Evaluation of Transmission of Bovine Viral Diarrhea Virus (BVDV) Between Persistently Infected and Naïve Cattle by the Horn Fly *(Haematobia irritans)*

by

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Abstract

Identifying transmission routes and reservoirs for bovine viral diarrhea virus (BVDV) are important in developing adequate prevention and control programs. The aim of our study was to evaluate BVDV transmission by the hematophagous horn fly (Haematobia irritans). Flies collected from four persistently infected cattle were placed in fly cages attached to principal (n=4) and control (n=4) BVDV-naïve calves housed individually in isolation rooms. Flies were able to feed on principal calves, but a barrier prevented fly feeding from control calves. Flies were tested for BVDV by RT-PCR and virus isolation at time of collection from PI cattle and after 48 h of exposure on BVDV-naïve calves. Blood samples were collected from calves and tested for BVDV infection. Virus was isolated from fly homogenates at collection from PI animals and at removal from control and principal calves. All calves remained negative for BVDV by virus isolation and serology throughout the study. Bovine viral diarrhea virus may be detected in horn flies collected from PI cattle, but horn flies do not appear to be an important vector for BVDV transmission.

It gives me great pleasure to dedicate this work to my beloved wife, Erin Donovan Chamorro, to my parents, Sixto E. Chamorro and Ruth S. Ortega, and to my brother and sister, Daniel E Chamorro and Catalina Chamorro, for their unfailing love, support, patience, and encouragement.

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Introduction

Disease in cattle as a result of bovine viral diarrhea virus (BVDV) occurs worldwide and is responsible for considerable economic losses in the cattle industry. Several countries have developed and implemented control or eradication programs for bovine viral diarrhea virus in cattle (Ridpath 2010). For successful control of BVDV, the viral ecology must be fully understood, which includes identifying reservoirs, recognizing routes of transmission, and assessing stability of BVDV in the host and environment. Although great strides have been made in understanding the pathogenesis, clinical manifestations, and control strategies regarding BVDV, limited information exists concerning the transmission of BVDV from infected to naïve cattle through routes other than direct contact. Persistently infected (PI) cattle shed large amounts of virus and are considered principal reservoirs for BVDV transmission within and between cattle herds (Houe 1999; Smith et al. 2004). Rapid BVDV transmission rates are reported after PI animals are born or introduced into cattle herds (Houe 1999); however, infections with BVDV in the absence of PI cattle have been described in seronegative herds (Moerman et al. 1993; Moen et al. 2005), indicating that transmission routes other than direct contact with PI cattle might be important in the epidemiology of the disease (Houe 1999). As control and eradication programs progress, all potential sources for the reintroduction of BVDV into cattle herds should be elucidated. In addition to PI cattle, other sources of BVDV include transiently (acutely) infected cattle, wild ruminants and non-bovine hosts, and BVDV-contaminated semen and embryos. Furthermore, the contamination of nose tongs, needles and vaccine vials was demonstrated to be

a source of BVDV (Gunn 1993; Niskanen et al. 2003). Studies have suggested flies as potential mechanical vectors of BVDV and a source of reintroduction into susceptible herds (Tarry et al. 1991; Gunn 1993; Thurmond 2005). Horse flies (*Hematopota pluvialis*) and stable flies (*Stomoxys calcitrans*) were able to transmit BVDV to naïve cattle after feeding on a PI steer (Tarry et al. 1991). The virus was isolated from susceptible cattle from 72 hours to 10 days after the flies had fed on them, and BVDV was recovered from the flies (*H. pluvialis* and *S. calcitrans*) 96 hours after feeding (Tarry et al. 1991). Additionally, BVDV was isolated from face flies (*Musca autumnalis*) that had previously fed on a PI bullock (Gunn 1993). Although the role of flies in the epidemiology of BVDV infections in cattle is uncertain, insects such as stable flies, horse flies, and face flies can potentially serve as a source of BVDV infection to naïve cattle (Tarry et al. 1991; Gunn 1993).

The horn fly (*Haematobia irritans*) is an obligate, bloodsucking ectoparasite of pastured cattle, and is the most common fly affecting cattle in the southern United States (Cupp et al. 1998). Commonly, adult horn flies spend their entire life on the same host, leaving only to lay their eggs in freshly passed feces; however, it has been reported that horn flies could fly long distances if necessary to find a new host (Byford et al. 1992; Cupp et al. 1998). The horn fly is able to migrate for distances up to 8 km, and trees or other physical barriers do not prevent migration to new herds (Kunz et al. 1983; Byford et al. 1992). The horn fly feeds frequently (20 to 40 times per day), for short periods of time, and is not able to ingest large volumes of blood per feeding compared with other bloodsucking flies of cattle such as the horse fly and the stable fly (Byford et al. 1987; Kunz et al. 1983). The horn fly is the intermediate host and biological vector of the filarial nematodes *Stephanofilaria stilesi* and *Parafilaria bovicola* in cattle (Bowman et al. 2004; Coetzer et al. 2004). Additionally, the horn fly is a mechanical vector for

Staphylococcus aureus, *Corynebacterium pseudotuberculosis*, and bovine leukemia virus (BLV) (Buxton et al. 1985; Owens et al. 1998; Spier et al. 2004); however, this conclusion was the result of experimental rather than natural conditions of disease transmission.

With respect to BVDV transmission, no controlled experimental trials have evaluated horn flies as vectors of BVDV. Therefore, the purpose of the research described in this thesis was to evaluate the potential of horn flies (*Haematobia irritans*) to transmit BVDV from PI cattle to naïve cattle. Understanding transmission routes besides direct contact with PI cattle is important for the prevention of reintroduction of the virus into BVDV-free cattle herd.

Chapter 1: Review of Literature

Introduction and History of Bovine Viral Diarrhea Virus (BVDV)

Bovine viral diarrhea was first described in 1946 after an outbreak of severe diarrhea that affected cattle herds in the state of New York, USA and in the province of Saskatchewan, Canada (Childs 1946; Olafson et al. 1946). The first reports on bovine viral diarrhea described an acute and severe condition, but also less severe and subacute cases were reported (Childs 1946; Olafson et al. 1946). Additional clinical signs described in affected cattle included signs of systemic illness such as depression, fever, and dehydration. Respiratory signs were reported in these outbreaks and included bilateral nasal discharge and elevated respiratory rates. Abortions and dramatic decreases in milk production were also reported (Olafson et al. 1946). Leukopenia was a consistent laboratory abnormality in affected animals. Ulceration and hemorrhage of mucous membranes of the gastrointestinal tract were described at postmortem examination of affected animals. Hemorrhages were also found in other tissues such as the epicardium and vaginal mucosa (Olafson et al. 1946). The disease was first termed "X disease" and subsequently "virus diarrhea" due to suspicions of a viral etiology for this new condition (Childs 1946; Olafson et al. 1946).

Eight years after the first report of this new transmissible disease of cattle, a viral etiology was confirmed (Baker et al. 1954). Also, a similar virus was subsequently associated with a new sporadically-occurring disease termed "mucosal disease" (Ramsey et al. 1953). Contrary to the first descriptions of bovine viral diarrhea, mucosal disease presented with low case attack rates and high case fatality rates (Ramsey et al. 1953). Clinical manifestations of mucosal disease were more severe than bovine viral diarrhea and included severe bloody diarrhea, erosions of mucous

membranes, erosions of other epithelial surfaces, and profuse salivation (Ramsey et al. 1953). Viral agents causing clinical cases of bovine viral diarrhea were isolated, and their effects in cell cultures were evaluated (Lee et al. 1957; Underdahl et al. 1957). Initial descriptions of the viral agents indicated that 2 forms existed, one that induced cytopathic effects (cell vacuolation and death) and one that was noncytopathic to cell cultures (Gillespie 1961). The viruses isolated from cases of bovine viral diarrhea were noncytopathic whereas the viruses isolated from cases of mucosal disease were cytopathic (Gillespie 1961). This led to the first classification of BVDV into cytopathic and noncytopathic biotypes. The discovery of different BVDV biotypes would initiate one of the most studied topics on BVDV infection, the pathogenesis of persistent infection and mucosal disease. Persistent infection was determined to be the result of an *in utero* infection with a noncytopathic strain of BVDV before 100 days of gestation (Liess 1984; McClurkin 1984). Studies determined that cattle persistently infected with noncytopathic BVDV developed mucosal disease after a superinfection with cytopathic BVDV (Bolin 1985; Brownlie 1987).

Persistently infected animals are currently considered the most important reservoir and source of transmission of BVDV within and between cattle herds (Houe 1999). The study of BVDV in the last two decades has rapidly increased the knowledge of the epidemiology and molecular biology of BVDV. A newly found viral isolate associated with severe acute disease and high mortality rates in cattle was determined to be genetically different from the initial BVDV isolate (Pellerin et al. 1994; Carman et al. 1998). The genetic dissimilarity between these isolates promoted the classification of BVDV in two genotypes (1 and 2) (Ridpath et al. 1994; Ridpath 2005).

Bovine Viral Diarrhea Virus Taxonomy and Prevalence

Bovine viral diarrhea viruses 1 and 2 are enveloped, single-stranded, RNA viruses in the genus *Pestivirus* from the family *Flaviviridae* (Ridpath 2005). Other viruses from the genus *Pestivirus* that are of veterinary importance include classical swine fever virus (hog cholera virus) and border disease virus of sheep. Based on viral effects on cell cultures, BVDV strains can be subclassified as cytopathic or noncytopathic, and this is referred to as biotype (Ridpath 2005; Ridpath et al. 2006). Cytoplasmic vacuolation and cell death are observed in cell cultures infected with cytopathic strains, while cell cultures remain intact in the presence of noncytopathic virus (Lee et al. 1957; Underdahl et al. 1957). A third biotype of BVDV has been recently reported (Ridpath et al. 2006). This biotype consists of a subpopulation of noncytopathic strains of BVDV capable of causing cytopathic effects in cultures of lymphocytes *in vitro* (Ridpath et al. 2006). The classification of BVDV by biotype is not related with virulence and pathogenicity of the virus *in vivo* (Fulton et al. 2000; Fulton et al. 2005). Cytopathic, noncytopathic, and lymphocytopathic strains can be equally pathogenic and capable of causing severe disease; however, only noncytopathic strains are able to produce persistently infected cattle (Fulton et. al 2000; Fulton et al. 2005, Ridpath et al. 2006). Noncytopathic strains of BVDV represent between 60 to 90% of the BVDV isolates from diagnostic laboratories, and it is believed that noncytopathic BVDV is the most common biotype in nature (Fulton et al. 2000; Fulton et al. 2005).

Based upon differences in viral nucleic acid sequences, two genotypes of BVDV have been described, BVDV 1 and BVDV 2 (Ridpath et al. 1994); however, genetic mutations and constant antigenic variation are common within BVDV viruses (Bachofen et al. 2008), and BVDV subgenotypes have been described for each BVDV 1 and BVDV 2 genotypes (Vilcek et

al. 2005). Twelve subgenotypes have been reported among BVDV 1 (BVDV 1a through BVDV 11) and 2 subgenotypes among BVDV 2 (BVDV 2a and BVDV 2b) (Vilcek et al. 2005). The distribution of BVDV subtypes within the United States cattle population involves 3 major subtypes, BVDV 1a, BVDV 1b, and BVDV 2a. Bovine viral diarrhea virus 1b is the predominating subtype found in the United States and accounts for 78% of persistently infected cattle in some studies in North America (Fulton et al. 2005; Fulton et al. 2006).

The natural host for BVDV is cattle, and prevalence of seropositive animals is influenced by management conditions, vaccination status, and the presence of persistently infected cattle (Houe 1995; Houe 1999). Serosurveys performed in cattle in North America have demonstrated seropositive rates between 40 and 90%. The percentage of herds with unvaccinated cattle yet seropositive to BVDV ranges from 28 to 53% depending on the geographic location of the herd (Durham et al. 1990; VanLeeuwen et al. 2005; Scott et al. 2006; VanLeeuwen et al. 2006). Prevalence of persistently infected cattle is considerably lower compared with seropositive rates found in individual animals in different studies and is estimated to be less than 1% of the cattle population (Wittum et al. 2001). Detection of persistently infected cattle within cow/calf operations and those arriving to feedlots in the United States varies from 0.1 to 0.4% (Wittum et al. 2001; O'Connor et al. 2005).

Although cattle are considered the natural host of BVDV, other species are susceptible to BVDV infection including pigs, sheep, goats, bison, deer, and camelids (Passler et al. 2010); however, implications of transmission between these species are still unknown. It is possible that the presence and identification of persistently infected individuals within heterologous species different from cattle may be of critical importance in the epidemiology of BVDV (Passler et al. 2010).

Clinical Manifestations of BVDV Infections in Cattle

Bovine viral diarrhea virus infection may result in a variety of clinical manifestations in cattle ranging from subclinical disease to acute fatal disease. Interactions between host, environmental, and viral factors can influence the clinical outcome of BVDV infections in cattle (Baker 1995). Host factors include the immune status, pregnancy status, gestational age of the fetus, and presence of concurrent infections with other pathogens. Environmental factors include severe weather and management-related factors such as commingling and transport. Viral factors include variation of viral biotype, genotype, and antigenicity (Houe 1995; Houe 1999). Both BVDV 1 and BVDV 2 can be associated with a wide range of clinical manifestations in cattle (Baker 1995; Ridpath et al. 2006); however, severe acute BVDV infection and the thrombocytopenic / hemorrhagic syndrome described in North America in the early 1990's were principally associated with BVDV 2 strains (Pellerin et al. 1994; Carman et al. 1998).

Three categories can be used to review the clinical manifestations of BVDV infections: 1) BVDV infection in immunocompetent cattle; 2) BVDV infection in the developing fetus; and 3) BVDV infection in immunotolerant cattle (Baker 1995).

BVDV Infection in Immunocompetent Cattle

Postnatal infections with BVDV in cattle that have the ability to respond immunologically to the virus are termed "acute" or "transient" and may vary from subclinical infections to peracute BVDV infection and hemorrhagic syndrome. In general, 70 to 90% of BVDV infections are subclinical or inapparent (Ames et al. 1986); however, animals affected with "inapparent" or subclinical infections can exhibit mild fever, leukopenia, decrease in feed intake, and decrease in milk production (Moerman et al. 1994). Acute BVDV infections are characterized by fever, diarrhea, depression, oculonasal discharge, anorexia, decreased milk

production, oral ulcerations, and leukopenia characterized by lymphopenia and neutropenia (Baker 1995).

Peracute BVDV infection reported in beef, dairy, and veal operations in the United States and Canada has been associated with severe clinical signs and higher case fatality rates (Pellerin et al. 1994; Carman et al. 1998). Severe and bloody diarrhea, fever, oral ulcerations, respiratory disease, abortions and decreased milk production have been described in cases of peracute BVDV infection (Pellerin et al. 1994; Carman et al. 1994). Histopathologic lesions of peracute BVDV cases were characterized by severe lymphoid depletion of Peyer's patches, necrosis of intestinal epithelium, and ulcerative lesions in the alimentary tract (Carman et al. 1998). Genomic analysis of the viral isolates associated with peracute BVDV infections revealed BVDV 2 genotypes (Carman et al. 1998). Hemorrhagic syndrome is another form of severe BVDV infection in cattle associated with noncytopathic isolates of BVDV 2 (Evermann et al. 2005). Clinical manifestations of hemorrhagic syndrome include severe thrombocytopenia, hemorrhage, bloody diarrhea, epistaxis, petechial hemorrhages, ecchymotic hemorrhages, bleeding from injection sites, and death (Rebhun et al. 1989; Bolin et al. 1992). Marked thrombocytopenia of affected cattle is characterized by altered function of platelets, thus quantitative and qualitative platelet defects contribute to the observed hemorrhagic diathesis (Walz et al. 2001). Another important contributing factor to thrombocytopenia induced by BVDV is the viral infection of megakaryocytes in the bone marrow (Walz et al. 2001).

Another important feature of BVDV infection in cattle is the ability of the virus to cause immunosuppression in acutely infected cattle. Decreases in the number of circulating immune cells, particularly B and T lymphocytes, lymphoid depletion in lymph nodes and Peyer's patches, and decreased function of cells from innate and adaptive immune response components have

been reported (Ellis et al. 1988; Welsh et al. 1995; Walz et al 2001). Leukopenia is common in acutely infected animals and is usually characterized by neutropenia and lymphopenia (T and B lymphocytes) (Ellis et al. 1988). The result of immunosuppression in acute BVDV infections is an increased susceptibility to polymicrobial infections, particularly with viruses such as bovine herpesvirus type 1 (BHV-1), and bacteria such as *Mannheimia hemolytica* and *Mycoplasma bovis* (Ridpath 2010). Infection with BVDV has also been reported to enhance other infectious conditions such as enteritis caused by *Salmonella* spp., colibacillosis, metritis, and mastitis (Ames 1987).

Infection of reproductive tract tissue with BVDV has been associated with decreased fertility and decreased conception rates in female cattle (Houe et al. 1993). Reduced conception rates in nonpregnant cows after acute BVDV infection have been related to infection and inflammation of the ovaries, reduced follicular growth, and decreased hormone production (Grooms et al. 1998). Both persistently and acutely infected bulls shed BVDV in semen, and the virus is transmitted to susceptible cows by natural breeding or artificial insemination (Paton et al. 1990; Kirkland et al. 1991). A combination of factors, including lower quality semen, ill-thrift of affected bulls, and effects of BVDV on the reproductive tract of exposed cows are responsible for lower conception rates when using semen from bulls acutely or persistently infected with BVDV (Grooms 2004). After acute BVDV infections in bulls, the virus may reside in the testes following transient infection and viremia. This phenomenon has been detected after natural and experimental infections and is referred to as prolonged testicular infection (Givens et al. 2009). Localized, prolonged testicular infections with BVDV have been experimentally produced following acute infection of peri-pubertal bulls with BVDV (Givens et al. 2003; Givens et al. 2007). Viral RNA was detected in semen for 2.75 years following BVDV exposure, and

infectious BVDV was isolated from testicular tissue for up to 12.5 months after BVDV exposure (Givens et al. 2009).

BVDV Infection in the Developing Fetus

Infection of the dam with BVDV during gestation may be clinical or subclinical since the majority of acute BVDV infections in immunocompetent cattle present only moderate clinical signs (Ames et al. 1986); however, infection of the fetus through the transplacental route appears to be highly efficient with BVDV (Dufell et al. 1985). The outcome of fetal infection with BVDV will depend on the biotype, the virulence of the virus and the gestational age of the fetus. In general, transplacental infection with BVDV may result in early embryonic death, abortion, mummification, congenital defects, stillbirths, normal calves born seropositive to BVDV, and persistently infected calves immunotolerant to BVDV (Grooms 2004).

Early embryonic death after BVDV infection in pregnant cows is an important cause of reduced reproductive performance in cattle herds (McGowan et al. 1995). Studies have confirmed that transplacental infection with BVDV during the pre-implantation period (29 to 41 days of gestation) results in considerable pregnancy losses in cattle (Carlsson et al. 1989, Grooms 2004). In contrast to dramatic pregnancy losses associated with transplacental infections with BVDV before day 41 of gestation, fetuses that survive infections with noncytopathic strains of BVDV during 45 to 125 days of gestation, develop persistent infection and immunotolerance to that specific BVDV strain (Grooms 2004).

Abortions caused by BVDV infections are usually concentrated during the first trimester of gestation between 50 and 100 days (Grooms 2004); however, although rare, mid and late term abortions and stillbirths have been reported after BVDV infection in cattle (Grooms 2004). Early fetal death can result in mummification, and expulsion of the fetus may take longer than 50 days

after BVDV exposure (McGowan et al. 1995). Abortions and reproductive failure are of critical importance in the economic losses associated with BVDV infections in cattle herds (Grooms 2004).

In utero infections with BVDV in pregnant cattle during the organogenesis period (100 - 150 days of gestation) can result in congenital malformations in newborn calves (Grooms 2004). Congenital abnormalities associated with BVDV infection appear to be frequently associated with defects in the development of the central nervous system and include cerebellar hypoplasia, hydrocephalus, hydrancephaly, optic neuritis, and microencephaly. Other congenital abnormalities include cataracts, retinal atrophy, brachygnathism, thymic aplasia, hypotrichosis, pulmonary hypoplasia, and growth retardation (Grooms 2004).

Fetuses that are infected with BVDV after development of immunocompetence (100 – 125 days of gestation) may appear normal at birth and are seropositive to BVDV (Dufell et al. 1985). Calves with precolostral serum antibody titers against BVDV have a higher risk of becoming severely ill during the first month of life, indicating that although they were able to survive BVDV infection during late gestation, they are susceptible to developing disease early in life (Munoz-Zanzi et al. 2003).

BVDV Infection in Immunotolerant Cattle (Persistently Infected Animals)

Persistently infected (PI) calves are the result of an *in utero* infection with noncytopathic BVDV during 45 to 125 days of gestation which corresponds to the gestational age before the development of immunocompetence (Grooms 2004). Immunotolerance of persistently infected animals is specific for the infecting noncytopathic strain of BVDV, and PI animals are able to respond immunologically and become seropositive to heterologous BVDV strains (Collen et al. 2000). The majority of PI calves are born weak, they usually fail to grow equivalent to their

cohorts, and 50% of them may die during the first year of life (Houe et al. 1993); however, some PI animals are born without abnormalities and are impossible to distinguish from normal healthy cattle (Baker 1995).

Persistently infected cattle usually have impaired immune responses and are more susceptible to opportunistic pathogens than their cohorts. This may be the reason why high mortality rates are observed in PI calves during the first year of life (Houe et al. 1995). Additionally, PI cattle are at risk of developing severe mucosal disease when they become "superinfected" with a cytopathic BVDV strain highly homologous to the noncytopathic BVDV strain responsible for the persistent infection (Bolin et al. 1984; Bolin 1995). Acute, chronic, and delayed onset mucosal diseases have been described (Bolin 1995). The severity and clinical features of each form of mucosal disease will depend on the degree of antigenic homology between the persistently infecting noncytopathic isolate and the superinfecting cytopathic isolate (Bolin 1995).

Acute mucosal disease develops when superinfecting cytopathic BVDV shares close antigenic homology with the PI noncytopathic BVDV strain and is considered fatal (Bolin 1995). Studies have suggested that acute mucosal disease results from genetic rearrangement and mutation of the persistently infecting noncytopathic BVDV strain and a *de novo* generation of a highly homologous cytopathic BVDV strain (Tautz et al. 1994). Clinical manifestations of acute mucosal disease include high fever, depression, fibrino-hemorrhagic diarrhea, mucopurulent oculonasal discharge, and erosions and ulcers in the gastrointestinal tract, coronary band, prepuce, and vulva (Evermann et al. 2005).

Chronic mucosal disease results from superinfection with a cytopathic BVDV strain that is heterologous with respect to the PI noncytopathic BVDV strain. Although chronic mucosal

disease might not be fatal, chronic clinical signs are observed (Baker 1995). The source of the heterologous strain is usually external and does not involve mutation or genetic rearrangement of the noncytopathic isolate (Bolin 1995). Clinical manifestations of chronic mucosal disease include diarrhea, weight loss, chronic bloat, lameness, and erosions on epithelial surfaces (Baker 1995). Recovery from clinical signs can be observed if the degree of heterology between strains is enough to induce an adequate immune response to clear the heterologous cytopathic BVDV infection; however, persistent infection with the original noncytopathic strain will still remain (Bolin 1995).

Delayed onset mucosal disease occurs when clinical manifestations of acute mucosal disease are observed several weeks or months after inoculation with a heterologous cytopathic BVDV isolate (Westenbrink et al. 1989). The source of the heterologous strain is external and has been associated with modified live viral vaccines (Ridpath et al. 1995). Some studies have suggested that recombination occurs between RNA from heterologous cytopathic BVDV and RNA from the PI noncytopathic BVDV, resulting in the creation of a new cytopathic BVDV isolate identical to the resident noncytopathic BVDV (Ridpath et al. 1995). Clinical signs are similar to those seen in acute mucosal disease.

Transmission of Bovine Viral Diarrhea Virus

Several direct and indirect routes of transmission have been described for BVDV within and between cattle herds (Thurmond 2005); however, the presence of persistently infected (PI) animals is considered the principal reservoir of the virus and the main source of BVDV transmission in cattle populations (Houe 1999). Direct contact of susceptible cattle with PI animals results in higher BVDV transmission rates compared with direct contact with acutely infected cattle (Thurmond 2005). Lower amounts and shorter duration of viral shedding have

been described in cattle acutely infected with BVDV compared to PI cattle (Dufell et al. 1985, Niskanen et al. 2000). Virus concentrations are high in oculonasal secretions, saliva, feces, urine, uterine discharges, semen, and milk from PI cattle (Houe 1995; Lindberg et al. 2004; Thurmond 2005).Therefore, shedding of BVDV in natural secretions from PI cattle results in constant contamination of the environment and increased transmission rates to susceptible naïve cattle (Houe 1999; Thurmond 2005). Passively acquired BVDV-specific antibodies from maternal colostrum provide protection against acute BVDV infection. In PI calves born from acutely infected dams, colostral antibodies reduce viremia and viral shedding, thus diminishing the amount and duration of infectiousness and affecting transmission (Thurmond 2005). Additionally, the presence of maternally-derived BVDV-specific antibodies in serum from PI calves decrease viremia and isolation of the virus in these animals (Brock et al. 1998; Thurmond 2005).

Rapid BVDV transmission occurs when PI animals are born or introduced into the herd (Houe 1999). In one study, all susceptible cattle that came in contact with a PI animal in the herd became seropositive in less than 3 months (Moerman et al. 1993). The purchase of a PI animal or of a pregnant heifer carrying a PI fetus represents the highest risk of introduction of BVDV into susceptible herds (Houe 1999; Van Campen 2010). Management practices of intensive cattle operations aid in the maintenance and dispersal of BVDV by purchasing pregnant heifers from facilities that commingle many cattle from different origins (Van Campen 2010). The risk of introducing a PI animal into the herd (including a PI fetus from a pregnant heifer) when buying 20 cattle of unknown origin and without testing is estimated to be 33% (Houe 1999). Introduction of BVDV to naïve herds through the purchase of acutely infected cattle can also result in BVDV transmission; however, factors such as virulence of the BVDV strain and

severity of clinical signs may affect the dynamics of viral spread and transmission (Houe 1999). The risk of introduction of an acutely infected animal when buying 20 cattle is estimated to be 8% (Houe 1999). Purchase of animals without testing or quarantine is a critical route of introduction of BVDV into susceptible herds (Thurmond 2005).

Contact with cattle from other herds while pasturing or in shows may also be an important route of introduction of BVDV into susceptible herds. Pasturing of cattle at a distance of less than 5 meters to cattle from another herd was moderately associated with BVDV seroconversion; however, cattle in these herds were not tested for presence of PI animals (Houe 1999). Housing PI animals within 1 to 10 meters in proximity with susceptible cattle resulted in BVDV infection (Niskanen et al. 2003).

Adequate biosecurity measures are a key management factor in the prevention of introduction of BVDV into susceptible herds as well as in the transmission of the virus within herds (Ezanno et al. 2008). All purchased cattle and their offspring should be isolated and tested for PI status before entry into the herd, semen only from test negative bulls should be used, animals leaving the herd for shows should be quarantined for 3 weeks before re-entry, and young stock should be separated from adults (Ezanno et al. 2008). Additionally, several studies emphasize the importance of improving herd immunity through vaccination and testing and removal of PI animals early in life to effectively clear BVDV infections from cattle herds (Cherry et al. 1998; Smith et al. 2010).

While persistently infected (PI) animals are the major source of virus transmission within and between cattle herds, transmission of BVDV in the absence of PI animals has been described (Moerman et al. 1993; Moen et al. 2005), indicating that other transmission routes are important in the epidemiology of the disease (Houe 1999). The birth of non-surviving PI fetuses, fence to

fence contact with infected cattle from other herds, contact with heterologous infected domestic livestock, contact with wildlife, use of contaminated semen or vaccines, use of contaminated fomites, airborne transmission, and hematophagous insects have been suggested as alternative routes of BVDV transmission within and between herds in absence of PI animals (Tarry et al. 1991; Gunn 1993; Houe 1999; Niskanen et al. 2002; Niskanen et al. 2003; Moen et al. 2005).

Bovine viral diarrhea virus can persist in tissues such as testes, ovaries, and white blood cells from acutely infected animals that have mounted an adequate and neutralizing immune response after the acute BVDV infection. Viral persistence in immune-privileged sites could contribute to BVDV transmission in cattle herds where the presence of PI animals has been determined to be absent (Houe 1999; Grooms et al. 1998a; Givens et al. 2003; Collins et al. 2009; Givens et al. 2009). Bulls acutely infected with BVDV were able to shed the virus in semen for 7 months after experimental induction of acute infection (Givens et al. 2003). Additionally, experimental inoculation of BVDV-naïve calves with semen samples from these bulls 5 months after induction of acute infection resulted in viremia and seroconversion in the calves (Givens et al. 2003). Experimental transmission of BVDV to susceptible cattle through semen from acutely infected bulls has been demonstrated under field conditions (Kirkland et al. 1997); however, the potential of BVDV transmission from bulls with prolonged testicular infections is not clear and appears to be low (Givens et al. 2009). Bovine viral diarrhea virus antigen has been detected in ovarian tissues up to 60 days after inoculation in cows (Grooms et al. 1998a). Whole blood from BVDV seropositive animals previously inoculated with BVDV 98 days earlier was transfused to BVDV naïve cattle which developed viremia and seroconversion after transfusion. Bovine viral diarrhea virus RNA was intermittently detected in white blood cells from initially seropositive calves (Collins et al. 2009).

Infections with BVDV are not limited to cattle but can also be found in many other species from the mammalian order Artiodactyla. Infections with BVDV in heterologous species may play a critical role in the epidemiology of BVDV in cattle (Passler et al. 2010). Epidemiological and experimental evidence exists for BVDV infections in diverse species such as sheep, goats, pigs, camelids, and deer (Passler et al. 2010). Persistently infected heterologous species raise concern about the implications of non-bovine species as a potential route of transmission of BVDV to susceptible cattle (Passler et al. 2010).

Indirect transmission of BVDV through contact with infected surfaces, embryos, fomites, vaccines, and flies has also been reported (Houe 1999; Thurmond 2005). Survival and infectivity of BVDV in the environment is short, and it is unlikely that the virus persists under dry conditions beyond 7 to 14 days (Baker 1987; Houe 1995). Translocation of BVDV naïve cattle to unclean, non-disinfected areas that were previously occupied by PI animals may result in BVDV transmission (Niskanen et al. 2003; Lindberg et al. 2004). Airborne transmission of BVDV was demonstrated when BVDV naïve calves were housed at 1.5 and 10 meters of distance from PI animals (Niskanen et al. 2003).

Transfer of embryos from or to PI or acutely infected cows with BVDV is an effective route of introduction of BVDV into susceptible cattle herds (Thurmond 2005). Use of contaminated fetal calf serum for washing procedures is also an effective route of BVDV transmission through embryo transfer (Houe 1999). Although it has been suggested that appropriate embryo washing eliminates the risk of BVDV contamination, one study demonstrated that recommended washing procedures were ineffective for removal of BVDV from an *in vitro* fertilization system (Houe 1999).

Several fomite vehicles such as hypodermic needles, nose tongs, rectal gloves, and the use of modified live or contaminated vaccines have been demonstrated to be associated with BVDV transmission within cattle populations (Gunn 1993; Houe 1999; Lindberg et al. 2004).

Although further research is necessary to determine their epidemiologic importance, insects such as stable flies, horse flies, and face flies can potentially serve as a source of BVDV infection to naïve cattle (Gunn 1993; Tarry et al. 1991). Horse flies (*Hematopota pluvialis*) and stable flies (*Stomoxys calcitrans*) were able to transmit BVDV to susceptible cattle after feeding on a persistently infected steer (Tarry et al. 1991). The virus was isolated from susceptible cattle from 72 hours to 10 days after the flies had fed on them, and BVDV was recovered from the flies (*H. pluvialis* and *S. calcitrans*) 96 hours after feeding (Tarry et al. 1991). Seroconversion was demonstrated in cattle exposed to *H. pluvialis* previously infected with BVDV (Tarry et al. 1991). In another study, BVDV was isolated from face flies (*Musca autumnalis*) that had fed on a PI bullock; however, experimental transmission with the face flies was not attempted (Gunn 1993). To the author's knowledge, evaluation of other insects such as horn flies, mosquitoes, midges, lice, and ticks, as potential routes for BVDV transmission has not been performed.

The Horn Fly (*Haematobia irritans*)

The horn fly *(Haematobia irritans)* is an obligate blood-sucking ectoparasite considered the most common fly affecting cattle in the southern United States. Horn flies affect mainly cattle but can also be found in horses, sheep and dogs (Bowman et al 2004). Economic losses associated with horn fly infestation in cattle are due to nuisance, irritation, and decreased feed intake. Blood loss can also be considerable when infestation is severe and anemia may result. Severely affected animals usually lose weight and develop general weakness (Cupp et al. 1998). When infestation with horn flies is greater than 100 flies per animal, weight gain may be reduced

by up to 0.5 lbs. per day. In lactating dairy cows that are heavily infested with horn flies, daily milk production can be reduced by 10 to 20% (Bowman et al 2004; Cupp et al. 1998). Commonly, adult horn flies spend their entire life on the same host leaving only to lay their eggs in freshly passed feces; however, it has been reported that horn flies could fly long distances if necessary to find a new host (Byford et al. 1992; Cupp et al. 1998). The horn fly is able to travel for distances up to 8 km and is able to pass several physical barriers seeking susceptible hosts (Kunz et al. 1983; Byford et al. 1992). The horn fly feeds frequently (20 to 40 times per day), for short periods of time, and is not able to ingest large volumes of blood per feeding compared with other bloodsucking flies of cattle such as the horse fly and the stable fly (Byford et al. 1987; Kunz et al. 1983). The life span of adult horn flies is 6.6 days or less. Daily mortality rates in adult females range from 0.09 to 0.34% (Taylor et al. 2007; Bowman 2004)

Female horn flies are capable of laying 300-400 eggs in batches of 20-30 eggs. At a temperature of 24 to 26 °C, the eggs hatch in 18-24 hours (Bowman 2004). There are three larval stages, and pupation occurs within the manure pat. The complete cycle is usually completed in 10-14 days; however in northern regions, the horn fly overwinters and hatching time of eggs is increased. Extreme heat in the summer dries the manure and decrease larval development (Byford et al. 1992; Cupp et al. 1998).

The horn fly has been identified as the intermediate host and biological vector of the filarial nematodes *Stephanofilaria stilesi*, and *Parafilaria bovicola* in cattle (Bowman 2004; Coetzer et al. 2004). These skin nematodes cause nodular, granulomatous, and ulcerative lesions of the subcutaneous tissue and skin of many parts of the body of cattle but principally of the ventral midline. The horn fly has also been reported as an effective mechanical vector of *Staphylococcus aureus* causing mastitis in dairy heifers (Owens et al. 1998). In one study,

experimental inoculation of mouthparts of 100 horn flies infected with bovine leukemia virus (BLV) into BLV seronegative calves resulted in BLV seroconversion in the naïve calves (Buxton et al. 1985). Another study identified the exotoxin (phospholipase D) from *Corynebacterium pseudotuberculosis* by RT-PCR testing in pools of horn flies (Spier et al. 2004). Experimental evaluation of the horn fly as a mechanical or biological vector of additional infectious agents of cattle has not been reported.

Chapter 2: Journal Article

Role of Flies in the Transmission of Infectious Diseases to Livestock

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Review Article

Introduction

Flies include more than 120, 000 arthropod species that belong to the order Diptera. Commonly, flies cause extensive production losses to the livestock industry due to constant nuisance and irritation to animals, decreased dry matter intake, and decreased productive performance. Fly infestations may also be associated with chronic blood loss, anemia, poor weight gain, myiasis, and transmission of infectious diseases (McClusky 2002; Cortinas et al. 2006). The economic losses associated with fly infestations in cattle operations in the United States have been estimated at approximately US$1,440 million annually. Approximately one-half of these losses (US$ 730 million) are attributed to a single fly, the horn fly (*Haematobia irritans*) (Byford et al. 1992; Catangui et al. 1997). Infectious diseases transmitted by flies to cattle and other livestock are responsible for economic losses of approximately US$4–5 billion per year in 37 countries in Africa (Eisler et al. 2003). Different fly species are usually found affecting livestock in individual farms. Flies such as the horn fly, the house fly *(Musca domestica),* the face fly *(Musca autumnalis),* the stable fly *(Stomoxys calcitrans*), the horse fly (*Tabanus* spp.), mosquitoes (*Culex* spp., *Aedex* spp.), black flies (*Simulium* spp.) and biting midges (*Culicoides* spp.) cause economic impact on livestock operations worldwide due to their ability to cause decreased production and transmit important infectious diseases (Byford et al. 1992; Catangui et al. 1997; Eisler et al. 2003).

Infectious diseases caused by viruses, bacteria, protozoa and helminths commonly affect commercial livestock populations and are associated with decreased productivity, considerable economic losses, and international trade restrictions. Some infectious diseases transmitted by flies are important zoonoses (Radostits 2001). The presence and maintenance of infectious diseases in livestock populations depends on the ability of the infectious agent to establish

reservoirs and enhance transmission to susceptible individuals. Although direct contact is the major route of transmission of infectious diseases within livestock species, living or inanimate vehicles can indirectly transmit infectious pathogens between affected and susceptible individuals (Radostits 2001). Living vehicles of infectious agents are known as vectors and are often involved in the transmission of infectious diseases to livestock. Arthropods such as flies and ticks are the most common vectors of infectious agents and, under certain environmental conditions, become effective transmitters of disease within livestock populations (Van den Bossche et al. 2010). The geographic distribution of vector-borne diseases of livestock is influenced by environmental conditions that affect host, pathogenic agent, and vector populations, and the interactions between them (Van den Bossche et al. 2010). Potential factors that influence vector disease patterns in livestock at the global, regional, and local levels include climate changes, demography, land tenure and use, and habitat fragmentation (Van den Bossche et al. 2010). Dispersal of vectors by warm winds and climatologic changes like global warming are considered responsible for the spread of pathogens to areas or regions previously free from the disease. It is believed that warm winds transporting *culicoides* species from Africa and South Europe are responsible for the introduction of Bluetongue virus to Northern Europe in 2006 (Mellor et al. 2008). In Africa, socioeconomic circumstances induce habitat fragmentation and movement of people and their livestock into endemic areas of tsetse flies, the principal vector of trypanosomosis, increasing the incidence of this zoonotic disease in animals and people (Van den Bossche et al. 2010).

Flies can transmit pathogenic microorganisms which are directly related to diseases in humans and animals (Forster et al. 2007). Flies are natural carriers of viruses, bacteria, protozoa, and helminths and play a critical role in the transmission of infectious pathogens to livestock

species (Meerburg et al. 2007; Forster et al. 2007). The ability of flies to transmit a pathogen depends on the complex interrelationship between the pathogen, the fly, the vertebrate host, and the influence of environmental conditions on each of them (Coetzer et al. 2004). Several factors determine the ability of fly vectors to transmit an infectious disease. For example, large fly vector populations are usually necessary before transmission can take place. The infection rate of fly vector populations, which is the number of individual flies that are infected with the pathogen within the fly population in the area, is also important for transmission of infectious agents. For example 26,780 Culicoides with an infection rate of 0.3% would be needed to effectively transmit bluetongue virus to a naive population of sheep, but only 173 Culicoides with an infection rate of 62% would be needed to effectively transmit bluetongue virus to the same naïve population of sheep (Coetzer et al. 2004). Other factors such as fly ecology, recovery of the pathogen from wild flies, and ability of flies to transmit the pathogen after a bite are also important to determine fly vector competence to transmit infectious pathogens (Coetzer et al. 2004).

Pathogenic microorganisms are acquired by flies through different pathways. Common routes of fly infection with pathogens are direct contact with blood of infected animals, direct contact with contaminated body secretions, contaminated surfaces, contaminated feces, feedstuffs or other materials (Meerburg et al. 2007).

Mechanical and Biological Transmission of Infectious Pathogens by Flies

Vector-borne transmission of infectious pathogens occurs when a living creature, because of its ecological relationship to others, acquires a pathogen from one living host and transmits it to another (Bowman 2004). Two major routes of transmission of infectious agents occur within vector and host populations in nature: mechanical and biological.

Mechanical Transmission

Mechanical transmission of infectious agents involves only the physical transport of pathogens from infected to susceptible hosts by the vector. Infectious agents are usually transported by attachment to the mouthparts and exoskeleton of the fly or after ingestion in the fly's gastrointestinal tract. Some pathogens are able to adhere and be carried temporarily in the salivary glands of the fly and be excreted in salivary secretions for a few hours after ingestion (Graczyk et al. 2005; Meerburg et al. 2007). Mechanical transmission of disease occurs after biting, regurgitating, and defecating of the infected fly on a susceptible host, or after dislodgment of the pathogen from the fly's exoskeleton on external body surfaces (Graczyk et al. 2005). Mechanical vectors do not support the absorption, replication, or alteration of infectious agents within their tissues; therefore, their ability to transmit disease is limited and short-lived (Radostits 2001; Graczyk et al. 2005). Mechanical transmission is restricted by the load of pathogen acquired, the ability of the pathogen to survive in the external or internal tissues of the fly, the lifespan of the fly, and by the ability of the fly to travel distances necessary to find a new host. Individual flies can travel as far as 20 miles; however, the vast majority do not travel more than 2 miles (Graczyk et al. 2005; Meerburg et al. 2007). Other factors that may influence mechanical transmission include persistence of feeding and fly size. Due to their larger meals (greater volume of blood per meal), larger flies that feed less frequently are more effective mechanical vectors compared with smaller and more frequently feeding flies (Coetzer et al. 2004). The total pathogen load transported by the fly is related to the amount of pathogen deposited or dislodged in the susceptible host tissues at fly feeding, influencing greatly the ability for pathogen transmission (Coetzer et al. 2004). Mechanical transmission is not a highly

effective route of transmission of infectious diseases when compared with biological transmission or direct transmission.

Biological Transmission

Biological vectors are those that support replication of the pathogen or development of the pathogen's life cycle stages within their tissues before transmission to susceptible hosts can take place. Biological vectors may also act as pathogen reservoirs maintaining a constant source of infection for susceptible hosts (Bowman 2004; Coetzer et al. 2004). Biological vectors transmit diseases more efficiently than mechanical vectors since a small pathogen dose may replicate and multiply in the vector tissues before transmission occurs (Scoles et al. 2008). In the biological transmission process, the pathogen is able to cross the intestinal epithelium of the fly's digestive tract and enter the circulatory system eventually reaching the salivary glands, the reproductive tract and other organs of the fly that will support the pathogen's replication. Three major forms of pathogen maintenance occur within biological vectors: colonization of salivary glands, transstadial transmission, and transovarial transmission (Bowman 2004). Upon colonization and replication within the salivary glands, the pathogen is constantly eliminated in salivary secretions during feeding of the fly on a susceptible host, as occurs with Bluetongue virus in *Culicoides* species (Carter et al. 2007). In transstadial transmission, infection is maintained in the vector as it develops between life stages. For example, a mosquito infected as larvae with *Setaria digitata*, the causative agent of Lumbar Paralysis of cattle in East Asia, will maintain the infection when it molts to the next stages through to the adult stage (Tung et al. 2004). Transovarial transmission is a form of vertical transmission in which the female vector passes the infectious agent through her eggs to the next generation. For example, eggs passed by a female black fly (*Simulium vittatum*) infected with vesicular stomatitis virus will hatch infected

larvae (Rosen 1981; Comer et al. 1991). Transovarial transmission is of critical importance in maintaining infectious agents within fly populations between inter-epidemic periods (overwintering) (Coetzer et al. 2004). Similar to the mechanical vector, competence of the biological vector for pathogen transmission and maintenance will depend on the size of the fly population, the infection rates in the fly population, and temperature conditions that are critical for virus replication within the fly tissues (Mellor et al. 2007). Low environmental temperatures are associated with slow replication and decreased vector competence (Mellor et al. 2007). For biological transmission, high vector competence and transmission require high levels of viremia in the host (Coetzer et al. 2004).

Infectious Pathogens Transmitted by Flies to Livestock

More than 329 species of flies may act as mechanical or biological vectors of viruses, bacteria, protozoa, and helminths affecting farm animals worldwide; however, the most important fly families associated with disease transmission in livestock are *Culicidae* (mosquitoes), *Simulidae* (black flies), *Ceratopoginadae* (culicoides or biting midges), *Psychodidae* (sand flies), *Tabanidae* (horse flies), and *Muscidae* (house flies, face flies, horn flies, and tsetse flies) (Bowman 2004; Coetzer et al. 2004). Disease transmission by flies and distribution in a determined geographic region is influenced by specific environmental conditions that allow the development and interaction of an infectious agent with its host and its vector(s).

Mechanical Transmission of Viruses

Porcine Respiratory and Reproductive Syndrome Virus (PRRSV)

Porcine reproductive and respiratory syndrome virus (PRRSV) is an RNA *Arterivirus* in the family Arteriviridae that causes abortions, mummifications, and stillbirths in sows and severe

respiratory disease in young pigs (Otake et al. 2002; Otake et al. 2003). Distribution of the disease is concentrated principally in North America and Europe where it causes considerable economic losses to producers (Radostits et al. 2007). Experimental studies evaluating mosquitoes (*Aedes vexans*) feeding on viremic pigs have demonstrated the ability of mosquitoes to mechanically transmit PRRSV to naïve pigs (Otake et al. 2002). Biological replication of the virus in mosquito (*Aedes vexans*) tissues was not demonstrated (Otake et al. 2002; Otake et al. 2003). Other experimental studies have demonstrated that *Musca domestica*, the house fly is also an effective mechanical vector of PRRSV between infected and naïve pigs. Two infected house flies were able to transmit PRRSV to a naïve pig in one study (Otake et al. 2004). Studies using PCR techniques demonstrated that PRRSV can survive up to 6 hours on the house fly exoskeleton and up to 12 hours in their gastrointestinal tract after feeding on a viremic pig (Otake et al. 2004; Pitkin et al. 2009). House flies are able to disperse PRRSV on average 1.7 kilometers, which may explain how PRRSV is seasonally transported between farms (Schurrer et al. 2004; Otake et al. 2004).

Classical Swine Fever Virus (CSFV)

Classical swine fever virus is an RNA *Pestivirus* in the family *Flaviviridae* associated with acute, chronic, or inapparent disease in pigs of all ages (Ribbens et al. 2004). Epidemics of the disease are associated with high morbidity and mortality rates in pig operations (Ribbens et al. 2004). Classical swine fever is considered a disease of worldwide distribution although the United States, Canada, Australia, New Zealand, and South Africa have not experienced disease for many years (Radostits et al. 2007). Acute disease is usually manifested by fever, severe depression, purplish discoloration of the skin, and peracute death. Nervous and reproductive forms have been described (Ribbens et al. 2004). The virus is considered endemic in South

American countries, certain parts of Europe, and East Asia. This disease is associated with major economic losses and interferes with trade commerce between countries. The mosquito *Aedes aegypti* is able to retain the virus of classical swine fever for 3 days, and inoculation of pools of infected mosquitoes into naïve pigs results in persistent viremia and chronic disease (Stewart et al. 1975). Other proposed mechanical vectors for classical swine fever virus (CSFV) are horse flies (*Tabanus* spp.), house flies (*Musca domestica*), and stable flies (*Stomoxys calcitrans*) (Morgan et al. 1976); however, the role of arthropods and other vectors in the spread of CSFV remains doubtful and active transmission by flies has never been demonstrated (Ribbens et al. 2004).

Aujeszky's Disease Virus (Pseudorabies)

Aujeszky's disease is caused by the Suid herpesvirus type 1. The virus primarily affects pigs but naturally occurring cases of pseudorabies in cattle, sheep, and horses have been described and although rare, are usually fatal (Radostits et al. 2007). Sporadic cases in cattle and sheep develop when these animals are close to pigs excreting the virus or when they are commingled with pigs (Radostits et al. 2007). The disease has a wide geographical distribution including North America, Europe, North Africa, Asia, South America, New Zealand, and Ireland (Medveczky et al. 1988). Clinical signs of disease in piglets are characterized by fever, incoordination, recumbency, convulsion, and death. Respiratory and reproductive signs may also be seen in pigs (Medveczky et al. 1988). Disease in cattle is characterized by intense pruritus, convulsions, altered mentation, opisthotonos, paralysis and death (Radostits et al. 2007). The house fly (*Musca domestica*) was demonstrated to be a potential mechanical vector for the Aujeszky's disease virus (Medveczky et al. 1988). Although the virus is not able to replicate in fly tissues, it may be carried and transmitted to susceptible pigs for short periods of time (less

than 24 hours) (Medveczky et al. 1988). The quantity of virus (5 x 10^5 pfu / ml) shed by a single housefly during feeding and vomiting on the cornea or abraded skin proved to be sufficient to cause infection in susceptible pigs, rabbits, and a lamb (Medveczky et al. 1988). It is possible that house flies could play a role in transmission of infection within herds; however, transmission between herds is much less likely (Medveczky et al. 1988).

Equine Infectious Anemia Virus (EIAV)

Equine infectious anemia (EIA) virus is an RNA *Lentivirus* of the family *Retroviridae* and subfamily *Lentivirinae*. The disease affects horses, mules, donkeys, and zebras. The disease has a worldwide distribution and has been diagnosed in all continents except the Antarctica (Olsen 2003). Infection with the virus may result in fever, edema, petechial hemorrhages, chronic weight loss, and abortion, or may be clinically inapparent. Infected animals are persistently infected with the virus for life (Olsen 2003). Transmission of EIA virus occurs predominantly through transfer of contaminated blood from an infected horse to a susceptible horse by biting flies (Hawkins et al. 1976; Foil et al.. 1983). Studies demonstrated that horse flies (*Tabanus fuscicostatus*), deer flies (*Chrysops flavidus*), and stable flies (*Stomoxys calcitrans*), are mechanical vectors of EIA virus (Hawkins et al. 1976; Foil et al. 1983). One hundred stable flies, 6 deer flies, and a single horse fly were able to transmit the virus from an acutely infected pony to susceptible ponies after feeding until gorging (Hawkins et al. 1976; Foil et al. 1983). Members of the *Tabanidae* family are effective mechanical vectors due to their ability to ingest large volumes of blood (10 nanoliters) (Hawkins et al. 1976; Foil et al. 1983).

Lumpy Skin Disease Virus (LSDV)

Lumpy skin disease virus is a *Capripoxvirus* in the family *Poxviridae*. The disease primarily affects cattle from the sub-Saharan region of Africa, Egypt, Madagascar, and the

Middle East with recent incursion into Israel (Yeruham et al. 1995). The disease has not been reported in North America, South America, or Europe. Cattle acutely infected with the virus develop fever, nodular lesions in the skin and mucous membranes, and lymphadenopathy (Yeruham et al. 1995). Economic losses are associated with abortion and reproductive failure (Yeruham et al. 1995; Chihota et al. 2001). Dairy breeds, particularly Jersey, Guernsey, and Ayrshire are more susceptible to clinical disease (Yeruham et al. 1995). Most cases are believed to result from mechanical transmission by a fly vector. The mosquito *Aedes aegypti* was able to mechanically transmit the virus to susceptible cattle for 2 to 6 days after feeding on contaminated nodular lesions of infected cattle (Chihota et al. 2001). Fourteen out of 17 dairy herds from a region of Israel became infected with LSD virus during a period of 17 days (Yeruham et al. 1995). Epidemiological investigation suggests that the original infection was brought to Israel by stable flies (*Stomoxys calcitrans*) carried by the wind from foci in northern Sinai, or from the Nile delta in Egypt (Yeruham et al. 1995).

Bovine Viral Diarrhea Virus (BVDV)

Bovine viral diarrhea viruses 1 and 2 are enveloped, single-stranded, RNA viruses in the genus *Pestivirus* from the family *Flaviviridae* (Ridpath 2005). Bovine viral diarrhea virus is an important infectious pathogen associated with health-related economic losses in beef and dairy herds worldwide (Houe 1999). The virus is associated with a variety of clinical syndromes in domestic and wild ruminants including subclinical disease, diarrhea, respiratory disease, hemorrhagic syndrome, reproductive failure, persistent infection, and mucosal disease (Baker 1995). Persistently infected (PI) animals are considered the most important reservoir and source for viral transmission within and between herds (Houe 1999); however, other routes of transmission have been described. Flies may act as mechanical vectors of BVDV (Tarry et al..

1991; Gunn 1993). The horse fly *Hematopota pluvialis* and the stable fly *Stomoxys calcitrans* were able to transmit BVDV to naïve cattle after feeding on a persistently infected steer (Tarry et al. 1991). The virus was isolated from naïve cattle from 72 hours to 10 days after the flies fed on them (Tarry et al. 1991), and was recovered from flies (*H. pluvialis* and *S. calcitrans*) 96 hours after an infective feeding (Tarry et al. 1991). Serology obtained from cattle exposed to horse flies (*H. pluvialis*) which had previously fed on a PI animal demonstrated steadily increasing BVDV antibody titers (Tarry et al. 1991). In another study, BVDV was isolated from face flies (*Musca autumnalis*) that were feeding on a PI bullock; however, experimental transmission with face flies was not attempted (Gunn 1993). Transmission of BVDV was not possible when groups of 50 horn flies (*Haematobia irritans*) previously feeding on different PI animals were transferred to 4 different BVDV naïve calves (Chamorro et al. 2011); however, BVDV was detected by PCR and virus isolation techniques in all horn fly homogenates (Chamorro et al. 2011).

Bovine Leukemia Virus (BLV)

Bovine leukemia virus is an RNA C-type *Oncovirus* in the family *Retroviridae* which had been identified as the causal agent of enzootic bovine lymphosarcoma (Radostits et al. 2007). The disease occurs worldwide and prevalence of infection varies between countries (Buxton et al. 1985; Weber et al. 1988). Infection of naïve cattle occurs through natural or iatrogenic transfer of lymphocytes containing virus (Burton et al. 2010). Following transmission to susceptible cattle, persistent infection and permanent antibody response is triggered. Around 30% of the cases develop persistent lymphocytosis (Hasselschewert et al. 1993; Burton et al. 2010). In general, neoplastic disease and clinical signs are not observed during the initial stages of infection (Burton et al. 2010). Lymphosarcoma develops in less than 5% of BLV infected cattle and a variety of clinical manifestations may be observed (Burton et al. 2010). Chronic

weight loss, anorexia, decreased milk production, and lymphadenopathy are some of the most common clinical signs observed in affected cattle (Burton et al. 2010). The neoplastic disease is fatal and causes economic losses to producers due to decreased production and slaughter of valuable animals. Horse flies and deer flies were identified as mechanical vectors of BLV (Buxton et al. 1985; Weber et al. 1988; Manet et al. 1989). Blood infected with BLV applied by capillary action to the mouthparts of 15 deer flies (*Chrysops flavidus*) and to a single horse fly (*Tabanus atratus*) that subsequently were allowed to feed on 2 naïve sheep, respectively, induced BLV seroconversion in the sheep. Horse flies (*Tabanus fuscicostatus*) were able to transmit BLV from a cow with persistent lymphocytosis to naïve beef and dairy calves (Hasselschewert et al. 1993). Transmission of BLV was accomplished with groups of 50 and 250 horse flies for beef calves and 75 and 250 horse flies for dairy calves (Hasselschewert et al. 1993). Studies demonstrated that stable flies (*Stomoxys calcitrans*) are able to carry BLV in their midgut and proboscis and could be considered potential mechanical vectors of BLV (Buxton et al. 1985; Freitas et al. 1991). Fifty stable flies that had previously fed on BLV viremic calves were able to transmit the virus to only 1 of 3 naïve BLV-seronegative calves (Weber et al. 1988). Other studies have demonstrated that BLV can be transmitted to naïve calves after inoculation of mouthparts of at least 50 BLV positive stable flies (*Stomoxys calcitrans*) or 100 BLV positive horn flies (*Haematobia irritans*) (Buxton et al. 1985); however, if inoculation of mouthparts is delayed greater than or equal to 1 hour after blood feeding, transmission does not occur (Buxton et al. 1985). While transmission of BLV has been documented via blood feeding insects, the significance of this route is unclear.

Other Viruses Mechanically Transmitted by Flies

The transmissible gastroenteritis (TGE) coronavirus that causes acute diarrhea in piglets has been detected by specific fluorescent antibody testing in house flies (*Musca domestica*) collected from a swine confinement facility in which TGE was enzootic (Gough et al. 1983). The authors proposed that TGE virus could be mechanically transmitted by house flies (Gough et al. 1983). The horn fly (*Haematobia thirouxi*) found in large amounts on buffalo in Africa has been implicated in the mechanical transmission of foot and mouth disease virus (FMDV), since buffalo are reservoirs of the virus (Coetzer et al. 2004). However, experimental transmission of FMDV by this route has been unsuccessful (Coetzer et al. 2004).

Mechanical Transmission of Bacteria and Rickettsia

Anaplasma spp.

Anaplasma spp. are obligate intra-erythrocytic rickettsia that cause anemia and abortion in ruminants. *Anaplasma marginale* is the causative organism of anaplasmosis in cattle and wild ruminants (Aubry et al. 2011). *Anaplasma ovis* is the causative agent in sheep and goats. *Anaplasma centrale* is closely related to *A. marginale* and causes mild anaplasmosis in cattle (Aubry et al. 2011). Anaplasmosis is common in tropical and subtropical regions and sporadic in temperate regions (Potgieter et al. 1981). Acute infection with *A. marginale* in cattle results in fever, anemia, icterus, abortion and death (Aubry et al. 2011). In sheep and goats, the infection is usually subclinical, but severe anemia may occur, particularly in goats. After acute infection, persistent infection with intermittent cycles of rickettsemia is common in affected animals (Aubry et al. 2011). Persistent infection is the main source and reservoir of anaplasmosis in cattle herds (Radostits et al. 2007). The disease is biologically transmitted by ticks in the genus *Boophilus* spp. and *Dermacentor* spp.; however, tabanid species have been incriminated in the mechanical transmission of *A. marginale* and *A. ovis* in central Europe (Hornok et al. 2008).

Studies have demonstrated that *Tabanus fuscicostatus* and *Tabanus nigrovittatus* are able to mechanically transmit *A. marginale* from acutely infected to naïve cattle (Wilson et al. 1966; Hornok et al. 2008). Additionally, evidence of mechanical transmission of *Anaplasma* spp. was determined after molecular characterization of *A. marginale* and rickettsial endosymbionts in *Tabanus bovinus* (Hornok et al. 2008). Experimental transmission of *A. marginale* to susceptible animals via stable flies (*Stomoxys calcitrans*) previously fed on splenectomized cattle acutely infected with *A. marginale* was successful in 1 of 3 attempts (Potgieter et al. 1981); however, in a more recent experimental trial, stable flies were not able to transmit *A. marginale* from an acutely infected calf to naïve calves (Scoles et al. 2008).

Salmonella spp.

The genus *Salmonella* belongs to the family *Enterobacteriaceae* and currently there are 2,463 serovars of *Salmonella* (Mohler et al. 2009). Two species have been described for the genus *Salmonella*, *Salmonella enterica* and *Salmonella bongori*. The subspecies *Salmonella enterica* subsp. *enterica* represents the majority (59%) of the 2,463 serovars of Salmonella (Olsen et al. 2000). Serotypes within this subspecies are considered important pathogens for human and animal health (Olsen et al. 2000; Mohler et al. 2009). Salmonella are facultative intracellular bacteria that survive in the phagolysosome of macrophages; thus evading the immune response (Mohler et al. 2009). Salmonella organisms may establish a host-adapted infection in specific hosts (Olsen et al. 2000). In the case of cattle, animals infected with *Salmonella dublin* become chronic carriers of the infection and are the main source of bacteria for their herdmates (Mohler et al. 2009). Clinical disease in cattle may manifest as a variety of clinical syndromes including inapparent or subclinical disease, septicemia in young calves, enterohemorrhagic diarrhea, septic arthritis, osteomyelitis, and abortion (Mohler et al. 2009).

Salmonella infections occur in all species and are spread by direct and indirect means (Olsen et al. 2000). Flies have been proposed as effective mechanical vectors of *Salmonella* spp. contributing to the environmental contamination and transmission of disease to humans and animals (Olsen et al. 2000; Winfield et al. 2003). Infection rates of adult house flies (*Musca domestica*) with *Salmonella* spp. on dairy farms can reach 67% (Winfield et al. 2003). Additionally, *Salmonella* is capable of surviving in fly tissues for up to 4 weeks, which is the entire lifespan of a house fly (Winfield et al. 2003). Thus, flies that come in contact with contaminated materials in dairies (i.e., manure, feed, and water) carry *Salmonella* organisms and increase the risk of transmission and infection to cattle and humans within farms (Winfield et al. 2005). An epidemiological study demonstrated that the presence of a large numbers of house flies (*Musca domestica*) in manure and feed in feedlot operations was an important risk factor for *Salmonella* shedding in cattle (Vanselow et al. 2007).

Escherichia coli O157:H7

Enterohemorrhagic *E. coli* O157:H7 is a verotoxin-producing, gram-negative organism that has become a worldwide public health concern because of its ability to cause food-borne poisoning and severe illness in humans (Ferens et al. 2011). Severity of clinical syndromes caused by *E. coli* O157:H7 in humans may vary from mild diarrhea to hemorrhagic colitis or hemolytic uremic syndrome (Ferens et al. 2011). Cattle are natural reservoirs of *E. coli* O157:H7 where clinical signs of disease are not commonly observed (Ahmad et al. 2007). The bacteria live in the intestines of healthy cattle and are constantly shed in their feces (Ahmad et al. 2007; Ferens et al. 2011). Contamination of carcasses and water sources with cattle feces are considered the most important routes of transmission and infection to humans (Ferens et al. 2011; Ahmad et al. 2007). Most of the epidemiological research in *E. coli* O157:H7 disease

outbreaks in humans were traced to the consumption of undercooked beef that was previously contaminated with bovine feces (Ferens et al. 2007). Studies demonstrated that one of the potential mechanisms of dissemination of the bacteria is through insects that develop in cattle manure such as house flies (*Musca domestica*) (Ahmed et al. 2007). Another study demonstrated that exposure of naïve calves to house flies (*Musca domestica*) inoculated with *E. coli* O157:H7 resulted in isolation of the bacteria in the calves' feces and drinking water after 1 day of exposure (Ahmed et al. 2007). Feces from all the calves remained positive to *E.coli* O157:H7 for 11 days, and in 62% of them, feces remained positive for 19 days (Ahmed et al. 2007). Therefore, the house fly (*Musca domestica*) is considered an effective mechanical vector for *E.coli* O157:H7, and it may be one of the routes of bacterial dissemination and exposure to human populations.

Moraxella bovis

Moraxella bovis are gram-negative bacteria identified as the causal agent of infectious bovine keratoconjuntivitis (IBK), although other infectious agents such as bovine herpesvirus type-1 and *Mycoplasma bovoculi* enhance the presentation of the disease (Kopecky et al. 1986; Brown et al. 1998). The disease affects only cattle and occurs in most countries of the world, especially in the summer and autumn (Brown et al. 1998). Clinical manifestations of acute infection in cattle include conjunctivitis, keratitis, corneal opacity, blepharospasm, and photophobia. Clinical signs are usually unilateral but both eyes may be affected (Brown et al. 1998). Cattle act as a reservoir and source of the infection through carrier animals that maintain the bacteria in their conjunctiva, nares, and vagina (Kopecky et al. 1986; Brown et al. 1998). The most important route of transmission of the bacteria from carrier, or affected cattle to susceptible cattle is through face flies (*Musca autumnalis*) (Gerhardt et al. 1982; Kopecky et al. 1986).

Moraxella bovis was isolated from face flies that had previously fed on the eyes of infected cattle in one study (Glass et al. 1984). Face flies remained infected with *M. bovis* for up to 3 days (Glass et al. 1984). Experimental transmission from cattle with IBK to susceptible cattle through face flies has also been demonstrated (Glass et al. 1984). Direct contact in absence of flies did not effectively transmit IBK from experimentally infected calves to naïve cattle (Kopecky et al. 1986). Spread of IBK in the summer months from affected to unaffected herds was observed once fly populations exceeded 10 face flies per animal (Gerhardt et al. 1982). The Asian face fly (*Musca bezzi*) is also considered an important vector for IBK (Radostits et.al 2007). Other factors associated with the presentation of the disease are the presence of dust and solar irradiation (Radostits et al. 2007).

Corynebacterium pseudotuberculosis

Corynebacterium pseudotuberculosis is a soil-borne, gram-positive rod that is the causative agent of caseous lymphadenitis in sheep and goats, ulcerative lymphangitis in cattle and horses, and external and internal abscesses in horses (pigeon fever) and cattle (Dorella et al. 2006). Additionally, it is considered a zoonotic microorganism (Dorella et al. 2006). Human infection can occur as a result of close contact with infected animals or consumption of nonpasteurized milk (Radostits et al. 2007). In Israel, a mastitic form of *Corynebacterium pseudotuberculosis* was described in dairy cattle (Yeruham et al. 1996). Teat skin lesions increase the risk of *C. pseudotuberculosis* contamination of the udder and development of the mastitic form (Yeruham et al. 1996). The disease in cattle and horses is uncommon, and cases in these species are seen mostly in the United States, North Africa, and Israel (Radostits et al. 2007). The organism possesses a cytotoxic surface lipid coat that facilitates intracellular survival and abscess formation and also produces a phospholipase exotoxin that contributes to its spread

and pathogenesis (Dorella et al. 2006). Direct contact and mechanical transmission by flies are considered the main routes of spread of the disease (Yeruham et al. 1996; Dorella et al. 2006). The house fly (*Musca domestica*) plays an important role in harboring and disseminating *C. pseudotuberculosis* in horse barns and dairy herds (Yeruham et al. 1996; Spier et al. 2004). The organism was isolated from flies feeding in lesions from cattle infected with *C. pseudotuberculosis* and in flies feeding in milk from cows with *C. pseudotuberculosis* mastitis (Yeruham et al. 1996; Braverman et al. 1999). The infected house flies excreted *C. pseudotuberculosis* in saliva from 5 minutes to 3 hours after feeding on lesions or contaminated milk from affected cows (Yeruham et al. 1996; Braverman et al. 1999). Bacteria were also isolated from the intestine and feces of 40 of 60 flies between 1 and 4 hours after feeding on contaminated milk (Yeruham et al. 1996; Braverman et al. 1999). Another study identified the exotoxin (phospholipase D) from *C. pseudotuberculosis* by RT-PCR testing of pools of house flies, stable flies, and horn flies trapped near horse barns containing affected horses (Spier et al. 2004).

Brucella abortus

Brucella abortus are gram negative bacteria that cause brucellosis in cattle (Carvalho et al. 2010). The disease has a worldwide distribution and is of major economic importance in developing countries. Brucellosis is the major cause of abortion in cattle in countries without a national control program (Radostits et al. 2007). Brucellosis is also an important zoonosis causing debilitating disease in humans (Carvalho et al. 2010). The organism is a facultative intracellular agent that causes a persistent infection and is continuously or intermittently shed in reproductive and mammary secretions (Carvalho et al. 2010). Acute infection manifests as abortion outbreaks in unvaccinated heifers and cows after the 5^{th} month of pregnancy, and as

orchitis and epididymitis in bulls (Carvalho et al. 2010). Synovitis has been described in other species (Radostits et al. 2007). Direct contact with infected uterine discharges is considered the main route of transmission between and within herds (Carvalho et al. 2010). The face fly (*Musca autumnalis*) has been reported as a mechanical vector of *B. abortus* for short periods of time (Cheville et al. 1989). *Brucella abortus* transiently persisted in the midgut and was eliminated in feces from face flies (Cheville et al. 1989). Bacterial replication in fly tissues was not observed, and *B. abortus* was recovered from face fly homogenates at 12 hours but not at 24 or 72 hours post experimental infection of the flies (Cheville et al. 1989). The bacterial excretion without midgut replication is consistent with transient but not long-term mechanical transmission of brucellosis in nature (Cheville et al. 1989).

Bacillus anthracis (Anthrax)

Bacillus anthracis is a gram positive spore-forming rod that causes anthrax outbreaks in cattle, small ruminants and horses (Coetzer et al. 2004a). Anthrax is also a cause of fatal disease in humans (Coetzer et al. 2004a). Spores of the bacteria survive in soil for many years and disease can be enzootic in certain regions. Pathogenic strains have plasmid-encoded virulence factors responsible for the lethal effects resulting from bacterial infection (Coetzer et al. 2004a). The disease originated in the sub-Saharan region of Africa but now has worldwide distribution. In tropical and subtropical regions with high annual rainfalls, bacteria persist in the soil and frequent outbreaks of anthrax are observed (Radostits et al. 2007). In temperate regions, sporadic outbreaks are seen commonly after accidental ingestion of contaminated feed. Clinical disease is characterized by severe septicemia and sudden death. Infective spores of the bacteria gain entrance to the body through ingestion and inhalation, or through the skin (Coetzer et al. 2004a). Flies and other insects have been found to harbor anthrax organisms, and some are able to

transmit it mechanically (Fasanella et al. 2010). Increases in the fly populations are often associated with anthrax epidemics in domestic animals (Fasanella et al. 2010). Experimental transmission of anthrax was demonstrated in guinea pigs and mice after the feeding of 2 to 4 stable flies (*Stomoxys calcitrans*), or 2 to 4 mosquitoes (*Aedes aegypti* or *Aedes taeniorhynchus*) on a bacteremic animal and subsequent feeding on susceptible naïve animals (Turell et al. 1987). Horse flies (*Tabanus* spp.) were also reported as effective mechanical vectors of *Bacillus anthracis* contributing to the natural spreading of the disease (Hugh-Jones et al. 2002). Another study demonstrated that house flies (*Musca domestica*) are infected with anthrax after feeding on contaminated carcasses or blood from animals dying from the disease (Fasanella et al. 2010). Additionally, sporulation of the *Bacillus anthracis* was detected in the midgut of the house flies revealing potential capabilities as mechanical vectors of anthrax (Fasanella et al. 2010). The potential for flies to mechanically transmit anthrax suggests that fly control should be considered as part of a program for control of epizootic anthrax (Hugh-Jones et al. 2002).

Bacterial Pathogens Causing Mastitis

Bovine mastitis is the most common and economically important disease affecting dairy cattle worldwide (Radostits et al. 2007). Mastitis is the inflammation of the mammary gland tissue usually as a consequence of bacterial infection (Radostits et al. 2007). Clinical mastitis is characterized by a range of physical and chemical changes in the milk and pathological changes in the glandular tissue (Owens et al. 1998). Contagious and environmental pathogens are incriminated in cases of mastitis in cattle (Owens et al. 1998). Contagious pathogens are transmitted at milking from infected to susceptible cattle and include *Staphylococcus aureus, Streptococcus agalactiae, Mycoplasma bovis,* and *Corynebacterium bovis*. Environmental pathogens are usually acquired from the environment and include coliforms (*E.coli, Klebsiella*

spp.), *Streptococcus uberis, Streptococcus dysgalactiae,* and *Arcanobacterium pyogenes* (Radostits et al. 2007). Flies can be involved in the mechanical transmission of contagious and environmental mastitis pathogens between affected and susceptible cattle (Madsen et al. 1992; Owens et al. 1998). Head flies (*Hydrotea irritans*) were allowed to feed on milk from cows experiencing summer mastitis caused by *Staphylococcus aureus*, *Streptococcus dysgalactiae,* and *Arcanobacterium pyogenes* (Chirico et al. 1997). Following feeding, head flies were exposed to 8 healthy and naïve heifers. Two teats in each heifer were deliberately damaged before fly exposure. One teat was cut and the other pricked with needles to mimic insect bites. Two of the heifers developed summer mastitis in the quarters where teats had been cut. The bacterial species isolated from these quarters corresponded to those that had previously been fed to the flies (Chirico et al. 1997). Experimental infection of horn flies (*Haematobia irritans*) with *Staphylococcus aureus* and subsequent exposure of flies to 4 uninfected teats of healthy heifers resulted in transmission of the bacteria (Owens et al. 1998). Head flies and horn flies are effective mechanical vectors of summer mastitis pathogens, and lesions in the teat skin may predispose to transmission and development of clinical disease (Chirico et al. 1997; Owens et al. 1998).

Other Bacteria Mechanically Transmitted by Flies

The house fly (*Musca domestica*) was incriminated as a mechanical vector of several bacteria including *Shigella* spp. and *Bartonella hensale*; however, experimental evaluation of mechanical transmission has not been reported (Coetzer et al. 2004). *Tabanus* species and mosquitoes may mechanically transmit *Clostridium chauvoei, Clostridium perfringens, Coxiella burnetii* (Q fever), *Erysipelothrix rhusiopathiae, Fusobacterium necrophorum, Listeria monocytogenes, Borrelia burgdorferi* (lyme disease), *Pasterella multocida, Mycoplasma ovis,*

Mycoplasma suis, and *Francisella tularensis* (Coetzer et al. 2004); however, experimental evaluation of transmission has not been described.

Mechanical Transmission of Protozoa

Cryptosporidium spp.

Cryptosporidiosis is caused by the coccidial parasites from the genus *Cryptosporidium*, of which there are at least 19 recognized species (Chako et al. 2010). *Cryptosporidium parvum* is the causal agent of cryptosporidiosis in cattle and other ruminants. In people, *Cryptosporidium hominis* is recognized as the principal causal agent of the disease (Chako et al. 2010); however, *Cryptosporidium parvum* is also an important cause of cryptosporidiosis in people when transmitted from cattle to humans. Cryptosporidiosis is a disease with worldwide distribution that affects primarily neonatal ruminants, but also has been reported in foals and piglets (Chako et al. 2010). Clinical manifestation of the disease in neonatal animals is associated with severe malabsorptive diarrhea (Chako et al. 2010). In people, severe non-responsive diarrhea is associated with cryptosporidiosis in immunosuppressed individuals, especially in those with acquired immune deficiency syndrome (AIDS) (Graczyk et al. 1999; Chako et al. 2010). Transmission of cryptosporidiosis occurs after oral ingestion of sporulated oocysts from contaminated environment, feed, or water, and by direct contact with fecal material from individuals or animals actively shedding oocysts (Graczyk et al. 1999; Chako et al. 2010). The house fly (*Musca domestica*) and other filth flies from the families *Calliphoridae* and S*acrophagidae* have been identified as mechanical vectors of *Cryptosporidium* spp. and *Giardia* spp. in farm animals (Graczyk et al. 2004; Conn et al. 2005). The breeding and feeding ecology of filth flies is considered a critical factor in the epidemiology and transmission of cryptosporidiosis between animals and humans (Conn et al. 2005). *C. parvum* oocysts in house

flies (*Musca domestica*) that had emerged from feces of infected cattle have been demonstrated using immufluorescent antibody tests (IFA) and fluorescent in situ hybridization (FISH) (Conn et al. 2005). The average number of infective *Cryptosporidium* spp. oocysts per adult house fly (*Musca domestica*) varied from 267 oocysts after 3 days of emersion to 14 oocysts at day 11 after emersion (Graczyk et al. 2004). Experimental induction of cryptosporidiosis in neonatal mice was performed using infective oocysts recovered from house flies (*Musca domestica*) that emerged from feces of diarrheic calves (Graczyk et al. 2004; Graczyk et al. 2005). Deposition of infective *C. parvum* oocysts by house flies through fecal spots and vomit drops on visited surfaces averaged 108 oocysts per cm^2 (Graczyk et al. 2004). The literature agrees that only 1 infective *C. parvum* oocyst is necessary to induce infection and disease in calves (Chako et al. 2010). Adult and larval stages of house flies (*Musca domestica*) having access to *C. parvum*-contaminated substrate mechanically carry oocysts and deposit them on visited surfaces, thus increasing the risk of disease in animals and humans (Graczyc et al. 1999; Graczyk et al. 2004).

Other Protozoa Mechanically Transmitted by Flies

Two species of filth flies, the house fly (*Musca domestica*) and the blow fly (*Chrysomia megacephala*) mechanically contaminated milk after exposure to cat feces containing oocysts of *Toxoplasma gondii*. Toxoplasma was also isolated from larvae and pupae of these flies after they had been reared in infectious cat feces (Wallace 1971); however, experimental transmission of the disease by flies has not been demonstrated. Another study described the ability of filth flies to naturally transport *Sarcocystis* spp. oocysts and other coccidia (Markus 1980).

Mechanical Transmission of Helminths

Gastrointestinal Nematodes of Pigs

The house fly (*Musca domestica*) was described as a mechanical vector of gastrointestinal parasites of pigs (Forster et al. 2009). In a single study, 224 house flies were caught at an organic pig farm and were examined for their potential as carriers of gastrointestinal parasites of pigs. Oocysts and larvae of 4 nematode species (*Ascaris suum*, *Strongyloides ransomi*, *Metastrongylus* spp., and Strongyles) were isolated from the exoskelelons and the intestines of house flies (Forster et al. 2009). The analysis of the pig feces revealed many eggs and larvae of nematodes found in the flies. Further experiments revealed the ability of the house fly (*Musca domestica*) to mechanically transmit *Ascaris suum* and *Trichuris suis* from contaminated to clean areas (Forster et al. 2009).

Biological Transmission of Viruses

Eastern, Western, and Venezuelan Encephalomyelitis

Alphaviruses of the family *Togaviridae* are RNA viruses that are the causal agents of eastern equine encephalomyelitis (EEE), western equine encephalomyelitis (WEE), and Venezuelan equine encephalomyelitis (VEE) (Radostits et al. 2007). Horses, as well as other mammals including humans, are accidental hosts of alphaviruses, while birds are considered the definitive hosts (Fulhorst et al. 1994). In the case of EEE and WEE, horses are dead-end hosts, and therefore transmission to insect vectors does not occur (Radostits et al. 2007). In the case of VEE, horses develop high titer viremia after infection and transmission to insect vectors can occur (Smith et al. 2007). Distribution of these viral pathogens was originally limited to the Americas; however, the diseases have been reported in other parts of the world (Fulhorst et al. 1994; Cupp et al. 2003). Whereas EEE and WEE are distributed predominantly in North and South America, VEE is more common in South America and Central America (Smith et al. 2007). Outbreaks of EEE, WEE, and VEE in horses usually affect multiple individuals,

especially young animals. The clinical presentation for these viral diseases includes fever and neurological signs such as ataxia, incoordination, circling, staggering, and paralysis (Hayes et al. 1977). Human disease is characterized by flu-like symptoms, and in 4 to 14% of patients neurological signs develop (Radostits et al. 2007). Mosquitoes of the genus *Culex* spp. and *Aedes* spp. are considered the most important biological vectors of EEE, WEE, and VEE and are responsible for the transmission and maintenance of the viruses between birds, horses, and other mammals (Fulhorst et al. 1994; Cupp et al. 2003). Vertical transmission is an important overwintering mechanism for alphaviruses, especially for EEE and WEE viruses. Replication of EEE, WEE, and VEE in mosquito populations (*Culex* spp. and *Aedes* spp.) has been described after ingestion of infected blood (Cupp et al. 2003; Wang et al. 2010). Additionally, the replication of EEE and WEE viruses in ovarian follicles from *Culex pipiens* suggests transovarial transmission to subsequent generations of mosquitoes (Fulhorst et al. 1994; Wang et al. 2010). The mosquito *Culiseta melanura* was identified as the most important vector of EEE (Cupp et al. 2003). Rapid viral replication of EEE in salivary glands of *Culiseta melanura* is a key factor in the successful transmission of the disease to susceptible animals (Scott et al. 1984; Cupp et al. 2003). Experimentally, the mosquito *Culex tarsalis* transmitted WEE virus from infected to naïve animals (Hayes et al. 1977). Experimental exposure of mosquitoes (*Culex (Melanoconion) cedecei*) to hamsters viremic with VEE resulted in high transmission and infection rates in mosquitoes (Turell et al. 2003). Studies in other mosquitoes such as *Aedes aegypti* and *Aedes taeniorhynchus* demonstrated their ability to harbor, disseminate, and transmit VEE virus to susceptible animals (Smith et al. 2007; Ortiz et al. 2008). Other flies such as *Culicoides* spp., *Simulium* spp., and *Tabanus* spp. were reported as potential biological or mechanical vectors of EEE, WEE, and VEE (Coetzer et al. 2004).

West Nile Encephalitis Virus (WNV)

West Nile virus (WNV) is an RNA *Flavivirus* in the family *Flaviviridae* that causes encephalitis in humans, horses, and other mammals (Bunning et al. 2002). The disease is enzootic in Africa, but spread to other regions has been described and include the Middle East, Israel, southern Europe, North America, and the Caribbean (Radostits et al. 2007). Clinical manifestations of the disease in horses are characterized by neurological signs including hyperexcitability, ataxia, paresis, and head tilt (Bunning et al. 2002). Infection in humans is manifested initially as flu-like febrile illness that may progress to neurological disease (Murray et al. 2011). The virus is maintained by cycling between amplifying hosts (birds) and mosquitoes (Murray et al. 2011). Transmission of infection to horses and humans occurs only through the bite of an infected mosquito (Bunning et al. 2002). Horses and humans are considered dead-end hosts in which the magnitude of the viremia is not sufficient to infect mosquitoes (Murray et al. 2011); however, titers of viral infection are usually high enough to cause transmission after a blood transfusion or organ transplant (Murray et al. 2011). Mosquitoes from the genus *Culex* are considered the most important biological vectors of WNV (Murray et al. 2011). Passerine birds are the most important non-vector reservoir of the infection (Bunning et al. 2002). Experimental studies demonstrated that the mosquito *Culex pipiens* shows the highest infection and transmission rates (87.5 and 74.2%, respectively) when allowed to feed on infectious blood meals (Jiang et al. 2010). Transovarial transmission of WNV in the mosquitoes *Aedes albopictus*, *Aedes aegypti,* and *Culex tritaeniorhynchus* was demonstrated after experimental inoculation of female mosquitoes with WNV and subsequent isolation of the virus from larvae and pupae from hatched eggs (Baqar et al. 1993). Experimental transmission of WNV was demonstrated after induction of WNV infection in *Culex univittatus* and subsequent exposure to naïve hamsters

(Cornell et al. 1989). Viral transmission rates to naïve hamsters in this study varied from 59 to 100% (Cornell et al. 1989). Experimental infection in horses was demonstrated after exposure to *Aedes albopictus* previously infected with WNV (Bunning et al. 2002). All horses developed neutralizing antibody titers > 1:80 after exposure. The stable fly (*Stomoxys calcitrans*) was described as a potential mechanical vector of WNV after isolating WNV from stable flies feeding on viremic pelicans (Johnson et al. 2010).

African Horse Sickness Virus (AHSV) and Other Orbivirus Infections in Horses

African horse sickness is a severe and fatal disease of horses, mules, donkeys, zebras, and camels caused by an RNA viscerotropic *Orbivirus* in the family *Reoviridae* (Mellor et al. 2004). Morbidity and mortality rates of the disease can be as high as 95% (Capela et al. 2003). The disease is enzootic in the sub-Saharan (south), central, and east Africa; however, outbreaks of the disease have been reported in Egypt, the Middle East, Spain, Portugal, Morocco, Pakistan, and India (Mellor et al. 2004). Some authors speculate that climatic changes such as global warming may be responsible for the spread of African horse sickness virus to regions previously free from the disease (Mellor et al. 2004). Dispersal of insect vectors (*Culicoides* spp.) over long distances through warm winds has been proposed (Mellor et al. 2004). Clinical manifestations of the disease are variable, and four clinical forms are described: pulmonary, cardiac, mixed, and mild. In general, animals affected by any form present with high fever, edema formation, and nasal discharge (Mellor et al. 2004). Transmission of AHSV is influenced by the presence of hematophagous insects that transmit the virus after a bite (Capela et al. 2003). Biting midges (*Culicoides spp*.) and mosquitoes are considered the most important biological vectors of the disease (Capela et al. 2003; Mellor et al. 2004). In endemic areas, AHSV is maintained by cycling between zebras and biological vectors such as the biting midges *Culicoides imicola* and

Culicoides bolitinos (Capela et al. 2003; Mellor et al. 2004; Venter et al. 2007). Infection rates after an infected blood meal are higher in *Culicoides imicola* and *Culicoides bolitinos* as compared to other culicoides species (Venter et al. 2007). In one study, oral infection of *Culicoides imicola* and *Culicoides bolitinos* after feeding on infected blood resulted in isolation of AHSV in vector pools until 10 days post-feeding (Venter et al. 2000); however, transovarial transmission of AHSV on *Culicoides spp*. has not been described (Venter et al. 2000; Mellor et al. 2004). Other culicoides species such as *Culicoides varipennis, Culicoides pulicaris,* and *Culicoides obsoletus* were described as competent biological vectors of AHSV due to their ability to maintain the infection over winter (Capela et al. 2003). Other possible vectors for AHSV include mosquitoes, ticks (*Hyalomma dromadarii* and *Rhipicephalus sanguineus*) and muscid flies (*Tabanus* spp. and *Stomoxys* spp.) (Mellor et al. 2004).

Equine encephalosis virus is also an *Orbivirus* in the family *Reoviridae* transmitted by *Culicoides spp*. that affects horses in the region of southern Africa and causes neurological disease (Paweska et al. 2004). Viral infection and replication in various *Culicoides* species including *C. imicola, C. bolitinos,* and *C. leucostictus* were described (Paweska et al. 2004). Recently, in Peru and Australia, another *Orbivirus* of the family *Reoviridae* was isolated from equids presenting with fatal neurological disease (78% case fatality rate) (Attoui et al. 2009). Molecular isolation of the same virus was demonstrated in *Culicoides* spp. and mosquitoes collected from the same region (Attoui et al. 2009). This virus belongs to a new species named the Peruvian horse sickness virus (PHSV) and is also known in Australia as Elsey virus (ELSV).

Vesicular Stomatitis Virus

Vesicular stomatitis virus is an RNA *Vesiculovirus* in the family *Rhabdoviridae* associated with severe vesicular disease in cattle, horses and pigs (Mead et al. 2009). There are 2

distinct serotypes of the virus: vesicular stomatitis New Jersey (VSV-NJ) and vesicular stomatitis Indiana (VSV-IN) (Walton et al. 1987). The most virulent and common serotype is VSV-NJ (Radostits et al. 2007). Disease caused by vesicular stomatitis viruses is important because it is indistinguishable from foot and mouth disease (FMD) (Walton et al. 1987). Additionally, VSV is associated with high morbidity (10-80%) and high production losses in cattle herds (Walton et al. 1987); however, overall case fatality rates are low (0-15%). The disease predominates in the western hemisphere and is endemic in Mexico, northern South America, and Ossabaw Island in the state of Georgia in the United States (Radostits et al. 2007). Sporadic outbreaks of the disease have been reported in Brazil, Argentina, and the United States (Radostits et al. 2007). Clinical manifestation of the disease is characterized by vesicular lesions and erosions on the oral mucosa, tongue, coronary band, and teats (Mead et al. 2009). Transmission of the disease involves insect vectors and direct contact or ingestion of contaminated secretions, feed, or water (Radostits et al. 2007). Black flies and sand flies are the biological vectors of the virus (Mead et al. 2009). Transovarial transmission of VSV has been described in black flies (*Simulium vittatum*) and sand flies (*Lutzomia shannoni*) (Braverman et al. 1994; Smith et al. 2009). Viral maintenance in enzootic areas is suspected to be through transovarial transmission in black and sand flies and through cycling between insects and susceptible hosts (Mead et al. 2009). Feral pigs are believed to be the reservoirs and amplifying hosts of VSV on Ossabaw Island (Radostits et al. 2007). Other studies proposed cattle as a potential amplifying host of VSV-NJ due to their ability to transmit the virus to uninfected black flies (*Simulium vittatum*) (Smith et al. 2010). Experimental studies demonstrated replication of the virus in the midgut and salivary glands of black flies (*Simulium vittatum*) after oral ingestion or intrathoracic inoculation with VSV-NJ (Howerth et al. 2002). Experimental transmission of VSV-NJ was demonstrated in cattle and

pigs after exposure of naïve animals to black flies (*Simulium vittatum*) that had previously fed on vesicular lesions of affected animals (Mead et al. 2004; Mead et al. 2009). Clinical disease and seroconversion to VSV-NJ in exposed animals confirmed the vector competence of black flies (Mead et al. 2004; Mead et al. 2009). Mechanical and biological transmission of VSV by other vectors including *Culicoides* spp., *Phlebotomus* spp., *Lutzomia* spp., and *Tabanus* spp. were also described (Walton et al. 1987; Kramer et al. 1990; Coetzer et al. 2004).

Akabane Virus (AKV) and Cache Valley Virus

Akabane virus and Cache Valley virus are RNA viruses in the genus *Bunyavirus* from the family *Bunyaviridae* and are associated with abortion and congenital arthrogryposis and hydrancephaly in ruminants (Jennings et al. 1989; Radostits et al. 2007). Akabane virus is thought to be endemic in East Asia, Japan, Australia, the Middle East, and Africa, while Cache Valley virus has been reported in North America (Jennings et al. 1989; Radostits et.al 2007). Great economic losses in cattle may be associated with abortions and abnormal calves after outbreaks of Akabane disease (Ogawa et al. 2007). Clinical manifestations in ruminants are associated with reproductive loss, arthrogryposis, and hydrancephaly in neonates (Jennings et al. 1989). Newborn calves are unable to stand, and usually have lack of coordination (Radostits et al. 2007). Morbidity of the disease ranges from 15% to 80% in endemic areas, and the case fatality rate is usually high in affected newborns (Ogawa et al. 2007; Kono et al. 2008). A few isolates, specifically the Iriki strain of Akabane virus can cause encephalomyelitis in calves and adult cattle (Kono et al. 2008). Some reports from Japan described neurological signs in affected cattle including tremors, ataxia, lameness, paralysis, nystagmus, opisthotonos, and hypersensitivity (Ogawa et al. 2007; Kono et al. 2008). The virus is maintained in nature by cycling in vector populations through transovarial transmission and overwintering (Jennings et

al. 1989). Biting midges from the genus *Culicoides* spp. and mosquitoes are considered the main reservoirs and biological vectors of the virus (Jennings et al. 1989). Biting midges (*Culicoides* spp.) support viral replication after experimental oral and intrathoracic inoculation of the virus (Mellor et al. 1981; Jennings et al. 1989). Additionally, *Culicoides* species are able to experimentally transmit the virus to naïve cattle 7 to 10 days after infection (Mellor et al. 1981; Jennings et al. 1989). *Culicoides oxystoma* is considered the principal vector in Japan (Radostits et al. 2007). *Culicoides brevitarsis* and *Culicoides nebeculosus* are the primary vectors in Australia, and *Culicoides imicola* can transmit Akabane virus in Africa (Radostits et al. 2007). Akabane virus was also isolated from the mosquitoes *Aedes vexans* and *Culex tritaeniorhynchus* in Japan and *Anopheles funestus* in Africa, and some authors believe that mosquitoes may play an important role in the biological transmission of Akabane disease (Ogawa et al. 2007; Radostits et al. 2007). Cache Valley virus has been isolated from mosquitoes and biting midges; however, only mosquitoes such as *Culex pipiens*, *Aedes aegypti*, and *Aedes triseriatus* become infected with Cache Valley virus and promote transmission of the infection to susceptible animals (Edwards et al. 1998; Radostits et al. 2007)

Rift Valley Fever Virus (RVFV)

Rift Valley fever virus is an RNA *Phlebovirus* in the family *Bunyaviridae* that causes acute disease in ruminants, principally cattle and sheep (Radostits et al. 2007). The disease is characterized by fever, incoordination, abortion and death. Mortality rates can approach 30% (Fontenille et al. 1998). The disease is zoonotic, and affected humans develop a transient illness; however, some cases may be complicated with hemorrhagic fever, retinal disease, and encephalitis (LaBeaud et al. 2010). Rift Valley fever virus was initially reported in the Rift Valley in Kenya, but now exists and occurs in the sub-Saharan region of Africa, Egypt,

Madagascar, and the Arabian Peninsula (Fontenille et al. 1998; Radostits et al. 2007). Outbreaks of RVFV are common in enzootic regions when environmental conditions promote the proliferation of vector populations. Mosquitoes from the genus *Aedex* and *Culex* are considered the most important biological vectors of RVFV (Fontenille et al. 1998; Moutailler et al. 2008). Studies have demonstrated that *Culex pipiens* and *Aedes aegypti* develop high infection and dissemination rates after experimental exposure to blood infected with $10^{8.5}$ plaque-forming units/mL of RVFV (Moutailler et al. 2008). *Culex pipiens* had infection and dissemination rates between 3.9 to 14.7% and *Aedes aegypti* up to 90% (Moutailler et al. 2008). Transovarial transmission in the vector population and cycling of the virus between wildlife, domestic animals, and mosquitoes are responsible for the maintenance of the virus in enzootic areas (Fontenille et al. 1998; Coetzer et al. 2004). Some authors believe that the virus has great potential to spread to other countries, and that the presence of vectors such as *Culex pipiens* may increase the risk of introduction of RVFV to regions free from the disease (Moutailler et al. 2008). *Aedes caspius* has been reported as an important vector of RVFV from cattle to humans (Fontenille et al. 1998). Transmission of RVFV has been associated with other vectors such as *Culicoides spp*., sand flies (*Phlebotomus spp*.), and stable flies (*Stomoxys calcitrans*) (Turell et al. 2010). *Stomoxys calcitrans* was able to mechanically transmit RVFV to susceptible hamsters (*Mesocricetus auratus*) after feeding on infected animals with high viral titers (Turell et al. 2010). Other *Stomoxys* species in Africa and elsewhere may play similar roles as mechanical vectors of RVFV.

Bovine Ephemeral Fever Virus (BEFV)

Bovine ephemeral fever virus is an RNA *Ephemerovirus* from the family *Rhabdoviridae* that causes an economically important disease in cattle and can also affect buffalo (Yeruham et

al. 2007). High morbidity (30-61%) and low mortality (0.6%) rates have been associated with outbreaks of BEFV in endemic regions (Yeruham et al. 2007). The disease is endemic in Africa, East Asia, the Middle East, and Australia (Radostits et al. 2007). Clinical manifestations of the disease are associated with high fever and depression, enlarged lymph nodes, decrease in milk production and in some cases abortion and infertility in bulls (Radostits et al. 2007). Mosquitoes are considered biological vectors and important reservoirs of BEFV (Yeruham et al. 2007). Direct contact with body secretions or aerosol droplets are not effective routes of transmission of BEFV (Coetzer et al. 2004; Yeruham et al. 2007). It has been suggested that maintenance of the virus in cattle populations is influenced by transovarial transmission of BEFV in mosquito (*Anopheles bancroftii*, *Culex annulirostris*, and *Anopheles annulipes*) populations (Yeruham et al. 2007; Finlaison et al. 2010). Studies reported *Anopheles bancroftii* as the most important biological vector of BEFV in Australia and Africa (Yeruham et al. 2007; Finlaison et al. 2010). Other reports suggested that the biting midges, *Culicoides brevitarsis* and *Culicoides imicola* are important biological vectors of BEFV (Finlaison et al. 2010); however, experimental transmission with these vectors has not been attempted.

Bluetongue Virus (BTV)

Bluetongue virus is an RNA *Orbivirus* in the family *Reoviridae* that affects ruminants and most commonly causes acute clinical disease in sheep (Osburn 1994). Morbidity and mortality of the disease in sheep may vary from 30 to 100% and to 0 to 70%, respectively (Mellor et al. 2008; Meiswinkel et al. 2006). Usually less than 5% of cattle develop acute clinical disease following an outbreak of BTV (Osburn 1994). Twenty four serotypes of BTV have been identified worldwide, but all serotypes do not exist in one single area (Ohashi et al. 1999; Takamasu et al. 2003). Acute clinical disease in sheep includes high fever, mucosal erosion and

ulceration, edema of the head and tongue, lameness, abortion, and arthrogryposis and hydrancephaly in newborns (Osburn 1994). In cattle, most infections are subclinical but infertility and decreased reproductive performance have been reported in affected herds (Osburn 1994). Bluetongue virus is found in many regions of the world including Africa, Asia, the Middle East, Australia, Europe, North America, and South America (Mellor et al. 2008). Bluetongue virus is not contagious and is rarely transmitted directly from vertebrate to vertebrate; however, transmission through semen and embryos has been reported in cattle and sheep (Osburn 1994; Sing et al. 1997). Biting midges of the *Culicoides* species are considered the biological vectors of the disease and the main source of transmission (Mellor et al. 2008). Distribution of the disease is limited to those sites where the C*ulicoides* vector is present (Mellor et al. 2008); however, recent incursions of BTV into regions previously free from the disease suggest changes in bluetongue virus epidemiology (Mellor et al. 2008). Climate changes, particularly the increase in global warming, have been associated with the recent outbreaks of BTV in North Europe due their effects on dispersal and distribution of *Culicoides* spp. (Mellor et al. 2008; Meiswinkel et al. 2006). Epidemiological research has demonstrated that bluetongue virus is able to persist from season to season in northern regions indicating virus overwintering (Takamasu et al. 2003); however, mechanisms of overwintering of the virus are not well understood (Takamasu et al. 2003; White et al. 2005). It is believed that bluetongue virus is maintained in nature cycling between biting midges (*Culicoides*) and ruminant species (Osburn 1994). Cattle are considered to be the host where BTV overwinters and a major reservoir of disease (Osburn 1994; Takamasu et al. 2003). Although some authors have suggested transovarial transmission of BTV in *Culicoides species* as an overwintering mechanism, this has not been definitively proven (Takamasu et al. 2003; White et al. 2005). Studies suggested that

BTV was able to overwinter in the vector's tissues and that some adult *Culicoides* were able to survive over the entire winter season (White et al. 2005; Mellor et al. 2008). Another study demonstrated that BTV was able to establish a persistent infection in ovine γ/δ T lymphocytes allowing the perpetuation of the virus through the winter season in sheep populations (White et al. 2005). Different *Culicoides* species are the biological vectors of BTV in different areas of the world. *Culicoides varipenis* var. *sonorensis* is considered the vector in North and South America, *C. imicola* in Africa and southern Europe, *C. obsoletus*/*C. scoticus* and *C. dewulfi* in northern Europe, *C. imicola* and *C. obsoletus* in the Middle East and Asia, and *C. brevitarsis* in Australia (Coetzer et al. 2004; Radostits et al. 2007; Mellor et al. 2008).

Another *Orbivirus* closely related to BTV is epizootic hemorrhagic disease virus (EHDV), also biologically transmitted by *Culicoides* spp. This virus predominantly affects cattle and deer (Ohashi et al. 1999). Geographic distribution of EHDV is similar to bluetongue because of the presence of shared *Culicoides* vectors (Radostits et al. 2007). Clinical disease is common in deer populations, and it causes clinical signs similar to BTV infection in sheep. Clinical disease is rare in cattle in North America, but EHDV is capable of causing severe disease in cattle in Asia, particularly in Japan (Ohashi et al. 1999). The Ibaraki strain of EHDV in Japan has been associated with ulcerative stomatitis, edema, abortion and reproductive failure in cattle (Ohashi et al. 1999).

Biological Transmission of Protozoa

Trypanosomes

Trypanosomosis is the term used to describe the disease in humans and domestic animals that results after infection with flagellated protozoa from the genus *Trypanosoma* spp. Infection in domestic animals including ruminants, horses, and pigs results in acute parasitemia and

invasion of other body fluid cavities depending on the species of the trypanosome (Coetzer et al. 2004). Fever, anemia, lethargy, weight loss, and cachexia are common clinical findings in affected animals (Batista et al. 2007); however, keratoconjuntivitis, acute hemorrhagic syndrome, abortions, and neurological signs have been described in cattle affected with certain *Trypanosoma* species (Batista et al. 2007; Magona et al. 2008). Clinical disease in humans is known as sleeping sickness and is caused principally by *Trypanosoma brucei* in African countries (Coetzer et al. 2004). In South America, Chagas'disease is the name used to describe the human form of the disease, and the organism is *Trypanosoma cruzi* (Radostits et al. 2007). *Trypanosoma congolense, T. vivax, T. brucei,* and *T. simiae* are the most common *Trypanosoma* species affecting cattle, small ruminants, horses and pigs, respectively (Radostits et al. 2007; Batista et al. 2007). Distribution of the disease is limited to the tropical areas of Africa and South America (Coetzer et al. 2004; Batista et al. 2007). African trypanosomosis in domestic animals is associated with infections with *T. vivax, T. congolense, T. brucei,* and *T. simiae* (Mekata et al. 2008). The presentation of disease in Africa is determined by the availability of a suitable habitat for tsetse flies (*Glossina spp*.), considered the principal biological vector of trypanosomosis (Gibson et al. 2003; Mekata et al. 2008). In tropical countries in Central and South America, *Trypanosoma vivax* is considered the principal causal agent of trypanosomosis in livestock species (Cuglovici et al. 2010). Transmission of trypanosomes in Central and South America relies on mechanical vectors such as horse flies and stable flies rather than biological transmission by other insects (Batista et al. 2007; Cuglovici et al. 2010). Mechanical transmission of *Trypanosoma congolense* from infected to naïve heifers was experimentally demonstrated after exposure of naïve heifers to horse flies (*Atylotus agrestis*). Twenty nine horse

flies (*Atylotus agrestis*) carrying *Trypanosoma congolense* were able to mechanically transmit trypanosomosis from an affected heifer to a naïve heifer (Desquesnes et al. 2003).

In Africa, tsetse flies (*Glossina spp*.) are endemic in 7 million hectares of savannah encompassing multiple African countries. In this area, 46 to 62 million cattle and other livestock species are considered high risk for developing trypanosomosis (Mekata et al. 2008). Detection of DNA from different *Trypanosoma* species in tsetse flies (*Glossina pallidipes*) by PCR techniques has identified high infection rates (1.1 to 5.8%) in these flies (Mekata et al. 2008). Trypanosomes undergo complex cycles of differentiation and multiplication in tsetse fly tissues, particularly in the salivary glands and the midgut, before transmission can occur (Gibson et al. 2003; Peacock et al. 2007). Studies demonstrated trypanosome differentiation and multiplication in the midgut of tsetse flies (*Glossina spp*.) using green fluorescent techniques to stain trypanosomes (Gibson et al. 2003). In South American countries, increases in the population of biting flies from the genus *Stomoxys* spp. and *Tabanus* spp. in determined regions have been correlated with outbreaks of trypanosomosis in cattle (Batista et al. 2007; Cuglovici et al. 2010). An increase in the seroprevalence of *Trypanosoma vivax* from 7.8% to 48% from 2007 to 2009 in the state of Minas Gerais (Brazil) was correlated with an increase in the population of stable flies (*Stomoxys calcitrans*) (Cuglovici et al. 2010).

Trypanosoma evansi is the causal agent of Surra (mal de caderas) in horses, camels, and buffalo in North Africa, Middle East, Asia, and Central and South America. *T. evansi* is mechanically transmitted by biting flies from the genus *Glossina* spp., *Tabanus* spp., and *Stomoxys* spp. (Coetzer et al. 2004). *Trypanosoma evansi* is incapable of undergoing cyclic development in tsetse flies (*Glossina spp*.); thus, biological transmission does not occur (Coetzer et al. 2004). The disease is of economic importance in the camel population of North Africa and

in the buffalo populations in Asia. In Indonesia, Surra is considered the third most important livestock disease (Radostits et al. 2007).

Biological Transmission of Helminths

Parafilariosis

Parafilariosis is a nodular dermatitis of horses and cattle caused by the nematodes *Parafilaria multipapillosa* and *Parafilaria bovicola*, respectively (Bowman 2004). The disease has been reported in Europe, East Asia, South America, North Africa, South Africa, and the Philippines (Losson et al. 2009). Adult parasites encyst in subcutaneous tissues forming nodules that ulcerate and bleed (Losson et al. 2009). Spontaneous recovery has been reported (Losson et al. 2009). The horn fly (*Haematobia irritans*) and the face fly *(Musca autumnalis*) are the biological vectors of *Parafilaria* spp. (Coetzer et al. 2004). The flies feed in the ulcerated skin nodules and become infected with microfilaria, which undergo cyclical development in fly tissues (Coetzer et al. 2004). Infective larvae are subsequently deposited in the skin of cattle and horses after fly feeding (Coetzer et al. 2004; Losson et al. 2009). Economic importance of these diseases is associated with carcass damage and condemnation.

Stephanofilariosis

Stephanofilariosis is a ventral abdominal dermatitis of cattle caused by the filarial nematode *Stephanofilaria stilesi* (Dies et al. 1985). Dermatitis in the neck, withers, legs, and eyes has been reported in cattle infected with different species of *Stephanofilaria* in several countries (Dies et al. 1985; Coetzer et al. 2004). The disease has a worldwide distribution and particularly affects cattle and buffalo. Clinical signs of the disease are associated with subcutaneous cysts and papules that coalesce and form lesions of 3 to 15 cm of diameter in the skin of the ventral abdomen (Dies et al. 1985). Development of cyclic life stages of infective

microfilaria from *Stephanofilaria stilesi* occur in the tissues of the horn fly (*Haematobia irritans*) and the buffalo fly (*Haematobia irritans exigua*) (Dies et al. 1985; Coetzer et al. 2004). Infection usually does not cause systemic compromise and productive performance is not negatively affected.

Elaeophorosis

Elaeophorosis is a disease caused by filarial nematodes that causes exudative dermatitis in sheep (Boyce et al. 1999). Clinically, *Elaeophora schneideri* is the most important species of this genus (Radostits et al. 2007). Mule deer are the natural host of *Elaeophora schneideri* in North America, but other deer species act as reservoir hosts. In sheep, infective larvae develop in the leptomeningeal arteries and subsequently migrate into the common carotid and internal maxillary arteries (Boyce et al. 1999; Bowman 2004). Reduced blood flow to the brain may result in blindness, deafness, and circling (Radostits et al. 2007). In sheep, microfilaria cause severe dermatitis and irritation of the skin which results in scratching, bleeding, and abscess formation (Boyce et al. 1999). Horse flies (*Tabanus spp*.) are considered the intermediate hosts and biological vectors of *Elaeophora schneideri* (Boyce et al. 1999).

Onchocercosis

Onchocerca spp. are nodule-dwelling filarial nematodes that localize in connective tissue of the skin and other organs of cattle and horses (Trees et al. 2000, Coetzer et al. 2004). Microfilaria migrate through the skin and other tissues causing pruritic dermatitis, severe hypersensitivity reactions, and formation of nodules (Trees et al. 2000). *Onchocerca gutturosa*, *O. gibsoni*, *O. ochengi*, and *O. lienalis* are common species affecting cattle, while *O. cervicalis* and *O. reticulata* are important in horses (Bowman 2004). Transstadial transformation of infective microfilaria from *Onchocerca* spp. has been demonstrated in black flies (*Simulium*

spp.) and biting midges (*Culicoides* spp.) (Fukuda et al. 2003). Bacteria from the genus *Wolbachia* were identified as important symbiotic organisms of *Onchocerca* spp. and have been targeted as a novel microfilaricidal therapy for onchocercosis (Trees et al. 2000; Nfon et al. 2006). Economic losses associated with onchocercosis in cattle are related to rejection and condemnation of beef carcasses.

Setaria digitata

Setaria digitata is a filarial nematode responsible for causing lumbar paralysis of cattle in East Asia and the Middle East (Tung et al. 2004). Cerebrospinal migration of microfilaria results in ataxia and recumbency in affected animals (Tung et al. 2004). Mosquitoes from the genus *Culex* spp. and *Aedes* spp. have been identified as important intermediate hosts and biological vectors of *Setaria digitata* in Japan and Taiwan (Tung et al. 2004). Vector efficiency of *Culex quinquefasciatus* and *Aedes aegypti* was evaluated after experimental infection with infective larvae from *Setaria digitata.* Fourteen days after feeding on infected blood, high numbers of infective larvae were still found in tissues from infected mosquitoes (Tung et al. 2004).

Thelazia spp. (eyeworm)

Thelazia spp. are filarial nematodes that localize in the conjunctival sac and tear ducts of cattle and horses (Giangaspero et al. 2004). These nematodes are distributed worldwide and clinical disease is manifested by excessive lacrimation, photophobia, conjunctivitis, keratitis, and corneal ulceration (Giangaspero et al. 2004). The disease, although rare, can affect animals in many different parts of the world, including countries from Asia, Europe, North America, and South America. One survey in the United States found that 43% of adult horses may be infected with *Thelazia* spp. (Lyons et al. 1986). The face fly (*Musca autumnalis*) is considered the intermediate host and biological vector of *Thelazia* spp. in cattle and horses (Lyons et al. 1986).

Transmission of eyeworms can occur via face flies that feed on the ocular secretions, tears and conjunctiva of infected and susceptible animals (Giangaspero et al. 2004).

Nematodes of the Stomach of Horses

Nematodes found in the stomach of horses include *Habronema muscae, Habronema majus (microstoma),* and *Draschia megastoma* and are collectively referred to as equine habronemiasis (Naem 2007). Equine habronemiasis is a disease distributed worldwide, but of greater importance in warm climates (Coetzer et al. 2004). Infective larvae are deposited by flies on the skin or wounds of horses causing severe inflammatory reactions. Alternatively, horses may develop gastric habronemiasis after aberrant larval migration through stomach tissues when flies carrying infective larval stages of *Habronema* spp. or *Draschia* spp. are ingested by the horse (Coetzer et al. 2004; Naem 2007). Larval migration through stomach tissues induces the formation of gastric granulomas (Naem 2007). Clinical disease in horses is characterized by gastritis, weight loss, and colic (Radostits et al. 2007). Cutaneous habronemiasis is common in the medial canthus of the eye and the abdomen where dermatitis with extensive deposition of granulation tissue and ulceration are usually seen (Radostits et al. 2007). Stable flies (*Stomoxys calcitrans*) and house flies (*Musca domestica*) are the intermediate hosts and principal biological vectors of the disease (Coetzer et al. 2004; Naem 2007). While the house fly is the biological vector for *Habronema muscae* and *Draschia megastoma*, the stable fly is the biological vector for *Habronema majus (microstoma)* (Bowman 2004; Naem 2007). Larvated eggs hatch in the manure of cattle and horses and are ingested by fly maggots in which they undergo cyclic development of life stages (Naem 2007).

Conclusion

Flies are present worldwide and are capable of occupying almost every ecosystem on earth. Flies are responsible for considerable physical damage, affecting normal performance, and transmitting infectious pathogens to human and animal populations. Transmission of infectious diseases to livestock species by flies has a great impact on the economy of several countries where agriculture represents a major percentage of total income. Adequate interactions between the environment, the fly, the pathogen, and the host are necessary before transmission of disease can occur. Enzootic and epizootic presentation of livestock diseases transmitted by flies can be influenced by climatic changes, alterations in the ecology of the fly, and regulations for land tenure and use (Van den Bossche et al. 2010). Flies are capable of transmitting infectious pathogens by mechanical or biological routes. The anatomical conformation and biological behavior of flies make them potential mechanical vectors of infectious pathogens. Almost every pathogen could be mechanically carried and transmitted by flies; however, mechanical transmission of disease by flies is short-lived, and factors such as the load of pathogen and its survival in the fly tissues limit the ability and competency of flies as mechanical vectors. Biological transmission of disease by flies is more efficient because pathogens are able to replicate or develop in the fly tissues. Biological transmission is generally of long duration because the fly is an integral part of the ecologic cycle of the disease, acting as a reservoir for susceptible individuals (Scoles et al. 2008). Several infectious agents including viruses, bacteria, protozoa, and helminths are biologically or mechanically transmitted by flies to livestock species; thus, the role that flies play in the epidemiology of vector-borne diseases of livestock populations in determined regions is of critical importance. Scientific research should be conducted for a better understanding of the interactions between flies and infectious agents that cause disease in livestock in specific geographic areas. Additionally, fly prevention and control

should be encouraged in production animal units to maintain biosecurity and decrease transmission of pathogens between animals and humans.

Chapter 3: Statement of Problem and Hypothesis

Disease in cattle as a result of bovine viral diarrhea virus (BVDV) occurs worldwide and is responsible for considerable economic losses. For successful control of BVDV, it is necessary to understand the ecology of BVDV which is the relationship of the virus with its environment. While persistently infected (PI) animals are the major source of virus transmission within and between cattle herds, transmission of BVDV in the absence of PI animals has been described, indicating that other transmission routes are important in the epidemiology of the disease. Flies are important mechanical and biological vectors of viral pathogens affecting cattle; however, their epidemiological significance in the transmission of BVDV is not completely understood. The overall goal of this research was to assess the role of horn flies *(Haematobia irritans)* in BVDV transmission. We hypothesized that horn flies could act as a mechanical vector for BVDV and would be capable of transmitting the virus to susceptible cattle.

Chapter 4: Journal Article

Evaluation of transmission of bovine viral diarrhea virus (BVDV) between persistently infected and naïve cattle by the horn fly *(Haematobia irritans)*

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Short Communication

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Abstract

Identifying reservoirs and transmission routes for bovine viral diarrhea virus (BVDV) are important in developing biosecurity programs. The aim of this study was to evaluate BVDV transmission by the hematophagous horn fly (*Haematobia irritans*). Flies collected from four persistently infected cattle were placed in fly cages attached to principal (n=4) and control (n=4) BVDV-naïve calves housed individually in isolation rooms. Flies were able to feed on principal calves, but a barrier prevented fly feeding from control calves. Flies were tested for BVDV by RT-PCR and virus isolation at time of collection from PI cattle and after 48 hours of exposure on BVDV-naïve calves. Blood samples were collected from calves and tested for BVDV infection. Virus was isolated from fly homogenates at collection from PI animals and at removal from control and principal calves. All calves remained negative for BVDV by virus isolation and serology throughout the study. Bovine viral diarrhea virus may be detected in horn flies collected from PI cattle, but horn flies do not appear to be an important vector for BVDV transmission.
Keywords: bovine viral diarrhea virus, BVDV, *Haematobia irritans*, horn fly, transmission

Introduction

Several countries have developed and implemented control or eradication programs for bovine viral diarrhea virus (BVDV) in cattle (Ridpath 2010). For successful control of BVDV, the viral ecology must be fully understood, which includes identifying reservoirs and recognizing routes of transmission. Persistently infected (PI) animals continuously shed large amounts of virus and are considered the main source of BVDV transmission within and between herds (Houe 1999; Smith and Grotelueschen 2004). However, infections with BVDV in the absence of PI cattle have been described in seronegative herds (Moen et al. 2005; Moerman et al. 1993). As control and eradication programs progress, all potential sources for the reintroduction of BVDV into cattle herds should be elucidated. In addition to PI cattle, other sources of BVDV include transiently infected cattle, wild ruminants and nonbovine hosts, and BVDV-contaminated semen and embryos. Furthermore, the contamination of nose tongs, needles and vaccine vials was demonstrated to be a source of BVDV (Gunn 1993; Niskanen et al. 2003). Although further research is necessary to determine their epidemiologic importance, insects such as stable flies, horse flies, and face flies can potentially serve as a source of BVDV infection (Gunn 1993; Tarry et al. 1991).

The horn fly *(Haematobia irritans)* is an obligate, bloodsucking ectoparasite of pastured cattle, and is the most common fly affecting cattle in the southern United States (Cupp et al. 1998). Commonly, male and female flies spend their entire life on the same host; however, horn flies have the ability to disperse over great distances (Oyarzun et al. 2008). The horn fly is able to migrate for distances up to 8 km, and trees or other physical barriers do not prevent migration to new herds (Byford et al. 1987; Kunz et al. 1983). Horn flies are considered vectors for the mastitis pathogen *Staphylococcus aureus* and the filarial nematode *Stephanofilaria stilesi* in

cattle (Oyarzun et al. 2008), but their role as viral vectors is uncertain. With respect to BVDV transmission, no controlled experimental trials have evaluated horn flies as vectors of BVDV; therefore, the objective of this study was to evaluate BVDV transmission from PI cattle to naïve cattle by the hematophagous horn fly.

Material and Methods

Eight 7-month-old male castrated Holstein calves that were BVDV-negative and BVDV seronegative and four BVDV-PI cattle were included in this study. Naïve calves were randomly assigned to principal (n=4) or control (n=4) groups, and each calf was housed separately in isolation rooms designed to prevent cross-contamination. The four PI cattle were located at the BVDV unit of Auburn University College of Veterinary Medicine which is geographically separate from the isolation facility and consists of isolated pastures where the cattle were housed. Separate personnel cared for the PI and BVDV-naïve cattle. During the study period, all cattle were given clinical examinations twice daily and were cared for under the guidelines of Auburn University Institutional Animal Care and Use Committee (PRN 2009-1558).

Horn flies were collected from each of four PI cattle (Table 3.1) using a vacuum collection system. For BVDV testing by RT-PCR and virus isolation (VI), an aliquot of horn flies from each PI animal was frozen at -80ºC for 1 hour. Aliquots of either 50, 25, or 10 horn flies were homogenized using a sterile mortar and pestle in 2 mL of culture medium consisting of minimum essential medium (MEM) containing 10% (vol:vol) equine serum (ES), sodium bicarbonate (0.75 mg/mL), L-glutamine (0.29 mg/mL), penicillin G (100 IU/mL), streptomycin (100 μg/mL), and amphotericin B (0.25 μg/mL). Fly homogenates were centrifuged for 10 min at 700 X g at 4 °C and the supernatant collected for BVDV detection.

A two-round rapid-cycle RT-PCR assay was performed to detect BVDV in fly homogenates. RNA was isolated from fly homogenate supernatants using the QIAamp® viral RNA mini kit (QIAGEN, Valencia, CA, and USA) according to the manufacturer's instructions. All steps of the RT-nPCR were performed in a single-tube reaction. In the first round, the outer primers, BVD 100 (5'-GGCTAGCCATGCCCTTAG-3') and HCV 368 (5'-CCATGTGCCATGTACAG-3') amplified a 290 base pair sequence of the 5' untranslated region of the viral genome. In the second round, the inner primers BVD 180 (5'-CCTGAGTACAGGGDAGTCGTCA-3') and HCV 368 amplified a 213 base pair sequence within the first amplicon. After completion of the PCR cycle, 5 µl of the RT-nPCR products were separated by 1.5% agarose gel electrophoresis. Visualization of the RT-nPCR product was performed by ethidium bromide staining and ultraviolet transillumination.

Fly homogenates were also assayed using ultracentrifugation and VI. Ultracentrifugation of samples prior to VI was previously noted to reduce sample cytotoxicity and lower BVDV-specific antibody concentrations in collected semen (Givens et al. 2006). Fly homogenate supernatants were centrifuged again for 10 min at 700 X g at 4 °C. Cell pellets were re-suspended in 6 mL of culture medium and then ultracentrifuged for 90 min at 80,000 to 90,000 X g at 4 ºC. The supernatant was decanted and the pellet resuspended in 8 mL of culture medium. The entire volume of re-suspended pellet was inoculated onto monolayers of Madin Darby bovine kidney (MDBK) cells in 150 cm^2 flasks. After a 1 h incubation period on an orbital shaker at 37 °C, 28 ml of culture medium were added to the flask. Cultures were incubated for 4 d prior to freezing at -80°C and thawing to release any intracellular virus. Lysates from this passage were assayed in triplicate by diluting 10 µL of cell lysate with 90 µL of culture medium and subsequently adding 50 µL of culture medium containing MDBK cells to the wells of a 96-

well culture plate. After 72 h of incubation, BVDV was detected using the immunoperoxidase monolayer assay as previously described (Walz et al. 2008).

A manual vector-transmission protocol was used to determine whether horn flies were capable of transmitting BVDV from PI cattle to naïve calves. This protocol was used previously to study transmission of bovine leukemia virus by stable flies *(Stomoxys calcitrans)* (Weber et al. 1988) and porcine respiratory and reproductive syndrome virus (PRRSV) by mosquitoes (*Aedes vexans*) and houseflies (*Musca domestica*) (Otake et al. 2003; Otake et al. 2004). To prevent inadvertent transfer of virus to BVDV-naïve calves by the collection equipment, horn flies were transported to the laboratory biohazard hood where they were transferred to clean, alcohol-disinfected transport tubes and enumerated prior to delivery to the fly cages on the BVDV-naïve calves. Personnel performing the transfer to transport tubes did not contact principal or control calves. Tubes containing flies were carried to isolation rooms and 50 flies were transferred to fly cages (25 flies per cage) in each principal and control calf. Fly cages were attached to a clipped area of the withers of control and principal calves. Following analgesia of the skin using 2% lidocaine HCl, two cylindrical fly cages (8.74 x 10 cm) were sutured to the prepared sites using non-absorbable suture material. Fly cages were covered on the side not contacting calves with a double layer of nylon screen with 64 holes per cm^2 to prevent fly escape. On the animal side, fly cages attached to principal calves were covered with one single layer of nylon screen of 1 mm diameter holes that allowed fly access and feeding on calf's skin. The bottom of fly cages on control calves was covered with a plastic lid that prevented fly contact and feeding on the calf. Horn flies remained on the experimental calves for 48 hours, and feeding of the flies was visually evaluated every 12 hours. After removal, the flies were immediately frozen at -80˚ C

and subjected to BVDV testing by RT-PCR and VI as described above. Calves remained in individual isolation rooms for 28 days.

Whole blood was collected from principal and control calves for virus isolation on days 0, 3, 6-10, 14, 21, and 28. The whole blood was processed by hypotonic lysis of the red blood cells yielding the white blood cell fraction. The isolated white blood cells were then resuspended in culture medium. The cell suspension underwent co-cultivation on 25 cm^3 flasks containing monolayers of MDBK cells and was incubated for 5 days at 37°C and 5% CO_2. Following cultivation, 50 µl of the cell culture supernatant was inoculated in triplicate into wells on 96-well microtiter plates containing monolayers of MDBK cells in culture medium. After 72 h of incubation, BVDV was detected using the immunoperoxidase monolayer assay as previously described (Walz et al., 2008).

Sera were collected on days 0, 14, 21, and 28 for virus neutralization procedures from principal and control calves. The virus neutralization tests were performed to detect antibodies directed against the BVDV strain from the PI animal which the horn flies fed on. After heat inactivation at 56°C for 30 minutes, serial 2-fold dilutions (1:2 to 1:4096) were made in 50 µL of culture medium. For each dilution, 3 wells of a 96-well plate were inoculated with an equal volume (50 µL) of culture medium containing 100 $TCID_{50}$ of the PI strain. After inoculation, the plate was incubated at 38.5°C in a humidified atmosphere of 5% CO_2 and air for 1 hour. Then, 2.5 x 10^3 Madin Darby bovine kidney (MDBK) cells in 50 µL of culture medium were added to each well. Plates were incubated for 72 hours and immunoperoxidase labeling of cell monolayers was performed to detect neutralization of the virus.

Results and Discussion

BVDV was isolated in high titers from serum of all PI animals used as horn fly donors in this study (Table 3.1). Aliquots of 25 and 10 horn flies from each of the 4 PI animals collected prior to transmission experiments were positive to BVDV by RT-PCR and VI (Table 3.1). At the time of collection of flies for the transmission experiment, fly homogenates from the PI animals were positive by RT-PCR and VI on three PI cattle. The fly homogenate collected from the fourth PI animal (AU-D) was negative, but only 20 flies were available from this animal for BVDV diagnostics.

During the transmission experiments, horn flies collected after feeding for 48 hours on principal calves remained alive, while all horn flies collected from control calves after 48 hours were dead. This observation likely indicated that horn flies fed on their principal calves, while control flies died due to the inability to feed. When horn fly homogenates comprising the 50 flies placed in the fly cages was subjected to BVDV testing, all fly homogenates, with exception of one fly homogenate from a principal calf, were positive for BVDV by RT-PCR and VI. BVDV transmission was not detected by virus isolation or virus neutralization in principal or control calves during the entire study period, nor did any principal or control calves present with signs of illness or abnormalities at daily physical examination.

Previous research indicated insects as a potential source of BVDV infections in cattle. Transmission of BVDV between a PI animal and naïve calves was demonstrated when 50 stable flies (*Stomoxys calcitrans*) were allowed to feed on the PI animal for 5 minutes and then fed on naïve calves 15 minutes later (Tarry et al. 1991). Virus was detected in one calf and seroconversion was detected in two other calves. Additionally, BVDV transmission occurred between a PI animal and naïve sheep when 50 horse flies (*Haematopota pluvialis*) were used as the experimental vector (Tarry et al. 1991). Horse flies and stable flies are the only insect species

that have been demonstrated to transmit BVDV from PI cattle to susceptible animals. Additionally, BVDV was detected in face flies (*Musca autumnalis*) and head flies (*Hydrotea irritans*) obtained from PI cattle (Gunn 1993; Tarry et al. 1991); however, their ability to transmit BVDV has not been established by experimentation. Our study evaluated the horn fly because it is one of the most widespread and economically important ectoparasites of cattle. Horn flies are hematophagous cattle pests that occur throughout Europe, North America, South America, and parts of Asia. Cattle are continuously annoyed by the feeding of horn flies, and individual cattle may suffer with horn fly infestations of up to 1000 flies per cow. In contrast to a previous study evaluating BVDV transmission by stable flies and horse flies (Tarry et al. 1991), horn flies were unable to transmit BVDV to susceptible cattle in the present study. Explanations for differences between results of the studies are likely attributable to the biology of the fly species chosen for study, but differences in experimental design exist between our study and previous work. Procedural and animal controls were instituted in the present study to avoid inadvertent BVDV transmission by means other than flies, whereas the previous study utilized the same equipment and personnel between PI and BVDV-naïve animals. This may have resulted in unintentional and undetermined BVDV transmission (Tarry et al. 1991).

The ability of insects to serve as mechanical vectors for BVDV are determined by the titer of BVDV in the host, the quantity and persistence of BVDV in the mouthparts of the insect vector, the infectiousness of BVDV at the site of insect bite, and the number and feeding frequency of the insects found on the host. Differences exist in the biology and feeding behavior of horn flies as compared to horse flies and stable flies, and these differences may contribute to the differences observed in BVDV transmission. The infective dose of BVDV on the mouthparts of 50 horn flies consuming blood from a PI animal may not be equivalent to the infective dose of

BVDV on mouthparts of 50 horse flies or stable flies as these fly species are larger, feed longer, and acquire larger blood volumes at each individual feeding. Furthermore, male and female horn flies feed frequently (up to 38 times per day) on cattle each day (Oyarzun et al. 2008), consuming smaller volumes of blood at each feeding (Kuramochi 2000). In contrast, male and female stable flies typically feed once or twice daily, taking a larger blood volume. Only female horse flies feed on livestock, and they feed sporadically but can consume up to 1 mL of blood per fly. Stable flies and horse flies are capable of contact with different animals since they do not spend their entire life cycle on a single host as does the horn fly, and this could favor mechanical transmission. The lack of BVDV transmission in our study did not appear to be caused by inadequate contact and feeding of horn flies on experimental calves. All horn flies placed on control calves where they were not allowed to feed died during the first 12 hours. In contrast, horn flies allowed to feed on principal calves remained alive during the entire 48 hours of exposure. This suggests that flies placed on principal calves survived due to blood feeding, and failure of viral transmission due to inadequate contact between flies and experimental calves is unlikely. To our knowledge, data are not available on the infectiousness of BVDV at the site of insect bites. Previous studies have documented that viral titers in PI cattle are dynamic and may vary over time (Brock et al. 1998); however, all PI cattle used in this study possessed high concentrations of BVDV in serum.

Horn fly homogenates were consistently positive for BVDV by RT-PCR and VI procedures. The utilized methods did not allow localization of the virus in the insect, but the positive results can likely be attributed to the presence of BVDV in the bloodmeal located within the gastrointestinal tract of the horn fly as part of the total fly homogenate. Data are not available on the amount of blood present on mouthparts of horn flies; however, measurements

made from electron micrographs obtained from stable flies indicate that the internal mouthparts would retain 0.03 nanoliters of blood (Weber et al. 1988). Horse flies (*Tabanus fuscicostatus*) have approximately 10 nanoliters of residual blood on mouthparts following an uninterrupted feeding (Foil et al. 1987). With such low volumes of blood present on mouthparts and viral titers in serum varying among PI animals, the ability of single flies to transmit BVDV is questionable. Furthermore, 50 flies carrying BVDV from a single PI animal to a single BVDV-naïve animal would be a difficult case scenario to reproduce in natural conditions. Therefore, flies in general probably do not represent an important epidemiological risk for the transmission of BVDV within and between cattle herds. Further research quantifying viral titers on mouthparts of single flies following uninterrupted feeding on PI animals would clarify potential for mechanical transmission of BVDV by insect vectors.

Conclusion

Although BVDV was consistently isolated from homogenates of horn flies obtained from PI cattle, transmission of BVDV did not occur between PI cattle and BVDV-naïve cattle using horn flies as the route of transmission. The results of this study suggest that the horn fly does not present a major epidemiological risk as a vector in the transmission of BVDV within and between herds.

Table 3.1 Results of virus isolation, RT-PCR, viral titration, and genotype obtained from four BVDV-PI cattle and the horn flies collected from each animal.

	PI animal	**AU-A**	**AU-B**	**AU-C**	**AU-D**
Preliminary Assessment	RT-PCR (25 flies)	+	+	+	+
	VI (25 flies)	+	+	+	+
	RT-PCR (10 flies)	+	+	+	+
	VI (10 flies)	+	+	+	+
Pre-feeding Assessment	No. of flies for virologic assessment	50	50	50	20
	RT-PCR	+	+	+	–
	VI	+	+	+	–
	Titer*	3.5×10^4/ml	6.2×10^4/ml	6.2×10^4/ml	3.5×10^4/ml
	BVDV genotype	1b	1b	1a	2
Post-feeding Assessment	No. of flies for virologic assessment	50	50	50	50
	RT-PCR and VI: Principal calf	–	+	+	+
	RT-PCR and VI: Control calf	+	+	+	+

KEY: *viral titer in serum from PI cattle; VI = virus isolation; RT-PCR = reverse transcription-polymerase chain reaction

Chapter 5: Summary and Conclusions

Disease in cattle as a result of bovine viral diarrhea virus (BVDV) occurs worldwide and is responsible for considerable economic losses. Great strides have been made in a number of countries to control or eradicate BVDV. For successful control of BVDV, it is necessary to understand the ecology of BVDV which is the relationship of the virus with its environment. Identification of reservoirs, understanding routes of transmission, and assessing stability of the virus in animals and the environment are important considerations for control. While persistently infected (PI) animals are the major source of virus transmission within and between cattle herds, transmission of BVDV in the absence of PI animals has been described, indicating that other transmission routes are important in the epidemiology of BVDV. As control and eradication programs progress, all potential sources for the reintroduction of BVDV into cattle herds should be elucidated. In addition to PI animals, other routes of transmission of BVDV include contact with acutely infected cattle or heterologous species such as sheep, goats, camelids, pigs, deer and other wild ruminants carrying acute or persistent BVDV infections. Semen, embryos, and fomites contaminated with BVDV were demonstrated to be a source of BVDV infection for susceptible cattle. Although two reports exist on BVDV transmission by flies, there still exist questions concerning their epidemiological significance with respect to BVDV. Flies are insects capable of successfully transmitting infectious diseases to cattle by biological or mechanical routes. A wide variety of viruses, bacteria, rickettsia, protozoa, and helminths are transmitted mechanically by flies to cattle populations worldwide; however, mechanical transmission of disease by flies is short-lived and not as efficient when compared to biological transmission.

The horn fly *(Haematobia irritans)* is the most common hematophagous ectoparasite affecting cattle in the southeastern United States. Heavy infestations of cattle with horn flies are reported to be as high as 1000 flies per animal causing devastating effects on productivity.

Commonly, adult horn flies spend their entire life on the same host leaving only to lay their eggs in freshly passed feces; however, horn flies have the ability to disperse over great distances to find a new host. The horn fly has been associated with the mechanical transmission of *Staphylococcus aureus*, *Corynebacterium pseudotuberculosis*, and bovine leukemia virus (BLV) in cattle; however, the ability of horn flies to mechanically transmit BVDV is unknown.

Previous research indicates that insects are a potential source of BVDV infections in cattle. Stable flies (*Stomoxys calcitrans*) and horse flies (*Haematopota pluvialis*) were able to effectively transmit BVDV between PI cattle and naïve calves (Tarry et al. 1991). In our study, although BVDV was detected by PCR and VI in fly homogenates collected from PI cattle, transmission of the virus to naïve calves was not detected by virus isolation or virus neutralization in principal or control animals during the entire study period, nor did any principal or control calves present with signs of illness or abnormalities at daily physical examination. Explanations for differences between results of our study and previous studies are likely attributable to the biology of the horn fly compared with the biology of the stable fly and the horse fly; however, differences in experimental design exist between our study and previous work. Procedural and animal controls were instituted in the present study to avoid inadvertent BVDV transmission by means other than flies, whereas the previous study utilized the same equipment and personnel between PI and BVDV-naïve animals. This may have resulted in unintentional and undetermined BVDV transmission (Tarry et al. 1991). Mechanical transmission of pathogens by flies is influenced by several factors including the titer of the pathogen in the host, the load of the pathogen transported by the fly, the ability of the pathogen to survive in the fly tissues, and the number and feeding frequency of the flies found on the host. Differences exist in the size and feeding behavior of horn flies as compared to horse flies and

stable flies, and these differences may contribute to the differences observed in BVDV transmission. The infective dose of BVDV on the mouthparts of 50 horn flies consuming blood from a PI animal may not be equivalent to the infective dose of BVDV on mouthparts of 50 horse flies or stable flies as these fly species are larger, feed longer, and acquire larger blood volumes at each individual feeding. Furthermore, stable flies and horse flies are capable of contact with different animals since they do not spend their entire life cycle on a single host as does the horn fly, and this could favor mechanical transmission. Under natural conditions, 50 horn flies carrying BVDV from a single PI animal to a single BVDV-naïve animal would be a very difficult case scenario to reproduce. Therefore, flies in general, and the horn fly in particular, although they are able to carry the virus, probably do not represent an important epidemiological risk for the transmission of BVDV within and between cattle herds.

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