinical findings of lymphadenopathy. Frequency of clinical signs and symptoms were different in our patients from various clinically published data of other regions, which could be due to disease duration, clinician experience and quality of equipment, which were used in physical examination. Frequency of clinical signs and symptom were significantly (<0.05) different in different clinical stages of Brucellosis (Table: 4.19). Buzgan *et al.,* (2010) and Shi *et al.,* (2018) also reported different clinical

manifestation in various stages of Brucellosis, which strongly support our current findings (Table: 4.19). Makis *et al.,* (2017) claimed that those patients should be evaluated for Brucellosis assays, who have fever, arthralgia, splenomegaly, hepatomegaly, positive history of risk factor and who do not respond to any therapeutic regimens. Liu and Zhao, (2017) stated that early assessment of Brucella associated bacteremia with compatible clinical symptoms like fever, arthralgia and with a known risk factor depends on collection of different clinical information, which is usually derived from various clinical and laboratory parameters like WBC count, measurement of ESR and CRP level. During the current study, various hematological abnormalities like anemia, leucopenia, leukocytosis, thrombocytopenia, thrombocytosis and pancytopenia were observed in women, who were positive for Brucellosis (Table: 4.21). Buzgan *et al.,* (2010) also claimed that mild hematological abnormalities usually occurred in Brucellosis infection, which support our current findings (Figure: 4.22), while, Citak *et al.,* (2010) claimed that various complication occur in 5-10% infected cases due to diagnostic delay and treatment of Brucellosis with nonspecific therapeutic regimen. Brucella may affect hematological system of the body and cause various hematological complications including hemolytic anemia (Eskazan *et al.,* 2014 and Makis *et al.,* 2017), leucopenia and thrombocytopenia (Guzel Tunccan *et al.,* 2014 and Makis *et al.,* 2017), leukocytosis (Fanni *et al.,* 2013), thrombocytosis (Sathyanarayanan *et al.,* 2011), pancytopenia (El-Koumi *et al.,* 2013, Makis *et al.,* 2017). During the current study, anemia was significantly (<0.05) observed in our patients (Table: 4.21). Guzel-Tunccan *et al.,* (2014) and Heydari, (2015) also claimed that mild to moderate anemia usually occurred in acute phase of Brucellosis. Buzgan *et al.* ,(2010), Behnaz *et al.,* (2011), Jia *et al.,* (2017), Shi *et al.,* (2018) and Jiang *et al.,* (2019) also reported anemia in 40.3%, 42%, 45.3%, 24% and 25.5% cases of Brucellosis, which strongly support our current finding (Table; 4.21). Anemia aggravates fatigue (Behnaz *et al.,* 2011), which was significantly (<0.05) observed during our clinical finding in Brucellosis patients (Table: 4.18). A change in TLC and DLC count was observed in infected women (Table: 4.21). According to Liu and Zhao, (2017), WBC and DLC count significantly (<0.05) changed, when infection becomes severe or titer of SAT assay increased in infected individuals. Leukocytosis is a common hallmark of many infections, which was also reported by Buzgan *et al.,* (2010), Jia *et al.,* (2017), Shi *et al.,* (2018) and Jiang *et al.,* (2019) in 9.0%, 15.4%, 6% and 5.6% cases of Brucellosis, which strongly support our current hematological findings (Table: 4.21). In acute bacterial infection, mature and immature neutrophils circulate in blood, while in chronic infection; there is a shift of elevation from neutrophils to lymphocyte in blood circulation. Sadia *et al.,* (2001) reported elevated WBCs with a lift shift in a case control clinical study of Brucellosis. Buzgan *et al.,* (2010) reported lymphomonocytosis in 28.2% cases of acute, sub-acute and chronic infection of Brucellosis, while Jia *et al.,* (2017), Shi *et al.,* (2018) and Jiang *et al.,* (2019) reported lymphocytosis in 33.4%, 19% and 34.7%

cases of Brucellosis. Leukocytosis indicates microbial infection, but other compatible clinical signs and symptoms, which are specific to particular a disease assist in accurate clinical diagnosis. A high count of different type of WBC can point out causes of leukocytosis (Berliner, 2012), while DLC is essential for evaluation of these causes. Exercise, trauma, surgery, emotional stresses, smoking, obesity, certain drugs, asplenia and chronic body inflammation are some other hidden factors, which can cause leukocytosis (Riley and Rupert, 2015). Therefore, during clinical examination, these hidden factors should be considered in mind for accurate diagnosis of disease and associated clinical complications. During the current study, various type of leucopenia was observed in infected women (Table: 4.21), which is strongly supported by studies of Abdi-Liae *et al.,* (2007), Buzgan *et al.,* (2010**),** Behnaz *et al.,* (2011), Jia *et al.,* (2017), Shi *et al.,* (2018) and Jiang *et al.,* 2019), who reported Brucellosis associated leucopenia in 13.6%, 10.9%, 8.5%, 9.3%, 12%, and 17.9% cases of Brucellosis. Sari *et al.,* (2008) claimed that leucopenia is a rare event in Brucellosis, while Buzgan *et al.,* (2010) stated that leucopenia commonly occurred during Brucellosis infection. According to Sadia *et al.,* (2001), Potts and Rothman, (2008) and Cerny and Rosmarin, (2012), mild to severe neutropenia may associate with rickettsial, typhoid and Brucellosis infection. Shi *et al.,* (2018) observed low lymphocytes in 0.5% infected cases, while Jiang *et al.,* (2019) reported lymphocythaemia, hypoeosinophilia and leucopenia in 34.7%, 26.9% and 17.9% cases of Brucellosis. During the current study, thrombocytopenia was significantly (<0.05) found in infected women, while thrombocytosis was observed with a low (>0.05) prevalence in infected women (Table: 4.21). A mild to moderate form of thrombocytopenia was globally reported in 5-40% cases by Citak *et al.,* (2010), Karaman *et al.,* (2016) and Aypak *et al.,* (2016) in Brucellosis endemic region, which support our current results (Table: 4.21). Guzel-Tunccan *et al.,* (2014) reported a mild form of thrombocytopenia in 1-26% infected cases, while Buzgan *et al.,* (2010), Behnaz *et al.,* (2011), Jia *et al.,* (2017), Shi *et al.,* (2018) and Jiang *et al.,* (2019) reported thrombocytopenia in 9.5%, 12%, 19.8%, 4% and 9.2% cases of Brucellosis, which support our current results (Table: 4.21). Tsolia *et al.,* (2002) reported thrombocytopenia in a mild to moderate form, which was subsided with Brucella specific therapeutic regimen. Heydari, (2015) claimed that a mild form of thrombocytopenia usually occur in Brucellosis patients, which could be due to bone marrow suppression, hemophagocytosis, hypersplenisem, immunologic reaction or disseminated intravascular coagulation. Beside thrombocytopenia, thrombocytosis was also observed (>0.05) in 10 (1.39%) infected women (Table: 4.21), which is rare in Brucellosis. Jiang *et al.,* (2019) support our current findings and reported 12.1% cases of thrombocytosis during Brucellosis infection, which is higher than our current findings (Table: 4.21), A mild form of pancytopenia was observed only in 8 (1.11%) infected women, which was associated with a low Hb, WBC and platelet count. Brucellosis associated pancytopenia have also been

previously reported by Starakis *et al.,* (2010), Buzgan *et al.,* (2010), Behnaz *et al.,* (2011) and Jiang *et al.,* (2019) in 4.76%, 4.9%, 1.5% and 2.7% infected cases, which strongly support our current finding (Table: 4.21). Abnormalities in various hematological parameters usually occurred during Brucellosis infection, but exact mechanisms and pathophysiology of these abnormalities are still unknown. Pappas *et al.,* (2005) proposed that Brucellosis associated pancytopenia is multi-factorial. Erdem *et al.,* (2011) and Demir *et al.,* (2012) found hemophagocytosis and formation of granulomas in bone marrows of infected patients. Various inflammatory cytokines like transforming growth factor (TGF-β) also play important role in infection associated hematological abnormalities. Mutations in TGF-β producer phenotype could also affect clinical outcomes during Brucellosis infection (Rafiei *et al.,* 2007). During the current study, various inflammatory biomarkers like ESR and CRP were determined in infected women (Table: 4.24). Elevated ESR and CRP were significantly (<0.05) observed in our patients (Table: 4.24). Buzgan *et al.,* (2010) also reported elevated ESR and CRP as prominent laboratory abnormalities in Brucellosis patients, which support our current results (Figure: 4.24). Other researcher like Roushan *et al.,* (2005), Afsharpaiman and Mamishi, (2008) and Tanir *et al.,* (2009) reported elevated ESR in 38-87% and CRP in 34-81% cases of Brucellosis. Bosilkovski *et al.,* (2007), Abdi Liae *et al.,* (2007) and Demroglu *et al.,* (2007) reported elevated ESR from 61% to 65.9% cases, which is consistent with our current finding (Table: 4.24). Buzgan *et al.,* (2010), Behnaz *et al.,* (2011), Jia *et al.,* (2017) and Shi *et al.,* (2018) also reported elevated ESR in 51.26%, 76.8%, 64.7% and 69.1% cases of Brucellosis, which strongly support our current findings (Table: 4.24). Similarly elevated CRP was reported by Buzgan *et al.,* (2010), Behnaz *et al.,* (2011), Jia *et al.,* (2017) and Shi *et al.,* (2018) in 58.4%, 77%, 44.2% and 39% patients of Brucellosis, which strongly support our current results (Table:4.24). According to Buzgan *et al.,* (2010), elevated level of ESR and CRP are prominent clinical laboratory indicators in diagnosis of acute and sub-acute cases of Brucellosis. Erdem *et al.,* (2014) reported a mild to moderate increases in ESR and CRP level, while Jia *et al.,* (2017) suggested that ESR and CRP are reliable indicators for clinical diagnosis and monitoring of disease. Early assessments of Brucellosis infection in those patients, who present with fever, rely on proper clinical and laboratory analysis of WBC, DLC, ESR and CRP. These clinical laboratory parameters lack specificity for early diagnosis, but are useful to point out consideration toward infection of Brucellosis in suspected clinical cases (Liu and Zhao, 2017). During the current study, assessment of important liver enzymes (ALT, AST) was also carried out to determine Brucellosis associated abnormalities in liver function in infected women (Table: 4.24). The level of serum AST and ALT was elevated (>0.05) in 30.22% and 33.28% infected women (Figure: 4.24), which show involvement of liver in Brucellosis infection in many patients. Transaminase (ALT, AST) is a basic biochemical assay, which is frequently used in clinical practice for monitoring of liver function

(Newsome *et al.,* 2018). Aspartate aminotransferase (AST) is commonly found in tissues of liver, kidney, lung, brain and skeletal muscle, while alanine aminotransferase (ALT) is present in cytoplasm of hepatocyte in liver. Whenever cell injury occurred in hepatocyte, permeability of cell membrane increases, which directly release AST and ALT into circulation of blood (Rej, 1978). Clinicians usually used AST/ALT ratio (AAR) for assessment of liver function and elevated level of AST and ALT indicate damages in liver cells. A high level of ALT in sera is a good indicator of liver damage as compared to AST, because AST also increased in extra-hepatic disease (Woreta and Alqahtani, 2014). Being a large site for reticuloendothelial tissues, liver is usually affected in Brucellosis in many patients (Buzgan *et al.,* 2010), as a result of which, a mild to moderate increase usually occur in ALT and AST level in serum of many infected patient (Young, 2005 and Doganay *et al.,* 2008). Starakis *et al.,* (2010) reported hepatic involvement and abnormalities in AST and ALT level in 50% cases of Brucellosis and claimed that level of these enzymes increased during infection of Brucellosis. A significant (<0.05) difference was found in AST and ALT abnormalities in acute, sub-acute and chronic cases of Brucellosis infection (Table: 4.25). Frequency of AST and ALT was increased in acute infection of Brucellosis, which slowly decreased in sub-acute and chronic cases of Brucellosis (Table: 4.25). Buzgan *et al.,* (2010) reported elevated level of liver enzymes in acute and sub-acute phases in 24.8% cases of Brucellosis, but they confirmed clinical hepatitis in only 2.7% infected cases, which support our current results (Table: 4.25). They suggested that all cases of high AST, ALT level should not be considered as clinical hepatitis. Similarly, Jia *et al.,* (2017) observed a high level of transaminase enzyme in 30.3% infected cases, while Shi *et al.,* (2018) found increase level of ALT and AST in 33% and 20% cases of acute Brucellosis, which strongly support our current findings (Table: 4.24) regarding elevated level of AST and ALT in Brucellosis patients.

5.6 Clinical manifestations, ultrasonographic findings and laboratory aspects of 881 women with zoonotic infection of Toxoplasmosis in Khyber Pakhtunkhwa province of Pakistan.

T. gondii is an obligate intracellular protozoan parasite that infects animals and human (Mohamed, 2020). A large number of human population belong to low socioeconomic status in Pakistan, due to which most of them are usually exposed to various zoonotic infections (Mujtaba *et al.,* 2016). Toxoplasmosis is globally a significant public health problem (Aguirre *et al.,* 2019). The parasite has adverse impact on human health and caused serious problems in immunocompromised individuals and pregnant women (Halonen and Weiss, 2013). Although various therapeutic regimens are commonly available for treatment, but rate of therapeutic failure is significantly high in infected individuals (Dunay *et al.,* 2018). Globally, one third of human population is infected by *T. gondii*, but infection is clinically not observed or caused different clinical symptoms, which usually depend on immunological status of infected individuals (Tian *et al.,* 2017). In most infected individuals, infection is usually self-limiting with no

obvious clinical symptoms, but parasite can cause severe disease in immuno-compromised individuals and in infants, when mothers are infected during pregnancy (Nissapatorn, 2009 and Oz, 2014). The parasite persist in tissues of host in a dormant cyst form for a long period, but cause no prominent clinical symptoms (Daryani, 2014), while some researcher believe that parasite cause some changes in personality during dormant cyst stage in infected persons (Flegr *et al.,* 2013 and Cook *et al.,* 2015). According to Alavi *et al.,* (2016), Toxoplasmosis is usually asymptomatic in immune-competent individual, but infection become life-threaten, whenever immune system shift to immunocompromised condition due to various other factors. Zoonotic infection of *T. gondii* is classified as asymptomatic, pregnancy infection, congenital infection, acquired or reactivated infection in immune-compromised or ocular disease. Tenter *et al.,* (2000) claimed that cysts of Toxoplasma parasite have high affinity for muscular and neural tissues and predominately located in patient eyes, CNS, cardiac and skeletal muscles. The cyst can be found in different visceral organs like lungs, liver and kidneys of infected individuals. It is believed that persistent cysts in tissues of various organs like muscles, brain and retina cause immunization, due to which 80% positive cases remained asymptomatic. The cysts reactivate in the body during immune-compromised condition, which cause serious infection and even death of infected individuals (Villard *et al.,* 2016). According to Gaballah *et al.,* (2018), persistent cysts in infected individuals do not give real protection from acute infection nor reduced colonization of cyst in brain after super-infection with another virulent strain of *T. gondii*, which indicate that those women, who were previously infected with *T. gondii* can develop congenital infection during pregnancy, whenever re-exposure to parasite. Contini, (2008) stated that due to mild, asymptomatic nature of infection, Toxoplasmosis remains undiagnosed in those infected individuals, who have healthy immune system. Persistent cyst in dormant condition can rupture in infected tissues, whenever immune systems become weak, as a result, parasite start to trigger severe sickness in chronically infected host. After reactivation of cysts, neuro-tropic complications may occur, which include brain abscess, encephalitis and death of infected person (Rostami *et al.,* 2014). Infection may cause various complications like myocarditis, encephalitis, chorioretinitis and pneumonitis in immunocompromised condition (Montoya *et al.,* 2010; Saadatnia and Golkar, 2012), but we did not observe such severe type of complications in our patients (Table: 4.20). Toxoplasmosis become dangerous and malignant in immunocompromised conditions, therefore urgent diagnosis and therapeutic management is usually necessary in infected individuals in endemic regions (Montoya and Liesenfeld, 2004; Montoya *et al.,* 2010; Saadatnia and Golkar, 2012). During the current study, various clinical symptoms were observed in our women patients, while most of them were also asymptomatic (Table: 4.20). Back pain, fatigue, headache and myalgia were significant (<0.05) and most prevalent clinical feature, which were followed by arthralgia, anorexia, malaise, nausea, sweating, fever, dry cough, chills

and weight loss (Figure: 4.20). These clinical manifestations were previously documented by many researcher in published clinical literature, but in very scare form. Symptoms like fever, arthralgia, headaches, myalgia, migran, nausea, respiratory problems and weight loss were previously reported by Carmo *et al.,* (2010), Abhilash *et al.,* (2013), Ogoina *et al.,* (2014) and Castilho-Pelloso *et al.,* (2007) in various clinical studies, which strongly support our current clinical findings (Table: 4.20). Abhilash *et al.,* (2013) reported fever with generalized arthralgia and weight loss in early stage of *T. gondii* infection in a case control study. Nunura *et al.,* (2010) and Cuomo *et al.,* (2013) reported fever and pneumonia, while Fernàndez-Sabé *et al.,* (2012) observed fever, respiratory, neurologic involvement and chorio-retinitis in cases of primary infection of *T. gondii* in organ transplanted patients. Montoya *et al.,* (2010) and Linguissi, (2012) stated that acute infection of *T. gondii* is usually asymptomatic in immune-competent women during pregnancy, but have regional lymphadenopathy, which was significantly (<0.05) observed in our infected patients (Table: 4.20). Many recent studies have shown that latent or acute infection of Toxoplasmosis is associated with reproductive disorder, infertility, behavioral changes and different neurological disorders in pregnant and non-pregnant women (Terpsidis *et al.,* 2009; Fallahi *et al.,* 2017). The disease usually show a mild and self-limiting flue like symptoms along with fever, myalgia and lethargy in 10% infected cases, which last for 4-6 weeks. These clinical symptoms rarely persist more than a year. Rafiqul and Faiz, (1989) stated that prolong fever is not a common clinical manifestation of Toxoplasmosis, but may occur in rare cases of infection. Many severe symptomatic cases have been reported from Brazil, French, Surinam, Guyana and Peru with different clinical manifestation, which include pneumonitis, myocarditis, hepatitis, polymyositis and encephalitis (Nunura *et al.,* 2010; Caselli *et al.,* 2012; Strabelli *et al.,* 2012), but no such severe and complicated clinical case was observed in our patients (Table: 4.20). Various clinical signs like lymphadenopathy, flue, splenomegaly, hepatomegaly (Figure: 4.21) and spleno-hepatomegaly were observed in women, who were positive for *T. gondii* antibodies. Lymphadenopathy was a significant (<0.05) clinical sign in our patients, while flue, splenomegaly and hepatomegaly were observed with a low (>0.05) frequency in our patients (Table: 4.20). Most researchers support our current clinical findings (Table: 4.20). Alavi and Alavi, (2010) and Pinto *et al.,* (2017) stated that *T. gondii* cause regional lymphadenopathy in immune-competent host, while Carmo *et al.,* (2010) reported that parasite cause lymphadenopathy and hepato-splenomegaly. Lymphadenopathy, splenomegaly and hepatomegaly were also reported by Fernàndez-Sabé *et al.,* (2012) in infected SOT recipients and by Abhilash *et al.,* (2013) in a case control study. Abhilash *et al.,* (2013) reported enlarged, non-tender cervical and axillary lymph nodes with moderate splenomegaly, which was palpable at 4 cm below left costal margin in a 32 year old immune-competent woman, while biopsy of left axillary lymph node showed reactive germinal centers, reactive follicular hyperplasia and focal distension

of sinuses with monocytoid cells. The woman was treated with pyrimethamine and clindamycin. She became afebrile and asymptomatic at beginning of treatment, but relapse in splenomegaly and hepatomegaly was observed after 3rd months of her treatment. During the current study, some of our patients developed (>0.05) hepatomegaly with an increased level of aminotransferase (Table: 4.26), which is consistent with studies of Nunura *et al.,* (2010), Caselli *et al.,* (2012) and Strabelli *et al.,* (2012), who stated that primary infection may develop hepatomegaly and hepatitis with moderate increase in amino transferase enzymes. According to Mahmood, (2016), tachyzoite of *T. gondii* can be seen in blood circulation and found in every body organ in infected individuals and disease has various clinical forms. They detected a significant (<0.05) relationship of Toxoplasmosis with hepatomegaly and abnormalities in liver functions, which support our current results (Table: 4.26). Blood lymphocytes synthesize some specific cytokines, which play important role in pathogenesis of disease. Abhilash *et al.,* (2013) stated that clinician should consider infection of Toxoplasmosis, whenever observe prolong febrile disease with splenomegaly and lymphadenopathy in suspected patients. During the current study, assessment of various hematological parameters was carried in women, who were positive for Toxoplasmosis (Table: 4.23)**.** Low and high values were observed in hemoglobin level, neutrophils, lymphocyte, monocytes, eosinophils, basophiles, and platelet counts (Figure: 4.23), while hematological parameters of many infected women were also found within normal ranges (Table: 4.23), which is in accordance with the study of Lappin, (1996) and Mahmood, (2018), who claimed that blood parameters do not change in some infected individuals and remains stable, while Huang *et al.,* (2004), Atmaca *et al.,* (2015) and Rafique, (2017) also observed similar results about hematological parameters in experimentally infected cats, gerbils mice and rats, which support our current results (Table: 4.23). In clinically published literature, there are controversial views about Toxoplasmosis associated abnormalities in various hematological parameters. Some researcher claimed that infection effect on various blood parameters, while other found no significant effect on hematology of infected individuals. Sandri *et al.,* (2020) proposed that change in WBC profile is additional diagnostic parameters, which must be considered for clinical diagnosis. Mahmood (2016) detected anemia in women, who were old, pregnant and had chronic infection of Toxoplasmosis. During the current study, anemia was significantly (<0.05) detected in our infected cases (Table: 4.23), but unfortunately, we have not investigated other possible factors of anemia like iron deficiency, gynecological problems or pregnancy in our patients. Hassen *et al.,* (2019) found that MCH, WBCs, granulocytes and platelets associated parameters like platelet count PCT%, MPV% and PDW% decreased in Toxoplasmosis, while lymphocytes and mid cells total count were increased in aborted women, who were positive for Toxoplasmosis. Abhilash *et al.,* (2013) observed low Hb, high WBC, neutrophils, lymphocytes, monocytes and low thrombocytes counts in a 32 year old woman, who was

positive for Toxoplasmosis. Mohamed, (2020) also reported a significant (<0.05) change in monocytes count, while WBCs, neutrophils, HCV, MCH and MPV were decreased in infected women. He further stated that anemia was significantly associated with chronic infection of Toxoplasmosis. During the current study, a significant (<0.05) increased was observed in lymphocytes and neutrophils count in most of our infected patients (Table: 4.23), which might be due to acute inflammatory response of the host body to proliferating tachyzoite of parasite. Sandri *et al.,* (2020) detected high lymphocytes and low neutrophils counts in acute infection of Toxoplasmosis, which support our current findings (Table: 4.23). According to Mahmood, (2016), tachyzoite of *T. gondii* can be seen in blood circulation and other body organ. Lymphocytes are blood cells that synthesize some specific cytokines, which play important role in pathogenesis of parasite. Low and high monocytes counts were observed in our patients, who were positive for Toxoplasmosis (Table: 4.23). According to Mahmood, (2016), monocytes counts increase in young infected women, while neutrophils decrease in older pregnant women during chronic infection of Toxoplasmosis. Ehmen and Lüder, (2019) stated that chronic infection in experimental mice has been associated with an increased counts of inflammatory monocytes. They observed that *T. gondii* parasite exerts a prolong effects on phenotype and reaction of monocytes, which have important role during innate immune responses against *T. gondii* and many other unrelated pathogens. Due to scarce data on hematological and biochemical abnormalities associated with infection of *T. gondii*, researchers have used various experimental animal models for assessment of these abnormalities. Advincula *et al.,* (2010) detected significant (<0.05) abnormalities in Hb, RBC, neutrophils and monocytes counts, while PCV, WBC, eosinophils and lymphocyte counts showed non-significant results in infected cat. Lappin, (1996) stated that hematological parameters remain unchanged during development of uncomplicated Toxoplasmosis, while Advincula *et al.,* (2010) claimed that these parameters significantly (<0.05) affected during active infection of *T. gondii* in serological positive cats. Atmaca *et al.,* (2015) observed a high count of neutrophils during an experimental study, which was performed on gerbils infected with *T. gondii*. Similarly Castello *et al.,* (2018) detected a significant (<0.05) increase in monocytes count in infected sheep during a case control study. During the current study, a low (>0.05) and high (<0.05) neutrophils count was observed in our patients (Table: 4.23). Low counts indicate impaired immunity, while high count may point out active infection in infected individuals (Stites *et al.,* 1984; Bliss *et al.,* 2001). The study of Bliss *et al.,* (2001) and Rajantie *et al.,* (1992) support our current clinical findings (Table: 4.23), who stated that neutropenia and neutrophilia occurred during infection of Toxoplasmosis, which depend on health of infected individual and many other aggravating factors. According to Bliss, (1999), Del Rio, (2001) and Bliss *et al.,* (2001), neutrophils are essential to control infection of *T. gondii* in mice, while Bliss (1999) stated that neutrophils also provide protection during Toxoplasmosis in

human host. During the current study, thrombocytopenia was also observed (>0.05) in some infected women (Figure: 4.23). Gürkan *et al.,* (2003) also claimed that *T. gondii* cause immune thrombocytopenia. Abhilash *et al.,* (2013) and Hassen *et al.,* (2019) also detected thrombocytopenia in patients, who were positive for *T. gondii* antibodies, which support our current findings (Table: 4.23). In clinical published literature, exact mechanism of Toxoplasmosis associated thrombocytopenia is unknown. There is still ambiguity between infection of *T. gondii* and its association with different abnormalities in hematological parameters, due to which it is suggested that further clinical studies under close monitoring system is most important on these aspects, which will provide comprehensive information related to various aspects of clinical and laboratory abnormalities that would improve patients' health and its clinical management. During the current study, assessment of selected parameters of liver profiles like AST and ALT was also carried out in women, who were positive for Toxoplasmosis (Table 4.26). Normal and elevated (>0.05) level of AST and ALT were found in infected women that suggest a mild effect of Toxoplasmosis infection on liver function in some infected individuals (Table: 4.26). In published clinical literature, there are controversial reports on hepatic abnormalities, associated with Toxoplasmosis. Some studies have shown effect of Toxoplasmosis on liver profile, while other found no significant (>0.05) effect on liver enzymes. Hassen *et al.,* (2019) reported a mild elevation in liver enzymes, which indicate that parasite may affect liver in such a way that these effects are not so sudden and sufficient to produce prominent clinical symptoms. Nunura *et al.,* (2010), Caselli *et al.,* (2012) and Strabelli *et al.,* (2012) claimed occurrence of moderate increase in liver aminotransferase enzymes in patients with hepatomegaly and hepatitis in primary infection of Toxoplasmosis, which support our current clinical findings (Table: 4.26). Similarly Pinon *et al.,* (1995) and Montoya and Liesenfeld (2004) detected increase level of liver enzymes in infected individuals, which reveals that liver tissues damage due to proliferation of parasite. El-Henawy *et al.,* (2015) reported necrosis in hepatic tissues during infection of *T. gondii.* Amany *et al.,* (2010), Al-Jowari and Hussein (2014) and Atmaca *et al.,* (2015) also reported involvement of liver and elevation of AST and ALT level in patients, who were infected with *T. gondii*. During the current study, a mild to moderate increase was observed in AST, ALT level (Figure: 4.25), which indicate that Toxoplasmosis have a mild effect on liver of some infected individuals and these parameters should be determined for accurate diagnosis, associated complications and clinical management of infection. During the current study, assessment of selected inflammatory markers (ESR and CRP) was also carried out in those women, who were positive for Toxoplasmosis (Table: 4.26). CRP was monitored in all infected women, but only 33.25% women were found, whose CRP was high from normal values (Table: 4.26), which suggest that beside specific diagnostic assays, CRP should also perform for monitoring and assessment of associated complication. Some low cost laboratory techniques like CBC, CRP and ESR are

easily available in most clinical laboratories in various developing countries, including Pakistan, which have important diagnostic role in various infections like Toxoplasmosis, when other expensive methods like ELISA, histopathological techniques, tissue culture and PCR are not frequently available. CRP is economical and important parts of non-specific immune response of human immune system and its increased level directly indicate infection and inflammation in body of infected individual (Sproston and Ashworth 2018). Mahboub *et al.,* (2013) stated that these changes in blood metabolites of infected individual can be used as an indicator for Toxoplasma and many other possible infections, which assist in diagnosis and detection of complication. Toxoplasmosis associated positivity of CRP was also reported by Birgisdottir *et al.,* (2006), who found a weak, but significant (<0.05) association between Toxoplasmosis and positivity of CRP in infected individuals, which strongly support our current results (Table: 4.26).

5.7 Treatment modalities for clinical management of Brucellosis, symptomatic improvement, therapeutic failure and various potential risk factors evaluated for therapeutic failure in women with Brucellosis infection in Khyber Pakhtunkhwa province of Pakistan.

Brucellosis is caused by intracellular bacteria of genus Brucella, which is a major zoonotic infection (Omolbanin Amjadi *et al.,* 2019) that manifests an acute febrile illness. Human infection is characterized by fever, chills and headache, which lead to chronic disease (Roushan and Ebrahimpour, 2015). The pathogen use various mechanisms and virulence factors for invasion and escaping from cellular immunity of host, which assists them to survive and replicate in intracellular environment of host tissues (Gopalakrishnan *et al.,* 2016). There is no vaccine for human protection, which is safe and approved by FDA, while treatment is also not more effective and reliable due to risk of therapeutic failure and relapse of infection (Omolbanin Amjadi *et al.,* 2019). Among 718 cases of Brucellosis, symptomatic improvement significantly (<0.05) occurred in 83.43% infected women, who were treated with various therapeutic regimens, while 16.57% women also showed persistent clinical symptoms and considered cases of therapeutic failure (Table: 4.27). The current results of our study is strongly supported by Franco *et al.,* (2007), who claimed that therapeutic failure occur in 5-15% cases of Brucellosis. It is essential to increase knowledge regarding pathogenesis of disease, diagnosis and therapeutic aspects of Brucellosis in order to manage and improve health of infected individuals. Being intracellular in nature**,** bacteria in genus Brucella invade and replicate within host cells, which protects them from immune responses, due to which bacteria persist and cause chronicity (Gomez *et al.,* 2013), therapeutic failure and relapse in infected individuals. For control of infection, prevention of complication, therapeutic failure and relapses of infection, it is critically important to treat infected individuals with those antibiotics for 8 weeks, which are orally more tolerable and effective Fanni *et al.,* (2013). Globally, various therapeutic regimens like

streptomycin, doxycycline, rifampicin, trimethoprim, sulfamethoxazole, gentamicin and ciprofloxacin are widely used for clinical management of Brucellosis in various geographical regions including Pakistan. More effective regimens for therapeutic management involve therapeutic combinations of two or three antibiotic. The clinician prescribes various therapeutic agents, which depend upon disease complexity and involvement of various body organs. According to WHO, (2006), treatment options for clinical management of Brucellosis include doxycycline 100 mg twice in a day for 45 days in combination with streptomycin 1g on daily basis for 15 days, while main alternative antibacterial therapy is doxycycline at 100 mg, twice a day for 45 days in combination with rifampicin (600-900 mg/day for 45 days. The data of current study (Table: 4.27) reveal inclusive clinical knowledge about various therapeutic regimens, symptomatic improvement and associated therapeutic failure, which provide a comprehensive clinical knowledge associated with various therapeutic regimens and in vivo efficacy of these antibiotics, which are recommended for clinical management of Brucellosis by clinicians, who are working in health sector in Pakistan. The current data related to therapeutic regimens (Table: 4.27) would help clinician to select effective therapeutic strategies for clinical management of Brucellosis in various endemic regions of Pakistan and other countries in the world. The study clearly revealed that various mono-therapeutic regimens like azithromycin, amoxicillin, ciprofloxacin, clarithromycin, ampicillin, cefixime, levofloxacin and tetracycline are still very effective for symptomatic improvement of Brucellosis in many patients, but therapeutic failure was also observed by these antibacterial agents as a mono-therapeutic agent (Table: 4.27), which indicate that some potential risk factors could persist behind therapeutic failure. Teker *et al.,* (2014) stated that bacteria of genus Brucella changes pH level in intracellular environment in host and those antibacterial therapeutic regimens must be given for effective therapeutic outcomes, which maintain maximum antibacterial activity in acidic conditions like doxycycline, rifampicin and trimethoprim/ sulfamethoxazole (TMP-SMX) combination, but we observed that many clinicians still preferred mono-therapeutic regimens for clinical management of Brucellosis (Table: 4.27). During the current study, doxycycline, streptomycin, ciprofloxacin, rifampicin and gentamicin were used in various therapeutic combinations for effective therapeutic outcomes (Table: 4.27)). Among 718 women patients, 1.41% patients were treated with therapeutic combination of doxycycline + rifampin + streptomycin, 1.81% with doxycycline + rifampicin + gentamycin and 3.06% with gentamicin + rifampicin + ciprofloxacin combination, which significantly (<0.05) improved clinical symptoms in all patients (100%) and no cases of therapeutic failure was observed, while among 3.62% infected cases, who were treated with therapeutic combination of doxycycline + streptomycin + ciprofloxacin, symptomatic improvement significantly (<0.05) occurred in 92.31% patients, while only 7.69% infected women were observed with therapeutic failure (Table: 4.27). Teker *et al.,* (2014) support results of our current study and reported a case, who

was allergic to TMP-SMX regimen, but she was successfully treated with therapeutic combination of rifampicin, ciprofloxacin and gentamicin, who was discharged with good clinical condition after treatment. During the current study, the efficacy of triple therapeutic regimens was significantly (<0.05) high (Table: 4.27). Kocagoz *et al.,* (2002) also support our current results (Table: 4.27) and claimed that single therapeutic regimens usually result in treatment failure, relapses and complications in infected individuals, which are identified by clinical examination and various other laboratory assays. They claimed that relapse occurred in 5-15% infected cases with use of traditional and single therapeutic regimen, which strongly support our current results (Table: 4.27). Ulu-Kilic *et al.,* (2014) recommended streptomycin for three week, after which doxycycline and rifampin should be started for achieving successful and more effective therapeutic outcomes in infected individuals. They claimed that streptomycin and doxycycline are antibacterial combination, which is most frequently used in clinical management of Brucellosis, but relapse usually occur in dual-therapeutic regimen. We observed a significantly (<0.05) high rate (15.39%) of therapeutic failure in those infected women, who were treated with dual therapeutic combination of doxycycline and streptomycin (Table: 4.27). To achieve results of successful and more effective therapeutic outcomes, Zheng *et al.,* (2018) stressed on prolong course of effective antibiotic and recommended that those individuals, who cannot tolerate or receive tetracycline due to pregnancy, trimethoprim and sulfamethoxazole should be used as alternative therapeutic combination, but we could not observe any infected woman, who was treated with therapeutic combination of trimethoprim and sulfamethoxazole. WHO recommend doxycycline and rifampicin in therapeutic combination for 42 days or streptomycin and doxycycline for 21 days, but we observed a significantly (<0.05) low rate of symptomatic improvement and high rate 15.39% of therapeutic failure in therapeutic combination of doxycycline, streptomycin, which was followed by 12.51% cases of drug failure in doxycycline, rifampicin combination, while rifampicin with doxycycline + streptomycin, doxycycline + gentamycin and gentamicin + ciprofloxacin showed a significantly (<0.05) high rate of symptomatic improvement (Table: 4.27). Rifampicin is still considered an effective therapeutic agent, which is very active in intracellular environment and is also used in clinical management of Tuberculosis. In published clinical literature, various studies showed that anti-Brucella activity of RIF was excellent due to strong cellular penetration power and effective synergism effect of drug with other therapeutic many other regimen. Sayan *et al.,* (2012) determined susceptibility of rifampicin against many isolates of *B. melitensis*. They found and reported that all identified isolates of Brucella were sensitive to RIF and only 2 isolates showed intermediate susceptibility, while no isolates was completely resistant to RIF. According to Yumuk and Dundar, (2012) and FAO, (2006), rifampicin is still more effective and used in clinical management of Brucellosis, which is widely used and accepted as a first-line therapeutic regimen

in clinical management of Brucellosis. Bao *et al.,* (2017) stated that rifampicin is also used with doxycycline for prophylaxis of diseases. Among 718 patients of Brucellosis, 3.34% cases were treated with therapeutic combination of doxycycline + rifampicin and 9.95% cases were clinically managed with doxycycline + streptomycin combination, which indicates that clinicians do not frequently prescribe these therapeutic combinations in various regions of Khyber Pakhtunkhwa province of Pakistan. Dizbay *et al.,* (2007) reported aminoglycoside, azithromycin and gentamicin as alternative therapeutic agent, but we observed with a high frequency of therapeutic failure (58.33%) in those women patients, who were clinically managed with azithromycin (Table: 4.27). During the current study, many patients were observed, who were treated with ciprofloxacin in single, dual and triple therapeutic regimen, but ciprofloxacin was significantly (<0.05) found more effective in dual and triple therapeutic regimens as compared to single therapeutic regimen (Table: 4.27). Many researchers like Trujillano-Martín *et al.,* (1999) and Kocagoz *et al.,* (2002) also stated that ciprofloxacin is more effective for drug resistance, antimicrobial toxicity and cases of relapse in Brucellosis. They further suggested that ciprofloxacin should not be used as a single therapeutic agent, but use in combination with rifampicin due to its good in vitro activity. Ciprofloxacin showed no therapeutic failure in combination with gentamicin and rifampicin, while rate of therapeutic failure was high 35.29% in single therapeutic regimen. Tanir *et al.,* (2009) reported effective treatment of Brucellosis with therapeutic combination of rifampicin, ciprofloxacin and gentamicin, which strongly support our current findings (Table: 4.27). Frequency of therapeutic failures was significantly (<0.05) different in various therapeutic regimens (Table: 4.27), which indicate that accurate therapeutic approach is essential for effective clinical management of Brucellosis, because bacteria of genus Brucella is intracellular in nature and less effective treatment can lead to significant complications, therapeutic failure and relapse of disease. It is necessary to treat patients of Brucellosis with more effective antibacterial drug, when drug failure, disease relapse and adverse reactions appeared during clinical management of Brucellosis with a particular antibiotic. During the current study, various parameters like therapeutic regimens, disease stage, hemoglobin level, obstetric status, ages and gravidity of patients were evaluated to determine risk factors of therapeutic failure (Table: 4.28). A significant (<0.05) association was found with therapeutic failure and various risk factors such as type of drug regimen, stages of disease and hemoglobin (Hb) level, while no significant (>0.05) association was found with other parameters like obstetric status, age and gravidity of women (Table: 4.28). During the current study, frequency of therapeutic failure was significantly (<0.05) high in those women, who were treated with single drug regimen (21.98%), chronic cases of Brucellosis (45.09%), and those women, who were anemic (23.15%). Similarly therapeutic failure was also found (>0.05) high in those women, who were pregnant 17.92%, more than 30 years old 17.16% and 17.31% cases in those

women, who were multigravida (Table: 4.28). The exact cause and mechanisms of therapeutic failure or relapse during Brucellosis is still not clear in published clinical literature, but some factors were assumed and considered as potential risk for therapeutic failure or relapse in disease. During a clinical study on 115 patients, Alavi *et al.,* (2009) found a significant association (<0.05) between relapse of Brucellosis and lymphopenia, high ESR and CRP level, delay in treatment, individuals with old age group and male sex, which strongly support our current results (Table: 4.28). They also associated drug failure with various strains of Brucella, host cellular immunity and type of infection (localized or systemic), while Ariza *et al.,* (1995) suggested that low dosage of antibiotic, prolonged positivity of blood culture, delay in treatment and thrombocytopenia were some predictive factors for therapeutic failure and relapse in Brucellosis. Effective treatment is only possible with antibacterial agents, which are intracellular effective and low risk of resistance. During the current study, frequency of therapeutic failure was significantly (<0.05) high in those infected women, who were treated with mono-therapeutic drug as compared to dual and triple drug regimen (Table: 4.28), which could be due to intracellular nature of Brucella, various therapeutic combination, different clinical stages of infection and immunity of patients. Al-Anazi and Al-Jasser (2016) also claimed that mono-therapy is usually associated with drug failure or relapse, while specific antibacterial combinations are more effective for successful recovery, which strongly support our current results (Table: 4.27). During the current study, dual drug combination of doxycycline and gentamycin was more effective as compared to doxycycline and streptomycin (Table: 4.27). WHO also claim that combination of doxycycline and gentamicin are more effective therapeutic regimen for clinical management of Brucellosis, which strongly support our results. According to CDC (2017), dual-therapeutic regimen should be administered for many weeks, but concerned clinician will decide duration and dosage of antibiotic. Yousefi-Nooraie *et al.,* (2012) stated that WHO also recommend drug combination of tetracycline with amino-glycoside or tetracycline with rifampicin. During the current study, only 1.81% women were treated with dual therapeutic combination of streptomycin and tetracycline by clinician in various geographical regions of the province and 15.38% of them showed therapeutic failure (Table: 4.27), which clearly indicate that due to clinical experience, physicians do not frequently recommend drug combination of streptomycin and tetracycline during their clinical practices in Khyber Pakhtunkhwa province of Pakistan (Figure: 3.1). They usually recommend doxycycline, gentamycin regimen, which showed a significantly (<0.05) low rate 6.52% of therapeutic failure and high rate 93.48% of symptomatic improvement (Table: 4.27). During the current study, a significantly (<0.05) high rate 45.09% of therapeutic failure was observed in chronic cases of Brucellosis (Table: 4.28). Kulakov, (2016) also support our current findings and claimed that existing drug combination schemes of streptomycin with rifampicin or tetracycline are not so effective in chronic cases of Brucellosis. Various

factors are involved in chronicity of Brucellosis, including lack of awareness and coordination among clinicians, non-availability of reliable and confirmatory diagnostic assays in rural area of Pakistan, due to which diagnosis of Brucellosis is either missed or delayed, which lead to delay in treatment of infected individuals and lead to chronicity of Brucellosis. According to Alavi *et al.,* (2009), therapeutic failure, relapse and chronicity are some specific characteristic of Brucellosis. Nonspecific and less-effective antibiotic, therapeutic failure and relapse usually lead disease from acute to sub-acute and chronic stages of infection. According to Yun *et al.,* (2017), lipopolysaccharide (LPS) in cells of bacteria in genus Brucella is a major virulence factor, which is also responsible for chronicity of infection. The LPS layers shows low toxicity and its strange structure was assumed to delay host immune response and favor bacteria for establishment of chronic infection (Yun *et al.,* 2017), which cause serious therapeutic challenges in infected individuals. Clinician recommend different therapeutic regimen for various clinical stages of Brucellosis. According to Ersoy *et al.,* (2005), WHO recommends therapeutic combination of doxycycline and rifampicin 600 mg for six weeks in acute stages of Brucella infection, but during the current study, a high rate of therapeutic failure was observed in our women patients, who were treated with therapeutic combination of doxycycline and rifampicin (Table: 4.27), which could be due to various factors like irregular and low use of antibacterial dosage, weak immunity, patient health and different strains of Brucella. Those patients, who were treated with therapeutic regimens like azithromycin, ciprofloxacin, cefixime and tetracycline showed different rate of therapeutic failure, which is strongly supported by other researcher like Tanyel *et al.,* (2007) and Ayaşlioğlu *et al.,* (2008), who reported different pattern of Brucella susceptibility to drugs like azithromycin, rifampicin, tetracycline and ciprofloxacin. For best clinical management of Brucellosis, effective antibiotics must be selected and used in various therapeutic combination, as various monotherapeutic regimen significantly (<0.05) showed a high rate of therapeutic failure (Table: 4.27). Al Johani (2014), Neto *et al.,* (2014) and Hashim *et al.,* (2014) claimed that regional variations occurred in antimicrobial susceptibility of Brucella strains. Therefore, clinicians should strongly recommend antibiotic susceptibility assay before recommending a particular antibacterial therapeutic drugs, which would reduce many challenges in therapeutic failure and relapses during clinical management of Brucellosis infection in infected individuals.

5.8 Conclusion: The current study showed an alarming situation of zoonotic diseases (Brucellosis, Toxoplasmosis) in women community in various geographical regions like Malakand, Peshawar, Hazara, Kohat, Mardan, D.I Khan and Bannu division of Khyber Pakhtunkhwa province of Pakistan, which indicate that Pakistan is endemic zone for these emerging zoonotic infections due to weak animal and public health system. Unfortunately, these infections are gradually transmitting from infected animals to women population due to various socio-demographic factors like resident in rural area, low educational

level, low socio-economic states, bad hygienic condition, lack of knowledge about zoonotic diseases, as well as persistent of various epidemiological factors like animals at homes, shearing of shelter with animals, exposure to animals, dealing and management of animal waste, shelters, assist animal births, abortion, stillbirth, cutting and handling of animals meat at home for cocking, drinking raw or unpasteurized milk, dairy products, processing animal milk and dairy products at homes, habits of eating roasted and under cocked meat, beef, eating of raw vegetables, salad and unwashed fruits. Moreover, most of families store animals manure in homes and near homes for agriculture purposes and drink water from agricultural channels and rivers, which are not safe for human health due to contamination with fecal materials of wild and domesticated animals. There is no vaccine of Brucellosis and Toxoplasmosis for human, which is safe and approved by FDA, while treatment is also not so effective and reliable due to risk of therapeutic failure and relapse of infection. There is lack of proper knowledge about these diseases among human community and co-existence of social-cultural and animal husbandry activities promote transmission of zoonotic infection in women community, while diagnostic challenges still persist in the region. The animal owners have still adopted old methods of animal husbandry practices, which facilitate spread of zoonotic diseases in the region. Due to poverty and lack of resources, most of the families in rural areas keep livestock animals in sleeping rooms in their home for protection from rains and cool weather during rainy and winter season. These zoonotic diseases are becoming a serious public health problem in women of reproductive age group, as these infection cause serious clinical complications such as anemia, various other hematological and biochemical abnormalities and adverse obstetric outcomes such as early or late abortion, intrauterine fetal death (IUFD), preterm birth, still birth, congenital anomalies like microcephales, macrocephales, mental retardation, blindness, ocular abnormalities, deepness and many other postnatal problems. Due to unauthorized medical practices, non-specific clinical symptoms and medications, lack of proper awareness and diagnostic facilities, these emerging zoonotic infections usually misdiagnosed and lead to chronicity in infected individuals, which cause various clinical, diagnostic and therapeutic challenges for clinician. Various cheap serological test kits are used for diagnosis of Brucellosis and Toxoplasmosis in local diagnostic laboratories in our province, which is not 100% sensitive and specific and cannot differentiate to diagnose false negative and positive cases of these emerging infections. ELISA and PCR are confirmatory assays for diagnosis of these infections, which is still not frequently available in local diagnostic centers in Pakistan. These assays are available in some private expensive diagnostic laboratories in Pakistan, where majority patient cannot access due to unawareness, expensive rates, poverty and living in rural areas, which compels clinician for relay on single serological assay. Advanced diagnostic assays are important for clinical differentiation and management of acute, sub-acute and chronic cases of Brucellosis and Toxoplasmosis

infection. The study also revealed a comprehensive clinical knowledge related to therapeutic modalities and failure of various antibacterial regimens frequently recommended for clinical management of Brucellosis infection. Monotherapy was not so effective, while dual and triple drug regimen has shown good clinical response. The data provide a comprehensive clinical knowledge related to in vivo efficacy of various antibiotics, which will help to select effective antimicrobial therapeutic strategies to improve patient's health. Various mono-therapeutic regimens are still effective in many patients, but therapeutic failure also occurred in some cases. Due to intracellular replicating nature, those antibiotics should be given, which can maintain maximum antibacterial activity. Moreover antibiotic susceptibility assay should be guided before prescribing a particular course of therapeutic regimen. Assessment of patients for disease stage, anemia, gravidity, pregnancy status, ages and body weight should be top priority of clinician before prescribing a particular course of antibiotic. Persistent clinical symptoms of Brucellosis after using a particular recommended therapeutic regimen for at least 2-3 week should be followed and confirmed through clinical and laboratory assays like SPAT, STAT, RBPT, ELISA or PCR assays for other possible and effective alternative therapeutic regimens. Human infection can be controlled by controlling disease in livestock animals through joint efforts of animal and public health authorities, technical and financial cooperation of various regional and international organizations. There is no screening program for surveillance, understanding epidemiology, awareness and control of such neglected zoonotic infections in Pakistan, which is a potential threat for pregnant women. Therefore we strongly recommend that WHO and other health authorities may launch awareness and screening program for proper control of these emerging zoonotic infections and associated adverse obstetric outcomes in Pakistan and other endemic countries of the world.

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Annexure: A

QUESTIONNAIRE

Date: Patient ID: _____ Age: _____Division/District:_____
Pregnancy statues: _____ No of Children:_____
Main Clinical complaint: _____
_____
_____Duration:_____
History of Adverse Obstetric outcome:_____
Clinical, Physical and abdominal sonographic Features/Findings:

_____
_____

Patients Socio-demographic characteristics:

1. Type of Residence: Urban/Rural 2. Educational status of Patients:_____
2. Occupation status_____ 4.Socio-economic statues of Patients:_____

5. Patients Hygienic statues: _____ 6. Knowledge of Zoonotic Infections:_____

Potential Risk Factors Associated with Zoonotic Diseases (Brucellosis, Toxoplasmosis)

1. Animals at home: Yes/No 2.Shearing shelter with animals during winter: Yes/No
3. Exposure to animals/cleaning animal waste/shelters:Yes/No
4. Assisted animal births/abortion/stillbirth:Yes/No
5. Cutting/ handling animals meat at Home for cocking:Yes/No
6. Proper Hand washing habit after cutting meat/milking and caring animals/other works:Yes/No
7. Previous, Recent History/Habit of Drinking/Raw/unpasteurized milk/dairy products:Yes/No
8. Processing animal Milk/dairy Products at Homes:Yes/No
9. Recent/past Family history of zoonotic infections (Brucellosis, Toxoplasmosis: Yes/No
10. Blood transfusion History:Yes/No
11. Habits of Eating roasted/under cocked meat/beef:Yes/No
12. Habits of eating raw vegetables/salad/unwashed fruits:Yes/No
13. Drinking Filter/boiled water at homes:Yes/No
14. Store animals waste/manure in homes/near homes for agriculture purposes: Yes/No

Diagnosis for Zoonotic Diseases:
Brucellosis: SPAT Test: _____ __ RBPT Test:_____ ELISA :
IgM_____IgG:_____PCR_____
Toxoplasmosis: ICT Test:_____ LAT Test:_____ ELISA: IgM_____IgG:_____
Hematological Parameters:_____
_____
Inflammatory Markers: ESR:_____CRP mg/L_____
Serum Biochemistry: AST U/L:_____ ALT U/L:_____
Treatment modalities for Brucellosis Management and its Associated Outcome Observed:

_____
_____

Tropical Medicine and Infectious Disease

MDPI

Article

Assessment of Geographical Distribution of Emerging Zoonotic *Toxoplasma gondii* Infection in Women Patients Using Geographical Information System (GIS) in Various Regions of Khyber Pakhtunkhwa (KP) Province, Pakistan

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Abstract: Toxoplasmosis is a zoonotic parasitic disease caused by *T. gondii*, an obligate intracellular apcomplexan zoonotic parasite that is geographically worldwide in distribution. The parasite infects humans and all warm-blooded animals and is highly prevalent in various geographical regions of the world, including Pakistan. The current study addressee prevalence of Toxoplasma infection in women in various geographical regions, mapping of endemic division and t district of Khyber Pakhtunkhwa province through geographical information system (GIS) in order to locate endemic regions, monitor seasonal and annual increase in prevalence of infection in women patients. Setting: Tertiary hospitals and basic health care centers located in 7 divisions and 24 districts of Khyber Pakhtunkhwa (KP) province of Pakistan. During the current study, 3586 women patients from 7 divisions and 24 districts were clinically examined and screened for prevalence of *T. gondii* infection. Participants were screened for Toxoplasma infection using ICT and latex agglutination test (LAT) as initial screening assay, while iELISA (IgM, IgG) was used as confirmatory assay. Mapping of the studied region was developed by using ArcGIS 10.5. Spatial analyst tools were applied by using Kriging/Co-kriging techniques, followed by IDW (Inverse Distance Weight) techniques. Overall prevalence of *T. gondii* infection was found in 881 (24.56%) patients. A significant (<0.05) variation was found in prevalence of infection in different divisions and districts of the province. Prevalence of infection was significantly (<0.05) high 129 (30.07%) in Kohat Division, followed by 177 (29.06%), 80 (27.87%), 287 (26.72%), 81 (21.21%), 47 (21.07%), and 80 (13.71%) cases in Hazara Division, D.I Khan Division, Malakand Division, Mardan Division, Bannu Division, and Peshawar Division. Among various districts, a significant variation (<0.05) was found in prevalence of infection. Prevalence of infection was significantly (<0.05) high 49 (44.95%) in district Karak, while low (16 (10.81%) in district Nowshera. No significant (>0.05) seasonal and annual variation was found in prevalence of Toxoplasma infection. LAT, ICT and ELISA assays were evaluated for prevalence of infection, which significantly (<0.05) detected *T. gondii* antibodies. LAT, ICT and ELISA assays significantly (<0.05) detected infection, while no significant (>0.05) difference was found between positivity of LAT and ICT assays. A significant difference (<0.05) was found in positivity of Toxoplasma-specific (IgM), (IgG) and (IgM, IgG) immunoglobulin by ICT and ELISA assay. The current study provides comprehensive information about geographical distribution, seasonal and annual variation of Toxoplasmosis infection in various regions of Khyber Pakhtunkhwa province of Pakistan. Infection of *T. gondii* in women shows an alarming situation of disease transmission from infected animals in the studied region, which is not only a serious

قائداعظم یونیورسٹی

QUAID-I-AZAM UNIVERSITY
Faculty of Biological Sciences
Bioethics Committee

No. #BEC-FBS-QAU2019-145 Dated: 22-03-19

Prof. Dr. Sarwat Jahan,
Department of Animal Sciences,
Faculty of Biological Sciences,
Quaid-i-Azam University, Islamabad
45320, Pakistan

Subject:- **"Molecular Epidemiology, Clinical Aspects and Adverse Obstetric Outcomes of Emerging Zoonotic Diseases in Women of Khyber Pakhtunkhwa, Pakistan."**

Dear Prof. Dr. Sarwat Jahan,

We wish to inform you that your subject research study has been reviewed and is hereby granted approval for implementation by Bio-Ethical Committee (BEC) of Quaid-i-Azam University, Your study has been assigned protocol **#BEC-FBS-QAU2019-145.**

While the study is in progress, please inform us of any adverse events or new, relevant information about risks associated with the research. In case changes have to be made to the study procedure, the informed consent from and or informed consent process, the BEC must review and approve any of these changes prior to implementation.

Sincerely,

Dr. Bushra Mirza
Department of Biochemistry

cc:
Dean, F.B.S

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