JIMMA UNIVERSITY

COLLEGE OF AGRICULTURE AND VETERINARY MEDICINE

SCHOOL OF VETERINARY MEDICINE

SEROPREVALENCE OF BOVINE VIRAL DIARRHEA VIRUS AND ITS POTENTIAL RISK FACTORS IN DAIRY CATTLE OF JIMMA TOWN, SOUTHWESTERN ETHIOPIA

MSc. THESIS

BY

TADELE TADESSE HIRPESA

SEPTEMBER, 2018

JIMMA, ETHIOPIA

JIMMA UNIVERSITY

COLLEGE OF AGRICULTURE AND VETERINARY MEDICINE

SCHOOL OF VETERINARY MEDICINE

Seroprevalence of Bovine Viral Diarrhea Virus and Its Potential Risk Factors in Dairy Cattle of Jimma Town, Southwestern Ethiopia

MSc. Thesis

By

Tadele Tadesse Hirpesa

A Thesis

Submitted to the College of Agriculture and Veterinary Medicine of Jimma University in Partial Fulfillment of the Requirements for the Degree of Master of Veterinary Science in Veterinary Epidemiology

Major Advisor: **Yosef Deneke (DVM, PhD, Associate Professor)**

Co-advisor: **Benti Deressa (DVM, MTADM, Associate Professor)**

September, 2018

Jimma, Ethiopia

DEDICATION

This thesis is dedicated to my beloved mother Adanech Muldhata for her laying foundation of my education.

DECLARATION

First, I declare that this thesis is my *bonafide* work and that all sources of material used for this thesis have been duly acknowledged. This thesis has been submitted in partial fulfillment of the requirements for Master of Veterinary Science (MVSc) degree at Jimma University, College of Agriculture and Veterinary Medicine and is deposited at the University/College library to be made available to borrowers under rules of the library. I solemnly declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma or certificate.

Brief quotations from this thesis are allowable without special permission provided that accurate acknowledgment of source is made. Requests for permission for extended quotation from or reproduction of this manuscript in whole or in part may be granted by the head of the School or the Dean of the College when in his or her judgment the proposed use of the material is in the interests of scholarship. In all other instances, however permission must be obtained from the author.

Name: Tadele Tadesse Hirpesa

Signature: _____

Place: Jimma University, College of Agriculture and Veterinary Medicine

Date of Submission: _____

LIST OF ABBREVIATIONS

BVDV	Bovine Viral Diarrhea Virus
CI	Confidence Interval
cp	Cytopathogenic
CSA	Central Statistics Authority
CSFV	Classical Swine Fever Virus
ELISA	Enzyme Linked Immunosorbent Assay
FBS	Fetal Bovine Serum
GDP	Gross Domestic Product
IFN	Interferon
JTLFRDO	Jimma Town Livestock and Fishery Resource Development Office
MD	Mucosal Disease
ncp	Non-cytopathogenic
NS	None Structural
OD	Optical Density
OIE	Office of International des Epizootic
OR	Odd Ratio
ORF	Open Reading Frame
PCR	Polymerase Chain Reaction
PI	Persistently Infected
RNA	Ribonucleic Acid
ROC	Receiver Operating Curve
TI	Transient Infection
TMB	Trimethylbenzimidine
TNF	Tumor Necrosis Factor
UTR	Untranslated Region

BIOGRAPHICAL SKETCH

Tadele was born in March 22, 1987 from his father Tadesse Hirpesa and his mother Adanech Muldhata in Wonchi district, Southwest Shoa zone, Oromia regional state, Ethiopia. He attended his elementary education (1-8) at Selam Gatiro Junior and elementary school. He also attended his high school (9-10) and preparatory education (11-12) at Dejazmech Geresu Dhuki Comprehensive Secondary School in Woliso town. After he completed his preparatory program, he joined Haramaya University College of Veterinary Medicine in 2007 and was awarded Doctor of Veterinary Medicine (DVM) Degree in Veterinary Medicine in June, 2011. After graduation, he was employed in Dandi Boru University College Jimma campus, as instructor from October, 2011-2014 for three consecutive years. Thereafter, she was also worked in Rift Valley University Jimma campus as Vice Academic Dean of the campus till he joined Jimma University; College of Agriculture and Veterinary Medicine to pursue Master of Veterinary Science (MVSc) degree in Veterinary Epidemiology in 2015. The author also married and a father of one daughter named Obsee.

ACKNOWLEDGMENTS

First and foremost I would like to thank my *Waaqa* (God) for his grace, immeasurable love and giving me strength and health throughout my life to finish this thesis successfully.

I would like to thank my advisors Dr Yosef Deneke and Dr. Benti Deresa for their noble help and unreserved professional advice in implementing the research design and critically reviewing the articles. I also thank them for their energetic encouragement, genuine and constructive comments, professional guidance and devotion of their time in correcting this thesis to produce the final version.

My special thanks go to also Jimma University, School of Veterinary Medicine staff members, for their positive cooperation during all my stay and research work. I want to express my deepest gratitude and appreciation to Jimma town dairy farm owners and attendants for their positive cooperation during sample collection and processing as well as National Veterinary Institute (NVI) staff members especially to Dr. Hundera Sori and Mrs. Wubet for their positive cooperation during laboratory analysis.

Finally, I wish to extend my sincere thanks and heartfelt gratitude to my wife Hafiza Jebal for her moral support that pave the way for the great success in my study. Lastly, but not the least, I would like to extend my great respect to my best classmates; Dereje Tulu, Tsegaye Gebre, Abiy Gojam and David Onafrou for their making our study period harmonious and unforgettable one.

TABLE OF CONTENTS

Contents **Page**

TABLE OF CONTENTS (*Continued*)

LIST OF TABLES

LIST OF FIGURES

LIST OF ANNEXES

SEROPREVALENCE OF BOVINE VIRAL DIARRHEA VIRUS AND ITS POTENTIAL RISK FACTORS IN DAIRY CATTLE OF JIMMA TOWN, SOUTHWESTERN ETHIOPIA

ABSTRACT

Bovine viral diarrhea virus (BVDV) is a highly contagious infectious agent of cattle populations across the world and causing a significant economic loss due to decreased performance, loss of milk production, reproductive disturbances and increased risk of morbidity and mortality. It is an envelope, positive-sense single-stranded (ss+) RNA-virus and belongs to the genus *Pestivirus* of the family *Flaviviridae*. The cross-sectional study was done form January, 2016 up to January, 2017 to estimate the seroprevalence of bovine viral diarrhea virus and its potential risk factors in dairy cattle of Jimma town, Southwestern Ethiopia. A total of 420 blood samples were collected from 45 dairy farms of the town. All sampled animals were identified by their sex, age, breeds, history of reproduction disorders (abortion, repeat breeding), parity status and history of farms by using questioner. The serum extracted from blood samples for the detection of BVDV antibody by using blocking ELISA. In this study, 51.7% (217/420) and 95.6% (43/45) seroprevalence of BVDV antibody was observed at individual and herd level, respectively. The higher seroprevalence of was observed inadult animals 55.1% (95% CI: 49.9-60.2%),dairy farms introduced new animals to their herds 100% (95% CI: 85.7-100%) and cows with history of repeat breeding as compared with cows with history of abortion 40.0% (95% CI: 24.6-57.7%)($P<0.05$). In this study, age (OR: 2.5; $P<0.05$), repeat breeder cows (OR: 2.4; $P<0.05$) and introduction of new animals to herds (OR: 1.6; $P<0.05$) were identified as potential risk factors for the seroprevalence of BVDV. This high seroprevalence result implies as BVD is widely distributed among Jimma town dairy farms and affecting production and productivity of farms. Thus, older and repeat breeder animals should be tested for BVD and properly managed as they act as potential source of infection in addition to awareness creation about BVD for the dairy owners.

Keywords: *Bovine Viral Diarrhea Virus (BVDV), Cattle, ELISA, Jimma, Prevalence, Risk Factors.*

1. INTRODUCTION

1.1. Background

Bovine viral diarrhea virus (BVDV) is a highly contagious infectious agent of cattle populations across the world and causing a significant economic losses due to decreased performance, loss of milk production, reproductive disturbances and increased risk of morbidity and mortality (Wernicki *et al.,* 2015). The broad nature of the disease, its transmittance and lack of treatment has made it a globally enzootic and one of the most significant cattle diseases (Khodakaram & Farjanikish, 2017). It was described for the first time in United States of America as a new transmissible disease in cattle during 1946 (Sarrazin *et al.,* 2013). The Office International des Epizootic (OIE) added bovine viral diarrhea to its list of reportable diseases in 2007, but the listing is as a reportable disease of cattle rather than as a reportable disease of multiple species (Walz *et al.*, 2010).

BVDV is an envelope, positive-sense single-stranded (ss+) RNA-virus of approximately 12.5kb in size and belongs to the genus *Pestivirus* of the family *Flaviviridae* (Liu *et al.*, 2009). There are two different genotypes; BVDV-1 and BVDV-2 with several sub-genotypes (Yazici *et al.,* 2012). The viruses in the two genotypes show considerable antigenic difference from each other and within their species. There are also two biotypes of BVDV, designated as cytopathogenic (cp) and non-cytopathogenic (ncp) strains. This designation depends on their effect on tissue culture cells, where the cytopathogenic strains will cause vacuolization and cell death (Walz, 2015).

Cattle is the primary host but serological evidence of *Pestivirus* infection has been found in over forty different species, for example sheep, pigs, goats, giraffe, kudu, nyala, oryx, water-buck, wild beest and African buffalo (Bachofen *et al.*, 2013; Braun *et al.*, 2013; Michael *et al.*, 2018). Nose-to-nose contact between a susceptible animal and a persistently infected individual is regarded as the most efficient route of transmission (Khodakaram & Farjanikish, 2017). However; the virus can utilize indirect routes as well through the use of contaminated equipment or through insemination with BVDV infected semen (Nahed *et al.,* 2012).

Bovine viral diarrhea viruses cause diarrhea, anorexia, pyrexia, oral erosion, decreased in milk production, abortion, congenital defects, poor growth, impaired reproductive performance, depression, fever, immune suppression and death. However; clinical presentations and severity of disease may vary with different strains of virus (Rypula *et al.*, 2011; Givens *et al.*, 2012). The environmental factors and knowledge of herd management which enhance the risks of BVDV infection would make better the ability to control and impede the transmission, minimizing the unfavorable effects of BVDV infection on herd health and productivity (Saa *et al.*, 2012).

There are two diagnostic approaches to detect BVDV infection; direct tests (detection of the virus or viral components) and indirect tests (detection of the immune response to BVDV) (Dubovi, 2013). The direct test includes virus isolation, antigen capture ELISA and polymerase chain reaction (PCR) (Lanyon *et al.*, 2014). Detection of virus specific antibodies by using different serological tests such as virus neutralization test and enzyme linked immunosorbent assay are an important ways for the indirect detection of the virus (Nahed *et al.*, 2012; OIE, 2015).

1.2. Statement of the Problem

Ethiopia has the largest livestock population in Africa which provides a livelihood for 65% of the population (FF, 2016). The livestock sector contributes about 16.5% of the national gross domestic product (GDP) and 35.6% of the agricultural gross domestic products. It also contributes 15% of export earnings and 30% of agricultural employment (Leta & Mesele, 2014). The predominant livestock production system in Ethiopia is extensive, where indigenous breeds are kept under low-input with low-output husbandry practices (CSA, 2016).

To increase the contribution of livestock to economic growth as well as to satisfy the increasing demand for livestock products (meat and milk), Ethiopia has given more attention to breed improvement, pasture development and health interventions (Shapiro *et al.*, 2015). In addition to these, the development of dairying at farmer's level is one of the activities that

given priority to increase the supply of milk from smallholder dairy farms. However; the dairy sector is still underdeveloped for several logistic and technical reasons including the predominance of infectious diseases that impede the productive and reproductive performance of dairy cattle (Asmare *et al.*, 2013). Infectious diseases are affecting reproduction in cattle can create losses all throughout the reproductive cycle by decreasing ovulation, fertilization, embryonic survival and fetal survival rates (Njiro *et al.*, 2011). Some of such diseases are caused by viruses including infectious bovine herpes virus (BHV-1), bovine viral diarrhea virus and schmallenberg virus (Pawaiya & Gupta, 2013).

Even though BVD has a global distribution with tremendous impact in the dairy industry, very much limited reports are available in Ethiopia. The first serological evidence for the presence of BVDV in Ethiopian cattle was reported by Nigussie *et al*. (2010) with 11.46% overall seroprevalence from serum samples collected for FMD national surveillance in three selected agro-ecological zones of Oromia regional state by using indirect ELISA kit. Two years later, Asmare *et al*. (2013) was reported 11.7% seroprevalence of BVDV exposure of dairy cattle in intensive farms of central and southern part of the country's by using competitive ELISA kit. Even though these reports were revealed the presence of BVDV in cattle population, there were no BVD vaccination activities done so far and no identified potential risk factors of the disease in cattle population solely.

Generally, knowledge about the prevalence and risk factors of the disease is crucial in establishing prevention, control or eradication plan which could minimize or eliminate the unfavorable effects of BVDV infection on herd health and productivity (Saa *et al.*, 2012). Such information is not only used to improve the health and welfare of livestock, but also has significant contribution to activities done to increase an income and wealth of farmers. Therefore, estimation of BVDV prevalence and assessing its potential risk factors in the study area is highly contributes to the establishment of an appropriate prevention and control methods of the disease; at the study area or at national level as well.

1.3. Objectives

- To estimates seroprevalence of BVDV infection in dairy cattle of Jimma town.
- To assess potential risk factors for BVDV infection

2. LITERATURE REVIEW

2.1. Etiology

Bovine viral diarrhea virus (BVDV) belongs to the *Pestivirus* genus in the *Flaviviridae* family and it causes serious clinical disease in cattle (Becher & Tautz, 2011). There are four recognized species within the genus; BVDV-1, BVDV-2, border disease virus and classical swine fever virus (hog-cholera virus) (Khodakaram & Farjanikish, 2017). However, there are four additional putative species including HoBi-like viruses, also known as BVDV-3 (Ridpath *et al.*, 2013; Ninnet *et al.*, 2017).The virus is relatively small (40-60nm), spherical, an envelope, positive-sense single-stranded (ss+) of approximately 12.5kb in size (Fig 2). The genome has a single open reading frame (ORF) flanked by two un-translated regions(UTR):5′ -N^{pro}, C, E^{rns}, E1, E2, p7, NS2-3, NS4A, NS4B, NS5A and NS5B-3′ (Pecora *et al.*, 2009; Factor *et al.*, 2016).

More than two decades ago BVDV isolates were segregated into BVDV-1 and BVDV-2 genotypes based on phylogenetic analysis of partial sequences (Ridpath, 2003). As the sequence conservation between BVDV-1 and BVDV-2 is high in the 5′ UTR, there are two short regions that are notable for their variability (Giammarioli *et al.*, 2015). Subsequent studies showed the existence of a growing number of BVDV-1 and BVDV-2 sub genotypes. After the description of BVDV-1 sub genotypes in the early 1990s, at least twenty one BVDV-1 sub genotypes (BVDV-1a to -1u) and four BVDV-2 sub genotypes (BVDV-2a to -2d) have been described to date (Deng *et al.*, 2015).

Bovine viral diarrhea virus of both genotypes may also classify as cytopathic (cp) and non-cytopathic (ncp) biotype, according to whether or not it produces visible change in cell cultures. Cytopathic biotype arises from rare mutations of the ncp strains. The ncp biotype is the predominant biotype in both BVDV species and can cross the placenta to establish persistent infections. Each biotype has a specific role in a variety of clinical syndromes acute, congenital and chronic infections (Nelson *et al.*, 2016). The ncp viruses are associated with the majority of BVDV infections (90%) and cause mild transient infection as well as

persistent infection. The cp biotype causes severe acute and per acute transient disease as well as mucosal disease in super infected PI animals (Walz *et al.*, 2010; Csaba *et al.*, 2015).

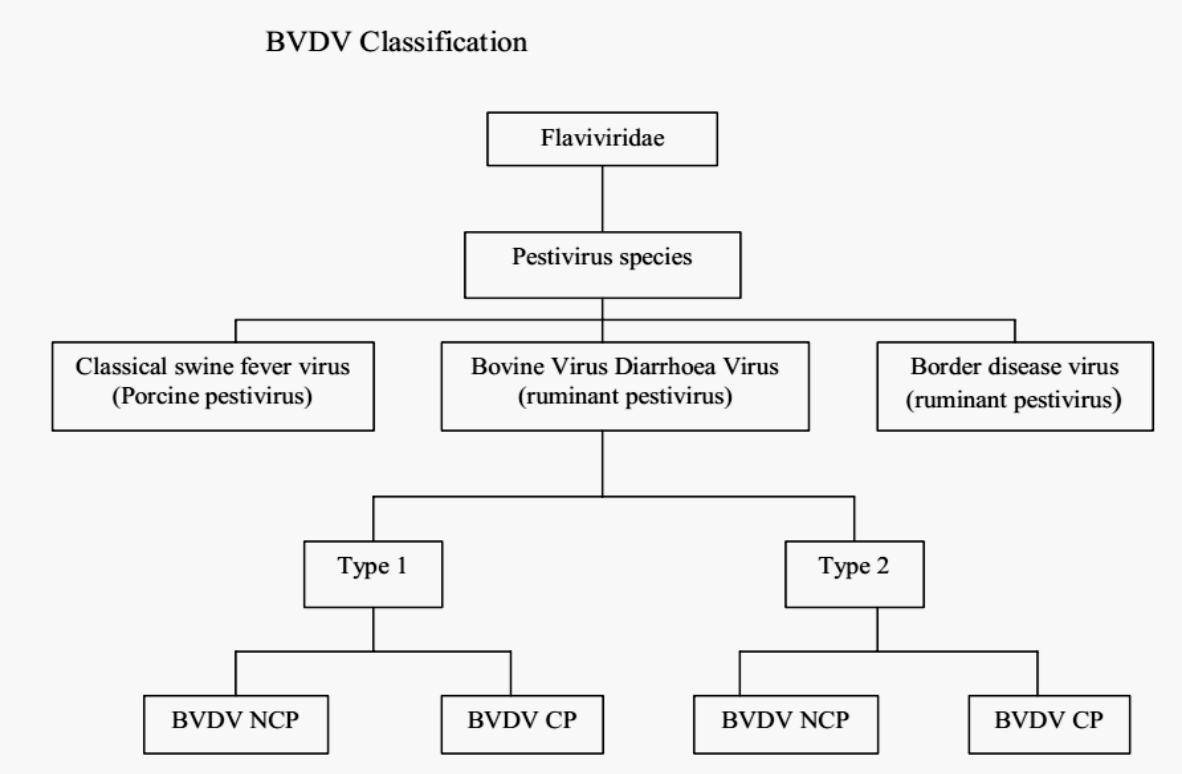

Figure 1: BVDV Classification (Brownlie, 2000)

The open reading frame encodes one large polyprotein, which is processed by cellular and viral proteases into four structural, and eight nonstructural proteins (Kadir *et al.*, 2017).With the exception of the first protein N^{pro}, which is a nonstructural viral auto-protease producing its own C-terminus, the BVDV genome is organized with the structural protein genes (the capsid (C) and 3 envelope glycoprotein) at the 5′ end of the ORF and the nonstructural protein genes (NS) occupying the last two thirds of the ORF (Tautz *et al.*, 2015).

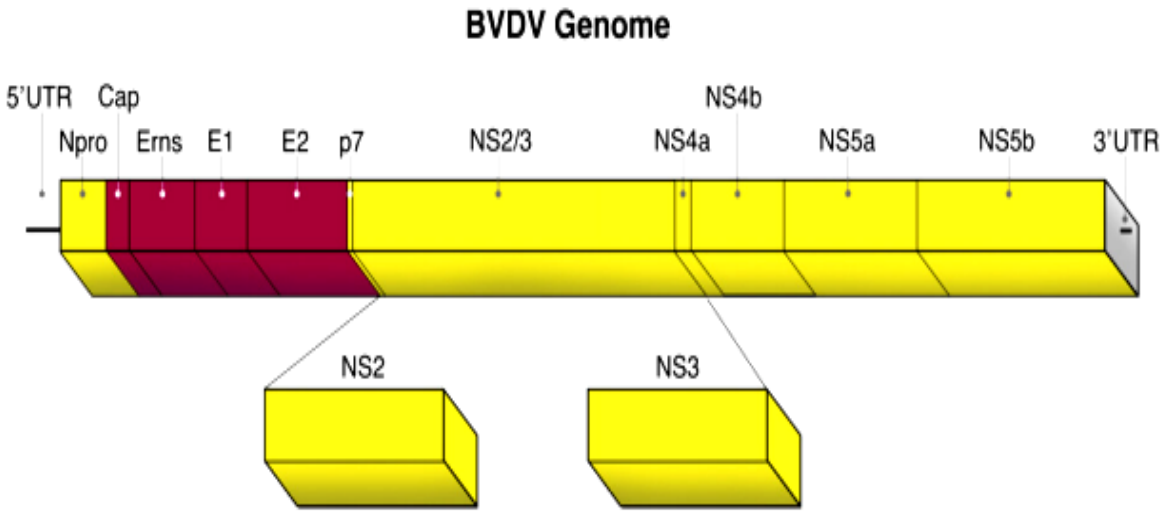

Figure 2: Genome Organization of BVDV (Neill, 2013)

2.1.1. Structural Proteins

The large ORF is translated as a polyprotein. The order of the individual viral proteins within the polyprotein is as follows: N^{pro}-C-E^{rns}-E1-E2-p7-NS2/3-NS4A-NS4B-NS5A-NS5B (Fig 2). The polyprotein is processed co-and posttranslational by host and viral proteases. The proteins associated with the mature virion (structural proteins) are: capsid (p14), E^{rns} (gp48), E1 (gp32), and E2 (gp53) (Raue *et al.*, 2011).

The capsid (C) is the virion nucleocapsid protein whereas E^{rns}, E1and E2 are associated with the outer envelope of the BVDV virion. These three proteins are highly glycosylated and possess the antigenic determinants of the virus (Becher & Tautz, 2011). It is not known whether the E^{rns} and E1 possesses neutralizing epitopes that are important in disease control. The E2 protein is the immunodominant structural protein and possesses neutralizing epitopes that function in disease control. Protective antibodies induced by killed vaccines are predominantly against the E2. Monoclonal antibodies (Mab's) produced against the E2 have been used to differentiate between BVDV-1 and BVDV-2 strains (Kadir *et al.*, 2017).

2.1.2. Non-structural Proteins

The first viral protein encoded by the BVDV open reading frame is the non-structural protein, N^{pro}. This protein is unique to the genus *Pestivirus* and auto catalytically releases itself from the polyprotein. This protein is not essential for virus replication in cell culture, but modulates interferon responses in infected cells. The next nonstructural proteinp7 follows the structural protein E2 in the polyprotein. While the role of this cell associated protein is unknown, it is hypothesized that it is required for production of infectious virus but not for RNA replication. The p7 protein is inefficiently cleaved from the E2 during processing of the polyprotein. This leads to two intracellular forms of E2 with different C termini (E2 and E2-p7). However, neither of p7 nor E2-p7 is found associated with infectious virus. Following p7 is the serine protease, NS2-3. In BVDV strains from the cp biotype the NS2-3 is cleaved to NS2 and NS3 (Ridpath *et al.*, 2010; Becher & Tautz, 2011).

Both the un-cleaved NS2-3 and the cleaved NS3 act as serine proteases that cleave the remaining nonstructural proteins from the polyprotein. The function of the NS2 is unknown. It is not required for RNA replication and its cleavage from the NS2-3 does not affect serine protease activity. Purified BVDV NS3 also possesses RNA helicase and RNA-stimulated NTPase activities and all three activities (serine protease, RNA helicase and RNA stimulated NTPase) are essential to virus viability (Xia *et al.*, 2007). While antibodies to the NS2-3 and NS3 do not neutralize infectivity, these proteins possess immune dominant epitopes. The NS2-3 and NS3 (but not the NS2), are strongly recognized by polyclonal convalescent sera and animals vaccinated with modified live vaccines have as nearly a strong antibody response to the NS2-3 and/or NS3 protein as to the E2 structural protein. In contrast, animals vaccinated with inactivated (killed) vaccines primarily react with structural proteins and not the NS2-3 or NS3. The difference in recognition of NS2-3 or NS3 may be useful in differentiating between immune responses to inactivated vaccines and immune responses to natural infection (Brian *et al.*, 2010).

The NS4A and NS4B proteins are similar in size, composition and hydrophobicity to the NS4A and NS4B proteins of other *Flaviviruses.* A NS4A acts as a co-factor for the NS2-3 and NS3 serine protease activity. Both NS4B and NS5A probably are replicase complex

components. RNA polymerase activity has been demonstrated for the NS5B protein (Mishra *et al.*, 2007, MacLachlan & Dubovi, 2011).

Bovine viral diarrhea virus is not stable in the environment. It is easily inactivated by heat and can only survive for one hour at 56°c. At room temperature, it survives for up to 5 days but the virus can be stored for up to 16 months at -40°c (Botner & Belsham, 2012). The virus survives best at pH range 5.7 up to 9.3 with maximum viability at pH 7.4. Common disinfectants like phenols and chlorhexidine readily inactivate the virus (MacLachlan & Dubovi, 2011).

2.2. Epidemiology

Bovine viral diarrhea virus is endemic in majority of the world (Ridpath *et al.*, 2010). There are many factors in the epidemiology of BVDV infections. Persistently infected animals, uncontrolled animal movement (livestock trade) and inter-species transfer are the main factors for the spread of infection. Persistently infected animals are a key cause of spreading the infection and hence, they represent a risk to the herd (Peterhans & Schweizer, 2013). Although *Pestiviruses* were initially designated according to their host of origin, BVDV infection can also occur in reservoir hosts; sheep, goats, swine and wild ruminants. All of these animals can act as reservoirs for the virus in nature and hence, as the source of cattle infection (Yazici *et al.*, 2012; Kurcubic *et al.*, 2015; Khodakaram & Farjanikish, 2017).

Because of the short duration of the infection and the intermittent shedding of virus, transiently infected animals have a minor importance in the epidemiology of BVDV (Heisman *et al.*, 2009). However, a persistently infected (PI) animal is the main risk factor for the spread of BVDV. This is due to the lifelong shedding of virus through their secretions and excretions (Safarpoor &Haghighi, 2012).

Contamination of fetal bovine serum (FBS) by the ncp biotype of BVDV has long been known and also remains a recognized risk factor for worldwide distribution of BVDV. Because FBS is used in the production of vaccines and other biological products, the global

trade of infected FBS products is a potential source of trans-boundary spread of BVDV (Strong *et al.*, 2013; Bauerman *et al.*, 2014).

Generally, the patterns of disease may vary substantially within and between herds, depending on herd immunity, including vaccination status as well as the presence or absence of persistently infected cattle in the herds. The complex epidemiology of BVDV partially lies in its ability to infect the fetus. If the infection occurs between the second and fourth month of gestation, the virus is able to cause a persistent infection of the fetus, which may result in the birth of a persistently infected (PI) calf (Peterhans *et al.*, 2010).

2.3. Mode of BVDV Transmissions

The main transmission route in infected herds is direct contact with a PI animal. The virus is transmitted by direct contact between a PI animal and a susceptible animal or transplacentally from dam to fetus (Radostits *et al.*, 2007).The horizontal transmission of BVDV may be direct or indirect via inhalation or ingestion of virus contaminated materials (Lanyon *et al.*, 2014). The common mechanisms of horizontal transmission include: fomites (feed, water, nose tongs, milk bottle nipples, needles), secretions and excretions (urine, feces, mucus, milk),crowding (can also increase transmission if animals are infected with the respiratory type of BVDV) and vectors(horse flies, stable flies, head flies, face flies) have also been shown to transmit BVDV (Lindberg *et al*., 2005; Safarpoor & Haghighi, 2012).

Transient infection (TI) is the most frequent route of BVDV infections through oro-nasal uptake of the virus. This type of infection occurs when a previously unexposed healthy animal (naive animal) becomes infected with BVDV (Pedrera *et al.*, 2012). Acute natural infections of BVDV seronegative cattle result in a transient viremia, starting 3 days' post infection. The duration of virus shedding by transiently infected animals likely depends on the virulence of BVDV strains and their efficiency to replicate and varies from less than 1day to 2 weeks (Lanyon *et al.*, 2014).Following the clearance of the virus from the blood and a systemic immune response, a prolonged spread of BVDV may still be possible, due to sequestration of the virus (Givens & Marley, 2013).

Vertical transmission often occurs when a dam that not persistently infected by ncp strain and when she experiences an acute infection by ncp strain. Persistently infected pregnant dams invariably transmit the virus to the fetus, resulting in new generation of persistently infected calves (Passler *et al.*, 2007). Persistently infected calves thus always result from congenital BVDV transmission, either by transmission from persistently infected dam to her fetus or via acute infection of the dam with ncp virus between day 30 and first trimester of gestation. In another way, semen from PI bulls contains large amounts of virus and breeding with such semen can result in acute infection of BVDV seronegative dams and possibly a persistently infected calf (Marley *et al.*, 2009).

In vertical transmission, the outcome of infection is determined by the infecting virus strain and stage of fetal maturation when exposed to the virus *in utero*. During the first 18 days of pregnancy; while embryo is unattached, no infection of the embryo occurs as BVDV does not penetrate the *zona pellucid* (Sayers *et al.*, 2015). Once cotyledons develop, viremia of the dam from days 29 to 41 post conceptions can result in embryonic infection leading directly to embryonic death and reduced pregnancy rates (Lanyon *et al.*, 2014).

Persistently infected (PI) calves are the result of *in utero* BVDV infection during the period of fetal development from gestation day 45 to gestation day 125 (Peterhans & Schweitzer, 2013). This gestational period is bracketed by the end of the embryonic stage and the development of fetal immune-competence. In these *feti*, the viral protein is recognized as self, resulting in an immune tolerant state and persistent viremia without seroconversion. However; if a different strain of the virus infects the PI animal (super infection), they can immunologically respond which resulting in seropositivity (Walz *et al.*, 2010).

Infection between 80 and 150 days of gestation can lead to teratogenic effects in the fetus. These include cerebellar atrophy, arthrogryposis, ocular degeneration and pseudo cyst formation in the brain, thymus and bone (Blanchard *et al.*, 2010; Webb *et al.*, 2012). If the fetus is infected in the third trimester of gestation (>180 days), the fetus is immunocompetent and will mount an immune response that may result in abortion, or the birth of weak, ill-thrifty calf which more susceptible to other infections. However, the calves' receiving

colostrum achieves a passive immunity against BVDV. The maternal antibodies in calve can be detected within few hours after the first meal and the level is declined at a rate of one half their remaining antibody titers every 21 days (Khezri, 2015). Therefore, it is very important to identify and remove them from the cattle herd.

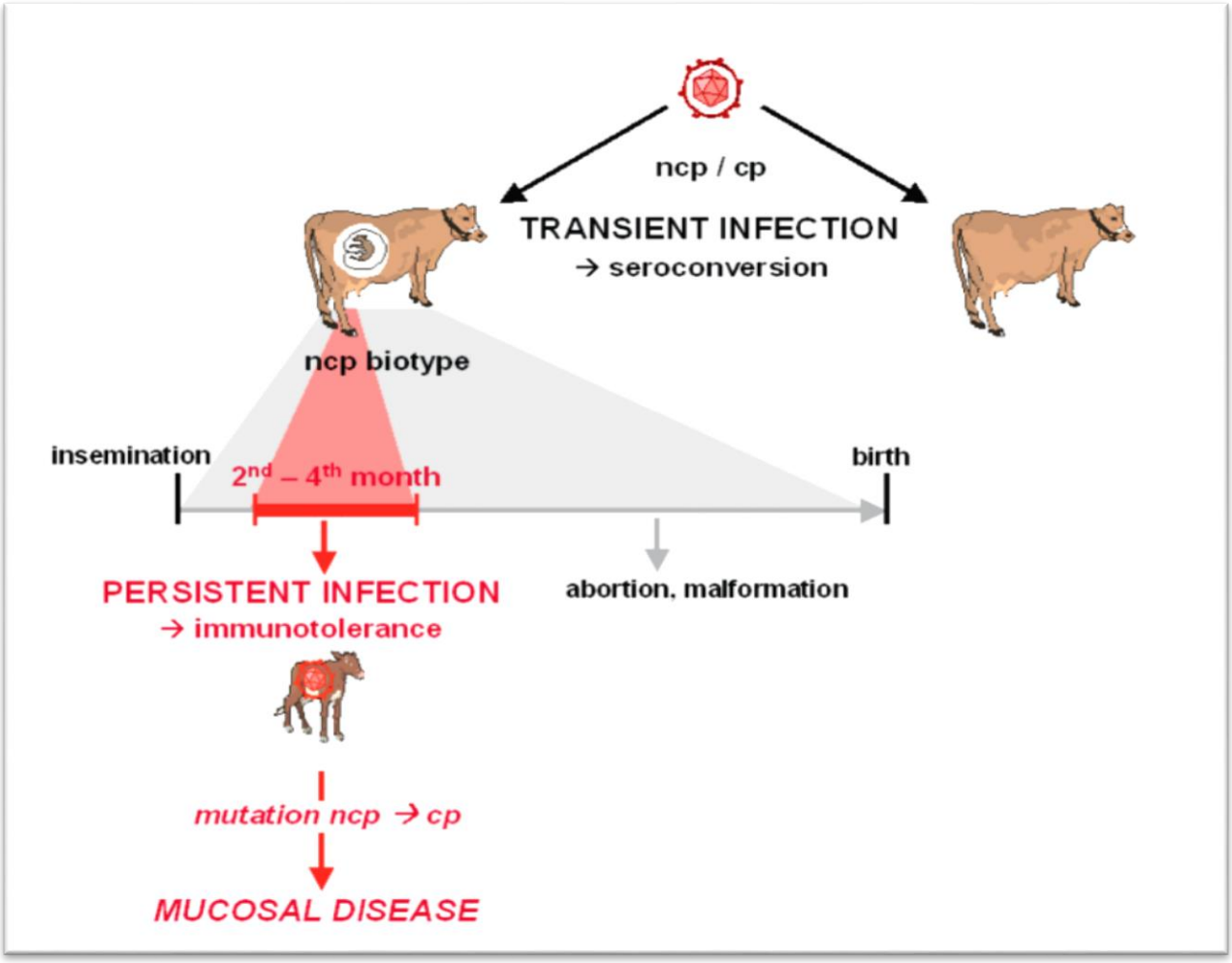

Figure 3: Persistent versus Transient Infection (Peterhans *et al.*, 2010)

The ability of ncp BVDV to inhibit the induction of type-I interferon in the fetus enables the virus to survive in the host and establish PI animals. These PI animals do not mount an antibody response or clear the virus and will shed large amounts of virus in all excretions and secretions (milk, semen, saliva, nasal secretions, urine, blood and aerosols) (Peterhans & Schweizer, 2013).

2.4. Pathogenesis of BVDV

Bovine viral diarrhea virus is naturally transmitting via the oro-nasal passages. The primary replication site of the virus is both the mucous membrane and tonsils. From these sites, it spread to the lymphocytes in the local lymph nodes and disseminates through the body via leukocyte circulation. To establish infection in vivo, viruses must replicate in the face of powerful innate and acquired immune response mechanisms. The innate immune response constitutes the first line of host defense against an invading virus and therefore, plays a crucial role in the early recognition and subsequent triggering of a proinflamatory response. For this purpose, the innate immune response represents two main mechanisms (interferon (IFN) production and induction of apoptosis) to limit the infection at the cellular level (Lindenbach *et al.*, 2007; Oguzoglu, 2012).

Based on BVDV studies, *Pestvirus* species appear to inhibit IFN synthesis; however, themechanism of inhibition of IFN synthesis by ncp *Pestvirus* strains in infected cells remains unclear. The ncp strains, but not cp strains, possess a function that inhibits IFN production in response to infection (Sayers *et al.*, 2015). Interferon regulatory factor-3(IRF-3) a transcriptional activatorresponsible for the increased transcription of IFN genes and closely associated with the induction of IFN-α/β is ubiquitously expressed in the cytoplasm and it is activated in response to viral infection (Oguzoglu, 2012).Many viruses, including *Pestvirus* species have mechanisms to interfere with theIRF-3 pathway, thus, inhibiting the induction of IFN-α/β. The N^{pro}, from both BVDVs and CSFs is essential for evading the cellular antiviral defense system. It targets IRF-3 for proteasomal degradation, significantly decreasing the amount of available IRF3 anddisrupting the IFN-α/β response (Bauhofer *et al.*, 2007).

Bovine viral diarrhea virus infections are associated with the direct effects on B-and T-cells and apoptosis (programmed cell death) of immune cells in gut-associated lymphoid tissue (Pedrera *et al.*, 2012; Sayers *et al.*, 2015).From a virological standpoint, apoptosis is an important aspect of the pathogenesis of viral infections. The primary purpose of apoptosis is to kill the virus-infected cell to prevent the virus from replicating, producing progeny and spreading to neighboring cells(Peterhans & Schweizer, 2013). Apoptosis is also a means to

kill the cell without inducing an inflammatory response that may damage the surrounding tissue (Bielefeldt*et al.*, 2008). Many viruses encode proteins to inhibit apoptosis until viral replication steps have been conducted, allowing production of maximal levels of progeny virus. Although no inhibitor of apoptosis has been specifically identified in the *Pestvirus* genomes, apoptosis has been induced in cultured cells with the cp strains (Bendfeldt *et al.*, 2007). In contrast, the ncp BVDV does not cause any visible alteration or induce the synthesis of IFN-α/β in its host cells, however; it does inhibit apoptotic cell death *in vivo*, as well as *invitro* (Oguzoglu, 2012).

A NS3 protease expressed by the cp BVDV results the induction of apoptosis (Gamlen *et al.*, 2010). Double stranded RNA is produced by the virus in infected cells triggering apoptosis by intrinsic and extrinsic pathways. Intrinsic pathways are regulated by the release of cytochrome from mitochondria inducing activation of the death regulator, apoptotic protease activating factor. External pathways include up-regulation of tumor necrosis factor alpha (TNF-α), a key cytokine participating in apoptosis execution. These changes occur primarily in the Peyer's patches leading to lymphoid depletion and atrophy. Micro villi disappear from the lamina propria over the Peyer's patches. Cell debris and mucous accumulate in dilated intestinal gland crypts giving the appearance of necrosis (Pedrera *et al.*, 2012).

Cytopathic BVDV localizes in the germinal centers of lymph nodes, tonsils and gut associated lymphoid tissue of Peyer's patches before spreading to gastrointestinal epithelium. It promotes monocyte activation and differentiation, while at the same time inhibiting antigen presentation to T-cells. This leads to uncontrolled inflammation and enhanced viremia, while impairing antiviral defenses (Pedrera *et al.*, 2012). Necrosis of keratinocytes in the stratum spinosum leads to disruption of intercellular junctions in the keratinized epithelium of the skin, muzzle, oral cavity, esophagus, rumen, reticulum and omasum. Normal wear and tear at the epithelial surface leads to erosion and ulceration of the weakened surface exposing underlying connective tissues. Leakage of fluid from the denuded surface of the gastrointestinal tract leads to diarrhea and dehydration, while bacterial infection and inflammation at the exposed sites results in secondary septicemia. Diarrhea, erosion and inflammation induce noticeable disease in affected animals bringing them to the attention of

the farmer and veterinarian. Death may occur within a few days or be protracted and take a few weeks (Lanyon *et al.*, 2014).

Bovine viral diarrhea virus's global success is credited to its ability in altering the mechanisms through which it establishes infection. While more virulent strain commonly initiates acute infections, most strains, particularly ncp strains, typically establish persistent infections during fetal development. BVDV has a unique and remarkable ability to bypass the adaptive immune system by gaining immune tolerance to both B- and T-lymphocytes, which is in addition to its ability to subvert components of the innate immune system (Lee *et al.*, 2009).

The appearance of a cp BVDV biotype arising from mutation of ncp BVDV that already circulating in the PI animal may result with the fatal form BVD called Mucosal Disease (MD). Mutations underlying the change in biotype include insertion of cellular sequences, gene duplication, deletion and single nucleotide changes. All cp biotype produce the non-structural (NS) protein NS3, whereas in ncp biotype only the un-cleaved form NS2/3 can be detected (Peterhans *et al.*, 2010; Lanyon *et al.*, 2014).

2.5. Clinical Signs

Bovine viral diarrhea virus is an economically important pathogen causing gastrointestinal, respiratory and reproductive disease in cattle. The clinical offering of BVDV infection is based on viral strain, the animal's immune status and stages of pregnancy at the time of infection (Givens *et al.*, 2012; Rodninga *et al.*, 2012).

Although acute infections with BVDV are often asymptomatic, clinical signs such as diarrhea, depression, oral erosion, loss of appetite, decreased milk production, fever, drooping ears, excessive lacrimation, nasal discharge, hyper salivation, hemorrhages of the gastrointestinal tracts, dehydration, embryonic death, abortion, teratogenesis, respiratory problems, immune system dysfunction, and death may occur (Rodninga *et al.*, 2012). In mild cases, diarrhea may not be prominent. Most of BVDV infections are sub-clinical and the course of the disease varies from 2-3 days up to 4 weeks; however, this results in measurable increases in antibody

levels. Calves with clinical BVD as dull, depressed, anorectic and mild bloat may occur. During the early period of infection, body temperature rises up to 41^{o}c but usually return to normal or below in 1-2 days and before diarrhea occur (Mohammad, 2015).

In calves that receive antibodies in colostrum, antibody disappears by 3-8 months of age and these animals may not show clinical signs after infection until colostral antibodies are lost. A biphasic fever and leucopenia occur in susceptible animals within an incubation period of 5-7 days after infection but the clinical course is usually mild (MacLachlan & Dubovi, 2011).

In acute mucosal disease cases, the infected animal will likely exhibit many of the aforementioned symptoms; notably exude excessive saliva and mucous due to the formation of ulcers within its nose and mouth. In severe cases, the animal will die within days of clinical onset. However, mucosal disease may also manifest chronically; in which case, animals often exhibit lameness, persistent weight loss, intermittent bouts of diarrhea, and respiratory disease (Rodninga *et al.,* 2012).

2.6. Diagnosis

As most BVDV infections occurs sub clinically and because of the broad range of clinical disease manifestations, the diagnosis of BVDV based upon clinical signs is not obvious and should therefore be supported by laboratory tests. Because of the insidious and complex nature of BVDV, laboratory diagnosis is critical in preventing and controlling BVDV infections. Fortunately, these tests are very reliable with their high sensitivity and specificity. There are two diagnostic approaches to detect BVDV infection, direct tests (detection of the virus or viral components) and indirect tests (detection of the immune response to BVDV) (Dubovi, 2013).

2.6.1. Virus-specific Antigen Detection

2.6.1.1. Virus Isolation (VI)

Virus isolation has been the "gold standard" for BVDV detection and will continue to be an important diagnostic test of BVDV. The virus is relatively easy to isolate from a variety of specimens including serum, buffy coats (white blood cells), nasal swabs and tissue samples (Dubovi *et al.*, 2013).Several factors must be considered in selecting the appropriate sample and method for virus isolation. Since BVDV appears to replicate best in lymphoid cells, samples that contain these cell types should be considered, especially when attempting to identify acute infections. These samples would include whole blood from which buffy coats can be isolated and lymphoid tissue such as Peyer's patches mesenteric lymph nodes, spleen and thymus from postmortem cattle or aborted fetuses. In cattle persistently infected with BVDV, virus can usually be isolated from serum, buffy coats and a majority of tissues (although lymphoid tissues are preferable) (Saliki & Dubovi, 2004).

Regarding the sample, the highest sensitivity is obtained with a blood sample from which viable mononuclear cells can be harvested. In this way, virus can even be isolated from sample containing antibodies. Viable cells come in close contact with the indicator cells permitting infection without the virus coming in contact with neutralizing antibodies. Freezing and thawing of the mononuclear cells to release the virus is unnecessary and permits the neutralization of the virus by antibodies, leading to false negative results (Dubovi *et al.*, 2013).

2.6.1.2. Antigen Captured ELISA (Ag-ELISA)

Antigen enzyme linked immunosorbent assays (Ag-ELISA) is used to detect the presence of BVDV antigen in various sample matrices such as non-coagulated blood, serum, skin biopsies (ear notches), tissue samples (spleen, lung, liver, and kidney) and milk (Lanyon *et al.*, 2014). Suitable viral proteins for detection are the envelope glycoproteins and the nonstructural protein. The Ag-ELISA is robust, simple and cost efficient but false negative test results may occur due to the presence of colostral antibodies that capture the antigens and make them

unavailable for the test (Fux & Wolf, 2012). Cross-reactivity with border disease virus has been observed in a commercially available BVDV Ag-ELISA (McFadden *et al.*, 2012).

2.6.1.3. Fluorescent Antibody (FA) Test

Fluorescent antibody (FA) test is another commonly used test for directly identification of BVDV antigen detection in fresh frozen tissues. It is most commonly used test because of its rapidity and for detection of acute infections. This assay is often used with smear preparations made from samples such as nasal swabs, lymph nodes, spleen and is often performed on gross necropsy samples as a first line screening assay for virus presence. The sensitivity and specificity of this test varies widely (Srinivas & Srikanth, 2012).

2.6.1.4. Immunohistochemistry (IHC) Test

Immunohistochemistry (IHC) test is used to identify BVDV antigen in frozen or formalin fixed tissues. This has clear advantages as tissue morphology is maintained which allows virus to be identified in conjunction with histopathological findings. Immunohistochemistry test is useful when investigating disease outbreaks that involve the respiratory, gastrointestinal or reproductive system where BVDV is suspected. Using immunohistochemistry, BVDV can be detected in properly fixed tissues for an extended period of time whereas the ability to isolate virus from fresh tissues can dissipates rapidly with time. This is especially advantageous when field samples cannot immediately be submitted to a diagnostic laboratory. Immunohistochemistry can also be used to look retrospectively for BVDV or other pathogens of interest in properly fixed tissues (Oguzoglu, 2012).

2.6.1.5. Reverse-Transcription Polymerase Chain Reaction (RT-PCR) Test

Reverse-transcription polymerase chain reaction test is used for direct identification of BVDV viral genomic RNA or a synthesized copy of the RNA called cDNA. It is widely accepted as the standard for BVDV diagnosis as it is faster and less expensive compared to virus isolation. It is 10 to 1000 times more sensitive than virus isolation and also suitable for a large variety of samples, including blood, milk, saliva, follicular fluid and tissue samples (Lanyon *et al.*, 2014).

The main advantages of RT-PCR are a high sensitivity, no interference of antibodies with the detection of the virus genome and the possibility to pool the samples as a consequence of the high sensitivity. By using specific primers specific to the 5′ un-translated region, it is possible to distinguish between BVDV-1 and BVDV-2 by using RT-PCR test. This may be useful in designing vaccine programs aimed at controlling different genotypes of BVDV (Khodakaram*et al.*, 2016).

2.6.2. Detection of BVDV Infection based on the Immune Response

Detection of antibodies in cattle is a valuable way of determining an individual animal's immune status and any previous exposure to the virus. A positive antibody test in unvaccinated individual will not only indicate that an animal has been previously exposed to BVDV, but that it is not PI. A positive result in a pregnant female will indicate the possibility that she is carrying a PI fetus. However, a negative antibody result in an individual does not confirm the animal as BVDV naive; further virus or antigen testing is required to confirm the animal is not PI (Lanyon *et al.*, 2014).

2.6.2.1. Virus Neutralization (VN) Test

The virus neutralization test is based on the inhibitory effect of antibodies on virus replication in cultured cells. Therefore, it requires cell culturing facilities and contamination control. It is suitable test for quantification of antibodies by titration. This test is also valuable using single serum samples from a group of animals to determine the infection status of a herd. It can also be used to evaluate the vaccination program (Srinivas & Srikanth, 2012). The sensitivity of the test depends on the cells used, the antigenic relatedness between the antibodies in the sample and the virus strains used in the test. As it is a labor intensive and expensive test, it is mostly used as the reference test. As an alternative to the virus neutralization test, indirect and competitive ELISAs are commonly used (Lanyon *et al.*, 2014).

2.6.2.2. Antibody ELISA (Ab-ELISA) Test

The ELISA systems use a color reaction measured as optical density (OD). Multiple ELISAs are commercially available for the detection of BVDV specific antibodies and have been validated for use in various samples, including serum, milk and bulk milk and will detect colostrums antibodies in suckling calves. In the competitive ELISA, the reaction is measured as percentage of inhibition relative to that of a negative control sample. Antibodies ELISA test have the advantage of being fast and inexpensive and they do not depend on cell culturing facilities. The OD values and percentage inhibition values may also be used as semi-quantitative measures (Fux & Wolf, 2012).

There are two principal types of Ab-ELISA test; indirect and competitive (blocking) one. In an indirect ELISAs, antibodies are trapped by immobilized antigen and detected using enzyme conjugated species-specific antiglobulins and a chromogenic substrate. The optical density (OD) is then measured, which will be higher in a positive sample than in a negative. In competitive ELISAs, virus specific antibodies in the sample block the binding of conjugated virus-specific antibodies to fixed viral antigen. In contrast to the indirect ELISA, a positive sample in a competitive ELISA will yield a weaker signal than a negative sample (Raue *et al.*, 2011).

Inference of vaccination with Ab-ELISA test is an issue as no marker vaccine to differentiate between infected and vaccinated animals is available up to date and no Ab-ELISA tests are able to differentiate vaccine-induced antibodies from antibodies following natural infections (Makoschey *et al.*, 2007; Raue *et al.*, 2011).

2.7. BVDV Risk Factors

Direct animal contact is the most efficient method of viral transmission from one animal to another. A herd can be considered as BVDV infected by the presence of BVDV seropositive animals following serologic examination or by the presence of virus-positive animals (Graham *et al.*, 2013). The main risk factors for introducing BVDV to a herd are housing and management system, communal pastures for grazing, contact with wild animals, herd size,

livestock trade (animal introduction), rearing cattle with small ruminants, uncontrolled animal movement and lack of separation of newly purchased cows from the herd (Saa *et al.*, 2012; Gates *et al.*, 2013; Sarrazin *et al*., 2013).

Different management techniques in different farms could be an important risk factor for the maintenance of the virus within herds. For example, in Croatia female calves on big farms were separated from the herds early after birth and stay separately until they reach sexual maturity. After artificial insemination heifers are placed they back into the herd. For these reasons, heifers are seronegative. When heifers come infected with the virus during the second trimester of pregnancy, the virus can infect the fetus and cause persistent infection (Bendevic *et al.*, 2013).

Bovine viral diarrhea is a contagious disease and can also transmit among inter-species of both domestic and wild animals (Pinior *et al.*, 2017). Transmission may also occur through the use of milk and other derivatives from infected cattle in pig feed (Deng *et al.*, 2012). Regular contact with goats and sheep has been shown to increase the risk of infection (Mischra *et al.*, 2009; Bachofen *et al.*, 2013; Kaiser *et al.*, 2017). Wildlife species act as disease reservoirs for domestic livestock they can seriously undermine the effectiveness of any disease control strategy in domestic species by escalating the number of susceptible animals in which the disease can persist, especially at the wildlife-livestock interface (Roman *et al*., 2008; Rodriguez *et al.*, 2016). Therefore, lack of biosecurity or communal pastures for grazing are risk for BVDV transmission within and or different species of animals (Khezri, 2015; Wolf *et al.*, 2017).

Increasing herd size emerged as a factor associated with disclosure of BVDV positive animals. Increasing herd size increases the probability of an individual animal becoming exposed to pathogens, becoming a carrier animal and is a well-recognized risk factor for the occurrence of disease. The higher density of animals and small area of farm, agglomeration and direct contact between the animals are favors the dissemination of BVDV. Direct contact is the most efficient way to transmit the virus, especially in the presence of PI animals in the herd (Marques *et al.*, 2016). Larger herds are likely to have biosecurity risks such as increased

purchase of animals and increased visitors (veterinary practitioners, technicians, contract workers), all of which will increase the risk of disease introduction and maintenance (Sayers *et al.*, 2015; Barrett *et al.*, 2018). Small herds are more likely to eliminate BVDV infections spontaneously. The probability of this self-clearance depends on the prevalence of susceptible animals in early pregnancy, which is lower in small herds (Sarrazin *et al.*, 2013).

Herd size has previously been documented as a significant risk factor associated with exposure to the BVDV virus in many countries. The larger number of animals within a farm is associated with an increased level of BVDV antibodies due to the high probability of PI animals' presence in the herds (Humphry *et al.*, 2012; Gates *et al.*, 2013). Almeida *et al*. (2013) found that a larger herd size was a risk factor for Brazilian dairy herds being BVDV seropositive when considering dairy herds BVDV seropositive based on bulk tank milk analysis. In Tanzanian also herd size was reported as the risk of BVDV introduction to cattle population with management system (Coletha *et al.*, 2017).

Purchase and exchange of animals from different sources are classic risk factors the occurrence and dissemination of the virus within and between herds (Marques *et al.*, 2016). Cuttance & Cuttance, (2014) identified the purchase of animals (introduction of replacement heifers) as a risk factor for being BVDV seropositive at the herd level, together with the farmer considering BVD was an issue on the farm and an increasing number of heifers on the farm. A larger number of heifers could suggest a higher probability of infecting at least one susceptible dam in early gestation, resulting in the birth of a PI animal.

2.8. Prevention and Control of BVDV

BVDV control strategies can be described as being either non-systematic or systematic (Lindberg *et al.*, 2006). A systematic approach implies that there is a goal oriented reduction in the incidence and prevalence of BVDV infections.Systematic control can be implemented either at the herd level or at theregional/national level.Three essential elements of systematic control approaches can be identified: a) biosecurity measures to prevent reintroduction, b) elimination of PI animals from infected herds, and c) monitoring the BVDV status. Non-systematic approaches lack one or more of these elements (Laureyns, 2014).

The implementation of a program to control the infection must be based on; first, the biosecurity measures have to implement in order toprevent reintroduction, direct contacts with possibly infected herds. However, due to the nature of BVDV with high mortality among PI calves, BVDV infected herd is cleared from infection without intervention in certain cases. Although biosecurity is not necessarily focuses on one particular infectious disease and aims at upgrading herd health in general, measures are based on the knowledge of the epidemiology of specific. Despite the fact that between pathogens there are considerable epidemiologic differences such as the reservoir, modes of transmission and incubation period, the basic principle is to reduce contact between disease agents and susceptible animals. Animate vectors such as pets, insects, rodents and wild birds can spread disease both between and within farms and specific measures should be implemented (Waltz *et al.*, 2010).

Vaccinationis an additional biosecurity measure may be advised in cattle-dense areas with intense cattle trading and a high BVDV prevalence to avoid infection.For vaccination to succeed, also the three essential measures for systematic BVDV control have to be implemented (Ridpath, 2013). Therefore, it represents an accompanying tool to prevent BVDV, but without removing PI animals it does not enable the elimination of the virus in a susceptible population. Incorrect use of vaccination can lead to incomplete protection and should be implemented in combination with the previously mentioned essential control measures (Meadows, 2010). The genetic variations described for BVDV-1 and BVDV-2 may be implicated in disease control as diagnostics and vaccines that work well against homologous strains can be less efficacious for genetically distinct viruses (Peleto *et al.*, 2012).

In areas where the disease is present and vaccination is not implemented, control strategies based on the analysis of bulk milk at dairy farms would greatly reduce the overall costs of testing while ensuring a good coverage and efficiency at the system level, as it has been done for other diseases (Muratore *et al.*, 2017). It can be used as the first step in a control strategy to evaluate the possibly infected and no infected dairy herds (Grooms *et al.*, 2014). In Switzerland, near-eradication has been achieved by serological testing of every head, followed by bulk milk surveillance of disease free farms (Thomann*et al.*, 2017).

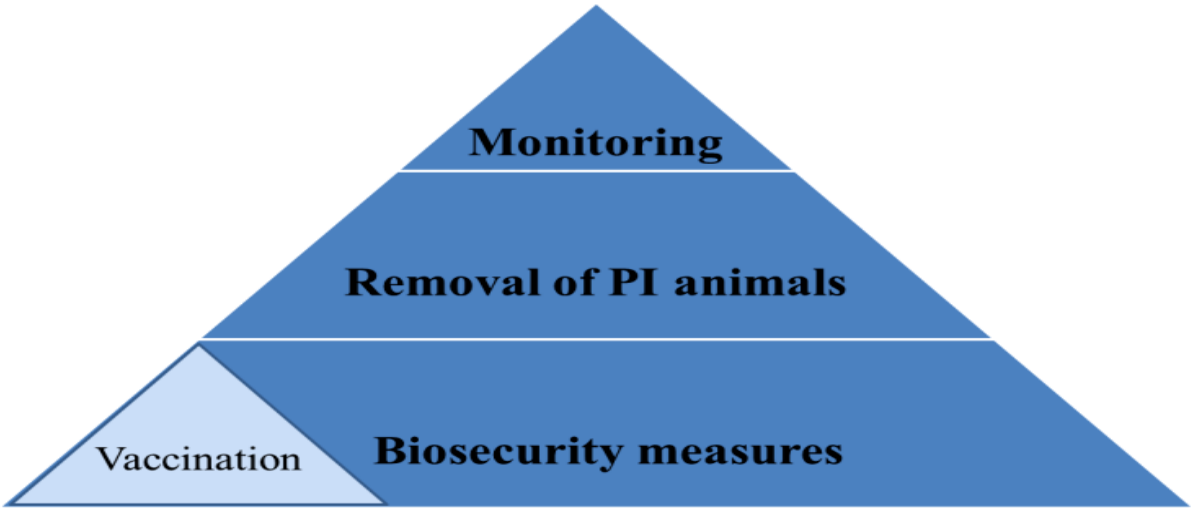

Figure 4: General Model for BVDV Control(Laurens, 2014)

Persistently infected animals have immunotolerance to the strain or strains with which they have been infected and commonly shed large quantities of BVDV throughout life, thus exposing herd mates and jeopardizing efforts to control and/or eradicate BVDV. Therefore, identification and elimination of PI animals is the key factor in BVDV control (Nelson *et al.*, 2016). Once BVDV is eliminated from a herd, a rigorous biosecurity program embraces all aspects of the prevention of pathogens entering and spreading within a group of animals. It can be divided into external biosecurity, which includes all measures preventing pathogens from entering a herd and internal biosecurity which embraces measures reducing the spread of pathogens within a herd (Villarreal*et al.*, 2007).

Monitoring or follow up of the BVDV status is a third essential measure for successful BVDV control and evaluates the effectiveness of elimination of BVDV in infected herds. Monitoring also serves for detection of new infections and therefore herds with no history of BVDV infections should be strongly encouraged to monitor the BVDV status, as re-infections with BVDV often occur. Whenever monitoring involves specific actions in case of a possible re-infection, this is defined as surveillance. Specific actions include for instance testing of newborn calves to early detect new PI animals or testing diseased animals for BVDV viremia (Lindberg *et al.*, 2006).

2.9. Economic Importance of BVDV Infection

Bovine viral diarrhea virus infection in ruminant has been reported by many countries worldwide and is listed by the Office International des Epizootic (OIE) as a notifiable in 2007 and priority cattle disease for international trade due to its economic importance (Walz *et al.*, 2010). It affects cattle populations around the world with significant financial consequences. The losses associated with BVD stem from reproductive loss (early embryonic death, abortion, reduced milk yield, congenital defects, growth retardation, extended calving intervals, reduced first service conception) are a direct losses. The occurrence of other diseases because of BVD related immunosuppression and increased control efforts are indirect losses (Lanyon & Reichel, 2013; Daves *et al.*, 2016; Pinior *et al.*, 2017).

The economic effects of BVDV infection highly depends on the risk of new infections and on the strain of virus involved. Epidemic outbreaks of BVDV in naive herds can be explosive and typically result from the introduction of the virus (usually a PI animal) into a highly susceptible population. The losses are self-limiting, as an increase in herd immunity will limit consequences in following years. However, even in endemically infected herds, in which a high level of immunity is common, consistent low level losses result in substantial (often unrecognized) losses for many years (Heuer *et al.*, 2007; Michael *et al.*, 2018).

The economic damage caused by BVDV can vary substantially because of the multiplicity and variations in severity of symptoms mentioned above and the interactions with other pathogens. Furthermore, management factors and structure of the herd play an important role. For example, the outcome of the BVDV infection can be disastrous in herds with a concentrated seasonal calving pathogen. In contrast, small herds can become self-cleared of the infection with hardly any damage (Stahl *et al.*, 2008).

3. MATERIALS AND METHODS

3.1. Study Area

The study was done in Jimma town dairy farms; southwestern part of Ethiopia from January, 2016-January, 2017. The town is located 352 km Southwest of Addis Ababa between, 7º41' N latitude and 36º50' E longitudes and has an altitude of 1704 meters above sea level. The study area receives a mean annual rainfall of about 1530 millimeters that comes from the long and short rainy seasons. The mean annual minimum and maximum temperatures are 14.4°c and 26.7°c respectively with dominant warm and humid weather condition (Nigussie *et al.*, 2010). Jimma town livestock populations were estimated at: 53,250 heads of cattle, 25,230 heads of sheep, 12,570 heads of goats, 10,030 heads of equine, 90,157 heads of poultry (JTLFRDO, 2017).

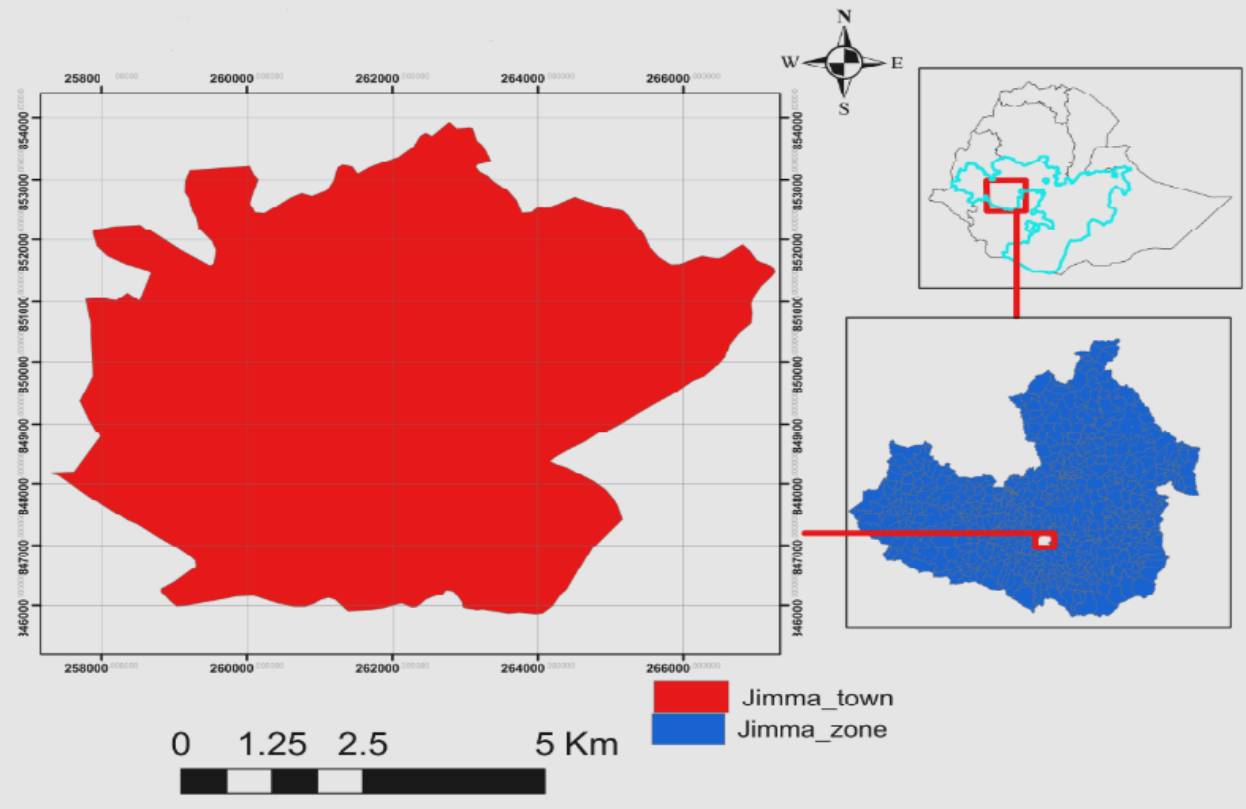

Figure 5: Map of Jimma town, Jimma zone, Ethiopia

3.2. Study Population and Their Management

The target populations were dairy herds' in Jimma town which are composed of Holstein-Friesian crosses breeds. Most of these smallholder dairy farms predominantly keep a small number of animals in a zero-grazing system to produce milk for both home use and sale. The farmers were keep animals in closed house, with corrugated iron sheet roofing, concrete, wooden and earthen floor structure with confined in poor ventilated, unhygienic and crowded stables both day and night. Most of the farmers had both feed and watering troughs in animal shelter. The feed on which the animals are feed include cut natural pasture, hay, *frushka*, milling byproducts, commercial and on farm formulated concentrate.

3.3. Study Design

Cross-sectional study design was used in 45 randomly selected dairy farms out of 61 registered dairy farms in Jimma town from January, 2016 up to January, 2017. All of the sampled animals were Holstein-Friesian crossbreed and were housed in which food supply is by cut and carry method of feeding. Cows are hand milked with twice per day milking frequency. A very few number of farms were used natural mating where as many farms were used AI breeding systems. There was no regular vaccination and spray/dipping, but farmers took their animals for treatment whenever diseases occurred. Only dairy calves above six months of age were included for this study. Relevant individual animal data and farm level information were collected using a semi-structured questionnaire.

3.4. Sampling Strategy and Sample Size Determination

A list of registered dairy farms was collected from Jimma town livestock and fishery resource development office and Jimma town dairy cooperative enterprise office. Depending on the herd sizes, herds were classified into two categories (I-herds with ≤5 animals and II- herds with >5 animals). Animals were also grouped into two age categories; young and adult. Thereafter, one stage cluster sampling method was used due to small number of individual cows per herds. Out of 61 registered dairy farms of the town, 45 farms were selected by

simple random sampling for this study. Thereafter, all animals within the randomly selected farms were included into the sample.

From the previous reports of bovine viral diarrhea virus in intensive dairy farms by Asmare *et al.* (2013), 11.7% expected prevalence was used to calculate the sample size. The minimum required sample size for this study was 159 cattle by using confidence level of 95% and 5% of precision (Thrusfeild, 2007).

$$n = \frac{Z^2 P_{exp}(1 - P_{exp})}{d^2}$$ Where, n = sample size

z = Confidence statistic

P_{exp} = expected prevalence

d = desired absolute precision

$$n = 1.96^2 \times \frac{0.117\ (1\text{-}0.117)}{0.05^2}$$

$$n = 3.84 \times \frac{0.117 \times 0.883}{0.0025} = 159$$

According to the above formula (without considering the design effect), the minimum number of animals to be sampled are 159. To account for the design effect, the calculated sample size (n) was multiplied by the design effect (D) of 2.64 which was calculated by using a formula D = ρ (n-1)+1, where n is average number of sampled cattle per cluster (5), an intra-cluster correlation coefficient of ρ = 0.41 was reported for BVD in cattle (Otte and Gumm, 1997). Thus, 420 cattle were selected to be enrolled in this study.

3.5. Sample Collection and Testing Procedures

Blood samples were collected from the jugular vein into 8ml vacutainer tubes by using sterile needles after cleaning the area with alcohol. After each of the samples were labeled with unique codes that corresponds to farm and individual animal identification codes, it putted in an icebox kit before transported to Jimma University Veterinary Microbiology Laboratory. Thereafter, blood samples were allowed to stand overnight at room temperature before being

centrifuged at 1000g for 10 minutes. Two milliliter of serum was poured into sterile micro tubes (cryovials), labeled accordingly and stored at -20°c in deep freezer until test was conducted. Finally, the samples were transported with an icebox kit to National Veterinary Institute (NVI); Bishoftu, for serology test. The detection of BVDV antibodies in samples was performed by using blocking ELISA kit (Ingezim Pestivirus Compac,12.BVD.K3, Madrid; Spain)according to the manufacturer's instructions (Annex 2).

3.6. Data Management and Analysis

Individual animal data and history of reproductive problem were collected by interviewing the farm owners or attendants by using a semi-structured questionnaire for this purpose (Annex 1). Individual animal level data (age, sex, breed, parity, history of reproduction problem) and farm level data (herd size, contacts with other herds, breeding methodsand introduction of new animals to herds) were obtained.

Data generated from questionnaire survey and laboratory investigations were recorded and coded using Microsoft® Excel for Windows 2007 and transferred to Statistical Package for the Social Sciences (SPSS)version 20.0 (IBM SPSS, 2011). The individual level seroprevalence was calculated as the number of seropositive samples divided by the total number of samples tested; whereas the herd level prevalence was calculated by dividing the number of positive herds by total number of herds tested.

Associations between an outcome (BVDV antibodies seropositive) and explanatory variables (risk factors) for all units of analysis were investigated by using logistic regression model. The strength of the association between outcome and explanatory variables was assessed using the crude and adjusted odds ratios (OR). The explanatory variables ($p<0.25$) were further checked for multicolliniarity using the variance inflation factor (VIF). Variance inflation factor values of greater than 10 or Tolerance less than 0.1 were considered the cut-off points for the collinearity diagnostics (Apeanti, 2016). Variables were also tested for interaction effects using cross-product terms. Multivariable logistic regression procedures were used to model the effects of potential risk factors on outcome variables (BVDV

antibodies). The backward elimination procedure was used to eliminate the factors that were not significant at $p<0.05$ in the overall model. Factors that were significant ($P<0.05$) were retained in the final model and model fit was examined by post-estimation goodness-of-fit tests, namely the Hosmer-Lemeshow test (Hosmer-Lemeshow, 2000). Finally, those variables with $P<0.05$ (adjusted OR, 95% CI) were considered as a significant potential risk factors for BVDV antibody seropositive results.

4. RESULTS

The individual level seroprevalence of bovine viral diarrhea virus in the 420 cattle tested was 51.7% (95% CI: 46.9-56.4%) in which 217 animals were found seropositive. The herd level prevalence of BVDV was 95.6% (95% CI: 85.2-98.8%) that of 43 farms have at least one seropositive for BVDV antibody out of 45 sampled dairy farms.

There was difference in serostatus of BVD among sex, age, parity, history of reproduction problems, herd size, introduction of new animals and breeding methodscategories.Prevalence of BVD was relatively higher in female animals 53.2% (95% CI: 48.4-58.1%), adults age 55.1% (95% CI: 49.9-60.2%), >2 parity 60.4% (95% CI: 53.7-66.6%), cows without history ofreproduction problems (95% CI: 56.8-67.9%), farms with more than five animals 100% (95% CI: 85.1-100%), farms introduced new animals to herds 100% (95% CI: 85.7-100%) and farms used AI 97.5% (95% CI: 87.1-99.6%) categories. In cows with history of repeat breeding, relatively higher prevalence observed compared to cows with history of abortion 40% (95% CI: 24.6-57.7%) (Table 1)

Table 1: Seroprevalence of BVDV antibodies in Jimma town dairy cattle of different categories from January, 2016-January, 2017

Variables	*Categories*	*Total Examined Animals or herds*	*Positive Animals*	*Prevalence (95% CI)*
Sex	Female	402	214	53.2 (48.4 58.1)
	Male	18	3	16.7 (5.8-39.2)
Age	Adult	361	199	55.1 (49.9-60.2)
	Young	59	18	30.5 (20.3-43.2)
Parity	>2 Parity	217	131	60.4(53.7-66.6)
	2 Parity	141	65	46.1(38.1-54.3)
	No Parity	44	18	40.9(27.7-55.6)
History of Reproduction Problems	Abortion	37	2	5.4 (1.5-17.7)
	Repeat Breeding	30	12	40.0 (24.6-57.7)
	No history of Reproduction Problems	291	182	62.5(56.8-67.9)
Herd Size	>5 Animals	22	22	100(85.1-100)
	≤5 Animals	23	21	91.3(73.2-97.6)
Introduction of New Animals	Yes	23	23	100 (85.7-100)
	No	22	20	90.9 (72.2-97.5)
Breeding Methods	Bull Service	5	4	80(37.6-96.4)
	AI	40	39	97.5(87.1-99.6)

There was statistically significant variation (P<0.05) in seroprevalence of BVD in age categories. Adult animals were two times (OR: 2.44; P=0.01) more likely to be infected with BVDV than young animals. Similarly, statistically significant difference in seroprevalence of BVD (P<0.05) was also observed among history of reproduction problems categories. Cows with history of repeat breeding age were almost three times (OR: 2.6; P=0.02) more likely to be exposed to BVDV than cows with no history of reproduction problems. There was also difference in seroprevalence (P=0.002) of BVD in cows with history of abortion compared to cows with no history of reproduction problems. In addition, there was association of BVD seroprevalence with dairy farms introduced animals to their herds (P<0.05). Dairy farms

introduced new animals to their herds were almost two times (OR: 1.6; P=0.04) more likely to be exposed to BVDV than dairy farms those did not new animals to their herds. However, sex, herd size, parity and breeding methods were not statistically associated with BVDVseroprevalence (P > 0.05) (Table 2).

Table 2: Univariable logistic regression analysis of risk factors of BVDV antibodies seropositive in Jimma town dairy herds from January, 2016-January, 2017

Variables	*Categories*	*Prevalence(95 % CI)*	*Univariable Analysis*	
			Crude OR (95% CI)	*P-value*
Sex	Female	53.2 (48.4-58.1)	3.1 (0.76-12.6)	0.11
	Male	16.7 (5.8-39.2)	*	*
Age	Adult	55.1 (49.9-60.2)	2.44 (1.2-4.8)	0.01
	Young	30.5 (20.3-43.2)	*	*
Parity	>2 Parity	60.4 (53.7-66.6)	1.07(0.7-1.7)	0.77
	2 Parity	46.1(38.1-54.3)	0.97(0.02-0.04)	0.97
	No Parity	40.9 (27.7-55.6)	*	*
History of	Repeat Breeding	5.4 (1.5-17.7)	2.6 (1.2-5.7)	0.02
Reproduction Problem	Abortion	40.0 (24.6-57.7)	0.08 (0.02-0.41)	0.002
	No Reproduction Problem	62.5(56.8-67.9)	*	*
Herd Size	>5 Animals	100(85.1-100)	0.78 (0.46-1.3)	0.34
	≤5 Animals	91.3(73.2-97.6)	*	*
Introduction of New	Yes	100 (85.7-100)	1.6 (1.02-2.4)	0.04
Animals	No	90.9 (72.2-97.5)	*	*
Breeding Methods	Bull Service	80(37.6-96.4)	0.41 (0.15-1.14)	0.09
	AI	97.5(87.1-99.6)	*	*

OR: Odd ratio CI: Confidence interval*: Reference

Variables with a P-value (<0.25) in the univariable logistic regression analysis with no multicollinearity were entered into the final multivariable logistic regression model. There was no significant interaction between variables. A Hosmer-Lemeshow goodness-of-fit value (P=0.56), indicated that the model was fit the data.The final multivariable logistic regression model of backward elimination method retained age, introduction of new animals and history of reproduction problems which were independently associated with (P<0.05) BVDV seroprevalence of Jimma town dairy cattle (Table 3).

Table 3: Multivariable logistic regression analysis of potential risk factors of BVDV antibodies in Jimma town dairy farms from January, 2016-January, 2017

Variables	*Categories*	*Multivariable Analysis*	
		Adj. OR (95% CI)	*P-value*
Age	Adult	2.5 (1.3-4.8)	0.01
	Young	*	*
Reproduction Problem	Repeat Breeding	2.4 (1.12-5.3)	0.024
	Abortion	0.08 (0.02-0.4)	0.002
	No Reproduction Problem	*	*
Introduction of New Animals	Yes	1.6 (1.0-2.5)	0.04
	No	*	*

OR: Odd ratio CI: Confidence interval*: Reference

5. DISCUSSION

The estimated individual level seroprevalence (51.7%) was the highest result yet reported in Ethiopian cattle. For example, 9.59% seroprevalence was previously reported by Nigusie *et al*. (2010) in Jimma zone by using indirect ELISA, 11.7% prevalence reported by Asmare *et al*. (2013) in central and southern parts of Ethiopia, 32.9% prevalence recently reported by Asmare *et al*. (2018) in Ethiopian dairy cattle with history of reproductive disorders by using competitive ELISA and 32.6% prevalence by Aragaw *et al*. (2018) in three milk sheds sample using competitive ELISA. This result was also higher than previous reports from other East African countries; 19.8% in Kenya (Callaby *et al.*, 2016) and 10.7% in Sudan (Saeed *et al.*, 2015). However; the high prevalence of BVD in this study be attributed to the fact that previously infected animals has served as source of infection for dairy animal's distribution around Jimma. In addition, it could also be related to the variation in management, study design, sample size, susceptibility and diagnostic tests performance used in the different study.

Many studies were also reported almost the same result in different countries. Among these, 51.1% prevalence reported in Brazil (Rego *et al.*, 2016), 51.75% (Haji & Seyfi, 2007), both 51.58% (Kish *et al.*, 2013) and 52% (Rezaeisaber *et al.*, 2013) in Iran and 51.1% in Bangladesh (Uddin *et al.*, 2017). Even though the result of this study was the highest seroprevalence yet reported in Ethiopia, it was smaller than 78.8% prevalence reported in Mexico (Sakhaee *et al.*, 2009), 77.9% in Iran (Feliciano *et al.*, 2016), 66.4% in Nigeria (Bello *et al.*, 2016) and 61.61% in Croatia (Bedekovic *et al.*, 2013). The antibodies detected in these countries might be due to vaccination as opposed to situation in Ethiopia where there is no vaccination. This indicated as the disease was neglected and remained as one of the economically important diseases highly affecting the health and production of cattle.

The variation of seroprevalence in different countries or regions could be attributable to the differences in management system (grazing practice, herd size, livestock trade, contact with other ruminants, biosecurity), types of tests used, sample size, study design and environmental condition.Many studies conducted in different countries reported that a herd is more likely to

have persistently infected cattle if they are simultaneously farming with small ruminants (Bachofen *et al.,* 2013; Kaiser e*t al.*, 2017) or contact with wild animals (Handel *et al.*, 2011; Malavika *et al*., 2017). Residing in an area where cattle density is high is likely to lead to increased antibody prevalence (Saa *et al.*, 2012). Many studies indicated that prevalence was higher in large herds than in small herds (Graham *et al.*, 2013; Sarrazin *et al.*, 2013). Contact between animals on pasture or over fences between neighboring farms is a risk factor for BVDV infection. Purchasing breeding cattle contributes to increase number of seropositive animals in herds (Handel *et al.*, 2011; Gates *et al.*, 2013).

In this study, the higher proportions of adult animals were seropositive compared to younger animals 55.1% (95% CI: 49.9-60.2%; P<0.05). This result was in line with other studies that reported higher prevalence of BVDV antibody in adult age than young age categories (Callaby *et al.,* 2016; Daves *et al.,* 2016). An increase of seroprevalence as age increases possibly due to an increase in an animal's risk of has been exposed to BVDV (Shirvani *et al.,* 2012). The lower seroprevalence in young could also be due to some of the young animals investigated might be PI animals which are known to be immunotolerant to the virus and do not produce antibody against the virus to be detected by the ELISA test (Abbas *et al.*, 2016; Daves *et al*., 2016). It should also be noted that relatively higher numbers of adult animals were included in this study than younger animals.

In this study, the higher seroprevalence was observed in cows with history of repeat breeding (possibly as a sequel to early embryonic death) compared to the cows with history of abortion (P<0.05). This result concurs with previously reported of higher prevalence of BVDV in animals with history of repeat breeding (88.9%) than animals with abortion (84.2%) history in Kenya (Okumu, 2014). BVD infection of naive pregnant cows and heifers has been reported to lead to reproductive disorders such as early embryonic death, fetal death and mummification, birth of calves with congenital defects, calves with poor growth rates, increased age at first calving and depressed ovarian function in affected herds (Heuer *et al.*, 2007; Altamarand *et al.,* 2013). Bovine viral diarrhea virus has also been reported to be fetopathogenic in cattle, thus leading to early embryonic death, repeat breeder syndrome and abortion in cattle (Yang *et al.,* 2012).

In dairy farms introduced new animals to their herds, a higher prevalence of BVD was found as compared with dairy farms not introduced new animals to their herds ($P<0.05$). This result concurs with previously reported purchasing breeding cattle significantly contributes to increased seropositivity compared to purchasing store cattle (Gates *et al.*, 2013). Increasing the number of cattle purchased as well as the number of source farms will significantly increase antibody prevalence (Gates *et al*., 2013).

In this study, adult animals were two times more likely to be infected than young animals. This result agrees with previous finding reported age as the risk factor for BVD infection (Nigusei *et al.*, 2010; Shirvani *et al.*, 2012; Aragaw *et al.*, 2018). This might reflect higher possibility of getting the virus from the environment which is shed by carrier animals. Obviously, older animals are more likely to have been exposed to the virus and as animals stay seropositive lifelong. Therefore, the herd seroprevalence is likely to be higher when many older animals are included in the sample.

In dairy farms with history of introduced new animals to their herds were two times more likely infected with BVDV than those with no history of new animals' introduction to their herds before. This result was in line with many reported from different parts of the world (Talafha *et al.*, 2009; Laurens*et al.*, 2010; Segura *et al.*, 2016). This could be due to the introducing of PI animals, dams carrying PI fetuses or contact between animals from infected and non-infected herds can be transmitting the virus to naive herds (Almeida *et al.*, 2013). In this study, all of the farmers introduced new animals to their herds were have no isolation room or conducted screening test for BVDV. This was implies as they might be purchase persistently infected animals which are the risk factor for the dissemination of virus within their herds.

This study showed that the risk of being infected by the BVDV was higher in cows with history of repeat breeding than cows with no history of repeat breeding. Cows with history of repeat breeding were three times more likely to be infected by BVDV than cows with no history of repeat breeding. This result is in line with previous findings in Ethiopia (Aragaw *et al.*, 2018; Asmare *et al.*, 2018). In Kenyan dairy cattle also the risk of having a positive

BVDV titter in repeat breeder was twice higher than no-repeat breeder (Okumu, 2014). The study conducted on 139 repeat breeding cows in Turkey reported BVDV to be the cause in 58.2% of the cows (Gür, 2011). If exposure and transient infection of the dam occurs prior to embryo attachment to the endometrium, infection is avoided as BVDV does not penetrate the *zona pellucida*. However, following attachment embryonic infection can occur and may lead to embryo loss with the dam returning to heat (Sayers *et al.,* 2015). Therefore, BVDV might be the cause of repeat breeding and further study is recommended for confirmation of the real cause.

The use of competitive ELISA for BVDV antibody testing in this study generally revealed the presence of BVDV in small scale dairy farms of Jimma town with a higher prevalence. However, based on the current study it is not possible to confirm PI status and tell the genotype of BVDV that might be predominant, whether BVDV-1 or BVDV-2. Knowing the genotype and sub-type of BVDV is very important in term of control of the infection through vaccination approaches. BVDV distribution reported globally has shown variation in genotype and sub-type. For example, the study by Lanyon *et al*. (2014) stated that BVDV-1 is predominant in Australia with sub-type 1c being the most prevalent, while in a study by Fulton *et al*. (2009) revealed that the most prevalent BVDV sub-type in affected beef cattle in south central of United State of America is type 1b followed by sub-type 1a and 2a.

6. CONCLUSION AND RECOMMENDATIONS

This study was found 51.7% and 95.6% seroprevalence at an individual level and herd level respectively. The higher seroprevalence was estimated in adult age categories, cows with history of repeat breeding compared to cows with history of abortion and farms introduced new animals to their herds. Among other suspected risk factors for BVDV infection, age, introducing of new animals to herd and animals with history of reproduction problems were potential risk factors for BVD in Jimma town dairy farms. The result of this study showed that presence BVDV antibody among dairy cattle of Jimma town as well as proofed the previous reports the presence BVDV antibodies in Jimma zone. Therefore, dairy farm owners have to isolate new animals before introducing to their herds, remove repeat breeder and old animals from herds in order to minimize the risk of viral spread in their herds. Further study needs to be done to evaluate and determine the overall prevalence status, local risk factors and economic significance of BVDV in Ethiopia. Based on the current study and previous studies, it is not possible to confirm PI status as well as tell the genotype of BVDV that might be predominant, whether BVDV-1 or BVDV-2. Knowing the genotype and sub-type of BVDV is very important in term of control of the infection through vaccination approaches.

Based on the findings of this study and conclusion, the following recommendations are forwarded:

- Awareness creation to the farmers about the diseases especially the risk of introducing new animals to herds should be given by professional in order to reduce the spread of the disease.
- Older and repeat breeder animals should be tested for BVD and properly managed as they act as potential source of infection.
- Further investigations should be carried out to identify biotypes of BVDV and to recommend appropriate vaccine.
- Cost-benefit analysis of BVD vaccination has to be done to identify appropriate control strategy.

7. REFERENCES

Abbas, J., Hajinezhad, M., Sadati, D., Jamshidian, A., Najimi, M., Ghalyanchi, A. (2016). Comparative prevalence of bovine viral diarrhea virus antibodies among native and imported cattle in north of Sistan and Baluchistan, Iran. *Iranian Journal of Virology*, **10**: 48-52.

Almeida, L., Miranda, I., Hein, H., Santiago, H., Costa, E., Marks, F., Roden, C., Canal, C., Corbellini, L. (2013). Herd-level risk factors for bovine viral diarrhea virus infection in dairy herds from Southern Brazil. *Research in Veterinary Science*, **95**: 901-907.

Altamarand, G., Kaiser, G., Mucci, C., Verna, E., Campero, M., Odeon, C. (2013). Effect of bovine viral diarrhea virus on the ovarian functionality and in vitro reproductive performance of persistently infected heifers. *Veterinary Microbiology*, **165**: 326-332.

Apeanti, W. (2016). Contributing factors to pre-service mathematics teachers' e-readiness for ICT integration. *International Journal of Research in Education and Science*, **2**: 223-238.

Aragaw, K., Sibhat, B., Ayelet, G., Skjerve, E., Gebremedhin, E., Asmare, K. (2018). Seroprevalence and factors associated with bovine viral diarrhea virus (BVDV) infection in dairy cattle in three milk-sheds in Ethiopia. *Tropical Animal Health and Production*. https://doi.org/10.1007/s11250-018-1624-5

Asmare, K., Regassa, F., Robertson, L., Martin, A., Skjerve, E. (2013). Reproductive disorders in relation to *Neospora caninum*, *Brucella spp*. and bovine viral diarrhea virus serostatus in breeding and dairy farms of central and southern Ethiopia. *Epidemiology & Infection*, **141**:1-9.

Asmare, K., Sibhat, B., Ayelet, G., Gebremedhin, E., Lidete, K., Skerve, E. (2018). Serological evidence of bovine herpesvirus-1, bovine viral diarrhea virus and schmallenberg virus infections in relation to reproductive disorders in dairy cattle in Ethiopia. *Acta Tropica*, **178**: 236-241.

Bachofen, C., Voget, H., Stalder, H., Mathys, T., Zanoni, R., Hilbe, M. (2013). Persistent infections after natural transmission of bovine viral diarrhea virus from cattle to goat and among goats. *BMC Veterinary Research*, **44**: 32.

Barrett, D., Parr, M., Fagan, J., Johnson, A., Tratalos, J., Lively, F., Diskin, M., Kenny, D. (2018). Prevalence of bovine viral diarrhea virus (BVDV), bovine herpes virus 1 (BHV-1), leptospirosis, neosporosis and associated risk factors in 161 Irish beef herds. *BMC Veterinary Research*, **14**: 1-10.

Bauermann, F., Falkenberg, S., Vander, B., Decaro, N., Brodersen, B., Harmon, A., Hessman, B., Flores, E., Ridpath, J. (2014). Generation of calves persistently infected with HoBi-like *pestivirus* and comparison of methods for detection of these persistent infections. *Journal of Clinical Microbiology*,**52**: 3845-3852.

Bauhofer, O., Summerfeld, A., Sakoda, Y., Tratschin, D., Hofmann, M., Ruggli, N. (2007). Classical swine fever virus N^{pro} interacts with interferon regulatory factor 3 and induces its proteasomal degradation. *Journal of Virology*, **81**: 3087-3096.

Becher, P. & Tautz, H. (2011). *Pestivirus* (*Flaviviridae*). In: Tidona, C., Darai, G. (eds.), Springer Index of Viruses, 2nd ed. Springer, Heidelberg, Germany, pp.483-488.

Bedekovic, T., Lemo, N., Barbic L., Cvetnic, Z., Lojkic, I., Benic, M., Cac, Z., Lojkic, M., Madi, J. (2013). Influence of category, herd size, grazing and management on epidemiology of bovine viral diarrhea in dairy herds, Croatia. *Acta Veterinaria Brno*, **82**:125-130.

Bello, S., Daneji, A., Chafe, U., Abubakar, M., Jibril, A., Festus, A. (2016). Detection of antibodies to bovine viral diarrhea virus in cattle presented for slaughter at Sokoto metropolitan abattoir, Nigeria. *Journal of Veterinary Medicine and Animal Health*, **8**: 11-14.

Bendfeldt, S., Ridpath, J., Neill, J. (2007). Activation of cell signaling pathways is dependent on the biotype of bovine viral diarrhea virus's type 2. *Virus Research*, **126**: 96-105.

Bielefeldt, H., Tolnay, E., Reisenhauer, C., Hansen, T., Smirnova, N., Van Campen, H. (2008). Transplacental infection with non-cytopathic bovine viral diarrhea virus types

1b and 2: viral spread and molecular neuropathology. *Journal of Comparative Pathology*, **138**: 72-85.

Blanchard, P., Ridpath, J., Walker, J., Hietala, S. (2010). An outbreak of late term abortions, premature births, and congenital deformities associated with a bovine viral diarrhea virus-1subtype b that induces thrombocytopenia. *Journal of Veterinary Diagnostic Investigation*, **22**: 128-131.

Botner, A. & Belsham, G. (2012). Virus survival in slurry: analysis of the stability of foot and mouth disease, classical swine fever, bovine viral diarrhea and swine influenza viruses. *Veterinary Microbiology*, **157**: 41-49.

Braun, U., Bachofen, C., Schenk, B., Hassig, M., Peterhans, E. (2013). Investigation of border disease and bovine virusdiarrhea in sheep from 76 mixed cattle and sheep farms in eastern Switzerland. *Schweiz. Arch. Tierheilkd.*, **155**: 293-298.

Brian, W., March, H., Van Regenmortel. (2010). *Desk Encyclopedia of Animal and Bacterial Virology*, Elsevier, Oxford, UK, pp. 77-83.

Brownlie, J., Thompson, I., Curwen, A. (2000). Bovine virus diarrhea virus: strategic decisions for diagnosis and control. *In Practice*, **22**: 176-187.

Callaby, R., Toye, P., Jennings, A., Thumbi, S., Coetzer, J., Conradie, J., Hanotte, O., Mbole, M., Bronsvoort, B., Kruuk, L., Woolhouse, M., Kiara, H. (2016). Seroprevalence of respiratory viral pathogens of indigenous calves in Western Kenya. *Research in Veterinary Science*, **108**: 120-124.

Central Statistical Agency (CSA) (2016). Livestock and livestock characteristics, agricultural sample survey. Addis Ababa, Ethiopia. *Statistical Bulletin*, **2**: 9-13.

Coletha, M., Klevar, S., Loken, T., Mdegela, H., Mwamengele, G., Skjerve, E., Godfroid, J., Stokstad, M. (2017).Reproductive infections in cattle in Tanzania- lessons for control priorities. *SOJ Microbiology and Infectious Diseases*, **5**: 1-9.

Csaba, K., Petra, F., Agnes, S., Mira, M., Akos, H., Conor, D. (2015). Seroprevalence of bovine viral diarrhea virus in Hungary situation before launching an eradication campaign. *Acta Veterinarian Hungarica*, **63**: 255-263.

Cuttance, W. & Cuttance, E. (2014). Analysis of individual farm investigations into bovine vira ldiarrhoea in beef herds in the North Island of New Zealand. *New Zealand Veterinary Journal*, **62**: 338-342.

Daves, L., Yimer, N., Arshad, S., Kazhal, S., Mohd, O., Rosnina, Y., Abdwahid, H. (2016). Seroprevalence of bovine viral diarrhea virus (BVDV) infection and associated risk factors in cattle in Selangor, Malaysia. *Open Veterinary Journal*, **1**: 22-28.

Deng, Y., Sun, Q., Cao, J., Lin, T., Yuan, S., Zhang, B., Zhai, L., Huang, L., Shan, L., Zheng, H., Wen, T., Tong, Z. (2012). High prevalence of bovine viral diarrhea virus 1 in Chinese swine herds. *Veterinary Microbiology*, **159**: 490-493.

Deng, M., Ji, S., Fei, W., Raza, S., He, C., Chen, Y., Chen, H., Guo, A. (2015). Prevalence study and genetic typing of bovine viral diarrhea virus (BVDV) in four bovine species in China. *PLOS ONE*, **104**:121.

Dubovi, E. (2013). Laboratory diagnosis of bovine viral diarrhea virus. *Biologicals*, **41**: 8-13.

Factor, C., Yus, E., Eiras, C., Sanjuan, M., Cervino, M., Arniaz, I., Dieguez, F. (2016). Genetic diversity of bovine viral diarrhea virus from the Galicia region in Spain. *Veterinary Records Open*, **3**:1-4.

Feed the Future (FF). (2016). Feed the Future Innovation Lab for Livestock Systems Ethiopia: Livestock Disease Management and Food Safety Brief. The Management Entity at the University of Florida, Florida, USA.

Feliciano, M., Ruben, H., Laura, H., Arcelia, A., Efren, D., Felix, M., Erika, G., Isabel, B., Horacio, Z. (2016). Seroprevalence and risk factors for reproductive diseases in dairy cattle in Mexico. *Journal of Veterinary Medicine and Animal Health*, **8**: 89-98.

Fulton, R., Whitley, E., Johnson, B. (2009). Prevalence of bovine viral diarrhea virus (BVDV) in persistently infected cattle and BVDV sub-types in affected cattle in beef herds in south central United States. *Canadian Journal of Veterinary Research*, **73**: 283-291.

Fux, R. & Wolf, G. (2012). Transient elimination of circulating bovine viral diarrhea virus by colostral antibodies in persistently infected calves: a pit falls for BVDV eradication program. *Veterinary Microbiology*, **161**: 13-19.

Gamlen, T., Richards, K., Mankouri, J., Hudson, L., McCauley, J., Harris, M., Macdonald, A. (2010). Expression of the NS3 protease of cytopathogenic bovine viral diarrhea virus results in the induction of apoptosis but does not block activation of the beta interferon promoter. *Journal of General Virology*, **91**: 133-144.

Gates, M., Woolhouse, M., Gunn, G., Humphry, R. (2013). Relative associations of cattle movements, local spread, and biosecurity with bovine viral diarrhea virus (BVDV) and seropositivity in beef and in dairy herds. *Preventive Veterinary Medicine*, **112**: 285-295.

Giammarioli, M., Ceglie, L., Rossi, E., Bazzuvchi, M., Casciari, C., Petrini, S., Demia, J. (2015). Increased genetic diversity of BVDV-1: recent findings and implication thereof. *Virus Genes*, **50**: 147-151.

Givens, M., Marley, M., Jones, C., Ensley, D., Galik, P., Zhang, Y., Riddell, K., Joiner, K. (2012). Protective effects against abortion and fetal infection following exposure to bovine viral diarrhea virus and bovine herpesvirus-1 during pregnancy in beef heifers that received two doses of multivalent modified live virus vaccine prior to breeding. *Journal of the Veterinary Medical Association*, **241**: 484-495.

Givens, M. & Marley, M. (2013). Immunology of chronic BVDV infections. *Biologicals*, **41**: 26-30.

Graham, D., Clegg, T., Lynch, M., More, S. (2013). Herd-level factors associated with the presence of bovine viral diarrhea virus in herds participating in the voluntary phase of the Irish national eradication program. *Preventive Veterinary Medicine*, **112**: 99-108.

Grooms, D., Brock, K., Bolin, S., Grotelueschen, D., Cortese, V. (2014). Effect of constant exposure to cattle persistently infected with BVDV on morbidity and mortality rates and performance of feedlot cattle. *Journal of the American Veterinary Medical Association*, **244**: 212-224.

Gür, S. (2011). Prevalence of bovine viral diarrhea, bovine herpesvirus type 1 and 4 infections in repeat breeding cows in Western Turkey. *Brazilian Journal of VeterinaryResearchand Animal Science*, **48**: 228-233.

Haji H. & Seyfi A. (2007). Serological study of bovine diarrhea virus infection of cattle in Ahvaz. *Journal of Veterinary Research*, **62**: 21-26.

Handel, I., Willoughby, K., Land, F., Koterwas, B., Morgan, K., Tanya, V., Bronsvoort, B. (2011). Sero-epidemiology of bovine viral diarrhea virus (BVDV) of Cameroon and use of the SPOT test to identify herds with PI calves. *PLOS ONE*, **6**: 11.

Heisman, B., Fulton, R., Sjeklocha, D., Murphy, T., Ridpath, J., Payton, M. (2009). Evaluation of economic effects and the health and performance of the general cattle population after exposure to cattle persistently infected with bovine viral diarrhea virus in a starter feedlot. *American Journal Veterinary Research*,**70**: 73-85.

Heuer, C., Healy, A., Zerbini, C. (2007). Economic effects of exposure to bovine viral diarrhea virus or dairy herd in New Zealand. *Journal of Dairy Science*, **90**: 5428-5438.

Hosmer, D. & Lemeshow, S. (2000). Applied Logistic Regression, 2nd ed. Wiley, New York, p. 392.

Humphry, R., Brulisauer, F., McKendrick, I., Nettleton, P., Gunn, G. (2012). Prevalence of antibodies to bovine viral diarrhea virus in bulk tank milk and associated risk factors in Scottish dairy herds. *Veterinary Record*, **171**: 445.

Jimma Town Livestock and Fishery Resource Development Office (JTLFRDO) (2017).

Kadir, Y., Gizem, A., Paul, B. (2017). Variability and global distribution of sub-genotypes of bovine viral diarrhea virus: Review. *Viruses*, **9**: 1-19.

Kaiser, V., Nebel, L., Schupbach, G., Zanoni, R., Schweizer, M. (2017). Influence of border disease virus (BDV) on serological surveillance within the bovine virus diarrhea (BVD) eradication program in Switzerland. *BMC Veterinary Research*, **21**: 1-13.

Khezri, M. (2015). Bovine viral diarrhea (BVD): A review emphasizing on Iran perspective. *Journal of Advanced Veterinary and AnimalResearch*, **2**: 240-251.

Khodakaram, A., Mohammadi, A., Farjanikish, G. (2016). Molecular characterization and phylogenetic analysis of bovine viral diarrhea virus in dairy herds of Farsi province, Iran. *Iran Journal of Veterinary Research*, **17**: 89-97.

Khodakaram, A. & Farjanikish, G. (2017). Persistent bovine viral diarrhea virus (BVDV) infection in cattle herds. Review Article. *Iran Journal of Veterinary Research*,**18**: 154-163.

Kish, G., Tafti, A., Mohammadi, A. (2013). Serological survey of bovine viral diarrhea virus by antigen capture ELISA in dairy herds in Farsi Province, Iran. *Bulgarian Journal of Veterinary Medicine*, **16**: 217-222.

Kurcubic, V., Petrovic, T.,Dokovic, R., Ilic, Z., Maskovic, P. (2015). Prevalence of BVD infection in ruminants in Serbia. *Journal of Central European Agriculture*, **16**: 130-147.

Lanyon, S.&Reichel, M. (2013). Understanding the impact and control of bovine viral diarrhea in cattle population. *Springer Science Reviews*, **1**:85-93.

Lanyon, S., Hill, F., Reichel, M., Brownlie, J. (2014). Bovine viral diarrhea: Pathogenesis and diagnosis. *The Veterinary Journal*, **199**: 201-209.

Laurens, J. (2014). "Challenges in the control of bovine viral diarrhea virus: Implications for a Belgian eradication program". Ghent University, Faculty of Veterinary Medicine, Merelbeke, Belgium. http://hdl.handle.net/1854/LU-4231850

Lee, D., Park, S., Choi, E., Lee, C. (2008). Investigation of the prevalence of bovine viral diarrhea virus in dairy cows in South Korea. *Veterinary Record*, **162**: 211-213.

Lee, S., Nanduri, B., Pharr, G., Stokes, J., Pinchuk, L. (2009). Bovine viral diarrhea virus infection affects the expression of proteins related to professional antigen presentation in bovine monocytes. *Biophysica Acta*, **1794**: 14-22.

Leta, S. & Mesele, F. (2014). Spatial analysis of cattle and shoat population in Ethiopia: growth trend, distribution and market access. *Springer Plus*, **3**: 310.

Lindberg, A. & Houe, H. (2005). Characteristics in the epidemiology of bovine viral diarrhea virus (BVDV) of relevance to control. *Preventive Veterinary Medicine*, **72**: 55-73.

Lindberg, A., Brownlie, J., Gunn, G., Houe, H., Moennig, V., Saatkamp, H., Sandvik, T., Valle, P. (2006). The control of bovine viral diarrhea virus in Europe: today and in the

future. *Revue Scientifique Technique - Office International des Epizooties*, **25**: 961-979.

Lindenbach, B., Thiel, H., Rice, C. (2007). *Flaviviridae*: the viruses and their replication. In: Fields Virology (Knipe, M & Howley, M, ed.), Lippincot-Raven Publishers, Philadelphia, PA, pp. 1101-1152.

Liu, L., Xia, H., Wahelberg, N., Belak, S., Baule, C. (2009). Phylogeny, classification and evolution insights into *Pestiviruses*. *Virology*, **385**: 351-357.

MacLachlan, N. & Dubovi, E., ed. (2011). *Fenner's Veterinary Virology*. 4th ed., London: Elsevier. pp. 470-475.

Makoschey, B., Sonnemans, D., Munozbielsa, J., Franken, P., Mars, M., Santos, L., Alvarez, M. (2007). Evaluation of the induction of NS3 specific BVDV antibodies using a commercial inactivated BVDV vaccine in immunization and challenge trials. *Vaccine*, **25**: 6140-6145.

Malavika, R., Mathew, M., Vanessa, E. (2017). Pathogen exposure in cattle at the livestock-wild life interface. *Ecohealth*,**14**: 542-551.

Marley, M., Tabor, J. & Givens, M. (2009). Bovine viral diarrhea virus is inactivated when whole milk from persistently infected cows is heated to prepare semen extender. *Veterinary Microbiology*, **134**: 249-253.

Marques, A., Assis, A., Simoes, S., Tolentino, M., Azevedo, S. (2016). Risk factors associated with bovine viral diarrhea virus (BVDV) infection in the semiarid of the state of Paraiba, in the northeast region of Brazil. *Semina: Ciências Agrárias, Londrina*, **37**: 3095-3106.

McFadden, A., Tisdall, D., Hill, F., Otterson, P., Pulford, D., Peake, J., Finnegan, C., La Rocca, S., Kokmun, T., Weir, A. (2012). The first case of a bull persistently infected with Border disease virus in New Zealand. *New Zealand Veterinary Journal*, **60**: 290-296.

Meadows, D. (2010). A study to investigate the use and application of BVDV vaccine in UK cattle. *Cattle Practice*, **18**: 202-215.

Michael, P., Reichel, I., Sasha, R., Fraser I. (2018). Perspectives on current challenges and opportunities for bovine viral diarrhea virus eradication in Australia and New Zealand. *Pathogens*, **7**: 1-10.

Mischra, N., Rajukumar, K., Tiwari, A., Nema,K., Behera, P. (2009). Prevalence of bovine viral diarrhoea virus (BVDV) antibodies among sheep and goats in India. *Tropical Animal Health and Production*, **41**:1231-1239.

Mishra, N., Dubey, R., Rajukumar, K., Tosh, C., Tiwari, A., Pitale, S., Pradhan, H. (2007). Genetic and antigenic characterization of bovine viral diarrhea virus type 2 isolated from Indian goats (*Capra hircus*). *Veterinary Microbiology*, **124**: 340-347.

Mohammad, K. (2015). Bovine viral diarrhea (BVD): A review emphasizing on Iran perspective. *Journal of Advanced Veterinary and AnimalResearch*, **2**: 240-251.

Muratore, E., Bertolotti, L., Nogarol, C., Caruso, C., Lucchese, L., Iotti, B., Ariello, D., Moresco, A., Masoero, L., Nardelli, S., Rosati, S. (2017). Surveillance of infectious bovine rhinotrachitis in marker-vaccinated dairy herds: Application of a recombinant gE ELISA on bulk milk samples. *Veterinary Immunology and Immunopathology*, **185**: 1-6.

Nahed, K., Eman, M., Wafaa, A., Hanaa, A. (2012). Field studies of bovine viral diarrhea virus (BVDV) in El-Sharkia and El-kalubia Governorates, 1st Conf. of An. Health Res. Inst. Assoc., p. 428-436.

Neill, J. (2013). Molecular biology of bovine viral diarrhea virus. *Biologicals*, **41**: 2-7.

Nelson, D., Duprau, J., Wolf, P., Evermann, J. (2016). Persistent bovine viral diarrhea virus infection in domestic and wild small ruminants and camelids including the mountain goat (*Oreamnos Americans*). *Frontiers Microbiology*, **6**: 1415.

Nigussie, Z., Tariku, M., Tefera, S., Tadele, T., Fekadu, R. (2010). Seroepidemiological study of bovine viral diarrhea (BVD) in three agro-ecological zones in Ethiopia. *Tropical Animal Health and Production*, **42**: 319-321.

Ninnet, G., Francisco, J., Antonio, V., Fernando, V., Julia, F. (2017). Genetic diversity of bovine viral diarrhea virus in cattle from Mexico. *Journal of Veterinary Diagnostic Investigation*, **29**: 362-365.

Njiro, S., Kidanemariam, A., Tsotetsi, A., Katsande, T., Mnisi, M., Lubisi, B., Potts, A., Baloyi, F., Moyo, G., Mpofu, J., Kalake, A., Williams, R. (2011). A study of some infectious causes of reproductive disorders in cattle owned by resource poor farmers in Gauteng province, South Africa. *Journal of the South African Veterinary Association*, **82**: 213-218.

Oguzoglu, T. (2012). A Review of Border Disease Virus Infect on in Ruminants: Molecular Characterization, Pathogenesis, Diagnosis and Control. *Animal Health, Production and Hygiene*, **1**: 1-9.

OIE Terrestrial Manual. (2015). Bovine viral Diarrhea (BVD). pp. 1-22.

Okumu, T. (2014). Infectious abortion and associated risk factors in dairy cattle farms in Nakuru district, Kenya. A thesis submitted in fulfillment of the requirements for the degree of Doctor of Philosophy. University of Nairobi, Faculty of Veterinary Medicine, Nairobi, Kenya.

Otte, J. & Gumm D. (1997). Intra-cluster correlation coefficients of 20 infections calculated from the results of cluster-sample surveys. *Preventive Veterinary Medicine*, **31**: 147-150

Passler, T., Walz, P., Ditchkof, S. (2007). Experimental persistent infection with bovine viral diarrhea virus in white tailed deer. *Veterinary Microbiology*, **122**: 350-356.

Pawaiya, R. & Gupta, V. (2013). A review on schmallenberg virus infection: a newly emerging disease of cattle, sheep and goats. *Vererinaria Medicina (Praha)*, **58**: 516-526.

Pecora, A., Aguirreburualde, M., Rodriguez, D. (2009). Development and validation of an ELISA for quantization of bovine viral diarrhea virus antigen in the critical stages of vaccine production. *Journal of VirologicalMethods*, **162**: 170-178.

Pedrera, M., Gomez, J., Risalde, M., Molina, V., Sanchez, P. (2012). Characterization of apoptosis pathways (intrinsic and extrinsic) in lymphoid tissues of calves inoculated with non-cytopathic bovine viral diarrhea virus genotype-1. *Journal of Comparative Pathology*, **146**: 30-39.

Peletto, S., Zuccon, F., Pitti, M., Gobbi, E., Marco, L., Caramelli, M., Masoero, L., Acutis, P. (2012). Detection and phylogenetic analysis of an atypical *Pestivirus*, strain IZSPLV-To. *Research in Veterinary Science*, **921**:147-150.

Peterhans, E., Bachofen, C., Stalder, H., Schweizer, M. (2010). Cytopathic bovine viral diarrhea viruses (BVDV): Emerging *Pestiviruses* doomed to extinction. *Veterinary Research*, **41**: 45-60.

Peterhans, E. & Schweizer, M. (2013). Bovine Viral Diarrhea Virus (BVDV): A *pestivirus* inducing tolerance of the innate immune response. *Biologicals*, **41**: 39-51.

Pinior, B., Firth, C., Richter, V., Lebl, K., Trauffler, M., Dzieciol, M., Hutter, S., Burgstaller, J., Obritzhauser, W., Winter, P., Kasbohrer, A. (2017). A systematic review of financial and economic assessments of bovine viral diarrhea virus (BVDV) prevention and mitigation activities worldwide. *Preventive Veterinary Medicine*, **137**: 77-92.

Radostits, M., Gay, C., Hinchcliff, W., Constable, D.(2007). VeterinaryMedicine. 10th ed. Philadelphia: Elsevier.

Raue, R., Harmeyer, S., Nanjiani, I. (2011). Antibody responses to inactivated vaccines and natural infection in cattle using bovine viral diarrhea virus ELISA kits: Assessment of potential to differentiate infected and vaccinated animals. *The Veterinary Journal*, **187**: 330-334.

Rego, M., Filho, A., Oliveira, P., Borges, J., Franca, C., Ribeiro, C., Pituco, E., Junior, J. (2016). Epidemiological analysis of infection by the bovine viral diarrhea virus on family farms in Brazil. *Semina: Ciências Agrárias, Londrina*, **37**: 4119-4130.

Rezaeisaber, A., Nikniaz, H., Davatgar, A. (2013).Comparison of bovine viral diarrhea virus infection between Sarabian and Holstein dairy cows in relation to abortion. *Annals of Biological Research*, **4**: 88-91.

Ridpath J. (2003). BVDV genotypes and biotypes: practical implications for diagnosis and control. *Biologicals*, **31**: 127-131.

Ridpath, J., Fulton, R., Kirkland, P., Neil, J. (2010). Prevalence and antigenic differences observed between bovine viral diarrhea virus sub genotypes isolated from cattle in

Australia and feedlots in the southwestern United States. *Journal of Veterinary DiagnosticInvestigation*, **22**: 184-191.

Rodninga, S., Givens, M., Marley, M., Zhang, Y., Riddell, K., Galik, P., Hathcock, T., Gard, J. (2012). Reproductive and economic impact following controlled introduction of cattle persistently infected with bovine viral diarrhea virus into a naive group of heifers. *Theriogeonology*, **78**: 1508-1516.

Rodriguez,V., Kukielka, D., Rivera-Arroyo, B., Martinez-Lopez, B.,Heras, A., Sanchez-Vizcaino, J., Vicente, J. (2016). Evidence of shared bovine viral diarrhea infections between red deer and extensively raised cattle in south-central Spain. *BMC Veterinary Research*, **12**: 1-11.

Roman, P., Eran, R., Thacker,H., Gregory, S. (2008). Prevalence and characterization of bovine viral diarrhea virus in the white-tailed deerpopulation in Indiana. *Journal of Veterinary DiagnosticInvestigation*, **20**:71-74.

Rypula, K., Kumala, A., Plenecz, K., Czopowic, M. (2011). Epidemiological aspects of concomitant BHV-1and BVD-MD virus infection in dairy cattle herd in Poland. *Central Europe Journal of Immunology*, **36**: 15-17.

Saa, L., Perea, A., Garcia, I., Arenas, A., Jara, D., Ramos, R., Carbonero, A. (2012). Seroprevalence and risk factors associated with bovine viral diarrhea virus (BVDV) infection in non-vaccinated dairy and dual purpose cattle herds in Ecuador. *Tropical Animal Health and Production*, **44**: 645-649.

Saeed, I., Ali, Y., Taha, K., Mohammed, N.,Nouri, Y., Mohammed, B., Mohammed, O., Elmagbool, S., Elghazali, F. (2015). First report of Bovine Viral Diarrhea Virus antigen from pneumoniccattle in Sudan. *Journal of Advanced Veterinary and AnimalResearch*, **2**:153-157.

Safarpoor, D. & Haghighi, N. (2012). Detection of bovine viral diarrhea virus in bovine and buffalo through conventional and real-time reverse transcriptase polymerase chain reaction. *Research Opinions in Animal and Veterinary Science*, **2**: 263-267.

Sakhaee, E, Khalili, M., Kazeminia, S. (2009). Serological study of bovine viral respiratory diseases in dairy herds in Kerman province, Iran. *Iranian Journal of Veterinary Research*, **10**: 49-53.

Saliki, J. & Dubovi, E. (2004). Laboratory diagnosis of bovine viral diarrhea virus infections. *Veterinary Clinic of North America-Food Animal Practice*, **20**: 73-75.

Sarrazin, S., Veldhuis, A., Meroc, F., Vangeel, I., Lauren's, J., Dewulf, J., Cay, A., Piepers, S., Hooyberghs, J., Ribbens, S. (2013). Serological and virological BVDV prevalence and risk factors analysis for herd to be BVDV seropositive in Belgium cattle herd. *Preventive Veterinary Medicine,* **108**: 28-37.

Sayers, G., Byrne, N., Doherty, E., Arkins, S. (2015). Prevalence of exposure to bovine viral diarrhea virus (BVDV) and bovine herpesvirus-1 (BHV-1) in Irish dairy herds. *Research in Veterinary Science*, **100**: 21-30.

Segura, J., Zapata, C., Jasso, J., Martinez, J., Lopez, R. (2016). Seroprevalence and risk factors associated with bovine herpesvirus-1 and bovine viral diarrhea virus in Northeastern Mexico. *Open Veterinary Journal*, **6**: 143-149.

Shapiro, B., Gebru, G., Desta, S., Negassa, A., Nigussie, K., Aboset, G., Mechal, H. (2015). Ethiopia livestock master plan. ILRI Project Report. Nairobi, Kenya: International Livestock Research Institute (ILRI).

Shirvani, E., Lotfi, M., Kamalzadeh, M., Noaman, V.,Bahriari, M., Morovati, H., Hatami, A. (2012). Seroepidemiological study of bovine respiratory viruses (BRSV, BoHV-1, PI-3V, BVDV, and BAV-3) in dairy cattle in central region of Iran (Esfahan province). *Tropical Animal Health and Production*, **44**: 191-195.

Srinivas, M. & Srikanth, N. (2012). Identification and control strategies of bovine viral diarrhea virus (BVDV): Review article. *International Journal of Pharma and Bio Sciences*, **3**: 874-884.

Stahl, K., Lindberg, A., Rivera, H., Ortiz, C., Moreno, J. (2008). Self-care from BVDV infection a frequent finding in dairy herd, in an endemically infected region in Peru. *Preventive Veterinary Medicine,* **83**: 285-296.

Strong, R., Errington, J., Cook, R., Ross-Smith, N., Wakeley, P., Steinbach, F. (2013). Increased phylogenetic diversity of bovine viral diarrhea virus type-1 isolates in England and Wales since 2001. *Veterinary Microbiology*, **162**: 315-320.

Talafha, A., Hirche, S., Ababneh, M., Al-Majali, A., Ababneh, M. (2009). Prevalence and risk factors associated with bovine viral diarrhea virus infection in dairy herds in Jordan. *Tropical Animal Health and Production*, **41**: 499-506.

Tautz, N., Tews, B., &Meyers, G. (2015). The molecular biology of *pestiviruses*. *Advances in Virus Research*, **93**: 47-160.

Thomann, B., Tschopp, A., Magouras, I., Meylan, M., Schupbach,G., Hasler, B. (2017). Economic evaluation of the eradication program forbovine viral diarrhea in the Swiss dairy sector. *Preventive Veterinary Medicine*, **145**: 1-6.

Thrusfeild, M. (2007). Veterinary Epidemiology, 3rd ed. Blackwell Science Ltd, Oxford, UK, pp. 228-246.

Uddin, M., Ahasan, A., Kamrul, I., Zohorul, I., Altaf, M., Ariful. I., Kazi, F., Abdul, A. (2017). Seroprevalence of bovine viral diarrhea virus in crossbred dairy cattle in Bangladesh. *Veterinary World*, **10**: 906-913.

Villarroel, A., Dargatz, D., Lane, V., McCluskey, B., Salman, M. (2007). Suggested out-line of potential critical control points for biosecurity and bio-containment on large dairy farms. *Journal of the American Veterinary Medical Association*, **230**: 808-819.

Walz, P., Grooms, L., Passler, T., Ridpath, J. F., Tremblay, R., Step, D. (2010). Control of bovine viral diarrhea virus in ruminants. *Journal of Veterinary Internal Medicine*, **24**: 476-486.

Walz, P. (2015). Diseases of the Alimentary Tract. I Bradford Smith (red.). *Large Animal Internal Medicine*. St. Louis: Elsevier, pp. 750-759.

Webb, B., Norrdin, R., Smirnova, N., Van Campen, H., Weiner, C., Antoniazzi, A., Bielefeldt-Ohmann, H., Hansen, T. (2012). Bovine viral diarrhea virus cyclically impairs long bone trabecular modeling in experimental persistently infected fetuses. *Veterinary Pathology*, **49**: 930-940.

Wernicki, A., Urban, R., Stegierska, D., Adaszek, L., Kalinowski, M., Puchalski1, A., Dec, M. (2015). Detection of the bovine viral diarrhea virus (BVDV) in young beef cattle in eastern and southeastern regions of Poland. *Polish Journal of Veterinary Sciences*, **18**: 141-146.

Wolff, C., Boqvist, S., Stahl, K., Masembe, C. Sternberg, S. (2017). Biosecurity aspects of cattle production in western Uganda and associations with seroprevalence of brucellosis, salmonellosis and bovine viral diarrhea. *BMC Veterinary Research*, **13**:382

Xia, H., Liu, L., Wahlberg, N., Baule, C., Belak, S. (2007). Molecular phylogenetic analysis of bovine viral diarrhea virus, a Bayesian approach. *Virus Research*, **130**: 53-62.

Yang N., Cui X., Qian W., Yu, S., Liu, Q. (2012).Survey of nine abortifacient infectious agents in aborted bovine fetuses from dairy farms in Beijing, China by PCR. *Acta Veterinaria Hunguarica*, **60**: 83-92.

Yazici, Z., Serdar, M., Gumusova, S., Albayrak, H. (2012). Molecular diagnosis and sero-epidemiology of *Pestiviruses* in sheep. *Veterinarian Arrive*, **82**: 35-45.

8. ANNEXES

Annex 1**.** Questioner **Nº --------**

FARM NAME: ----------BREED: ----------SEX: ----------AGE: ----------

Production System (free-range/zero-grazed) ----------

Breeding Method:----------

Herd Size: ----------

Parity: ----------

Medical History:----------

History of Reproductive Problems:----------

Annex 2. Competitive ELISA Test Procedures Procedure

Antibody detection test was done by competitive ELISA kit (Ingezim Pestivirus Compac, 12.BVD.K3, Madrid, Spain). This is an enzyme linked immunoassay that detects antibodies directed to the p80/p125 protein. The test was used according to the instructions provided by the manufacturer.

1. All reagents (except conjugate) were become to room temperature before used.

2. First, 80µl of diluents was added to each well, followed by adding 20µl of positive control to two wells and 20µl of negative control to two wells.

3. After this 20µl of serum sample was added into the remaining wells, the contents homogenized by lightly tapping the plate, and then the plate covered and left to incubated for 60 minutes at 37^{o}c.

4. Without removing the serum samples, 50µl of conjugate (prepared at 1/100 diluents; 60µl of conjugate in 6 ml of diluents for one plate) was added to each well and plates were shake carefully to homogenize the components and left for 60 minutes at room temperature ($+25^{o}$c).

5. Each plate was washed 5 times by adding 300µl of washing solution (prepared at 40 ml of concentrate solution to 960 ml of deionized water) on each well. In between each washing step the plate was tapped on to paper to remove remaining fluid.

6. Thereafter, 100µl of TMB substrate solution was added to each well and the plate covered and left in the dark place at room temperature for 15 minutes.

7. This was then followed by adding 100ml of stop solution to each well.

8. Then the optic density of each well measured (reader) with spectrophotometer at450nm within 5 minutes after the stop solution was added.

The validity of the positive and negative controls was calculated. The mean of the negative control needed to be higher than 0.8 to be valid, and the mean of positive control needed to be less than 0.4 to be valid. For invalid assays, the assay was not repeated due to shortage of material. The positive cut off value was calculated by multiplying the mean of negative control (Negative control OD) by 0.5 and negative cut off value by multiplying the mean of negative control (Negative control OD) by 0.55. All samples with OD higher than negative Cut Off values were considered as negatives and all samples with OD values lower than positive Cut Off values were considered as positives. Samples with OD values between both cut-offs were considered as doubtful that need re-assay after 3 weeks.