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Epidemiological Investigation of Brucellosis, Toxoplasmosis and Coxiellosis Associated with Reproductive Disorders in Small Ruminants

Islam, Md. Hemayatul
University of Rajshahi

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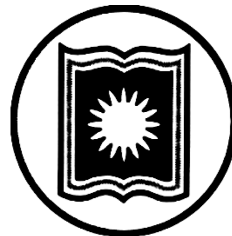

THESIS SUBMITTED FOR THE DEGREE
OF
DOCTOR OF PHILOSOPHY
IN THE
INSTITUTE OF BIOLOGICAL SCIENCES
UNIVERSITY OF RAJSHAHI
BANGLADESH

BY

MD. HEMAYATUL ISLAM
DVM; MS in Obstetrics
Institute of Biological Sciences
University of Rajshahi

DECEMBER, 2015

INSTITUTE OF BIOLOGICAL SCIENCES
UNIVERSITY OF RAJSHAHI
RAJSHAHI-6205
BANGLADESH

Dedicated

To

My Beloved Parents

Ph.D
Thesis

Epidemiological Investigation of Brucellosis, Toxoplasmosis and Coxiellosis Associated with Reproductive Disorders in Small Ruminants

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RAJSHAHI-6205
BANGLADESH

DECLARATION

I the undersigned hereby solemnly declare that the entire works submitted in the thesis titled **"Epidemiological Investigation of Brucellosis, Toxoplasmosis and Coxiellosis Associated with Reproductive Disorders in Small Ruminants''** to the Institute of Biological Sciences (IBSc), University of Rajshahi, Bangladesh for the Degree of Doctor of Philosophy (Ph.D) is the result of the original investigation carried out by researcher. This work was Supervised by **Professor Dr. Md. Jalal Uddin Sarder**, Department of Animal Husbandry and Veterinary Science, University of Rajshahi and Co-supervised by **Professor Dr. Md. Siddiqur Rahman**, Department of Medicine, Bangladesh Agricultural University, Mymensing, Bangladesh.

To the best of my knowledge and belief it contains no material previously published or written by any other person except when due reference is made in the text of the thesis.

December, 2015

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The Author

Md. Hemayatul Islam

ABBREVIATIONS AND ACRONYMS

Ab neg/ Ab (- ve)	Negative Antibody
Ab Pos/ Ab (+ ve)	Positive Antibody
ABTS	2, 2'- azino-bis-(3- ethylbenzothiazoline-6- sulphonic acid)
Ag	Agglutination
B	*Brucella*
BAU	Bangladesh Agricultural University
BVD	Bovine Viral Diarrhoea
C	*Coxiella /* Control
C. burnitii	*Coxiella burnitii*
C-ELISA	Competitive-Enzyme Linked Immunosorbent Assay
CFT	Compliment Fixation Test
CP	Calculate Percentage
DF/ df	Degrees of Freedom
DLS	Director of Livestock Services
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleoside triphosphates
EC	European Commission
EEC	European Economic Council
ELISA	Enzyme Linked Immunosorbent Assay
et al.	et alii (and others)/ et alia
EU	European Union
FAO	Food and Agriculture Organization
FPA	Fluorescent Polarization Assay
GDP	Gross Domestics Product
H_1	Alternate Hypothesis
H_o	Null Hypothesis
I-ELISA	Indirect Enzyme Linked Immunosorbent Assay
IFAT	Indirect Fluorescents Antibody Test
IgA	Immunoglobin A
IgE	Immunoglobin E
IgG	Immunoglobin G
IgM	Immunoglobin M
IHA	Indirect Hemagglutination
INRA	Institute National Research Agronomique
LAT	Latex Agglutination Test

MAT	Modified Agglutination Test
MCD	Microscopic Cyst Detection
MEAT	Mercaptoethanol Agglutination Test
$MgCl_2$	Magnesium chloride
Ml	Milliliter/s
MLE	Multilocus Enzyme Electrophoresis
MLST	Multilocus Sequence Typing
MQ PCR	Multiplex Quantitative Polymerase Chain Reaction
MR	Microplate Rider
MRI	Magnetic Resonance Imaging
MRT	Milk Ring Test / Milk Ring Test
MS	Microsatellite
MT	Metric ton
N	Negative
NBT	Nothern Barind Tract
NC	Negative Control
nPCR	Nested Polymerase Chain Reaction
NPV	Negative Predictive Value
NRL	National Reference Laboratory
NS	Non-Significant
OB	Ovine Brucellosis
OD	Odd Ratio/ Optimal Density
OIE	Office International Des Epizooties
OT	Ocular Toxoplasmosis
P	Positive / Probability
PAT	Plate Agglutination Test
PBS	Phosphate Buffer Saline
PC	Positive Control
PCR	Polymerase Chain Reaction
PP	Percent Positivity
PPV	Positive Predictive Value
PVC	Poly Vinyl Chloride
Q fever	Queensland Fever
qPCR/ Q PCR	Quantitative Polymerase Chain Reaction
R	Correlation Coefficient/ Reference Category
RAPD	Random Amplification Polymorphic DNA
RB	Repeat Breeding

RBT	Rose Bengal Test
RD	Reproductive Disease(s) / Reproductive Disorder/s
RFLP	Restriction Fragment Length Polymorphism
RP	Retained placenta
RSPAT	Rapid Serum Plate Agglutination Test
RT PCR	Real Time Polymerase Chain Reaction
RU	University of Rajshahi
SAG	Surface Antigen
SAT	Serum Agglutination Test/ Slow Agglutination Test
SD	Standard Deviation
S-LPS	Specific Lipopolysaccharide
SNP	Single Nucleotide Polymorphism
SP	Serum Positivity
SPSS	Statistical Package for Social Science
SR	Small Ruminants
STAT	Standard Tube Agglutination Test
STAT	Serum *Toxoplasma* Antibody Test
T	*Toxoplasma*
T. gondii	*Toxoplasma gondii*
TAT	*Toxoplasma* Antibody Test/Tube Agglutination Test
TBE	Tris Borate EDTA
TE	Toxoplasmic Encephalitis
TMB	Tetra Methyl Benzidine
UTI	Urinary Tract Infection
VP	Vaginal Prolapse
YTC	Youth Training Center
χ^2	Chi Square Test
%	Percentage (S)

ABSTRACT

Islam, M.H. (2015). Epidemiological Investigation of Brucellosis, Toxoplasmosis and Coxiellosis Associated with Reproductive Disorders in Small Ruminants. Ph.D Thesis. Institute of Biological Sciences, University of Rajshahi, Rajshahi-6205, Bangladesh, pp:1-282.

The present study was to evaluate the "Epidemiological Investigation of Brucellosis, Toxoplasmosis and Coxiellosis Associated with Reproductive Disorders in Small Ruminants" (SR) at Northern Barind Tract (NBT) in Bangladesh from January, 2012 to June, 2015. A total 2667 small ruminants were selected and recorded 270 (10.2%) reproductive disorder cases, also 475 blood samples were collected from those animals. The serum sample was then subjected for testing of *Brucella* and *Toxoplasma*. The common *Brucella* & *Toxoplasma* positive and abortion occurred during the time of study were about 91 serum samples plus aborted tissue (20) were tested for *Coxiella* by using ELISA & PCR. The whole research was divided into 4 Experiments to achieve the goals successfully.

Experiment I: Retrospective study of reproductive diseases in small ruminants

This study was to determine the pattern of reproductive diseases at NBT in Bangladesh. Records of 2667 clinical cases of small ruminants (2394 goats, 273 sheep) from questionnaires report under this study, during July 2012 to June 2013 were analyzed to assess the importance of existing diseases. The small ruminants were significantly ($P<0.05$) suffering from various diseases and disorders. The maximum 816 (30.6%) small ruminants were affected by infectious diseases, whereas, reproductive disease prevalence was 10.2%. The highest prevalence of diseases (89.8%) was observed in goat than in sheep (10.2%). The highest & lowest prevalence of reproductive disorders in relation to sex were 32.5% & 1.9% in anoestrus and posthitis. The age had significant effect ($P<0.05$) and highest value were 20.4%, 7.9% & 4.5% observed in anoestrus of young, adult and old ages, respectively. The season had significant effect ($P<0.05$) and the overall disease prevalence was higher in rainy season (40.4%), but the individual highest value observed in anoestrus 14.7% in winter season. Among the total diseases approximately, 10% reproductive diseases and disorders were recorded in SR. Moreover, the gout species, female animal, young age, rainy season were greater chance to attack RD, particularly an anoestrus problem of small ruminants at NBT in Bangladesh.

Experiment-II: Investigation on Brucellosis associated with reproductive disorders in small ruminants

A seroepidemiological study of *Brucella* infections was in small ruminants at NBT in Bangladesh and a cross-sectional multi-stage sampling technique was employed to select 475 small ruminants, 396 goats and 79 sheep from the target populations. Serum was collected from the animals, and serially tested using Rose Bengal Test and iELISA according to the protocol recommended by the manufacturer. The overall prevalence of brucellosis was 12.0%, 9.3%, and 2.7% in SR, goats and sheep, respectively. Brucellosis was insignificantly ($P>0.05$) highest positive reactors value in species, age, parity, flock size and location were 5.6%, 8.9% in older goat and sheep; 7.6%, 5.5% and 15.7% 1st parity in small ruminants, goat and sheep, 8.2% small flock of small ruminants; 5.9%, 1.5%, 2.3% and 2.3% into Rajshahi, Chapai Nawabgonj, Natore and Naogaon district at NBT in Bangladesh. The brucellosis was higher insignificant ($P>0.05$) negative correlation with Local breed (6.9%); heavy body weight (6.1%) and rainy seasons

(6.1%) of small ruminants in study area. Brucellosis was positive and highly significant effect (P<0.01) of female animal (9.5%) but negative effect with traditional biosecurity (8.2%) and grazing (8%) in SR. The seropositive rate of *Brucella* was higher in non-pregnant (4%) than pregnant (4%) and aborted (2.1%) animals with reproductive disease concern at NBT in Bangladesh.

Experiment-III: Assessment of Toxoplasmosis linked with reproductive disorders in small ruminants

The 3rd experiment was carried out on 475 of SR serum samples were subjected to test for *Toxoplasma* anti-*T. Gondii* antibodies using kit for human Toxo test MT (Eiken Kagaku, Japan) according to the protocol recommended by the manufacturer. The overall seroprevalence of toxoplasmosis was 56%. The highest prevalence of toxoplasmosis was recorded in goat (45.7%), local breed (31.2%), female animal (34.3%), older age (26.1%), 1st parity (31.4%), medium body weight (23.7%), small size flock (38.7%), traditional biosecurity (30.9%), grazing habit (26.9%), summer season (20.0%), Rajshahi region (30.9%), non-pregnant (49.3%) in SR and abortion (10.7%) cases according to their influencing factors. The prevalence *Toxoplasma gondii* opportunity increased in female, Local breed, goat species, older age, smaller flock size, traditional biosecured farm, grazing habit, rainy season, aborted condition and Rajshahi distract (more urbanization area) at NBT in Bangladesh.

Experiment-IV: Survey of Coxiellosis related to reproductive disorders in small ruminants

The experiment-4 was designed to find out the other zoonotic diseases present in collected samples. For that, the positive *Brucella, common Brucella and Toxoplasma* positive and randomly selected (location wise) total 91 serum samples were send to National Reference Laboratory (NRL) in Germany to identify *Coxiella* in small ruminants. Serological test was performed by iELISA on goat and sheep database created with Microsoft Office software Excel 2007 (Microsoft®, Redmond) and SPSS program. An overall seroprevalence of *Coxiella* was found 11%. Goats was significantly (P<0.01) lower seroprevalence than sheep, respectively 5.6% and 30.0%. Small ruminants had significantly (P<0.05) higher chance of *Coxiella* in animals reared at smaller size flock (13.1%) and city adjacent to border (Rajshahi, Naogaon & Chapai Nawabjong) than their corresponding groups. The seropositivity was higher in Local breed (7.7%), female animals (7.7%), older age (6.6%), medium & heavy body weight (5.5%), traditional biosecurity (6.6%) and rainy season (7.7%) than others groups and had insignificant effect (P>0.01). The parity and pregnancy status of SR were negative correlation but significant effect (P<0.01) with Q fever infection in experimental area. The animals from the stall feeding (4.4%) had negative but insignificant (P>0.01) association and lower seroprevalence than animals from the grazing on field (6.6%). First report, the seroprevalence of *Q* fever in goat and sheep in Bangladesh is established now. Female Local breed from goat and sheep having lower body weight, older age, > 3rd parity, traditional biosecurity farms and grazing in field were more chance to Q fever attack.

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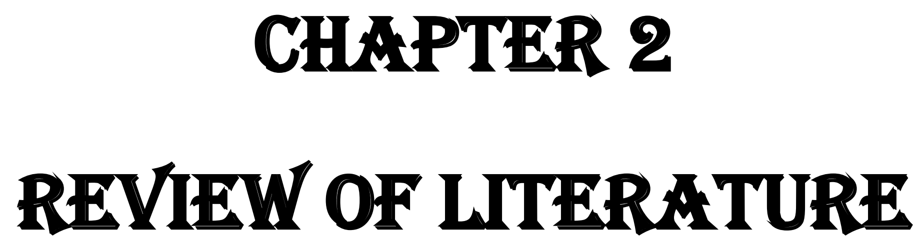
CHAPTER 1
INTRODUCTION



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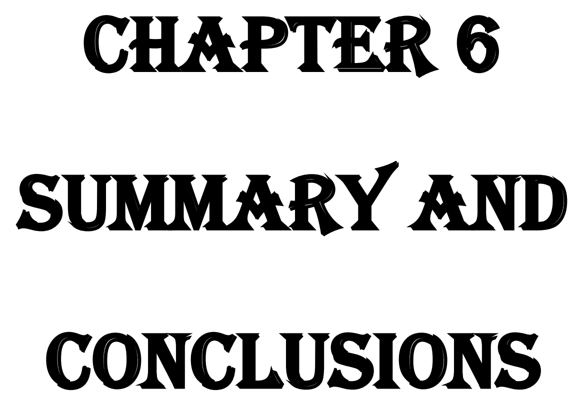

CHAPTER 6

SUMMARY AND CONCLUSIONS

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CHAPTER 1

INTRODUCTION

The livestock sub-sector provides full time employment for 20% of the total population and part-time employment for another 50% (Begum *et al*., 2011). The GDP contribution of this sub-sector has been a modest 2.6% annually in the 1990s (IMF, 2005) which was lower than the previous estimates of 5% of total and 10% of agricultural GDP during the 1970s and 1980s (FAO, 1990). The world total of small ruminants is 1940.1 million about and the specific numbers of goats and sheep were 861.9 and 1078.2 million, respectively, i.e. there is about one goat to approximately 1.25 sheep in the world (FAOSTAT, 2008). In Asia total small ruminants is 966.7 million and it is about 50 percent world SR population. There is an estimated 51.67 million livestock population in which 23.12 million cattle, 1.39 million buffaloes, 24.15 million goats, 3.00 million sheep reared in Bangladesh (Bangladesh Economic Review, 2012). Whereas Bangladesh the total number of small ruminants population is 58 million and it is about 3 percent of world total SR population (FAOSTAT, 2008). Small ruminants are especially important to woman, children and the aged, who are often most vulnerable members of the society. Goats were among the first farm animals to be domesticated. As indicated by the archaeological evidence, they have been associated with man in a symbiotic relationship for up to 10,000 years (Ensminger and Parker, 1986). Small ruminants disseminated all over the world because their great adaptability to varying environmental conditions and the different nutritional regimes under which they were evolved and subsequently maintained. They proved useful to man throughout the ages due to their productivity, small size, and non-competiveness with him for food. There are many things can disrupt a healthy pregnancy in a doe and ewe. While it is common for about 25% of embryos to die or reabsorbed the first three weeks of pregnancy up to the time of implantation, these are the most crucial in establishing healthy pregnancies.

The nutritional requirements of ewes during early gestation is only slightly more than maintenance requirements, but it is essential that the flock not be exposed to any undue stresses. It appears normal for about 1.5 to 2.0% (up to 5%) of the ewes in a flock to abort. The small ruminant's i.e. goats were among the first farm animals to be domesticated. As indicated by the archaeological evidence, they have been associated with man in a symbiotic relationship for up to 10,000 years (Ensminger and Parker, 1986). Goats disseminated all over the world because their great adaptability to varying environmental conditions and the different nutritional regimes under which they were evolved and subsequently maintained. They proved useful to man throughout the ages due to their productivity, small size, and non-competiveness with him for food. In many parts of the world where the geophysical properties of the terrain are not suitable for other livestock species, goats seem to be the best choice. The role of goats in supplying food to humans has been well stated by many researchers (Devendra, 1985). Based on the accumulated information on goat characteristics, it can be stated that goats have a specific place in the animal agricultural economy of many countries. These characteristics can be summarized in the following points:

1. Goats can withstand heat stress and can endure prolonged water deprivation. They have additionally great adaptability to adverse climatic and geophysical conditions, where cattle and sheep cannot survive.

2. They can efficiently utilize poor quality forage and cover long distances looking for food. Their peculiar feeding habits make it easier to choose diets to meet their requirements.

3. Goats are the most prolific domesticated ruminants. Faster reproduction contributes to the genetic progress that can be achieved and enables their owners to recover quickly.

4. Farmers and pastoralists are increasingly relying on goats as means of survival and a way of boosting their income (Peacock, 2005). The

increasing frequency of droughts, with long-term environmental degradation is causing pastoralists to change from cattle or sheep to camels or goats.

5. Overgrazing makes rangelands increasingly suitable for browsing species such as goats.
6. The widespread decline in services supplied by governmental agencies encourages farmers to move from keeping cattle to goats.
7. Goats provide their owners with a broad range of products and socio-economic services and have played an important role in the social life of many people being used as gifts, dowry, in religious rituals and rites of passage (Peacock, 1996).

Goats, especially dairy ones, are an ideal species for poverty reduction and economic development for the poor in developing countries. Several reasons make goats particularly attractive for poverty reduction and improvement of family food security and livelihood of the poor in developing countries:

a) Goats are easily acquired by the poor as they require modest starting capital.
b) They can easily be tended by the weak, women or children.
c) They provide people by valuable nutrients.
d) Many people cannot drink cow milk as they are allergic to it. Several studies indicated that people with cow's milk allergy could tolerate goat's milk (Restani, 2004).
e) The growing demand for goat meat presents an opportunity for goat fattening.

The goat is an important source of meat and milk in Asia and Africa.In Bangladesh, goats occupy the second largest livestock population and is called poor men's cow. It plays an important role not only in the rural economy but

also in the national economy of Bangladesh. The current goat population cannot meet the available demand. The availability of ready market for goats, short generation lengths, potentially high reproductive rates and production efficiency make goats the best alternative to cattle production in Bangladesh especially in areas where land is limited. Consequently, many farmers, especially small-scale farmers have adopted goat rearing. Sheep is an essential component among livestock in agriculture based production system. They sustain the employment and incomes of millions of people in rural areas contribute food and cash security available to many poor people. Economic value of sheep is accounted for their good quality tender meat, prolificacy, higher fertility, early sexual maturity, good quality wool and skin. As rearing sheep is very economic, they are also called the poor men's cow. A substantial amount of foreign currency is earned every year by exporting skin and other by products.

In Bangladesh, sheep occupies a very significant position after goat as animal source. There are about 3 million sheep in Bangladesh (Bangladesh Economic Review, 2013). Among the livestock populations sheep still now occupies the third position and about 80% sheep reared by rural farmer in small scale in Bangladesh. They have risen usually under free-range system or in adjunct to crop production. The sheep in Bangladesh are mainly utilized for meat purposes but also important for good quality leathers and source of income to rural people. The sheep can significantly play an important role in the economic well being of the resource-poor farmer of Bangladesh. According to Rahman *et al.* (2014) for sustainable growth of the livestock industry, Bangladesh has to face the challenges on several fronts, such as the lack of capital, inadequate availability of inputs, inadequate institutional credit, guaranteed and profitable markets for output, quality control and certification of livestock product, value added in different stages of marketing of livestock products, disease control.

Regular and successful reproduction is the key to profitable goat production. This entails early attainment of sexual maturity and rising of two crops of kids per year with a twinning rate of 10%–30% during a doe's reproductive life. High reproductive efficiency is very much important for achieving the maximum return from this animal. But the production is hampered due to various reproductive disorders. Research on reproductive system of the goat has got paramount importance from the standpoint of national development. Any structural and functional abnormalities in reproductive system may interrupt animal production. Reproductive disorders of sheep can lead to economic losses in term of reduced fertility, longer interlambing/ interkidding or interval, and increased expense on medication in farm (Samad *et al*., 2001). Ultimately its effects fall on the economic traits of this species. When sufficient information about reproductive status of ewe will be available then these disorders could only be minimized. Management of sheep reproductive disorders is deeply related to the increased production of sheep meat, skin and wool (Dhanani and Samo, 1987). Problems associated with sheep and goat reproduction represent an important economic loss in terms of lost milk yield and meat production and in lower stock replacement rate. The major reproductive disorders include abortion, still birth, low or no milk production, mastitis, uterine infection, delivery problems and lamb/kid mortality. Many of the above problems are associated with systemic diseases that lower the overall performance of the animal, while others specifically cause fetal mortality, abortion or male infertility **(**Hamito, 2011).

To make the farm profitable, it is essential to know the pattern of occurrence of reproductive diseases in in small ruminants including risk factors influencing those diseases. This will definitely help farmers to reduce the occurrence of such diseases resulting in economically viable this type of industry in Bangladesh. Small ruminants fetus could be exposed to viral (bluetongue virus, pest viruses and caprine arthritis encephalitis virus), bacterial (*Brucella spp*., *Salmonella spp.*), protozoa (*Toxoplasma gondii*) and

rickettsial (*Coxiella burnetii*) infection from early embryonic term to the end of the gestation period. These diseases cause abortion, fetal loss and congenital abnobrmalities in lambs and kids (Aydin, 1997; Burgu *et al*., 1992; Fieni *et al*., 2003). Moreover, it is likely that occurrence of reproductive diseases in small ruminants may be influenced, among others, by body condition score, feeding practice, suckling, milk yield and parity. Considering that above mention facts, the present study was conducted to Epidemiology is the basic discipline for a rational approach to prevent and control of diseases (Burridge, 1981). Moreover, it is likely that occurrence of reproductive diseases in small ruminants may be influenced, among others, by body condition score, feeding practices, suckling, milk yield and parity. Considering the above mentioned facts, the present study was conducted to 'Epidemiological Investigation of Brucellosis, Toxoplasmosis and Coxiellosis associated with reproductive disorders in small ruminants'.

To achive, targets the whole study has been divided into 4 (four) Experiment and Discuss below with their specific Objectives:

1. Retrospective study of reproductive diseases in small ruminants.
2. Investigation on Brucellosis associated with reproductive disorders in small ruminants.
3. Assessment of Toxoplasmosis linked with reproductive disorders in small ruminants.
4. Survey of Coxiellosis related to reproductive disorders in small ruminants.

1.1. Experiment 1: Retrospective study of reproductive diseases in small ruminants

Reproductive efficiency is always considered to be the most vital factor ensuring increase in productivity to a certain environmental condition (Hossain *et al*., 2004). Increased production efficiency can be obtained from goats since they have a high reproductive efficiency with the potential for increased litter size and shorter generation interval and they have a relatively higher fertility rate in comparison to other farm animals (Haque *et al*., 2013). Reproductive performance of goats is a major determinant of productivity and economic viability of commercial goat farms. The goats' reproductive performance is an indicator of their adaptation to the adverse conditions. Reproduction is a complex composite trait influenced by many components including puberty, estrus, ovulation, fertilization, embryo implantation, pregnancy, parturition, lactation, and mothering ability. Reproductive efficiency in female goats is determined by many different processes (Shelton, 1978). These processes include, for example, the length of the breeding season, cyclic activity, ovulation rate, fertilization rate, the post-partum anoestrous period and the growth and viability of the offspring.

Reproductive traits are economically important characters which could be improved by selection of local breeds (Mourad, 1993). The number of individuals born per parturition makes a much greater contribution to the total weight of individuals weaned than the growth rate of individuals (Bradford, 1986). Thus reproductive rate is an economically important trait in small ruminant production enterprises. Both biological and economic traits are improved with high levels of flock reproduction. Improvement of reproductive traits can have more economic impact than improving growth rate (Dickerson, 1978). Reproduction is a major contributing factor to efficiency of meat production and makes an important contribution by influencing the number of surplus animals which may be utilized for meat and contributing to current and

future production through culling (Song, 2003). One of the most favorable attributes of the Black Bengal goat as a meat producing animal is its high rate of reproduction and the fact that it has an extended breeding, especially as reproduction is a major contributing factor to the efficiency of meat production. The level of reproductive performance of goats is dependent on genetic and environmental factors, but this performance is particularly sensitive to the latter (Devendra and Burns, 1970; Riera, 1982; Song *et al*., 2006). Although this breed has an excellent ability to accommodate and adapt to fluctuation in environment, this often involves some degree of reproductive failure (Devendra and Burns, 1983). The goat is the most prolific of all domestic ruminants under tropical and sub-tropical conditions and certain breeds are able to breed throughout the year (Devendra and Burns, 1983), while other breeds like, for example, the Angora have a restricted breeding season (Shelton, 1978; Van der Westhuysen, 1980). There has been a growing interest and necessity for more knowledge concerning the reproductive characteristics of farm animals, with the widespread application of artificial insemination in domestic animals. The genetic effect on each component of reproduction varies (Safari *et al*., 2005). Within a production or management system, the phenotypic variation of a composite trait is influenced by the level of variability among its component traits and their interactions (Snowder, 2008). Although component traits of reproduction are under the influence of many genes, a limited number of major genes associated with separate components of reproduction have been reported (Piper and Bindon, 1982; Bradford *et al*., 1986). Expressions of the genetic effects on reproduction are affected by numerous environmental factors such as season, climatic conditions, management, health, nutrition, breeding ratio, age and weight of doe, and libido of buck and fertility. Because genetic and environmental factors interact, genetic improvement of reproduction is very complicated (Snowder, 2008). So there is a lot of prospects to rear small ruminants in that area and several reports have been written on livestock diseases in the country,

but with very little attention to small ruminant's diseases especially reproductive diseases. These studies have been emphasised to the analysis of prevalence rate and evaluation of several reproductive diseases trends in small ruminants with the following objectives.

Objectives:

- To evaluation the prevalence of reproductive diseases in small ruminants.
- To study the effect of age, sex, and seasons on reproductive diseases in small ruminants.

1.2. Experiment 2: Investigation on Brucellosis associated with reproductive disorders in small ruminants

Brucellosis is an important zoonosis. It affects a wide variety of mammals causing significant reproductive failure and enormous economic losses. In humans, it is associated with chronic debilitating infection (CFSPH, 2007). The epidemiology of brucellosis in goats is more complex as several extrinsic factors such as flock size, mangemental and ecological conditions and socioeconomic factors play important poor defined roles (Abd El-Razik *et al*., 2007). The global incidence of human brucellosis is estimated at more than 500,000 infections per year (Pappas and Akritidis, 2006). But, the true incidence has been estimated to be 25 times higher than the reported incidence because of the lack of essential statistics, disease reporting and notification systems in many countries (Mantur and Shinde, 2007). *Brucella* organisms are usually transmitted between animals by contact with the placenta, fetus, fetal fluids and vaginal discharges from an infected animal. Entry into the body occurs by ingestion and through the mucous membranes, broken skin and possibly intact skin (WHO, 2006). Brucellosis in human beings is caused by exposure to livestock and livestock products. Infection can result from direct contact with infected animals and can also be transmitted to consumers through raw milk and milk products. Brucellosis spreads between animals in a herd and the disease is a systemic infection that can involve many organs and tissues. Once the acute period of the disease is over, symptoms of brucellosis are mostly not pathognomonic, and the organism can be chronically located in the supramammary lymphatic nodes and mammary glands of 80% of infected animals. Thus they continue to secrete the *Brucella* organism in their body fluids. In Bangladesh, approximately 80% of people live in villages, and rural income is largely dependent on livestock; the people are in close contact with livestock on a daily basis. 6.5% of national income and 3.5% gross domestic product come from livestock. Sheep and goats brucellosis (excluding infection

which is not pathogenic for humans) is a zoonotic infection with important effects on both public health and animal health and production and is widespread in many areas of the world. It was first isolated by Bruce in 1887 (Alton, 1990) from the spleens of soldiers dying of Mediterranean fever on the island of Malta. Bruce called it *Micrococcus melitensis*. Brucellosis in sheep and goats used to cause mainly by bacteria, known as *Brucella melitensis* although, *Brucella abortus* caused clinical brucellosis. *Brucella ovis* is a cause of epididymitis of rams but it has also been associated with abortions and infertility. Brucellosis is a disease that can also affect humans. *B. melitensis* infection causes a disease in humans (undulant or Malta fever) characterized by intermittent fever, depression, fatigue, night sweats, muscle and joint pain whereas, *B. abortus* causes a mild disease. Bone inflammation/pain is a common complication in human brucellosis. The major importance of *Brucella* ovis is as a cause of epididymitis in rams, but it also causes late-term abortions, still births, and birth of weak lambs. *B melitensis* is rare in the USA but causes abortion in areas where it is found. *B abortus* occasionally causes abortion in sheep. *Brucella* abortions occur late in gestation, resulting in placentitis with edema and necrosis of the cotyledons and thickened, leathery intercotyledonary areas. Many fetuses aborted due to *B ovis* are alive at the beginning of parturition, although fetuses can be mummified or autolyzed. Most fetuses aborted due to *B melitensis* or *B abortus* are autolytic. Culture of the placenta, abomasal contents, and the dam's vaginal discharge are diagnostic. A vaccine for *B melitensis* is available in some countries. *B melitensis* and *B abortus* are zoonotic. Bacterial zoonoses still represent a serious medical problem (Karabay *et al*., 2004; Holt *et al*., 2011; Bardon *et al.,* 2011). Brucellosis, a widely spread disease in the developing countries (Ahmed *et al.,* 2010) and also known as undulant or Malta fever (Karabay *et al.,* 2004; Tzaneva *et al.,* 2009), is an important zoonotic disease caused by six species of the bacterial genus *Brucella* (Kreeger *et al*., 2004; Al-Majali *et al.,* 2009; Holt *et al.,* 2011). These bacteria exist in the reproductive and internal

organs, as well as in the blood (Holt *et al*., 2011), and can be divided into nine biovar (Al-Majali *et al*., 2009). Brucellosis is a significant public health and food safety concern (Mikolon *et al*., 1998a) and considered an important economic impact on food production because majority of food-producing animals worldwide are ruminants, which are susceptible to this disease (Al-Majali *et al.,* 2009; Ahmed *et al.,* 2010; Gomo *et al.,* 2011; Holt *et al.,* 2011). Most cases of brucellosis infection are inapparent and lack clinical symptoms. The first evident manifestation is abortion, which frequently occurs 3 to 4 months after gestation. The amniotic fluid, placenta, and secretion of aborted ewes have special infectivity, aside from the fur, excrement, and urine of these ewes that also infected with the bacteria (Al-Majali *et al*., 2009). Clinical symptoms of infected rams are orchitis, arching, anorexia, emaciation, and gradual loss of capacity for hybridization. Other symptoms also include mastitis, broncheaitis, and arthritis, among others (Tzaneva *et al*., 2009). Brucellosis in sheep caused by *Brucella ovis,* one of the most virulent species of *Brucella*, is a widespread zoonosis, especially in Mediterranean and the middle-east regions where it also constitutes a hazard for humans (Jacques *et al.,* 1998). This microorganism is the main etiological agent of sheep brucellosis in Turkey (Arda *et al.,* 1987; Leyla *et al.,* 2003). Sheep and their products remain the main source of infection, but *Brucella melitensis* in cattle has emerged as an important problem in some southern European countries, Israel, Kuwait, and Saudi Arabia. *Brucella melitensis* infection is particularly problematic because *Brucella abortus* vaccine do not protect effectively against *Brucella melitensis* infection; the *Brucella melitensis* vaccine has not fully evaluated for use in cattle. Thus, bovine *Brucella melitensis* infection is emerging as an increasingly serious public health problem in some countries. Worldwide, brucellosis remains a major source of disease in humans and domesticated animals. Although reported incidence and prevalence of the disease vary widely from country to country, bovine brucellosis caused mainly by *B. abortus* is still the most widespread form. In humans, ovine/caprine

brucellosis caused by *Brucella melitensis* is by far the most important clinically apparent disease. The disease has a limited geographic distribution, but remains a major problem in the Mediterranean region, west-tern Asia, and parts of Africa and Latin America. Recent reemergence in Malta and Oman indicates the difficulty of eradicating this infection (Amato Gauci, 1995). Ovine brucellosis (OB) caused by the bacteria *Brucella ovis* is a venereal disease in sheep. *Brucella ovis* is an infection of the genital tract that may cause lesions which affects the quality of the semen and the fertility of the effected ram. The organism can enter the body through any mucous membrane. Transmission caused by rams and it is riding each other or rams serving could infect ewes. The ewe has not a significant role in the transmission of *B. ovis* outside of the mating season. Therefore, the focus is on the detection in the ram. *Brucella ovis* can be eradicated by a test and cull procedure. Infected rams detected by scrotal palpations and serum testing. Usually, several tests needed to make sure that infected animals detected. In addition, the infection can spread rapidly between rams making repeat tests necessary.

The importance of brucellosis not known precisely, but it can have a considerable impact on human and animal health, as well as socioeconomic impacts, especially in which income relies largely on livestock breeding and dairy products (Islam *et al*., 1983). Brucellosis in human beings caused by exposure to livestock and livestock products. Infection can result from direct contact with infected animals and the animals transmitted to consumers through raw milk and milk products. Brucellosis spreads between animals in a herd and the disease is a systemic infection that can involve many organs and tissues. Once the acute period of the disease is over, symptoms of brucellosis are mostly not pathognomonic, and the organism can be chronically located in the supramammary lymphatic nodes and mammary glands of 80% of infected animals. Thus, they continue to secrete the *Brucella* organism in their body fluids (Cordes and Carter, 1979; Redkar *et al*., 2001). A lot of undiagnosed

cases of abortion, still birth and retained placenta were reported in sheep and goats which must be resulted from brucellosis. Brucellosis is an important constraint for the development of livestock in Bangladesh. The importance of brucellosis in Bangladesh is not known precisely, but it may have a considerable impact on both human and animal health. In Bangladesh, brucellosis was first detected in cattle in 1967 (Mia and Islam, 1967), in buffalo in 1997 (Rahman *et al.,* 1997). In human and goats brucellosis was first reported in 1983 (Rahman *et al.,* 1983). However, scant information is available about the seroprevalence of brucellosis by agglutination tests in human beings, cattle, sheep, and goats (Ahasan *et al*., 2010; Amin *et al*., 2004 and 2005; Matyas and Fujikura, 1984; Rahman *et al*., 1983, 2006, 2010 and 2011 and Rahman and Rahman 1981). Therefore, the present study was carried out for the following objectives.

Objectives:

- ❖ To identify the seroprevalence of Brucellosis in small ruminants by Rose Bengal Test (RBT).
- ❖ To confirmation of RBT positive cases by ELISA.
- ❖ To know the effects of species, breed, sex, age, parity, body weight, flock size, biosecurity, feeding habit, seasons, region, pregnancy status and reproductive diseases with co-infection on prevalence of Brucellosis in small ruminants.

1.3. Experiment 3: Assessment of Toxoplasmosis linked with reproductive disorders in small ruminants

Toxoplasmosis is one of the most common parasitic zoonoses worldwide caused by *Toxoplasma gondii,* which establishes long-lasting infections in humans and animals (Dubey, 2010; Butcher *et al.,* 2011). The parasite infects one third of the human population Worldwide (Montoya and Liesenfeld, 2004) and among food animals' sheep and goats have well known sources of human infection (Dubey, 2010). Toxoplasmosis is one of the most common parasitic infections of man and other warmblooded animals in most parts of the world (Hill *et al.,* 2002). The disease is a major cause of ovine abortion in many countries (Christensen *et al.,* 2009). Seroprevalence studies show high rates of infection in farm animals. Major importance of disease in farm animals is its zoonotic potential. Source of infection for sheep, and cattle is the oocyst passed in the feces of the cats. Cats shed oocysts by their feces. Cats infected by ingesting tissues of intermediate hosts (Radostits *et al.,* 2008). Ingestion of undercooked goat meat and unpasteurized goat milk are source of human infections (Skinner *et al.,* 1990; Lindsay and Dubey, 2007; Samra *et al*., 2007; Jones *et al.,* 2009; Dubey, 2010; Dubey *et al*., 2011). *Toxoplasma gondii* leads to a considerable disease burden and ranked second in a list of 86 emerging zoonotic pathogens in The Netherlands (Havelaar *et al*., 2010). Toxoplasmosis of The central nervous system reported as an important complication in AIDS patients in Ethiopia (Amogne *et al.,* 2006). Among food animals, goats appear to be more susceptible to clinical toxoplasmosis, and adult goats reported to have died of acute toxoplasmosis (Dubey and Beattie, 1988; Dubey, 2010). The disease is a common cause of abortion (Tenter *et al.,* 2000; Lindsay and Dubey, 2007; Dubey, 2010) and neonatal mortality in goats (Dubey, 2010). Seroepidemiological investigation of *T. gondii* infection in goats is essential in order to assess long lasting antibodies and provides the basis for the analysis of the potential risk of meat and milk for human infection, especially for

countries like Ethiopia where consumption of raw or undercooked goat meat is popular tradition. In Ethiopia, five serological surveys carried out in goats over the last three decades reported seroprevalence ranging from 22.9% to 74.8% (Bekele and Kasali, 1989; Demissie and Tilahun, 2002; Negash *et al.,* 2004; Teshale *et al.,* 2007; Yibeltal, 2008). The coccidian protozoan *Toxoplasma gondii* is a highly ubiquitous and prevalent parasite. It causes toxoplasmosis, a potentially very serious disease to humans and other warm-blooded animals. Infection has in many studies been shown to be rather common in the Nordic countries also, where its prevalence both in domestic animals and wildlife can be explained by contacts with cats and their faeces, cats and wild felids being the only definitive hosts of the parasite known. The prevalence of seropositivity for *Toxoplasma* antibodies varies with geographic location, flock and country (Tutuncu *et al.,* 2003). Acute acquired toxoplasmosis is most commonly asymptomatic, but it can range from mild symptomatic in the normal host to fulminante and fatal illness in the immunocompromised host. Toxoplasmosis is associated with the occurrence of embryonic death and absorption, fetal death and mummification, abortion, still birth and neonatal mortality (Dubey, 2009). Moreover, toxoplasmosis has harmful effects on the health and performance of ewes and does after parturition and sometimes leads to their death (Radostits *et al.,* 2007). Therefore, toxoplasmosis leads to major economic losses in livestock production (Freyre *et al.,* 1999; Maki *et al.,* 1996). Munday and Mason (1979) were the first to describe toxoplasmosis as an important cause of reproductive losses in goats. Although often unnoticed, this infection can cause significant damage in both young and adult animals (Dubey, 1987). The main route of Infection is ingestion of the parasite's sporulated oocysts present in the environment (Dubey and Beverley, 1988). Risk factors for *T. gondii* infection in goats include age, number of cats in the farm, and either no use of feeding troughs or use of wooden feeding troughs (Cavalcante *et al.,* 2008). Rorman *et al*., 2006 studied to detect the presence of

T. gondii in serum samples of experimental goat and sheep using ELISA well as serological techniques.

Toxoplasmosis infection has established as a major economic threat for sheep and goat farming industry particularly due to abortion all over the world (Buxton *et al*., 2007; Innes *et al*., 2009). This disease may also cause the monitory losses in terms of the reproductive disorders such as abortions, still birth, or weakness of neonates that results in death (Bueno *et al*., 2004; Soares *et al*., 2009) or adult mortality, reduced productivity in small ruminants with lower quality of wool and leather (Parthiban *et al*., 2005). According to the findings of Kijlstra and Jongert (2009), T. gondii is a food hazard as well.

As the survival of T. gondii is affected by environmental factors (Chacin-Bonilla and Sanchez-Chavez, 2000; Hill *et al*., 2005). The researcher chose to conduct the current study to ascertain the variations in the rates of infections in the small ruminant's population at Northern Barind Tract in Bangladesh. The present study based on the research conducted to following objectives.

Objectives:

- To determine, the seroprevalence of Toxoplasmosis in goat-sheep by latex agglutination test (LAT).
- To come across the effects of species, breed, sex, age, parity and body weight of animals in relation with Toxoplasmosis.
- To find out the consequence of *Toxoplasma* infection with flock size, biosecurity, feeding habit, seasons, location, pregnancy status and reproductive diseases with in small ruminants.

1.4. Experiment 4: Survey of Coxiellosis related to reproductive disorders in small ruminants

Q (Query or Queensland) fever is a zoonotic infection affecting a variety of animals. Though rare, it can cause abortion in sheep and goats. Q fever caused by *Coxiella burneti*. *C. burnetii* can transmit by aerosols or direct contact; it also spread by ingestion of an infected placenta, other reproductive discharges or milk. Organisms localize in the mammary glands, supramammary lymph nodes, uterus and placenta in domestic ruminants and other susceptible species; bacteria can shed in milk, the placenta and reproductive discharges during subsequent pregnancies and lactations. C. burnetii can also found in the feces and urine. Ticks seem to spread infections among ruminants and sometimes people. Q (for Query) fever is a ubiquitous zoonosis caused by an obligate intracellular bacterium *Coxiella burnetii.* It has reported from all over the world except Antarctica and possibly New Zealand (Maurin and Raoult, 1999; Angelakis and Raoult, 2010). The primary reservoirs of *C. burnetti* are cattle, sheep and goat. However, the infection has reported in other mammals (humans, cats, dogs, rodents, rabbits, horses, swine, camels, water buffalo and marine mammals), ticks and other arthropods, birds, fish and reptiles (Babudieri, 1959; Porter *et al.,* 2011). *Coxiella burnetti* is capable of producing resistant, spore-like forms that can survive for months to years in extreme environmental conditions and chemicals (Franz *et al.,* 1997). The common manifestations of Q fever in ruminants are abortion, still birth, premature delivery and delivery of weak offspring (Angelakis and Raoult, 2010). However, these clinical manifestations usually observed in sheep and goats. In cattle, Q fever is mostly asymptomatic. Clinically infected cows may develop infertility, metritis and mastitis (To *et al.,* 1998).

It is believed that *C. burnetii* survives in nature maintaining two different cycles, the wild cycle (involving ticks and wild animals) and the domestic

cycle (ruminants and pets such as dogs and cats as main reservoirs) (Maurin and Raoult, 1999; Arricau and Rodolakis, 2005).

The infected mammals spread the pathogen by bacterial shedding in their body secretions (milk, feces, urine, and vaginal mucous and birth products). The most common route of infection for humans is the inhalation of dust contaminated by infected animal secretions, followed by ingestion, particularly of unpasl: eurized dairy products (Maurin and Raoult, 1999; Porter *et al.,* 2011). In humans, Q fever is mostly asymptomatic but may be responsible for acute or chronic disease conditions such as influenza-like illness, pneumonia, hepatitis, meningoencephalitis, myocarditis, endocarditis and chronic fatigue syndrome in persistently infected patients and may contribute to abortion and still birth (Wildman *et al.,* 2002; Angelakis and Raoult, 2010). The acute Q fever is usually benign and spontaneous recovery occurs within 2-3 weeks in majority of acute cases even without treatment. The farmers, veterinarians and abattoir workers those in contact with dairy products and laboratory personnel performing *Coxiella burnetii* culture and more importantly working with C. *burnetii* infected animals are at high risk for Q fever (Maurin *et al.,* 1999). Diagnosis of Q fever in animals based on to detect bacteria, bacterial DNA or antibodies (Rodolakis, 2006). Although this bacteria can grow in axenic (host cell- free) media, isolation is time consuming and hazardous for the laboratory workers (Omsiand *et al.,* 2013). Bacterial DNA can detected by using Polymerase Chain Reaction (PCR), which was an expensive, and resource intensive (Rousset *et al.,* 2010). Indirectly, C. *burnetii* exposure in animals can screen by serological tests. The Complement Fixation Test (CFT) (OlE recommended test) and Enzyme-Linked Immunosorbent Assay (ELISA) (EU recommended test) are the two most commonly used to tests in this purpose. Thus, CFT protocol is complex and fails to detect antibodies in sheep or goats (Kovacova *et al.,* 1998). The ELISA reported to be highly sensitive and specific for the diagnosis of Q fever (Paul *et al.,* 2012). Thus, ELISA can be used to detect antibodies in bulk milk (easy to collect and cheaper than blood

analysis) and individual animal serum. Although Q fever is present world-wide, its status in animals, humans, arthropods, birds, wild animals and other reservoirs in Bangladesh is not yet known. However, the reproductive diseases in dairy cattle (Talukder *et al.,* 2005; Khair *et al.,* 2013; Sarker *et al.,* 2013) and pyrexic diseases in humans are endemic in Bangladesh (Ahmed *et al.,* 2005; Ram *et al.,* 2007; Haque *et al.,* 2009; Zaman *et al.,* 2011). In a context where there is no idea about the existence of Q fever, it is preferable to know initially the herd level status of this disease. So, the experiment was looked-for the realization of the following objectives.

Objectives:

- To determine the seroprevalence of Q fever in goat-sheep by ELISA.
- To identify the Q fever by Multiplex Quantitative PCR from aborted fetal samples of doe and ewe.
- To evaluate the prevalence of Q fever in relation with species, breed, sex, age, parity and body weight of small ruminants animals.
- To study the effect of flock size, biosecurity, feeding habit, season of year, location of farm, pregnancy status and reproductive diseases on prevalence on Q fever.

CHAPTER 2

REVIEW OF LITERATURES

The main goal of small ruminant's husbandry is to provide sustainable protein supply to consumers. Many factors affect the protein production and reproductive health of the small ruminants. One of the most important production limiting factors is poor reproductive performance as efficiency of small holder goat and sheep farming depends greatly on reproductive performance. Transition goat and sheep are more prone to reproductive problems resulting in economic losses. Major reproductive problems include abortion, still birth, retained fetal membranes, dystocia, vaginal prolapsed, uterine prolapsed, metritis, pyometra, anestrus, repeat breeders, orchitis, epididymitis, posthitis, urolithiasis and urinary tract infection which pose a great threat to production and reproduction efficiency of animals. Many authors have investigated the various factors affecting the prevalence of these reproductive disorders orient problems; some significant studies are summarized below under specific experiments.

2.1. Experiment 1: Retrospective reproductive diseases in small ruminants

Over the years, the demand for animal protein has been increasing in Bangladesh, which has resulted into increased importation of meat and milk products into the country to supplement the local shortfalls. There exists several factors that affect the physiology and reproductive performance of farm animals which include diseases, environmental temperature, poor husbandry practices and nutrition (Drazen, 2012) leading to huge economic losses (Waziri, 2006). Retrospective epidemiological studies provide useful information on disease patterns that could be useful for prevention and policy formulation for their management. Disease may be defined as inability to perform physiological functions at normal levels even though nutrition and other environmental requirements are provided on adequate levels (Radositis

et al., 2007). Disease conditions always impair livestock production (Akerejola *et al.,* 1979; Lamorde, 1996). Apart from this, several other factors such as environment and nutrition, especially inadequate protein intake (Kumidiake *et al.,* 1981; Smith and Somade, 1994), decrease reproductive performance. However, technological difficulties in these countries hinder extensive use of modern diagnostic techniques in disease surveillance (Nwanta *et al.,* 2000). Diseases and climatic problems have blamed as the main limitation to modern animal husbandry in tropical areas (Ameen and Ajayi, 2013). The diseases and reproductive disorders observed in the different systems of small ruminants were subdivided on the basis of influencing factors, which were below:

2.1.1. General diseases pattern

The general diseases were gastrointestinal, respiratory, musculoskeletal system, female reproductive disorder, integumentary involvement, disease of Sense organ, infectious disease, deficiency syndrome, poison, disorder of male sex organs and surgical cases. Ali *et al.* (2011) observed among individual diseases the prevalence of worm infestation was highest followed by 5 diseases were more prevalent viz. enteritis, indigestion, pneumonia and pneumonitis, mange and ephemeral fever. The prevalence of other diseases was comparatively low. Among diseases of different systems those affecting reproductive system constituted highest occurrence is anestrous.

2.1.2. Occurrence reproductive diseases in small ruminants

The reproductive disorders were abortion, still birth, retained fetal membranes, dystocia, vaginal prolapsed, uterine prolapsed, metritis, pyometra, anestrus, repeat breeders, orchitis, epididymitis, posthitis, urolithiasis and urinary tract infection. The occurrence above disorders is classified on the basis of:

2.1.3. Species

Peter *et al.* (2014) showed the prevalence of reproductive disorder based on species and a total of 308 (90.6%) sheep were affected which was significantly ($p<0.05$) higher than goats with 32 (9.4%). Umaru *et al.* (2009) also observed

that higher prevalence of reproductive disease conditions in small ruminants (sheep and goat) over cattle has been recorded. Umrah *et al.* (2009) founded sheep had the highest prevalence of 51 (57.95 %), followed by goats 32(36.36 %) then cattle 5(5.68 %).

2.1.4. Age

Ali *et al.* (2011) observed all 6 cases of arthritis were found in age group A_1 (0-2 months) in case of goat. Deficiency and metabolic diseases are more common in age group A_1. Diseases of integumentary system (Skin diseases) were found more common 282 cases (74.80%) in age group A_1. In the study 45 sheep of different aged were recorded for investigating of 6 no. of enlisted diseases related with gastrointestinal system, respiratory systems as well as metabolic and deficiency. It was observed that, sheep of 0-2 years aged were more susceptible than that of older aged. The study also revealed that sheep of older aged gave fully negative response against the diseases of pneumonia as well as zinc deficiency.

2.1.5. Sex

Peter *et al.* (2014) showed the prevalence of reproductive disorders based on the sex of the animals and season of examination. Female sheep and goats had higher prevalence ($p<0.05$) of reproductive disorders with 283 (21.9%) and 32 (21.5%) compared with the males having 25 (3.1%) and 0 (0%) respectively.

Umaru *et al*. (2009) also found that reproductive disease condition were more in females probably due to their unique position as essential reproductive vessels and the fact that females are reared for a longer periods than males. Waziri *et al*. (2006) observed in Maiduguri and was report consistent result in relation of sex.

2.1.6. Disease condition

Peter *et al*. (2014) got results of his study that dystocia, pregnancy toxaemia, mastitis and retained placenta were the most common reproductive disorders encountered (Wosu and Anene, 1990; Waziri *et al*., 2006; Umaru *et al*., 2009

and Neils *et al*., 2009). However, the overall prevalence was higher (14.5%) compared to the 4.07% by Waziri *et al.,* and 9.1% by Williams *et al.* (2005) from the same study area. The reason for this variation is unclear, but may likely be due to the total quantum of caseloads analysed or the period of the study. Dystocia has been reported to be common in primigravid than in multigravida sheep and goats. The high prevalence of dystocia observed in this study could have been due to such and also due to early pregnancy when such animals are not physically mature for normal kidding/lambing at parturition. The prevalence rates in pregnancy toxaemia and mastitis were 19.7% and 14.4%, respectively. Pregnancy toxaemia is caused by low glucose concentrations in the blood and excessive break down of fats to compensate.

Umrah *et al*. (2009) also got dystocia 23 (26.13 %) was the most prevalent reproductive condition, that is followed by pregnancy toxaemia 11 (12.50 %), than retained placenta 10 (11.36 %), mastitis 9 (10.23 %); paturient paresis 7 (7.95%); abortion 6 (6.82 %); surgical 5 (5.68 %); vaginal prolapsed 4 (4.55%); still birth 4 (4.55%); orchitis 3 (3.41 %); uterine prolapse 3 (3.41 %); balanoposthitis 2 (2.27 %); and phimosis 1 (1.14 %).

2.1.7. Seasonal effect

Islam *et al.* (2015) observed that the general frequency of the disease randomly distributed throughout the year, but relatively more cases were encountered during rainy season followed by winter season than dry or summer season.

Experiment 2: Investigation on Brucellosis associated with reproductive disorders in small ruminants

The disease brucellosis remained of the origin a mystery for nearly 20 years until it was discovered that goats were the source of infection for human populations. Human brucellosis is widely distributed all over the world, with regions of high endemicity such Mediterranean, Middle East, Latin America and parts of Asia (Corbel, 1997; Lopez-Merino, 1989). The true incidence of human brucellosis is unknown. Reported incidence in endemic-disease areas varies widely, from <0.01 to >200 per 100,000 population (Lopez-Merino, 1989). Humans are accidentally infected and almost always dead-end hosts of *Brucella* infections. The disease is primarily an occupational risk in exposed professions, i.e. veterinarians, farmers, laboratory technicians, abattoir workers, and others who work with animals and their products. People living near infected premises may also contract infection. The primary source is the animal and infection is contracted either by direct or indirect contact through the skin or mucous membranes or ingestion of contaminated products, especially fresh dairy products. The maximum danger is therefore during the lambing or kidding period. Dairy products are the 8 main source of infection for people who do not have direct contact with animals. Much of the milk which is consumed now rendered safe by pasteurisation or boiling, but cheese made from sheep and goat milk is preferably prepared from untreated milk and by the use of rennet from lambs and kids that may have come from *Brucella* infected animals. During the course of cheese manufacture, any *Brucella* organism present in the milk become trapped in the clot and thus concentrated in the cheese, although bacteria may subsequently be inactivated by manufacturing or ripening processes. Cream and ice cream prepared from goat milk has also been incriminated (Flores-Castro and Baer, 1980). The prevalence of human brucellosis acquired from dairy products is seasonal, reaching a peak soon after kidding and lambing. Abattoir workers handling

infected animals are also at risk, especially from the contents of uteri and udders. The handling of raw wool has been identified as a potential source of infection of workers involved. Finally, *Brucella melitensis*is was acquired by laboratory infection easily. Humans are susceptible to *B. melitessis*, *B. abortus* and *B.suis*. *B. Susi* and *B. melitensis* often give rise to a severe and long lasting form of the disease. After an incubation period of 8 to 20 days, illness occurs in different forms. Asymptomatic infection is frequent and mainly due to *B. abortus*. It is characterised by antibody formation in persons with no history of symptoms consistent with brucellosis. The acute form of the disease is also common and symptoms include lassitude, headache and muscular or joint pain, and drenching sweats, especially at night. The manifestations of brucellosis are sometimes more pronounced or limited to a specific system or organ. This is then termed a complication when it occurs in the course of acute infection, or localised brucellosis when occurring in the absence of other signs of systemic illness. The most common localisations are spondylitis, peripheral arthritis, especially of the hip, knee and shoulder, or epididymo-orchitis. Nervous, genitourinary, hepatosplenic and cardiovascular complications may also be observed. Brucellosis is termed chronic when it includes one or more of the signs described above and persists or recurs over a period of six months or more. Finally, B*rucella* dermatitis has traditionally been ascribed to "allergy" to *Brucella*.

The disease presents a great variety of clinical manifestations, making it difficult to diagnose clinically. Therefore, the diagnosis must be confirmed directly by isolation of *Brucella*, mostly from blood culture, or indirectly by the detection of immune response against its antigens. The diagnosis of brucellosis based exclusively upon *Brucella* isolation presents several drawbacks (Orduna *et al*., 2000). The slow growth of *Brucella* in primary cultures means that diagnosis may take more than 7 days (Ariza, 1996, Rodríguez-Torres *et al*., 1987, Yagupsky, 1999). Besides, blood culture sensitivity is often low, ranging from 50% - 90% depending on disease stage,

Brucella species, culture medium, number of circulating bacteria and the culture technique employed (Gotuzzo *et al.,* 1986, Yagupsky *et al*., 1999). Hence, serological tests play a major role in diagnosis when the agent cannot be detected by blood culture. Yet, the interpretation of these tests is often difficult, particularly in patients with chronic brucellosis, in re-infections and relapses, and in endemic areas where a high portion of the population carries antibodies against brucellosis (Orduña *et al.,* 2000). Various serological tests have been used for the diagnosis of human brucellosis. Orduña *et al.* (2000) stated that the most common *Brucella* tests used are serum agglutination test (SAT), Coombs anti *Brucella* test, Rose Bengal test (RBT) and complement fixation test (CFT). During the last decade, radioimmunoassays (Hewitt and Payne, 1984, Parrat *et al*., 1977) and in particular enzyme-immunoassays (Ariza *et al*., 1992; Gazapo *et al*., 1989, Saz *et al*., 1987) have also been was used for detection of brucellosis. Other tests have proved useful in some patients, such as the indirect immunofluorescence test, Brucellin counter-electrophoresis and passive haemagglutination test, but their value in clinical practice is still under assessment. Allergic tests reveal a delayed-type hypersensitivity; using conventional antigen preparations. Brucellin-INRA, an S-LPS free product was reported as reliable and innocuous.

2.2.1. General occurrence

Brucellosis is one of the world's major zoonotic problems. Though it has been eradicated from many developed countries in Europe, Australia, Canada, Israel, Japan and New Zealand (Geering *et al*., 1995) but, almost all domestic species can be affected with brucellosis except cats which are resistant to *Brucella* infection. Brucellosis has recorded in Bosnia, Herzegovina, Mediterranean basin, Middle East, Central Asia and Latin America (Gul and Khan, 2007). The highest rate (72.9%) of infection has reported in the Palestinian and the second highest (71.42%) in mules from Egypt (Shuaibi, 1999). In Pakistan, Brucellosis is endemic in cattle, buffalo, sheep and goat

populations (Ahmed and Munir, 1995). In cattle and buffalo, it has reported that the incidence of brucellosis is 3.25% and 4.40%, respectively, in different areas of Pakistan (Masoumi *et al*., 1992).

Neculita *et al*. (2006) conducted an epidemiological study on *Brucella ovis* infection of rams in Value County, Romania between 2000 and 2005. Blood samples collected and analyzed by CFT, which establish the incidence and seroprevalence of brucellosis. Results showed that the highest incidence was observed in 2000 and 2002 with 123 (1.33%) and 155 (1.46%) cases, respectively. The incidence of *Brucella ovis* infection in Romania between 2000 and 2005 was 8.97%.

Reviriego *et al.* (2000) evaluated risk factors for ovine and caprine brucellosis in the Avila region (center of Spain) using data obtained from a cross-sectional study of the most important diseases of small ruminants in this Spanish region during 1996 and 1997. Questionnaire data from 56 herds (35 ovine and 21 caprine) used. Sixteen (29%) flocks (3 caprine and 13 ovine) found to be brucellosis seropositive.

In Bangladesh, brucellosis first identified in cattle in 1967 (Mia and Islam, 1967), buffalo in 1997 (Rahman *et al*., 1997) with others reported brucellosis in one or two species of livestock as well as humans (Amin *et al*., 2005; Uddin and Rahman, 2007; Rahman *et al*., 2009 & 2010; Ahasan *et al*., 2010; Muhammad *et al*., 2010). Rahman *et al*. (2011) reported that the prevalence of brucellosis was determined in the ruminants (buffaloes, cattle, sheep and goats) of five different districts viz. Bagerhat, Bogra, Gaibangha, Mymensingh and Sirajgonj of Bangladesh. Before 1945, the India and Bangladesh was the same country and brucellosis was first recognized in India in 1942 (Renukaradhya *et al.,* 2002). So, historically, in this Indian subcontinent, the credit of first investigation of contagious abortion in livestock, associated with brucellosis, goes to the Imperial Veterinary Research Institute (now Indian Imperial Veterinary Research Institute), Muketswar, in northern India

(Anonymyous, 1918). Mia and Islam (1967) reported that 37% of our adult cows were infertile and that bovine infertility causes an economic loss of 40.46 crores of rupees in East Pakistan (Now Bangladesh). It was very probable that brucellosis plays an important role in causing infertility in Bangladesh. Current review, the prevalence of Brucellosis in animals was 3.7%, 4.0%, 3.6%, 7.3% and 2.5-18.6% in, cattle, buffalo, goats, sheep, and the livestock owners, respectively (Rahman *et al*., 2014) in Bangladesh.

Rahman and Mia (1970) conducted a study and got incidence (18.4%) of brucellosis studied in Bangladesh Agricultural University Dairy Farm and in surrounding areas based on tube agglutination test. Later on human, caprine and bovine brucellosis identified in Bangladesh (Rahman *et al*., 1983; Amin *et al*., 2005). Rahman *et al*. (1978) reported positive reactions to the *Brucella* milk ring test (MRT) 11.44% in Savar, 16.66% from Tangail and 4.19% from Bangladesh Agricultural University (BAU) dairy farm. Higher incidence of disease observed among cows of organized farms. Rahman and Rahman (1981) studied the incidence of *Brucella* infection in subclinical mastitis infected udder, collecting milk samples from the dairy farm at BAU, Central Breeding and Dairy farm, Savar, Dhaka and domestic holdings of adjacent villages of BAU campus. Card screening test perform for subclinical mastitis and milk ring test for brucellosis. The MRT revealed 5.5%, 11.44% and 0.00% of brucellosis in cattle on BAU dairy farm, Central Breeding and Dairy farm, savar and rural areas respectively. With the help of bacteriological technique, it was possible to isolate and identify *Brucella* organism from 2% samples of BAU dairy farm, 3.4% from Central Breeding and Dairy farm, savar and none from BAU campus adjacent villages. Pharo *et al*. (1981) studied the prevalence of bovine brucellosis in the Pabna milk-shed area of Bangladesh. By using Milk Ring test they showed that in individual herd the prevalence was 62.5% , 30.7% of MRT positive cows were found to be RBPT (rose Bengal plate test) positive. Rahman and Rahman (1982) carried out a study on the prevalence of brucellosis in cows in organized farms and domestic

holdings in Bangladesh. It was observed that 11.52% of a total of 425 milk samples from cows belonging to Baghabari milk shed area, Pabna, 2.92% of 3.6 milk samples of Takerhat, Faridpur, and 2% milk samples from different villages of Bogra district were positive to MRT. Serological test in order to determine the prevalence of *Brucella* agglutination in sera samples of MRT positive cows showed that 8.47%, 1.63% and 0.41% of milk samples from Pabna, Faridpur and Bogra respectively were also positive for RBPT. Higher incidence of disease (about 4%) was observed among cows of farms particularly in exotic and crossbreds.

Islam *et al.* (1983) reported that economic losses due to brucellosis among cattle in Bangladesh is due to abortion, loss of calf production, reduced milk yield, infertility, disposal of vectors and also occasional mortality. The total monetary loss from milk, calves was calculated to be Taka 0.88 million per 1000 heads of cows per year. On conservative estimation these amount may be accepted as taka 0.15 million for 1000 crossbred cows and taka 85.00 million for 1000 exotic breed cows.

2.2.2. Transmission

There are 27 strains of *Brucella spp.* all of the *B. melitensis* species, were isolated. The etiological agent was of human brucellosis were *B. melitensis,* bio-variety-I clearly being the most prevalent. Mishal *et al.* (1999) reported that there were no significant differences with respect to having cuts on hands, working in the cowshed without gloves.

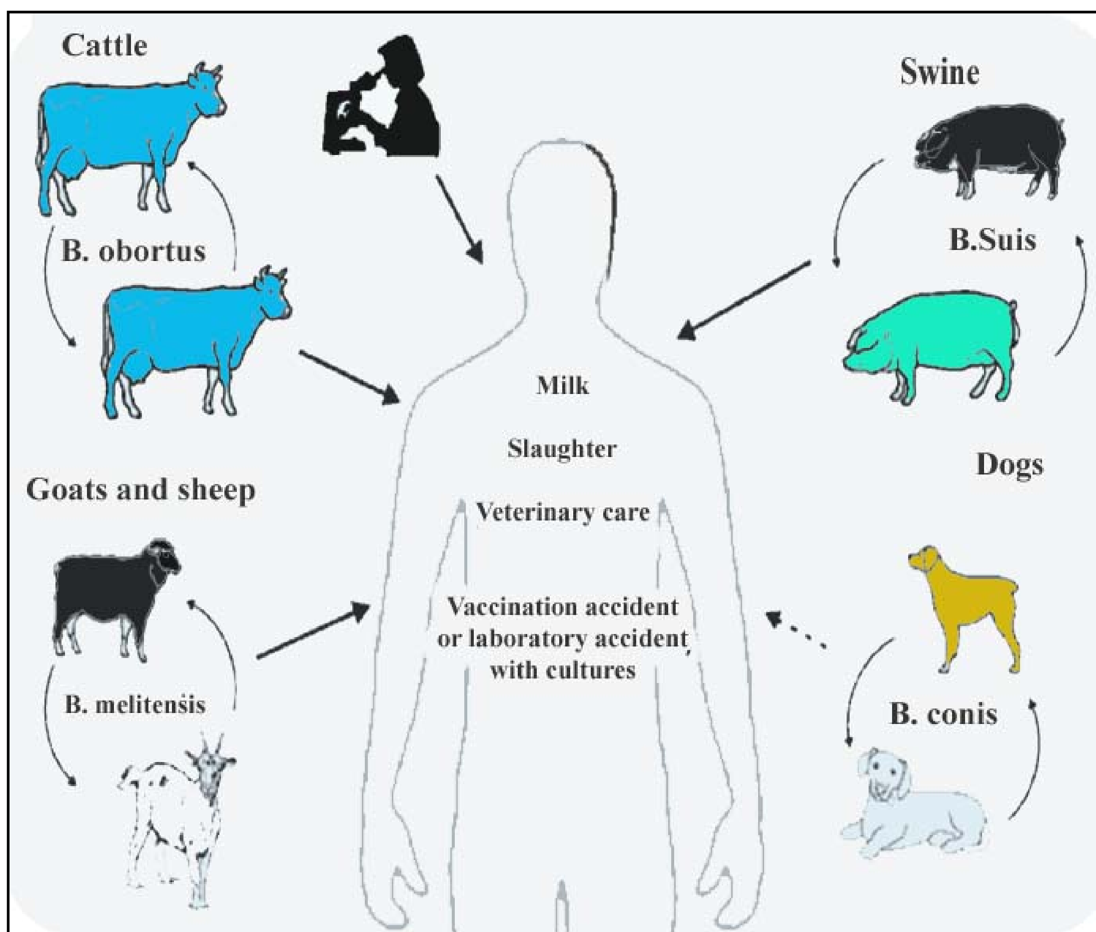


Plate 1. Transmission cycle of brucellosis.

In addition, the two major risk factors are working in the cowshed and consumption of unpasteurized milk. As the cows were affected *by B. melitensis* (which usually affects flocks of goats and sheep rather than cows), the microb was probably transmitted to the cowshed from neighboring flocks by wandering dogs, and then to the infected humans.

Grillo *et al.* (1999) reported that females do not play significant role in maintaining *Brucella* infection and direct transmission of infection from female to male by venereal route has reported as a rare event. Buddle (1995) reported that direct female-to-male venereal transmission has been occurred to be a rare occurrence in the epidemiology of *Brucella* infection. Grillo *et al.* (1997) stated that the transmission of *B. abortus* from infected dam to offspring has well documented in cattle and the calves remain sero-negative

for month's even years.

Cheville *et al.* (1995) reported that in bovine brucellosis, infection acquired through suckling infected dam has been suggested as one of possible modes of transmission. Marco *et al.* (1994) showed that, the importance of female animal in the transmission of infection has not fully clarified in sheep.

George (1994) stated that the normal route of invasion of *Brucella* to animals is by the oral route from licking aborted fetuses, infected placentas, or vaginal discharges or ingestion of contaminated feed or water. After invasion, the *Brucella* ingested by local phagocytes, which enter the lymphatics, having caused them to localize temporarily in the lymph nodes draining the invasion sites. *Brucella* multiplies in the cytoplasm of phagocytic cells, eventually killing and rupturing them. Than the organisms ingested by new phagocytes and repeating the cycle. In the presence of adequate host defense the infection may localized in local lymphnodes and eventually may eliminated. Generally, the organisms escape the lymph nodes and set up a general bacteremia as free bacterial cells and in the cytoplasm of circulating phagocytic cells.

Nielsen and Duncan (1990) stated that generally rat infections seem to be from areas where there are a large number of infected cattle, suggesting that for the rat infection, cattle are the important infection source through direct or indirect contact. The transmission of *B. abortus* from infected dam to offspring has well documented in cattle, and the calves remain sero-negative for month's even years.

Mukasa-Mugerwa (1989) described that *Brucella* organism can picked up by rest of the herd through drinking water and feed or may enter through the lacerated mucous membrane of the nose and eye when uninfected animals come in close contact of the potential reservoirs. Hughes, (1972), Grillo *et al.* (1997), Burges, (1982) and Bulgin, (1990) suggested the possibility of congenital transmission in sheep and goats.

Galloway (1960) stated that the bacteria, *Brucella* after entering into the body,

transmit via blood to different organs like bursae, scrotum, joint capsule etc. and finally localizes there to produce the characteristic symptoms of disease of new infected animals, which can infect the rest of the herd from discharges of infected genital tract through an aborted fetus.

Leyla *et al.* (2003) studied the Polymerage Chain Reaction (PCR) as the promising option for the diagnosis of brucellosis. During three successive lambing seasons, 126 aborted fetus samples was each of different flocks and location examined. *Brucella* strain were isolated from 39 (31%) of the samples and all of the strains were identified as *B. melitensis* by biochemical characteristics, agglutination test with nonspecific A and M sera and PCR. 37 of 39 *melitensis* isolates were biotyped as biotype 3 and 2 isolates as biotype 1. From 38 of 39 cultures positive fetal stomach contents *B. melitensis* specific DNA was to detect by PCR. PCR found negative in all of the culture negative samples. Compared with culture, sensitivity and specificity of PCR were determined as 97.4 and 100%, respectively. The results indicate that this PCR procedure has a potential for use in routine diagnosis of sheep.

Refai (2002) stated that in countries of the Near East region (Saudi Arabia, Kuwait, Oman, Iraq, Iran, Sudan, Egypt, Libya, United Arab Emirates and Somalia), brucellosis reported in almost all domestic animals, particularly cattle, sheep and goats. *Brucella melitensis* biovar 3 is the most commonly isolated species from animals in Egypt, Jordan, Israel, Tunisia and. Turkey. *B. melitensis* biovar 2 reported in Turkey and Saudi Arabia, and *B. melitensis* biovar 1 in Libya, Ond Israel. *B. abortus* biovar 1 reported in Egypt, biovar 2 in Iran, biovar 3 in Iran and Turkey, and biovar 6 in Sudan.

Renukaradhya *et al.* (2002) stated in India that brucellosis recognized first in 1942 and is now endemic throughout the country. The disease reported in cattle, buffalo, sheep, goats, pigs, dogs and humans. *B. abortus* biotype-1 in cattle and buffaloes and *B. melitensis* biotype-1 in sheep, goats and man are the predominant infective biotypes.

Ghani *et al.* (1998) stated that several epidemiological factors, such as age, sex, breed, lactation number, herd size and living conditions influence the sero-prevalence of brucellosis.

2.2.3. Susceptibility

While it is obvious that susceptible factors influence the outcome of *Brucella* infection in small ruminants (self-limited infections, acute infection, latent persistence, etc.) no studies have demonstrated the exact nature and role of individual susceptible factors compared with other favouring or unfavourable factors. The susceptible factors were:

2.2.4. Species

The goat was originally considered the principal host of *Brucella melitensis*, notably in Latin America, where sheep are not significantly infected even when kept in close contact with goats. In many other areas the disease is more important in sheep. There are several reasons for this difference Species behaviour is also considered as a favouring factor. Ewes generally gather together at lambing or at night, while goats do not. Excretion from the vagina in goats is more copious and prolonged than in cows and lasts for at least 2-3 months. In this animal, about two thirds of acute infections acquired naturally during pregnancy lead to infection of the udder and excretion of the organisms in the milk during the next lactation. Excretion may cease during lactation. Infection in goats results in a greater reduction in milk production than is the case in cattle (Alton, 1985). To the best of knowledge, there is no published comparative report of the sero-prevalence of brucellosis in various livestock species, which takes most of the livestock species in Bangladesh into consideration.

Rahman *et al.* (2012) stated that positive reactors or suspects by CELISA was 2 out of 135 (1.48%) in buffaloes, 1 out of 465 (0.22%) in cattle, 5 out of 230 (2.17%) in goats and 15 out of 170 (8.82%) in sheep.

Bokaie *et al.* (2008) investigated the prevalence rate of brucellosis in human, sheep, goats and cattle in Iran during 2002-2006. The prevalence of human brucellosis was 37/100 000, 340/10,000 in sheep and goats and 56/10 000 in cattle. Statistical analysis showed that Pearson correlation coefficient of cattle and sheep brucellosis (r=+0.746), cattle and human (r=+0.228), human and sheep (r=+0.304) were positive but incomplete. Coelho *et al.* (2008) stated that herd sire and production type might have an impact on brucellosis seroprevalence in sheep and goat.

Kebede *et al.* (2008) was undertaken a cross sectional study to determine the prevalence of bovine brucellosis on 1116 cattle and to assess risk factors that could promote its transmission to man in 111 smallholder farms in central Ethiopia. Using CFT, herd and individual animal prevalence were 45.9 % (51/111) and 14% (12S/1136), respectively, while using RBT, herd and individual animal seroprevalences were 46.8% (S2/111) and 12.5% (142/1136), respectively. Valarmathy *et al.* (2007) randomly selected 17 villages of Udham Singh Nagar and Bageshwar districts of Uttarakhand, India. Total 326 serum samples (from 213 goats and 113 sheep) tested by RBT, STAT and i-ELISA. The overall seroprevalence was 26.99%. Species wise seroprevalence in sheep and goats was 12.38, 5.30, 21.23% and 14.55, 9.85, 30.04% by RBT, STAT and indirect ELISA, respectively. Emslie and Nel (2002) mentioned that *B. melitensis* is a Gram-negative bacterium whose primary hosts are goats and sheep. Like the other *Brucella* spp., with the exception of *Brucella ovis,* it is not particularly host specific as it is pathogenic for a variety of other mammal species including humans. McDermott and Arimi (2002) found that brucellosis is an important disease among livestock and people in sub-Saharan Africa. In general, the prevalence is the highest in pastoral production systems and decreases as herd size and size of landholding decreases. The prevalence of risk factors for infections best understood for bovine brucellosis and to a lesser extent for ovine and caprine brucellosis.

Burriel *et al.* (2002) tested 250 sheep and 250 goats from 25 and 26 flocks respectively in Greece for brucellosis and 16.8% of sheep and 13.1% of goats were positive to *Brucella* infection.

Ahl *et al.* (1993) tested serum samples from 161 goats and 53 sheep of the University of Virgin Islands flock and seroprevalence of *B. melitensis* antibodies was 11.3% for sheep and 2.5% for goats.). Mahboub *et al.,* 2013 observed goats (36.84%) had a higher *B. melitensis* seroprevalence than sheep(12.26%).

2.2.5. Breed

Bandey *et al.* (1989) performed a sero-epidemilogical study on brucellosis in exotic (Merino) sheep in Kashmir valley and tested 8034 serum by the serum agglutination test for antibodies to *B. abortus* and found that 258 (3.2%) were positive. Islam *et al*. (1983) reported the prevalence of brucellosis among different breeds of native bovine species and their crosses in Bangladesh. They found the prevalence rate in indigenous, are less than crossbred. . First, whereas most breeds of goat are fully susceptible to infection, a great variation in the susceptibility of different breeds of sheep has been reported. Thus, sheep milking breeds appear more susceptible than those kept for meat production (Corbel and Brinley-Morgan, 1984). Maltese and South American sheep breeds appear very resistant, whereas the fat-tailed sheep of Southwest Asia and Mediterranean breeds are very susceptible and form a reservoir of infection that gives rise to widespread infections of man. Therefore in most countries bordering the Mediterranean Sea and in Southwest Asia, the brucellosis problem largely centres on sheep, while in Latin America goats are chiefly involved. Second, in the Mediterranean sheep are the predominant species, being often kept in large flocks, in conditions that favour the spread of infection.

2.2.6. Sex

In view of the significance of this disease, the present study designed to detect prevalence of Brucellosis, and to analyses risk factors, especially in small ruminants and in livestock farmers. Yesuf *et al*., 2011 conducted a cross-sectional study in south Wollo zone, northeast Ethiopia from October 2008 to March 2009 aimed to determine the sero-prevalence and to identify potential risk factors of ovine brucellosis. A total 800 sheep sampled were from two districts (Kalu and Harbu). Over all seroprevalence of 1.5% (12 of 800) ovine brucellosis was observed. Seroprevalence was higher in female sheep compared to male sheep. Seroprevalence calculated between sexually immature and sexually mature sheep, between animals kept under extensive and semi-intensive management system, and between animals of the two districts. Higher levels of sero-prevalence was observed in sexually mature sheep, in animals kept under extensive management system, and in sheep of Kalu with level of 1.54%, 1.6% and 1.58%, respectively. However, there was no statistically significant difference between the sero-prevalences of brucellosis in the different study groups of sheep.

Gul *et al.,* 2014 experiment to got result in relation to sex, sero-prevalence of brucellosis in goats, prevalence of brucellosis was higher in bucks as compared to does and the difference was statistically significant through all tests except RBPT. In sheep, sero-prevalence of brucellosis was lower in ewes as compared to rams and the difference was statistically significant (P<0.001) through all four tests.

Rahman *et al*. (2012) Sex related seroprevalence of brucellosis is relatively higher prevalence was found in female than in male cattle, goats, and sheep, whereas higher prevalence of brucellosis was found in male than in female in case of buffaloes. It declared in goats, male's sera found positive reactor. In case of female goats, average seroprevalence were 3.13% (6 out of 192) by TAT, 2.60% (5 out of 192) by C-ELISA and FPA. In case of sheep, females

only showed an average prevalence, 9.66% (14 out of 145) by TAT, 10.34% (15 out of 145) and 8.28% (12 out of 145) by C-ELISA, respectively.

Rahman *et al*. (2011) stated in goats, 3.57% of male sera found to be positive reactors. The average sero-prevalence was 4.04% in female goats. In sheep, 0.0% prevalence found with every test performed in males but in females, the average prevalence was 2.61% with I-ELISA.

Siriwardane and Ortmann (2010) showed the prevalence of ovine brucellosis in Sri-Lanka was approximately 2% and varied from 1.2 to 20% in various areas. Brucellosis in goats diagnosed once in 1968 and has not have detected in Sri-Lanka since 1995. The control program comprised testing and elimination of reactors, supplemented by S19 vaccination.

Valarmathy *et al.* (2007) randomly selected 17 villages of Udham Singh Nagar and Bageshwar districts of Uttarakhand, India. A total 326 serum samples (from 213 goats and 113 sheep) were tested and observed prevalence was higher in females than in males.

Tome (1995) reported that an abortion storm observed in a herd of 2200 animals on a farm in Argentina was observed in 26% aborted the females. Serological studies using Rose Bengal test, tube agglutination test, and 2-mercaptoethanol tests showed a prevalence of brucellosis of 68.5%. Two strains of *B. melitensis* biovar 1 were isolated, one from milk and 1 from colostrum.

Ogundipe *et al.* (1994) observed that the infection rates of brucellosis were apparently higher in female goats than male.

2.2.7. Age

Gul *et al.* (2014) trial to observed in goats, the difference in sero-prevalence among these four age groups was statistically significant ($P<0.002$) through RBPT, while it was non-significant through all other tests. In sheep, the difference among different age groups was statistically non-significant through

all tests except c-ELISA, where difference was statistically significant (*P*<0.04).

Rahman *et al.* (2012) pragmatic the seroprevalence of brucellosis in goats of less than 24 -month of age was 1.52% (3 out of 197). Nevertheless, in goats over 24 month of age, the prevalence of brucellosis was 9.09% (3 out of 33). In case of sheep of over 24 month, the prevalence of brucellosis was 35.0% (14 out of 33).

Rahman *et al.* (2011) observed in goats in the age group of over 24 months, the prevalence of brucellosis was 18.18% (two out of 11). In the case of sheep, in 110 sheep of less than 24 months of age, the prevalence of brucellosis was 0.00% but in the age group of over 24 months, the prevalence of brucellosis was 15.0% (three out of 20) in the five different regions of Bangladesh.

Gebretsadik-Berhe *et al*. (2007) carried out an epidemiological study on brucellosis. A statistically significant increase of brucellosis was recorded with increasing age (*P*<0.01) but not parity (*P*>0.05).

Yesuf *et al*. (2011) conducted a cross-sectional study 800 sheep were sampled from two districts, Kalu and Harbu. All sheep above six months of age with no history of previous vaccination against brucellosis selected. Rose Bengal Plate Test (RBPT) utilized as a screening test for *Brucella* agglutinins while Complement Fixation Test (CFT) (Addlestone, United Kingdom) used to confirm the reactors by RBPT.

Amin *et al*. (2004) demonstrated that brucellosis was more prevalent in cows older than 4 years of age.

Andrewartha and Elliott (1990) conducted a survey in 1986 to establish the prevalence of ovine brucellosis in 53 flocks not previously tested for the disease. All rams over 12 months old tested for *B. ovis* antibody using a complement fixation test (CFT). The prevalence was 0.14% in 718 samples tested and the flock prevalence was 1.9% in the 53 flocks surveyed.

2.2.8. Parity

Gebretsadik-Berhe *et al*. (2007) carried out an epidemiological study on brucellosis. A statistically significant increase of brucellosis was recorded with increasing age ($P<0.01$) but not parity ($P>0.05$).

2.2.9. Body weight

Gul *et al.* (2014) trial the difference in sero-prevalence of brucellosis in goats and sheep, statistically the difference between two groups was significant only through RBPT and prevalence was higher in animals having higher body weights.

Mahboub *et al.* (2013) exposed that effects of brucellosis on animal performance revealed that young goat and sheep the (Mean±SE) were (2.00±0.45) & (0.67±0.45) had significantly reduced with *Brucella meletensis* compared to adult and their (Mean±SE) were (50.00±3.61) & (31.33±3.78), respectively.

2.2.10. Flock size

Lithg-Pereira *et al*. (2004) conducted a case-control study in brucellosis low prevalence area of Spain to determine factors associated small ruminants brucellosis prevalence in 1998. The introduction of replacement animals into the flock, the presence of older farmers, a higher flock seroprevalence in the town were positively associated with case flocks. Megersa *et al*. (2012) stated that the herd level prevalence was 51.7% (30/58) for cattle, 15.0% (16/107) for camels and 13.3% (13/98) for goats. The mean within-herd prevalence was 15.5% (range 4.8–50.0%) for cattle, 8.9% (4.4–33.3%) for camels and 10.5% (5.0–25%) for goats.

Adugna *et al.* (2013) practically showed the univariable logistic regression analysis of the putative risk factors showed statistically significant ($P<0.05$) difference on *Brucella* reactivity between small ruminants with small and large

flock size. This signifies that brucellosis has significant economic implication in its ability to bring about morbidity at flock level.

Mahboub *et al.* (2013) revealed that small size flocks shown more seropositive for *Brucella* infection than large flocks (P<0.003).

Gebretsadik-Berhe *et al*. (2007) carried out an epidemiological study on brucellosis. Significant increment of seropositivity was also observed as herd size increases from small to medium (P<0.05) and then to large sizes (P<0.001).

2.2.11. Biosecurity

Ramos *et al*. (2008) observed that 645 serum samples analyzed by the CFT. A 4.0% frequency was found (26/645) in patients scrum and among those 4.1% (23/551) were slaughterhouses employees and 8.1% were rural workers. Of the total positive samples, there (2.0%) were women and (13 4.7%) were men, (2.9%) were between the ages of 18 and 30, six (3.4%) were between 31 and 40, and 9 (8.0%) were above 41 years of age. Gebretsadik-Berhe *et al*. (2007) carried out an epidemiological study on brucellosis. A significantly higher seroprevalence found in animals in the low land than those in the high land agro-climatic zones.

Teshale *et al.* (2006) conducted a seroprevalcnee study of small ruminant in two sheep and goat rearing pastoral regions of Ethiopia, namely Afar and Somali, from November 2004 to April 2005. The study revealed higher prevalence of *Brucella* antibodies (9.7%) in the absence of *Brucella* vaccination. Rajesh *et al.* (2003) reported the oral route, contamination of the udder during milking and contact with aborted fetuses and infected newborn lambs considered common methods of spread, also the venereal transmission of the disease occur due to infected male or contaminated semen. Infected tissues and contaminated materials handled under (biosafty 3) conditions. Transmission could be either by contaminated food, invasion by intact skin,

inhalation of aerosols containing the bacteria and aerosol contamination of the conjunctiva.

Rodriguez *et al.* (2001) investigated that brucellosis outbreak, occurred in a slaughterhouse Zaragoza, Spain between 2 December 1998 and 4 May 1999, affecting 28 employees. There were no significant differences in risk factors involving working in a risk area, use of protective measures and presence of cuts and wounds.

Casalinuovo *et al.* (1996) tested serologically on 269335 sheep and goats on 7163 farms, 11342 (4.2%) animals, and 924 (12.9%) farms were positive. When materials from serologically positive animals examined bacteriologically 40 *Brucella* strain from sheep and goats isolated.

2.2.12. Feeding habit

Mahboub *et al.* (2013) revealed that non-grazed flocks shown more seropositive for *Brucella* infection than grazed flocks ($P<0.001$).

Yesuf *et al*. (2011) conducted a cross-sectional study a total of 800 sheep were sampled from two districts (Kalu and Harbu) and he was calculated seroprevalence between sexually immature and sexually mature sheep, between animals kept under extensive and semi-intensive management system, and between animals of the two districts. Higher levels of sero-prevalence was observed in sexually mature sheep, in animals kept under extensive management system, and in sheep of Kalu with level of 1.54%, 1.6% and 1.58%, respectively. Darwish and Benkirane (2001) reported the epidemiological status of brucellosis in cattle and small ruminants in Syria from 1990 to 1996. In sheep and goats, brucellosis seroprevalence fluctuated in the two sectors, but was higher in the private sector where husbandry is principally extensive. Bacteriological investigations led to the isolation of *Brucella melitensis* biovars 2 and 3 in sheep and *Brucella abortus* biovar 9 in cattle. Kabagambe *et al.* (2001) investigated cross-sectional prevalence and risk factors For *Brucella* seropositivity in goats in Uganda. The most-

important herd-level risk factors identified were use of a hired caretaker as the primary manager of the operation compared to owner/family members, keeping sheep in addition to goats compared to having no sheep, and free browsing, when compared to tethering or zero-grazing. Using the TAT, 10% (141/1446) of the goats tested positive. The positives distributed in 43% (63/145) of the herds. Free browsing when compare to tethering or zero grazing and lack of veterinary care were the most-important factors identified in the multivariable model for *B.* seropositivity *melitensis* herd. Omer *et al.* (2000) collected samples from 2427 cattle, 661 goats and 104 sheep from Eritrea for screening *Brucella* infections by the Rose Bengal test and positive reactors confirmed by the complement fixation test during 1997- 1998. The highest individual seraprevalence was in dairy herds kept under the intensive husbandry system, with an individual prevalence of 8.2% and unit (herd) sero-prevalence of 35.9%. Individual prevalence of 3.8% (goats) and 1.4% (sheep) and unit prevalence of 33.3% (goats) and 16.7% (sheep) found. Megersa *et al*., (2012) stated that illustrates village-level seropositivity to *Brucella* infection by animal species. Seropositive of animals were be found in 93.8% (15/16), 43.8% (7/16) and 18.8% (3/16) of the villages with at least one, two and all three positive animal species, respectively. A village-level seropositive factor was more frequently detect in cattle (93.3%) than in camels (56.3%) and goats (37.5%). The average number of positive animals per positive herd was generally low and comparable in the three species, cattle (1.5), goats (1.5) and camels (1.2), suggesting a slow within-herd spread of the disease.

2.2.13. Seasons

Akakpo and Bornarel (1987) described those factors such as climate and type of husbandry played a much greater role in prevalence of brucellosis than strictly intrinsic factors.

Radwan *et al.* (1983) studied the results of an extensive serological survey for brucellosis antibodies using the standard plate agglutination procedure on

14,000 serum samples from native domestic animals and imported livestock over a five-year period are be reported. The prevalence of brucellosis was highest (11.6%) in small ruminants reared intensively in breeding establishments, next highest (2.6%) in imported animals sacrificed during the Hajj season and somewhat lower (1.5%) in local livestock sacrificed during the Hajj season. The prevalence was very low among the following groups of livestock: small ruminants raised on desert ranges (0.5%), small ruminants raised in small groups around individual homes (0.4%) and in commercial dairy herds (0.2%).

Pandey and Desai (1973) carried out a study and concluded that the highest prevalence of the disease (13%) had been have found to be associated with areas having heavy rainfall with moderate temperature. Practice of breeding with artificial insemination also found to play contributory role in the prevalence of bovine brucellosis.

2.2.14. Location

Adugna *et al.,* 2013 studied Sero-prevalence of small ruminants' brucellosis in four districts of Afar National Regional State, Northeast Ethiopia. He saw epidemiology of the disease at individual and herd level show wider spread of the disease in different species of animals. In Afambo and Assayita, districts of zone one, animals kept in confinement around cultivation fields than the other two districts, as the districts largely dominated by agricultural irrigation using Awash River. This may be responsible for the high prevalence in zone one as infection easily transmitted within the entire herd under this management system. Teru and Awura districts are mostly pastoralist settings and dominated by free-range management system. Shehu *et al.* (1999) reported a prevalence of 6.6% in sheep in Nigeria. However, Yesuf *et al.* (2010) conducted a cross-sectional study in sheep from two districts (Kalu and Harbu) in Ethopia and reported a higher seroprevalence of 1.5% in south Wollo. Teshale *et al.* (2006) and Ashenafi *et al*. (2007) reported seo-prevalence of 14.6% and 3.2% in Mille

and Dalifage districts of Afar region and in Afar region, respectively. In other countries, Bale *et al.* (1982) reported 15.9% prevalence in a study conducted in Northern Nigeria. Higher prevalence in goats compared to this finding was reported by Teshale *et al.* (2006) (16.45%), Bale *et al.* (1982) (34.8%) and Ojo *et al.* (2007) (45.75%) in Afar region of Ethiopia, northern Nigeria and Abeokuta, respectively. However, a lower prevalence of 5.8% reported by Ashenafi *et al.* (2007).

2.2.15. Pregnancy status

The preponderance of seropositive reactors in mature and pregnant animals suggests that sexually mature and pregnant animals are at higher risk of infection with *Brucella spp.* (Muma *et al*., 2007). Higher prevalence of brucellosis in animals with a history of abortion constitutes a significant risk for transmission of brucellosis to the uninfected animals since they are known to shed massive number of *Brucella* from the uterus at subsequent normal parturitions (Islam *et al*., 2013).

2.2.16. Reproductive diseases

Brucellosis in relation with reproductive diseases was described by various authors in their studies.

The researcher Tobias *et al*. (1993) reported that in both domestic and wild hosts *Brucella* colonization of the gravid reproductive tract can lead to severe placental damage, fetal infection and fetal death. This influence could be explained by the fact that brucellosis is essentially a disease of the sexually mature animals, the predilection site being the reproductive tract, especially the gravid uterus (Abubakar *et al.,* 2012). Rahman *et al.* (1988) conducted a study on sero-prevalence of caprine and human brucellosis in some selected areas of Bangladesh and observed higher prevalence of the disease in goats with reproductive disorders.

Brucellosis due to decreased milk production, abortions, weak off springs, weight loss, infertility and lameness, it is one of the most serious diseases of

livestock. It is also a major impediment for the trade. Death may occur because of acute metritis, followed by retained fetal membranes (Radostits *et al.,* 2000). From public health viewpoint, brucellosis considered an occupational disease that mainly affects slaughterhouse workers, butchers, and veterinarians. Transmission typically occurs through contact with infected animals or materials with skin abrasions.

The *Brucella* may enter the body through digestive tract, lungs or mucosal layers and intact skin. Then it may spread through blood and the lymphatic system to any other organ where it infects the tissues and causes localized infection (Lapaque *et al.,* 2005). There are so many factors, which can affect the prevalence of brucellosis in various species of livestock. Prevalence of brucellosis can vary according to climatic conditions, geography, species, sex, age and diagnostic tests applied.

A study carried out on seroprevalence of human and animal brucellosis in Bangladesh by Rahman *et al.* (1983). Higher occurrence of the brucellosis observed in cows of organized farms. Rahman *et al.* (1988) reported the seroprevaelnce of brucellosis among goats of domestic holding of some selected areas of Bangladesh. Higher incidence of the disease observed in goats with reproductive disorders. In addition, the occurrence of *Brucella* agglutination in individuals who were in direct contact with goat population revealed higher occurrence of the disease.

Sandhu *et al.* (2001*)* suggested that interspecies transfer of infection poor management conditions prevailing for brucellosis at the farms. They also suggested that brucellosis is the major etiological agent of abortions in the farm animals.

Tittarelli *et al*. (2005) studied that the persistence of infection in 46 ewes experimentally infected with *B. melitensis* biovar 3 and monitored through three subsequent reproductive cycles. The entire experimental period lasted for 151 weeks. Infection of ewes and elimination of *Brucella* milk, or its presence

in vaginal discharges, persisted throughout the duration of the trail as demonstrated by recurrent elimination of *Brucella* in milk and vaginal discharges. *B. melitensis* recovered from the tissue of one ewe killed at the end of the trail.

Ocholi *et al.* (2004) isolated *Brucella* from aborted fetuses, hygroma fluids, milk and vaginal swabs obtained from aborting cattle, sheep, goats, pigs, and horses in Nigeria. A total 25 isolates, obtained mainly from cattle, sheep and horses, were biotyped. All strains belonged to one species, *B. abortus* biovar 1.

Al-Ani *et al.* (2004) examined between 1996 and 1998, a total 1,594 samples of animal blood, collected from 1,050 sheep from 20 flocks, and 544 goats from eight herds. The serum samples tested using the Rose Bengal test, the tube agglutination test, the complement fixation test and an enzyme-linked immunosorbent assay (ELISA). Moreover, a complete history compiled from each flock/herd. The rate of abortions in sheep due to brucellosis ranged from 0.5% to 56%, with a mean of 33.2%. The goats had a higher abortion rate. Thirty-four aborted sheep fetuses collected from these 20 flocks bacteriologically and pathologically examined. A pure culture of *B. melitensis* biotype 3 was isolated from 21 of the aborted fetuses.

Al-Talafhah *et al.* (2003) isolated the *Brucella* organisms from aborted fetuses and vaginal swabs were characterizing as *B. melitensis* biotype 3.

Chand *et al*. (2002) reported the involvement of *Brucella* in sheep in several cases of epidimyo-orchitis in breeding rams to an organized sheep farm in northern India. Clinical examination of the rams revealed a marked enlargement and pendulous appearance of the scrotum.

Samad (2001) stated that in case of brucellosis usually death fetus aborted. Weak fetus may be parturated but death may occur immediately after parturition. Abortion usually occurs at the later stage of the pregnancy and retention of placenta is a common feature. In male, the main clinical manifestation is orchitis and epididymitis.

Karaman *et al.* (1993) reported that in lambing periods of 1989-1992 the bacteriological examination of 156 aborted fetuses resulted in the isolation of *B. melitensis* from 34 (22%) and of the 4658 ewes examined serologically after abortion, 647 (14%) were positive for antibodies for brucellosis.

Tobias *et al*. (1993) reported that in both domestic and wild hosts *Brucella* colonization of the gravid reproductive tract can lead to severe placental damage, fetal infection and fetal death.

O'Hara (1987) stated that a voluntary control program for *Brucella ovis* practiced. Of 72736 ovine serum samples tested, 8% had significant titres and 0.9% suspicious titres. Of 86443 rams examined clinically, 2.6% had palpable lesions of epididymitis.

2.3. Experiment 3: Assessment of Toxoplasmosis linked with reproductive disorders in small ruminants

Toxoplasma gondii is an obligate intracellular protozoan parasite that has an extremely wide host range and that can survive in all nucleated cells of mammals, including humans (Pfefferkorn, 1990). The establishment of *T. gondii* within a modified host cell vacuole leads to chronic infection and the production of cysts 8 in skeletal muscle and the central nervous system (Remington and Cavanaugh, 1965). The domestic sheep and goats are the vital source of milk, meat, fibers and pelts in almost all the countries of the world. These livestock animals are vulnerable to different parasitic diseases (Gebremedhin *et al*., 2013) including toxoplasmosis (Othman and Al-Azuheir, 2014) that causes a range of reproductive problems such as prenatal and postnatal mortality, still births or abortion (Edwards and Dubey, 2013) leading to the economic losses and threats to human health via zoonotic transmission (Othman and Al-Azuheir, 2014). The livestock animals in general and small ruminants in particular are exposed to a variety of infectious and noninfectious diseases of parasitic origin (Akhter and Arshad, 2006; Siddiki *et al*., 2010) leading to the reduction in productivity (Haileleul, 2002). Parasitic infections pose threats to health and limit the productivity due to the associated morbidity and mortality (Nwosu *et al.,* 2007) or due to early death in the embryonic life, mummification, abortion, still birth (Panadero *et al.,* 2010) and in some cases the neonatal or postnatal death (Edwards and Dubey, 2013). These infections have become a serious universal threat for livestock (Lashari and Tasawar, 2011).

Toxoplasmosis is deleterious in terms of both economy of a country and health of its people (Hill *et al.,* 2005) and approximately thirty three percent of animals and human population of the world has been estimated to be infected with *T. gondii* at an average (Sensini, 2006) ranging between 1 and 99% rates of infections (Olivier *et al.,* 2007). The open-air meat markets considered as a

potential source of infestation of human by *T. gondii.* While being transported from slaughterhouses, the meat by far contaminated with oocysts of *T. gondii* hence resulting as a risk factor of zoonosis to human beings. The incidence of *T. gondii* seroprevalence in different species of animals has been have studied in many countries of the world with results varying from country to country, region to region, herd to herd and season to season. Sometimes even these results vary with variation of methodology utilized on the same herd (Yu *et al.,* 2007). The open-air markets are common in Rahim Yar Khan and Rajan Pur regions of southern Punjab, Pakistan for creating a potential source of pathogen contamination. Where stray cats and dogs usually laid around the markets, which, being a natural reservoir of *T. gondii*, add many millions of oocysts of parasite through their excreta just within a couple of week after getting infected (Dabritz *et al.,* 2007). Oocysts of *T. gondii* exhibit a characteristic resistance against a broad spectrum of disinfecting agents due to structural stability principally in warm and humid regions of the world, nevertheless having poor.

2.3.1. Overall occurrence

Toxoplasmosis caused by *Toxoplasma gondii* (hereafter referred as *T. gondii* or/and *Toxoplasma*) belonging to class Coccidia of phylum Apicomplexa (Ferguson, 2002; Kopecna *et al*., 2006). *Toxoplasma gondii* is considered as the most successful parasitic pathogen worldwide. Cats (domestic and wild), the definitive hosts of *T. gondii,* are epidemiologically important animals because they shed environmentally resistant oocysts in the faeces (Dubey, 2010). Warm-blooded vertebrates including humans, rodents, birds, livestock and marine mammals are intermediate hosts (Dubey, 2010). *T. gondii* is widespread in distribution and can be considered as one of the most successful protozoan parasites (Doskaya *et al.,* 2006) that can inflict serious diseases in all the endothermic animals (Doskaya *et al.,* 2006; Dubey, 2009) such as mammals including small ruminants and humans (Aspinall *et al.,* 2002). Since

Toxoplasma is transmitted through zoonosis particularly from sheep and goats (Sevgili *et al.,* 2005), there is dire need to screen the animals whose meat is used by human as food so that the human health is ensured (Hill and Dubey, 2002; Lhafi *et al.,* 2004). The seroprevalence in goat is 19.7%, but several researcher observe that the Bekele and Kasali (1989) from Central Ethiopia (22.9%), Tilaye and Getachew (2002) from Debre-Birhan, North Shewa (34%) and Negash *et al.* (2004) from Nazareth, East Shewa (24%). Teshale *et al.* (2007) in goats (62-84%) from South Omo, North Omo (Southern Ethiopia) and East Shewa Zones (Central Ethiopia). The differences in the seroprevalence could be due to differences in the relative cat densities and the access of goats to contaminated feed and water, the geographical variability, the serological tests used and the cut-off value reported. According to the review of Dubey (2010), seroprevalence ranging from 3.2% in Mexico (by ELISA) to 90.9% in the Netherlands (by latex agglutination test) were reported.

2.3.2. Transmission

The transmission of *toxoplasma* with its life cycle was described according to Gebremedhin (2014) description in below:

The life cycle includes intestinal-epithelial (entero epithelial) and extra intestinal stages in domestic cats and other felines but only extra intestinal stages in other hosts. Sexual reproduction occurs in the intestine of cats, and only asexual reproduction is seen in various tissues of intermediate hosts (all warm-blooded animals including most livestock and humans) (Plate. 2) (Buxton, 1998; Marquardt *et al*., 2000; OIE, 2008; Tenter, 2009; Dubey, 2010). There are three infectious stages, i.e. tachyzoites (rapidly multiplying stage), bradyzoites (contained in tissue cysts and slowly multiplying stage) and sporozoites (contained in sporulated oocysts) that are infectious for both intermediate and definitive hosts (Sibley *et al*., 2009; Tenter, 2009). Cats acquire infection by eating meat containing bradyzoites in tissue cysts of intermediate hosts, such as birds and rodents or by ingesting infective oocysts.

Upon ingestion of tissue cysts proteolytic enzymes in the cat's stomach and intestine degrade the wall of the tissue cysts and bradyzoites are released. Bradyzoites then penetrate the intestinal epithelial cells of the cat where they initiate the development of numerous generations of *T. gondii* (Dubey, 2010). Five morphologically distinct types of *T. gondii* (A to E) develop in intestinal epithelial cells before gametogony begins (Dubey, 2010). The sexual cycle starts two days after ingestion of tissue cysts by the cat, eventually producing millions of oocysts that are released in the cat's faeces for 2-3 weeks (Montoya and Liesenfeld, 2004; Dubey, 2010). Following sporulation that takes between 2 and 5 days, oocysts can survive for months to years under moderate environmental conditions and their resistance to destruction assures potential widespread contamination of food and water supplies (Dubey, 2004 & 1998). Although bradyzoites within tissue cysts are less resistant to environmental conditions than oocysts, they remain viable over a wide range of temperatures, including mild freezing conditions (Gajadhar *et al*., 2006). Cats shed oocysts with a short prepatent period (3 to 10 days) after ingesting bradyzoites, whereas after ingestion of tachyzoites or oocysts, the prepatent period is longer (≥14 days) (Dubey *et al*., 1998; Dubey, 2010). Intermediate hosts, including felids, can acquire *T. gondii* by ingesting either tissues of infected animals, food or drink contaminated with sporulated oocysts or by transplacental transmission (Tenter *et al*., 2000; Radostits *et al*., 2006). After ingestion, bradyzoites released from tissue cysts or sporozoites from oocysts penetrate intestinal tissues, transform to tachyzoites, multiply locally, and are disseminated in the body via blood or lymph to start the extraintestinal stage of the parasite. After a few multiplication cycles, tachyzoites give rise to bradyzoites in a variety of tissues due to development of host immune response. Bradyzoites may have a lifelong persistence in the host (Montoya and Liesenfeld, 2004). The feline intestinal tract is the only source for the production of *T. gondii* oocysts. Transmission to humans usually occurs through the ingestion of oocysts from contaminated sources (such as soil, cat

litter, garden vegetables, and water) or the ingestion of tissue cysts in undercooked meat from infected animals. Vertical transmission through tachyzoites from mother to fetus has also been reported in many species (Dubey and Beattie, 1988; Dubey, 2010). Although fetal infection most often occurs after acute *T. gondii* infection in a pregnant woman, it also can occur after the reactivation of latent infection in an immuno-compromised pregnant woman (Remington *et al.,* 2006; Dubey, 2010). Numerous variables influence whether congenital transmission will occur. Many of these factors are recognized but poorly understood.

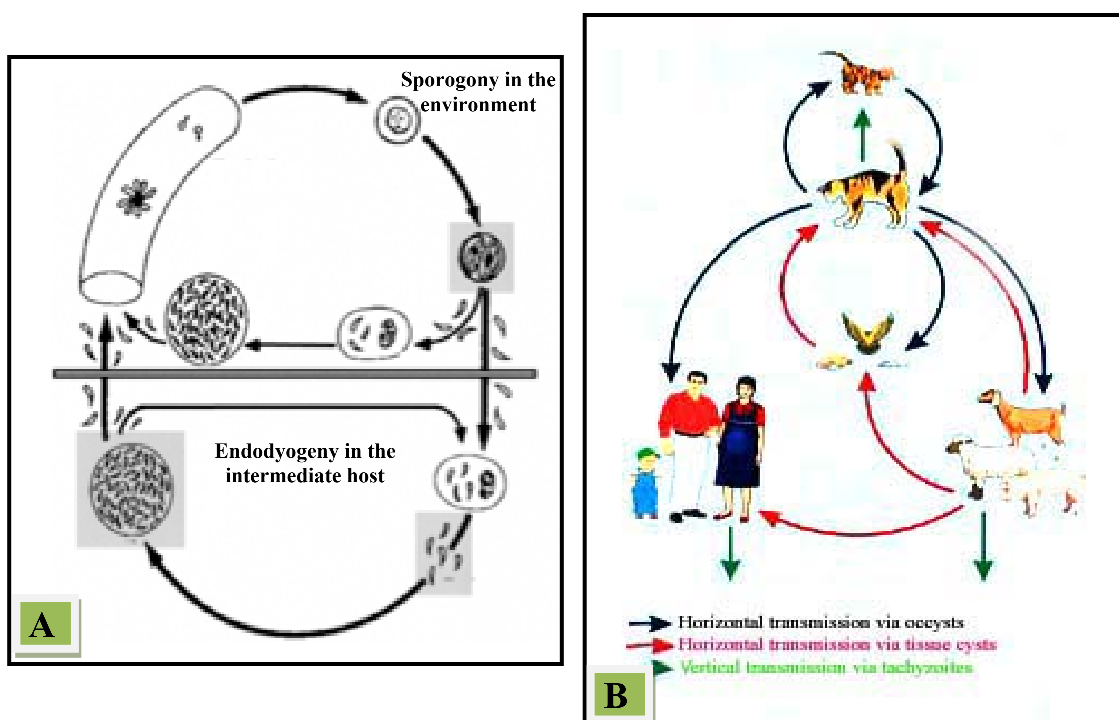


Plate 2. Pathways for *Toxoplasma gondii* infection [Adapted from: Jones *et al.*, 2003 (A) and Tenter *et al.*, 2000 (B)]

They include the strain and virulence of *T. gondii,* inoculums size, route of infection, time during gestation, and immuno-competence of the pregnant woman. All of these also pertain to infection of the fetus and its outcome in the newborn thereafter (Remington *et al.*, 2006). Horizontal transmission through ingestion of unpasteurized sheep and goat milk has also been reported (Dubey and Beattie, 1988; Dubey, 2008). Oocysts in soil can be mechanically

transmitted by invertebrates such as flies, cockroaches, dung beetles, and earthworms, which can spread oocysts into human food and animal feeds (Hill *et al*., 2005). Transmission through blood transfusion, accidental laboratory infection and organ transplant are also less common horizontal transmissions (Das, 1992; Tenter *et al*., 2000; Remington *et al*., 2001; Dubey, 2004; Montoya and Liesenfeld, 2004). Although strict herbivores are primarily infected by oocysts, oral transmission via ingestion of tissue cysts probably contributes to the spread of *T. gondii* infection through the food chain owing to carnivorous or omnivorous feeding. These different modes of transmission may contribute to the extremely broad host range of *T.gondii* (Sibley *et al*., 2009). The possible mode or routes of transmission and source of infections of toxoplasmosis are summarized in Plate 2 (A and B).

2.3.3. Effect of species

Shahiduzzama *et al.* (2011) research about *Toxoplasma gondii* Seroprevalence in Domestic Animals and Humans in Mymensingh District, Bangladesh and demonstrated that a high seroprevalence found in (40%) sheep in Bangladesh than in (32%) goat. Mahboub *et al.* (2013) observed *T. gondii* seroprevalence was higher in sheep (31.01%) than goats (17.11%). Gebremedhin and Gizaw 2014 found a result that odds of acquiring *T. gondii* infection was significantly higher in sheep (Odds Ratio (OR) = 2.6, 95% CI: 1.16-5.81; P = 0.028) than goats.

Dubey and Jones (2008) reported that prevalence of *T. gondii* in lambs could high but the role of ingestion of infected lamb in the epidemiology of toxoplasmosis in human’s remains to be determined.

2.3.4. Effect of breed

Mahboub *et al.* (2013) discovered that serological screening of the breeds revealed that the prevalence rate of *Toxoplasma gondii* among sheep was higher in Baladi breeds 35.42% (119 out of 336, *P*=0.001) than other sheep breeds. There was a no significant difference among sheep breeds, could detect

for brucellosis. All samples collected from Barki sheep had no detectable *Toxoplasma* infection. Seroprevalence for 76 goat breeds showed that Zarabi had higher *Toxoplasma* infection than other breeds.

2.3.5. Effect of sex

Yin *et al.* (2015) studied seroprevalence and risk factors of *Toxoplasma gondii* in Tibetan sheep in Gansu province, Northwestern China and observed the prevalence in females (19.2%, 95% CI = 17.01 – 21.46) were lower than in males (22.8%, 95% CI = 19.22 – 26.36).

Gebremedhin *et al.* (2014) observed this study used samples from abattoir where the majority of the slaughtered animals are young (55.7%, 350/ 628) and male (95.9%, 602/638). The significantly high prevalence in adult sheep than young sheep is due to high chance of exposure to the source of infection as the age increases and suggests that most sheep acquire the infection post-natal.

2.3.6. Effect of age

Gebremedhin and Gizaw (2014) found a result that odds of acquiring *T. gondii* infection was significantly higher in adult sheep (OR = 8.55, 95% CI: 2.79-26.15; P < 0.001) than young sheep.

Moizur *et al.* (2014) observed we calculated the seroprevalence of *T. gondii* in sheep, goats and cattle according to their age. They got seroprevalence in goats and cattle less than 1-year-old appeared to be lower than that of the older animals. In contrast, no clear age-specificity observed in sheep. To examine this more objectively, differences in the young (<1 year old) and adult (≥1 year old) animals were analyzed by Fisher's exact test. They found that seroprevalence in the young goats was statistically lower than that in the adult goats. They also found no statistically significant difference between seropositivity in the young and adult cattle (*P*=0.078). There was no significant difference in seropositivity between the young and adult sheep.

Considering that the oldest goat, they examined just 5-year-old, it is most likely that acquired infection occurs rapidly over only a few years in the goats.

Gebremedhin and Gizaw (2014) stated that *Toxoplasma gondii* seroprevalence was significantly higher in adult goats (> 1 year age) (22.5% than in the young age group (≤ 1 year) (11.4%). Increased risk of Toxoplasmosis in adults is likely due to increased opportunities of exposure to several predisposing factors or sources of infections from the environment. Therefore, this difference in prevalence among age group can explained by the cumulative effect of age (Hall *et al*., 2001; Dubey, 2010) and suggests that most goats in Ethiopia acquire the infection after birth.

Shahiduzzaman *et al.* (2011) research about *Toxoplasma gondii* seroprevalence in domestic animals and humans in Mymensingh district, Bangladesh and showed goats and sheep relatively high seroprevalence (32 and 40%, respectively). It was reporting more than 10 years ago that 12.8% of goat in this district was seropositive. The goat seroprevalence did not decrease in these 10 years. The results strongly suggest that control of *T. gondii* infection among domestic animals in this area has not been going well. When differences in seroprevalence between cattle vs goat and cattle vs sheep were examined by Chi-square test with Bonferroni correction, a statistical difference was found between cattle vs sheep ($P<0.05$). Mix grazing of cattle, goats and sheep at bank of river is common rearing style in this area. However, goat and sheep more frequently ranged on the streets. It might result in an increasing opportunity to access domestic cat feces.

Yin *et al.* (2015) studied seroprevalence and risk factors of *Toxoplasma gondii* in Tibetan sheep in Gansu province, Northwestern China and observed positive samples found in all four age groups, varied from 18.6% to 21.2%. In addition, the highest prevalence was detected in Tibetan sheep of the between 1 and 3 years of age (21.2%, 95% CI: 17.89-24.51) and the univariate analysis showed that the difference was not significant ($P>0.05$). Thus, there was no

correlation between the seroprevalence and age in Tibetan sheep. This result indicated that age was not a crucial risk factor for *T. gondii* infection in Tibetan sheep in Gansu province.

2.3.7. Effect of parity

Yin *et al.* (2015) studied seroprevalence and risk factors of *Toxoplasma gondii* in Tibetan sheep in Gansu province, Northwestern China and observed the numbers of parturition of female Tibetan sheep ranged between zero pregnancy and above 3 pregnancies. Moreover, the *T. gondii* seroprevalence varied in female Tibetan sheep with different numbers of pregnancies, ranging from 19% to 21.5% with the highest seroprevalence (21.5%, 95% CI: 17.03-25.87) in 3 or higher pregnancies group.

2.3.8. Effect of body weight

Mahboub *et al.* (2013) exposed that Effects oftoxoplamosis on animal body weight performance were revealed that young goat and sheep the Mean±SE were 1.33±0.32 & 2.33±0.45were significantly (*P<0.05*) *at* different letters size compared to adult and their Mean±SE were 42.33±2.56& 38.67±3.78, respectively.

2.3.9. Effect of flock size

Mahboub *et al.* (2013) revealed that large flocks size animals had higher *Toxoplasma* infection than small size grazed flocks (*P*=0.0001).

2.3.10. Effect of biosecurity

Saghir *et al.* (2015) demonstrated the populated where biosecurity measure did not properly take that observed the significant (*P*-Value= 0.000). It was regionally varying prevalence of *Toxoplasma* infection consistent (Yang *et al.*, 2013) who was reporting same picture in China and (Sechi *et al.,* 2013) whose results demonstrated the association between toxoplasmosis and still water sources contaminated by cats' access to water consumed by small ruminants

one of the important determinants in three different regions in the present study area.

Shahiduzzaman *et al.* (2011) research about *Toxoplasma gondii* seroprevalence in domestic animals and humans in Mymensingh district, Bangladesh and showed the relatively high seroprevalence of these small ruminants. Considering goats and sheep commonly used for meat production in this area and pork meat religiously forbidden food for majority of Bangladesh people, these small ruminants are potentially an important source of *T. gondii* for human infection.

2.3.11. Effect of feeding habit

Mahboub *et al.* (2013) revealed that grazed animals had higher *toxoplasma* infection than non-grazed flocks (P=0.001).

Shahiduzzaman *et al*. (2011) research about *Toxoplasma gondii* seroprevalence in Domestic Animals and Humans in Mymensingh District, Bangladesh and showed Goats and sheep showed relatively high seroprevalence (32 and 40%, respectively). Mix grazing of cattle, goats and sheep at bank of river is common rearing style in this area. However, goat and sheep more frequently ranged on the streets. It might result in an increasing opportunity to access domestic cat feces. The relatively high seroprevalence of these small ruminants might be due to the rearing system.

2.3.12. Effect of Season

Yin *et al.* (2015) studied seroprevalence and risk factors of *Toxoplasma gondii* in Tibetan sheep in Gansu province, Northwestern China and observed the seroprevalence in different season ranged from 16.5% in winter to 23.6% in summer.

Gebremedhin *et al*. (2014) observed this study sheep sampled during the dry season (December to March) have four time more chance of seropositivity (P = 0.005) as compared to those sampled during wet season (April to November).

2.3.13. Effect on Location

Considerable geographical differences exist in prevalence of toxoplasmosis. Differences in the epidemiology of the infection in various geographical areas and between population groups within the same area may be explained by differences in exposure to the two main sources of the infection: the tissue cyst (in meat of animals) and the oocyst (in soil contaminated by cat feces) (Remington *et al.,* 2006). Cultural habits with regard to food probably are the major cause of the differences in frequency of *T. gondii* infection from one country to another, from one region to another in the same country, and from one ethnic group to another in the same region (Remington *et al*., 2006). Higher prevalence of toxoplasmosis in warm and moist areas compared to cold and dry areas was attributed to the longer viability of *T. gondii* oocysts in moist or humid environments (Van der Puije *et al.,* 2000). Ahmad and Tasawar (2015) studied Toxoplasmosis in small ruminants from varied habitats and cemented that sometimes even prevalence of *Toxoplasma* results vary with variation of methodology utilized on the same herd (Yu *et al.,* 2007). Shahiduzzaman *et al*. (2011) studied in *Toxoplasma gondii* seroprevalence in domestic animals and humans in Mymensingh district, Bangladesh and observed the seroprevalence among women in Mymensingh district might be lower than Dhaka.

2.3.14. Effect on pregnancy ststus

Shahiduzzaman *et al*. (2011) studied in *Toxoplasma gondii* seroprevalence in domestic animals and humans in Mymensingh district, Bangladesh and observed initial exposure to *T. gondii* during pregnancy can cause spontaneous abortion and congenital defects (Pappas *et al.,* 2009). However, for pregnant women previously infected with *T. gondii,* sub- sequent infection rarely causes such birth defects.

2.3.15. Effect of Reproductive disorders

Dubey (2009) stated toxoplasmosis is associated with the occurrence of embryonic death and absorption, fetal death and mummification, abortion, stillborn and neonatal mortality Radostits *et al.* (2007) and moreover, toxoplasmosis has harmful effects on the health and performance of ewes and does after parturition and sometimes leads to their death.

2.4. Experiment 4: Survey of Coxiellosis related to reproductive disorders in small ruminant

The Q fever is a very important neglated zonnotic disease caused by *Coxiella burnetii*. The effect of *Coxiella* discuss under following several sections.

2.4.1. Transmission

Q fever organism is shed in vaginal fluids, faeces and milk, and is present in products of abortion. Excretion in vaginal fluids occurs at normal parturitions.

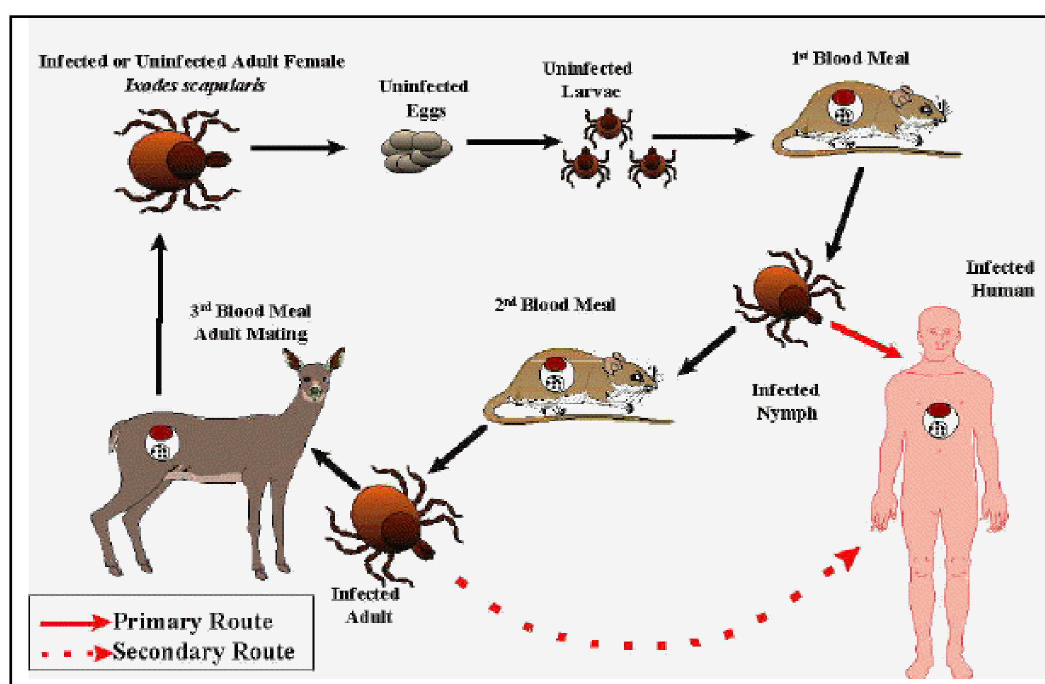


Plate 3. Transmission cycle of Q fever.

Infection via the oropharynx is followed by multiplication in regional lymph nodes and a bacteraemia lasting 5–7 days. The organism localizes in the mammary gland and placenta of pregnant ewes. *C. burnetti* spores survive well in the environment and can be transmitted to new hosts by indirect exposure via fomites, such as hay, straw, wool and manure mainly by inhalation. The organism may be airborne and disseminated on wind for more than a kilometer.

2.4.2. Overall seroprevalence

Coxiella burnetii can be isolated in the laboratory. Several serological tests are available. Placentitis is the most characteristic sign in ruminants. The placenta is typically leathery and thickened and may contain large quantities of white-yellow, creamy exudate at the edges of the cotyledons and in the intercotyledonary areas. The primary significant of Q fever is its zoonotic potential. In livestock, the disease is usually subclinical. Occasional abortions outbreaks have been have reported in goats; less commonly in sheep. Susceptible pregnant females develop placentitis. Abortion and still birth may occur in late gestation because of damage to the placenta. After initial abortions or infection, animals become immune. For treatment, tetracycline is the drug of choice. Q fever can transmit to humans by ingesting milk from infected animals and having contact with placenta or feces. Symptoms are flu-like. The organism killed by pasteurization.

2.4.2.1. Prevalence of Q fever

Prevalence of Q fever in cattle, goats and sheep estimated by indirect ELISA only is reviewing, as it is relevant to this study.

2.4.2.1 (1). Seroprevalence of Q *fever* in goats

The seroprevalence of Q fever in goats in published literatures by different authors varied from 8.7 to 60.4% (Table 1).

Table 1. Seroprevalence of Q fever in goats.

Reference	Test	Cut-off value	Sample	Number of tested animals	Individual level Prevalence
Arserim *et al.,* 2011	ELISA	-	Serum	700	38.6
Schimmer *et al.,* 2011	ELISA	-	Serum	2,828	21.4
Rodriguez *et al.,* 2010	ELISA	-	Serum	733	60.4
Ruiz-Fons *et al.,* 2010	ELISA	OD > 0.4	Serum	115	8.7
Cekani *et al.,* 2010	ELISA	OD > 0.4	Serum	64	18.7
Masala *et al.,* 2004	ELISA	- OD > 0.5	Serum	2,155	13.0
Schelling *et al,* 2003	ELISA	- OD > 0.4	Serum	134	13.0
Salinas-Mele`dez *et al., 2002*	ELISA	OD> 0.4	Serum	60	35.0

2.4.2.1 (2). Seroprevalence of Q fever in sheep

Asadi *et al.* (2013) studied about seroprevalence of Q fever in sheep and goat flocks with a history of abortion in Iran between 2011 and 2012 and resulted that a total of 215 sheep (19.5%; 95% CI: 17-22%) and 49 goats (27.2%; 95% CI: 21-34%) had antibodies specific to *C. burnetii*. There was a significant difference in seropositivity between sheep and goats (p=0.02). The highest prevalence in sheep and goats was 23.8% and 40.8% in Central Iran, respectively. The seroprevalence of *C. burnetii* infection in sheep populations has been estimated in several other countries such as USA 10% (McQuiston and Childs, 2002), Spain 21% (Ruiz-Fons *et al*., 2010), Cyprus 18.9% (Psaroulaki *et al*., 2006) and Germany 1.3% (Hellenbrand *et al.,* 2001). The seroprevalence of Q fever in sheep varied from 0 to 40%. Most of the authors used ELISA test cut-off OlD> 0.4 but few used OD>0.2 and >0.5.

Table 2. Seroprevalence of Q fever in sheep.

Reference	Test	Cut-off value	Sample	Number of tested animals	Individual level Prevalence (%)
Esmaeili *et al.,* 2014	ELISA	-	Serum	253	33.6
Arserim *et al.,* 2011	BLISA	-	Serum	612	25.4
Vaidya *et al.,* 2010	ELISA	-	Serum	43	9.3
Kennerman *et al.,* 2010	ELISA	OD>0.2	Serum	743	20.0
Ruiz-Fons *et al.,* 2010	ELISA	OD>0.4	Serum	1379	11.8
Dorko *et al.,* 2010	ELISA	-	Serum	269	37.22
Rodriguez *et al.,* 2010	ELISA	-	Serum	369	31.7
Garci'a-Perez *et al.,* 2009	ELISA	OD>0.4	Serum	1011	8.9
Banazis *et al.,* 2009	ELISA	OD>0.4	Serum	50	0.0
Cekani *et al.,* 2008	ELISA	-	Serum	293	3.1
Masala *et al.,* 2004	ELISA	OD>0.5	Serum	7194	9.0
Schelling *et al.,* 2003	ELISA	OD>0.4	Serum	142	11.0
Salinas-Mele'dez *et al.,* 2002	ELISA	-	Serum	90	40.0
Hilbink *et al,* 1993	ELISA	OD>0.2	Serum	30	0.0

2.4.2.2. History

Q fever first described by Edward Holbrook Derrick (Derrick, 1983) in abattoir workers in Brisbane, Queensland, Australia. The “Q” stands for “query” and applied at a time when the causative agent was unknown; it chosen over suggestions of “abattoir fever” and “Queensland rickettsial fever,” to avoid directing negative connotations at either the cattle industry or the state of Queensland (Marrie, 1990). The pathogen of Q fever discovered in 1937, when Frank Macfarlane Burnet and Mavis Freeman isolated the bacterium fromone of Derrick’s patients (Burnet and Freeman, 1937). It originally identified as a species of *Rickettsia*. H.R. Cox and Gordon Davis isolated it from ticks in Montana, USA in 1938 (Davis and Cox, 1938 7). *Coxiella burnetii* is no longer regarded as closely related to Rickettsiae, but as similar to *Legionella* and *Francisella*, and is a proteobacterium.

2.4.2.3. Epidemiology

Q fever is a worldwide zoonosis. The reservoirs are extensive but only partially known and include mammals, birds, and arthropods, mainly ticks. While an important reservoir seems to be small wild rodents, the most commonly identified sources of human infection are farm animals such as cattle, goats, and sheep. Pets, including cats (Higgins and Marrie, 1990), rabbits, and dogs, had have also been demonstrated to be potential sources of urban outbreaks. Cats suspected as an important reservoir of *C. burnetii* in urban areas and may the source of urban outbreaks (Marrie, 1990; Morita *et al.*, 1994). In Canada, 6 to 20% of cats have anti-*C. burnetii* antibodies (Higgins and Marrie, 1990). Wild rats have been suspected as an important reservoir in Great Britain (Webster *et al.*, 1995). All these mammals, when infected, shed the desiccation-resistant organisms in urine, feces, milk, and, especially, birth products (Kazar, 1996). Reactivation of infection occurs in female mammals during pregnancy. Q fever causes abortions in goats and, less frequently, sheep and causes reproductive problems in cattle (Zeman *et al.*, 1989). High concentrations of *C. burnetii* (up to 109 bacteria per gram of tissue) is founded in the placentas of infected animals. Due to its resistance to physical agents, probably related to its sporulation process (McCaul, 1991), *C. burnetii* survives for long periods in the environment. In humans, infection results from inhalation of contaminated aerosols from amniotic fluid or placenta or contaminated wool. Therefore, Q fever is an occupational hazard. At greatest risk, persons who contact with farm animals, but also at risk are laboratory personnel who work with infected animals. When looking for the source of *C. burnetii* exposure, the investigator should search for contact with a parturient or newborn animal. Mammals also shed *C. burnetii* in milk, and thus, consumption of raw milk could be a source of infection (Fishbein and Raoult, 1992). Sexual transmission of Q fever had has been demonstrated in the mouse (Kruszewska and Tylewska-Wirzbanowska, 1992) and has been suspected in humans (Mann, *et al.,* 1986). Sporadic cases of human-to-human

transmission following contact with an infected parturient woman had have been reported and had been suspected to occur by direct aerosol transmission. It has also proved to occur via transplacental transmission, resulting in congenital infections (Raoult and Stein, 1994), via intradermal inoculation, and via blood transfusion (Raoult and Marrie, 1995). Ticks transmit *C. burnetii* to domestic mammals but not to humans (Kazar, 1996). *C. burnetii* may persist asymptomatically in humans throughout life. However, pregnancy, a cardiac valvular abnormality, a vascular aneurysm or prosthesis, hemodialysis (Leonetti, 1994), and immunodeficiency, including AIDS (Raoult *et al*., 1992), may promote reactivation of dormant *C. burnetii*. In Europe, acute Q fever cases are more frequently be reported in spring and early summer. They may occur at all ages, but they are more frequent in men than in women. Q fever is usually benign, but mortality occurs in 1% to 11% of patients with chronic Q fever (Raoult, 1990). *C. burnetii* is endemic in every part of the world except New Zealand (Kaplan and Bertagna, 1955). Since the clinical presentation is very pleomorphic and nonspecific, the incidence of Q fever among humans is probably underestimated, and diagnosis particularly relies upon the physician's awareness of the symptoms of Q fever and the presence of a reliable diagnostic laboratory. In southern France, 5% to 8% of cases of endocarditis are due to *C. burnetii*, and the prevalence of acute Q fever is 50 cases per 100,000 inhabitants (Dupont *et al*., 1994). Seroepidemiological surveys have shown that 18.3% of blood donors in Morocco, 26% in Tunisia (Letaief *et al*., 1995), 37% in Zimbabwe (Kelly *et al*., 1993), 44% in Nigeria (Blondeau *et al*., 1990), 10 to 37% in northeast Africa, and 14.6% to 36.6% in different areas of Canada (Brouqui *et al*., 1993) had anti-*C. burnetii* antibodies. Large outbreaks of Q fever had have also been reported in the Basque country in Spain (Errasti *et al*., 1984), in Switzerland (Dupuis *et al*., 1987), in Great Britain (Guigno *et al*., 1992), and in Berlin, Germany (Schneider *et al*., 1993). In addition, a large number of Q fever cases had has been reported in The Netherlands since 2007, with over 3700 human cases reported through March 2010. Infected dairy goat

farms are believed to be the source of the outbreak, and most human cases have been reported in the southern region of the country (Schimmer *et al*., 2008).

2.4.2.4. Diagnosis of *Coxiella burnetii* infection in animals

Coxiella burnetii is a highly virulent bacteria and biosafety level 3 laboratory is a pre-requisite to handle contaminated specimens and cultivate this organism from clinical samples. The diagnosis of *Q* fever is based on direct and indirect methods. The direct methods identify the presence of the organism or of its components by coloration and direct visualization, immunohistochemistry, bacterial culture and Polymerase Chain Reaction (PCR). On the other hand, indirect diagnostic methods measure specific humoral or cellular immunity in response to *C. burnetii* infection, which includes Complement Fixation Test, Enzyme-Linked Immunosorbent Assay, Immunofluorescence Assay etc. (Porter *et al.,* 2011). In animals, Q fever is routinely diagnosed by examination of fixed impressions or smears prepared from placenta and stained by the Stamp, Gimenez or Machiavello techniques along with serological tests (Angelakis and Raoult, 2010). Isolation of *C. burnetii* is not usually done for routine diagnosis in veterinary medicine. Although this bacteria does grow in axenic (host cell-free) media, isolation is time consuming and hazardous for the laboratory workers (Omsland *et al.,* 2013). Immunohistochemistry can be used to detect *C. burnetii* in placental tissues fixed in paraffin or acetone smears (Raoult *et al.,* 1994). PCR based diagnostic techniques including conventional, nested and realtime PCRs are also available to detect *C. burnetii* DNA in cell culture and clinical samples (Bern *et al.,* 2003; Klee *et al.,* 2006). However, PCR techniques are resource intensive and depend on the actual presence of bacterial DNA (Rousset *et al.,* 2010). Therefore, indirect methods of diagnosis i.e. serological tests were mostly used as screening tests at animal or herd level. The Compliment Fixation Test or CFT (OIE recommended test) and ELISA (EU recommended test) are the two most commonly used tests for

this purpose. However, CFT analysis protocol is complex and fails to detect antibodies in sheep or goats (Kovacova *et al.,* 1998). The ELISA was reported to be highly sensitive and specific for the diagnosis of Q fever (Paul *et al.,* 2012). Moreover, ELISA is used to detect antibodies in bulk milk (easy to collect and cheaper than blood analysis) and individual animal serum. The reported sensitivity (S/P cut-off 40) of milk and blood ELISA at animal level were 86% (95% Confidence interval (CI): 76-96) and 84% (95% CI: 75-93) and an equal specificity of 99% (Paul *et al.,* 2012).

2.4.3. Effect of species

The prevalence of *C. burnetii* is slightly higher in sheep than in goats and beef cattle (Ruiz-Fons *et al*., 2010; Khalili and Sakhaee, 2009). The various researchers observed the prevalence in goat and sheep in various years are given in Table 3.

Among the domestic animals especially cattle with reproductive disorders has the highest percentage of seroprevalence of coxiellosis (Hirai-To, 1998). In many countries, goats are the most common source of human infection due to their extensive raising and close contact with humans (Berri *et al.,* 2007).

Table 3. Prevalence of Q fever in goat and sheep by various authors.

Reference	Individual Level seroprevalence (%)	
	Goat	**Sheep**
Arserim *et al.,* 2011	38.6	25.4
Ruiz-Fons *et al.,* 2010	8.7	11.8
Rodriguez *et al.,* 2010	60.4	31.7
Masala *et al.,* 2004	13.0	9.0
Schelling *et al.,* 2003	13.0	11.0
Salinas-Mele'dez *et al.,* 2002	35.0	40.0

Goats and sheep are highly susceptible to abortion (Arricau *et al.,* 2003; Bern *et al.,* 2007). The frequency of occurrence of Q fever abortions in goats is

more important than in sheep with up to 90% of females being affected (Berri *et al.,* 2007). Klaasen *et al.,* 2014 had have the seropossitive effect of Q fever among Sheep 18.5% had a significantly lower risk of being seropositive as compared to 24.2 % goats (OR 0.65, p = 0.02).

2.4.4. Effect of breed

Klaasen *et al.* (2014) studied about *Coxiella burnetii* seroprevalence in small ruminants in the Gambia. He showed practically all animals sampled in the villages belonged to the indigenous goats and Djallonke sheep, whereas at Abuko abattoir 81.1% of the animals were exotic breeds. At Farafenni abattoir, the proportion indigenous to imported breeds was 55.7% to 44.3%.

High seroprevalence of Q fever was found in dairy breed (Ryan *et al.,* 2011; Alvarez *et al.,* 2012). Indigenous breed has the highest prevalence of Q fever. The native breed and third parity animals on individual level were considered the most important risk factors for *C. burnetii* infection. Seropositivity increased with parity and it is highest in third parity (Asadi *et al.,* 2012). Seropositivity of Q fever increased with Holstein breed, increasing number of parity and high milk protein contents, but decreased with increasing milk yield and high milk fat contents (Paul *et al.,* 2012). However, increased prevalence of Q fever was reported in Friesian breed (McCaughey *et al.,* 2010).

2.4.5. Effect of sex

Qassim (2012) studied 500 serum samples, only 80 samples of small ruminants were positive for ELISA anti- *Coxiella burnetii* as it was showed non-significant effect of sex on examined animals on Q fever. Seroprevalence of Q fever 15% (male), 16.3% (female) in small ruminants, which sumarized as 18.7%, 19.6% of male & female sheep, respectively and 7.5%, 8.1% of male & female goat respectively.

Klaasen *et al.* (2014) stated that serum samples were obtained from 490 goats and 398 sheep, during the four weeks of sampling at Farafenni abattoir, the

number of sheep offered for slaughter was limited to 66 sheep. As to the sex distribution: 68.6% of the goats and 80.4% of the sheep were female.

2.4.6. Effect of age

Klaasen *et al.* (2014) experiment that *Coxiella burnetii* seroprevalence in small ruminants in the Gambia and observed the higher seroprevalence increasing with the age and it were 9.8% (256), 26.1% (379) and 27% (244), respectively. Seroprevalence of Q fever varies with the age group (Bo *et al.,* 2011). The seroprevalence of Q fever in dairy cows aged 3 years is slightly higher and it was slightly lower in dairy cows that aged 4 years (Bo *et al.,* 2011). The seropositive cases of Q fever in sheep occur between 3 to 4 years of ages (Esmaeili *et al.,* 2014). The primiparous ewes of 1 year old have higher antibodies rates than newborn sheep of aged less than 10 months or biparous ewes of 2 years old (Kennerman *et al.,* 2010). The prevalence of Q fever in older ewes is significantly greater than that of yearlings or replacement lambs (Garcf`a-Perez *et al.,* 2009).

2.4.7. Effect of parity

Seropositivity increased with parity and it is highest in third parity (Asadi *et al.,* 2012). Seropositivity of Q fever increased with Holstein breed, increasing number of parity and high milk protein contents, but decreased with increasing milk yield and high milk fat contents (Paul *et al.,* 2012). However, increased prevalence of Q fever was reported in Friesian breed (McCaughey *et al.,* 2010).

2.4.8. Effect of body weight

Body weight of animal's relation with diseases prevalence such as Gul *et al. (*2014) trial the difference in sero-prevalence of brucellosis in goats and sheep, statistically the difference between two groups was significant only through RBPT and prevalence was higher in animals having higher body weights. In Q fever relation with body weight was not direct effect, but the infection animal's losses its body weight. Maurin and Raoult (1999) studied Q fever and they

were finding out relation between pregnancy and Q fever. Q fever during pregnancy had been associated with abortion, premature birth, and low weight in newborn babies (Maurin and Raoult, 1999).

2.4.9. Effect of flock size

The prevalence of Q fever was higher in large herd size (Ryan *et al.,* 2011; McCaughey *et al.,* 2010). The occurrence of Q fever in sheep was higher in larger flocks than in medium and small flocks (Kennerman *et al.,* 2010). Among the herd level factors, herd size, tie stall housing system, quarantine of newly purchased animals and good hygienic precautions taken by the veterinarian before entering into the stable are significantly associated with seropositivity of *C. burnetii* (Paul *et al.,* 2012).

Lange *et al*. (2015) observed Q fever-affected areas and areas not affected by Q fever, in the years 2003 through 2004 and 2008 through 2010 based on flock density of sheep and goat and obtained results in Table 4.

Table 4. Effect of flock size in goat and sheep by Lange *et al*. (2015).

Species	Density	Experiment Year			
		2003	2004	2008	2010
Goat	Low	11 649 (27.1%)	46 963 (42.4%)	18 829 (31.9%)	73 678 (45.9%)
	Medium	9577(22.3%)	39 763 (35.9%)	8812 (14.9%)	51 179 (31.9%)
	High	21 783 (50.6%)	24 057 (21.7%)	31 408 (53.2%	35 564 (22.2%)
Sheep	Low	17 893 (41.6%)	51 111 (46.1%)	28 589 (48.4%)	75 135 (46.8%)
	Medium	17 810 (41.4%)	36 943 (33.4%)	20 016 (33.9%)	51 945 (32.4%)
	High	7306(17.0%)	22 729 (20.5%)	10 444 (17.7%)	33 341 (20.8%)

2.4.10. Effect of biosecurity

Cantas *et al*. (2011) examined that Q fever abortions in ruminants and associated on-farm risk factors in northern Cyprus and observed in presence and absence of Rodents in Animal Housing, Ticks on aborted Animals,

Houseflies on Farm, Pigeons on Farm & Presence of Carnivores at Farm reviled (9% & 16%), (2% & 23%), (4% & 21%), (3% & 22%) and (4% & 21%), respectively.

2.4.11. Effect of feeding habit

Cantas *et al*. (2011) examined that Q fever abortion in ruminants, associated on-farm risk factors in northern Cyprus, and observed PCR positive of *Coxiella burnetii* on feed farm made feed and commercial feed are 19% and 3%.

2.4.12. Effect of Season

Prevalence of Q fever varies from season to season with the highest prevalence in the month of June. Up to 9% increase in prevalence occurs due to seasonal variation (Hubert *et al,* 2012). Cows were at a higher risk of infection with Q fever during summer than other seasons (Paul *et al.,* 2012).

Cantas *et al.* (2011) practical the lowest registered number of abortion cases in the general database was in November while the lowest occurrence of *C. burnetii* abortions was in December. However, this occurrence gradually increased from January to another peak in February and then decreased towards March. The *C. burnetii* abortions and presence of ticks on abortion cow cases seem to follow the gradual fall in temperature as the season transition from autumn to winter

2.4.13. Effect of location

Hasan *et al.* (2011) stated that Q fever in there region includes Northbound Region, Border Region and Karpas Region. Q fever infection occur in presence of parasite likes tick on farm management risk factor in northern Cyprus, on the other hand Good hygiene practices are an important way of reducing the risk of spread of infectious diseases, and the findings of this study agree with this notion. The higher the frequency of litter cleaning (5<×<10 and ×>10 times/year) on farm the more protective (OR = 0.3; P = 0.05 and OR = 0.09; P

= 0.05) it was against the risk of Q fever. Studies done in rural areas have all indicated that poor hygiene could be an exacerbating factor in the spread of *C. burnetii* (Lyytikäinen *et al.,* 1998).

Klaasen *et al*. (2014) studied the serological survey of *C. burnetii* seroprevalence in small ruminants in The Gambia demonstrates a considerable prevalence of current or past infection in the sheep and goat population. The species and age of the animals as well as their location and origin are of influence on the seropositivity of *C. burnetii.* Although a direct link between the human and veterinary data could not be demonstrated, there were clear zoonotic implications. *C. burnetii* was highly contagious and very resistant in the environment.

2.4.14. Effect of pregnancy status

Maurin and Raoult (1999) studied Q fever and they were finding out relation between pregnancy and Q fever. They observed Q fever during pregnancy has been associated with abortion, premature birth, and low weight in newborn babies

2.4.15. Effect of Reproductive diseases

It has reported that Q fever associated with abortion, still birth, premature delivery and delivery of weak offspring (Angelakis and Raoult, 2010). These reproductive disorders were usually seeing in sheep and goats. Q fever was frequently subclinical in cattle but infected cows may develop infertility, metritis, and mastitis (Hirai-To *et al.,* 1998). Moreover, *C. burnetii* found to be significantly associated with placentitis (Bildfell *et al.,* 2000; Hansen *et al.,* 2011). In the first pregnancy, the highest seroprevalence of Q fever found in dairy cows (Bo *et al.,* 2011). The organism can be isolated from the blood, milk and urine and localized in the kidneys, udder and the placenta after experimental infection in sheep. Ewes might occasionally shed the organism at successive parturitions (Welsh *et al.,* 1959; Berri *et al.,* 2002) indicates that infection was persistent and that pregnancy enhances multiplication of the

organism but the specific location of the organism where they persists during the non-pregnant period and the mechanisms that initiate its active multiplication in the placenta are not clearly understood. The immunosuppressive effects of pregnancy may be responsible for the increased multiplication of the organism in the placenta (Polydorou, 1981).

Chapter 3

MATERIALS AND METHODS

3.1. Study area

The aim of the study was to identify the Brucellosis, Toxoplasmosis and Coxiellosis associated with reproductive disorders in small ruminants at Nothern part of Bangladesh, particularly Nothern Barind Tract (NBT). The Barind Tract is largest Pleistocene physiographic unit of the Bengal basin, covering an area of about 7,770 sq km. It was long been recognised as a unit of old alluvium, which differs from the surrounding floodplains. In Bangla, it called to spelled and pronounced as Varendra Bhumi. Geographically this unit lies roughly between latitudes 24.20°N and 25.35°N and longitudes 88.20°E and 89.30°E (Banglapedia). The Barind Tract covers most parts of the greater Dinajpur, Rangpur, Joypurhat opf Rangpur division and Pabna, Rajshahi, Bogra and Naogaon districts of Rajshahi division. Northern Barind Tract includes Rajshahi, Naogaon, parts of Natore and Chapi Nawabjong districts (Banglapedia). The present study was carrried out from July 2011 to June 2015 in Northern Barind Tract. The sampling was performed at four areas comprising different sites in the Barind region.

The research has been performed into 4 experiments. Firstly, General materials and methods were discussed below in different heading.

3 (1). Selection of animals

The 396 goats and 79 sheep were selected at Northern Barind region in Bangladesh. The selection was done this location about 2667 closed questionarie methods. The questionneries consist of various information that was attached in appendex 3. From them 475 reproductive diseases of small ruminants were selected 267 in Rajshahi, 80 in Chapai Nawabjong, 59 in Natore and 69 in Naogoan district. Each year in March to May months, it was expected that the infection status of the small ruminants were stabilized,

following the period of August to November during which most parturitions occur.

3 (2). Data collection procedure

The primary data were collected by randomly selected location in each district of retrospective survey from the Veterinary and Vaccination camp of the study area. The Health and Vaccination awareness camp was taken by primary questionnaire were filled up. The Vaccination camps were in Upazilla Livestock Office, Nachal and Chapai Nawabjong; Youth Tanning Centre, Naogaon; Moukhara High School field, Natore and Parila Primary School field, Paba, Rajshahi. The others data were collected from record book of Veterinary Clinic and Artificial Insemination Center in the Department of Animal Husbandry and Veterinary Science, University of Rajshahi. Records of 2667 clinical cases of small ruminants (2394 goats, 273 sheep) questionnaires report under this study, from July 2012 to June 2013 were analyzed to assess the importance of existing diseases. The results were analyzed into 11 major diagnostic groups in small ruminants, which were:

Group 1: Gastrointestinal (diseases involved in digestive system)

Group 2: Respiratory (diseases involved in respiratory system)

Group 3: Musculoskeletal (diseases involved in musculoskeletal system)

Group 4: Integumentary (diseases involved in Integumentary system)

Group 5: Disease of sense organ (diseases involved in sense organ)

Group 6: Infectious disease (diseases relation with batcterail, viral, parasitic and protozoal infection)

Group 7: Deficiency syndrome (diseases relation with vitamin and mineral deficiency)

Group 8: Poison (diseases relation with poison)

Group 9: Female reproductive (diseases involved in female reproductive organ or system)

Group 10: Male reproductive (diseases involved in male reproductive organ or system)

Group 11: Surgical (Surgical case of animals)

3 (3). Grouping of animals

The indigenous small ruminants (goat and sheep) breeds are the local and crossbred. Again, the goats were divided as local or Black Bengal and cross were local, Black Bengal, Jomunapari, Beetal and their crossbred goats. Sheep were short tail breed were denoted as local and long tail were said as cross. The age of each animal was determined by asking the owner and by dentition. Diagnosis of these cases was made based on signalment (age, sex and breed), clinical history and clinical examinations. To avoid overlapping of these diseases, certain adjustments were made so that each disease was counted under only one group. The data on the occurrence of clinical diseases and disorders were analyzed into 11 major diagnostic groups

3 (4). Management of population

The traditional and still widely practiced livestock system in The Barind was agropastoralism, a low-input mixed crop-livestock system with extensive grazing and a low level of integration. In this type of production system livestock are dependent on natural forage and left overs of the cropping season. During the dry season, sheep and goats were left free for grazing whereas in the rainy season (cropping season) the sheep and goats are either tethered approximately the village or herded to avoid crop damage. Most of the rural households own a few small ruminants which serve as savings or emergency cash (e.g. to meet up current need), provide protein (meat or milk) or non-food commodities (manure, hides) and were used in religious celebrations.

3 (5). Study Design

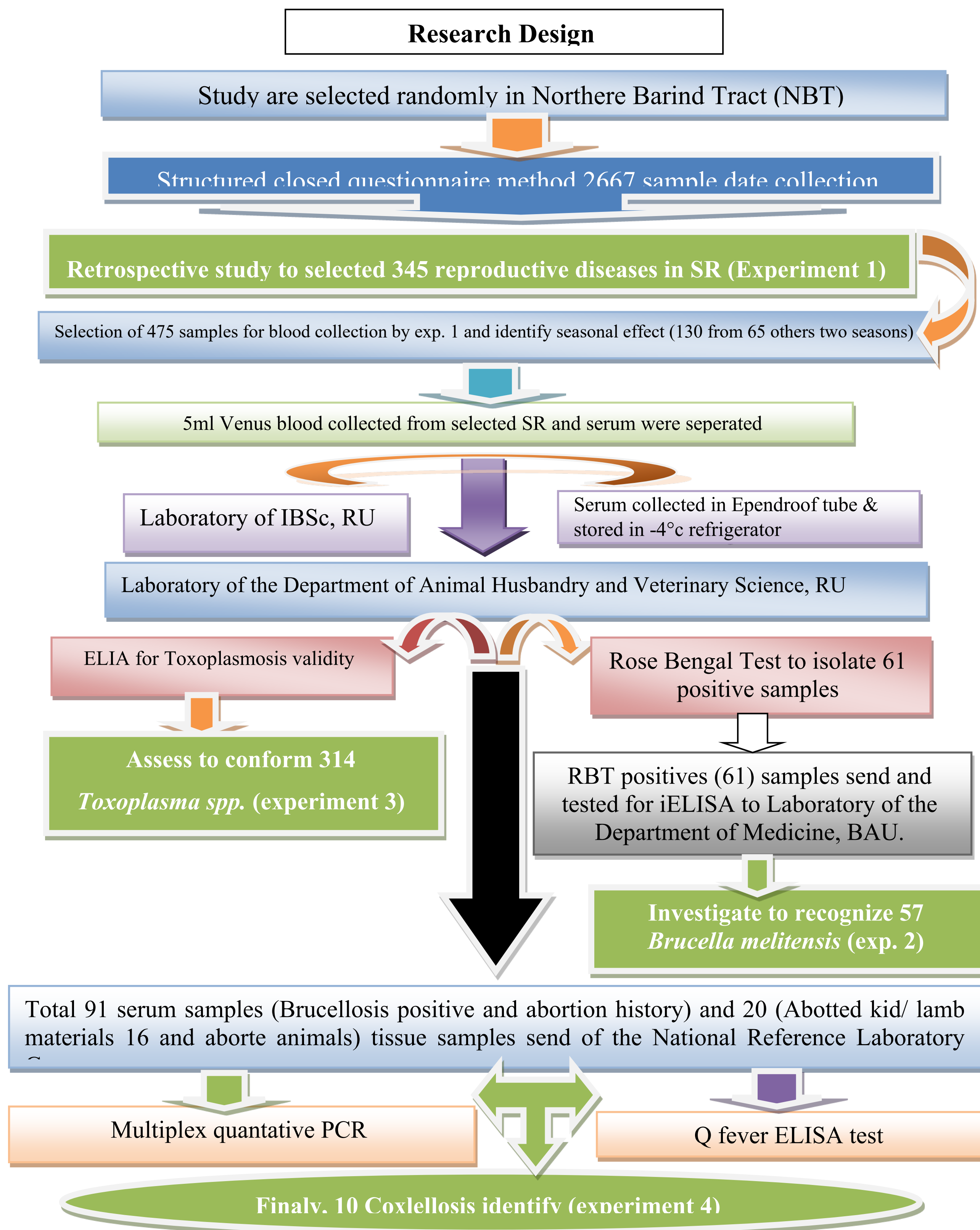


Plate 4. Research design at a glance.

Preceding the sampling, introductory meetings were held in the four Upazilla Livestock Office, Nachol & Chapai Nawabjong and Veterinary Clinic and eight villages (Meherchondi, Parila school para, Hat parila, Paba & Nimtoli, Godagari, Rajshahi; Hackrol, Nachol & Station para, Amnura, Chapai Nawabjong; Mouchara, Natore and Moshorpur, Naogaon) with the community elders and owners. All owners gave consent for their animals to be sampled for the study. In that location randomly selected 2667 closed structured questionary (Appendix-3) were fulfill. A total 475 reproductive abnormal small ruminants cases from 79 were sheep and 396 were goats for sampling choosing by retrospective study. This sample size, calculated using Win Episcope 2.0, was expected to enable detection of a 30% difference in seropositivity between different subpopulations, for instance sheep versus goats or young versus old animals. The research design is given plate 4.

3 (6). Sampling

A structured closed questionnaire was used to record data including the name of the owner (villages), species, breed, sex, estimated age, lactating or not, and, if lactating, the suckling lamb(s) or kid(s) were also sampled and their relationships were documented. In the villages aborted fetus samples taken from the aborted dams, which included in the PCR sampling. Age estimated by dentition as defined earlier (Goossens *et al*., 1998).

Blood samples were collected from the jugular vein in evacuated blood collecting tubes of 3 ml using 5ml disposable syringe used. The tubes left at ambient temperature for circa 1 hour and then stored in a cool box on ice and/or in a refrigerator. Samples were centrifuged within 18 hours (2500g,10 min) and serum samples were then stored frozen.

Abomesal content of fetus and limph node from aborted doe and ewes were taken in a sterial Poly vinyle chloride (PVC) tube. The aborted samples were stored in frozen (-20^{0}c) until testing.

3 (7). Variables

Several variables were considered for the study and this are mentioned below:

3 (7.1). Species

Two type species were selected for study.

Group-I: Goat (n=396)

Group-II: Sheep (n=79)

3 (7.2). Breed

The present study breeds were classified on the basis on genetic composition.

In gaot,

Group-I: Local or Black Bengal (n=215)

Group-II: Black Bengal, Beetal, Jamunapari crosses (n=181)

In sheep,

Group-I: short tail (n=41)

Group-II: Long tail (n=38)

3 (7.3). Sex

Sex was determined by the phesical characteristics of animals and have 2 groups:

Group-I: male (n=197)

Group-II: female (n=278)

3 (7.4). Age

Age was determined by dentition and birth registration kept by the farmers. Small ruminants were different age group and those were divided in the following 3 groups:

Young: below 1 year (n=88)

Adult: 1years to 2 years (n=177) and

Older: more than 2 years (n=210)

3 (7.5). Parity

Parity means how many times the kid/ lamb has given birth. Doe and ewe were different parities and up to 4 parities of doe and ewe were considered for the study. The doe and ewe were divided in the following groups considering parity:

Group-I (1st time giving birth): Parity-1 (n=133)

Group-II (2nd time giving birth): Parity-2 (n=76)

Group-III (3rd time giving birth): Parity-3 (n=44)

Group-IV (> 3 times giving birth): Parity- 4 (n=25)

3 (7.6). Body weight

The gross body weight of the doe or ewe were measured using the following formula by McNitt (1983):

$$W = L \times \frac{G^2}{300} \text{ lbs}$$

where, W= gross body weight,

L = length of the body (in inches from the point of shoulder to the pin-bone) and

G = Heart girth (in inches).

The recorded weight in ibs was converted to kg dividing the value by 2.204.

Small ruminants were different body weight which was measured in kg and those were divided in the following groups.

Light: <10 kg body weight (n=323)

Medium: 10 to 15kg body weight (n=100)

Heavy: >15 kg body weight (n=52)

3 (7.7). Flock size

Flock size ware classified based on small ruminants housed in one farms, flock size was divided into 3 categories.

Small: < 5 small ruminants in a farm (n=323)

Medium: 5 to <10 small ruminants in a farm (n=100)

Large: > 10 small ruminants in a farm (52)

3 (7.8). Biosecurity

The biosecurity of the farm scored up to 0 to 10 on the basis of farmer sanitary education, preventive medication use, pest control, equipment cleaning, contract of purchase and sales, construction plan of shed, health monitoring program, operational hygiene, control and inspection. The allocated marks distribution were divided in to 3 groups

Traditional: It means traditional management practice which rank between 0- 4 (n=253)

Moderate: It means moderate management practice which rank between > 4 to 7 (n=125)

Good: It means good management practice which rank between >7 to to 10 (n=97)

3 (7.9). Feeding habit

Small ruminants had having different feeding habit. The small ruminants were divided in the following groups considering feeding habit.

Grazing: Small ruminant animals graze in field some extra feed either provides or not (n=297)

Stall-feeding: It means animal reared in confined place, grazing should prohabitate (n=178)

3 (7.10). Seasons

The whole year divided into 3 seasons that based on months of the year.

Summer: It includes the monthe of March to June (n=170)

Rainy: It includes the monthe of July to October (n=177)

Winter: It includes the monthe of November to Feburary (n=128)

3 (7.11). Location

Location group in four categories in the Northern Barind Tract, it includes:

Group 1: Rajshahi (n=267)

Group 2: Chapai Nawabgonj (n=80)

Group 3: Natore (n=59)

Group 4: Naogaon (n=69)

3 (7.12). Pregnancy status

It based on pregnancy ststus of an animal and divided into 2 catagories.

Group 1: pregnant

Group 2: non-pregnant group

3 (7.13). Reproductive diseases

It classified based on the deseases involved in reproductive organs of animals which were as follows:

Abortion, still birth, retained fetal membranes, dystocia, vaginal prolapsed, uterine prolapsed, metritis, pyometra, anestrus, repeat breeders, orchitis, epididymitis, posthitis, urolithiasis and urinary tract infection.

3 (7.14). Serology test result

The serological diagnosis of brucellosis, toxoplasmosis and coxiellosis established according to the test result described in specific chapter. The results of test was denoted as

Group 1: Positive

Group 2: Negative

Plate 5. Map of Bangladesh red demarcation and (B.T.Q) has shown the positive samples present in Northern Barind Tract (red arrow show).

3 (8). History

The history was helped to create an appropriate list of diagnostic hypotheses. The following information helped:

- Number, proportion, type, age and source of animals aborting;
- Plotting out on a calendar when abortions occurred (clustering) and gestational age;
- Recent introductions to the flock/herd (even virgin replacement females) or sharing of animals in the last year including rams or bucks;
- Previously diagnosed abortions or illness on the farm;
- Vaccination history, including abortion vaccines and timing and frequency of administration;
- Nutritional and grazing history, e.g. salt / mineral supplementation, silage feeding or toxic weed exposure;
- Potential exposure to toxins including drugs, e.g. an anthelmintic with a known teratogenic effect;
- Environmental factors, e.g. extreme heat during early pregnancy, stress, predation, presence of cats, rats on the property;
- Clinical illness in the individual ewe / doe before, during or after abortion.

3 (9). General Inspection

Assess the environment in which the animals were housed. Judge the animals in good body condition, evidence of diarrhea, access to water / not and palatable free-choice salt / mineral. Evidence of rodent, cat feces was in vicinity.

3 (10). Clinical Examination

The Adult

Examine both the pregnant at-risk animals as well as aborted. If the females were ill, this might support some hypotheses such as abortion due brucellosis or toxoplasmosis and Q fever. Often the females look healthy while aborting but may have had a history of being transiently off-feed such as with *Campylobacter spp*.

The Fetus

Determine how premature the fetus is (size, crown-rump length, evidence of wool/hair coat). The fetus was alive when aborted. Aborted fresh, macerated or mummified. Look for skin lesions that may indicate a mycotic or bacterial infection. Congenital defects such as arthrogryposis, spinal bifida, cleft palate, microopthalmia, etc was or not and the fetus meconium stained or not.

The Placenta

Examine the cotyledons for evidence of inflammation (swelling, hyperemic, purulent debris), necrosis or calcification. Check the inter cotyledonary space for evidence of inflammation, thickening, necrosis, hyperemia, etc. Normal inter cotyledonary placenta should be transparent and thin, e.g. can read the paper through it.

Submission for diagnostic testing

In almost all cases laboratory support was necessary to make a definitive diagnosis. Have the producer gather up all the abortions and placentas available. They should be placed in a clean, water-proof sac (e.g. garbage bag) and kept cool and away from scavenging animals (e.g. rodents, cats, and dogs) prior to submission. Instruct the client regarding the zoonotic risk and to wear gloves and protective clothing. Submission of placenta is critical. When submitting, it was very helpful to indicate a list of diagnostic hypotheses and to direct the pathologist on what you consider the most likely cause(s) of the abortion. In many diagnostic laboratories, the pathologist cannot run tests unless you request them. For this reason, your input was critical for the success of the diagnostic investigation.

Submission of Entire Fetuses and Placentas

Gently remove debris but do not wash off; submit all specimens available; do not freeze before submitting but do keep chilled; submit in leak-proof clean containers.

Necropsy and Submission of Specimens from Fetuses and Placentas

It was not always possible to submit entire fetuses and placentas. In this case, perform a gross necropsy, make note of any abnormal findings, and submit as outlined below or follow your state diagnostic labs recommendations on appropriate samples to submit. Record the weight of the fetuses, estimated gestational age, and history.

Submit a separate tissue sample for each lab section, i.e. bacteriology, virology and mycoplasmology in separate, sealed and labeled Whirl-Pak bags. Keep fresh tissues chilled using ice packs in insulated leak-proof containers. Submit tissues showing typical lesions.

Placenta formalin-fixed: At least 2 cotyledons + intercotyledonary area. Include areas with obvious lesions.

Placenta – fresh: Place into separate sealed bag. *Coxiella burnetii* may be present in large numbers on the placenta and should be carefully handled. PCR for *C burnetii* and *Chlamydophia abortus.*

Fetal Tissues - Formalin-fixed: Eyelid, skeletal muscle, thyroids (submit with tracheal section), thymus, lung, myocardium, liver, kidney, adrenal, spleen, jejunum, spiral colon (with meconium), and brain. Immunohistochemistry can be requested for several of the disease agents, e.g. *Toxoplasma gondii.*

Fresh in one bag: Lung, spleen, liver, thymus and thyroid for isolation of Border Disease virus (BDV).

Stomach content (in sterile leak-proof container)**:** for culture.

Fresh in serum tube: fetal thoracic fluid or heart blood (serum) for analysis for titres to BDV and *Toxoplasma gondii.*

3 (11). Serum from reproductive diseases involvement

Serology was not as rewarding as a titre doesn't always indicate causality and it usually costly. However, if aborted fetuses and placenta can't be obtained, serology may offer some clues as to why the abortions occurred. Sample all aborting females and a portion (minimum of 10%) of pregnant ewes/does. Submit paired sera - acute and convalescent (10 to 21 days after acute sample) to demonstrate a rising titre.

3 (12). Managing the aborting flock

While developing a therapeutic plan, there were actions that may influence the severity of the outbreak or may reduce the risk to humans. Remove the pregnant females from aborted females, which should remain in the contaminated pen or pasture. If you have a working diagnosis, it may be prudent to initiate specific control measures before the diagnosis is confirmed. If the aborted females are to be culled, they should be sent directly to slaughter once the vaginal discharge has cleared, to avoid the risk of being taken into another flock as a breeding animal.

3 (13). Zoonotic risks

Be aware of the zoonotic risks from many of the infectious agents. Advise the producer and others working with the animals to wear gloves, boots and protective clothing that were changed before managing the rest of the flock. These clothing items should never go in the house but should remain in the barn and should only be used when managing the aborting animals. Fitted N95 respirators were also recommended as some of the disease agents can be aerosolized. These masks should be worn when assisting a birth or removing an abortion or cleaning the barn. Pregnant women or immune compromised people should not assist at birthing and should, if possible, not have contact with the pregnant, aborting or newly lambed /kidded females and offspring.

3 (14). Ethics Statement

The study described in this thesis conducted in compliance with legislation on animal experimentation and practicing veterinary medicine of the University of Rajshahi, Bangladesh and ethical committe of Institute of Biological Science. The study was not an animal experiment, but an epidemiological study in the field using common sampling methods for routine diagnostic purposes.

3 (15). Analytical technique

The collected data dealing specifically with the diseases were divided in to gastrointestinal, respiratory, musculoskeletal system, intugmentary, sense organ, infectious, deficiency, poisonous, surgical and reproductive diseases. The reproductive diseases were abortion, retained fetal membranes, dystocia, anestrus, repeat breeders, cervicitis, mastitis, orchitis, posthitis, urolithiasis and urinary tract infection were analyzed.

3 (16). Statistical Analysis

Data were statistically analyzed by Statistical Package for Social Science (SPSS) software 17.0 version. Correlation, regression of coefficient analysis, student t-test, chi-square and F-test was used to know the association between different groups in respective cases.

Model for χ^2test

Researcher supposed that null hypotheisis (H_0): There was no relationship between *Brucella*, *Toxoplasma* and *Coxiella* with species, breed, body weight, age, sex, parity, density of population, biosecurity feeding habit, seasons, location, reproductive diseases and pregnancy. The alternate hypothesis (H_1): There was relationship between *Brucella*, *Toxoplasma* and *Coxiella* with species, breed, body weight, age, sex, parity, flock size, biosecurity feeding habit, seasons, location, reproductive diseases and pregnancy. If the calculated

value was greater than the tabulated value than the hypothesis was reject and vice versa.

Logistic Regression Model:

Logistic regression was a statistical method for analyzing a dataset in which there were one or more independent variables that determine an outcome. The outcome was measured with a dichotomous variable (in which there are only two possible outcomes).

In logistic regression, the dependent variable was binary or dichotomous, i.e. it only contains data coded as 1 (positive or presence of diseases etc.) or 0 (Negative or absence of diseases etc.).

The goal of logistic regression was to find the best fitting (yet biologically reasonable) model to describe the relationship between the dichotomous characteristic of interest (dependent variable = response or outcome variable) and a set of independent (predictor or explanatory) variables. Logistic regression generates the coefficients (and its standard errors and significance levels) of a formula to predict a logit transformation of the probability of presence of the characteristic of interest:

$$\text{logit}(p) = b_0 + b_1 X_1 + b_2 X_2 + b_3 X_3 + \ldots + b_k X_k$$

Where p is the probability of presence of the characteristic of interest. The logit transformation is defined as the logged odds:

$$\text{odds} = \frac{p}{1-p} = \frac{\textit{probability of presence of characteristic}}{\textit{probability of absence of characteristic}}$$

and

$$\text{logit}(p) = \ln\left[\frac{p}{1-p}\right]$$

Simple logistic regression finds the equation that best predicts the value of the Y variable for each value of the X variable. What makes logistic regression different from linear regression is that the Y variable is not directly measured; it is instead the probability of obtaining a particular value of a nominal

variable. If you were studying SR population who had zoonotic diseases, the values of the nominal variable would be "did have a zoonotic diseases" vs. "didn't have a zoonotic diseases. The Y variable used in logistic regression would then be the probability of having zoonotic diseases. This probability could take values from 0 to 1. The limited range of this probability would present problems if used directly in a regression, so the odds, Y/ (1-Y), is used instead. (If the probability of a zoonotic diseases is 0.25, the odds of a zoonotic diseases are 0.25/(1-0.25)=1/3. In gambling terms, this would be expressed as "3 to 1 odds *against* having zoonotic diseases.") Taking the natural log of the odds makes the variable more suitable for a regression, so the result of a logistic regression is an equation that looks like this:

$$\text{Ln}\,[Y/(1-Y)]=a+bX$$

The slope (b) and intercept (a) of the best-fitting equation in a logistic regression are found using the maximum-likelihood method, rather than the least-squares method used for linear regression. Maximum likelihood is a computer-intensive technique; the basic idea is that it finds the values of the parameters under which you would be most likely to get the observed results. The odds ratio (O.R.) for the independent variable X_i and it gives the *relative* amount by which the odds of the outcome increase (O.R. greater than 1) or decrease (O.R. less than 1) when the value of the independent variable was increased by 1 units.

There are several different ways of estimating the *P*-value. The Wald chi-square was fairly popular, but it may yield inaccurate results with small sample sizes. The likelihood ratio method may be better. It uses the difference between the probability of obtaining the observed results under the logistic model and the probability of obtaining the observed results in a model with no relationship between the independent and dependent variables.

The specific materials and methods have been described in different experiment.

3.1. Experiment 1: Retrospective reproductive diseases of small ruminants

The aim of the experiment was to identify the reproductive cases of small ruminants for the selection of animals to collecte samples for the experiment 2-4. For experiment 1 following procedure were maintained.

3.1.1. Geographical description

The Barind tract is situated in the Northern belt of Bangladesh. It lies between latitude 5° 4' and 6° 3' N and longitude 6° 15'and 7° 34' E. The area was 200m above sea level except for elevations associated with the uplands (Ofomata, 1975). It has an annual rainfall of about 1700mm to 2500mm, which was concentrated almost entirely between March and October. Average humidity was about 80%, with up to 85% occurring during the rainy season. The mean daily maximum air temperatures range from 28°C to 35°C, while the mean daily minimum ranges from 19°C to 24°C. Small ruminants are allowed to roam throughout the seasons, thriving on indigenous browses growing in compound bushes and farm fallows with additional supplementation from kitchen wastes (Okoli *et al*., 2003).

3.1.2. Sources of information

For retrospective study records of 2667 clinical cases of small ruminants (2394 goats, 273 sheep) questionnaires report under this study, from July 2012 to June 2013 were analyzed to assess the importance of existing diseases. The results analyzed into 11 major diagnostic groups in small ruminants (discussed in general materials and methods chapter).

Data on clinical cases of reproductive abnormality (male and female) were again classified into Abortion, retained fetal membranes, dystocia, anestrus, repeat breeders, cervicitis, orchitis, posthitis, urolithiasis and urinary tract infection. The disease was diagnosed at the Health and Vaccination Campaign by the Veterinarian was usually based on flock history, clinical sign and symptoms listed in below.

3.1.3. Clinical history

The history and symptoms was helped to create an appropriate list of diagnostic hypotheses. The information was discussed under general materials and methods chapter.

3.1.4. Data Analysis

All the data were stored in SPSS Microsoft windows program and subsequently. Overall, yearly, monthly and seasonal trends were computed using descriptive and quantitative analyses. The former involved the use of simple averages to determine trends across years, months and three seasons namely, hot and dry period (March to June), wet and hot period (July to October), cold and dry period (November to February).

3.1.5. Statistical Analysis

Data were statistically analyzed from Statistical Package for Social Science (SPSS) software 17.0 version. Student t-test, chi-square and F-test was used to know the association between different groups in respective cases.

3.2. Experiment 2. Investigation on Brucellosis associated with reproductive disorders in small ruminants

The aim of the experiment was to investigate the brucellosis associated the risk factors including reproductive disorders in below way.

3.2.1. Experimental design

Venous blood samples collected aseptically from randomly selected 475 goat-sheep in Rajshahi, Chapai Nawabgonj, Naogaon and Natore districts of Bangladesh. Sera separated from all the collected blood samples and tested by Rose Bengal Plate Test. The samples positive in RBT subjected to i-ELISA for more conformation.

3.2.2. Plan of action

The aim of present study was to sero prevalence of *Brucella* organism of small ruminants animals specially sheep and goat at NBT in Bangladesh. The study carried out in private goat and sheep farm under Rajshahi, Natore, Chapai Nawabgonj, Naogaon, district of Bangladesh. The serum samples tested in Laboratory of the Department of Animal Husbandry and Veterinary Science, University of Rajshahi, Laboratory in the Institute of Biological Sciences, University of Rajshahi. ELISA tested was performed in the Laboratory of the Department of Medicine at Bangladesh Agricultural University, Mymensingh, Bangladesh.

3.2.3. Definition of variables

Grouping of the selected animals: Various classification of animal based on their specific character (discussed in General materials and methods chapter).

Plate 6. Photograph A: Blood collection from the Dept of Animal Husbandry and Veterinary Science of goat and sheep farm, RU; B: Blood placed in test tube for serum separation; C: Blood collection from vaccination and health camp at YTC, Naogoan and D: Venupuncture for blood collection from sheep**.**

Photo A : Serum sample.

Photo B: Rose Bengal test (RBT) antigen from Daesun Microbiological lab .

Photo C: RBT at RU AHVS veterinary clinic and AI centre Narikelbaria unit.

Photo D: Interpretation of RBT test.

Plate 7. Brucellosis RBT procedure in the laboratory under the Institute of Biological Sciences at University of Rajshahi.

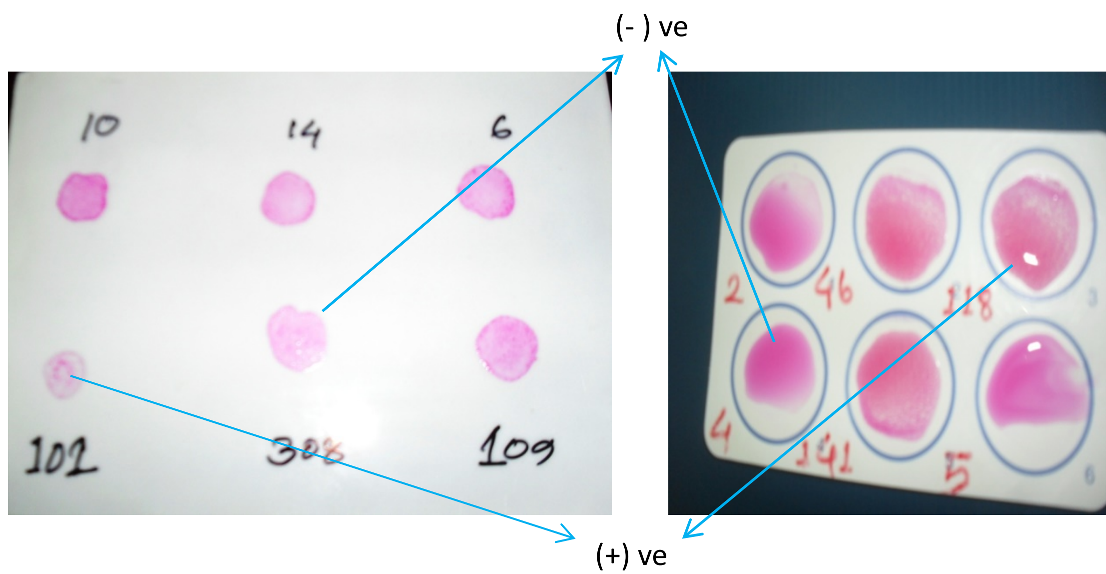


Plate 8. Positive and negative reactions of Rose Bengal test (RBT).

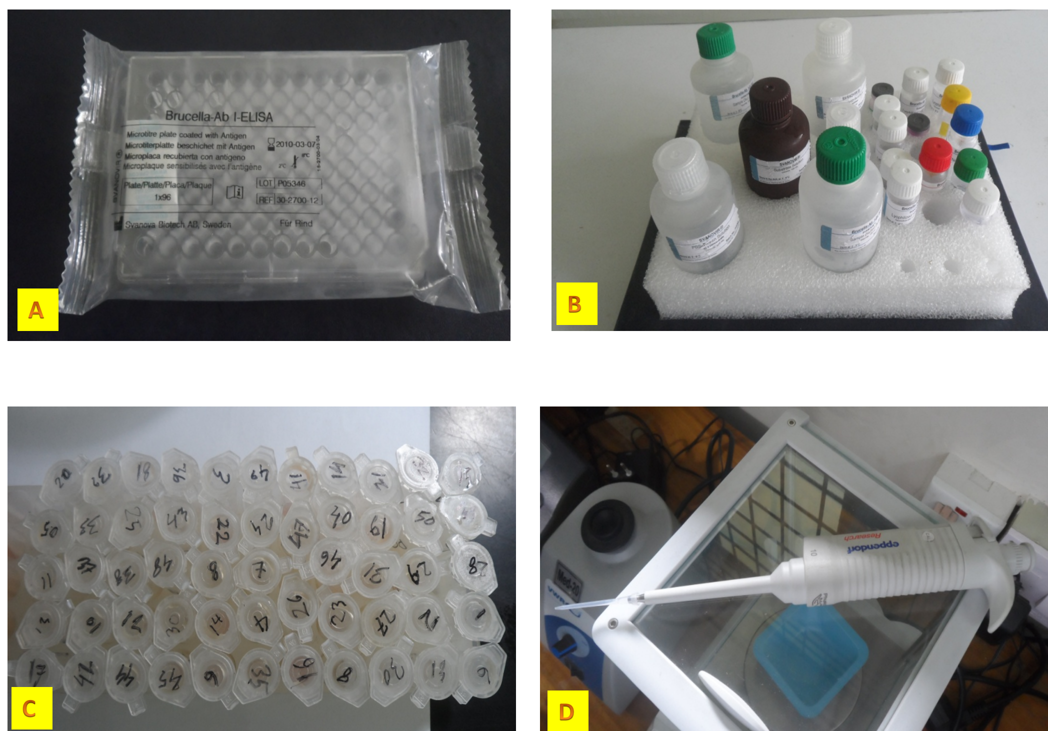


Plate 9. Photograph A: indirect ELISA plate; B: test regents; C: RBT possotive serum samples and D: Test materials.

Plate 10. Photograph A: ELISA whole number in sheet; B: iELISA test procedure; C: ELISAplate after test complete and D: ELISA reader.

3.2.4. Laboratory analysis

The collected sera were screened for the presence of antibodies against *Brucella* antigens by using the Rose Bengal Plate Test "RBPT" and Positive samples were conformed a commercially available indirect enzyme linked immunosorbent assay (iELISA) (J OVAC, Jordan).

The percent positivity values (PP)/ seroprevalence calculated using the following formula:

$$PP = \frac{\text{Test sample or Neg C (OD)}}{\text{Positive control (OD)}} \times 100$$

Where, OD= Optimal Density

The assay calibrated against the OIE ELISA Standard sera and Standardized against the EU derivatives (64/432/EEC). The PP value of >40 was considered as positive.

3.2.5. Statistical Analysis

The results compiled in a database created with Microsoft Office software Excel 2007 (Microsoft®, Redmond) and SPSS program. The amplification sensitivity of the assay defined as the percentage of results correctly identified as positive among all the replicas made and the specificity of the results as the percentage of results correctly identified as negative from the total number of samples processed. To evaluate significant differences between the tabulated and calculated value at degrees of freedom obtained from cosstab analysis, correlation and regression of coffecient with computer statistical program for the Social Sciences version 17.0 (SPSS ® Inc., Chicago, IL) and a *P* value < 0.05/ 0.01/ 0.1 considered statistically significant.

Model Building

Simple logistic regression model has been undertaken to estimate the impact of various reproductive diseases such as abortion, still birth, retained fetal membranes, dystocia, vaginal prolapsed, uterine prolapsed, metritis, pyometra, anestrus, repeat breeders, orchitis, epididymitis, posthitis, urolithiasis and urinary tract infection with control group and ELISA test for brucellosis in small ruminants in NBT in Bangladesh. In this analysis to create a dependent variable as well as disease presence, variable researcher has calculated the presence and absence of diseases by ELISA test. Then this variable has been coded as 0 (zero) for negative and 1for possitive. To do literally meaningful the new variable treated as index of diseases presence or absence in small ruminants in study area. The dependent variable "Disease (*Brucella* ELISA)" was shown to be binary or dichotomous one. The presence and absence of diseases in small ruminants in NBT in Bangladesh was estimated by the created index from the logistic model analysis. When it takes the value 1 the probability will be *P* (say) if the respondent contains "Possitive" and 0 with probability (1-*P*) if it contains "Negative." The independent variable of the analysis are categorical as well as indicator variables as to handle in simple

logistic regression analysis the individual category of a variable is converted into the present and absence of a characteristic, usually denoted by 1 and 0, often called dummy variables. Explanations of the variables are shown in Table 5.

Table 5. Description of variables appearing in the simple Logistic regression analysis of *Brucella*.

Variables	Value Labels	*N*
Dependent Variable		
Disease (*Brucella* ELISA)= Y	Negative = 0 $\Rightarrow$ Y= 0 Positive = 1 $\Rightarrow$ Y = 1	475
Independent (Explanatory) Variables		
Reproductive diseases of SR=X_5	Abortion = 1 $\Rightarrow X_1 = x_{11}$ Still birth = 2 $\Rightarrow X_2 = x_{12}$ Retained placenta = 3 $\Rightarrow X_3 = x_{13}$ Dystocia =4 $\Rightarrow X_4 = x_{14}$ Vaginal prolapsed =5$\Rightarrow X_5 = x_{15}$ Metritis =6 $\Rightarrow X_6 = x_{16}$ Anestrus =7 $\Rightarrow X_7 = x_{17}$ Repeat breeding =8 $\Rightarrow X_8 = x_{18}$ Cervicitis =9 $\Rightarrow X_9 = x_{19}$ Orchitis =10 $\Rightarrow X_{1,10} = x_{1,10}$ Postitis =11 $\Rightarrow X_{1,11} = x_{1,11}$ Urinary tract infection=12 $\Rightarrow X_{1,12} = x_{1,12}$ Epididymitis^r $\Rightarrow X_{1,12}^{\{r\}} = 0$	475

Logistic Regression Model for Variables of Table-5

The explanations of the model corresponding to disease groups were as abortion, still birth, retained fetal membranes, dystocia, vaginal prolapse/ uterine prolapse, metritis or pyometra, anestrus, repeat breeders, cervicitis orchitis, epididymitis, posthitis, urolithiasis and urinary tract infection in realation with ELISA test of *Brucella* in small ruminants at NBT in Bangladesh variables in Table 5 are as follows:

Expression of P_i is given by

$$P_i = E\left[Y_i = 1 \middle| \begin{matrix} X_{11} = x_{11}, X_{12} = x_{12}, X_{13} = x_{13}, X_{14} = x_{14}, X_{15} = x_{15}, X_{16} = x_{16}, X_{17} \\ = x_{17} X_{18} = x_{18}, X_{19} = x_{19}, X_{1,10} = x_{1,10}, X_{1,11} == 0 \end{matrix} \right]$$

(Here the values of the variables corresponding to the reference category are considered as '0')

That is,

$$E(Y_i = 1 | X_i) = P_i = \frac{e^{(a+bx)}}{1 + e^{(a+bx)}} = \frac{1}{1 + e^{-(a+bx)}}$$

and

$$1 - P_i = \frac{e^{-(a+bx)}}{1 + e^{-(a+bx)}}$$

Therefore,

$$\frac{P_i}{1 - P_i} = e^{(a+bX)}.$$

Hence the simple binary logistic regression model is given by

$$\log_e \frac{P_i}{1-P_i} = a + b_{11}X_{11} + b_{12}X_{12} + b_{13}X_{13} + b_{14}X_{14} + b_{15}X_{15} + b_{16}X_{16} + b_{17}X_{17} + b_{18}X_{18} + b_{19}X_{19} \quad \text{.. (B1)}$$

3.3. Experiment 3. Assessment of Toxoplasmosis linked with reproductive disorders in small ruminant

To assess the toxoplasmosis linked with reproductive disorders in small ruminants at NBT in Bangladesh the below procedure was approved for the experiment 3.

3.3.1. Blood and sera sample collection

At first the owner and attendant controlled the animals and then the site of blood collection at jugular furrow was soaked with Tincture of iodine. About 5-7 ml of blood samples were randomly and aseptically obtained from sexually mature goats, and sheep of both sexes. It was collected from jugular vein of each goat-sheep with the help of sterile disposable syringe and needle and was kept undisturbed on a tray for at least 30 minutes at room temperature in a slightly inclined position to facilitate clotting and separation of serum. The study also recorded required clinical and reproductive information. After this period, the clotted blood samples with sera was transfer to refrigerator at 4 °C and kept overnight. Later on, the sera were poured into the separate test tube from each labeled syringe and the test tube was marked with same number by permanent marker. Then the serum was centrifuged at 2500 rpm for 10 min after centrifugation clear sera was found and then the sera were transferred to the vial. The vial was stored in ice chamber at -20 °C for future use. During sampling an another short questionnaire form including information on species, breed, age, sex, density of population, body weight, parity, reproductive diseases, biosecurity, feeding habit, seasons, pregnancy status and reproductive problems such as repeat breeding, previous abortion, retention of placenta and management. When collections of sample the selected area arrange a free vaccination camp were reproductive problem related small ruminants samples were chosen.

3.3.2. Sample preparation

The blood samples were collected from the small ruminants linked with reproductive problem. Each sample was kept at 4ºC until used. Serial dilutions of the tachyzoites to 1:10, 1:100, 1:1,000 and 1:10,000 were performed. Each 100 µl amniotic fluid sample was added to 100 µl of tachyzoites using a blinded technique. Seven samples of 200 µl amniotic fluid each were pre-pared as negative samples. The forty-one added samples and seven negative samples were kept at -20ºC until the ELISA protocol was performed.

Plate 11. Photograph A: Serum samples for test; B: Centifuged serum collection at IBSCs, RU; and C & D: *Toxoplasma* ELISA at AHVS Dept. laboratorty.

3.3.3. Laboratory analysis

The collected sera were screened for the presence of antibodies against *Toxoplasma* antigens by using the anti-*T. gondii* antibodies by using diagnostic kit for human, Toxotest-MT (Eiken Kagaku, Japan).

Definition of variables:

Grouping of the selected animals: The variables were classified according to their respondent was described in general materials and methods section.

3.3.4. Serology

Serologic determinations was performed with the kit VIDAS Toxo IgG and IgM (Bio Mérieux, Paris, France), and the manufacturer's directions were followed.

3.3.4.1. Test of Toxoplasmosis (Toxotest-MX 'Eiken'): Contents

1. Antigen *T. gondii* antigen coated polystyrene latex beads----- 10% vol.

2. Buffer fluid 2-amino-2- methyl-1-plopanol ------------------------------ 0.2 mol/L

3. Positive control serum ———————————————— dry

Caution:

1. Sample sera were kept at less than -20 until usage. Repeating freezing and thawing should be avoided. Fresh sera were also available.
2. In the case of frozen samples, samples should be warmed up near room temperature.
3. Glucose, albumin and hemoglobin did not affect the result, at least up to 8%.
4. Control serum, buffer fluid and latex beads were settled for each rot. Did not use any contents in this kit with contents in another rot.

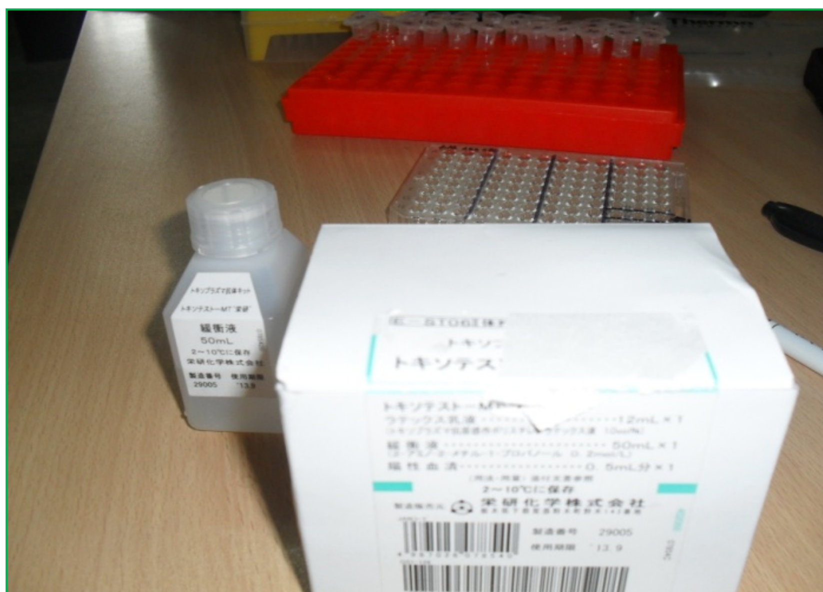


Plate 12. anti-*T. Gondii* antibodies using kit for human Toxotest MT (Eiken Kagaku, Japan).

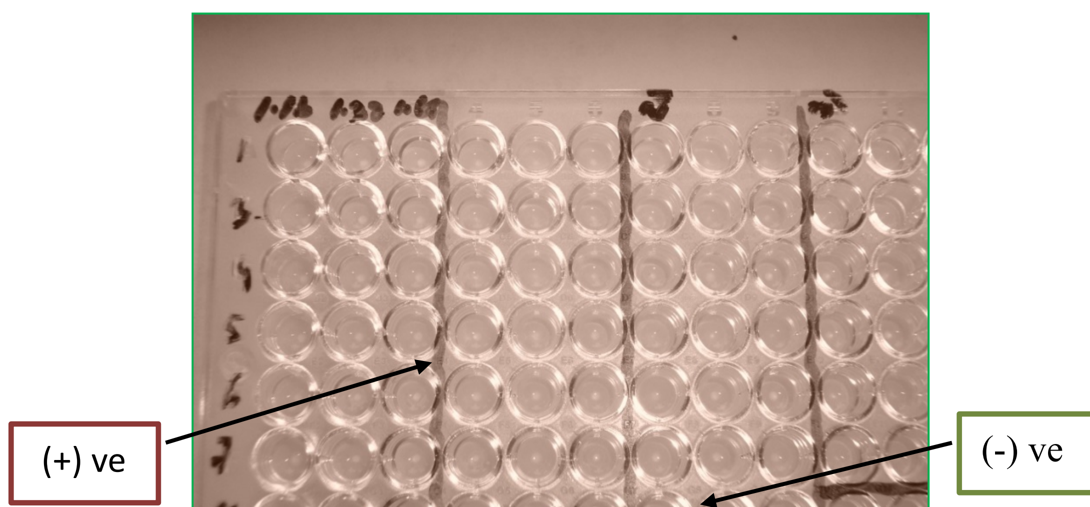


Plate 13. Interpretation of ELISA test of toxoplasmosis.

3.3.4.2. Protocol

Toxoplasmosis ELISA test procedure as below:

3.3.4.2 (1). Controls

Controls were positive and negative control and their preparation is in following procedure.

a. Preparation of positive control

Add 500 ml of buffer fluid to positive control serum bottle. Once dissolved, the positive control serum was kept in 4ºC up to 3 weeks. The dissolved serum by the protocol is available 8 × diluted control serum. If the diagnosis kit was kept under good condition, the control serum should show positive at 128×256×2048.

According to Takashima (Shahhiduzzaman *et al.*, 2014)

Buffer fluid, beads and unsolved positive control serum can be kept in refrigerator for few months (of cause, it was better to finish up once opened contents as soon as possible.). However, once the positive control serum was solved, it could be kept only 3 weeks. In the case that positive control serum has finished, you could use other seropositive animal serum of which antibody titer was known.

b. Preparation of negative controls

As negative control, add 25 ml of buffer fluid in a well. As positive control, add 25 ml of buffer fluid and 25 ml of dissolved positive control serum in a well. After suspending several times using pipet, discard 25 ml of the diluted positive control serum. At this moment, 25 ml each of negative and positive control has been prepared.

3.3.4.2 (2). Preparation of sample sera

To prepare 8 times diluted serum samples, dilute 50 ml of each sample serum with 350 ml of buffer fluid.

Comment of Takashima (Shahhiduzzaman *et al.*, 2014)

The manual recommends diluting each sample to 1:8 in this step and then preparing 2 × serial dilutions in the next step. This is official protocol to minimize pipet-induced error. However, if you can prepare 2 × serial dilutions from 1:16 correctively in the next step, this step can be passed or modified (e.g. Dilute 10 ml of sample with 70 of buffer fluid).

3.3.4.2 (3). Preparation of serial dilutions

Add 25 ml of buffer fluid to each well. Then add 25 ml of the 1:8 diluted sample serum to well on the first lane (50 ml of 1:16 diluted sample has been prepared in well on the first lane). After suspend several times using pipet, transfer 25 ml of the 1:16 diluted sample into well on the second lane (50 ml of 1:32 diluted sample has been prepared in well on the second lane). Repeating this procedure, make 2 × serial dilutions from 1:16 to 1:2048. At this moment, 25 ml of sample is prepared in each well from the first lane to seventh lane, and 50 ml of 1:2048 samples is prepared in well on the eighth lane. Discard 25 ml of the 1:2048 diluted sample in well on the eight lane.

Comment of Takashima (Shahhiduzzaman *et al.*, 2014)

Dilution to 1:2048 was not always essential. Only a small portion of samples show positive at 1:1024 and/or 1:2048. In my experience, data obtained using serial dilution until 1:16 to 1:256 was accepted without any problem for publication on international journal.

3.3.4.2 (4). Addition of polystyrene latex beads and incubation

Shake the bottle of polystyrene latex beads gently to prepare homogenously-distributed emulsion of polystyrene latex beads, because the beads easily sink down in the bottle. Add 25 ml of the homogenously-distributed emulsion to each sample and control well. Tapping the plate gently, mix properly sample in each well. Keep the plate over night at room temperature.

3.3.4.2 (5). Reading result

Determine aggregation level according to the following standard. Level 0 and Level 0.5 should be judge as negative. More than Level 1 should be judge as positive.

Table 6: Determination of agglutination test result.

Level 3	Surrounding area of aggregated beads coming unstuck. Form of the sunken beads is not regular circle form but irregular form
Level 2	Latex beads spread widely on bottom of well
Level 1	Latex beads spread on bottom of well
Level 0.5	A little bigger dot than Level 0
Level 0	Small clear dot

Comment 1 of Takashima (Shahhiduzzaman *et al.*, 2014)

Judgment between Level 0.5 and Level 1 was sometimes vary depend on investigator. However, such variation of each investigator's criterion for judgment could not affect severely on the final result (See the next step). Comment 2 of Takashima: Latex beads don't make clear 'negative small dot' in scratched or dirty well. Therefore, re-use of plate could not recommended. Disposable plate was better.

3.3.4.2 (6). Final determination

Judge the result of each sample as seropositive or seronegative according to the following criteria (Shahiduzzaman *et al*., 2014).

Table 7: ELISA test result of toxoplasmosis**.**

Dilution	Interpretations
< 1:16	Negative (Seronegative)
1:16	Positive
>1:32	Negative
1:32	Positive (Seropositive)

3.3.5. Statistical Analysis

The results compiled in a database created with Microsoft Office software Excel 2007 (Microsoft®, Redmond) and SPSS program. The amplification sensitivity of the assay defined as the percentage of results correctly identified as positive among all the replicas made and the specificity of the results as the percentage of results correctly identified as negative from the total number of samples processed. The standard curves obtained for the individual real-time PCR assays, regression equations and correlation coefficients generated in Microsoft® Excel 2007. To evaluate significant differences between the tabulated and calculated value at degrees of freedom obtained from cosstab analysis, correlation and regression of coffecient with computer statistical program for the Social Sciences version 17.0 (SPSS® Inc., Chicago, IL) and a p value <.05/ 0.01/ 0.1 considered statistically significant.

Model Building

Simple logistic regression model has been undertaken to estimate the impact of various reproductive diseases such as abortion, still birth, retained fetal membranes, dystocia, vaginal prolapsed, uterine prolapsed, metritis, pyometra, anestrus, repeat breeders, orchitis, epididymitis, posthitis, urolithiasis and urinary tract infection with Control group and ELISA test for brucellosis in small ruminants in NBT in Bangladesh. In this analysis to create a dependent variable as well as disease presence, variable researcher has calculated the presence and absence of diseases by ELISA test. Then this variable has been coded as 0 (zero) for negative and 1for possitive. To do literally meaningful the new variable treated as index of diseases presence or absence in small ruminants in study area. The dependent variable “Disease (*Toxoplasma* ELISA)” was shown to be binary or dichotomous one. The presence and absence of diseases in small ruminants in NBT in Bangladesh was estimated by the created index from the logistic model analysis.

Table 8: Description of variables appearing in the simple logistic regression analysis of *Toxoplasma*.

Variables		Value Labels	*N*
Dependent Variable			
Disease (*Toxoplasma* ELISA)= Y		Negative = 0 ⇒ Y= 0 Positive = 1 ⇒ Y = 1	475
Independent (Explanatory) Variables			
	Reproductive diseases of SR=X_5	Abortion = 1⇒ $X_1 = x_{11}$ Still birth = 2⇒ $X_2 = x_{12}$ Retained placenta = 3 ⇒ $X_3 = x_{13}$ Dystocia =4 ⇒ $X_4 = x_{14}$ Vaginal prolapsed =5⇒ $X_5 = x_{15}$ Metritis =6 ⇒ $X_6 = x_{16}$ Anestrus =7 ⇒ $X_7 = x_{17}$ Repeat breeding =8 ⇒ $X_8 = x_{18}$ Cervicitis =9 ⇒ $X_9 = x_{19}$ Orchitis =10 ⇒ $X_{1,10} = x_{1,10}$ Postitis =11 ⇒ $X_{1,11} = x_{1,11}$ Urinary tract infection=12 ⇒ $X_{1,12} = x_{1,12}$ Epididymitis^r ⇒ $X_{1,12}^{\{r\}} = 0$	475

When it takes the value 1 the probability will be *P* (say) if the respondent contains “Possitive” and 0 with probability (1-*P*) if it contains “Negative.” The independent variable of the analysis are categorical as well as indicator variables as to handle in simple logistic regression analysis the individual category of a variable is converted into the present and absence of a characteristic, usually denoted by 1 and 0, often called dummy variables. Explanations of the variables are shown in Table 8.

The explanations of the model corresponding to disease groups of as abortion, still birth, retained fetal membranes, dystocia, vaginal prolapsed/uterine prolapsed, metritis/ pyometra, anestrus, repeat breeders, cervicitis, orchitis, epididymitis, posthitis, and urinary tract infection in realation with ELISA test of *Toxoplasma* in small ruminants at NBT in Bangladesh variables in Table 8

are as follows:

Expression of P_i is given by

$$P_i = E\left[Y_i = 1 \left| \begin{array}{l} X_{11} = x_{11}, X_{12} = x_{12}, X_{13} = x_{13}, X_{14} = x_{14}, X_{15} = x_{15}, X_{16} \\ = x_{16}, X_{17} = x_{17} X_{18} = x_{18}, X_{19} = x_{19}, X_{1,10} = x_{1,10}, X_{1,11} == 0 \end{array} \right. \right]$$

(Here the values of the variables corresponding to the reference category are considered as '0')

That is,

$$E(Y_i = 1|X_i) = P_i = \frac{e^{(a+bx)}}{1+e^{(a+bx)}} = \frac{1}{1+e^{-(a+bx)}}$$

and

$$1 - P_i = \frac{e^{-(a+bx)}}{1+e^{-(a+bx)}}$$

Therefore,

$$\frac{P_i}{1-P_i} = e^{(a+bX)}.$$

Hence, the simple binary logistic regression model is given by

$$\log_e \frac{P_i}{1-P_i} = a + b_{11}X_{11} + b_{12}X_{12} + b_{13}X_{13} + b_{14}X_{14} + b_{15}X_{15} + b_{16}X_{16} + b_{17}X_{17} + b_{18}X_{18} + b_{19}X_{19} \cdots \text{(T1)}$$

3.4. Experiment 4: Survey of coxiellosis related to reproductive disorders in small ruminant

To conduct the survey about coxiellosis related with reproductive disorders on SR at NBT in Bangladesh following materials and methods were conducted.

3.4.1. Study area

The present study was conducted during the period from July 2011 to June 2015 at NBT in Bangladesh. The serum and aborted fetal materials samples were send to the National Reference Laboratory in Germany. The aim of the research was to know the prevalence of Q fever in small ruminants in Bangladesh based on iELISA test and RT PCR.

3.4.2. Study design and sampling

A structured questionnaire was used to record data including the name of the owner (villages) or trader (abattoirs), species, breed, sex, estimated age, lactating or not, and, if lactating, the suckling lamb(s) or kid(s) were also sampled and their relationships were documented. In the villages, aborted samples were taking from the kidding and lambing dams, which were stored for PCR, testing sampling.

Blood samples were collected from the jugular vein in evacuated blood collecting tubes of 5 ml (Greiner Bio-One, Kremsmu¨nster, Austria), using 20 G_6 1.5 Multi-sample Blood collection needles (Greiner Bio-One, Kremsmu¨ nster, Austria). The tubes were left at ambient temperature for circa 1 hour and then stored in a cool box on ice and/or in a refrigerator. Samples were centrifuged within 18 hours (2500 g,10 min) and serum samples were then stored frozen. After cleaning the teats with disinfectant wipes and forest-ripping, milk samples were collected in 15 ml polystyrene milk tubes (Greiner Bio-One, Kremsmu¨nster, Austria). Milk samples were preserved with Broad Spectrum Microtabs II (D&F Control Systems, Norwoord, USA), containing bronopol and natamycin. The samples tested were found positive in *Brucella*, and abortion history was selected for Q fever test in Germany.

3.4.3.1. Serum sample collection

Serum samples were randomly collecting to study brucellosis positive in sheep and goats in different part at NBT in Bangladesh. The samples were collected and sera were separated by centrifugation 11000 r.p.m for 5 minutes at 4ºC (by cooled centrifuge) and serum samples were preserved at freezing stored at the laboratory of Institute of Biological Sciences, University of Rajshahi until used. Finally, there were 91 serum samples were send to the National Reference Laboratory, Germany with maintain cool chain for further testing.

3.4.3.2. Herd and animal level data collection

Animal-level data on age, breed, sex, pregnancy status and herd-level data on herd size, herd composition and location of the herd were collected from available database of serum samples. For tissue samples (aborted materials), the location of the farm and number of lactating small ruminants in herd were collected from the aborted SR.

3.4.4. Indirect ELISA test

Serological testing methods available for the detection of *C. burnetii* in animals include complement fixation (CF), enzyme-linked immunosorbent assay (ELISA) indirect IFA tests and Real Time Polymerage Chain Reaction (RT PCR). Indirect ELISA test was performing in following procedure followed.

3.4.4.1. Preparation of wash solution

The wash congugate was brought to 18-26°C and mixed to ensure dissolution of any precipitated salts. The wash concentrate was diluted at 1:10 with deionized water before use. The solution was stored at 2-8°C.

3.4.4.2. Enzyme-linked immunosorbent assay (ELISA)

This technique has a high sensitivity and a good specificity (Kittelberger *et al.,* 2009; Rousset *et al.,* 2007; 2009). It is easy to perform in laboratories that have the necessary equipment (a spectrophotometer) and reagents. The ELISA is preferred to IFA and CFT, particularly for veterinary diagnosis, because it is convenient for large-scale screening and, as it is a reliable technique for

demonstrating *C. burnetii* antibody in various animal species (Jaspers *et al.,* 1994; Soliman *et al.,* 1992). Ready-to-use kits are commercially available and can detect anti-phase II antibodies or both anti-phase II antibodies and I. Wells of the microplate were coated with *C. burnetii* whole-cell inactivated antigen. Diluted serum samples were added to the wells and react to antigens bound to the solid support. Unbound material was removed by washing after a suitable incubation period. Conjugate (horseradish-peroxidase-labelled anti-ruminants Ig) reacts with specific antibodies bound to the antigen. Unreacted conjugate was removed by washing after a suitable incubation period. Enzyme substrate was added. The rate of conversion of substrate is proportional to the amount of bound antibodies. The reaction was terminated after a suitable time and the amount of colour development is measured spectrophotometrically.

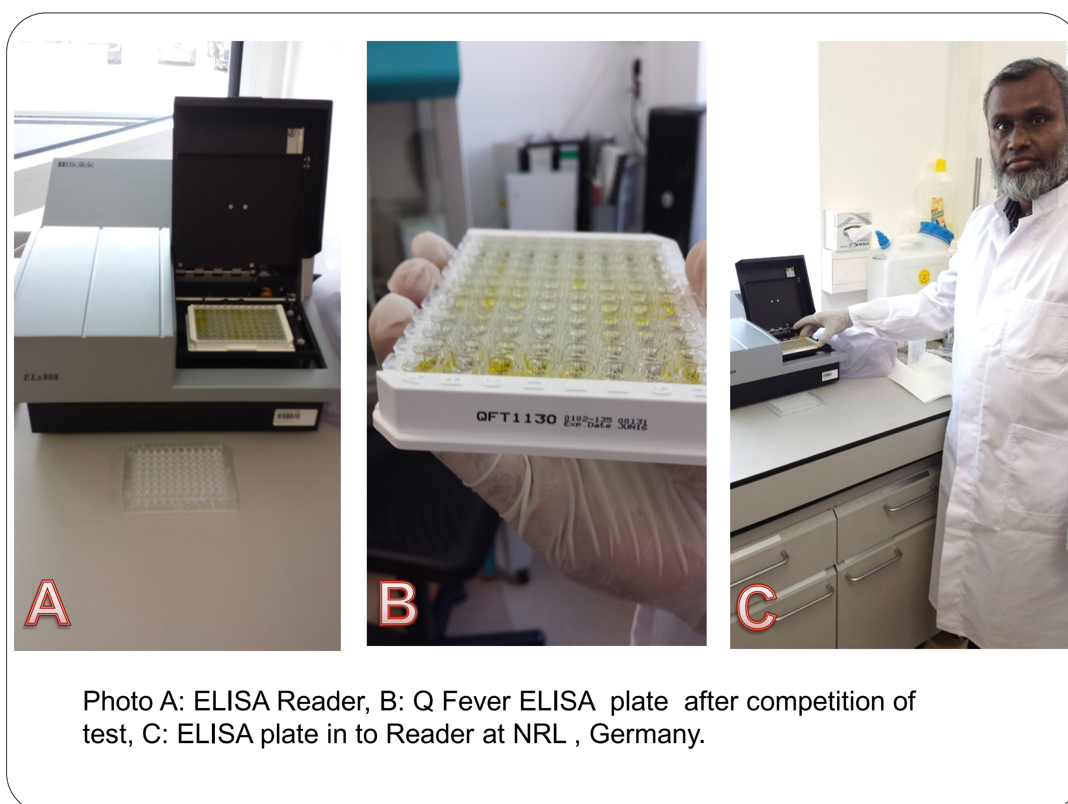


Photo A: ELISA Reader, B: Q Fever ELISA plate after competition of test, C: ELISA plate in to Reader at NRL , Germany.

Plate 14. Q fever ELISA tests at National Reference Laboratory, Germany.

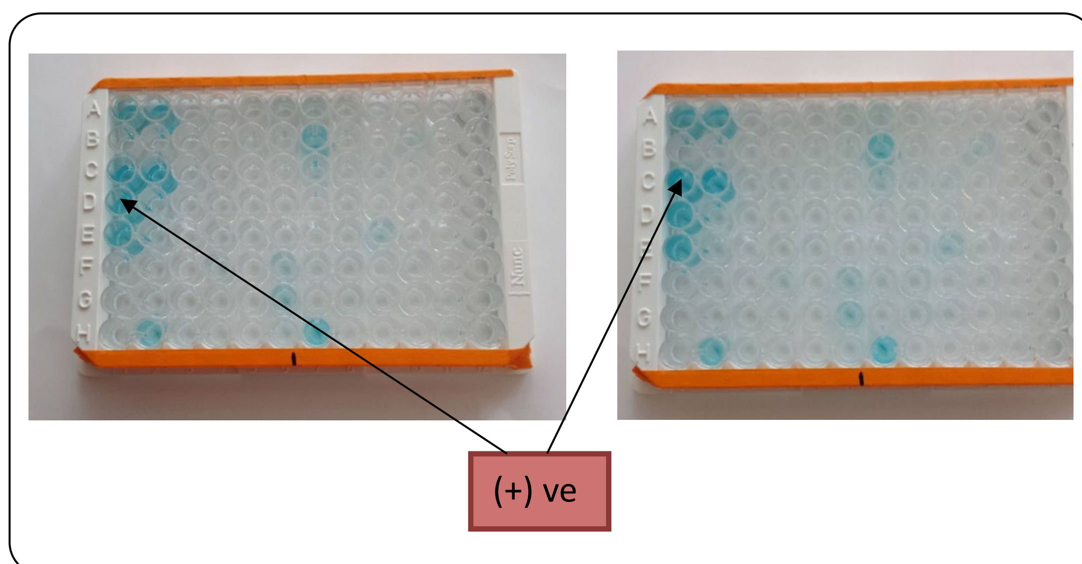


Plate 15. Q fever ELISA test result interpitation.

3.4.4.3. Materials and reagents

1. Microtitre plates with 96 flat-bottomed wells, freshly coated or previously coated with Q fever antigen;
2. Microplate reader (spectrophotometer; 405 and/or 450 and/or 492 nm filters);
3. 37°C humidified incubator; 8- and 12-channel pipettes with disposable plastic tips; microplate shaker (optional).Positive and negative control sera;
4. Conjugate (ruminants anti-immunoglobulin labelled with peroxidase);
5. Tenfold concentration of diluent (PBS–Tween); distilled water;
6. Substrate or chromogen (TMB [tetramethylbenzidine], ABTS [2, 2'-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid)] for peroxidase) and
7. Hydrogen peroxide (H_2O_2).

3.4.4.4. Testing procedure

i) Dilute the serum samples, including control sera, to the appropriated dilution (usualy 1/100) and distribute 0.1 ml per well in duplicate. Control sera are positive and negative sera provided by the manufacturer and an internal

positive reference serum from the laboratory in order to compare the titres between different tests.

ii) Cover the plate with a lid and incubate at room temperature for 30–90 minutes. Empty out the contents and wash three times in washing solution at room temperature.

iii) Add the appropriate dilution of freshly prepared conjugate to the wells (0.1 ml per well).

iv) Cover each plate and incubate as in step ii. Wash again three times.

v) Add 0.1 ml of freshly prepared chromogen substrate solution to each well (for example: TMB in 0.1 M acetic acid and 30% H_2O_2 solution [0.2 µl/ml]; or 0.25 mM ABTS in citrate phosphate buffer, pH 5.0, and 30% H_2O_2 solution [0.1 µl/ml]).

vi) Shake the plate; incubate according to the manufacturer recommendations, stop the reaction by adding stopping solution to each well, e.g. 0.05 ml 2 M sulphuric acid for TMB or 10% sodium dodecyl sulphate for ABTS.

vii) Read the absorbance of each well with the microplate reader at 405 nm (ABTS) or 450 nm (TMB). The absorbance values were used to calculate the results.

3.4.4.5. Interpretation of the results

For commercial kits, interpretations and values were provided with the kit.

For example: calculate the mean absorbance (Ab) of the sample serum and of the positive (Abpos) and negative (Abneg) control sera, and for each serum, calculate the percentage (CP)

$$\text{CP} = \frac{Ab - Ab(-ve)}{Ab - Ab(+ve)} \times 100$$

Interpret the results as follows:

Ab <30% negative serum

Ab 30–40% doubtful serum

Ab >40% positive serum

3.4.5. Diagnosis Q fevers by Multiplex Quantitative PCR Systems

Between the years 2011-2015, a total of 20 tissue samples where 16 abomasal contents of the aborted fetuses consisting of 8 goats, 8 sheep and 4 sheep aborted fetal materials were sent to the national reference laboratory, Germany under sterile conditions and cold chain. At the time of arrival, DNAs were extracted from all samples.

3.4.6.1. DNA Extraction

DNA extracted from positive strain containing the gene coding phase II antigen was kindly obtained from Federal Research Institute for Animal Health (Friedrich-Loeffler-Institut, Naumburger Str. 96a, 07743 Jena), Germany. DNAs from all abomasal and fetal materials contents were extracted by commercial DNA isolation kit (DNA easy Tissue Kit, Qiagen, Germany) according to the manufacturer's instructions. DNAs were stored at -20°C until used. Water and 3-µl template. Cycling parameters is as follows: initial denaturation at 95°C for 2 min followed by 5 cycles of denaturation at 94°C for 30 sec, 66 to 61°C (the temperature was decreased by 1°C between consecutive steps) for 1 min, extension 72°C for 1 min and final extension 72°C for 10 min. Trans-PCR was performed using Thermal Cycler (Arktik, Thermoscientific, Germany).

3.4.6.2. Agarose Gel Electrophoresis

PCR products were electrophoresed on a 1.5% agarose gel in TBE buffer (Thermo Scientific, Vilnius, Lithuania) containing 0.5 µ/ml of ethidium bromide at 100 V for 45 min and visualized under UV light.

3.4.6.3. Primers

Trans-1 and trans-2 primers, specific to the *IS1111*fragment, a transposon-like repetitive region were targeted for the detection of *C. burnetii* by Trans-PCR.

Primers as previously described by Hoover *et al*. (1992) consisted of the following sequences: Trans 1; 5'-TAT GTA TCC ACC GTA GCC AGT C-3' and Trans-2; 5'-CCC AAC AAC ACC TCC TTA TTC-3'. Expected amplicon size was 687 bp.

3.4.6.4. MxPro-Mx3005P PCR

Each reaction had a volume of 25 µl including, 22 µl reaction mixture containing 2.5 µl 10× PCR buffer (without MgCl2), 0.5 µl dNTP (10 mM), 1.5 µl MgCl2 (25 mM), a 1 µl of each primer (10 pmol/µl), 0.25 µl Taq DNA polymerase (5 U/ µl) (Fermantas, Vilnius, Lithuania), 15.25 µl deionized.

3.4.6.5. Multiplex Quantitative PCR run

Federal Research Institute for Animal Health (Friedrich-Loeffler-Institut, Naumburger Str. 96a, 07743 Jena), Germany on August 08, 2014 run Multiplex Quantitative PCR Systems. The Quantitative PCR - Text report K:\AGr130\MX3000\Mertens\2014\PCR-2014-CB-08082014.mxp
Filter gain factors: CY5 x8 ROX x1 HEX-JOE x4 FAM x8.

3.4.7. Definition of variables

Serum samples were selected by positive *Brucella* and abortion history of 91 small ruminants from 71 goats and 20 sheep of different sexes and classified according to their factors (described as general materials and method).

3.4.8. Statistical analysis

The results compiled in a database created with Microsoft Office software Excel 2007 (Microsoft®, Redmond) and SPSS program. The amplification sensitivity of the assay defined as the percentage of results correctly identified as positive among all the replicas made and the specificity of the results as the percentage of results correctly identified as negative from the total number of samples processed. The standard curves obtained for the individual real-time PCR assays, regression equations and correlation coefficients generated in

Microsoft® Excel 2007. To evaluate significant differences between the tabulated and calculated value at degrees of freedom obtained from cosstab analysis, correlation and regression of coffecient with computer Statistical Program for the Social Sciences version 17.0 (SPSS ® Inc., Chicago, IL) and a *p* value <.05/ 0.01/ 0.1 considered statistically significant.

Model Building

Simple logistic regression model has been undertaken to estimate the impact of various reproductive diseases such as abortion, still birth, retained fetal membranes, dystocia, vaginal prolapsed, uterine prolapsed, metritis, pyometra, anestrus, repeat breeders, orchitis, epididymitis, posthitis, urolithiasis and urinary tract infection with control group and ELISA test for Q fever in small ruminants in NBT in Bangladesh. In this analysis to create a dependent variable as well as disease presence, variable researcher has calculated the presence and absence of diseases by ELISA test. Then this variable has been coded as 0 (zero) for negative and 1for possitive. To do literally meaningful the new variable treated as index of diseases presence or absence in small ruminants in study area. The dependent variable "Disease (Q fever ELISA)" was shown to be binary or dichotomous one. The presence and absence of diseases in small ruminants in NBT in Bangladesh was estimated by the created index from the logistic model analysis. When it takes the value 1 the probability will be P (say) if the respondent contains "Possitive" and 0 with probability ($1-P$) if it contains "Negative." The independent variable of the analysis are categorical as well as indicator variables as to handle in simple logistic regression analysis the individual category of a variable is converted into the present and absence of a characteristic, usually denoted by 1 and 0, often called dummy variables. Explanations of the variables are shown in Table 9.

Table 9: Description of variables appearing in the simple logistic regression analysis of Q fever.

Variables		Value Labels	*N*
Dependent Variable			
Disease (Q fever ELISA)= Y		Negative = 0 ⇒ Y= 0 Positive = 1 ⇒ Y = 1	91
Independent (Explanatory) Variables			
	Reproductive diseases of SR=X_5	Abortion = 1⇒ $X_1 = x_{11}$ Still birth = 2⇒ $X_2 = x_{12}$ Retained placenta = 3 ⇒ $X_3 = x_{13}$ Dystocia =4 ⇒ $X_4 = x_{14}$ Vaginal prolapsed =5⇒ $X_5 = x_{15}$ Metritis =6⇒ $X_6 = x_{16}$ Anestrus =7⇒ $X_7 = x_{17}$ Repeat breeding =8⇒ $X_8 = x_{18}$ Cervicitis =9⇒ $X_9 = x_{19}$ Orchitis =10⇒ $X_{1, 10} = x_{1, 10}$ Postitis =11⇒ $X_{1, 11} = x_{1, 11}$ Urinary tract infection=12⇒ $X_{1, 12} = x_{1,12}$ Epididymitis^r ⇒ $X_{1,12}^{\{r\}} = 0$	91

The explanations of the model corresponding to Disease groups of as abortion, still birth, retained fetal membranes, dystocia, vaginal prolapsed, uterine prolapsed, metritis, pyometra, anestrus, repeat breeders, orchitis, epididymitis, posthitis, urolithiasis and urinary tract infection in realation with ELISA test of Q fever in small ruminants at NBT in Bangladesh variables in Table 1 are as follows:

Expression of P_i is given by

$$P_i = E\left[Y_i = 1 \middle| \begin{array}{l} X_{11} = x_{11}, X_{12} = x_{12}, X_{13} = x_{13}, X_{14} = x_{14}, X_{15} = x_{15}, X_{16} \\ = x_{16}, X_{17} = x_{17} X_{18} = x_{18}, X_{19} = x_{19}, X_{1,10} = x_{1,10}, X_{1,11} == 0 \end{array} \right]$$

(Here the values of the variables corresponding to the reference category are considered as '0')

That is,

$$E(Y_i = 1 | X_i) = P_i = \frac{e^{(a+bx)}}{1 + e^{(a+bx)}} = \frac{1}{1 + e^{-(a+bx)}}$$

and

$$1 - P_i = \frac{e^{-(a+bx)}}{1 + e^{-(a+bx)}}$$

Therefore,

$$\frac{P_i}{1 - P_i} = e^{(a+bX)}.$$

Hence the simple binary logistic regression model is given by

$$\log_e \frac{P_i}{1-P_i} = a + b_{11}X_{11} + b_{12}X_{12} + b_{13}X_{13} + b_{14}X_{14} + b_{15}X_{15} + b_{16}X_{16} + b_{17}X_{17} + b_{18}X_{18} + b_{19}X_{19} \quad ..(C1)$$

Chapter 4

RESULTS

Epidemiological investigation of Brucellosis, Toxoplasmosis and Coxiellosis associated with reproductive disorders in small ruminants discussed under four experiments are as follows:

4.1 Experiment 1: Retrospective study of Reproductive diseases of small ruminants

The results of retrospective study of reproductive diseases of small ruminants at NBT in Bangladesh is discussed in relation with species, sex, age and seasons in different section.

4.1.1 Effect of species

The diseases in the different systems and reproductive disorders observed in small ruminants are shown in the Table 10-13. A total 2667 cases were presented to the study, and of these, 270 (10.2%) were reproductive case. The percentage distribution of diseases according to species showed goat with the highest number of cases 2394 (89.8%), then sheep 273 (10.2%) (Table10). In the study it was found that small ruminants were significantly suffering from various diseases and disorders, where maximum 816 (30.6%) of small ruminants were affected by infectious diseases. The lowest number 18 (0.7%) small ruminants affected with poisonous diseases. The other number of diseases involve in small ruminants were highest to lowest rate 444 (16.6%), 354 (13.3%), 345 (12.9%), 231 (8.7%), 162 (6.1%), 102 (3.8%), 84 (3.1%), 72 (2.7%) and 39 (1.5%) were in gastrointestinal, respiratory, surgical affection, female reproductive disorder, deficiency syndrome, Integumentary involvement, diseases of sense organ, musculoskeletal system and disease of male sex organ, respectively.

Table 10. Distribution of diseases of small ruminants of study area.

Diseases involved in organs / systems	Small ruminants		Total
	Goat	Sheep	
Gastrointestinal	372 (13.9%)	72 (2.7%)	444 (16.6%)
Respiratory	327 (12.3%)	27 (1.0%)	354 (13.3%)
Musculoskeletal	69 (2.6%)	3 (0.1%)	72 (2.7%)
Integumentary	93 (3.5%)	9 (0.3%)	102 (3.8%)
Disease of sense organ	78 (2.9%)	6 (0.2%)	84 (3.1%)
Infectious disease	750 (28.1%)	66 (2.5%)	816 (30.6%)
Deficiency syndrome	153 (5.7%)	9 (0.3%)	162 (6.1%)
Poison	12 (0.4%)	6 (0.2%)	18 (0.7%)
Female reproductive	183 (6.9%)	48 (1.8%)	231 (8.7%)
Male sex organ	39 (1.5%)	0 (0%)	39 (1.5%)
Surgical affection	318 (11.9%)	27 (1.0%)	345 (12.9%)
Total	2394 (89.8%)	273 (10.2%)	2667 (100%)
F value	7.882	Significant	

4.1.2. Effect on sex on reproductive diseases in small ruminants

The overall incidence rate of reproductive disorders were 10.2%, where 8.7% in female and 1.5% in male sex involvement. The incidence rate (highest to lowest) of reproductive disorders involvement with relation to sex were 32.8%, 15.8%, 10.2%, 7.9%, 6.8%, 5.7%, 4.5% in anestrus, abortion, dystocia, retained placenta, cervicitis, mastitis and repeat breeding in case of female, but in male it was 5.7%, 5.3%, 2.6% and 1.9% in urinary tract infection, ureolithiasis, posthitis and orchitis, respectively (Table 11).

Table 11. Distribution of reproductive diseases of small ruminants associated with sex.

Legend	Sex of small ruminants		Total
	Male	**Female**	
Abortion	-	42 (15.8%)	42 (15.8%)
Retained placenta	-	27 (10.2%)	27 (10.2%)
Dystocia	-	21 (7.9%)	21 (7.9%)
Anoestrus	-	87 (32.8%)	87 (32.8%)
Repeat breeding	-	12 (4.5%)	12 (4.5%)
Cervicitis	-	18 (6.8%)	18 (6.8%)
Mastitis	-	15 (5.7%)	15 (5.7%)
Orchitis	7 (2.6%)	-	7 (2.6%)
Posthitis	5 (1.9%)	-	5 (1.9%)
Urolithiasis	14 (5.3%)	-	14 (5.3%)
Urinary tract Infection	15 (5.7%)	2 (0.8%)	17 (6.4%)
Total	41 (15.5%)	224 (84.5%)	265 (100%)
Chi-Square Test	Calculated value	Tabulated value	Significant P< 0.05
	251.506	18.307	

4.1.3. Effect of age of small ruminants in relation with reproductive diseases

The study also revealed that the reproductive disorders of small ruminants were varied with age. The incidence rate of abortion, retained placenta, dystocia, anestrus, repeat breeding, cervicitis, mastitis, orchitis, posthitis, urolithiasis and urinary tract infection were 8.7%, 5.7%, 4.5%, 20.4%, 1.1%, 5.7%, 2.3%, 1.9%, 4.2%, 1.9% & 1.9% in young; 5.7%, 2.3%, 1.1%, 7.9%, 2.3%, 1.1%, 2.3%, 0.8%, 0%, 3.4% & 1.5%, and in adult 1.5%, 2.3%, 2.3%, 4.5%, 1.1%, 0%, 1.1%, 0%, 0%, 0% & 0.8% in older respectively (Table 12).

Table 12. Distribution of reproductive diseases of small ruminants associated with age.

Legend	Age of the small ruminants			Total
	Young	Adult	Old	
Abortion	23 (8.7%)	15 (5.7%)	4 (1.5%)	42 (15.8%)
Retained placenta	15 (5.7%)	6 (2.3%)	6 (2.3%)	27 (10.2%)
Dystocia	12 (4.5%)	3 (1.1%)	6 (2.3%)	21 (7.9%)
Anestrus	54 (20.4%)	21 (7.9%)	12 (4.5%)	87(32.8%)
Repeat breeding	3 (1.1%)	6 (2.3%)	3 (1.1%)	12 (4.5%)
Cervicitis	15 (5.7%)	3 (1.1%)	0 (0%)	18 (6.8%)
Mastitis	6 (2.3%)	6 (2.3%)	3 (1.1%)	15 (5.7%)
Orchitis	5 (1.9%)	02 (0.8%)	0 (0%)	7 (2.6%)
Posthitis	5 (1.9%)	0 (0%)	0 (0%)	5 (1.9%)
Urolithiasis	5 (1.9%)	9 (3.4%)	0 (0%)	14 (5.3%)
Urinary tract infection (UTI)	11 (4.2%)	4 (1.5%)	02 (0.8%)	17(6.4%)
Total	154 (58.1%)	75 (28.3%)	36(13.6%)	265 (100%)
Chi-Square Test	Calculated value	Tabulated value	Not Significance P>0.05	
	36.279	31.410		

Young = 0 to < 12 months, Adult= 12 to 24 months & Older= > 24 months.

4.1.4. Effect of seasons of the year with reproductive diseases in small ruminants

The seasonal influence also diverse with the reproductive disorders of small in case of abortion, retained placenta, dystocia, anestrus, repeat breeding, cervicitis, mastitis, orchitis, posthitis, urolithiasis and urinary tract infection were 3.4%, 1.1%, 2.3%, 9.1%, 0.0%, 1.1%, 3.4%, 1.9%, 0.8% 1.9% & 2.3% in summer; 6.8%, 6.8%, 5.7%, 9.1%, 1.1%, 3.4%, 2.3%, 0.0%, 1.1%, 1.5% & 2.6% in rainy and 5.7%, 2.3%, 0%, 14.7%, 3.4%, 2.3%, 0%, 0.8%, 0%, 1.9% & 1.5% in winter seasons, respectively (Table 13).

Table 13. Distribution of reproductive diseases of small ruminants associated with Season.

Reproductive disorders	Seasons			Total
	Summer	Rainy	Winter	
Abortion	9 (3.4%)	18 (6.8%)	15 (5.7%)	42 (15.8%)
Retained placenta	3 (1.1%)	18 (6.8%)	6 (2.3%)	27 (10.2%)
Dystocia	6 (2.3%)	15 (5.7%)	0 (0%)	21 (7.9%)
Anestrus	24 (9.1%)	24 (9.1%)	39 (14.7%)	87 (32.8%)
Repeat breeding	0 (0.0%)	3 (1.1%)	9 (3.4%)	12 (4.5%)
Cervicitis	3 (1.1%)	9 (3.4%)	6 (2.3%)	18 (6.8%)
Mastitis	9 (3.4%)	6 (2.3%)	0 (0%)	15 (5.7%)
Orchitis	5 (1.9%)	0 (0%)	2 (0.8%)	7 (2.6%)
Posthitis	2 (0.8%)	3 (1.1%)	0 (0%)	5 (1.9%)
Urolethiasis	5 (1.9%)	4 (1.5%)	5 (1.9%)	14 (5.3%)
Urinary tract Infection	6 (2.3%)	7 (2.6%)	4 (1.5%)	17 (6.4%)
Total	72 (27.2%)	107 (40.4%)	86 (32.5%)	265 (100%)
Chi-Square Test	Calculated value	Tabulated value	Significant P<0.05	
	62.917	31.410		

4.2. Experiment 2: Investigation on Brucellosis associated with reproductive disorders in small ruminants

The results of investigation on Brucellosis associated with reproductive disorders in small ruminants were discussed with influencing factors.

4.2.1. Overall seroprevalence of brucellosis

Overall seroprevalence of brucellosis in small ruminants is 17.4% and 12% through RBPT and iELISA respectively (Table-14). Initial screening through RBPT showed that highest seroprevalence was in goats, followed by sheep.

Table 14. Crosstab of serological status of diseases and *Brucella* serology test.

Serological status of diseases	***Brucella* serology test**		Total
	Negative	**Positive**	
Brucella	0 0%	10 2.1%	10 2.1%
Toxoplasma	222 46.7%	0 .0%	222 46.7%
Brucleea, *Toxoplasma* and *Coxiella*	0 .0%	11 2.3%	11 2.3%
Brucella and *Toxoplasma*	0 .0%	26 5.5%	26 5.5%
Brucella and *Coxiella*	0 .0%	10 2.1%	10 2.1%
Toxoplasma and *Coxiella*	8 1.7%	0 .0%	8 1.7%
Completely negative	188 39.6%	0 0%	188 39.6%
Total	418 88.0%	57 12.0%	475 100.0%

The samples tested for *brucella* relation with other diseases and observed the prevalence 2.1%, 0%, 2.3%, 5.5%, 0%, and 0% sole *Brucella* positive, *Toxoplasma* positive, common three diseases positive, *brucella* & *Toxoplasma* positive, *Brucella* & *Coxiella*, *Toxoplasma* & *Coxiella* positive case and completely negative samples. It was supposed that the null hypothesis (H_0): There was no association between serological status of diseases (*Brucella*) and influencing factors and an alternative hypothesis (H_1): There was association between serological status of diseases (*Brucella*) and influencing factors.

The Pearson chi-square statistics was 214.649 and the *p*-value was < 0.0001, thus the null hypotheses see independent variables would be rejecte. Thus, we could conclude that there was a significance association between Serological status of diseases and *Brucella* serology under the study area.

Table 15. Statistical analysis of brucellosis serology.

Chi-Square Tests			
Test catagory	Value	Degrees of Freedom	Asymp. Sig. (2-sided)
Pearson Chi-Square	214.649[a]	3	P< 0.0001
Likelihood Ratio	175.393	3	P< 0.0001
Valid Cases (N)	475		

a. 1 cells (12.5%) have expected count less than 5. The minimum expected count is 1.92

4.2.2. Effect of species on the brucellosis on ELISA test in small ruminants

Seroprevalence of *brucella* is highest in goat than sheep is shown from Table 16 & Figure 1. The goat possage 9.3% and sheep 2.7% of *Brucella* positives cases, but within species the percentages were 11.1% and 16.5%. The species was statistatically insignificant and had positive correlation (r= 0.074) between species and *Brucella* in small ruminants at Nothern Barind Tract in Bangladesh.

Table 16. Seroprevelance of brucellosis corelartion with species of SR.

Test of Brucellosis	Goat	Sheep	Small ruminant
Positive	44 9.3%	13 2.7%	57 12%
Negative	352 74.1%	66 13.9%	418 88%
Total	396 83.4%	79 16.6%	475 100%
Correlation (r)	0.074 ns		

ns = Non Significant

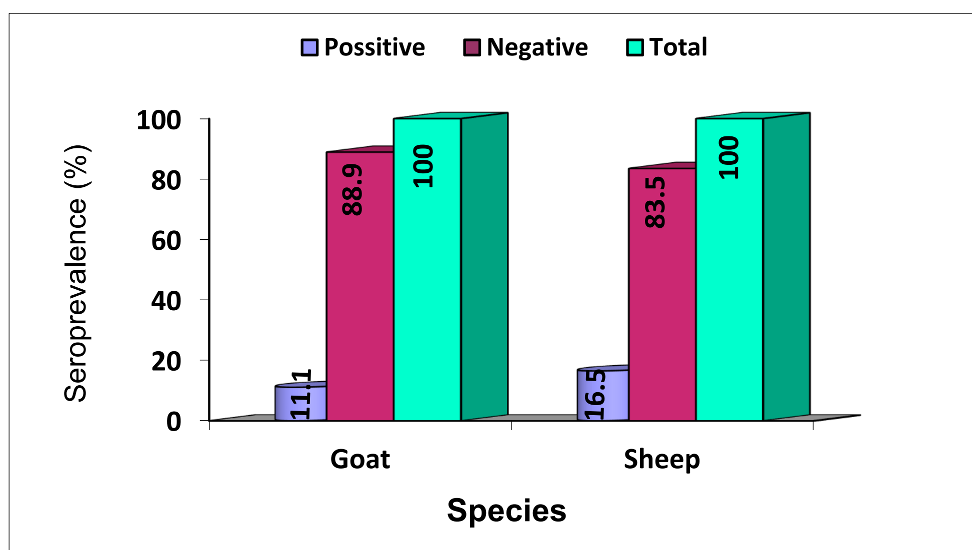


Figure 1. Brucellosis results shows in relation with species of small ruminants.

4.2.3. Effect of breed on brucellosis test

The breed of small ruminants and brucellosis test result are presented in Table 17 and Figure 2. Seropositiveness of the diseases got 6.9% in Local breed, but highest 10.1% in sheep for crossbred and goat 7.1% in local breed. The disease and breed had negatives correlation (-0.026) and statistically not significant (P>0.05).

Table 17. *Brucella* association with breed of small ruminants.

Test of Brucellosis	Breeds		
	Local	**Crossbred**	**Total**
Positive	33 6.9%	24 5.1%	57 12%
Negative	223 46.9%	195 41.1%	418 88%
Total	256 53.9%	219 46.1%	475 100%
Correlation (r)	-0.026 ns		

Correlation is significant at the 0.01 level (2-tailed). **

Correlation is significant at the 0.05 level (2-tailed)* and Non significant (NS)

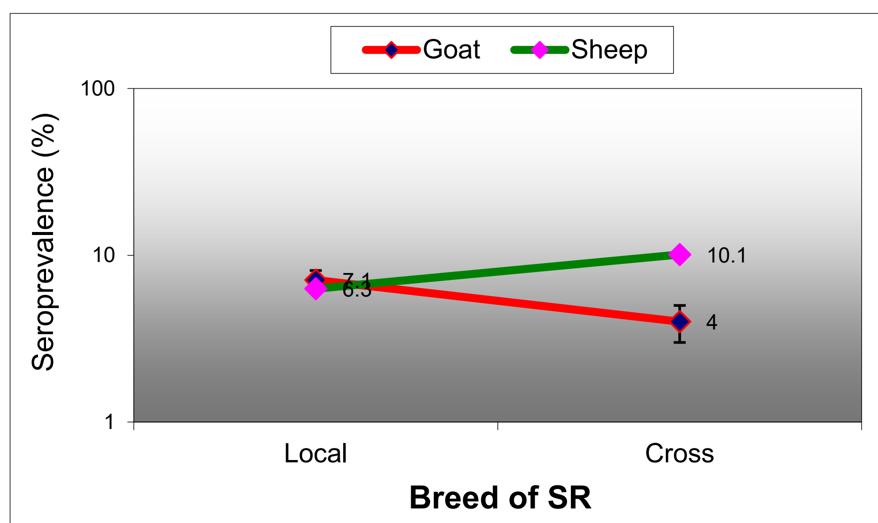


Figure 2. Graphical represent of breed of SR on the prevalence of brucellosis.

4.2.4. Brucellosis relation withtin sex of small ruminants

The Table 18 and Figure 3 is observed the seroprevalence of brucellosis in association with sex of small ruminants. Sex wise prevalence of brucellosis revealed that prevalence in male was 2.5%. On the other hand, prevalence in female was record as 9.5%. Prevalence of brucellosis in female ruminant animals was higher than male animals.

Table 18: Sex of ruminants animals' relationship with brucellosis

Test of Brucellosis	Sex		
	Male	**Female**	**Total**
Positive	12 2.5%	45 9.5%	57 12%
Negative	185 38.9%	233 49.1%	418 88%
Total	197 41.5%	278 58.5%	475 100%
Correlation (r)	**0.0173****		

Correlation is significant at the 0.01 level (2-tailed). **

Correlation is significant at the 0.05 level (2-tailed)* and Non significant (ns)

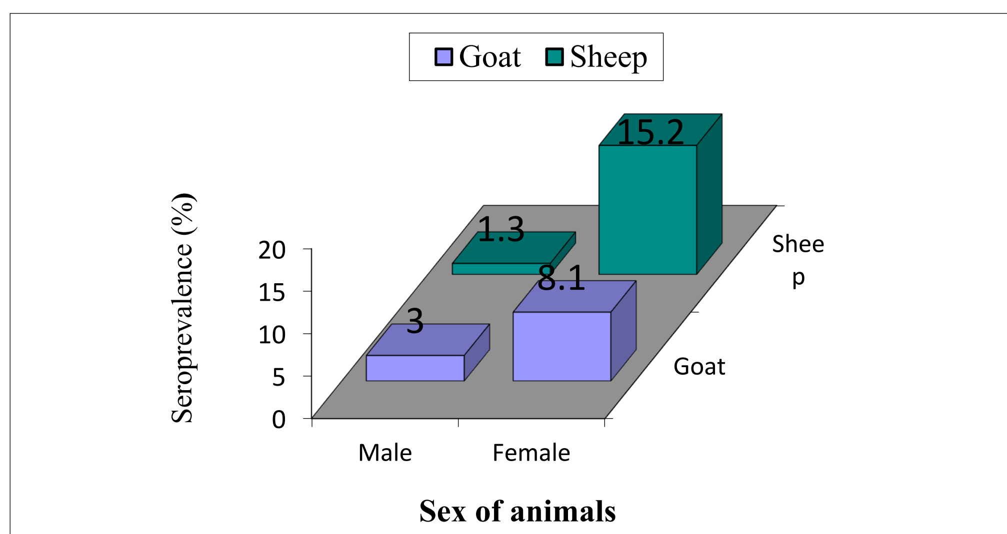


Figure 3. Sex wise seroprevalence of brucelosis in SR.

The occurrence of brucellosis had highly significant (p< 0.01) relationship with sex of small ruminants. The prevalence of brucellosis in goat was found to be higher in female 8.1% I-ELISA than male 3.0%, where as sheep, the share was 15.2% female than 1.3% male.

4.2.5. Brucellosis relation with age of small ruminants

Prevalence of brucellosis in relation with age is shown on the Table 19 and Figure 4.

Table 19. Age of ruminants relation with brucellosis.

Test of Brucellosis	Age of ruminants animals			
	Young	**Adult**	**Older**	**Total**
Positive	9 1.9%	19 4.0%	29 6.1%	57 12%
Negative	79 16.6%	158 33.3%	181 38.1%	218 88%
Total	88 18.5%	177 37.3%	210 44.2%	475 100%
Correlation (r)	0.033 ns			

Correlation is significant at the 0.01 level (2-tailed). **

Correlation is significant at the 0.05 level (2-tailed)* and Non significant (ns)

Young = 0 to < 12 months, Adult= 12 to 24 months & Older= > 24 months.

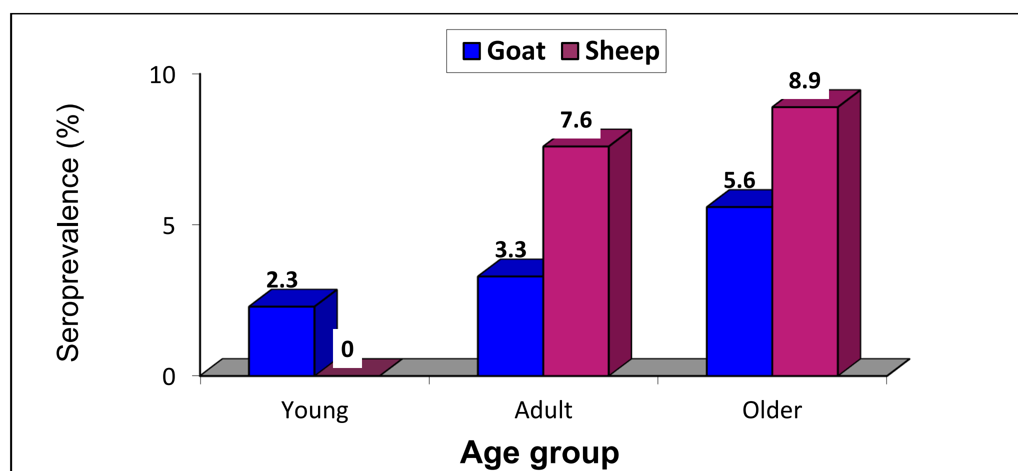


Figure 4. Seroprevelence of brucellosis in relation with age of animals.

The prevalence of brucellosis was lowest in younh small ruminants and highest in older age Table-19. The highest rate of infection observed in older goat (5.6%) and sheep (8.9%) in respect to others age group. These were 2.3% & 0% and 3.3% & 7.6% in goat & sheep at young and adult age. Statistically, there was existed a positive association and not significant (*P*>0.05) between breed of small ruminants and the prevalence of brucellosis.

4.2.6. Brucellosis relation with parity of small ruminants

The parity of small ruminants influence on brucellosis (serological observation) are distributed in Table 20 and Figure 5. The highest percentages of *Brucella* infection obtained at first parity 7.6%, 5.5% and 15.7% in small ruminants, goat and sheep, respectively.

Table 20. Serological test result of brucellosis in relative with parity of small ruminants.

Serological test		Parity				Total
		1st	2nd	3rd	>3rd	
Brucellosis	Positive	21 7.6%	10 3.6%	9 3.2%	5 1.8%	45 16.2%
	Negative	112 40.3%	66 23.7%	35 12.6%	20 7.2%	233 83.8%
Total		133 47.8%	76 27.3%	44 15.8%	25 9.0%	278 100.0%
Correlation (r)		0.031 ns				

Correlation is significant at the 0.01 level (2-tailed). **
Correlation is significant at the 0.05 level (2-tailed)* and Non significant (ns)

The lowest rate were at >3rd parity 1.8% and 1.7% in of small ruminants and goat, but in sheep it was at 2nd, 3rd and >3rd parity and possage same value 1.7%. The parity of small ruminants had positive asoociation and statically had no significant (*P*>0.05) effect on brucellosis at northern barind tract, Bangladesh.

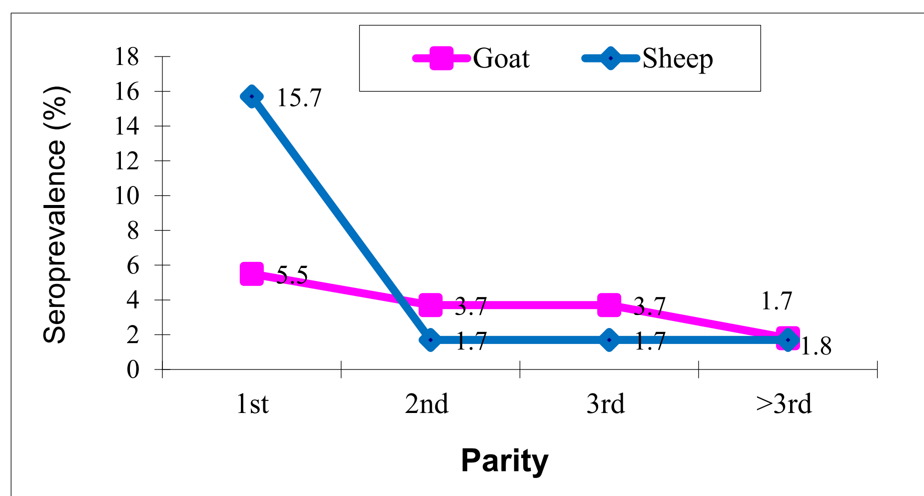


Figure 5. Serological status of *Brucella* in case of parity of small ruminants.

4.2.7. Brucellosis relation with body weight

Brucellosis in small ruminants in relation with body weight is expressed in Table 21 and Figure 6.

Table 21. Test of *Brucella* relation with body weight of small ruminants.

Test of Brucellosis	Body weight			
	Light	**Medium**	**Heavy**	**Total**
Positive	10 2.1%	18 3.8%	29 6.1%	57 12%
Negative	77 16.2%	147 30.9%	194 40.8%	418 88%
Total	323 68%	100 21.1%	52 10.9%	475 100%
Correlation (r)	-0.038 ns			

Correlation is significant at the 0.01 level (2-tailed). ** Correlation is significant at the 0.05 level (2-tailed)*, Non-significant (ns); Light = <10 kg body weight, Medium= 10 to 15kg body weight & Heavy= >15 kg body weight.

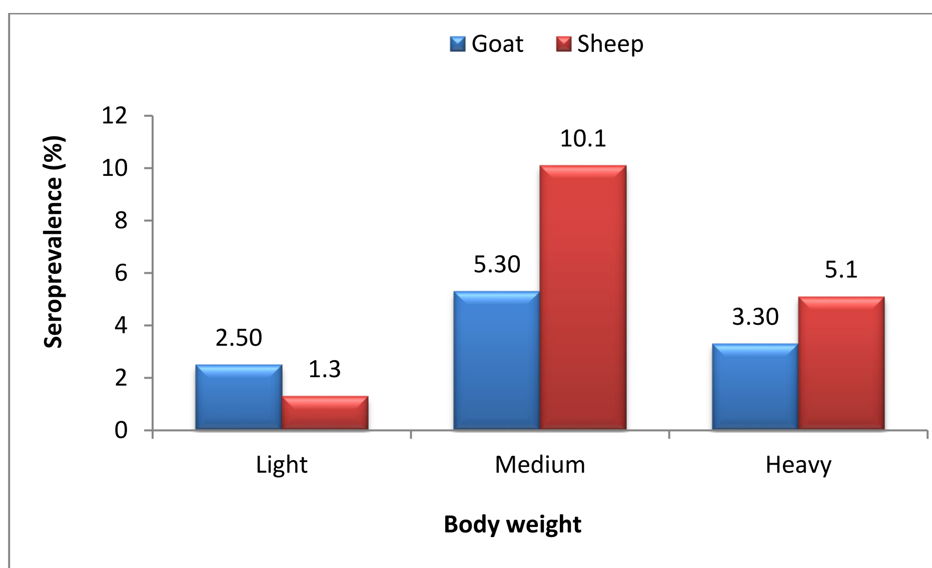


Figure 6. Body weight of SR with serological result of *Brucella*.

In small ruminants, the highest and lowest frequencies were 6.1% & 2.1% at heavy and light body weight and others was 3.8% in medium body weight group. Similarly, the highest and lowest value in case of goat and sheep were 5.3% & 10.1%; 2.5% & 1.3% in medium and light body weight, respectively. The body weight of small ruminants and *Brucella* was negative relationship and had not significant (P>0.5) when statistaclly tested.

4.2.8. Brucellosis relation with flock size of the farm at Northern Barind Tract in Bangladesh

Flock size prevalence of brucellosis is shown on the Table 22 and Figure 7. Prevalence of brucellosis was height in small size flock (8.2%). On the other hand, the prevalence of brucellosis was recorded in SR animals relatively lower (2.1%) in medium size flock, and lowest in large size flock (1.7%). Statistically, there was existed non-significant (P>0.5) association between flock size of SR and the prevalence of brucellosis. From the Figure 7, the highest and lowest prevalence in goat were 8.8% and 0.5% in small and large size flock, but in case of sheep it was reverse the highest and lowest rate and there were 7.6% in large size flock and 1.8% in medium size flock, respectively.

Table 22: The prevalence of brucellosis in ruminants in relation with density.

Test of Brucellosis	Flock size			
	Small	**Medium**	**Large**	**Total**
Positive	39 8.2%	10 2.1%	8 1.7%	57 12%
Negative	284 59.8%	90 18.9%	44 9.3%	418 88%
Total	323 68%	100 21.1%	52 10.9%	475 100%
Correlation (r)	0.055 ns			

Correlation is significant at the 0.01 level (2-tailed). **

Correlation is significant at the 0.05 level (2-tailed)*and Non significant (ns)

Small = <5 animals, Medium = 5 to 10 animals and Large = >10 animals in a flock

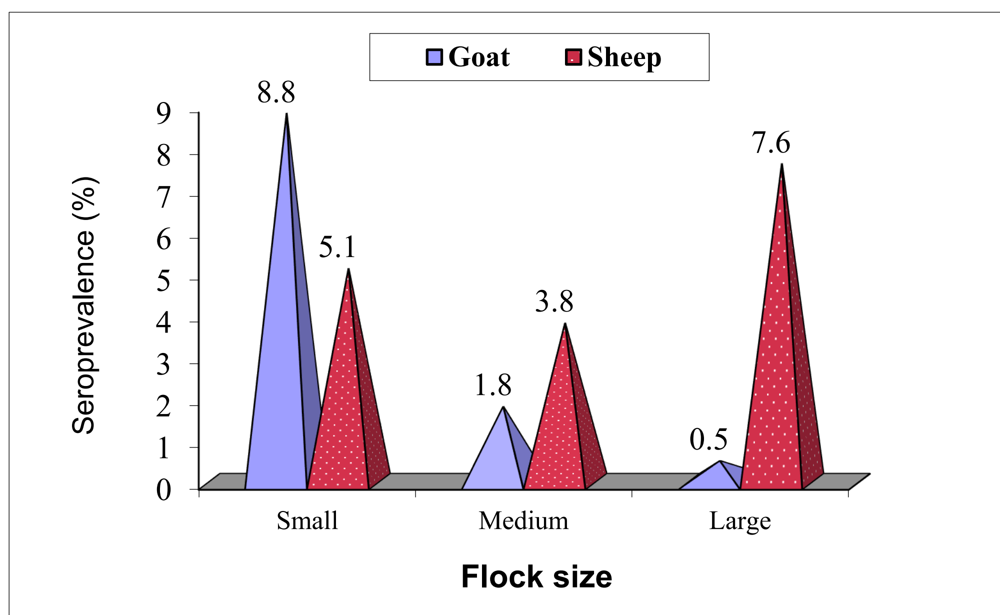


Figure 7. Prevalence of brucellosis in goat and sheep depend on flock size of animal population.

4.2.9. Effect of biosecurity of farms on the basis of *Brucella* infection presence

The biosecurity of small ruminants accordance with the prevalence of brucellosis is shown on the Table 23. Prevalence of brucellosis was highest in traditional (8.2%) level of biosecurity, lower (1.3%) in moderate level of biosecurity farms and other was 2.5% in good management level of biosecurity farms. On the other hand, the prevalence of brucellosis in goat and sheep was demonstrat in Figure 8. The highest, lowest and other prevelance of brucellosis incase of goat were 8.8%, 1% and 1.3% in traditional, good and moderate level of bio security maintained farms. Where as sheep, it was record highest, lowest and other prevelance of brucellosis were 10.1%, 1.3% and 5.1% in good, traditional and moderate management practiced farms. Statistically, there was a significant ($P<0.05$) but negative association between bio security of ruminants animals and the prevalence of brucellosis.

Table 23: The prevalence of brucellosis on small ruminants in relation with biosecurity.

Test of Brucellosis	Biosecurity			
	Traditional	**Moderate**	**Good**	**Total**
Positive	39 8.2%	6 1.3%	12 2.5%	57 12%
Negative	214 45.1%	119 25.1%	85 17.9%	218 88%
Total	253 53.3%	125 26.3%	97 20.4%	475 100%
Correlation (r)	-0.048*			

Correlation is significant at the 0.01 level (2-tailed). **

Correlation is significant at the 0.05 level (2-tailed)* and Non significant (ns)

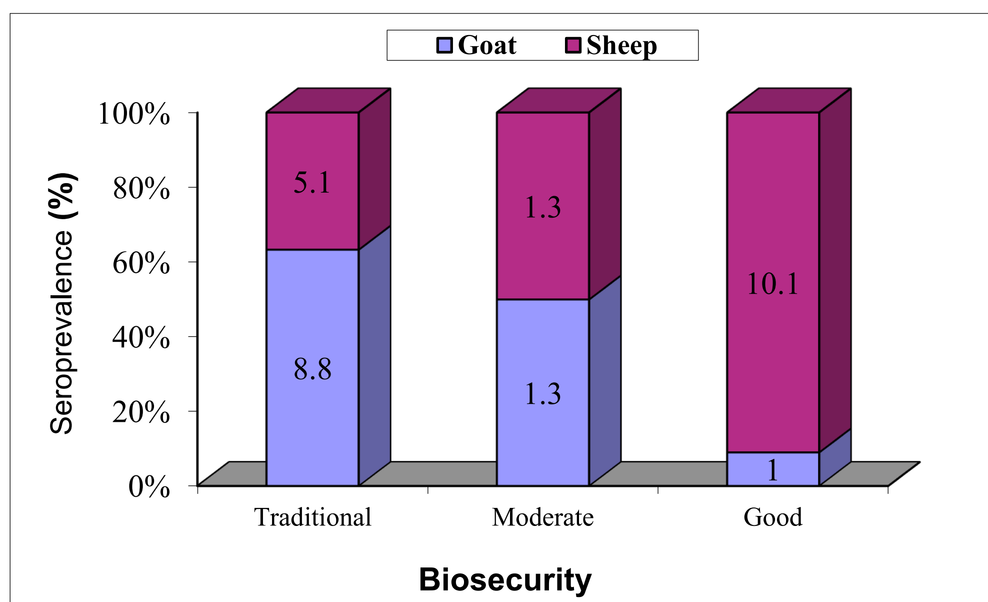


Figure 8. Biosecurity of farms reveal the seropositiveness of *Brucella.*

4.2.10. Brucellosis relation with feeding habit of the farm

Grazing effect on brucellosis in small ruminants is shown in the Table 24.

Table 24. The prevalence of brucellosis in ruminants animal's relation with grazing practice.

Test of Brucellosis	Feeding habit		
	Grazing	**Stall feeding**	**Total**
Positive	38 8%	19 4%	57 12%
Negative	259 54.5%	159 33.5%	418 88%
Total	297 62.5%	178 37.5%	475 100%
Correlation (r)	**-0.052***		

Correlation is significant at the 0.01 level (2-tailed). **

Correlation is significant at the 0.05 level (2-tailed)* and Non significant (ns)

The prevalence of brucellosis was higher (8%) in grazing small ruminants than stall-feeding (4%). Statistically, there was significant effect (P<0.05) on grazing behavior of ruminant animals and brucellosis. The r-value was -0.052, so that thre was a negative relation between dependent and independent variable. When the grazing results observed in goat and sheep indididually, it was 6.6% & 4.5% and 15.2% & 1.3% grazing and stall-feeding value.

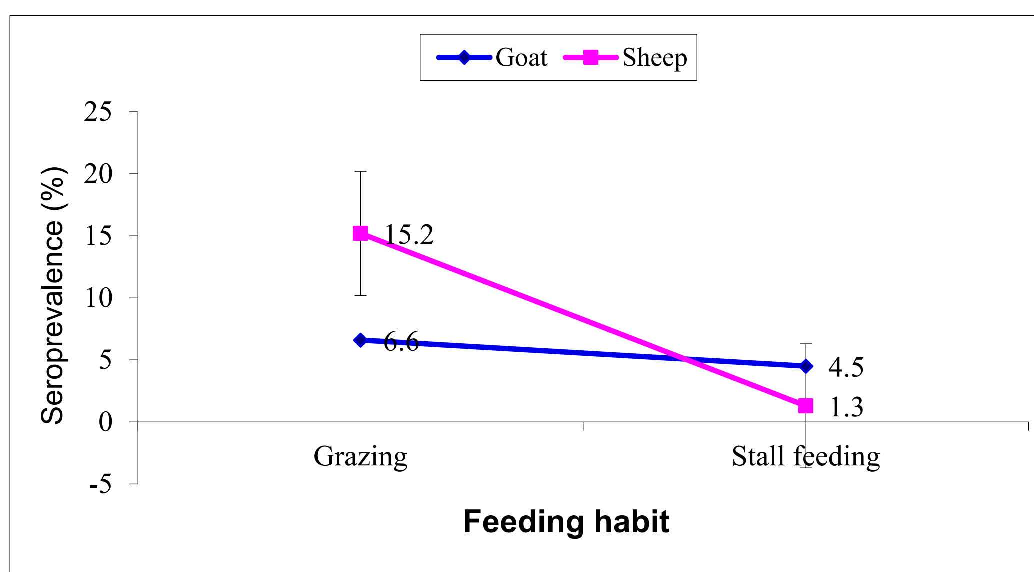


Figure 9. Grazing and stall-feeding value in relation with brucellosis in small ruminants.

4.2.11. Brucellosis relation with season of the year

Seasonal effect in relation with *Brucella* in small ruminants are described in Table 25 and 10 Figure. From that table it was define that the percentages of *Brucella* positive case in small ruminants were 3.4%, 6.1% and 2.5% in summer, rainy and winter seasons of the year. There was negative correlation (r= -0.09) between *Brucella* and season that had significant effect (P>0.05/0.1). The proportion of *Brucella* in summer rainy and winter was 3.3%, 5.3% & 2% and 3.8%, 8.9% & 3.8% in goat and sheep, respectively.

Table 25. Brucellosis association with seasonal effect of the years.

Brucellosis test results		Seasons			Total
		Summer	Rainy	Winter	
	Positives	16 3.4%	29 6.1%	12 2.5%	57 12.0%
	Negatives	154 32.4%	148 31.2%	116 24.4%	418 88.0%
	Total	170 35.8%	177 37.3%	128 26.9%	475 100.0%
	Correlation (r)	-0.09 (NS)			

Correlation is significant at the 0.01 level (2-tailed). **
Correlation is significant at the 0.05 level (2-tailed)* and Non significant (NS)

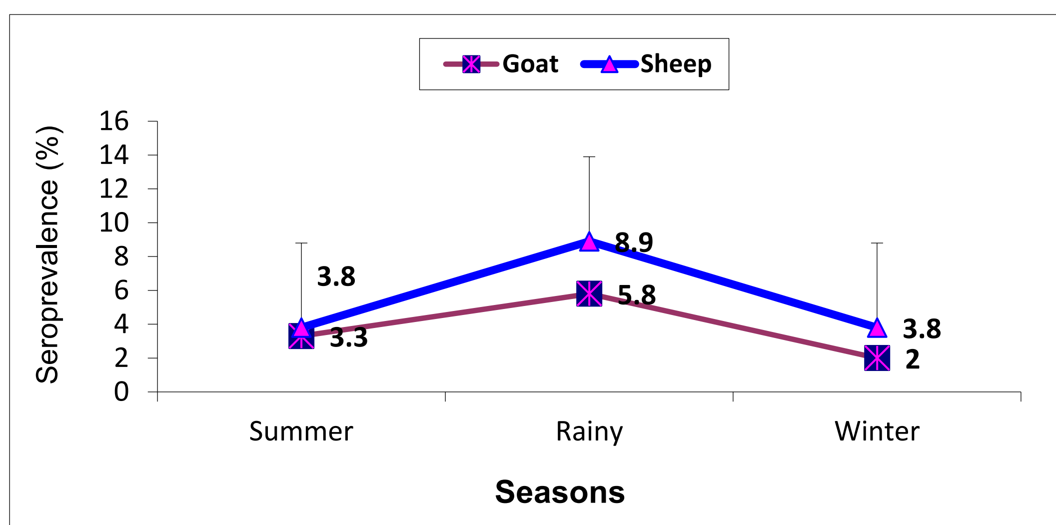


Figure 10. Brucellosis relation with seasons of the year.

4.2.12. Brucellosis relation with farm location

Seroprevalence of brucellosis in relation to location at NBT in Bangladesh are publicized in Table 26 & 11 Figure. The upbeat part of *Brucella* organism were 5.9%, 1.5%, 2.3% and 2.3% into Rajshahi, Chapai Nawabjong, Natore and Naogaon region in small ruminants. *Brucella* and location had possage positive relationship in SR and the r-value was 0.092. Thre was no significant effect on *Brucella* and location. In goat & sheep, the fraction of *Brucella* were

6.3%, 1%, 1.5% & 2.3% & 3.8%, 3.8%, 5.1% and 3.8% in Rajshahi, Chapai Nawabgonj, Natore and Naogaon districts.

Table 26. Seroprevalence of brucellosis in relation to location at NBT in Bangladesh.

		Districts				Total
Brucellosis		**Rajshahi**	**Chapai Nawabgonj**	**Natore**	**Noagoan**	
	Positive	28 5.9%	7 1.5%	11 2.3%	11 2.3%	57 12.0%
	Negative	239 50.3%	73 15.4%	48 10.1%	58 12.2%	418 88.0%
	Total	267 56.2%	80 16.8%	59 12.4%	69 14.5%	475 100.0%
	Correlation (r)		0.092 (ns)			

Correlation is significant at the 0.01 level (2-tailed). **

Correlation is significant at the 0.05 level (2-tailed)* and Non significant (ns)

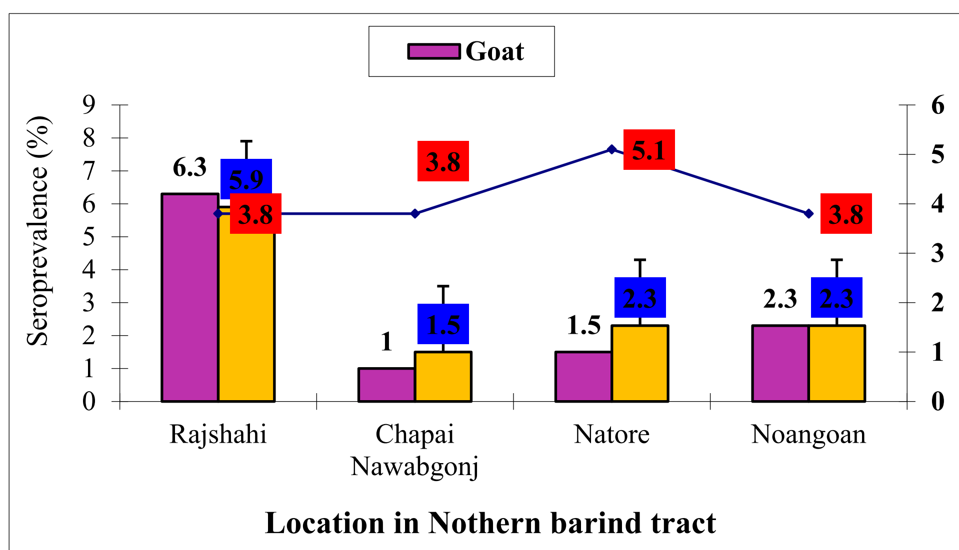


Figure 11. Location wise *Brucella* test result in small ruminants.

4.2.13.Effect of pregnancy status on *Brucella* of small ruminants

The prevalence of brucellosis in small ruminants according to pregnancy status is presented in Table 27 and Figure 12.

Table 27. Seroprevalence of *Brucella* in relation with pregnancy ststus of doe and ewe

Brucellosis test results		**Pregnancy status**		**Total**
		Pregnant	**Non pregnant**	
	Positives	8 4%	17 8.6%	25 12.6%
	Negatives	23 11.6%	150 75.8%	173 87.4%
	Total	31 15.7%	167 84.3%	198 100.0%
Chi square test	Calculated Value 5.78	Tabulated value 3.85 at 1 Degree of freedom (Significant $P<0.05$)		

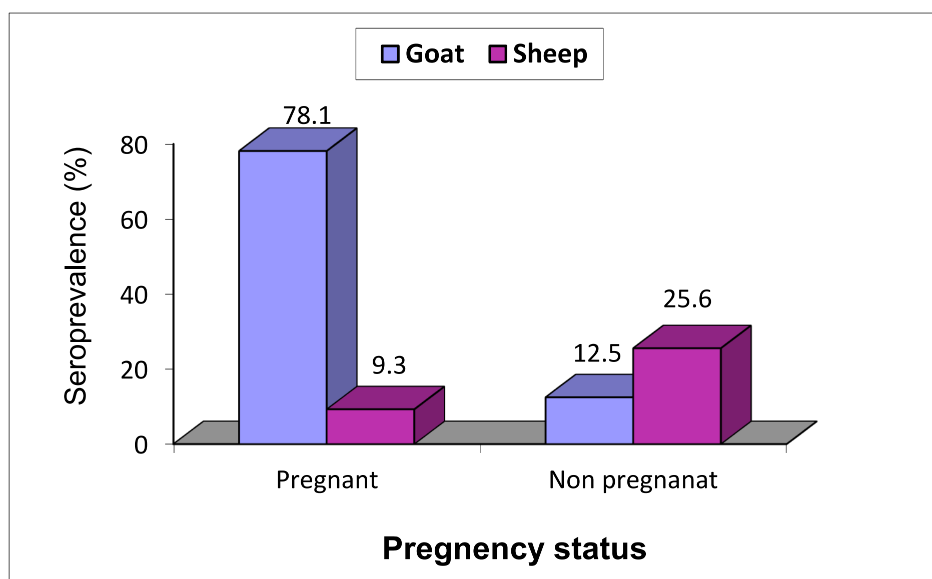


Figure 12. Serological condition of *Brucella* and pregnancy status of small ruminants.

The over all prevalence of brucellosis in relation with pregnant status of SR was recorded 12.6% but pregnant 4% and in non-pregnant 8.6%. It was

showing that higher prevalence record in pregnant cases (9.3% & 78.1%) than non-pregnant (12.5% & 25.6%) in goat and sheep. The prevalence of brucellosis had significant (P<0.5) relationship with pregnancy status in small ruminants at Northern Barind Tract in Bangladesh.

4.2.14. Brucellosis in relation with various reproductive diseases on SR

The incidence rate of *Brucella* in relation with reproductive diseases in small ruminants are summarized in Table 28 & 29 and Figure 13. The seropositive rate of *Brucella* in coordination with abortion, still birth, retained placenta, dystocia, cervicitis, vaginal prolapse or uterine prolapse, endometritis or pyometra, anestrus, repeat breeding, orchitis, epididymitis, posthitis and urinary tract infection were 2.1%, 0.4%, 1.7%, 0.6%, 1.9%, 0.6%, 0.4%, 1.1%, 0.6%, 0.2%, 0.4%, 0.2% & 1.7% respectively. The result in logistic regression analysis of equation (B1) has given in Table 28. From Table 28 shows that the logistic regression model of the test was significant at 11 degrees of freedom at 10% level. That means researcher may reject the null hypothesis and finally commented that there was effect of *Brucella* on reproductive diseases in small ruminants. In the logistic regression analysis, thirteen RDs in SR at NBT in Bangladesh had taken as indepedendable variable. The thirteen RDs in SR at Northern Barind Tract in Bangladesh had been taken as indepedendable variable were abortion, still birth, retained fetal membranes, dystocia, vaginal prolapse/ uterine prolapse, metritis/ pyometra, anestrus, repeat breeders, orchitis, posthitis & urinary tract infection and epididymitis was the control group, respectively. To the analysis, control was assuming the reference category. The retained placenta, anoestrus and cervicities of logistic regression coefficients were found to be positive and statistically significant (P<0.01), abortion, vaginal prolapse/ uterine prolapse at (P<0.05) and dystocia, orchitis (P<0.10) level. The still birth and posthitis were insignificant (P>0.10). The odds ratios of abortion, still birth, retained placenta, dystocia, cervicitis, vaginal prolapse or uterine prolapse, endometritis or pyometra, anestrus, repeat

breeding, orchitis, epididymitis, posthitis and urinary tract infection were 4.49, 3.65, 7.52, 4.03, 11.1, 6.20, 0.03, 5.74, 7.75, 5.17, 1.0 1.94, and 1.24 respectively. It means affected rate of brucellosis in small ruminants were 4.49, 3.65, 7.52, 4.03, 11.1, 6.20, 0.03, 5.74, 7.75, 5.17, 1.0 1.94 and 1.24 time more chance of infection than control group (Epididymitis).

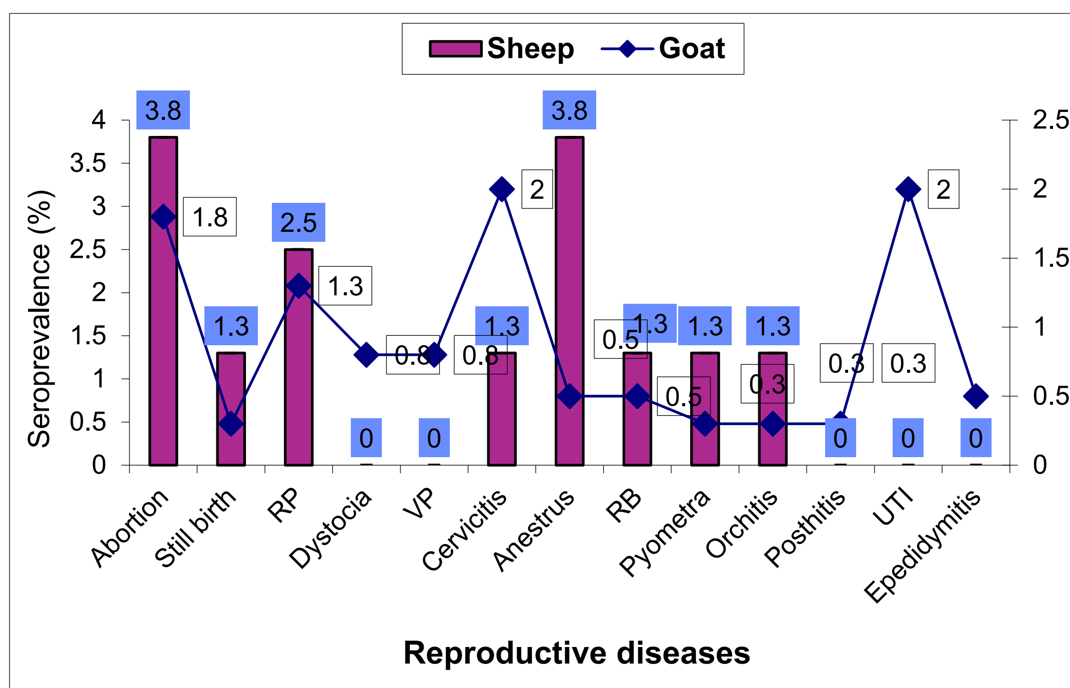
Table 28. The prevalence of brucellosis in ruminants animal's relation with various reproductive diseases.

Reproductive diseases of SR	Brucellosis		
	Positive	**Negative**	**Total**
Abortion	10 2.1%	69 14.5%	79 16.6%
Still birth	2 0.4%	17 3.6%	19 4.0%
Retained placenta	8 1.7%	33 6.9%	41 8.6%
Dystocia	3 0.6%	23 4.8%	26 5.5%
Cervicitis	9 1.9%	25 5.3%	34 7.2%
Vaginal prolapse/ Uterine prolapse	3 0.6%	15 3.2%	18 3.8%
Endometritis/ pyometra	2 0.4%	12 2.5%	14 2.9%
Anoestrus	5 1.1%	27 5.7%	32 6.7%
Repeat breeding	3 0.6%	12 2.5%	15 3.2%
Orchitis	1 0.2%	16 3.4%	17 3.6%
Epididymitis	2 0.4%	62 13.1%	64 13.5%
Posthitis	1 0.2%	25 5.3%	26 5.5%
Urinary tract infection	8 1.7%	82 17.3%	90 18.9%
Total	57 12.0%	418 88.0%	475 100.0%
Chi square test	Calculated value 18.596	Tab value at 10DF	Significant

Table 29. Logistic Regression Estimates of the Odds Ratios of Selected *Brucella* infection and reproductive diseases in small ruminants.

Independent Variables / **Reproductive diseases of SR**	**B**	**S.E.**	**Exp(B)**
Abortion	1.502	.794	4.493**
Still birth	1.294	1.037	3.647
Retained placenta	2.017	.819	7.515*
Dystocia	1.397	.945	4.043***
Cervicitis	2.412	.817	11.160*
Vaginal prolapse/ Uterine prolapse	1.825	.957	6.200**
Endometritis/ Pyometra	-3.434	.718	.032
Anoestrus	1.748	.868	5.741*
Repeat breeding	2.048	.966	7.750*
Orchitis	1.642	1.049	5.167***
Posthitis	.661	1.256	1.937
Urinary tract infection	.215	1.247	1.240
Constant (Epididymitis[r])	1.107	1.000	1.000

r = Reference Category; B = Logistic Regression Coefficient; and Exp (B) = Odds Ratio‘*’ = Significant at 1% level; ‘**’ = Significant at 5% level; and ‘***’ = Significant at 10% level



RP= Retained Placenta, VP= Vaginal Prolapse, RB= Repeat Breeding and UTI= Urinary Tract Infection

Figure 13. Serological test status of *Brucella* and reproductive diseases in small ruminants.

4.3. Experiment 3: Assessment of Toxoplasmosis linked with reproductive disorders in small ruminants

To assess the *Toxoplsma* in small ruminants with influencing factors were discussed under specific sub heading.

4.3.1. Overall sero-prevalence of toxoplasmosis

Overall sero-prevalence of toxoplasmosis in small ruminants was 56% through ELISA serology (Table-30). Initial screening through serum test shows that highest sero-prevalence is in goats, followed by sheep.

Table 30. Crosstab of serological status of diseases and *Toxoplasma* serology test.

Serology test combination		***Toxoplasma* serology test**		**Total**
		Negative	**Positive**	
Serological status of diseases	*Brucella*	10	0	10
		2.1%	.0%	2.1%
	Toxoplasma	1	221	222
		.2%	46.5%	46.7%
	Brucleea, *Toxoplasma* and *Coxiella*	0	11	11
		.0%	2.3%	2.3%
	Brucella and *Toxoplasma*	0	26	26
		.0%	5.5%	5.5%
	Brucella and *Coxiella*	10	0	10
		2.1%	.0%	2.1%
	Toxoplasma and *Coxiella*	0	8	8
		.0%	1.7%	1.7%
	Totally negative	189	0	189
		39.8%	.0%	39.8%
Total		209	266	475
		44.0%	56.0%	100.0%

The samples test for serology of three diseases and their prevalence 0%, 46.5%, 2.3%, 5.5%, 0%, and 1.7% and 0% in *Brucella* positive, *Toxoplasma* positive, common three diseases positive, *Brucella* & *Toxoplasma* positive,

Brucella & *Coxiella* , *Toxoplasma* & *Coxiella* positive case and totally negative samples uses for srology. The researcher supposes that the null hypothesis (H_0): There was no association between serological status of diseases (*Toxoplasma*) and influencing factors and an alternative hypothesis (H_1): There was association between serological status of diseases (*Toxoplasma*) and influencing factors. Now the Pearson chi-square statistics was 213.03 at three degrees of freedom (P<0.0001), thus the null hypothesis could be rejected.

Therefore, researcher could conclude that there was a significance association between serological status of diseases and *Toxoplasma* serology under the study area.

Table 31. Statistical analysis of toxoplasmosis serology.

Chi-Square Tests			
Test catagory	**Value**	**df**	**Asymp. Sig. (2-sided)**
Pearson Chi-Square	213.030[a]	3	P< 0.0001
Likelihood Ratio	185.165	3	P< 0.0001
Valid Cases (N)	475		

a. 1 cells (12.5%) have expected count less than 5. The minimum expected count is 2.05.

4.3.2. Prevalence of toxoplasmosis in relation with species of the small ruminants

The seroprevalence of toxoplasmosis in relative with species are focussed to explain under the Table 32 & 14 Figure. The positive and negative percentages of *Toxoplasma* were 45.7% & 37.7% in goat and 10.3% & 6.3% in sheep. When compare within species the infection rate was 45.2% & 54.8% in goat and 38% & 62% in sheep. Pearson's correlation coefficient (r) value was 0.089 and statiscally insignificant (P>0.05). Thus, the correlation coefficient was a measure of linear (with a positive slope) association between *Toxoplasma* and species under the working area.

Table 32: Seroprevalence of toxoplasmosis in small ruminants relation with species

	Result of ELISA test	Species		
		Goat	Sheep	Small ruminants
Toxoplasmosis	Positive	217 45.7%	49 10.3%	266 56%
	Negative	179 37.7%	30 6.3%	209 44%
	Total	396 83.4%	79 16.6%	475 100%
	Correlation (r)	0.089 ns		

Correlation is significant at the 0.01 level (2-tailed). **

Correlation is significant at the 0.05 level (2-tailed)* and Non significant (ns)

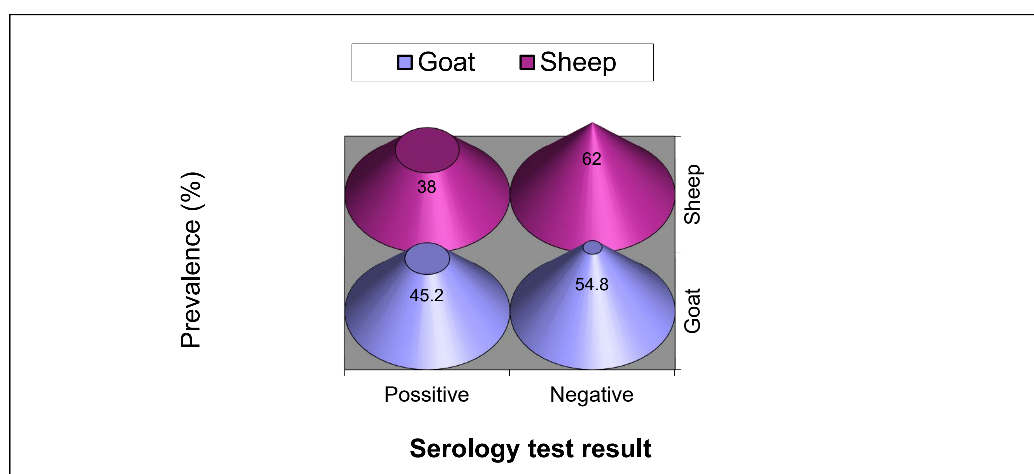


Figure 14. Seroprevalence of toxoplasmosis in goat and sheep relation with species.

4.3.3. Prevalence of toxoplasmosis in relation with breeds of the small ruminants NBT in Bangladesh

Seroprevalence of toxoplasmosis in small ruminants relation with breed is presented in Table-33 & Figure-15.

Table 33. Seroprevalence of toxoplasmosis in small ruminants relation with species

	Result of ELISA test	Breed		
		Local	**Crossbred**	**Total**
Toxoplasmosis	Positive	148 31.2%	118 24.8%	266 56%
	Negative	108 22.7%	101 21.3%	209 44%
	Total	265 53.9%	219 46.1%	475 100%
	Correlation (r)	-0.025 ns		

Correlation is significant at the 0.01 level (2-tailed). **

Correlation is significant at the 0.05 level (2-tailed)* and Non significant (ns)

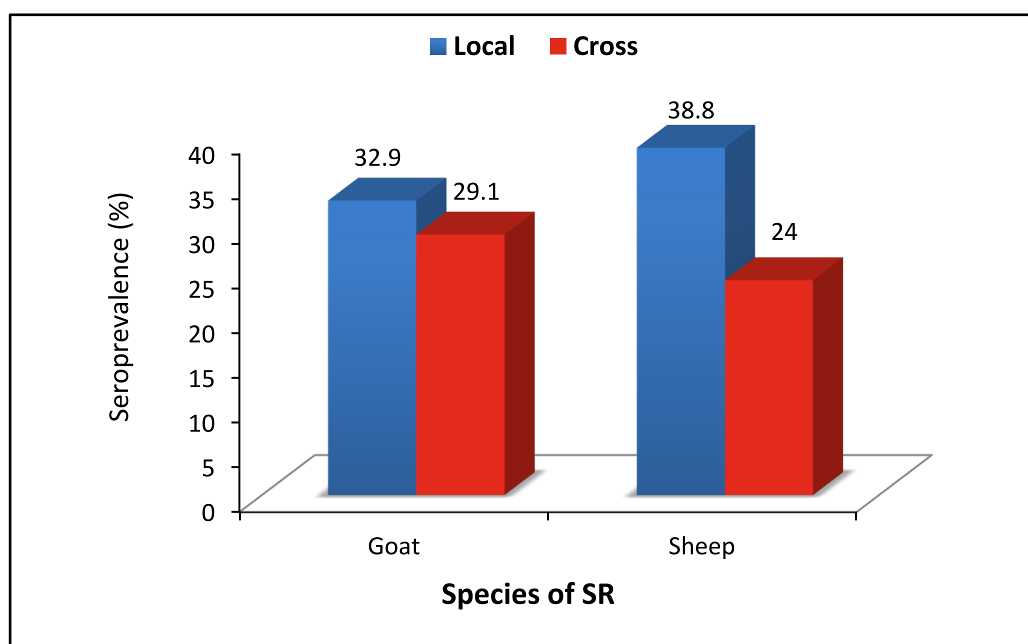


Figure 15. Seropevalence of *Toxoplasma* with breed in goat and sheep.

The highest (31.2%) result observes in local breed than crossbred (24.8%). Seropositiveness of the *Toxoplasma* infection possibility in got 32.8%

forlocal breed, and 29.1% for crossbred. Where compared in sheep, the infection possibility was 38.8% for Local breed and 24% for crossbred.

Pearson's correlation coefficient (r) value was -0.025 and statistically insignificant (P>0.1/0.05). Thus, the correlation coefficient was a measure of linear (with a negative slope) association between *Toxoplasma* and breed under the working area.

4.3.4. Prevalence of toxoplasmosis in relation with sex of the small ruminants

Sex wise seroprevalence of toxoplasmosis is reveale at Table 34. The female animal observed highest percentage (38.9%) of sensitive cases. On the other hands prevalence in male was recorded as 26.3%. There was a significant association between sex of SR and the prevalence of toxoplasmosis.

Table 34. Seroprevalence of toxoplasmosis due to sex involment in SR.

	Result of ELISA test	**Sex**		
		Male	**Female**	**Total**
Toxoplasmosis	Positive	103 21.7%	163 34.3%	266 56%
	Negative	94 19.8%	115 24.2%	209 44%
	Total	197 41.5%	278 58.5%	475 100%
	Correlation (r)	-0.025 ns		

Correlation is significant at the 0.01 level (2-tailed). **

Correlation is significant at the 0.05 level (2-tailed)* and Non significant (ns)

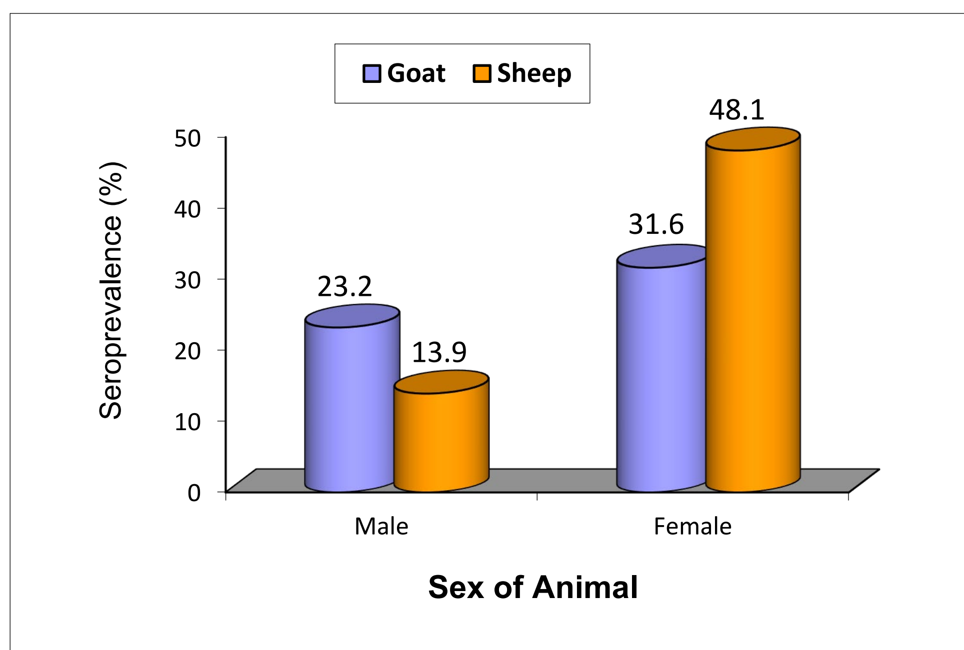


Figure 16. Prevalence of toxoplasmosis in goat and sheep relation with sex.

In the Figure 16 is presented the prevalence rate of *Toxoplasma* in goat and sheep. In goat and sheep, the infection possibility was 23.2% and 13.9% for male and 31.6% & 48.1% for female, respectively. The disease and sex had negatives correlation (-0.025) and statically not significant (*P*>0.1/0.05) effect on toxoplasmosis in small ruminants at NBT in Bangladesh.

Pearson's correlation coefficient (r) value was -0.025 and statistically insignificant. Thus, the correlation coefficient was a measure of linear (with a negative slope) association between *Toxoplasma* and breed under the working area.

4.3.5. Prevalence of toxoplasmosis in relation with age of the small ruminants NBT in Bangladesh

Prevalence of brucellosis relation with age is showed on the Table 35 and Figure 17.

Table 35. Effect of toxoplasmosis on age of Small ruminants.

	Result of ELISA test	Age			
		Young	Adult	Old	Total
Toxoplasmosis	Positive	44 9.3%	98 20.6%	124 26.1%	266 56%
	Negative	44 9.3%	79 16.6%	86 18.1%	209 44%
	Total	88 18.5%	177 37.3%	210 44.2%	475 100%
	Correlation (r)	-0.025 ns			

Correlation is significant at the 0.01 level (2-tailed). **

Correlation is significant at the 0.05 level (2-tailed).* Non Significance (ns)

Young = 0 to < 12 months, Adult= 12 to 24 months & Older= > 24 months.

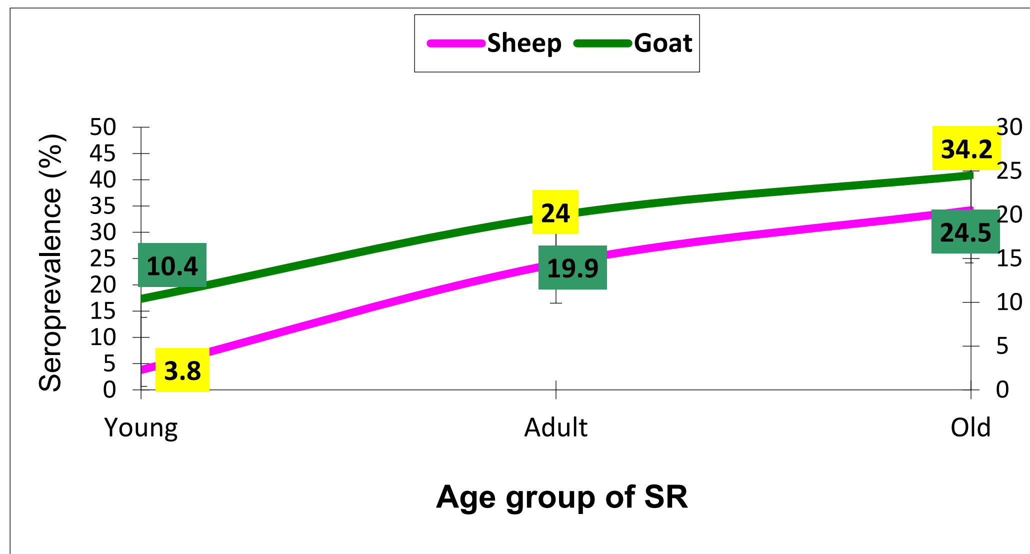


Figure 17. Age of goat and sheep shows the relationship with *Toxoplasma*.

Prevalence of brucellosis was lowest (9.3%) in young small ruminants and highest (26.1%) in older age and the other was 20.6% in adult age of animals. When compare it separately, the highest rate of infection was 24.5% & 34.2%

in older goat and sheep in respect to others age group. The others were 19.9% & 24% and 10.4% & 3.8% in goat & sheep at young and adult age, respectively. Statistically, there was existed a negative association and was not significant ($P>0.05$) between age of small ruminants and the prevalence of toxoplasmosis.

4.3.6. Prevalence of toxoplasmosis in relation with parity of the small ruminants

The parity of goat and sheep association between seroprevalencce and infection of toxoplasmosis are shown in the Table-36 & Figure-18. The highest to lower percentage of positive case of toxoplasmosis were 23.7%, 17.6%, 11.2% and 6.1% in 1st, 2nd, 3rd and >3rd parity, respectively in small ruminants.

Table 36. Effect of toxoplasmosis on parity of small ruminants

	Test result	**Parity**				**Total**
		1st	**2nd**	**3rd**	**>3rd**	
Toxoplasmosis	Positive	66 23.7%	49 17.6%	31 11.2%	17 6.1%	163 58.6%
	Negative	67 24.1%	27 9.7%	13 4.7%	8 2.9%	115 41.4%
	Total	133 47.8%	76 27.3%	44 15.8%	25 9.0%	278 100.0%
	Correlation (r)	0.207**				

Correlation is significant at the 0.01 level (2-tailed). **
Correlation is significant at the 0.05 level (2-tailed).* and Non Significance (ns)

Pearson's correlation coefficient (r)-value was 0.207 and statistically significant ($P<0.1$). Thus, the correlation coefficient was a measure of linear (with a positive slope) association between *Toxoplasma* and parity under the working area. The Figure-18 observe that the parity wise (1st to >3rd)

contamination rate were 19.2%,10.2%, 8.5% & 5.1% in case of goat and 40.7%, 19.9%, 11.6% & 6.4% in sheep at NBT in Bangladesh.

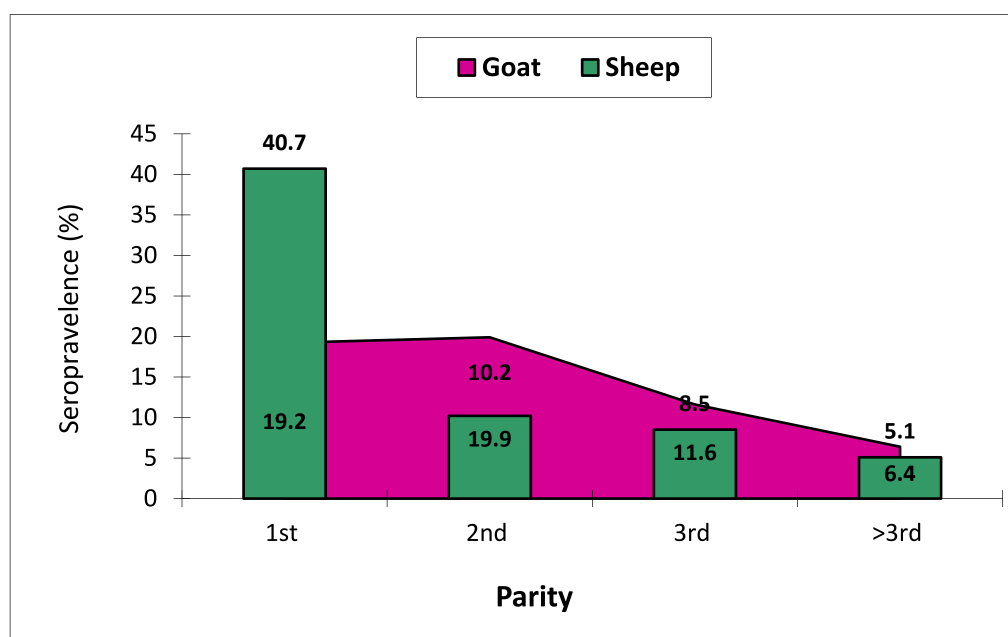


Figure 18. Figure showed parity wise prevalence of toxoplasmosis in goat and sheep.

4.3.7. Prevalence of toxoplasmosis in relation with body weight of the small ruminants

Seroprevalencce of toxoplasmosis in relation with body weight of SR is placed in Table 37. The highest (25.9%) prevalence of toxoplasmosis in SR was observe at medium (10 to<15 Kg) body weight group and lowest at heavy (21.1%) body weight group. On the other hands the seroprevalence of toxoplasmosis of SR was (9.1%) in light (<10 Kg) body weight group. Pearson's correlation coefficient (r) value was 0.046 and statistically insignificant (*P*>0.05). Thus, the correlation coefficient was a measure of linear (with a positive slope) association between *Toxoplasma* and body weight at the operational area. In Figure 19, the researcher shows that goat trends infection of *Toxoplasma* (lower to higher) 10.9%, 19.9% & 24% in light, heavy and medium body weight groups. Whereas in sheep this trends

were 2.5%, 26.6% & 32.9% in light, heavy and medium groups at NBT in Bangladesh.

Table 37. Effect of toxoplasmosis on body weight of Small ruminants.

	Result of ELISA test	Body weight			
		Light	**Medium**	**Heavy**	**Total**
Toxoplasmosis	Positive	43 9.1%	123 25.9%	100 21.1%	266 56%
	Negative	44 9.3%	100 21.1%	65 13.7%	209 44%
	Total	87 18.3%	223 46.9%	165 34.7%	475 100%
	Correlation (r)	0.046 ns			

Correlation is significant at the 0.01 level (2-tailed). ** Correlation is significant at the 0.05 level (2-tailed)*, Non-significant (ns); Light = <10 kg body weight, Medium= 10 to 15kg body weight & Heavy= >15 kg body weight.

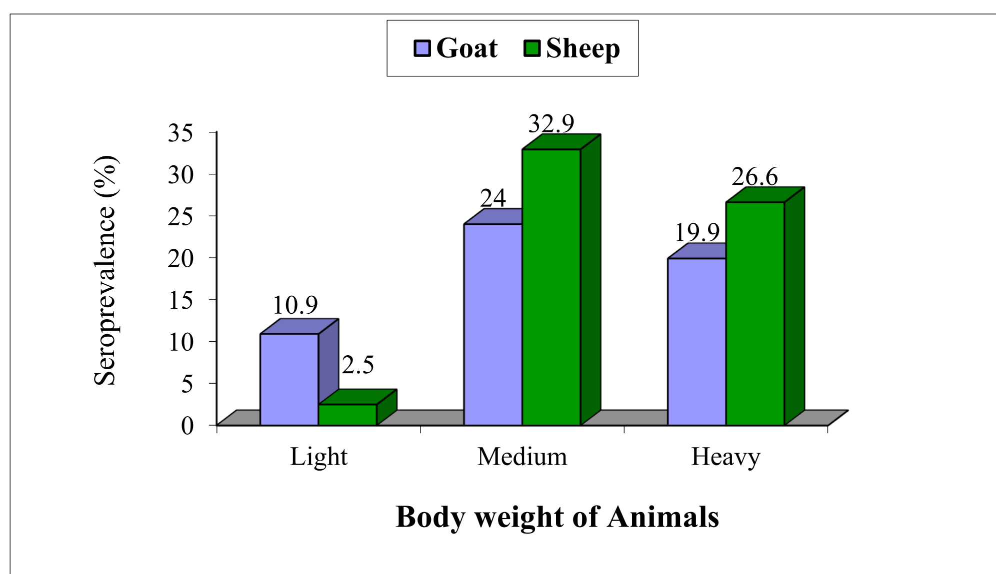


Figure 19. Body weight of animals (goat & sheep) shows bond with *Toxoplasma gondii.*

4.3.8. Prevalence of toxoplasmosis in relation with flock size of the small ruminants

Flock size seroprevalencce association with *Toxoplasma* is showed on the Table-38 and Figure-20. Seroprevalence of toxoplasmosis was highest 38.7% in SR of small density (<5 heads flock) populated farm than medium (11.8%) and large (5.5%) density populated farms. Surprisingly observe that the seroprevalence of toxoplasmosis in goat and sheep individually, the goat showed highest infection rate in which group sheep observed lowest percentage in same groups. Similarly lowest were vice versa.

Table 38. Seroprevalence of *Toxoplasma* in coordination with flock size.

	Result of ELISA test	**Flock Size**			
		Small	**Medium**	**Large**	**Total**
Toxoplasmosis	Positive	184 38.7%	56 11.8%	26 5.5%	266 56%
	Negative	139 29.3%	44 9.3%	26 5.5%	209 44%
	Total	323 68.0%	100 21.1%	52 10.9%	475 100%
	Correlation (r)	0.011 ns			

Correlation is significant at the 0.01 level (2-tailed). **
Correlation is significant at the 0.05 level (2-tailed).* Non Significance (ns)
Small = <5 animals, Medium = 5 to 10 animals and Large = >10 animals in a flock

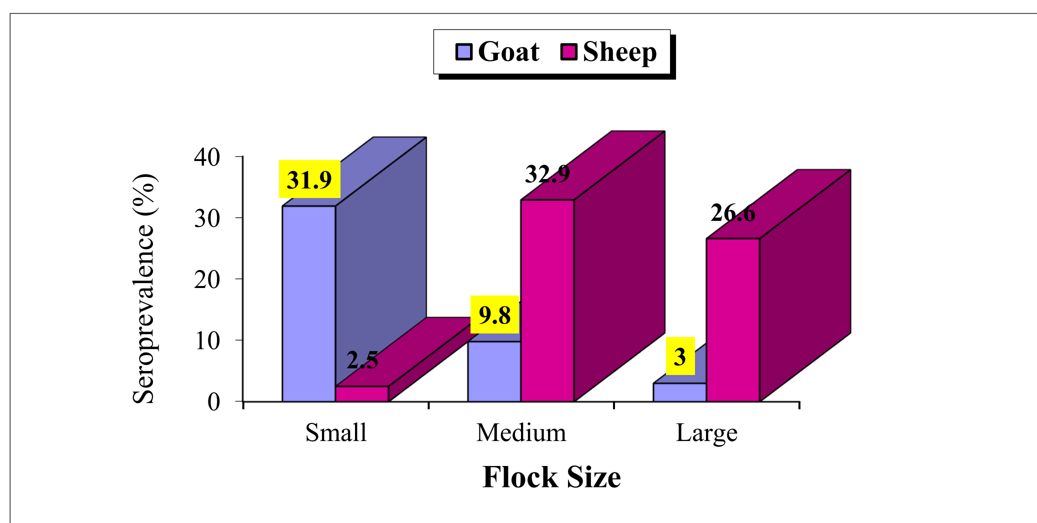


Figure 20. Flock size of animal population bears the involvement with *Toxoplasma*.

The values of goat highest to lowest were 31.9%, 9.8% & 3% in small, medium and large density populated goat farms. Similarly, in sheep there were 32.9%, 26.6%, 2.5% in medium, large and small density farms. Statistically, there was no significant (P>0.05) association between density of SR and toxoplasmosis, but they were possage positive relationship between them.

4.3.9. Prevalence of toxoplasmosis in relation with biosecurity

The biosecurity of small ruminants accordance with the seroprevalencce of toxoplasmosis is summarized in the Table-39 & Figure-21. The seroprevalence of toxoplasmosis increased with level of biosecurity that was traditional, moderate and good. On the other hand, the seroprevalence of toxoplasmosis was recorded in SR relatively lower in moderate (13.3%) biosecured maintained farms, lowest good (12.0%) biosecuried and highest in traditional (30.9%) level of biosecurity farms at observation area.

Pearson's correlation coefficient (r) value was 0.043 and statistically insignificant (P>0.05). Thus, the correlation coefficient was a measure of linear (with a positive slope) association between *Toxoplasma* and biosecurity of farms under the operational the area.

Table 39. Effect of toxoplasmosis on biosecurity of Small ruminants.

	Result of ELISA test	Biosecurity of Farm			
		Traditional	Moderate	Good	Total
Toxoplasmosis	Positive	147 30.9%	62 13.3%	57 12%	266 56%
	Negative	106 22.3%	63 21.1%	40 8.4%	209 44%
	Total	253 53.3%	125 26.3%	97 20.4%	475 100%
	Correlation (r)	0.043 ns			

Correlation is significant at the 0.01 level (2-tailed).**
Correlation is significant at the 0.05 level (2-tailed).* ns= Non Significance

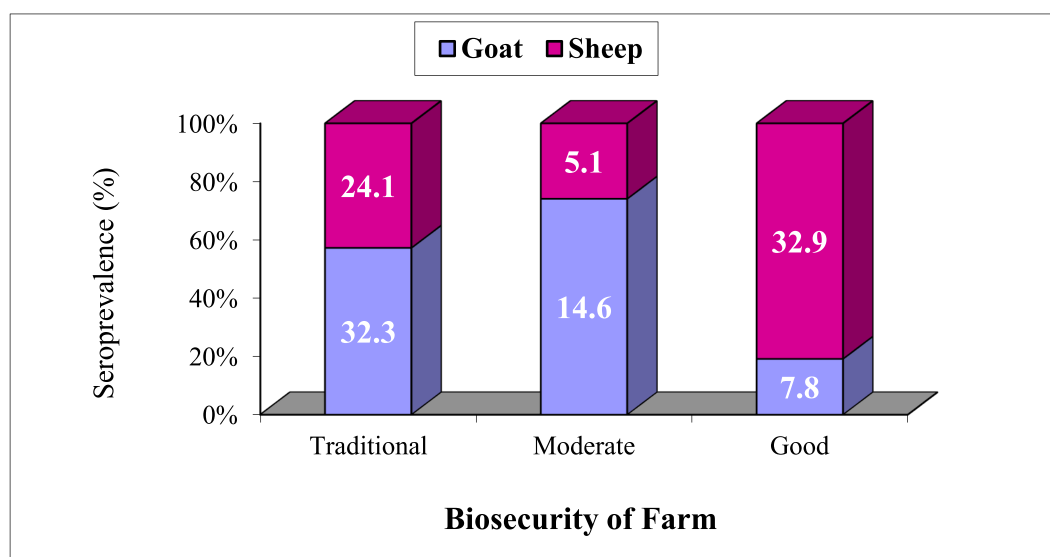


Figure 21. Prevalence of *Toxoplasma gondii* in association with biosecurity.

4.3.10. Prevalence of toxoplasmosis in relation with feeding habit

Feeding habit of small ruminants on toxoplasmosis is shown on the Table-40 & Figure-22. Seroprevalence of toxoplasmosis in small ruminants was higher 26.9% at graze habit animals than stall-feeding (17.1%) groups of observe animals. The grazing and stall-feeding values were 33.1% & 21.7%, 48.1% &13% in case of goat and sheep, correspondingly.

Table 40. Toxoplasmosis affected with feeding habit of small ruminants

Toxoplasmosis	Result of ELISA test	Feeding Habit		
		Grazing	Stall feeding	Total
	Positive	169 26.9%	81 17.1%	266 56.0%
	Negative	128 19.8%	115 24.2%	209 44%
	Total	297 62.5%	178 37.5%	475 100%
	Correlation (r)	-0.102 ns		

Correlation is significant at the 0.01 level (2-tailed).

**, Correlation is significant at the 0.05 level (2-tailed) & * Non-Significance (ns)

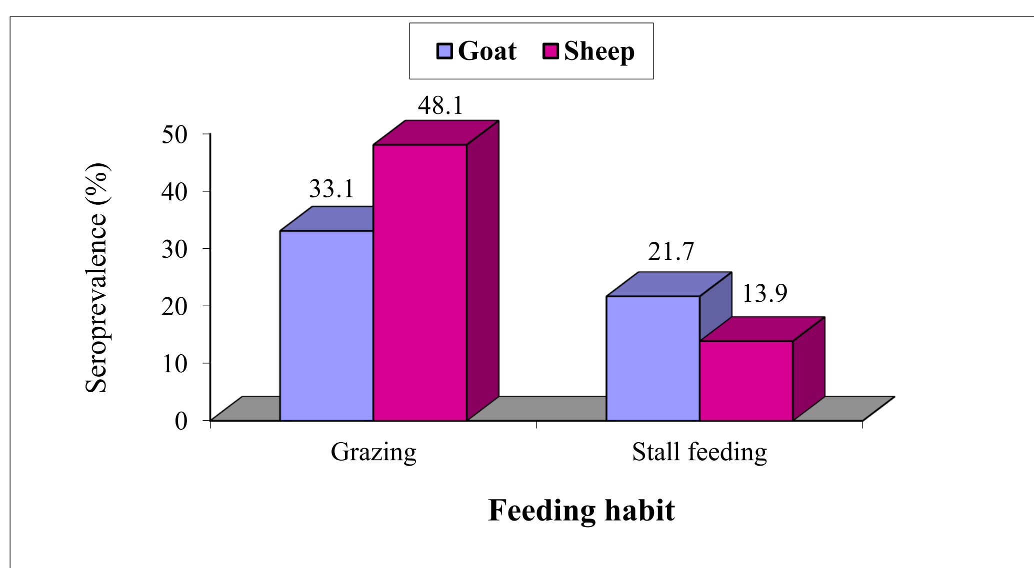


Figure 22: Prevalence of feeding habit on toxoplasmosis in small ruminants.

Pearson's correlation coefficient (r) value was -0.102 and statistically insignificant (*P*>0.05). Thus, the correlation coefficient was a measure of linear (with a negative slope) association between *Toxoplasma* and feeding habit at the functioning area.

4.3.11. Prevalence of toxoplasmosis in relation with season of the year

Seasonal effects in relation to *Toxoplasma* in small ruminants are described under the Table-41 and Figure-23.

Table 41. Result of *Toxoplasma* ELISA in accordance with seasons in small ruminants.

	Result of ELISA test	Season of the Year			
		Summer	Rainy	Winter	Total
Toxoplasmosis	Positive	95 20.0%	92 19.4%	79 16.6%	266 56%
	Negative	75 15.8%	85 17.9%	49 10.3%	209 44%
	Total	170 35.8%	177 37.3%	128 26.9%	475 100%
	Correlation (r)	0.114 ns			

Correlation is significant at the 0.01 level (2-tailed).

**, Correlation is significant at the 0.05 level (2-tailed) & *Non-Significance (ns)

From that table it was defined that the percentages of *Toxoplasma* upbeat case in small ruminants were 20%, 19.4% and 16.6% in summer, rainy and winter seasons of the year. The proportion of *Toxoplasma* in summer rainy and winter were 22.2%, 17.9% & 14.6% and 8.9%, 26.6% & 26.6% in goat and sheep, respectively. Pearson's correlation coefficient (r) value was 0.114 and statistically insignificant. Thus, the correlation coefficient was a measure of linear (with a positive slope) association between *Toxoplasma* and season of the year at the examined area.

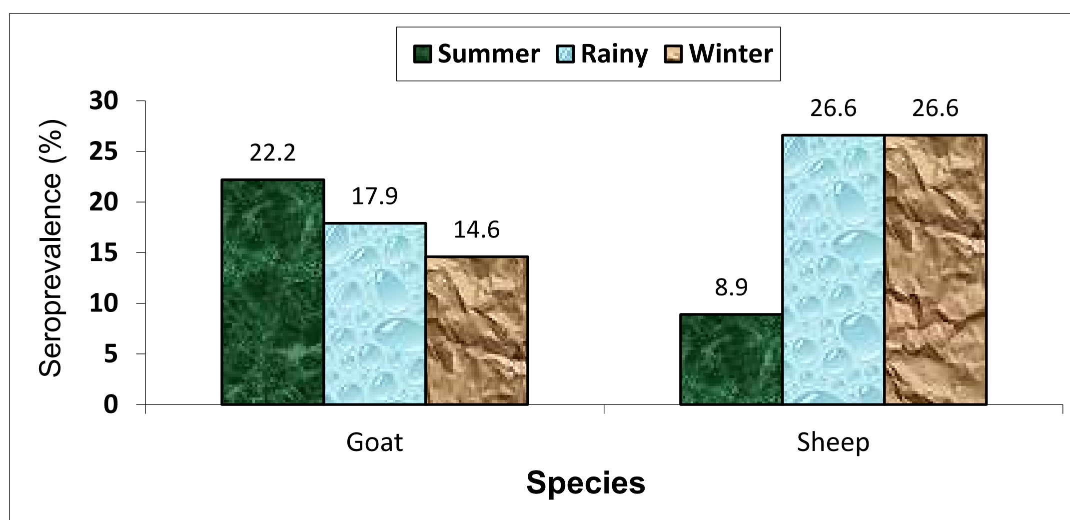


Figure 23. Effect of seasons due to toxoplasmosis in small ruminants.

4.3.12. Prevalence of toxoplasmosis in relation with location of farm

Seroprevalence of toxoplasmosis in relation with location are publicized in Table-42 & Figure-24 at NBT in Bangladesh.

Table 42. *Toxoplasma* Serology consequence relation in location at NBT in Bangladesh

	Result of ELISA test	Location				
		Rajshahi	**Chapai Nawabjong**	**Natore**	**Naogaon**	**Total**
Toxoplasmosis	Positive	147 30.9%	47 9.9%	31 6.5%	41 8.6%	266 56%
	Negative	120 25.3%	33 6.9%	28 5.9%	28 5.9%	209 44%
	Total	267 56.2%	80 16.8%	59 12.4%	69 14.5%	475 100%
	Correlation (r)	0.013 ns				

Correlation is significant at the 0.01 level (2-tailed)
**, Correlation is significant at the 0.05 level (2-tailed)* & Non-Significance (ns)

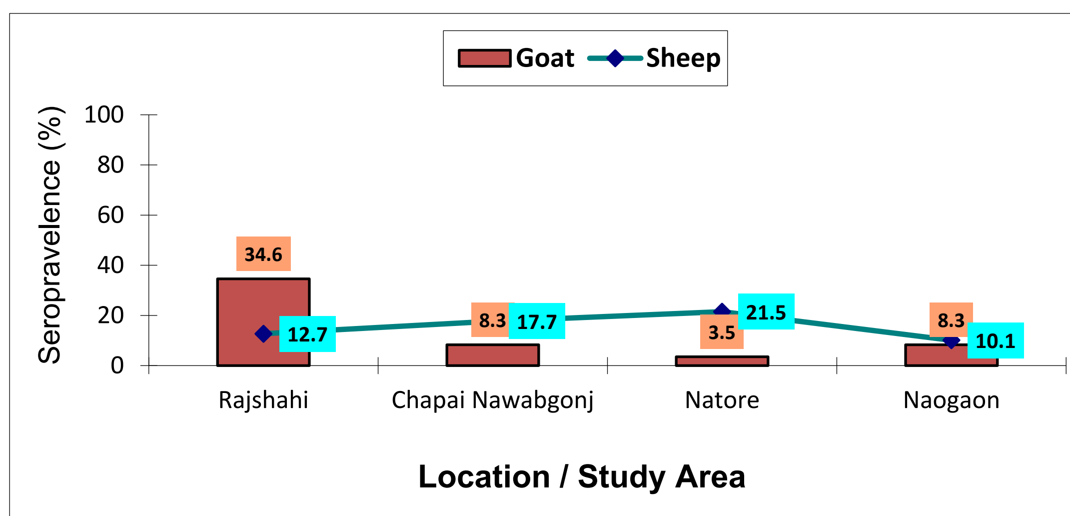


Figure 24. Seroprevalence of toxoplasmosis association with location at NBT.

The positive parts of *Toxoplasma* organism highest to lowest were 30.9%, 9.9%, 8.6% and 6.5% into Rajshahi, Chapai Nawabjong, Naogaon and Natore region in small ruminants. In goat & sheep the part of *Toxoplasma* were 34.6% & 12.7%; 8.3% & 17.7%; 3.3% & 21.5% and 8.3% & 10.1% in Rajshahi, Chapai Nawabjong, Natore and Naogaon districts, correspondingly. Pearson's correlation coefficient (r) value was 0.013 and statiscally insignificant (P>0.05). Thus, the correlation coefficient was a measure of linear (with a positive slope) association between *Toxoplasma* and location at NBT in Bangladesh.

4.3.13. Prevalence of toxoplasmosis in relation with pregnancy status of SR

Prevalence of toxoplasmosis in SR according to pregnancy status is presented in Table-43 and Figure-25. The prevalence of toxoplasmosis in relation with pregnant status of SR recorded as 12.2%, in non-pregnant 87.8%, in pregnant. This calculation showed that higher prevalence recorded in non-pregnant (49.3%) cases than pregnant (9.4%) in small ruminants. Pearson's correlation coefficient (r) value was -0.017 and statistically insignificant (P>0.05). Thus, the correlation coefficient was a measure of linear (with a negative slope) association between *Toxoplasma* and pregnancy status at NBT in Bangladesh.

Table 43. Effect of abortion due to toxoplasmosis on pregnancy status of SR.

	Test Results	Pregnancy status small ruminants		Total
		Pregnant	Non pregnant	
Toxoplasmosis	Positive	26 9.4%	137 49.3%	163 58.6%
	Negative	8 2.9%	107 38.5%	115 41.4%
	Total	34 12.2%	244 87.8%	278 100.0%
	Correlation (r)	-0.017 ns		

Correlation is significant at the 0.01 level (2-tailed).
**, Correlation is significant at the 0.05 level (2-tailed).* and Non Significance (ns)

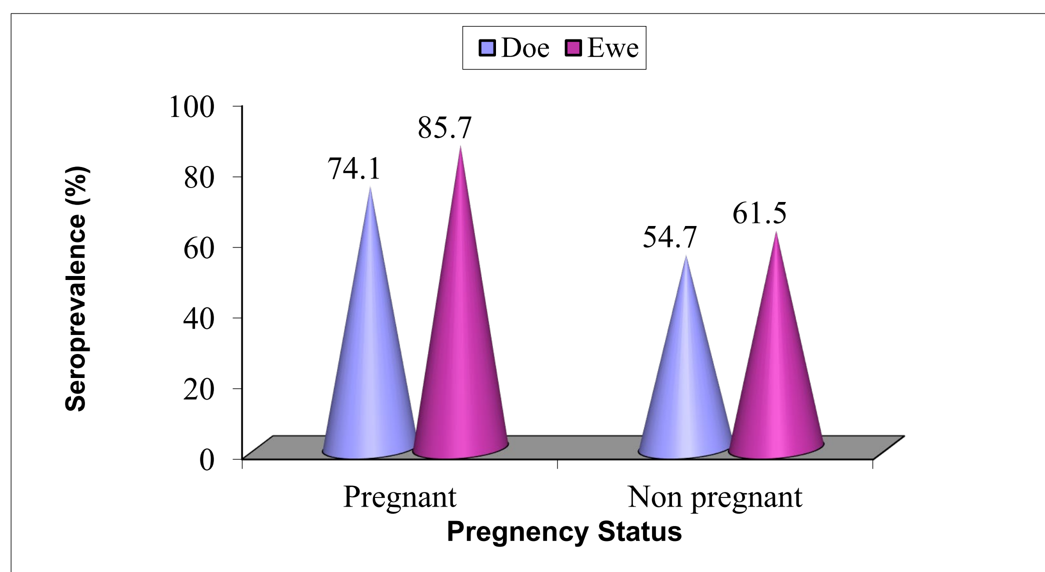


Figure 25. *Toxoplasma* test relation correlation with pregnancy status of doe and ewe.

The Figure 25 investigator observed that the prevalence of *Toxoplasma* in goat and sheep were 74.1% & 85.7% and 54.7% & 61.5% at pregnant and non-pregnant doe and ewe at NBT in Bangladesh.

Table 44. Effect of toxoplasmosis on reproductive diseases in small ruminant.

	Serological test results of toxoplasmosis	**Positive**	**Negative**	**Total**
Reproductive diseases	Abortion	51 10.7%	28 5.9%	79 16.6%
	Still birth	8 1.7%	11 2.3%	19 4.0%
	Retained placenta	24 5.1%	17 3.6%	41 8.6%
	Dystocia	16 3.4%	10 2.1%	26 5.5%
	Cervicitis	17 3.6%	17 3.6%	34 7.2%
	Vaginal prolapse/ Uterine prolapse	9 1.9%	9 1.9%	18 3.8%
	Endometritis/ pyometra	10 2.1%	4 .8%	14 2.9%
	Anoestrus	18 3.8%	14 2.9%	32 6.7%
	Repeatbreeding	10 2.1%	5 1.1%	15 3.2%
	Orchitis	10 2.1%	7 1.5%	17 3.6%
	Epididymitis	32 6.7%	32 6.7%	64 13.5%
	Posthitis	20 4.2%	6 1.3%	26 5.5%
	Urinary tract infection	41 8.6%	49 10.3%	90 18.9%
Total		266 56.0%	209 44.0%	475 100%
Chi-Square value (χ^2)		Calculated value 17.07	Tabulated value 6.304 at 12 DF	Significant ($p<0.15$)

4.3.14. Prevalence of toxoplasmosis in relation with reproductive diseases

The seroprevalence rate of *Toxoplasma* in association with reproductive diseases in small ruminants is explained in Table-44 & 45 and Figure-26. The seropositive rate of *Toxoplasma* in coordination with abortion, still birth,

retained fetal membranes, dystocia, cervicitis, vaginal prolapse/uterine prolapse, metritis/pyometra, anestrus, repeat breedering, orchitis, epididymitis, posthitis, and urinary tract infection were 3.6%, 1.7%, 5.1%, 3.4%, 3.6%, 1.9%, 2.1%, 3.8%, 2.1%, 2.1%, 6.7%, 4.2% & 8.6%, respectively. The equation of (T1) logistic regression analysis is given in Table 44.

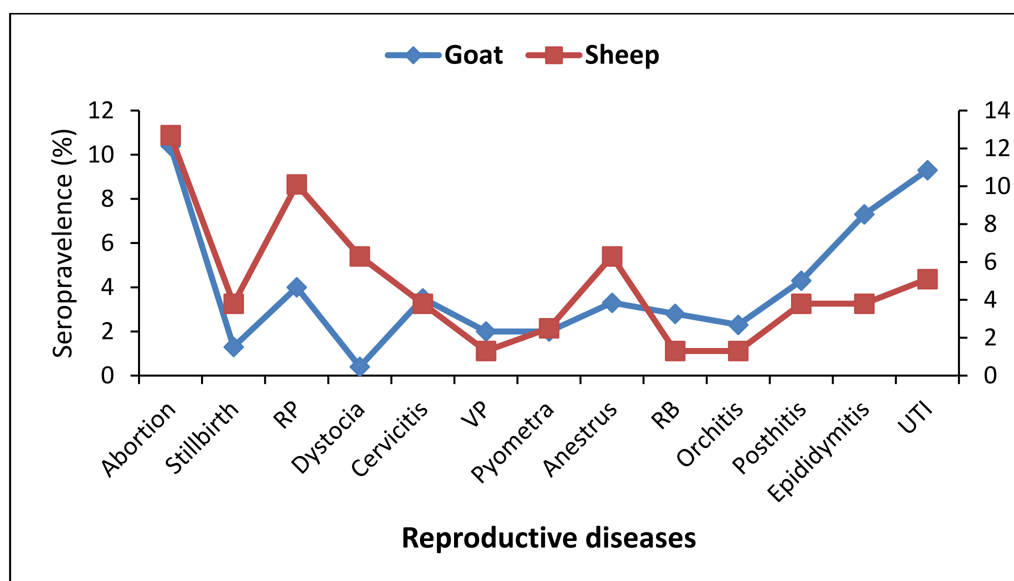
Table 45. Logistic regression estimates of the odds ratios of selected reproductive diseases and serological test (Toxoplasmosis) result in small ruminants at Northern Barind Tract of Bangladesh.

Independent Variables	**B**	**S.E.**	**Exp(B)**
Reproductive diseases of SR			
Abortion	.600	.343	1.821**
Still birth	-.318	.528	.727***
Retained placenta	.345	.404	1.412***
Dystocia	.470	.474	1.600***
Cervicitis	.000	.424	1.000
Vaginal prolapse/ Uterine Prolapse	.000	.534	1.000
Endometritis/ Pyometra	.916	.642	2.500**
Anoestrus	.251	.435	1.286
Repeat breeding	.693	.602	2.000***
Orchitis	.357	.553	1.429
Posthitis	1.204	.528	3.333*
Epididymitis[r]	.000	.250	1.000
Urinary tract infection	-.178	.328	.837

r = Reference Category; B = Logistic Regression Coefficient; and Exp (B) = Odds Ratio‘*’ = Significant at 1% level; ‘**’ = Significant at 5% level; and ‘***’ = Significant at 10% level

The cervicitis, anestrus, vaginal / uterine prolapse, orchitis and urinary tract infection were insignificant (P>0.10). The odds ratios of abortion, still birth, retained fetal membranes, dystocia, cervicitis, vaginal prolapse or uterine prolapse, metritis or pyometra, anestrus, repeat breeders, orchitis, epididymitis,

posthitis, and urinary tract infection were 1.8, 1.0, 1.4, 1.6, 1.0, 1.0, 2.5, 1.2, 2.0, 1.4, 3.3, 0.8 and 1.0 respectively.



RP= Retained Placenta, VP= Vaginal prolapse, RB= Repeat Breeding and UTI= Urinary Tract Infection

Figure 26. Reproductive diseases in relation with *Toxoplasma* in goat and sheep.

It means prevalence rate of *Toxoplasma* in small ruminants' were1, 1.8, 1.4, 1.3, 2, 1.6, 1, 1, 2.5, 1.4, 3.3 and 0.8 time more chance of infection than control group (Epididymeditis).From Figure 24 the prevalence rate of *Toxoplasma* in goat and sheep were 10.4% & 12.7%; 1.3% & 3.8%; 4.0% & 10.1%; 0.4% & 6.3%; 3.5% & 3.8%; 2.0% & 1.3%; 2% & 2.5%; 3.3% & 6.3%; 2.8% & 1.3%; 2.3% & 1.3%; 4.3% & 3.8%; 7.3% & 3.8% and 9.3% & 5.1% in abortion, still birth, retained fetal membranes, dystocia, cervicitis, vaginal prolapse, uterine prolapse, metritis, pyometra, anestrus, repeat breeders, orchitis, epididymitis, posthitis, urolithiasis and urinary tract infection. It means prevalence rate of *Toxoplasma* in small ruminants' were1, 1.8, 1.4, 1.3, 2, 1.6, 1, 1, 2.5, 1.4, 3.3 and 0.8 time more chance of infection than control group (Epididymitis).

4.4. Experiment 4: Survey of Coxiellosis related to reproductive disorders in small ruminants

The 91 serum samples from 71 goats and 20 sheep were tested on ELISA and 20 abomasal samples half from goat and half sheep plus 4 were sheep fetal materials tested by Multiplex Quantitative PCR for Q fever.

4.4.1. Q fever PCR test result

The PCR test results of all the samples were found to be negative and amplification is seen only in positive control (Figure-27). The serum samples test results had discussed under specific headings were as follows:

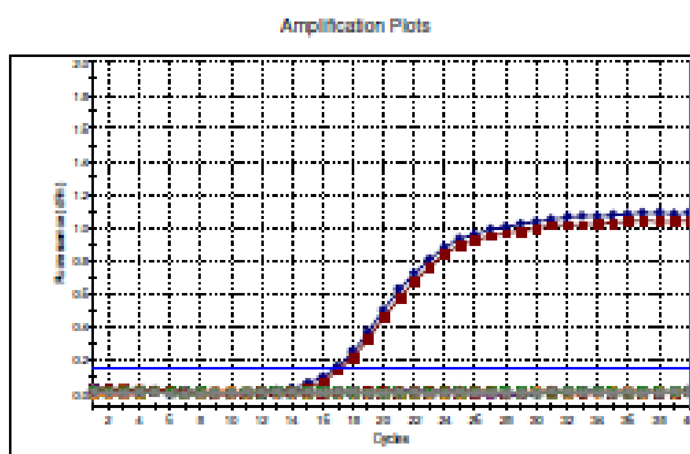


Figure 27. Amplification of Plots in Multiplex Quantative PCR with small ruminants aborted tissue.

4.4.2. Overall seroprevalence of Q fever

Overall sero-prevalence of coxiellosis in small ruminants was 11% through iELISA serology (Table-46 & 47). Final serum test shows that highest sero-prevalence is in sheep followed by goat. The samples test for serology of three diseases and their prevalence 1.1%, 0%, 2.2%, 4.4%, 0%, 3.3% and 0% in sole *Brucella Toxoplasma* & *Coxilla*, common three diseases positive (*Brucella*, *Toxoplasma* & *Coxiella*), *Brucella* & *Toxoplasma, Brucella* & *Coxiella* positive case and totally negative samples uses for srology.

Table 46. Crosstab of serological status of diseases and Q fever ELISA.

	Combination of diseases	Q Fever ELISA		Total
		Negative	Positive	
Serological status of diseases	*Brucella*	17	1	18
		18.7%	1.1%	19.8%
	Toxoplasma	2	0	2
		2.2%	0%	2.2%
	Coxiella	0	2	2
		0%	2.2%	2.2%
	Brucella, *Toxoplasma* and *Coxiella*	0	4	4
		.0%	4.4%	4.4%
	Brucella and *Toxoplasma*	32	0	32
		35.2%	.0%	35.2%
	Brucella and *Coxiella*	0	3	3
		0%	3.3%	3.3%
	Totally negative	30	0	30
		33.0%	0%	33.0%
Total		81	10	91
		89.0%	11%	100.0%

Table 47. Statistical analysis of coxiellosis serology.

Category	Value	Degrees of freedom	Asymp. Sig. (2-sided)
Pearson Chi-Square	81.345[a]	6	$P< 0.0001$
Likelihood Ratio	55.300	6	$P< 0.0001$
Valid Cases (N)	91		

a. 11 cells (78.6%) have expected count less than 5. The minimum expected count is .22.

The investigator asumpt that the null hypothesis (H_0): There was no association between serological status of diseases (*Coxiella*) and influencing factors and an alternative hypothesis (H_1): There was association between serological status of diseases (*Coxiella*) and influencing factors. Now the Pearson chi-square statistics was 81.345 at six degrees of freedom ($P<0.0001$) thus the null hypothesis could be reject. Therefore, researcher could conclude that there was a significance association between serological status of diseases (*Coxiella*) and influencing factors under the study area.

4.4.3. Occurrence of Q fever in relation with species of SR

The seroprevalence of Q fever in relative with species are focus to explain under the Table-48 & Figure-28 (A & B). The positive and negative percentages of *Toxoplasma* were 4.4% & 73.6% in goat and 6.6% & 15.4% in sheep. When compare within species the infection rate was 6% in goat and 30% in sheep.

Pearson's correlation coefficient (r) value was 0.392 and statiscally significant ($P<0.01$). Thus, the correlation coefficient was a measure of linear (with a positive slope) association between Q fever and species under the working area.

Table 48. Seroprevalence of Q fever in small ruminants relation with species.

	Result of ELISA test	Species		
		Goat	Sheep	Small ruminants
Q Fever	Positive	4 4.0%	6 6.1%	10 11%
	Negative	67 73.6%	14 15.4%	81 89.0%
	Total	71 78%	20 22%	91 100%
	Correlation (r)	0.392**		

Correlation is significant at the 0.01 level (2-tailed) **,
Correlation is significant at the 0.05 level (2-tailed)* and Non Significance (ns)

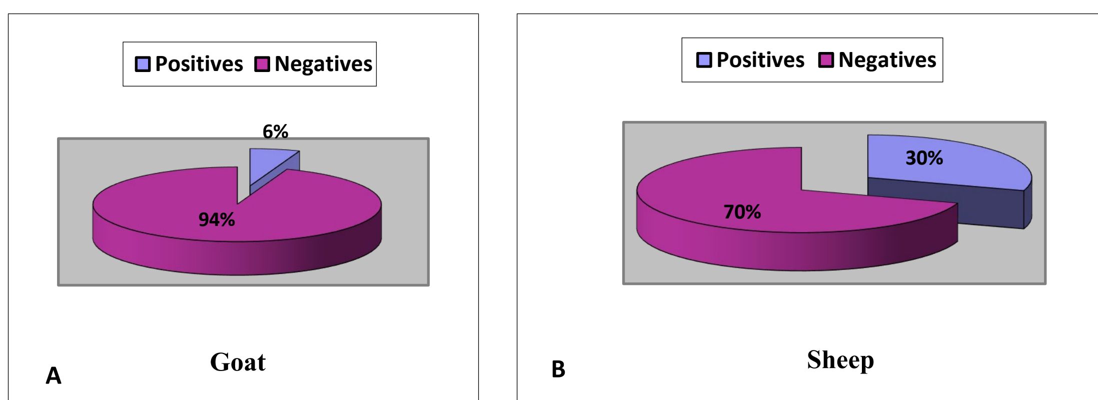


Figure 28 (A & B): Q fever positive and negative ratio in goat and sheep.

4.4.4. Occurrence of Q fever in relation with breed of SR

The breed of small ruminants and Q fever test results are shown in Table 49 and Figure-29. Seropositiveness of the Q fever was 7.7% in Local breed, 3.3% in crossbred. In goat the prevalence was 4.2% & 1.4% in Local and crossbred

Table 49. Breed relation with coxiellosis in SR**.**

	Result of ELISA test	**Breed**		
		Local	**Cross**	**Total**
Q Fever	Positive	7 7.7%	3 3.3%	10 11%
	Negative	41 45.1%	40 44%	81 89%
	Total	48 52.7%	43 47.3%	91 100%
	Correlation (r)	0.105 ns		

Correlation is significant at the 0.01 level (2-tailed) **,
Correlation is significant at the 0.05 level (2-tailed)* and Non Significance (ns)

But in sheep the percentages of Q fever infection was 20.0% & 10.0% in local and cross one, respectively. Pearson's correlation coefficient (r) value was 0.105 and statiscally insignificant (*P*>0.01/0.05). Thus, the correlation coefficient was a measure of linear (with a positive slope) association between Q fever and breed at tested samples of the study area.

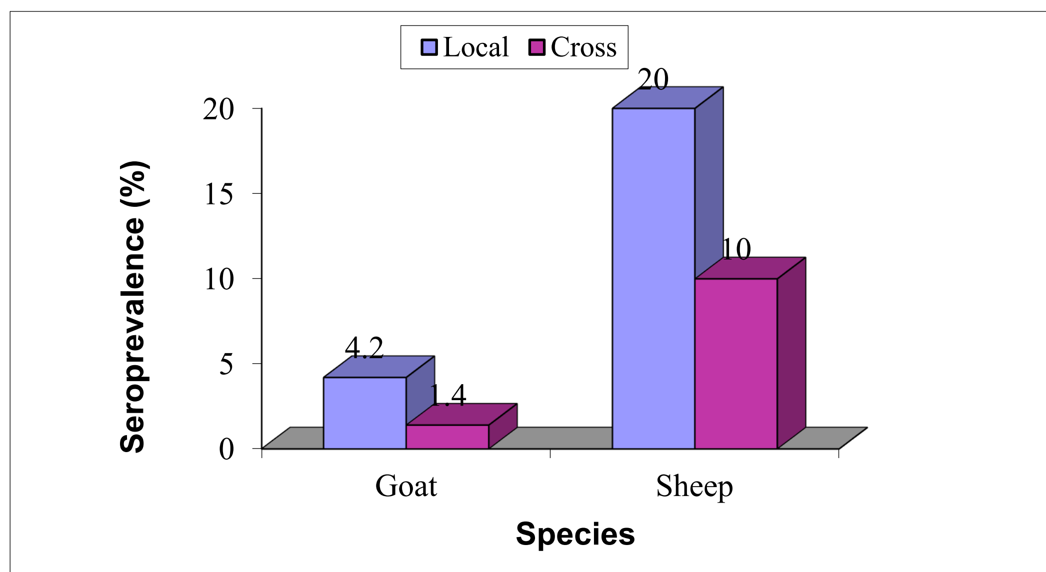


Figure 29. Seroprevalence of Q fever accordance with goat and sheep.

4.4.5. Occurrence of Q fever in relation with sex of SR

The Table-50 and Figure-30 is showed the seroprevalence of Q fever in association with sex of small ruminants.

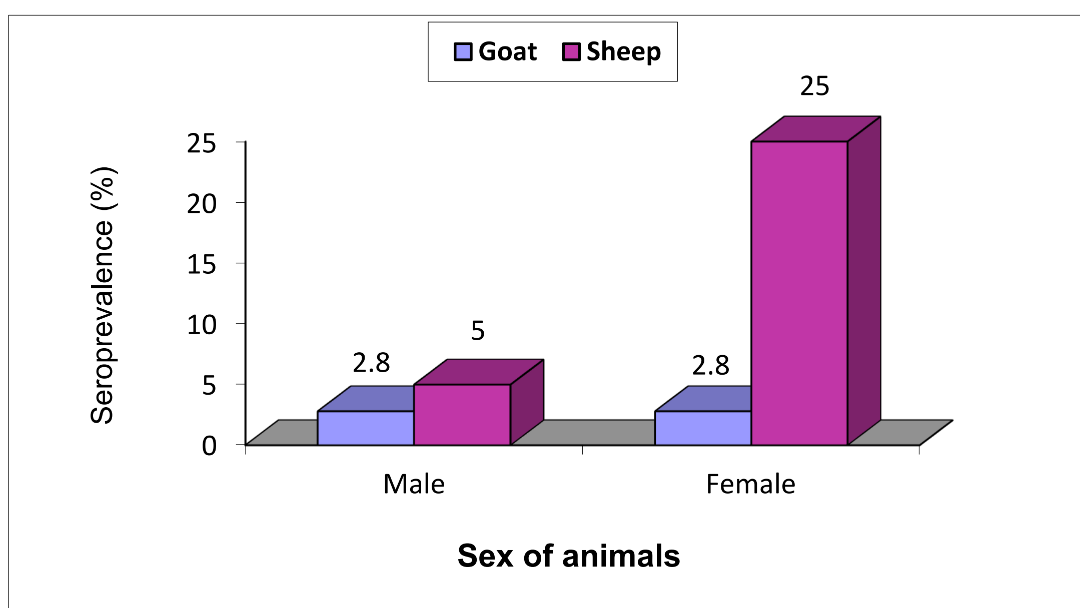


Figure 30. Seroprevalence of Q fever relation with sex of goat and sheep.

Table 50. Seroprevalence due to Q fever on connection with sex of small ruminants.

	Result of ELISA test	Sex		
		Male	Female	Total
Q Fever	Positive	3 2.4%	7 7.6%	10 11%
	Negative	40 44.0%	41 45.1%	81 89.0%
	Total	43 47.3%	48 52.1%	91 100%
	Correlation (r)	0.104 ns		

Correlation is significant at the 0.01 level (2-tailed) **,
Correlation is significant at the 0.05 level (2-tailed)* and Non Significance (ns)

Sex wise prevalence of Q fever revealed that prevalence in male was 3.3%. On the other hands prevalence in female was record as 7.7%. The prevalence of coxiellosis in female ruminants animals was higher than male animals. Statistically, the occurrence of coxiellosis was highly significant (P<0.01) relationship with sex of small ruminants. The prevalence of Q fever in goat founded no difference in female 2.8% i-ELISA than male 2.8%, but in sheep, prevalence rate in female was 25% that was higher than male animals (5%).

Pearson's correlation coefficient (r) value was 0.104 and statiscally insignificant (P>0.01/0.05). Thus, the correlation coefficient was a measure of linear (with a positive slope) association between Q fever and sex under the working area.

4.4.6. Occurrence of Q fever in relation with age of SR

Prevalence of Q fever relation with age is showing the Table-51 and Figure-31.

Table 51. Effect of coxiellosis on age of SR.

Q Fever	Result of ELISA test	Age			Total
		Young	Adult	Old	
Q Fever	Positive	1 1.1%	3 3.3%	6 6.6%	10 11%
	Negative	17 18.7%	34 37.4%	30 33%	81 89.0%
	Total	18 19.8%	37 40.7%	36 39.6%	91 100%
	Correlation (r)	0.057 ns			

Correlation is significant at the 0.01 level (2-tailed) **,
Correlation is significant at the 0.05 level (2-tailed)* and Non-Significance (NS); Young= 0 to < 12 months, Adult= 12 to 24 months & Older= > 24 months

Prevalence of Q fever was lowest 1.1% in young small ruminants and others were 3.3% in adult age and 6.6% in older age. The highest rate of infection in older goat was 4.2%. However, the highest rate in sheep 15% at older sheep than that of others groups. The others infection rate in young and adults age groups were 0% & 1.4% for goat and 5% & 10% for sheep.

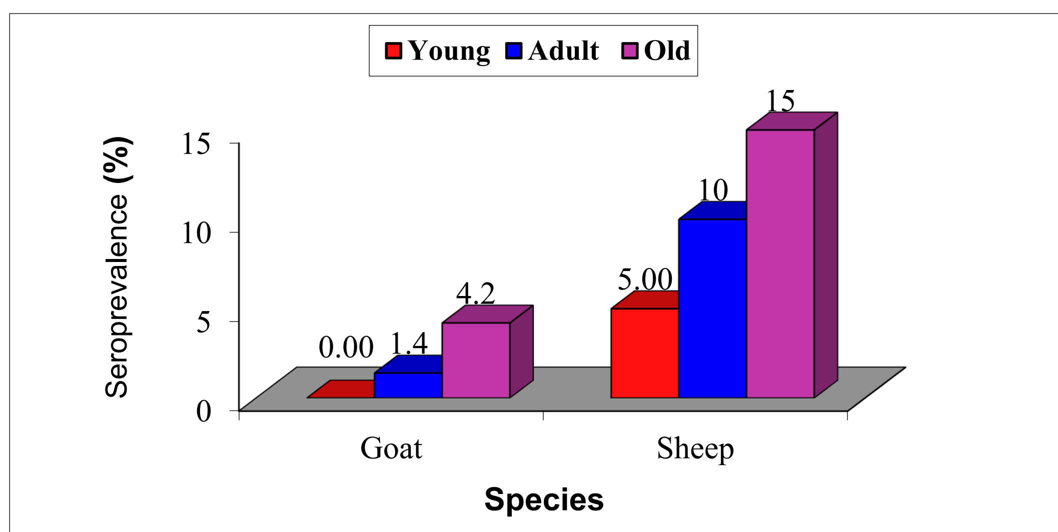


Figure 31. Occurrence of Q fever in goat and sheep on age variation.

Pearson's correlation coefficient (r) value was 0.057 and statistically insignificant (*P*>0.01/0.05). Thus, the correlation coefficient was a measure of linear (with a positive slope) association between Q fever and age of small ruminants under the working area.

4.4.7. Occurrence of Q fever in relation with parity of SR

The parity of goat and sheep and the seroprevalencce of Q fever are shown in the Table-52 & Figure-32.

Table 52. Effect of coxiellosis on parity of SR.

	Test result	**Parity**				**Total**
		1st	**2nd**	**3rd**	**>3rd**	
Q Fever	Positive	2 4.2%	1 2.1%	1 2.1%	3 6.2%	7 14.6%
	Negative	18 37.5%	9 18.8%	9 18.8%	5 10.4%	41 85.4%
	Total	20 41.7%	10 20.8%	10 20.8%	8 16.7%	48 100%
	Correlation (r)	-0.176*				

Correlation is significant at the 0.01 level (2-tailed)**,
Correlation is significant at the 0.05 level (2-tailed)* and Non Significance (ns)

The highest to lower percentage of positive case of Q fever were 6.2%, 4.2%, 2.1% and 2.1% in >3rd, 1st, 2nd, and 3rd parity, respectively. In goat percentage of Q fever were 6.7% only in >3rd parity and others are 0% in 1st, 2nd, 3rd parity, respectively observe. Nevertheless, in sheep the rate were 11.1% in 1st parity and others 2nd, 3rd and >3rd parity was 5.6%.

Pearson's correlation coefficient (r) value was -0.176 and statistically significant (*P*<0.01). Thus, the correlation coefficient was a measure of linear (with a negative slope) association between Q fever and parity under the working area.

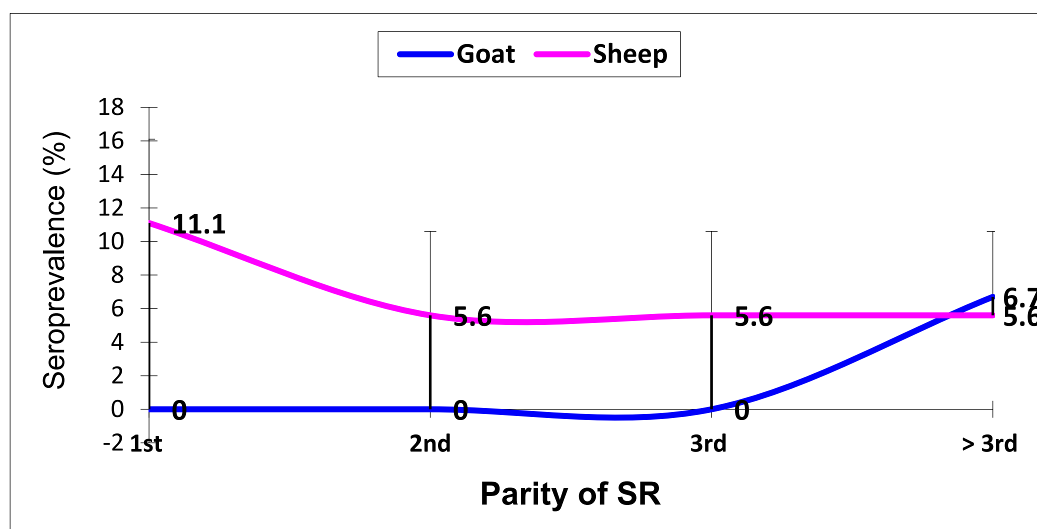


Figure 32. Shows parity of doe and ewe in association with seroprevelance of Q fever in SR.

4.4.8. Occurrence of Q fever in relation with body weight of SR

Seroprevalencce of Q fever in relation with body weight of SR is shown in Table 53 and Figure 33.

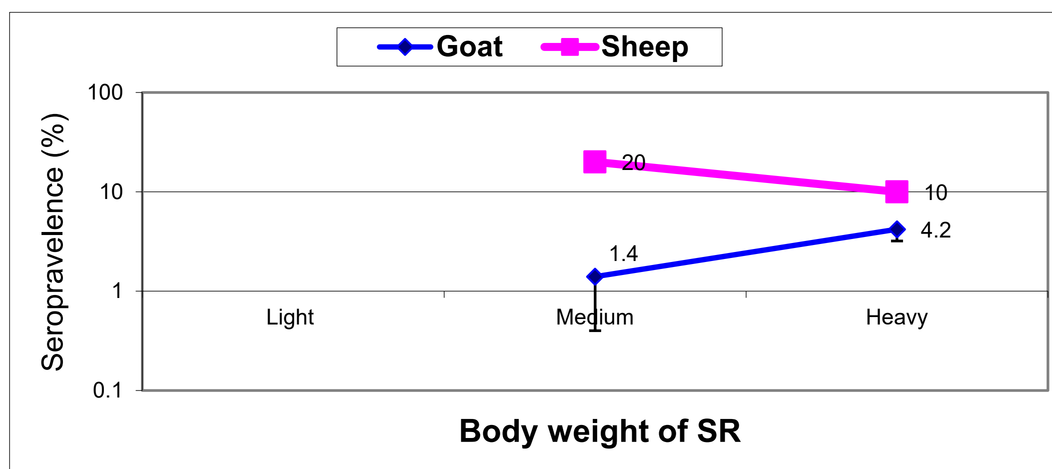


Figure 33. Shows the body weight of animals in relation with Q fever.

The seroprevalence of Q fever relation with body weight was similar trend in medium and heavy both body weight that bear 5.5% than light body weight group, which bear zero value. When seroprevalence of Q fever compare in goat and sheep individually, the percentages were 0 % & 0%, 1.4% & 20% and 4.2% & 10% in light, medium and heavy body weight.

Table 53. Effect of coxiellosis on body weight of small ruminants**.**

	Result of ELISA test	Body weight			
		Light	Medium	Heavy	Total
Q Fever	Positive	0 0%	5 5.5%	5 5.5%	10 11%
	Negative	21 23.1%	38 41.8%	22 24.2%	81 89.0%
	Total	21 23.1%	43 47.3%	27 29.7%	91 100%
	Correlation (r)	0.064 ns			

Correlation is significant at the 0.01 level (2-tailed). ** Correlation is significant at the 0.05 level (2-tailed)*, Non-significant (ns); Light = <10 kg body weight, Medium= 10 to 15kg body weight & Heavy= >15 kg body weight.

Pearson's correlation coefficient (r) value was 0.064 and statiscally significant (*P*>0.01/0.05). Thus, the correlation coefficient was a measure of linear (with a positive slope) association between Q fever and body weight of small ruminants under the working area.

4.4.9. Occurrence of Q fever in relation with flock size

The flock size is shown (Table-54 and Figure-34) the prevalence of Q fever on Seroprevalence of Q fever was height in small size flock (8.8%) small ruminants reared farm. On the other hand, the seroprevalence of Q fever was record relatively lower (1.1%) in medium size flock and (1.1%) in large size flock small ruminants reared farms in Northern Barind Tract. From the Figure 31, the prevalence fraction in goat & sheep were placed 5.6% & 20%; 0% & 5% and 0% & 5% in small, medium and large size flock. Pearson's correlation coefficient (r) value was 0.149 and statistically significant (*P*<0.01). Thus, the correlation coefficient was a measure of linear (with a positive slope) association between Q fever and density of animal population under the working area.

Table 54. Effect of density of animal population with Q fever ELISA results.

	Result of ELISA test	Flock size			Total
		Small	Medium	Large	
Q Fever	Positive	8 5.2%	1 3.1%	1 1.8%	10 11%
	Negative	53 58.2%	16 17.6%	12 13.2%	81 89.0%
	Total	61 67%	17 18.7%	13 14.3%	91 100%
	Correlation (r)	0.149**			

Correlation is significant at the 0.01 level (2-tailed).**,
Correlation is significant at the 0.05 level (2-tailed).* and ns=Non Significance.
Small = <5 animals, Medium = 5 to 10 animals and Large = >10 animals in a flock.

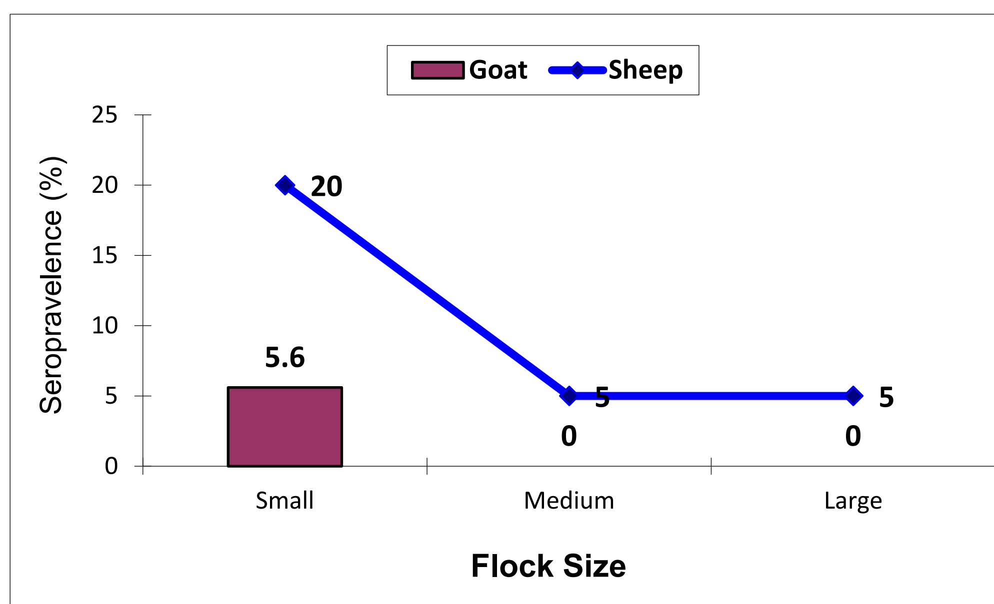


Figure 34. Figure shown the flock size of farms relation with Q fever.

4.4.10. Occurrence of Q fever in relation with biosecurity

The biosecurity of small ruminant accordance with the prevalence of Q fever is summarized on the Table-55 and Figure-35.

Table 55. Effect of Q fever on biosecurity of small ruminants.

	Result of ELISA test	Biosecurity of Farm			
		Traditional	**Moderate**	**Good**	**Total**
Q Fever	Positive	6 6.6%	2 2.2%	2 2.2%	10 11%
	Negative	45 49.5%	14 15.4%	22 24.2%	81 89.0%
	Total	51 56.0%	16 17.6%	24 26.4%	91 100%
	Correlation (r)	0.035 ns			

Correlation is significant at the 0.01 level (2-tailed) **, Correlation is significant at the 0.05 level (2-tailed)* and Non Significance (ns)

Prevalence of Q fever was highest in traditional (6.6%) level of biosecurity maintained farms, lowest (2.2%) in good and moderate level of biosecurity maintained farms. On the other hand, the prevalence of Q fever in goat and sheep demonstrated in Figure-35. The highest prevalence of Q fever incase of goat was in 5.6% in traditional farms than moderate and good both level of bio security farms, there accrue zero percentage of chance of infection. Whereas sheep, it was record that the prevalence were similar and 10% in among three groups of biosecured farms at Northern Barind Tract.

Pearson's correlation coefficient (r) value was 0.035 and statistically insignificant (*P*>0.01/0.05). Thus, the correlation coefficient was a measure of linear (with a positive slope) association between Q fever and bio security level of farms under the working area.

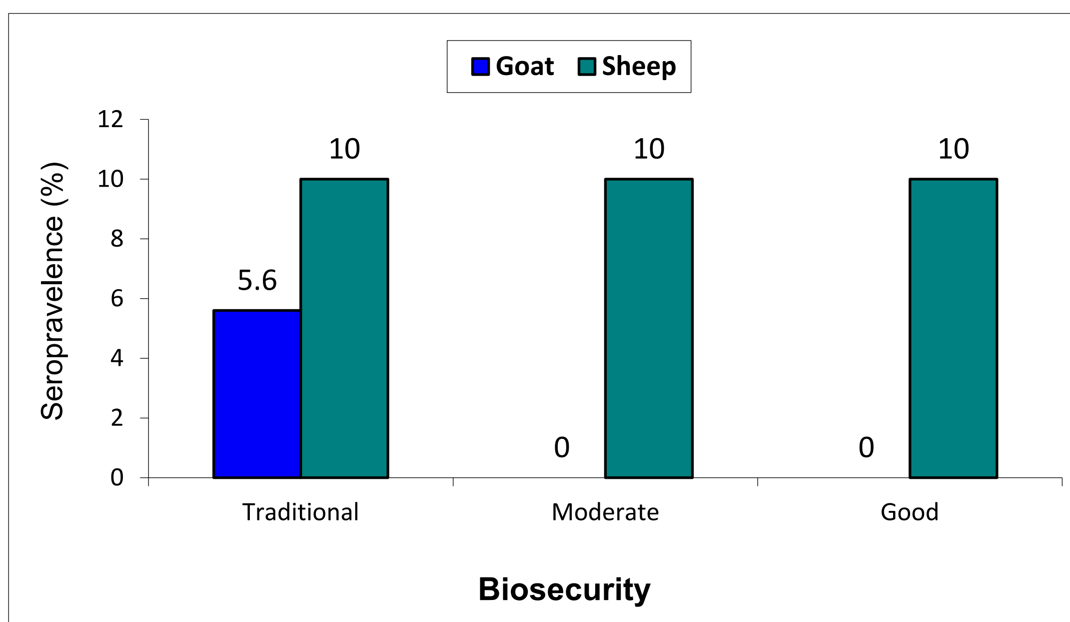


Figure 35. Effect of coxiellosis on biosecurity of small ruminants animals.

4.4.11. Occurrence of Q fever in relation with feeding habit of SR

Feeding habit on Q fever in small ruminants shows in the Table-56 and Figure-36.

Table 56. Feeding habit on Q fever in small ruminants.

	Result of ELISA test	Feeding Habit		
		Grazing	Stall feeding	Total
Q Fever	Positive	6 6.6%	4 4.4%	10 11%
	Negative	54 59.3%	27 29.7%	81 89.0%
	Total	60 65.9%	31 34.1%	91 100%
	Correlation (r)	-0.100 ns		

Correlation is significant at the 0.01 level (2-tailed) **,
Correlation is significant at the 0.05 level (2-tailed)* and Non Significance (ns)

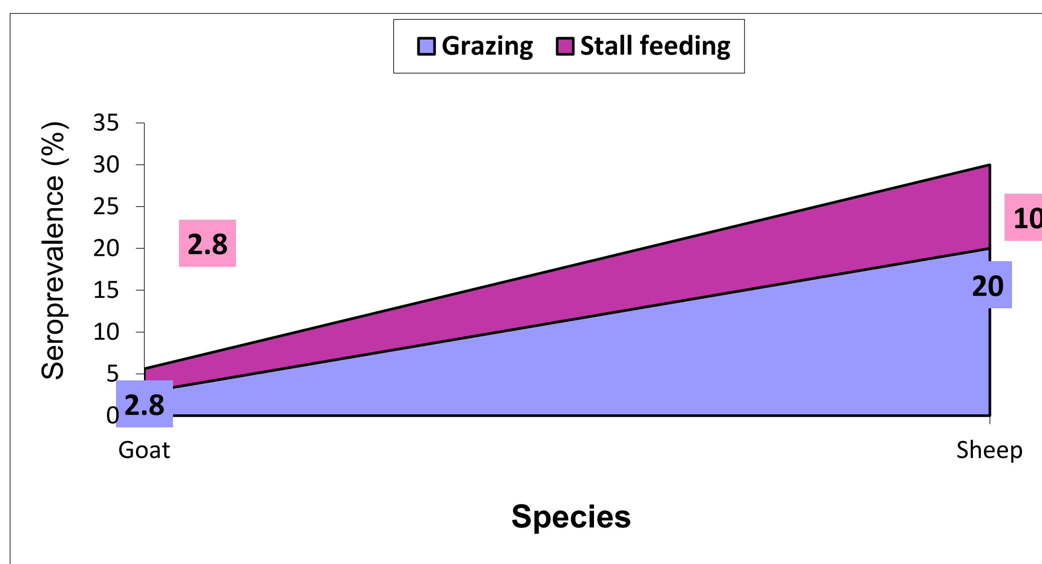


Figure 36. Shows feeding habit of small ruminants and seroprevalence of Q fever.

Prevalence of Q fever was higher (6.6%) in grazing small ruminants than stall-feeding (4.4%). When the grazing results observe in goat & sheep separately, it got 2.8% & 20% and 2.8% & 10% for grazing and stall-feeding habit. Pearson's correlation coefficient (r) value was -0.100 and statiscally insignificant (*P*>0.01/0.05). Thus, the correlation coefficient was a measure of linear (with a negative slope) association between Q fever and feeding habit in experimental area.

4.4.12. Occurrence of Q fever in relation with season of the year

Seasonal effect in relation with Q fever in small ruminants are described on Table-57 and Figure-37. From the Table (57) it was defined that the percentages of *Coxiella* positive case in small ruminants were 1.1%, 7.7% and 2.2% in summer, rainy and winter seasons of the year. The proportion of *Coxiella* in goat and sheep were 0% & 5%; 4.2% & 20% and 1.4% & 5% in summer rainy and winter, respectively. Pearson's correlation coefficient (r) value was 0.075 and statistically insignificant (*P*>0.01/0.05). Thus, the correlation coefficient was a measure of linear (with a positive slope) association between Q fever and seasons at NBT in Bangladesh.

Table 57. Effect of Q fever in small ruminants due to seasonal variation.

	Result of ELISA test	**Season of the Year**			**Total**
		Summer	**Rainy**	**Winter**	
Q Fever	Positive	1 1.1%	7 7.7%	2 2.2%	10 11%
	Negative	29 31.9%	29 31.9%	23 25.3%	81 89.0%
	Total	30 33.0%	36 39.6%	25 27.4%	91 100%
	Correlation (r)	0.075 ns			

Correlation is significant at the 0.01 level (2-tailed) **,
Correlation is significant at the 0.05 level (2-tailed)* and Non Significance (ns)

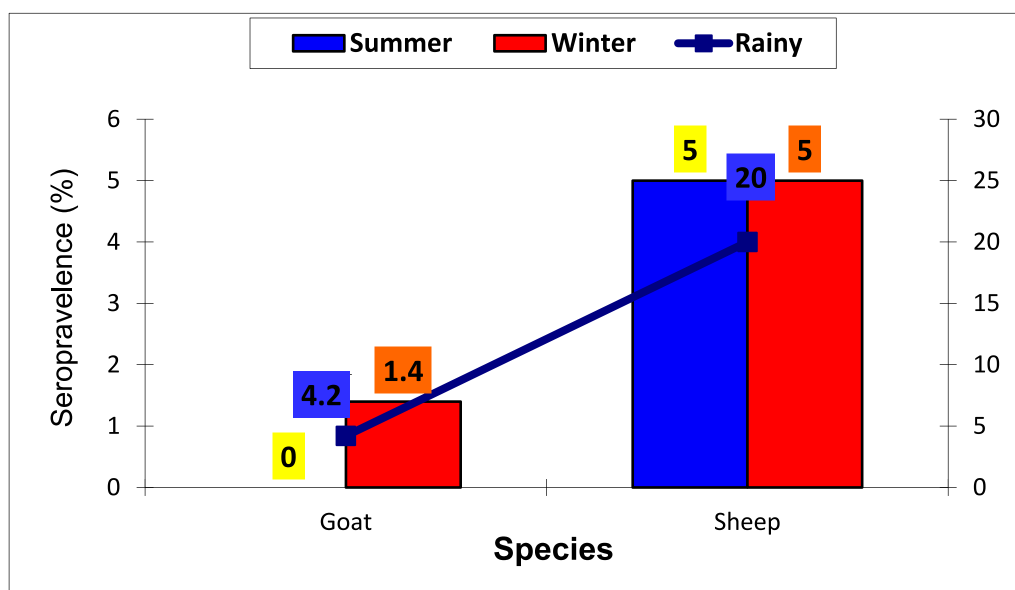


Figure 37. Shows season wise seroprevalence in Q fever at study region.

4.4.13.Occurrence of Q fever in relation with location of the farm

Seroprevalence of Q fever in relation with location is furnished in Table-58 & Figure-37 at NBT in Bangladesh.

Table 58. Effect of location on *Coxiella* at NBT in Bangladesh.

Q Fever	Result of ELISA test	Location				Total
		Rajshahi	Chapai Nawabjong	Natore	Naogaon	
	Positive	3 2.4%	3 2.4%	1 1.8%	3 3.4%	10 11%
	Negative	34 37.4%	15 16.5%	15 16.5%	17 18.7%	81 89.0%
	Total	37 40.7%	18 19.8%	16 17.6%	20 22.0%	91 100%
	Correlation (r)	0.223**				

Correlation is significant at the 0.01 level (2-tailed) **,
Correlation is significant at the 0.05 level (2-tailed)* and Non Significance (ns)

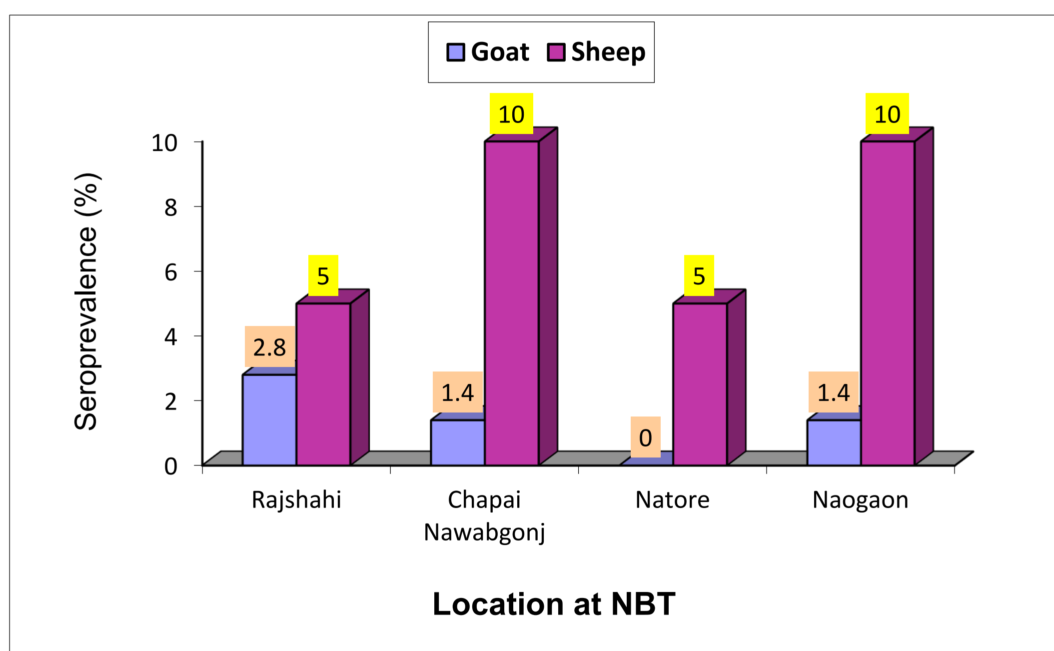


Figure 38. Various regions show the seroprevalence of *Coxiella*.

The upbeat part of *Coxiella* organism were 3.3%, 3.3%, 1.1% and 3.3% into Rajshahi, Chapai Nawabjong, Natore and Naogaon region in small ruminants. In goat & sheep the fraction of Q fever were 2.8%, 1.4%, 0% & 1.4% and 5%,

10%, 5% & 10% in Rajshahi, Chapai Nawabjong, Natore and Naogaon districts. Pearson's correlation coefficient (r) value was 0.223 and statiscally significant ($P<0.01$). Thus, the correlation coefficient was a measure of linear (with a positive slope) association between *Coxiella* and location under the working area.

4.4.14. Occurrence of Q fever in relation with pregnancy ststus of SR

The prevalence of *Coxiella* in SR according to pregnancy status is shown on Table-59 and Figure-39. The prevalence of Q fever in relation with pregnancy status of SR was evaluating as 10.4% in pregnant and 2.1% in non-pregnant small ruminants. Particularly in goat and sheep, it evaluation was 3.3% & 22.2% in pregnant and 0 & 5.6% in non-pregnant group of animals at NBT in Bangladesh.

Table 59. Effect of pregnancy status due to *Coxiella* infection in small ruminants.

	Test Results	Pregnancy status		Total
		Pregnant	Non pregnant	
Q Fever	Positive	5 10.4%	1 2.1%	6 12.5%
	Negative	24 50.0%	18 37.5%	42 87.5%
	Total	29 60.4%	19 39.6%	48 100%
	Correlation (r)	-0.171*		

Correlation is significant at the 0.01 level (2-tailed) **,
Correlation is significant at the 0.05 level (2-tailed)* and Non Significance (NS)

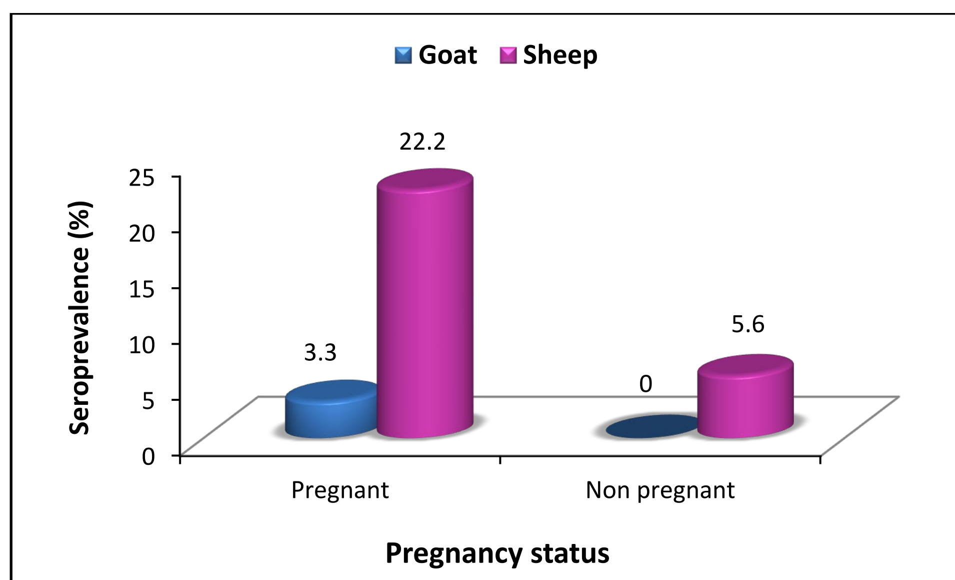


Figure 39. Shows the seroprevelance of coxiellosis relation with pregnancy status of goat and sheep.

Pearson's correlation coefficient (r) value was -0.171 and statistically significant (P<0.05). Thus, the correlation coefficient was a measure of linear (with a negative slope) association between Q fever and pregnancy status under the working area.

4.4.15. Occurrence of Q fever in relation with reproductive diseases of SR

The seroprevalence rates of *Coxiella* in relation with reproductive diseases in small ruminants are explained on Table-60 & 61 and Figure-40. The seropositive rate of Q fever in coordination with abortion, still birth, retained placenta, dystocia, cervicitis, vaginal prolapse or uterine prolapse, endometritis or pyometra, anestrus, repeat breeding, orchitis, posthitis, epididymitis, urinary tract infection and were 2.2%, 1.1%, 1.1%, 1.1%, 0%, 0%, 1.1%, 1.1%, 0%, 0%, 1.1%, 2.2% and 0% respectively. The equation of logistic regression analysis (C1) has given in Table-61. From Table-61 shows that the complete logistic regression model of the test was significant at 12 degrees of freedom at 10% level. That means researcher can reject the null hypothesis and finally comment there was effect of *Coxiella* on reproductive diseases in small

ruminants. In the logistic regression analysis, thirteen RDs of SR at NBT in Bangladesh had taken as indepedendable variable.

Table 60. Effect of *Coxiella* on reproductive diseases of Small ruminants.

Serological test results Q Fever		Positive	Negative	Total
Reproductive diseases	Abortion	2 2.2%	11 12.1%	13 14.3%
	Still birth	1 1.1%	1 1.1%	2 2.2%
	Retained placenta	1 1.1%	8 8.8%	9 9.9%
	Dystocia	1 1.1%	3 3.3%	4 4.4%
	Cervicitis	0 0%	7 7.7%	7 7.7%
	Vaginal prolapse/ Uterine prolapse	0 0%	3 3.3%	3 3.3%
	Endometritis/ pyometra	1 1.1%	1 1.1%	2 2.2%
	Anoestrus	1 1.1%	4 4.4%	5 5.5%
	Repeat breeding	0 0%	3 3.3%	3 3.3%
	Orchitis	0 0 %	3 3.3%	3 3.3%
	Epididymitis	2 2.2%	10 11.0%	12 13.2%
	Posthitis	1 1.1%	3 3.3%	4 4.4%
	urinary tract infection	0 0 %	24 26.4%	24 26.4%
	Total	10 11%	81 89.0%	91 100%
Chi square test	Calculated value 47.278*	Tabulated value 26.217	Degrees of Freedom 12	Significant (p<0.01)

The thirteen-indepedendable variable were abortion, still birth, retained fetal membranes, dystocia, vaginal prolapse, uterine prolapse, metritis, pyometra, anestrus, repeat breeders, orchitis, epididymitis, posthitis, urolithiasis and

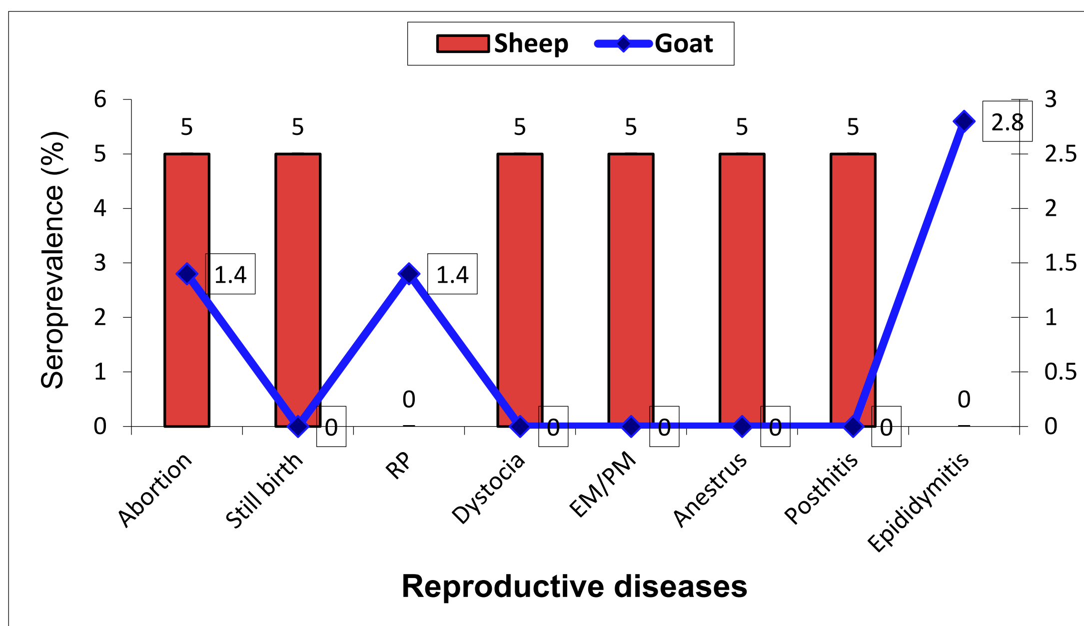
urinary tract infection and control group, respectively. To the analysis, control was assuming the reference category.

Table 61. Logistic Regression Estimates of the Odds Ratios of Selected Reproductive diseases and Serological test (Q fever) results in SR.

Independent Variables	B	S.E.	Exp(B)
Reproductive diseases of SR			
Abortion	1.315	1.091	3.725***
Still birth	2.385	1.612	10.857*
Retained placenta	1.728	1.313	5.630**
Dystocia	.693	1.390	2.000**
Cervicitis	2.028	1.519	7.600**
Vaginal prolapse/ Uterine prolapse	1.240	2.321	3.455**
Endometritis/ Pyometra	2.251	1.612	9.500**
Anoestrus	1.979	1.360	7.238*
Repeat breeding	2.134	2.321	8.444*
Orchitis	2.251	2.321	9.500*
Posthitis	2.028	2.321	7.600***
Urinary tract infection	1.903	1.390	6.706***
Epididymitis^{r}	-	-	1.000
Constant	-3.638	0.775	.026

r = Reference Category; B = Logistic Regression Coefficient; and Exp(B) = Odds Ratio; '*' = Significant at 1% level; '**' = Significant at 5% level; and '***' = Significant at 10% level

The anoestrus, repeat breeding, still birth and orcitis of logistic regression coefficients were found to be positive and statistically significant ($P<0.01$), cervicitis, retained placenta, dystocia, vaginal prolapse/ uterine prolapse & pyometra significant at ($P<0.05$) and abortion, posthitis and urinary tract infection are significant at (P<0.10) level.



RP= Retained Placenta, EM= Endometritis, PM= Pyumetra

Figure 40. Shows the prevalence of Q fever in goat and sheep individually with history of reproductive problems**.**

The odds ratios of abortion, still birth, retained fetal membranes, dystocia, cervicitis, vaginal prolapse, uterine prolapse, metritis, pyometra, anestrus, repeat breeders, orchitis, epididymitis, posthitis, urolithiasis and urinary tract infection were 3.7, 11.0, 5.6, 2.0, 7.6, 3.5, 9.5, 7.2, 8.44, 9.5, 7.6 and 6.7 respectively. It means prevalence rate of *Coxiella* in small ruminants were 3.7, 11.0, 5.6, 2.0, 7.6, 3.5, 9.5, 7.2, 8.44, 9.5, 7.6 and 6.7 time more chance of infection than control group (Epididymitis).

Prevalence of Q fever in goat and sheep individually with history of reproductive problems is showing in Figure-40. The prevalence of reproductive problems in goat & sheep were abortion (1.4% & 5%), still birth, (0% & 5%), retained placenta (1.4% & 0%), dystocia (0% & 5 %), endometritis/ pyometra (0% & 5%), anestrous (0% & 5.0 %), posthitis (0% & 5%) and epididymitis (2.8% & 0%), respectively.

Chapter 5

DISCUSSION

Epidemiological Investigation of Brucellosis, Toxoplasmosis and Coxiellosis associated with reproductive disorders in small ruminants have been discussed under separate experiment in related with their various factors were as given below.

The retrospective study provided preliminary information on the occurrence and pattern of the reproductive disorders prevalent at the Northern Barind tract in Bangladesh. The frequency of reproductive disorders randomly distributed among the species. However, there were more cases affecting small (goat and sheep). This can be explained since small ruminants are in absolute terms, more numerous and it is relatively cheaper to rear small ruminants within the Barind area (urban area) where they are simply allowed to roam freely and scavenge for food. Sera samples had collected from 475 small ruminants from NBT including the districts Rajshahi, Natore, Naogaon and Chapai Nawabjong.

5.1. Experiment 1: Retrospective study of reproductive diseases in small ruminants

The influencing factors in relation with retrospective study of reproductive diseases in small ruminants at NBT in Bangladesh were as follows:

5.1.1. Diseases in small ruminants

In the study it was found that small ruminants were significantly suffering from various diseases and disorders, where maximum 816 (30.6%) were affected by infectious diseases and minimum 18 (0.7%) connection with poison. The other number of diseases involved in small were highest to lowest rate 444 (16.6%), 354(13.3%), 345 (12.9%), 231 (8.7%), 162 (6.1%),102 (3.8%), 84 (3.1%),72 (2.7%) and 39 (1.5%) were in gastrointestinal, respiratory, surgical, female reproductive disorder, deficiency syndrome,

Integumentary involvement, diseases of sense organ, musculoskeletal system and disease of male sex organ, respectively. The relationship between nutritional / management factors, poor feeding and present in infectious agent in the environment would be the second position of abortion. Twining poor feeding and management as a cause of dystocia (Arthur *et al*., 1998; Dryendahl *et al*., 1977; Bendixen *et al*., 1987 & Islam *et al*., 2013) might be associated with the relatively high incidence of dystocia and retained placenta observed in the study.

5.1.2. Species

The diseases and reproductive disorders observed in the different systems of small ruminants was discussed and total 2667 cases were presented to the study, and of these, 270 (10.2%) were reproductive case. The percentage distribution of diseases according to species shows goat with the highest number of cases 2394 (89.8%) then sheep 273 (10.2%).

5.1.3. Sex

The overall incidence rate of reproductive disorder was 10.2%, where 8.7% in female and 1.5% in male sex involvement. The incidence rate (highest to lowest) of reproductive disorders involvement with relation to sex were 32.8%, 15.8%, 10.2%, 7.9%, 6.8%, 5.7%, 4.5% in anoestrus, abortion, dystocia, retained placenta, cervicitis, mastitis and repeat breeding in case of female, but in male it was 5.7%, 5.3%, 2.6 and 1.9% in urinary tract infection, ureolithiasis, posthitis and orchitis, respectively. The occurrence of diseases was predominate to be more in female than male (Wazari *et al*., 2006) due to the presence of higher number of female animals in the study area (Ali *et al*., 2011). This is consistent with the fact that females are kept for longer periods in the herd for breeding or milk supply than the males. Also, the peculiarity of the female's vulnerability could be a contributing factor and observed high frequency of anoestrus.

5.1.4. Age

The study also revealed that the reproductive disorders of small ruminants were varied with aged. The incidence rate of abortion, retained placenta, dystocia, anestrus, repeat breeding, cervicitis, mastitis, orchitis, posthitis, urolithiasis and urinary tract infection were 8.7%, 5.7%, 4.5%, 20.4%, 1.1%, 5.7%, 2.3%, 1.9%, 4.2%, 1.9% & 1.9% in young; 5.7%, 2.3%, 1.1%, 7.9%, 2.3%, 1.1%, 2.3%, 0.8%, 0%, 3.4% & 1.5%, and in adult 1.5%, 2.3%, 2.3%, 4.5%, 1.1%, 0%, 1.1%, 0%, 0%, 0% & 0.8% in older respectively.

5.1.5. Seasons

The effect of seasons with the reproductive disorders of small in case of abortion, retained placenta, dystocia, anestrus, repeat breeding, cervicitis, mastitis, orchitis, posthitis, urolithiasis and urinary tract infection were 3.4%, 1.1%, 2.3%, 9.1%, 0.0%, 1.1%, 3.4%, 1.9%, 0.8% 1.9% & 2.3% in summer; 6.8%, 6.8%, 5.7%, 9.1%, 1.1%, 3.4%, 2.3%, 0.0%, 1.1%, 1.5% & 2.6% in rainy and 5.7%, 2.3%, 0%, 14.7%, 3.4%, 2.3%, 0%, 0.8%, 0%, 1.9% & 1.5% in winter seasons, respectively. Similarly the high incidence of urinary tract infection in male would be associated with dry weather of the region and neglected of the infection. Similarly the general frequency of the disease was randomly distributed throughout the year, but relatively more cases were encountered during the month of July to October (rainy season) followed by month of November to February (winter season) than the month of March to June (dry or summer season) which partially agree with Khair *et al.* (2013) and disagree with Waziri *et al*. (2006) The rainy season lies highest frequency may be due to the lack or scarcity of fodder and lack of grazing.

5.2 Experiment 2: Investigation on Brucellosis associated with reproductive disorders in small ruminants

Seroprevalence for *Brucella* exposure was essential for its control and many countries have eradication program to control brucellosis. Economic losses can be heavy due to abortion, infertility, and subsequent culling, so that the herd would monitor for the presence of infection. Despite eradication programs, vaccination, testing and slaughter out, brucellosis remains a major zoonosis worldwide (WHO, 1986; Kakoma *et al*., 2003; Baek *et al*., 2003) and the disease had remained prevalent in many areas in the world. Each year half of a million cases of brucellosis reported worldwide but according to WHO (1986), these numbers greatly under estimated. In recent years, many countries have eradicated brucellosis from their herd, and many other countries had significantly reduced the prevalence of the infection among their livestock populations. Even so, brucellosis was distributing throughout the world wherever livestock could reared. Likewise, in many, less developed countries and in developing countries, brucellosis continues to cause major losses in livestock and poses a serious threat to people (Crawford *et al*., 1990). The distribution of the disease was geographically limited, but it remains a major problem in parts of Africa and Latin America, Western and Southern Asia including Bangladesh.

The seroprevalence of brucellosis in small ruminants studied 475 serum samples were subjected to RBPT test and the positive samples again conform by I-ELISA. The positive samples were discuss on the basis of influencing factors were species, breed, sex, age, parity, body weight, flock size, biosecurity, feeding habit, seasons, location, pregnancy status and reproductive disease involvement in small ruminants.

5.2.1. Overall seroprevalence

Overall seroprevalence of brucellosis in small ruminants was 12.0% through I-ELISA. Seroprevalence of brucellosis in relation with other tested disease either single and / or combination of *Brucella*, *Toxoplasma* and *Coxiella* were

2.1%, 0%, 2.3%, 5.5%, 0%, and 0% sole *Brucella* possitive, *Toxoplasma* positive, common three diseases positive, *Brucella* & *Toxoplasma* positive, *Brucella* & *Coxiella*, *Toxoplasma* & *Coxiella* positive case and totally negative samples. In Kenya 6.01% in sheep and goats (Waghela, 1976), Kaoud *et al.* (2010) observed 26.66%, 18.88% and 17.22%in cattle, goat and sheep, respectively.

5.2.2. Species

The goat passage 9.3% and sheep 2.7% of *Brucella* positives cases, but within species the percentages were 11.1% and 16.5% at Northern Barind Tract in Bangladesh. Brucellosis also reported in ruminants from different parts of the world. The prevalence brucellosis were 1.7% in sheep and 1.5% in goats Sudan (Abdalla, 1966); 2.8% to 5.29% in goats and 7.2% in sheep in Somalia (Falade, 1997); 3.8% in goats and 1.4% in sheep in Eritrea (Omer, 2000); 4% in goats and 1% in sheep in eastern Sudan (El - Ansary, 2001); 6.6% in sheep and 4.75% in goats in Nigeria. From 255 sheep and 289 goats slaughtered at an abattoir of New Delhi India, brucellosis diagnosed in 9.02%, 4.31%, 27.45% & 10.95% sheep and 1.73%, 1.38%, 7.27% & 18.34% in goats using RBPT, Standard Tube Agglutination Test (STAT), Complement Fixation Test (CFT) and dot - ELISA, respectively.

Bangladesh, brucellosis was reported cattle in 1967 (Mia and Islam, 1967), buffalo in 1997 (Rahman *et al.,* 1997). Bangladesh also human brucellosis was first reported in 1983 (Rahman *et al.,* 1983) as well as a very few study in sheep and goat (Rahman *et al.,* 1978; Rahman and Rahman, 1981; 1984; Rahman *et al.,* 1983; Amin *et al*., 2005; Rahman *et al*., 2006; Uddin and Rahman, 2007; Ahasan, 2009). The numbers of positive reactors or suspected by FPA was 2 out of 135 (1.48%) in buffaloes, 0 out of 465 (0.00%) in cattle, 5 out of 230 (2.17%) in case of goats, 12 out of 170 (7.06%) in sheep. Rahman *et al*. (2006) reported an animal-level seroprevalence of brucellosis in cattle, 2.4-18.4% while the herd occurrence in cattle was 62.5%. In case of goats, the prevalence was 3.15% by i-ELISA, which was higher than 1.98%, reported by

Ahasan *et al*., (2010) and 2.33% reported by Uddin and Rahman (2007), but it is lower than that of Rahman *et al*., (1988) who reported 14.57% positive cases of brucellosis in caprine in different areas of Bangladesh. In Bangladesh, there are sample opportunities for intermixing of species, grazing lands and composite smallholdings of livestock maintained by nearly 80% of the rural population. Recent study (Rahman *et al*., 2011) reported that among all the livestock species in Bangladesh, overall serological prevalence was 2.87% in buffaloes; 2.66% in cattle, 3.15% in goats and 2.31% in sheep, beside goats were found predominantly infected with brucellosis. In contrary, in this study, researcher found the evidence of exposure to *Brucella spp*. was relatively high in sheep (8.24%). The prevalence and severity of the disease may vary with breed, geographic location, and type of diagnostic test, husbandry and environmental factor (Amin *et al*., 2005).

5.2.3. Breed

The breed had direct relation with brucellosis. In this research it had negatives correlation and statically not significant. Sero*positiveness* of the diseases got 6.9% in local breed and 5.1% in crossbred. In goat the local breed found more infection than cross and exotic one, but sheep reverse the result (Islam *et al*., 1983), that was cross possaged highest than local breed. This was due to herdleness nature of sheep. Bandey *et al.* (1989) performed a sero-epidemiological study on brucellosis in exotic (Merino) sheep in Kashmir valley found that 258 (3.2%) were positive. Corbel & Brinley-Morgan (1984) also foud sheep milking breeds appear more susceptible than those kept for meat production.

5.2.4. Sex

In this study, a significant involvement ($P<0.05$) was observed in sero-prevalence of brucellosis on the basis of sex in small ruminants which disagreed to the results previously reported that the prevalence of brucellosis appeared not to be associated with sex and disease prevalence was as frequent in males as in females (Akbarmehr and Ghiyamirad, 2011; Asmare *et al.,*

2013). In case of caprine and ovine brucellosis, prevalence was higher in females as compared to males and had also been expressed by Khan *et al.* (2009), Omer *et al.* (2010), Junaidu *et al.* (2011); Rahman *et al*. (2012) and Ogundipe *et al.* (1994). However, these results contradict with previous reports, where it was stated that prevalence in males was significantly higher than female stated by Rahman *et al.* (2011) and Gul *et al.* (2014).

5.2.5. Age

An age knew as one of the intrinsic influencing factors the sero- positivity of brucellosis (Megersa *et al.,* 2011 & Bekele *et al.,* 2011). The current experiment existed a positive association and not significant between breed of small ruminants and the prevalence of *Brucella.* The rate of infestation increased with the age of small ruminants, similar trand certified by Sanogo *et al,* (2012); Gebretsadik-Berhe *et al*. (2007); Amin *et al*. (2004); Andrewartha and Elliott (1990). Younger animals tend to be more resistant to *Brucella* infections; however, latent infections can occur in these animals also expert openion by Gul *et al.* (2013). Older goat and sheep was higher percentages 5.6 and 8.9 similar result was found by Rahman *et al.* (2011 & 2012) and pragmatic the seroprevalence of brucellosis in goats of less than 24 -month of age was 1.52% (3 out of 197). However, in goats over 24 month of age, the prevalence of brucellosis was 9.09% (3 out of 33). In case of sheep of over 24 month, the prevalence of brucellosis was 35.0% (14 out of 33).

5.2.6. Parity

An insignificant difference (*P*>0.05) was observed in sero-prevalence of brucellosis based upon parity through ELISA in small ruminants. The highest percentages of *Brucella* infection obtained 7.6%, 5.5% and 15.7% first parity in small ruminants, goat and sheep, respectively. The lowest rate were in 1.8% and 1.7% in >3rd parity of small ruminants and goat, but in sheep it was1.7% in second, third and more than third parity. Sero-prevalence based upon parity had increased in animals with increasing parity. It was due to the reasons that

prevalence of brucellosis increased with repeated exposure to parturition and other physiological stresses during gestation (Matope *et al.,* 2011; Hadush *et al.,* 2013; Gul St *et al*., 2014). Some researchers disagreed and they got a statistically significant increase of brucellosis was recorded with increasing age (P<0.01) but not parity (Gebretsadik-Berhe *et al*., 2007).

5.2.7. Body weight

In relation to body weight, seroprevalence of brucellosis in small ruminants highest and lowest frequency was 6.1% & 2.1% at heavy (>15Kg) and light (< 10 Kg) body weight and others is 3.8% in medium (10 to <15 Kg) body weight animals. Similarly, the highest and lowest value in case of goat and sheep are 5.3% & 10.1%, 2.5% & 1.3% in medium and light body weight animals, respectively. This might be because the body weight of mature animals was higher as compared to immature or younger kids and lamb, so the prevalence was higher in these animals. Mahboub *et al.* (2013) exposed that effects of brucellosis on animal performance had revealed that young goat and sheep the Mean±SE were 2.00±0.45 & 0.67±0.45 had significantly reduced with *Brucella meletensis* compared to adult and their Mean±SE (50.00±3.61 & 31.33±3.78). Gul *et al.* (2014) trial the difference in seroprevalence of brucellosis in goats and sheep, the difference between two groups was significant only through RBT and prevalence was higher in animals having higher body weights.

5.2.8. Flock size

Prevalence of brucellosis was height (8.2%) in small density (<5 heads) flock of animals population, similar results was observed by Mahboub *et al.* (2013). The result partially approved and Gebretsadik-Berhe *et al*. (2007) carried out an epidemiological study on brucellosis. Significant (*P*<0.001) increment of seropositivity was also observed as herd size increased from small to medium (*P*<0.05) and then to large sizes. On the other hand, the prevalence of brucellosis was recorded in SR animals relatively lower (2.1%) in medium (5 to 10 head) flock, large (>10 heads) density populated flock was 1.7%.

Statistically, there existed a no significant (*P*>0.001) association between flock size of SR and the prevalence of brucellosis. Practically, when compared with the goat highest and lowest prevalence were 8.8% and 0.5% in small and large flock. However, in case of sheep, it was reverse the highest and lowest rate were 7.6% in large flock and 1.8% in medium size flock. The others researchers practically certify the observation (Adugna *et al.,* 2013) showed an univariable logistic regression analysis of the putative risk factors showed statistically significant (*P* < 0.05) difference on *Brucella* reactivity between small ruminants with small and large size flock. This signifies that brucellosis had significant economic implication in its ability to bring about morbidity at flock level.

5.2.9. Biosecurity

Prevalence of brucellosis was highest in traditional (8.2%) level of biosecurity, lower (1.3%) in moderate biosecurity level and other was 2.5% in good management of biosecurity maintained farms. On the other hand, the prevalence of brucellosis in goat and sheep had demonstrated. The highest, lowest and other prevalence of brucellosis in goat were 8.8%, 1% and 1.3% traditional, good and moderate biosecurity of management farms. Whereas sheep, it was record highest, lowest and other prevalence of brucellosis were 10.1%, 1.3% and 5.1% in good, traditional and moderate management practiced farms. Statistically, there was a significant (*P*<0.05) negative association between biosecurity of small ruminants animals and the prevalence of brucellosis. Ramos *et al*. (2008) observed that 645 serum samples had analyzed by the CFT. A 4.0% frequency was found (26/645) in patients serum and among those 4.1% (23/551) were slaughterhouses employees and 8.1% were rural workers. Of all the total positive samples, there (2.0%) were women and (4.7%) were men, (2.9%) were between the ages of 18 and 30, six (3.4%) were between 31 and 40, and nine (8.0%) were above 41 years of age. Gebretsadik-Berhe *et al*. (2007) carried out an epidemiological study on

brucellosis. A significantly higher seroprevalence founded in animals in the low land than those in the high land agro-climatic zones.

Teshale *et al.* (2006) conducted a seroprevalcnee study of small ruminants in two sheep and goat rearing pastoral regions of Ethiopia, namely Afar and Somali, from November 2004 to April 2005. The study revealed higher prevalence of *Brucella* antibodies (9.7%) in the absence of *Brucella* vaccination. Rajesh *et al.* (2003) reported the oral route, contamination of the udder during milking and contact with aborted fetuses and infected newborn lambs were considering common methods of spread, also the venereal transmission of the disease occur due to infected male or contaminated semen. Infected tissues and contaminated materials must be handling under (biosafty 3) conditions. Transmission could be either by contaminated food, invasion by intact skin, inhalation of aerosols containing the bacteria and aerosol contamination of the conjunctiva.

Casalinuovo *et al.* (1996) tested serologically on 269335 sheep and goats on 7163 farms, 11342 (4.2%) animals, and 924 (12.9%) farms were positive. When materials from serologically positive animals had been examined bacteriologically 40 *Brucella* strain from sheep and goats were isolated.

Rodriguez *et al.* (2001) investigated that brucellosis outbreak, occurred in a slaughterhouse Zaragoza, Spain between 2 December 1998 and 4 May 1999, affecting 28 employees. There were no significant differences in risk factors involving working in a risk area, use of protective measures and presence of cuts and wounds

5.2.10. Feeding habit

Prevalence of brucellosis was higher (8%) in grazing small ruminants than stall-feeding (4%). Statistically, there was significant (P<0.01/0.05) effect on grazing behavior of small ruminants animals and brucellosis. The r-value was -0.052, so that there was a negative relation between dependent and independent variable. When the grazing results observed in goat and sheep

individually, it was 6.6% & 4.5% and 15.2% & 1.3% grazing and stall-feeding value. Goats in grazing systems had higher risk of testing positive to brucellosis compared to goats in zero-grazing systems. Mahboub *et al.* (2013) and Montiel *et al*. (2013) reported that grazing was very popular among small-scale goat farmers, and the only possible way for many farmers to keep a flock because feeding was free. Goats often graze on communal land where other goats and cattle graze too. The pattern of positiveness was alike in Yesuf *et al*. (2011). He conducted a cross-sectional study a total of 800 sheep were sampled from two districts, Kalu and Harbu and he was calculated seroprevalence between sexually immature and sexually mature sheep, between animals kept under extensive and semi-intensive management system, and between animals of the two districts. Higher levels of seroprevalence was observed in sexually mature sheep, in animals kept under extensive management system (Darwish and Benkirane; 2001) and in sheep of Kalu with level of 1.54%, 1.6% and 1.58%, respectively. Kabagambe *et al.* (2001) investigated cross-sectional prevalence and risk factors. For *Brucella* seropositivity in goats in Uganda. The most-important herd-level risk factors identified were use of a hired caretaker as the primary manager of the operation compared to owner/family members, keeping sheep in addition to goats compared to having no sheep, and free browsing, when compared to tethering or zero-grazing. Using the TAT, 10% (141/1446) of the goats tested positive. Omer *et al.* (2000) dis agreed the current observation and he found higher prevalence in intensive system of managements.

5.2.11. Seasons

The highest percentage of *Brucella* positive case in small ruminants was 6.1% rainy seasons of the year. This finding supported to the author Pandey and Desai (1973). He also carried out a study and concluded that the highest prevalence of the disease (13%) founded to be associated with areas having heavy rainfall with moderate temperature. There was negative correlation (r= -0.09) between *Brucella* and season and had (*P*>0.05/ 0.1) significant effect.

5.2.12. Location

The upbeat part of *Brucella* organism was 5.9%, 1.5%, 2.3% and 2.3% into Rajshahi, Chapai Nawabjong, Natore and Naogaon region in small ruminants. *Brucella* and location had possage positive relationship with SR and the r-value was 0.092. Thre was no significant effect on *Brucella* and location. In goat & sheep the fraction of Brucella was 6.3%, 1%, 1.5% & 2.3% & 3.8%, 3.8%, 5.1% and 3.8% in Rajshahi, Chapai Nawabjong, Natore and Naogaon districts. Among four districts, urban area was less chance than others located in rural place. Adugna *et al.* (2013) studied seroprevalence of small ruminants' brucellosis in four districts of Afar National Regional State, Northeast Ethiopia. He agreed with current research findings and observed epidemiology of the disease at individual and herd level show wider spread of the disease in different species of animals. In Afambo and Assayita, districts of zone one, animals kept in confinement around cultivation fields than the other two districts, as the districts largely dominated by agricultural irrigation using Awash River. This might be responsible for the high prevalence in zone one as infection easily transmitted within the entire herd under this management system. Teru and Awura districts were mostly pastoralist settings and dominated by free-range management system. In sheep, the study was fairly agreement with different findings. Shehu *et al*. (1999) reported a prevalence of 6.6% in sheep in Nigeria. However, the findings disagree with that of Yesuf *et al*. (2010) who reported a seroprevalence of 1.5% in south Wollo, Teshale *et al*. (2006) and Ashenafi *et al*. (2007) who reported seoprevalence of 14.6% and 3.2% in Mille and Dalifage districts of Afar region and in Afar region, respectively. Such differences might attribute to methodologies followed by number of animals and geographical and management differences. In other countries, Bale *et al*. (1982) reported 15.9% prevalence in a study conducted in Northern Nigeria. Higher prevalence in goats compared to this finding was reported by Teshale *et al*. (2006) (16.45%), Bale *et al.* (1982) (34.8%) and Ojo *et al*. (2007) (45.75%) in Afar region of Ethiopia, northern Nigeria and

Abeokuta, respectively. However, a lower prevalence of 5.8% reported by Ashenafi *et al*. (2007). The high prevalence and wide distribution were not surprising since small ruminants not vaccinated against brucellosis, coupled with the traditional practice of communal grazing in most part of the region.

5.2.13. Pregnancy status of animals

Prevalence of brucellosis in small ruminants according to pregnancy status had significant positive relationship. The overall prevalence of brucellosis in relation with pregnancy status of SR was recorded 12.6%. However, the prevalence rate in pregnant 4% and in non-pregnant 8.6%. It had showed that higher prevalence record in pregnant (9.3% & 78.1%) cases than non-pregnant (12.5% & 25.6%) in goat and sheep. The researcher Tobias *et al*. (1993) reported that in both domestic and wild hosts *Brucella* colonization of the gravid reproductive tract (Abubakar *et al.,* 2012) can lead to severe placental damage, fetal infection and fetal death.

5.2.14. Reproductive Diseases

The seropossitive rate of *Brucella* in coordination with abortion, still birth, retained placenta, dystocia, cervicitis, vaginal prolapse or uterine prolapse, endometritis or pyometra, anestrus, repeat breeding, orchitis, epididymitis, posthitis and urinary tract infection were 2.1%, 0.4%, 1.7%, 0.6%, 1.9%, 0.6%, 0.4%, 1.1%, 0.6%, 0.2%, 0.4%, 0.2% & 1.7% respectively. The Cervicitis, retained placenta, anestrus of logistic regression coefficients were found to be positive and statistically significant ($P<0.01$), abortion, vaginal prolapse/ uterine prolapsed at ($P<0.05$) and dystocia, orchitis ($P<0.10$) level. The still birth and posthitis was insignificant ($P>0.10$). The odds ratios of abortion, still borth, retained placenta, dystocia, cervicitis, vaginal prolapse or uterine prolapse, endometritis or pyometra, anestrus, repeat breeding, orchitis, epididymitis, posthitis and urinary tract infection were 4.49, 3.65, 7.52, 4.03, 11.1, 6.20, 0.03, 5.74, 7.75, 5.17, 1.0 1.94, and 1.24 respectively. It means affected rate of brucellosis in small ruminants were 4.49, 3.65, 7.52, 4.03,

11.1, 6.20, 0.03, 5.74, 7.75, 5.17, 1.0 1.94 and 1.24 time more chance of infection than control group (Epididymitis). It means affected rate of brucellosis in small ruminants were 11, 4.5, 7.5, 5.55, 4, 3.5, 6, 5, and 2 time more chance of infection than control group (Epididymitis). The highest result observed in abortion (Karaman *et al.,* 1993; Samad, 2001; Ocholi *et al*., 2004; Al-Ani *et al,*.2004; Al-Talafhah *et al.*, 2003) case and lowest in vaginal prolapsed and pyometra in female animals in small ruminants. In male animals, highest rate was epididymitis (Samad, 2001; O'Hara, 1987) and lowest in posthitis and orchitis (Samad, 2001). Chand *et al*. (2002) partially agreed the current research and reported the involvement of *Brucella* in sheep in several cases of epidimyo-orchitis in breeding rams to an organized sheep farm in northern India. Clinical examination of the rams revealed a marked enlargement and pendulous appearance of the scrotum.

5.3. Experiment 3: Assessment of Toxoplasmosis linked with reproductive disorders in small ruminants

Under experiment 3 a total 475 serum samples collected from 2667 questionnaire basis. This 475 sample tested for *Toxoplasma* ELISA. The seroprevalence findings combined and individual's results were discussed under separate headings.

5.3.1. Overall seroprevalence of toxoplasmosis

Toxoplasma was a significance association between serological statuses of diseases. Overall seroprevalence of toxoplasmosis in small ruminants was 56% through ELISA serology. The seroprevalence findings were vary by researchers to researchers. The several researcher observe that the Bekele and Kasali (1989) from Central Ethiopia (22.9%), Tilaye and Getachew (2002) from Debre-Birhan, North Shewa (34%) and Negash *et al.* (2004) from Nazareth, East Shewa (24%). Teshale *et al.* (2007) in goats (62-84%) from South Omo, North Omo (Southern Ethiopia) and East Shewa Zones (Central Ethiopia). The differences in the seroprevalence could be due to differences in the relative densities of other animal population and the access of goats to contaminated feed and water, the geographical variability, the serological tests used and the cut-off value reported. According to the review of Dubey (2010), seroprevalence ranging from 3.2% in Mexico (by ELISA) to 90.9% in the Netherlands (by latex agglutination test) were reported. Initial screening through serum test shows that highest seroprevalence is in goats, followed by sheep. The samples test for serology of three diseases and their prevalence 0%, 46.5%, , 2.3%, 5.5%, 0%, and 1.7% and 0% in *Brucella* possitive, *Toxoplasma* positive, common three diseases positive *Brucella* & *Toxoplasma* positive, *Brucella* & *Coxiella*, *Toxoplasma* & *Coxiella* positive case and totally negative samples uses for serology. Hill *et al*. (2005) partially agreed and approximately thirty-three percent of animals and human population of the world had estimated to infect with *T. gondii* at an average (Sensini, 2006) ranging between 1 and 99% rates of infections (Olivier *et al*., 2007).

5.3.2. Species

Species had linear positive correlation with *Toxoplasma* protozoan infection. Seroprevalence of *Toxoplasma* in goat and in sheep were 54.8% and 62%. Various researchers various area demonstrated and seroprevalence in sheep 31.45% was steady with the previous reports from Ethiopia (22.9% - 34%) (Bekele and Kasali, 1989; Demissie and Tilahun, 2002; Gebremedhin *et al*., 2012 and 2013), Morocco 27.6% (Sawadogo *et al*., 2005), similar reported from Ethiopia 56% (Negash *et al*., 2004), Zimbabwe 67.9% (Hove and Mukaratirwa, 2005) and Egypt 47.5% (Barakat *et al.,* 2009) and 98.4% (Ghoneim *et al.,* 2009). Seroprevalence was lower than current study also reported from South Africa 5.6% (Samra *et al.,* 2007). Similarly, the seroprevalence reported in goats was 15% in accord with the 11.6% (Bekele and Kasali, 1989) and 19.7% (Zewdu *et al*., 2013), Ethopia & Tanzania, 19.3% (Swai and Kaaya, 2013), 35% (Demissie and Tilahun, 2002) and 74.8% (Teshale *et al*., 2007). Seroprevalence reported in Uganda, 31% (Bisson *et al*., 2000) and 41.7% (Ghoneim *et al.,* 2009) and 59.4% (Barakat *et al.,* 2009) in Egypt. Compared to the current results reported by Kamani *et al*. (2010) much lower seroprevalence of 4.6% from goats in Nigeria.

5.3.3. Breed

Breed had negative linear correlation with Seroprevalence of toxoplasmosis in small ruminants in current study. The seroprevalence of small ruminants was 57.8% and 53.9% in local and crossbred. Nevertheless, in goat and sheep it was 32.9% & 38.8% and 29.1% & 24%. . Sheep cross/ exotic / high yielding were more susceptible than local/ meat type (Corbel & Brinley-Morgan, 1984; Islam *et al*., 1983 and Mahboub *et al*., 2013). Bandey *et al.* (1989) reported sheep in Kashmir valley and local sheep tested and found that 3.2%.

5.3.4. Sex

Sex wise seroprevalence of toxoplasmosis revealed that a significant association in small ruminants. The female animal observed highest percentage (38.9%) in small ruminants. Similarly, she or nanny goat and ewe also obtained higher infection rate of *Toxoplasma* than their male. Yin *et al.* (2015) bearded similar trend but lower in percentages. He studied seroprevalence and risk factors of *Toxoplasma gondiii* in Tibetan sheep in Gansu province, Northwestern China and observed the prevalence in females (19.2%) lower than in males (22.8%). Gebremedhin *et al.* (2014) disagreed and observed this study used samples from abattoir where the majority of the slaughtered animals were young (55.7%, 350/ 628) and male (95.9%, 602/638).

5.3.5. Age

Statistically, age existed a negative association and insignificant between age of small ruminants and the prevalence of toxoplasmosis. Prevalence of *Toxoplasma* was lowest (9.3%) in young small ruminants and highest (26.1%) in older age and the other was 20.6% in adult age of animals. When compare it separately, the highest rate of infection in older goat and sheep (Gebremedhin and Gizaw 2014; Moizur *et al.,* 2014) in respect to others age group. The others were 19.9% & 24% and 10.4% & 3.8% in goat & sheep at young and adult age, respectively. Toxoplasmosis in adults was likely due to increased opportunities of exposure to several predisposing factors or sources of infections from the environment. Therefore, this difference in prevalence among age group could be explained by the cumulative effect of age (Hall *et al*., 2001; Dubey, 2010). Our finding was in conformity with other reports on caprine toxoplasmosis from Ethiopia (Teshale *et al*., 2007; Yibeltal, 2008) and from other countries (Dubey, 1990; Opel *et al*., 1991; Dorny *et al*., 1993; Jittapalapong *et al*., 2005; Carneiro *et al*., 2009; Chikweto *et al*., 2011). Shahiduzzaman *et al.* (2011) agreed with current experiment, but showed lower in prevalence (Yin *et al.,* 2015) showed goats and sheep had 32% and 40%, respectively. Researcher obtained significantly high prevalence in adult

sheep than young sheep. This outcome might be observed due to high chance of exposure to the source of infection and suggests that most sheep acquire the infection post-natal.

5.3.6. Parity

The parity of small ruminants, goat and sheep with seroprevalencce of toxoplasmosis had statically significant (with a positive slope). The highest to lowest percentage of positive case of toxoplasmosis were 23.7%, 17.6%, 11.2% and 6.1% in 1st, 2nd, 3rd and >3rd parity, respectively in small ruminants. The infection rate decreased with the increasing parity of small ruminants. Yin *et al.* (2015) also established it. However, in goat and sheep the parity wise (1st to $>3^{rd}$) contamination rate decrease were 19.2%,10.2%, 8.5% & 5.1% in case of goat and 40.7%, 19.9%, 11.6% & 6.4% in sheep at NBT in Bangladesh.

5.3.7. Body weight

Seroprevalencce of toxoplasmosis in relation with body weight of small ruminants had positive association and statically in significant (*P*<0.01/).05). The highest (25.9%) prevalence of toxoplasmosis in SR was observe at medium (10 to<15 Kg) body weight group and lowest was heavy (21.1%) body weight group. On the other hand, the seroprevalence of toxoplasmosis was SR, 9.1 percentages in light (< 10 Kg) body weight group. The researcher showed that goat trends in infection *Toxoplasma* (lower to higher) 10.9%, 19.9% & 24% in light, heavy and medium body weight groups. Whereas in sheep this trends are 2.5%, 26.6% & 32.9% in light, heavy and medium groups at NBT in Bangladesh. The similar opinion was by Mahboub *et al.* (2013) exposed that effects of toxoplamosis on animal body weight. Performance were revealed that young goat and sheep the Mean±SE were 1.33±0.32 & 2.33±0.45 were significantly (*P<0.05*) *at* different letters size compared to adult and their Mean±SE were 42.33±2.56 & 38.67±3.78, respectively.

5.3.8. Flock size

Density of animal population based on flock size had no significant correlation between *Toxoplasma* and occurrence of small ruminants. Seroprevalence of toxoplasmosis was highest (38.7%) in SR at small density (<5 heads flock) than medium (11.8%) and large (5.5%) density of animals in farms. Surprisingly observe that the seroprevalence of toxoplasmosis in goat and sheep individually, the goat showed highest occurrence in large size flock and sheep lowest prevalence was in small size flock, similarly vice versa. Researcher Mahboub *et al*. (2013) was not approving this result. He revealed that large flocks size animals had higher *Toxoplasma* infection than small size grazed flocks (*P*=0.0001). The value of goat was highest to lowest 31.9%, 9.8% & 3% in small, medium and large density populated goat species farms. Similarly, in sheep there was 32.9%, 26.6%, 2.5% in medium, large and small density farms. The variation in goat and sheep might be due to the associative nature of the sheep.

5.3.9. Biosecurity

The biosecurity of small ruminants accordance with the seroprevalencce of toxoplasmosis had a positive effects and statically in significant. The trends of seroprevalence of toxoplasmosis increased with the level of biosecurity, i.e., traditional, moderate and good. On the other hand, the seroprevalence of toxoplasmosis was recorded in SR relatively lower (13.3%) in moderate biosecured maintained farms, lowest (12.0%) good biosecuried and highest (30.9%) in traditional level of biosecurity farms at observation area. Saghir *et al.* (2015) partially agreed the current study and demonstrated the populated where biosecurity measure did not taken properly. They observed the significant (*P*-Value= 0.0001) regional variations in prevalence of *Toxoplasma* infection consistent with (Yang *et al.,* 2013) who reported same picture in China (Sechi *et al.,* 2013) whose results demonstrated the association between toxoplasmosis and still water sources. However, occurrence value of goat and

sheep varied due to rearing system (Shahiduzzaman *et al.,* 2011) and trading nature.

5.3.10. Feeding habit

Feeding habit of small ruminants had negative correlation with seroprevalence of *Toxoplasma*. Seroprevalence of toxoplasmosis in small ruminants was higher (26.9%) at graze habit animals than stall-feeding (17.1%) groups of survey animals. The grazing and stall-feeding values was 33.1% & 21.7% and 48.1% & 13% in case of goat and sheep, correspondingly. Mahboub *et al.* (2013) strongly support our consequences and he got significance (P<0.001). The other researchers such as Shahiduzzaman *et al*. (2011) partially support the current conclusion. Shahiduzzaman *et al*. (2011) research about *Toxoplasma gondii* Seroprevalence in Domestic Animals and Humans in Mymensingh District, Bangladesh and showed Goats and sheep showed (32 and 40%, respectively) mix grazing of cattle, goats and sheep at bank of river was common rearing style in this area. However, goat and sheep more frequently ranged on the streets.

5.3.11. Seasons

Seasonal had positive but insignificant effect on *Toxoplasma* in small ruminants. The percentages of *Toxoplasma* upbeat case in small ruminants were 20%, 19.4% and 16.6% in summer, rainy and winter seasons of the year. The proportion of *Toxoplasma* in summer rainy and winter were 22.2%, 17.9% & 14.6% in goat and 8.9%, 26.6% & 26.6% in sheep, respectively. Summer had more prevalence than that of rainy and winter. Partially decided with Gebremedhin *et al*. (2014) and sheep sampled during the dry season (December to March) have four time more chance of seropositivity (P<0.005) as compared to those sampled during wet season (April to November). This might be a reflection of fluctuations or differences in rate of transmission between seasons in that more infections taking place in the preceding wet months (where the climate was more suitable for survival of the oocysts) were

carrying to dry season. Since IgG antibodies to *T. gondii* was long lasting in the body of the animals, the high positivity seen in dry season might partly be due to the carry over effect from preceding wet season infections (i.e. it did not necessarily mean that seropositive animals sampled in dry season infected in same dry season). Yin *et al.* (2015) studied and similar conclusion, seroprevalence and risk factors of *Toxoplasma gondii* in Tibetan sheep in Gansu province, Northwestern China and observed the seroprevalence in different season ranged from 16.5% in winter to 23.6% in summer.

5.3.12. Location

Regional (location) difference was effect the seroprevalence of toxoplasmosis in small ruminants. The positive parts of *Toxoplasma* organism highest to lowest were 30.9%, 9.9%, 8.6% and 6.5% into Rajshahi, Chapai Nawabjong, Naogaon and Natore region in small ruminants. In goat & sheep the part of *Toxoplasma* were 34.6% & 12.7%, 8.3 % & 17.7%, 3.3% & 21.5% and 8.3% & 10.1% in Rajshahi, Chapai Nawabjong, Natore and Naogaon districts, correspondingly. Pearson's correlation coefficient (r) value was 0.013 and had insignificant positive association between *Toxoplasma* and location at NBT in Bangladesh. This variation might be due to climatic condition, variation of methodology (Ahmad and Tasawar, 2015; Yu *et al.,* 2007) and cultural eating habits because the consumption of raw and rare meat was not normally practiced in Bangladesh (Shahiduzzaman *et al*., 2011).

5.3.13. Pregnancy status

Pregnancy had direct effect on *Toxoplasma* in small ruminants. Pearson's correlation coefficient (r) value of toxopalsma and pregnancy was -0.017 and statistically insignificant. Thus, the correlation coefficient was a measure of linear (with a negative slope) association between *Toxoplasma* and pregnancy status at NBT in Bangladesh. The prevalence of toxoplasmosis in relation with pregnant SR was 12.2% and non-pregnant was 87.8%. Researcher also observed that the prevalence of *Toxoplasma* in goat and sheep are 74.1% &

85.7% and 54.7% & 61.5% at pregnant and non-pregnant doe and ewe at NBT in Bangladesh. In pregnancy stage, the variation of seroprevalence would have effect on abortion and birth defect (Pappas *et al.,* 2009 & Shahiduzzaman *et al*., 2011).

5.3.14. Reproductive diseases

The seropositive rate of *Toxoplasma* in coordination with abortion (Dubey, 2009), still birth, retained fetal membranes, dystocia, cervicitis, vaginal prolapsed/uterine prolapsed, metritis/pyometra, anestrus, repeat breeding, orchitis, epididymitis, posthitis, and urinary tract infection were 3.6%, 1.7%, 5.1%, 3.4%, 3.6%, 1.9%, 2.1%, 3.8%, 2.1%, 2.1%, 6.7%, 4.2% & 8.6%, respectively. The posthitis of logistic regression coefficients were found to be positive and statistically significant ($P<0.01$), abortion, endometritis/ pyometra at ($P<0.05$) and retained placenta, repeat breeding, dystocia and still birth at (P<0.10) level. The cervicitis, anestrus, vaginal / uterine prolapsed, orchitis and urinary tract infection were insignificant ($P>0.10$). The prevalence rate of *Toxoplasma* with abortion, still birth, retained fetal membranes, dystocia, cervicitis, vaginal prolapsed or uterine prolapsed, metritis or pyometra, anestrus, repeat breeders, orchitis, epididymitis, posthitis, and urinary tract infection were 1.8, 1.0, 1.4, 1.6, 1.0, 1.0, 2.5, 1.2, 2.0, 1.4, 3.3, and 0.8 than control (epididymitis) group.

Dubey (2009) agreed this research and stated toxoplasmosis was associated with the occurrence of embryonic death and absorption, fetal death and mummification, abortion, stillborn and neonatal mortality Radostits *et al.* (2007). Moreover, toxoplasmosis had harmful effects on the health and performance of ewes and does after parturition and sometimes leads to their death.

5.4. Experiment 4: Survey of Coxiellosis related to reproductive disorders in small ruminants

In this study, the prevalence of Q fever in small ruminants includes goats and sheep estimated by using indirect ELISA test. As Q fever was a zoonosis and it exists in animals of Bangladesh, it was also supposed to be present in humans. Due to lack of reporting, awareness and nonspecific influenza-like symptom of this disease in humans, it might overlook and remained undiagnosed in human diagnostic laboratories. Due to lack of reporting from animals, the physicians are also unaware about this disease in humans. As a result, physicians usually do not refer flu-like cases for Q fever diagnosis. About 91 serum samples from goat and sheep tested for indirect ELISA of Q fever in the National Reference Laboratory in Germany. The prevalence calculated in conjugation with *Toxoplasma* and *Brucella* estimated and place in separate section.

5.4.1. Seroprevalence

The overall seroprevalence of coxiellosis in small ruminants was 11% through I-ELISA serology. Final serum test showed that highest seroprevalence is in sheep followed by goat. The samples test for serology of three diseases and their prevalence 1.1%, 0%, 2.2%, 4.4%, 0%, 3.3% and 0% in sole *Brucella Toxoplasma* & *Coxilla*, common three diseases positive (*Brucella*, *Toxoplasma* & *Coxiella*), *Brucella* & *Toxoplasma, Brucella* & *Coxiella* positive case and totally negative samples uses for serology. The reported sensitivity (S/P cut-off 40) of milk and blood ELISA at animal level were 86% (95% Confidence interval (CI): 76-96) and 84% (95% CI: 75-93) and an equal specificity of 99% (Paul *et al.,* 2012).

5.4.2. Species

Species had direct effect on seroprevalence of Q fever in small ruminants. The positive and negative percentages of *Coxiella* were 4.4% & 73.6% in goat and 6.6% & 15.4% in sheep. When compare within species the infection rate was 6% in goat and 30% in sheep. Asadi *et al*. (2013) got higher percentages than

studied trial. He studied about seroprevalence of Q fever in sheep and goat flocks with a history of abortion in Iran between 2011 and 2012 and resulted that a total of 215 sheep (19.5%; 95% CI: 17-22%) and 49 goats (27.2%; 95% CI: 21-34%) had antibodies specific to *C. burnetii*. Pearson's correlation coefficient (r) value was 0.392 and statically significant ($p<0.01$). Thus, the correlation coefficient was a measure of linear (with a positive slope) association between *Coxiella* and species under the working area. The seroprevalence of *C. burnetii* infection in sheep populations had been estimated in several other countries such as USA 10% (McQuiston & Childs, 2002), Spain 21% (Ruiz-Fons *et al*., 2010), Cyprus 18.9% (Psaroulaki *et al*., 2006) and Germany 1.3% (Hellenbrand *et al*., 2001).

5.4.3. Breed

The breed of small ruminant's positive correlation with Q fever at tested samples of the study area. Seropositiveness of the Q fever was 7.7% in local breed 3.3% in crossbred. In goat the prevalence was 4.2% & 1.4% in Local and crossbred. But in sheep the percentages of Q fever infection was 20% & 10% in Local and cross one, respectively. Pearson's correlation coefficient (r) value was 0.105 and statistically insignificant ($P>0.01/\ 0.05$). Thus, the correlation coefficient was a measure of linear (with a positive slope) association between Q fever and breed at tested samples of the study area. Klaasen *et al.* (2014) obtained comparable pattern but variation in prevalence. Klaasen *et al.* (2014) studied about *Coxiella burnetii* seroprevalence in small ruminants in the Gambia. Researcher showed practically all animals sampled in the villages belonged to the indigenous goats and Djallonké sheep, whereas at Abuko abattoir 81.1% of the animals were exotic breeds. At Farafenni abattoir, the proportion indigenous to imported breeds was 55.7% to 44.3%. Other researchers such as Ryan *et al.* (2011) and Alvarez *et al.* (2012) found high seroprevalence of Q fever was found in dairy breed but Asadi *et al.* (2012) observed indigenous breed has the highest prevalence of Q fever.

5.4.4. Sex

Sex wise prevalence of Q fever reveals that prevalence in male was 3.3%. On the other hand, prevalence in female was record 7.7%. Prevalence of brucellosis in female ruminants animal was higher than male animals (Klaasen *et al.,* 2014). Statistically, the occurrence of coxiellosis had highly significant (p< 0.01) relationship with sex of small ruminants. Sex was direct influenced on seroprevalence of Q fever, Qassim (2012) disagreed but opposite opinion and was showed non-significant effect on sex of examined animals on Q- fever prevalence 15% (male), 16.3% (female) in small ruminants, which summarized as 18.7% & 19.6% of male and female sheep, respectively and 7.5% & 8.1% of male and female goat respectively).

The prevalence of Q fever in goat was found to be similar in male and female 2.8% by i-ELISA. Similarly, sheep was 25% female than 5% male. Pearson's correlation coefficient (r) value was 0.104 and insignificant (*P*>0.01/0.05) positive association between Q fever and sex under the working area.

5.4.5. Age

Age of small ruminants had positive correlation with Q fever. Prevalence of Q fever was lowest (1.1%) in young small ruminants and highest (6.6%) in older age group. Therefore, infection rate of Q fever increasing with the age. This prevalence pattern of age agreed with several workers, such as Klaasen *et al.* (2014), Kennerman *et al.* (2010), Garcf`a-Perez *et al.* (2009) and partially relation with Esmaeili *et al.* (2014), but dissimilar with Bo *et al. (*2011). The highest rate of infection in older goat was 4.2%. However, the highest rate in sheep (15%) at older sheep than that of others. These were 1.4% & 0% in adult and young goat and 10% in young & 5% in adult sheep. Pearson's correlation coefficient (r) value was 0.057 and statically insignificant (*P*>0.01/ 0.05). Thus, the correlation coefficient was is a measure of linear (with a positive slope) association between Q fever and age of small ruminants under the

working area. The variation in results might due to older have higher antibodies rates than newborn goat & sheep.

5.4.6. Parity

The highest to lower percentage of positive case of Q fever were 4.2%, 2.1%, 2.1% and 6.4% in 1st, 2nd, 3rd and 4th parity, respectively. In goat and sheep the highest to lower percentage of Q fever were 0% & 0%; 0% & 6.7%; 11.1% in 1st parity and 5.6% in 2nd, 3rd and 4th parity.

Pearson's correlation coefficient (r) value was -0.176 and significant (P<0.01) with a negative association between Q fever and parity in current study area. So parity had negative influence but statically significant and prevalence decreased with increasing parity (Paul *et al.,* 2012). Asadi *et al.* (2012) partially support it and with his result, seropositivity increased with parity and it was highest in third parity.

5.4.7. Body weight

Body weight of small ruminants had significant (P<0.01/0.05) relation with Q fever (Gul *et al.,* 2014). The correlation coefficient was a measure of linear positive association with Q fever and under the research area. The highest (5.5%) prevalence of Q fever in SR was observed in medium and heavy body weight group. When seroprevalence of Q fever compare in goat and sheep individually, the percentages was 0 % & 0%, 1.4 % & 20% and 4.2 % & 10% in light, medium and heavy weight animals. This variation might be because the body weight of mature animals was higher as compared to immature or younger kid and lamb. Q fever during pregnancy has been associated with abortion, premature birth and low weight in newborn babies (Maurin and Raoult, 1999). Therefore, Q fever had not direct correlation with body weight, but affected animals delivered low weight babies (Maurin and Raoult, 1999).

5.4.8. Flock size

Q fever infections directly depended on animal population and their density of a farm. Seroprevalence of Q fever was highest in SR of small (<5 heads) size flock (8.8%). On the other hand, the seroprevalence of Q fever recorded in SR relatively lower (1.1%) in medium (5 to 10 head) flock and large (> 10 heads) sized flock group. The prevalence in goat & sheep were 5.6% & 20%, 0% & 5% and 0% & 5% in small, medium and large size populated farms (Kennerman *et al.,* 2010).

Pearson's correlation coefficient (r) value was 0.149 and significant ($P<0.01$) positive association between Q fever and density of animal population. The rate of infection was increasing the density that was small flock less prone than larger flock. Ryan *et al.* (2011) and McCaughey *et al.* (2010) gave same opinion. Paul *et al.* (2012) also same result that was significantly association with density (heard size). Lange *et al*. (2015) partially consent and observed that descriptive statistics of all births in Q fever-affected areas and areas not affected by Q fever, in the years 2003 through 2004 and 2008 through 2010 on the basis of Flock density of sheep and goat and obtained results.

5.4.9. Biosecurity

The effect of biosecurity with Q fever prevalence had positive but insignificant association (Paul *et al.,* 2012) in NBT in Bangladesh. Prevalence of Q fever was highest in traditional (6.6%) level of biosecurity maintained farms and lowest was 2.2% in moderate & good level of biosecurity maintained farms. On the other hand, the prevalence of Q fever in goat and sheep demonstrated the highest, lowest and other prevalence of Q fever in goat were 5.6%, 0% and 0% traditional, moderate and good biosecurity maintained farms. Whereas sheep, it was record the prevalence of Q fever was same as 10% for traditional, moderate and good biosecurity maintained farms at Northern Barind Tract. Cantas *et al.* (2011) studied in different situation in Q fever of ruminants associated with on-farm risk factors in Northern Cyprus and observed in

presence and absence of Rodents in Animal Housing, Ticks on aborted Animals, Houseflies on Farm, Pigeons on Farm & Presence of Carnivores at Farm reviled (9% & 16%), (2% & 23%), (4% & 21%), (3% & 22%) and (4% & 21%), respectively.

5.4.10. Feeding habit

Consequence of feeding habit had negative relation Q fever in small ruminants but statistically significant ($P<0.01$). Prevalence of Q fever was higher (6.6%) in grazing small ruminants than stall-feeding (4.4%). When the grazing results observe in goat and sheep separately, the researcher obtained 2.8% & 20% and 2.8% & 10% for grazing and stall-feeding habit. Cantas *et al.* (2011) study with feeding habit but they have different factors on-farm risk factors in northern Cyprus and observed PCR positive of *Coxiella burnetii* on feed farm made feed and commercial feed were 19% and 3%.

5.4.11. Seasons

Seasonal effects in relation with Q fever in small ruminants were partial. The percentages of *Coxiella* positive case in small ruminants were 1.1%, 7.7% and 2.2% in summer, rainy and winter seasons of the year. The proportion of *Coxiella* in goat and sheep were 0% & 5%; 4.2% & 20% and 1.4% & 5% in summer rainy and winter, respectively. The season had not significant ($P>0.01/0.05$) effect on prevalence with Q fever.

Prevalence of Q fever varies from season to season with the highest prevalence in the month of June. Up to 9% increase in prevalence occurs due to seasonal variation (Hubert *et al.,* 2012). A cow was at a higher risk of infection with Q fever during summer than other seasons (Paul *et al.,* 2012).

Cantas *et al.* (2011) practical the lowest registered number of abortion cases in the general database was in November while the lowest occurrence of *C. burnetii* abortions was in December. However, this occurrence gradually increased from January to another peak in February and then decreased towards March. The *C. burnetii* abortions and presence of ticks on abortion

cow cases seem to follow the gradual fall in temperature as the season transition from autumn to winter.

5.4.12. Location

The location wise effect on seroprevalence of Q fever and Pearson's correlation coefficient (r) value was 0.223 and significant ($P<0.01$) positive association between *Coxiella* and location. The upbeat part of *Coxiella* organism were 3.3%, 3.3%, 1.1% and 3.3% into Rajshahi, Chapai Nawabjong, Natore and Naogaon region in small ruminants. In goat & sheep the fraction of Q fever were 2.8%, 1.4%, 0% & 1.4% and 5%, 10%, 5% & 10% in Rajshahi, Chapai Nawabjong, Natore and Naogaon districts. The seroprevalence was more in rural part than urban. This finding was agreed with Hasan *et al.* (2011). He stated that Q fever in there region includes Northbound Region, Border Region and Karpas Region. Q fever infection occur in presence of parasite likes tick on farm management risk factor in northern Cyprus, on the other hand Good hygiene practices are an important way of reducing the risk of spread of infectious diseases, and the findings of this study agree with this notion. The higher the frequency of litter cleaning ($5 < \times < 10$ and $\times > 10$ times/year) on farm the more protective (OR = 0.3; P = 0.05 and OR = 0.09; P = 0.05) it was against the risk of Q fever. Studies done in rural areas have all indicated that poor hygiene could be an exacerbating factor in the spread of *C. burnetii* (Lyytikainen *et al.,* 1998).

Klaasen *et al*. (2014) studied the serological survey of *C. burnetii* seroprevalence in small ruminants in The Gambia demonstrates a considerable prevalence of current or past infection in the sheep and goat population. The species and age of the animals as well as their location and origin was of influence on the seropositivity of *C. burnetii.* Although a direct link between the human and veterinary data could not be demonstrated, there was clear zoonotic implications. *C. burnetii* was highly contagious and very resistant in the environment.

5.4.13. Pregnancy status

Pregnancy was negative relation with seroprevalence of Q fever in small ruminants at NBT in Bangladesh. The prevalence of Q fever in relation with pregnancy status of SR was evaluating as 10.4% in pregnant and 2.1% in non-pregnant small ruminants. Particularly in goat and sheep it evaluation was 3.3% & 22.2% in pregnant and 0 & 5.6% in non-pregnant group of animals. The correlation coefficient (r) value was -0.171 and significant (P<0.05) negative association between fever and pregnancy. Researcher had negative correlation, but Maurin and Raoult (1999) observed positive relationship between Q fever and pregnancy and he obtained Q fever during pregnancy had been associated with abortion, premature birth, and low weight in newborn babies.

5.4.14. Reproductive diseases

The seropossitive rate of Q fever in coordination with abortion, still birth, retained placenta, dystocia, cervicitis, vaginal prolapse or uterine prolapsed, endometritis or pyometra, anestrus, repeat breeding, orchitis, posthitis, epedidymitis, urinary tract infection and were 2.2%, 1.1%, 1.1%, 1.1%, 0%, 0%, 1.1%, 1.1%, 0%, 0%, 1.1%, 2.2% and 0% respectively. The anoestrus, repeat breeding, still birth and orchitis of logistic regression coefficients were found to be positive and had significant at (P<0.01) and cervicitis, retained placenta, dystocia, vaginal prolapse/ uterine prolapse & pyometra significant at (P<0.05) and also abortion, posthitis and urinary tract infection significant at (P<0.10) level. The prevalence of reproductive problems in goat & sheep were abortion (1.4% & 5%), anestrous (0% & 5.0%), retained placenta (1.4% & 0%), dystocia (0% & 5 %), still birth & (0% & 5%) endometritis/ pyometra (0% & 5%), posthitis (0% & 5%) and epididymitis (2.8% & 0%), respectively.

Q fever had reported to be associated with abortion, still birth, premature delivery and delivery of weak offspring (Angelakis and Raoult, 2010). These reproductive disorders usually had seen in sheep and goats. Q fever was

frequently subclinical in cattle but infected cows might develop infertility, metritis, and mastitis (To *et al.,* 1998). Moreover, *C. burnetii* founded to be significantly associated with placentitis (Bildfell *et al.,* 2000; Hansen *et al.,* 2011). In the first, pregnancy the highest seroprevalence found in dairy cows (Bo *et al.,* 2011). The organism could be isolated from the blood, milk and urine and localized in the kidneys, udder and the placenta after experimental infection in sheep. Ewes may occasionally shed the organism at successive parturitions (Welsh *et al.,* 1959; Berri *et al.,* 2002) indicates that infection was persistent and that pregnancy enhances multiplication of the organism but the specific location of the organism where they persists during the non-pregnant period and the mechanisms that initiate its active multiplication in the placenta are not clearly understood. The immunosuppressive effects of pregnancy may be responsible for the increased multiplication of the organism in the placenta (Polydorou, 1981).

Chapter 7

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Appendices

Appendix-1

Publications

The articles and abstracts already published in National and International conference and Journal are placed in below:

Article (Full Length Paper):

- **Islam, MH**., Sarder, M.J.U., Rahman, M.S., Haque, M.A., Islam, MA., Jahan, S.S.J. and Khaton, R. **2015.** Retrospective Study of Reproductive Diseases of Small Ruminants in Northern Barind Tract in Bangladesh. Animal and Veterinary Sciences. 3(5): 136-140; doi: 10.11648/j.avs. 20150305.13.

Abstract (Presented in Different Conference):

- **Md. Hemayatul Islam,** Md Jalal Uddin Sarder and Md. Alamgir Hossain Sarkar 2015. Reproductive disease frequencies in small ruminants in relation with age, sex and seasons. International Symposium on Dairy Animal Reproduction (ISDAR), Lahore, Pakistan.
- **Md. Hemayeatul Islam**, Md. Jalal Uddin Sarder, Md. Shofinur Rahman, Md. Atiar Rahman and Subroto Kumar Paul 2015. Evaluation of breed, age and sex responsible for seroprevalence of brucellosis in small ruminants at Northern Barind Tract. International Conference on Environment and Ecology (ICEE 2015), Kolkata, India.
- **MH Islam,** MJU Sarder, MS Rahman and MS Islam 2014. Epidemiological study of diseases in Small ruminants at Rajshahi University veterinary clinic- BSVER conference, Mymensing. Bangladesh
- **MH Islam,** MJU Sarder, MA Haque, and MS Rahman 2013**.** Suroprevalance of Brucellosis in Small Ruminants in northern part of Bangladesh. One Health Dhaka Conference 21-23 November 2013

Appendix-2

Correlation of Brucellosis, Toxoplasmosis and Coxiellosis

Correlation (*r*)	Animal ID	Districts	Breed of Small ruminants	Various age of SR	Sex of SR	Flock size of SR	Body weight of SR	Parity of SR	Biosecurity of SR	Grazing behaviour of	Seasonal effect of SR	Pregnancy status	*Toxoplasma* serology test	*Brucella* serology	Q Fever ELISA	Serological status
Animal ID	1	.267**	.037	.116*	.162**	.368**	.151**	-.157*	.303**	-.157**	.188**	.025	.089	.074	.392**	.074
Districts		1	.374**	.008	-.036	.028	.034	-.159*	-.057	-.168**	.041	-.149*	.013	.092	.223**	.097
Breed of Small ruminants			1	-.046	.088	.002	.024	-.146*	-.053	.062	-.007	.084	-.025	-.026	.105	.000
Various age of SR				1	.119*	.052	.654**	.045	.099	.070	.023	-.030	.011	.033	.057	.013
Sex of SR					1	.125*	.147**	.c	-.022	-.063	.015	.c	.031	.173**	.104	.106
Flock size of SR						1	.089	.050	.457**	-.263**	.035	-.004	.087	.055	.149**	.039
Body weight of SR							1	-.018	.196**	.184**	.042	.117	.046	-.038	.064	.025
Pariety of SR								1	.074	-.082	.113	-.142*	.207**	.031	-.176*	.165*
Biosecurity of SR									1	-.055	.140*	.165*	.043	-.048	.035	-.074
Grazing behaviour of SR										1	-.064	.037	-.102	-.052	-.100	-.109*
Seasonal effect of SR											1	.011	-.014	-.009	.075	-.008
Pregnancy status												1	-.017	-.629**	-.171*	-.341**
Toxoplasma serology test													1	-.194**	-.074	.613**
Brucella serology test														1	.381**	.316**
Q Fever ELISA															1	.138*
Serological status of diseases																1

Appendix-3

Questionnaire (data collection)

(Epidemiological Investigation of Brucellosis, Toxoplasmosis and Coxiellosis in Association with Reproductive Disorders in Small Ruminants)

1. **Farmers details**: i. Name:

   ii. Address:

   iii. Mobile No.:

General information of farm owners (Put mark)

2. Owners occupation: i. Service holders ii. Business iii. Jobs seeker iv. Agriculture v. Others.

3. Farming goal: i. Main business ii. Side business

4. Education: 0-Class 5 i. Class 6-10 iii. Secondary school level iv. Higher secondary level v. Above higher secondary level.

5. Land size (Acre): i. 0-0.5 ii. 0.5-1.0 iii. 1.1-2.0 iv. 2.1-5 v. Above 5

Specific information of farm (Put mark) or mention

6. Typre of Fram: i. Small ruminants ii. Goat iii. Sheep iv. Mixed (ruminants)

7. Breed involvement of Individual animals: i. Local ii. Crossbred

8. Sex involvement in the farm animals: i. Male ii. Female

9. Age Involment of Individual animals: i. Below 12 Months ii. 12- 24 months iii. Above 24 months

10. Parity invloment of individual's animals: Numbers of Kidding / Lambing: i. 1st Parity= First time kidding or Lambing ii. 2nd Parity= one Second times kidding or Lambing iii. 3rd Parity=Third times kidding or Lambing iv. 4th Parity= Above Third times kidding or Lambing

11. Body weight individual's animals: i. Light = <10 kg body weight ii. Medium= 10 to 15kg body weight iii. Heavy= >15 kg body weight.

12. Herd size (Goat or Sheep): i. 0-5 ii. 5-10 iii. Above 10

13. Management Information (Put mark)

Where the Small ruminants rear to feed

a. Farm level (Stall feeding) b. Traditional (Grazing)

14. Housing system (Biosecurity level for maintaining rearing)

    i. Traditional= It means traditional management practice which rank between 0- 4
    ii. Moderate: It means moderate management practice which rank between > 4 to 7
    iii. Good: It means good management practice which rank between >7 to to 10

15. Month of sample (Mention the name of month): Jan/Feb/Mar/Apr/May/Jun/Jul/Aug/Sep/Oct/Nov/Dec

16. How do you manage health related problems?

    By vets/ veterinary field assistants/ village doctors/ethno veterinary/...

17. How frequently you tit-worm your animals? As recommended by

    vets/when sick/never/…

18. Have you faced any diseases involment with your animals during last 18 months? (Put mark): Gastrointestinal/Respiratory/ Musculoskeletal/ Integumentary/ Disease of sense/Infectious disease/ Deficiency syndrome/ Poison/ Female reproductive/ Male sex organ/ Surgical

19. Have you faced any reproductive problems with your animals during last 18 months? (Put mark): Abortion/still birth/retained fetal membranes/dystocia/ vaginal prolapsed or uterine prolapse/metritis or pyometra/anestrus/repeat breeders/orchitis/epididymitis/posthitis/urolithiasis/urinary tract infection

20. Have the animal pregnant? : i. yes ii. No

21. Sample No. i. Sample materials: a. Blood b. Tissue

22. Remarks

Age: groups are

For, Goat: Young = below 1 years, Adult= 1years to 2 years and Older= more than 2 years; for Sheep: Young = below 1 years, Adult= 1years to 2 years and Older= more than 2 years

Biosecurity Scale (0-10) by following informations

a. Where do your animals graze? Fallow land / char/ haor/ bill /tethering in road side or field /zero grazing/….

b. How do you breed your animals? Natural (own bulls, bucks/ neighbors' animals)/ artificial insemination

c. Type of animal house used: kaccha floor/brick floor/ cemented/...

d. Level of sanitation in the animal shed: poor/moderate/good

e. How many animals you purchased during last 12 months?

f. How many animals you sold during last 12 months?

g. How many animals died last 12 months?

h. Do you keep any animal inside your house? Yes/no

If yes, please name the species and number of animals: goat/ sheep/ others

i. The fate of aborted animal: kept in herd/sold/slaughtered

j. How do you dispose aborted materials? Burial / burning none/ disinfectant used after cleaning/ no use of disinfectants

Signature of information collector: Date:

Appendix -4

Chi-Square (χ^2) Distribution Table

df	$\chi^2_{.995}$	$\chi^2_{.990}$	$\chi^2_{.975}$	$\chi^2_{.950}$	$\chi^2_{.900}$	$\chi^2_{.100}$	$\chi^2_{.050}$	$\chi^2_{.025}$	$\chi^2_{.010}$	$\chi^2_{.005}$
1	0.000	0.000	0.001	0.004	0.016	2.706	3.841	5.024	6.635	7.879
2	0.010	0.020	0.051	0.103	0.211	4.605	5.991	7.378	9.210	10.597
3	0.072	0.115	0.216	0.352	0.584	6.251	7.815	9.348	11.345	12.838
4	0.207	0.297	0.484	0.711	1.064	7.779	9.488	11.143	13.277	14.860
5	0.412	0.554	0.831	1.145	1.610	9.236	11.070	12.833	15.086	16.750
6	0.676	0.872	1.237	1.635	2.204	10.645	12.592	14.449	16.812	18.548
7	0.989	1.239	1.690	2.167	2.833	12.017	14.067	16.013	18.475	20.278
8	1.344	1.646	2.180	2.733	3.490	13.362	15.507	17.535	20.090	21.955
9	1.735	2.088	2.700	3.325	4.168	14.684	16.919	19.023	21.666	23.589
10	2.156	2.558	3.247	3.940	4.865	15.987	18.307	20.483	23.209	25.188
11	2.603	3.053	3.816	4.575	5.578	17.275	19.675	21.920	24.725	26.757
12	3.074	3.571	4.404	5.226	6.304	18.549	21.026	23.337	26.217	28.300
13	3.565	4.107	5.009	5.892	7.042	19.812	22.362	24.736	27.688	29.819
14	4.075	4.660	5.629	6.571	7.790	21.064	23.685	26.119	29.141	31.319
15	4.601	5.229	6.262	7.261	8.547	22.307	24.996	27.488	30.578	32.801
16	5.142	5.812	6.908	7.962	9.312	23.542	26.296	28.845	32.000	34.267
17	5.697	6.408	7.564	8.672	10.085	24.769	27.587	30.191	33.409	35.718
18	6.265	7.015	8.231	9.390	10.865	25.989	28.869	31.526	34.805	37.156
19	6.844	7.633	8.907	10.117	11.651	27.204	30.144	32.852	36.191	38.582
20	7.434	8.260	9.591	10.851	12.443	28.412	31.410	34.170	37.566	39.997
21	8.034	8.897	10.283	11.591	13.240	29.615	32.671	35.479	38.932	41.401
22	8.643	9.542	10.982	12.338	14.041	30.813	33.924	36.781	40.289	42.796
23	9.260	10.196	11.689	13.091	14.848	32.007	35.172	38.076	41.638	44.181
24	9.886	10.856	12.401	13.848	15.659	33.196	36.415	39.364	42.980	45.559
25	10.520	11.524	13.120	14.611	16.473	34.382	37.652	40.646	44.314	46.928
26	11.160	12.198	13.844	15.379	17.292	35.563	38.885	41.923	45.642	48.290
27	11.808	12.879	14.573	16.151	18.114	36.741	40.113	43.195	46.963	49.645
28	12.461	13.565	15.308	16.928	18.939	37.916	41.337	44.461	48.278	50.993
29	13.121	14.256	16.047	17.708	19.768	39.087	42.557	45.722	49.588	52.336
30	13.787	14.953	16.791	18.493	20.599	40.256	43.773	46.979	50.892	53.672
40	20.707	22.164	24.433	26.509	29.051	51.805	55.758	59.342	63.691	66.766
50	27.991	29.707	32.357	34.764	37.689	63.167	67.505	71.420	76.154	79.490
60	35.534	37.485	40.482	43.188	46.459	74.397	79.082	83.298	88.379	91.952
70	43.275	45.442	48.758	51.739	55.329	85.527	90.531	95.023	100.425	104.215
80	51.172	53.540	57.153	60.391	64.278	96.578	101.879	106.629	112.329	116.321
90	59.196	61.754	65.647	69.126	73.291	107.565	113.145	118.136	124.116	128.299
100	67.328	70.065	74.222	77.929	82.358	118.498	124.342	129.561	135.807	140.169