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CENTER FOR GRAIN AND ANIMAL HEALTH RESEARCH (CGAHR)
ARTHROPOD-BORNE ANIMAL DISEASES RESEARCH UNIT (ABADRU):

CONSIDERING RIFT VALLEY FEVER AND OCCUPATIONAL BIOSAFETY AND
HAZARD PRACTICES

by

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Abstract

The Master of Public Health (MPH) capstone experience results in numerous personal and academic gains. Through my MPH capstone experience, I was able to utilize knowledge gained from previously related classes. I gained exposure and understanding of the risks and hazards of zoonotic disease research, especially as it relates to attenuated Rift Valley fever virus (MP-12) in a Biosafety Level (BSL)-2 Research Facility. I was also able to achieve an understanding of what procedures should be in place to protect the occupational safety of workers in the laboratory or research setting. Finally, in a Frontier trip to the United States-Mexico Border Port of Entry in Santa Teresa, New Mexico, I was able to observe the real life activities, issues, and actions, which occur on a daily basis to protect and defend this nation's public health and food supply.

The risk of foreign animal or zoonotic disease outbreaks are high today and may be rising. The reasons involved include increased international trade and travel, population growth, the changing patterns of human-animal contact, an increased demand for animal protein, increased wealth, environmental changes and human encroachment of farm land and previously undisturbed wildlife habitat. Infectious disease outbreaks can cause human suffering/death and substantial economic losses to the agricultural sectors resulting in disrupted agricultural commodity markets. Zoonotic diseases such as those caused by Rift Valley fever virus (RVFV) can spread from humans to animals and vice-versa, raising an additional dimension of vulnerability to human health. Rift Valley fever virus is not considered an immediate risk to the continental United States; however, it is considered a possible threat and, if established, could result in similar consequences as West Nile Virus (WNV) in 1999. Its socioeconomic impact

and significant effect on human health and livestock could resemble the economically crippling effect seen in countries, such as those in Africa. This impact is seen in the form of stressed health care services, decreased animal reproduction, and financial loss due to human and animal morbidity and mortality, and national as well as international trade restrictions.

Through the understanding of RVFV's global importance and the application of zoonotic agent laboratory research, I was able to produce two PowerPoint presentations, which will be useful as training exercises for new laboratory workers starting with the Arthropod-Borne Animal Disease Research Unit (ABADRU). Additionally, I was able to participate in the preliminary occupational risk evaluation of an intradermal needle-free injection device, for the potential use in future ABADRU work.

Report Organization

This report is arranged with an abstract, general introduction, literature review, occupational biosafety review, field experience review, one individual paper, and three appendixes. Two of the appendixes are prepared PowerPoints for future laboratory workers at the USDA CGAHR ABADRU and the last is a daily log of activities during the field experience. The paper was conducted for in-house purposes as preliminary research and is organized as follows: An abstract, introduction, materials and methods, results, discussion/conclusion. References cited in the literature review and respective chapters are located at the end of each section, after the tables and figures. All reference citations follow the American Psychological Association (APA) format.

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Dedication

This report is dedicated to my father, Walter Roof, my mother, Toni Roof, my brother, Levi Roof, and last, but not least, my sister, Emily Roof. I am truly blessed to have them as a part of my life. Without their support, love, understanding, and confidence, this work would not have been possible. I am appreciative of and thankful for their support and encouragement to pursue my aspirations of a second Master's degree. I would also like to dedicate this work to my close friends and classmates, especially Trista Mills, for her support and feedback. Your contributions and patience helped with the completion of this document.

Chapter 1 - General Introduction

Introduction

The zoonotic threat of Rift Valley fever virus (RVFV) has the potential to be both financially and epidemically devastating (Clements, *et al*, 2007; Sindato, *et al*, 2011). Rift Valley fever is an arthropod-borne infection causing acute hemorrhagic fever resulting in viral disease among humans and ruminants (Gerdes, 2002; Gerdes, 2004; Hartley, *et al*, 2011; Ikegami & Makino, 2011; Musser, *et al*, 2006). This virus is a medically and agriculturally important pathogen in Africa (Hughes-Fraire, *et al*, 2011; Schamlljohn & Hooper, 2001). Rift Valley fever virus periodically causes outbreaks in humans and in ruminants, including sheep, cattle, and goats (Hartley, *et al*, 2011; Ikegami & Makino, 2011; Mullen & Durden, 2009; Sindato, *et al*, 2011). Rift Valley fever epidemics are characterized by livestock mortality and abortion in pregnant animals (Clements, *et al*, 2007; Woods, *et al*, 2002). A RVFV outbreak results in significant human morbidity, livestock mortality and major economic disruption – largely due to livestock losses and trade restrictions (Clements, *et al*, 2007; Hartley, *et al*, 2011; Hughes-Fraire, *et al*, 2011; Sindato, *et al*, 2011; Terrell, *et al*, 2008). In the Kenya, Africa RVFV outbreak of 2006/2007, the total economic losses from livestock mortality in this outbreak were estimated to be \$7.6 million with national economic losses estimated to be \$26 million (MacMillian, 2010). Traders and slaughterhouses are affected by movement bans on livestock and decreased consumer demand for meat, which greatly affects sales of live animals and products (MacMillian, 2010; Mohamed, *et al*, 2010; Sindato, *et al*, 2011).

Rift Valley fever virus is one of the most important veterinary pathogens in the world and has the potential to cause lethal epidemics in sheep and cattle, as well as severe human disease epidemics (Archer, *et al*, 2011; CDC, 2012; Murphy, *et al*, 1999; Strauss & Strauss, 2008). It

can also cause high mortality in new-born animals and abortion in pregnant animals (Bird, *et al*, 2009; Swanepoel and Coetzer, 2004). From 1977-1978, an epizootic in Egypt infected 25-50% of cattle and sheep in some areas, killing hundreds of thousands of sheep, goats, and cattle; causing food shortages for many years (Otte, *et al*, 2004; Strauss & Strauss, 2008). In addition, the Egyptian epizootic resulted in 200,000 reported human cases and resulted in at least 600 deaths (0.3%) (Clements, *et al*, 2007; Otte, *et al*, 2004; Schamlijohn & Hooper, 2001; Strauss & Strauss, 2008; WHO, 2010). After this outbreak, RVFV extended its range and in 1987, Mauritania and Senegal experienced a large epidemic which caused more than 200 human deaths (WHO, 2010).

In 1997-1998, East Africa received the largest rainfall documented in 35 years, 60-100 times the normal precipitation in some areas (Anyamba, *et al*, 2009; Mondet, *et al*, 2005; Wood, *et al*, 2002). This phenomenon resulted in high arthropod populations and a subsequent large epidemic involving 5 countries with approximately 90,000 human infections; including 478 deaths, a loss of approximately 100,000 domestic animals, and a significant economic impact due to a ban of livestock exports from the region (Anyamba, *et al*, 2009; Clements, *et al*, 2007; Gerdes, 2002; Mondet, *et al*, 2005; Wood, *et al*, 2002). Hundreds of human hemorrhagic fever cases were recorded and sheep and goat mortalities reached 70%, while 20-30% of cattle and camels were lost (Clements, *et al*, 2007; CDC, 2012; Hartley, *et al*, 2011; Strauss & Strauss, 2008). In 2000, Saudi Arabia and Yemen had an outbreak resulting in more than 3,500 human cases, including 208 fatalities (WHO, 2010). Rift Valley fever virus later hit the Horn of Africa in 2006 causing 350 human deaths (WHO, 2010; Hughes-Fraire, *et al*, 2011). Furthermore, in 2007-2008, an outbreak in Sudan resulted in 230 human deaths (WHO, 2010; Hughes-Fraire, *et al*, 2011).

The National Institute for Communicable Diseases (NICD) recently published information on a Rift Valley fever outbreak occurring in South Africa during 2010. This outbreak resulted in 238 human infections, including 26 human deaths, 14,342 animal cases, and 8,877 laboratory-confirmed deaths across 8 of the 9 South African provinces (NICD, 2011). However, these numbers only represent laboratory confirmed RVFV cases and only symptomatic people were tested; it is estimated that greater than 80% of RVFV infections are asymptomatic (NICD, 2011). Additionally, NICD data suggests approximately 82% of the reported cases worked with occupations in direct contact with animals (NICD, 2011). Contact with livestock has been statistically associated with acute infection of RVFV, which indicates direct contact during an outbreak is an important means of disease spread to humans (Archer, *et al*, 2011; Gerdes, 2002; Mullen & Durden, 2009; Strauss & Strauss, 2008). Furthermore, the virus is potentially infectious by aerosol and can replicate in a wide range of mosquito species including *Aedes mcintoshi*, *Aedes vexans*, *Culicoides sonorensis*, as well as other *Aedes*, *Culex*, *Eretmapoites*, and *Mansonia* species as well as other vectors such as sand flies (Fontenille, *et al*, 1998; Gaff, *et al*, 2007; Hartley, *et al*, 2011; Turell, *et al*, 2008).

Rift Valley fever virus is restricted to certain national laboratories for all research and diagnostic procedures to prevent human exposure (Hughes-Fraire, *et al*, 2011; Murphy, *et al*, 1999). It is important that research be conducted in secure laboratories to safely develop and produce control measures and perform diagnostics to protect public health as well as the U.S. livestock industries (Hughes-Fraire, *et al*, 2011; Turell, *et al*, 2008). As can be seen from the above reports, RVFV constitutes an important public health problem as well as a serious socioeconomic burden in Africa and surrounding areas, with the potential to become globally significant.

Chapter 2 - Literature Review

Importance

The introduction of West Nile virus (WNV) in 1999 and its spread across North America has demonstrated the ability of an exotic arbovirus to be introduced and rapidly spread across North America (Mandell & Flick, 2011; Nash, *et al*, 2001; Turell, *et al*, 2008). Within six years of its introduction, WNV became endemic across the continental United States (U.S.) (Murray, *et al*, 2010). The successful spread of WNV was due to a competent mosquito vector present in the U.S. (Hartley, *et al*, 2011; Turell, *et al*, 2008). There is little known about the potential for North American mosquitoes to transmit Rift Valley fever virus (RVFV) should it be introduced into North America (Hartley, *et al*, 2011; Turell, *et al*, 2008). However, there are a number of studies that have been conducted in order to evaluate selected mosquitos' ability to transmit and serve as potential vectors for RVFV (Gargan, *et al*, 1988; Turell, *et al*, 2008).

Rift Valley fever virus is viewed as a major disease threat to the U.S. because it has become endemic in a number of countries that previously were disease-free (Clements, *et al*, 2007; Hughes-Fraire, *et al*, 2011; WHO, 2010). There are researchers who believe humans returning from an infected part of the world could be incubating RVFV and, once within our borders, develop a full-blown viremia (Kurkjian, *et al*, 2006). Mosquitos, could then theoretically pick up and transmit the virus to other humans and ruminant livestock, much as what happened with WNV (Kurkjian, *et al*, 2006). Infectious disease experts fear RVFV could come to the U.S. or Europe and cause major human suffering and devastate the livestock industry in affected areas (Kurkjian, *et al*, 2006; Ikegami & Makino, 2011; UTMB, 2012).

Due to the potential catastrophic side effects, including human morbidity, livestock mortality, and significant economic damage RVFV is a reportable zoonotic virus to the World

Organization of Animal Health also called the Office of International Epizootics (OIE) (Clements, *et al*, 2007; Musser, *et al*, 2006). The virus is also a restricted pathogen and importation or possession of RVFV is prohibited by law or regulation by most national governments (Clements, *et al*, 2007; Murphy, *et al*, 1999; Spickler & Roth, 2006). Because of these regulations, RVFV is restricted to certain national laboratories for all research and diagnostic procedures to prevent human exposure (Hughes-Fraire, *et al*, 2011; Murphy, *et al*, 1999). An overview for laboratory workers at ABADRU can be seen in Appendix B.

History of Disease

Rift Valley fever virus is a member of *Bunyaviridae*, one of the largest families of enveloped viruses, with 350 members, including the well-known Hantaviruses (Clements, *et al*, 2007; Sindato, *et al*, 2011; Turell, *et al*, 2008). Bunyaviruses are normally carried and transmitted by arthropods or rodents (Ikegami & Makino, 2011; Ikegami & Makino, 2009; Miller, 2008). These arthropods include mosquitoes, ticks, culicoides, or flies (Murphy, *et al*, 1999). Often Bunyaviruses cause transient infection in their vertebrate hosts and lifelong persistent infections in their arthropod vectors (Ikegami & Makino, 2011; Murphy, *et al*, 1999). Most Bunyaviruses never impact humans or domestic animals, but there are three which cause important diseases ranging from encephalitis and hepatitis to acute respiratory disease (Ikegami & Makino, 2011; Murphy, *et al*, 1999). These diseases include Rift Valley fever virus (RVFV), Crimean-Congo Hemorrhagic fever virus (CCHF) (disease transmitted via ticks to humans), and Nairobi Sheep Disease virus (NSDV) (disease transmitted via ticks to sheep and goats in Africa) (Murphy, *et al*, 1999). Other arthropod-borne viruses in other families of importance include Bluetongue Virus (BTV) (disease of sheep characterized by edema and hemorrhage), Epizootic

Hemorrhagic Disease Virus (EHDV) (disease considered closely related to BTV, strongly affecting wild ruminants), and Cache Valley Virus (CVV) (disease of sheep characterized by fetal death) (Ikegami & Makino, 2011; Murphy, *et al*, 1999). These viruses have gained both national and international disease control agencies' attention (Ikegami & Makino, 2011; Murphy, *et al*, 1999).

Though there are many exotic arboviruses affecting humans and animals alike, of particular concern is Rift Valley fever virus, which has been responsible for numerous outbreaks of severe disease in humans and ruminants in sub-Saharan Africa over the past 70 years (Hartley, *et al*, 2011 Ikegami & Makino, 2011; Turell, *et al*, 2008). The geographic distribution of Rift Valley fever virus covers much of Africa, having been documented in Kenya, Somalia, Tanzania, Egypt, Sudan, Madagascar, Mauritania, Senegal, Yemen, and Saudi Arabia (Clements, *et al*, 2007; Schamlljohn & Hooper, 2001; Swanepoel, & Coetzer, 1994; WHO, 2010). Outbreaks were confirmed in Yemen and Saudi Arabia in 2000; marking the first cases documented outside of Africa and raising concerns that it could extend into other parts of Asia and to Europe (Clements, *et al*, 2007; Ikegami & Makino, 2011; WHO, 2010).

Since 1930, the virus has caused large outbreaks of disease in humans and livestock, these outbreaks were primarily confined to Africa until the first outbreak reported in Yemen and Saudi Arabia (Clements, *et al*, 2007; CDC, 2012; Musser, *et al*, 2006; Schamlljohn & Hooper, 2001). This spread is believed to be the consequence of more abundant mosquito populations most likely due to flooding in the Asir Mountains (Hartley, *et al*, 2011; Musser, *et al*, 2006). Furthermore, the virus is potentially infectious by aerosol and can replicate in a wide range of mosquito species including *Aedes mcintoshi*, *Aedes vexans*, *Culicoides sonorensis*, as well as other *Aedes*, *Culex*, *Eretmapoites*, and *Mansonia* species (Fontenille, *et al*, 1998; Gaff, *et al*,

2007; Hartley, *et al*, 2011; Turell, *et al*, 2008). Additionally, other vectors such as sand flies readily transmit RVFV (Fontenille, *et al*, 1998; Hartley, *et al*, 2011; Turell, *et al*, 2008). The ability of the virus to be aerosolized increases the possibility of its use as a bioweapon (Ikegami & Makino, 2011; Hartley, *et al*, 2011; Mandell & Flick, 2011). The recent spread of the virus outside of the African continent, demonstrates its ability to move northward to RVFV-free regions (Europe and Northern America) (CDC, 2012; Clements, *et al*, 2007; Musser, *et al*, 2006; Turell, *et al*, 2008). These fears fuel the international demand for reliable and validated diagnostic tools for rapid diagnosis and control of RVFV (Clements, *et al*, 2007).

In 1931, Daubney and Hudson reported an outbreak of enzootic hepatitis in a herd of ewes which caused abortion and mortality on a farm in the Rift Valley near Lake Naivasha, Kenya, Africa (Flick & Bouloy, 2005; Ikegami & Makino, 2009; Musser, *et al*, 2006). Though first isolated out of a lamb during the investigation in 1930, the virus was probably present in tropical Africa as early as the 1800s (Daubeny, *et al*, 1931). The investigators observed a number of abortions in ruminants and the presence of a hyper-acute lethal infection, characterized by necrotic hepatitis in lambs and proposed the name Rift Valley fever for the disease (Easterday, *et al*, 1962; Easterday, *et al*, 1965; Hartley, *et al*, 2011; Ikegami & Makino, 2009). Pregnant ruminants infected with RVFV are subject to high rates of abortion, fetal malformations, and subclinical-to-fatal febrile illness (Bird, *et al*, 2009; Ikegami & Makino, 2011).

Etiology: Rift Valley Fever Virus

Rift Valley fever virus is an enveloped, single-stranded, negative, and ambisense RNA virus considered to be the cause of large zoonotic outbreaks of acute illness in humans and

livestock (Foster & Walker, 2002; Getsch & Bishop, 1979; Mullen & Durden, 2002; Murphy, *et al*, 1999; Turell, *et al*, 2008). The virus is spherical, 80-120 nm in diameter, and is readily inactivated by lipid and acid solvents/conditions <6 pH (Bishop, *et al*, 1980; Gerdes, 2002; Gerdes, 2004). Rift Valley fever virus is an antigenically distinct member of the family *Bunyaviridae* of the genus *Phlebovirus* and causes abortions and death (Clements, *et al*, 2007; Gerdes, 2002; Sindato, *et al*, 2011; Spickler & Roth, 2006). It is a typical Bunyavirus with a tripartite, negative-stranded RNA, genome consisting of S-, M-, and L- segments each containing a separate nucleocapsid within the virion (Gerdes, 2002; Ikegami & Makino, 2009; Murphy, *et al*, 1999). The nucleocapsids include the S-segment (1,000-3,000 nucleotides), which encodes N and NSs in an ambisense manner, the L-segment (6,500-12,000 nucleotides) that is responsible for the RNA dependent RNA polymerase gene, and the M-segment (3,600-4,900 nucleotides) which encodes the glycoproteins to make up the virus envelope (Figure 2.1) (Ikegami & Makino, 2011; Gerdes, 2002; Gerdes, 2004). This virus replicates in many cell types and antigen has been demonstrated in most areas of the spleen, liver, renal glomeruli, adrenocortical cells, and the walls of vessels (Gerdes, 2002; Gerdes, 2004; Van Der Lugt, *et al*, 1995).

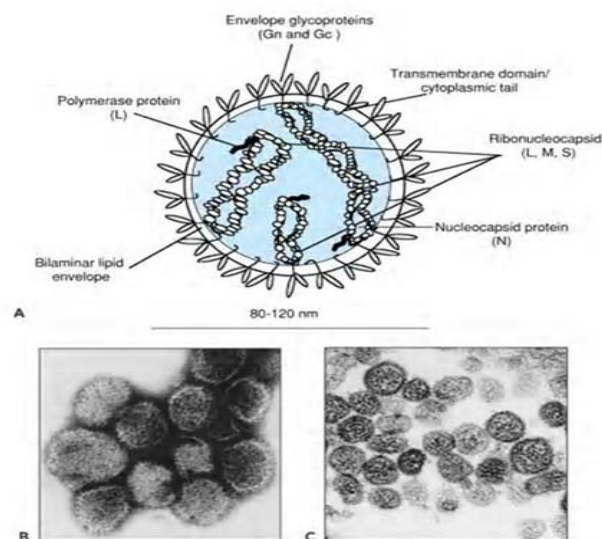


Figure 2-1: Morphology of Rift Valley fever virion adapted from Geisbert, *et al*, 2001

Diagnosis

Due to the potential of RVFV to impact a wide geographic area, especially in areas where animal husbandry is extensive, the laboratory confirmation of the virus is treated as a diagnostic emergency (Archer, *et al*, 2011; Clements, *et al*, 2007; Murphy, *et al*, 1999). Humans and animals of all ages and production systems can be impacted by this hemorrhagic fever (Clements, *et al*, 2007). Diagnosis depends on virus detection of live virus or viral nucleic acids by real time polymerase chain reaction (RT-PCR) or isolation in mice or cell culture (Clements, *et al*, 2007; Murphy, *et al*, 1999; NICD, 2011). Rift Valley fever virus has the ability to replicate in a variety of cell cultures (Murphy, *et al*, 1999; Spickler & Roth, 2006). The general method for isolation of RVFV is either intracranial inoculation of suckling mice or infection of susceptible cell culture lines (Schamlljohn & Hooper, 2001; Spickler & Roth, 2006). Common cell cultures include Vero E6 (African green monkey) and BHK-21 (baby hamster kidney) cells (Ikegami & Makino, 2009; Murphy, *et al*, 1999). Due to the rapid cytopathic nature of the virus, plaques form quickly in cell cultures and fatal infections in mice occur after intracranial challenge (Ikegami & Makino, 2009; Schamlljohn & Hooper, 2001). Thin section electron micrographs show the virus to have a typical overall morphology with predominantly spherical or ovoid particles of about 90 - 100 nm in diameter with surface fringe projections of about 6 - 7.5 nm (Schamlljohn & Hooper, 2001). The virus is distinct when viewed by negative staining with sharply defined surface structures of small round morphologic units about 9.5 nm in diameter with a visible central hole (Martin, *et al*, 1985).

The use of immunoassay methods are used to confirm the identity of isolates (Mohamed, *et al*, 2010; Murphy, *et al*, 1999). Serologic diagnosis is done by IgM or IgG capture enzyme-linked immunoassay (ELISA) on acute sera or by ELISA or hemagglutination inhibition (HAI)

assays on paired sera from surviving animals (Mohamed, *et al*, 2010; Murphy, *et al*, 1999; NICD, 2011). In the absence of hemorrhages or specific organ manifestations, Rift Valley fever is clinically difficult to diagnose (Murphy, *et al*, 1999). Rapid laboratory confirmation of cases is therefore essential for timely execution of supportive treatment, appropriate case management, infection control, and tracing of exposed contacts (Archer, *et al*, 2011; Mohamed, *et al*, 2010).

Differential clinical diagnosis of RVFV varies depending on the region in question (Schamlljohn & Hooper, 2001). The disease should be suspected in: RVFV endemic regions following abnormally high precipitation, in outbreaks of increased rates of abortion in livestock, and in outbreaks of acute influenza-like illness in individuals with close contact with potentially infected livestock (Archer, *et al*, 2011; Schamlljohn & Hooper, 2001; Spickler & Roth, 2006). Veterinarians, as well as field and laboratory workers, should use caution during postmortem examination of animals or while processing diagnostic materials in the laboratory in order to not become infected with the virus (Archer, *et al*, 2011; Murphy, *et al*, 1999; NICD, 2011). Some researchers believe a human becomes infected primarily from contact with infected tissues of livestock or wild (game) animals, and less frequently from mosquito bites (NICD, 2011).

Virologic diagnosis is usually quite simple given the high viremia present throughout the acute phase of illness and the ease of growth of the RVFV when inoculated intracranially into suckling mice or in susceptible cell cultures (Swanepoel, & Coetzer, 1994). Serologic testing is also straightforward, particularly if paired sera (one taken acutely and the other 1-2 weeks later) are available (Mohamed, *et al*, 2010; Schamlljohn & Hooper, 2001; Swanepoel, & Coetzer, 1994). IgM and IgG ELISA tests using inactivated RVFV infected cell lysates or slurries have shown to be highly beneficial in outbreak investigations (Morvan, *et al*, 1992; NICD, 2011; Woods, *et al*, 2002).

Transmission

Rift Valley fever virus is transmitted to human hosts and livestock primarily by biting vectors – mosquitos, culicoides, and sand flies – and handling of infected animals by individuals (Archer, *et al*, 2011; Gerdes, 2002; Hartley, *et al*, 2011; Mullen & Durden, 2009; Sindato, *et al*, 2011). This occurs through direct exposure to infected animals during slaughter or through veterinary and obstetric procedures (Abu-Elyazeed, *et al*, 1996; Archer, *et al*, 2011; CDC, 2012; Chambers & Swanepoel, 1980). Additionally, aerosol transmission has occurred in the laboratory setting (CDC, 2012). The disease caused by RVFV in humans is self-limiting and humans rarely die of infection; however, they may develop severe illness (Mullen & Durden, 2002; Mullen & Durden, 2009). No human-to-human transmission of RVFV has been documented (NICD, 2011).

In Africa, competent vectors include numerous mosquitoes including *Aedes* and *Culex* sp. which generally prefer to feed on livestock outdoors at night (Gerdes, 2002; Hartley, *et al*, 2011; Ratovonjato, *et al*, 2011). Vector competence studies conducted by Turell (2008), *et al*, have found competent vectors in both genera exist in the United States (U.S.). In other countries, such as Madagascar, regional virological studies have been conducted to identify the mosquito species that could act as RVFV vectors (Ratovonjato, *et al*, 2011). In Egypt, retrospective studies showed RVFV had not been enzootic before 1977 (Meegan, 1979). Rift Valley fever virus activity disappeared after 1981 only to be reintroduced in 1993, which suggests that although there are mosquitos capable of acting as epizootic vectors (*Culex* sp.) existing in Egypt, mosquitos capable of RVFV transovarial transmission are probably lacking (Arthur, *et al*, 1993; Schamlijohn & Hooper, 2001). Researchers believe that RVFV was most likely introduced into Egypt from enzootic areas in Sudan (Meegan, 1981; Peters, 1997).

Outbreaks of RVFV tend to follow large rainfalls and climate variability in affected regions, which are associated with the periodic rise and fall of the disease (Davies, *et al*, 1985; Linthicum, *et al*, 1999; Sinato, *et al*, 2011). The outbreaks occur at irregular intervals of years, some believe 8-10 years (NICD, 2011; Sinato, *et al*, 2011). Following heavy rains breeding of the mosquito vector is favored and can recur over a succession of unduly wet seasons, which may not be seen for decades during drier spells (NICD, 2011; Sinato, *et al*, 2011; WHO, 2010). Researchers have documented a correlation between heavy rain and increased mosquito activity furthering the spread of the virus (Davies, *et al*, 1985; Linthicum, *et al*, 1999; NICD, 2011; Sinato, *et al*, 2011). Post-excessive rainfall, mosquito eggs, usually of the genus *Aedes* and *Culex* sp., hatch (Davies, *et al*, 1985; Gerdes, 2002; Mondet, *et al*, 2005). These mosquito eggs are transovarially infected with the RVFV and the resulting mosquitoes transfer the virus to their hosts (Ratovonjato, *et al*, 2011).

Initially, all transmission is by mosquitos; however, viremic livestock can transmit directly and by aerosol to humans and other animals (NICD, 2011; Ratovonjato, *et al*, 2011). Once viremia is established in a host, additional species of mosquitoes can become infected and spread the disease (Musser, *et al*, 2006; Ratovonjato, *et al*, 2011). Vertical transmission from infected female mosquitos to offspring is largely driving the RVFV outbreaks (Seufi & Galal, 2010; Sinato, *et al*, 2011). Observations in the field indicate RVFV is maintained vertically during dry periods in the eggs of *Aedes* sp. mosquitoes; however, this has not been observed in laboratory settings (Hartley, *et al*, 2011). Mondet (2005), *et al*, documented *Aedes* spp. produced hardy eggs which remained viable in African soil for many years; therefore, if RVFV were introduced into the U.S., eradication would be extremely difficult (Hartley, *et al*, 2011; Musser, *et al*, 2006). The reason the disease caused by RVFV has not spread more widely from Africa is

unknown because mosquitos capable of transmitting the virus exist in most parts of the world (Hartley, *et al*, 2011; Musser, *et al*, 2006; Turell, *et al*, 2008).

Disease Cycle: Epidemiology

Rift Valley fever virus is considered one of the most prolific viruses in the world (Hartley, *et al*, 2011; Sindato, *et al*, 2011). It typically occurs as explosive epidemics of hemorrhagic disease in animals (Sindato, *et al*, 2011). However, the ecology and epidemiology of Rift Valley fever virus is complex and poorly understood (Gaff, *et al*, 2007; Schamlljohn & Hooper, 2001). The infections in livestock are often recognized by the onset of abortion “storms” which sweep through livestock followed by acute febrile disease in humans (Schamlljohn & Hooper, 2001). Rift Valley fever virus is transmitted to human and livestock hosts primarily by arthropod vectors and handling of infected animals (Hartley, *et al*, 2011). The virus enters via a mosquito bite, *Aedes* and *Culex* sp. or through the respiratory system via aerosolization (Gerdes, 2002; Hartley, *et al*, 2011; Murphy, *et al*, 1999). The virus enters its target tissues and replicates very quickly to high viremia (Gerdes, 2002; Gerdes, 2004; Murphy, *et al*, 1999). The virus can affect many species including, cattle, sheep, goats, water buffalo, camels, monkeys, gray squirrels, and other rodents (Mullen & Durden, 2002). However, cattle and sheep are considered the primary amplifying hosts (Sindato, *et al*, 2011). During the incubation time, the virus invades the parenchyma of the liver and the reticuloendothelial organs, causing severe cytopathology often resulting in organ failure (Anderson, *et al*, 1987; Murphy, *et al*, 1999).

At necropsy, it is not uncommon to find almost complete hepatocellular destruction (Gerdes, 2002; Murphy, *et al*, 1999). Furthermore, spleen enlargement and hemorrhage is seen

in the gastrointestinal tract and the subserosal tissue (Gerdes, 2002; Murphy, *et al*, 1999). Evidence of encephalitis can be detected by the presence of neuronal necrosis and perivascular inflammatory infiltration seen in animals that survive the hepatic infection (Murphy, *et al*, 1999; Spickler & Roth, 2006). In sheep, hepatic necrosis and hemorrhagic complications are the primary cause of death (Anderson, *et al*, 1987; Murphy, *et al*, 1999; Spickler & Roth, 2006). Survivors recover rapidly and maintain long lasting immunity (Hartley, *et al*, 2011; Murphy, *et al*, 1999). In the laboratory setting, the virus infects a wide variety of laboratory and domestic animals and is often lethal to both. Hepatitis and encephalitis syndromes are seen most commonly in these settings (Anderson, *et al*, 1987; Murphy, *et al*, 1999). Viremia without severe disease can be seen in adult cats, dogs, horses, and some monkeys, while rabbits, pigs, guinea pigs, chickens and hedgehogs the most resistant (Daubney, *et al*, 1931; Easterday, 1965; Ikegami & Makino, 2011; Scott, 1963; Scott, *et al*, 1963; Spickler & Roth, 2006; Weiss, 1957; Walker, *et al*, 1970a; Walker, *et al*, 1970b). Severe disease/mortality can occur in newborn puppies and kittens (Walker, *et al*, 1970a; Walker, *et al*, 1970b).

Human Signs of Clinical Disease

Similar to animals, the clinical signs of Rift Valley fever vary and may be asymptomatic (NICD, 2011; Sindato, *et al*, 2011). The incubation period ranges from 2-6 days, but can be as short as 12 hours (Murphy, *et al*, 1999; Spickler & Roth, 2006). Humans experience influenza-like syndromes, hemorrhagic pyrexia (37.8-40°C), strong headaches, body pain, dizziness, nausea, epigastric discomfort, photophobia-retinitis, anorexia, petechia, and hemorrhage from body cavities (Deutman & Klomp, 1981; Swanepoel & Coetzer, 2004; Sindato, *et al*, 2011; Spickler & Roth, 2006).

Additionally, blindness and encephalitis have been documented to occur in 1-2% of affected individuals with a case fatality risk of 10-20% (Otte, *et al*, 2004; Schamlijohn & Hooper, 2001; Sindato, *et al*, 2011; Swanepoel & Coetzer, 2004). Asymptomatic infection or a relatively mild illness can be observed with pyrexia, chills, headache, “back breaking” myalgia, diarrhea, vomiting, hemorrhages, and hepatitis (Murphy, *et al*, 1999; Swanepoel & Coetzer, 2004; Sindato, *et al*, 2011). Recovery from Rift Valley fever is considered to occur within 4-7 days (Murphy, *et al*, 1999; Spickler & Roth, 2006). A small percentage of cases – less than two percent – can progress from illness to death; however, in patients with hemorrhagic disease fatalities are considered to reach 10% (Murphy, *et al*, 1999; Sindato, *et al*, 2011). A small percentage of cases – less than one percent – can progress to meningoencephalitis with a subsequent extremely low case-fatality rate (Murphy, *et al*, 1999). Other causes for these symptoms must be considered to facilitate timely treatments, prevention, and control for other infections including malaria, brucellosis, and Crimean-Congo hemorrhagic fever (Murphy, *et al*, 1999; NICD, 2011; Sindato, *et al*, 2011).

Animal Signs of Clinical Disease

Clinical signs vary with age, species, and breed of animal and are most severe in young animals (Sindato, *et al*, 2011). The incubation period is 12-36 hours in newborn lambs (who are most susceptible) and up to 72 hours in sheep, cattle, and dogs (Easterday, *et al*, 1962; Murphy, *et al*, 1999; Spickler & Roth, 2006). Lambs experience pyrexia (40-42°C), anorexia, lymphadenopathy, weakness, and usually death within 36 hours of inoculation (Mandell & Flick, 2011; Murphy, *et al*, 1999; Sindato, *et al*, 2011). It is not unusual to observe 90-100% mortality in affected animals (Mandell & Flick, 2011; Murphy, *et al*, 1999; Sindato, *et al*, 2011). Adult

sheep also experience pyrexia (40-42°C); however, mucopurulent nasal discharge, hemorrhagic, and/or foul-smelling diarrhea, jaundice, unsteady gait may also be observed; mortality is typical in 20-60% of adults (Sindato, *et al*, 2011; Spickler & Roth, 2006). Abortion occurs in 90-100% of affect ewes (Mandell & Flick, 2011; Murphy, *et al*, 1999). The disease is similar in goats but is considered milder in cattle (Murphy, *et al*, 1999). Calves experience pyrexia (40-41°C), anorexia, depression, and death within 36 hours of inoculation, and 10-70% mortality is observed in affected animals (Murphy, *et al*, 1999; Sindato, *et al*, 2011). Adult cattle also experience pyrexia (40-41°C); however, it is not uncommon to observe excessive salivation, anorexia, weakness, fetid diarrhea, and decrease in milk production (Mandell & Flick, 2011; Murphy, *et al*, 1999; Gerdes, 2002). Like ewes, abortion in cattle is expected in 90-100% of dams (Mandell & Flick, 2011; Murphy, *et al*, 1999; Gerdes, 2002). The mortality rate in adult animals is usually less than 10% (Murphy, *et al*, 1999; Sindato, *et al*, 2011). Other causes for these clinical signs must be considered to facilitate timely treatments, prevention, and control for other infections including Bluetongue virus (BTV), Wesselsbron, ephemeral fever, enterotoxemia of sheep, ovine enzootic abortion, *Campylobacter* or *Salmonella* infection, brucellosis, vibriosis, trichomoniasis, Nairobi sheep disease, heartwater, or other causes of abortion (NICD, 2011; Sindato, *et al*, 2011; Spickler & Roth, 2006).

Prevention, Control, and Treatment

Immunization of livestock is considered to be the most effective way to prevent human Rift Valley fever cases and epizootic outbreaks (NICD, 2011; Schamlljohn & Hooper, 2001; Spickler & Roth, 2006). Currently there are no human or veterinary RVFV vaccines available commercially in the U.S. (Mandell & Flick, 2011; Hartley, *et al*, 2011). Vaccines for veterinary

use are available in other countries, however, these may cause birth defects and abortion in sheep and induce only low-level protection in cattle (CDC, 2012; Schamlljohn & Hooper, 2001).

There are attenuated and killed vaccines produced in mice brains and embryonated eggs, which are considered effective and inexpensive, but are thought to cause abortion in pregnant ewes (Hartley, *et al*, 2011; Murphy, *et al*, 1999). Killed vaccines, such as the Smithburn Attenuated vaccine, Clone 13 Attenuated vaccine, Onderstepoort Biological Products (major deletion in NSs gene that inhibits interferon), and the costly GALVmed Vaccine out of Kenya, confer lifelong immunity to vaccinated animals (Sindato, *et al*, 2011; CDC, 2012). Inactivated vaccines, such as the formalin-inactivated wild-type virus used in Egypt and South Africa, produced in cell cultures avoid the problem of abortion, but are considered expensive and induce only short-lived immunity (El-Karamany, *et al*, 1981; Murphy, *et al*, 1999).

Both live and attenuated of vaccines have been produced in large volumes in Africa (Murphy, *et al*, 1999). The human live-attenuated MP-12 vaccine produced by Pfizer has shown promise in laboratory trials in domestic animals, but more research is needed before it can be used in the field (CDC, 2012; Ikegami & Makino, 2011; Morrill, *et al*, 1997; Schamlljohn & Hooper, 2001). Hunter (2002), *et al*, described pregnant ewes vaccinated with MP-12 at 28 to 56 days of gestation either miscarried or produced teratogen lambs. This is due to the antibodies evoked by MP-12 being identical to those produced in response to infection by full-strength RVFV (UTMB, 2012). This response could also be an issue in the event of an outbreak, because public health officials would be unable to tell humans or animals vaccinated with MP-12 from naturally infected individuals, making it impossible to map the spread of an outbreak and respond effectively (CDC, 2012; Ikegami & Makino, 2011; UTMB, 2012).

Ideally, to be effective, the RVFV vaccine must be delivered in a systematic way to entire animal populations, preferably on a regular schedule, before the start of the mosquito season (Hunter, *et al*, 2002; Murphy, *et al*, 1999). However, vaccination for RVFV is problematic because 1) viral movement can be so rapid that once an epidemic has been detected, it is difficult – if not impossible – to administer enough vaccine fast enough, 2) even when vaccine is delivered quickly, there is often not enough time for protective immunity to develop in the animals – need fourteen days minimum, and 3) control of this disease is expensive, ineffective, and very demanding in both human and monetary resources (Ikegami & Makino, 2011; Sindato, *et al*, 2011; Murphy, *et al*, 1999). Due to this cost, farmers and ranchers are resisting vaccination protocols in most areas of southern Africa (Murphy, *et al*, 1999).

Other ways to mitigate the spread of RVFV involves control of the vector through widespread use of mosquito repellants in the human population to protect against their bites (Gerdes, 2002; Hartley, *et al*, 2011; NICD, 2011; Murphy, *et al*, 1999). Use of larvacides at mosquito breeding sites are considered the most effective form of vector control as long as these sites can be clearly identified and are limited in size and extent (Hartley, *et al*, 2011; NICD, 2011; Murphy, *et al*, 1999). During periods of flooding, the number and extent of breeding sites may be too high for larvicide applications to be feasible (NICD, 2011). Additionally, the interruption of the epizootic/epidemic transmission of RVFV would require the effective and wise use of mosquito adulticides to eliminate infected females when amplifying host animals are viremic (Hartley, *et al*, 2011). Mosquito control is impossible in most areas of Africa due to the wide range of vector species with different habitats and econiche preferences, long breeding seasons in wide geographic areas (Murphy, *et al*, 1999). These variables contribute to large

mosquito populations resulting in widespread epidemics (Gerdes, 2002; Gerdes, 2004; Murphy, *et al*, 1999).

Epidemics usually are limited in duration to 3 to 4 months, but may persist in wetter areas for 1 to 3 years (Gerdes, 2002; Gerdes, 2004; NICD, 2011). It is important to identify which species are competent vectors in the natural transmission cycle because control measures vary (Gargan, *et al*, 1988; Turell, *et al*, 2008). Close monitoring and surveillance of vectors is essential for control of the Rift Valley fever infection in animal and human populations (CDC, 2012; Mandell & Flick, 2011; NICD, 2011).

There is no current medical treatment available for humans infected with RVFV; often only supportive care is available (NICD, 2011; Schamlljohn & Hooper, 2001). Some researchers believe the antiviral drug ribavirin may be of some benefit in the treatment of RVFV, although the human efficacy data is considered to be lacking (Peters, *et al*, 1986; Schamlljohn & Hooper, 2001). However, NICD (2011) does not recommend ribavirin or corticosteroids for the treatment of RVFV.

Early dialysis for patients with renal failure is considered to improve outcome (NICD, 2011; Schamlljohn & Hooper, 2001). Isolation or barrier nursing is not necessary with suspected individuals; however, theoretical risk of transmission is considered possible (Mandell & Flick, 2011; NICD, 2011). Standard precautions should be implemented to avoid contact with infected blood and tissues (Hartley, *et al*, 2011; Mandell & Flick, 2011; NICD, 2011). Additionally, the treatment of nosocomial (hospital) infections should be considered and treated promptly (Hartley, *et al*, 2011; NICD, 2011). Sanitation and vector controls should be attempted when dealing with RVFV, but often do not control the spread of disease (NICD, 2011; Spickler & Roth, 2006). Rift Valley fever virus is inactivated by ether, chloroform, strong solutions of

sodium or calcium hypochlorite (chlorine should exceed 5000 ppm), low pH (<6.8), and detergents that break up the virus (Spickler & Roth, 2006). It is recommended that carcasses of previously infected animals be buried or burned (Murphy, *et al*, 1999; Spickler & Roth, 2006). Workers at risk for exposure should wear personal protective equipment (PPE) to avoid contact with potentially infectious materials and aerosols (Hartley, *et al*, 2011; Murphy, *et al*, 1999; Schamlijohn & Hooper, 2001).

In 2011, a group at the Department of Agricultural Economics at Texas A&M University conducted an economic assessment of the U.S. agricultural and human vulnerability and consequence of a RVFV epidemic outbreak (Hughes-Fraire, *et al*, 2011). Because a Rift Valley fever outbreak has never been seen in the U.S. the economic model is based on previously seen diseases, including WNV, and models previously conducted by Hartley, *et al* (2009) and Gaff, *et al* (2007) (Hughes-Fraire, *et al*, 2011). An integrated epidemic/economic model to examine the extent of RVFV spread in the Southeast Texas Livestock population indicated a potential loss to the U.S. of \$120 million – \$2.3 billion with combined livestock and human national costs (Hughes-Fraire, *et al*, 2011). This estimated cost includes healthcare, control, surveillance, and animal product losses (Hartley, *et al*, 2009; Hughes-Fraire, *et al*, 2011). The reported cost shows the economic damages of an outbreak in the first year to be approximately three times greater in the livestock population compared to the human population (Hughes-Fraire, *et al*, 2011). However, Hughes-Fraire, *et al* (2011) believes the cost estimates of both the human and livestock populations are likely underestimated. Future research should be conducted to follow up the report because additional variables were not included, such as the domestic damages of decreased beef demand due to food safety concerns and trade bans, all susceptible livestock

(goats and swine), as well as the impact of damages to society and tourism, in an endemic country (Hartley, *et al*, 2009; Hughes-Fraire, *et al*, 2011; Mandell & Flick, 2011).

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Chapter 3 - Occupational Biosafety

Introduction

Biosafety is the consistent application of safety measures to minimize or prevent exposure to the agent handler, lab and building occupants, the community, and the environment (CDC, 2009b; Harding & Byers, 2006; Lord, *et al*, 1999; Pedrosa & Cardoso, 2011; Singh, 2009). Biosafety is often achieved through good microbiological work practices, safety and containment equipment, and facility design consideration (CDC, 2009b; Harding & Byers, 2006). Another common term in the laboratory is biosecurity. Biosecurity and biosafety are related concepts and are sometimes confused with each other (CDC, 2009b). However, biosecurity prevents the loss, theft or misuse of microorganisms, biological materials, and research related information through limiting access to facilities and research materials and information (CDC, 2009b; Pedrosa & Cardoso, 2011).

Biosafety applications are applied in many types of laboratories and are commonly used in research involving human and animal pathogens, select agents (bioterrorism), toxins of biological origin, rDNA research, human gene transfer, plants, and animals, shipping, transport, import, export, permits, training, infection control, shipping, BSL-3, and work practices, and lastly in field work/work abroad (feral animal, insects, and arthropods) (Baron & Miller, 2008; CDC, 2009b; Harding & Byers, 2006; Ikegami & Makino, 2011; Singh, 2009). Often, a breakdown in biosafety procedures is the result of a breakdown in good microbiological work practices (Baron & Miller, 2008; Pike, 1979). This breakdown in microbiological practices has been identified by epidemiologists to be the cause of 90% of laboratory acquired infections (LAI) (Baron & Miller, 2008; CDC, 2009b; Harding & Byers, 2006; Kruse, *et al*, 1991).

In the last 150 years, laboratory workers have occasionally become infected by the microorganisms they were working with and some of these cases have resulted in fatalities (CDC, 2009b; Pike, 1979; Singh, 2009). Only a small number of LAIs involve a specific incident, a majority of them involve non-specific associates such as being in a laboratory working with agents (Harding & Byers, 2000; Harding & Byers, 2006). Attempts have been made to define the extent of the problem, determine the cause of the accidental infections, and to devise safe equipment and procedures to prevent them (Harding & Byers, 2006; Pedrosa & Cardoso, 2011; Pike, 1979; Singh, 2009). This safety equipment includes the use of mechanical pipettors which were first used as early as 1907 to prevent LAI (Reinhardt, 1918). The use of a protective microbiological cabinet was first reported in Germany in 1919 (Fricke, 1919; Phillips, 1961). In contrast, the use of protective microbiological cabinets in the U.S. was not seen until the 1940s when they began to be used at the National Institutes of Health (NIH), Naval Biological Laboratory (NBL), and Fort Detrick (Kruse, *et al*, 1991; Phillips, 1961). Today there are several classes (I-III) of safety cabinets with various features to allow safe handling and bench work with aerosol containment (Kruse, *et al*, 1991; Miller, 2008; Wedum, *et al*, 1957; Wedum, 1963; Wedum, 1997). Additionally, personal protective equipment (PPE) involving gloves, laboratory coats, and safety glasses/shields are now commonly used in all laboratories (CDC, 2009b; Wedum, 1963).

When discussing occupational biosafety for the laboratory worker, the recognition of the risks and dangers of pathogens causing accidental infection is important (Kruse, 1991; Pedrosa & Cardoso, 2011; Singh, 2009; Sulkin & Pike, 1951). Efforts to secure and maintain pure cultures of infectious agents has been around for quite some time, but it was not until laboratory acquired infections (LAI) appeared that any attention was given to the protection of the worker and

surrounding personnel (CDC, 2009b; Kruse, *et al*, 1991). The risk of LAIs has been associated with the isolation, identification, collection, and research of pathogenic agents (Kruse, *et al*, 1991; Phillips & Bailey, 1965; Singh, 2009). This risk has also been associated with transmission hazards, such as mouth pipettes, syringes and needles, spills and splashes, bites of animals or ectoparasites, as well as aerosolization, which have been recognized and reported since the late 1800's (Kruse, *et al*, 1991; Phillips & Bailey, 1965; Wedum, 1963). Laboratory-acquired infections represent an occupational hazard unique to laboratory workers, especially those in the microbiology laboratory (Pedrosa & Cardoso, 2011; Singh, 2009). Exposures can occur inadvertently, or may be due to lapse in technique leading to accidental inoculation (Pedrosa & Cardoso, 2011; Pike, 1979; Singh, 2009). Fortunately, not every exposure results in LAI (Hanson, *et al*, 1967; Pedrosa & Cardoso, 2011; Pike, 1979; Singh, 2009). Nevertheless, the detection of hazardous risks while working with biological agents has shaped the implication of safety equipment, laboratory design, and laboratory protocols and procedures (CDC, 2009b; Lord, *et al*, 1999; Singh, 2009; Sulkin & Pike, 1951; Phillips & Bailey, 1965).

Procedures and regulations for biological laboratories first were established in the 1950's, with biosafety levels being established later. In 1956 West Germany had published regulations for laboratories that 1) prohibited mouth pipetting, and 2) forbid food, drink, tobacco, and chewing gum in the laboratory setting, both of which are still regulations in place today (Phillips, 1961; Wedum, 1997). Currently, it is highly recommended laboratory workers be vigilant when working with infectious agents should be especially attentive to hazardous procedures including making slides, inoculating biochemical agents, working with infected animal tissue suspensions and arboviruses, other bloodborne pathogens, using a centrifuge, and anything that capable of generating an aerosol (Beekmann & Henderson, 2005; Hanson, *et al*, 1967; Miller, 2008;

Pedrosa & Cardoso, 2011; Pike, 1979; Wedum, 1963). It is also common practice for laboratories to have standard operating procedures (SOP), use biological safety cabinets (BSC), vaccinate workers in high risk situations, and have established procedures with medical response plans for each agent (Miller, 2008; Pedrosa & Cardoso, 2011; Pike, 1979).

Brief History of Biosafety

It is believed that even though scientific application of disinfectants, sterilants, and preservatives contributing to biosafety are limited to the past 150 years, empirical practices go back to ancient times (Block, 2001; Pike, 1979; Singh, 2009). Seymour Block, an Environmental Microbiologist, is credited with researching the historic use of disinfectants or infection control (Block, 2001). He cites that one of the oldest recordings of decontamination was Homer's account of the use of sulfur to gas decontaminate a home in his novel *The Odyssey* (Block, 2001). When Odysseus returned home and killed his wife's suitors, he says to his nurse "bring me some disinfectant sulfur and make me a fire so I can fumigate the house" (Block, 2001; Rieu, 1952). Block (2011) also states that *The Bible* contains references for infection control, waste control, decontamination and patient isolation and that Aristotle advised Alexander the Great to boil water and bury excrement (Kruse, *et al*, 1991).

One of the founding principles of biosafety today is credited to Susruta, a Hindu physician more than 1500 years ago, who stated "disinfect surfaces or equipment before and after use" (Block, 2001). The Black Plague, *Yersinia pestis*, in the Middle Ages brought forth several applications of protective measures and devices, even though the people at the time did not understand the full conceptual pathology of the disease process (Block, 2001; CDC, 2009b). Preventive measures included burning victims' clothing, burning bodies, and using unique

personal protective equipment (PPE) worn by physicians (Block, 2001; CDC, 2009b). In 1438, the Venice Magistry of Health held cargo and mail from ships out at sea at a “safe distance from port” until they could gas decontaminate items before they were brought to the main land (Block, 2001). Today, organizations that protect the U.S. from agricultural and ecological damage, United States Department of Agriculture (USDA) and Animal Plant Health Inspection Service (APHIS), use similar measures to quarantine and inspect materials before they are brought in to the country (Block, 2001). Other individuals such as Oliver Wendell Holmes, Louis Pasteur, Joseph Lister, and Robert Koch have made significant contributions to biosafety and the preservation of public health (Block, 2001; CDC, 2009b; Ikegami & Makino, 2011; Kruse, *et al*, 1991).

Laboratory-Acquired Infections

The first recognized laboratory acquired infections (LAI) with biological agents were reported in the late 1880’s (CDC, 2009b). Biological agents such as brucellosis (1887), glanders, diphtheria, cholera, tetanus (1893), and Typhoid fever (1885) were known to be the cause of accidental laboratory infections (CDC, 2009b; Chosewood & Wilson, 2009; Kruse, *et al*, 1991; Pike, 1979; Wedum, *et al*, 1957). The epidemiological review of LAIs had a slow start; thirty years after the first reported LAI Typhoid fever infection, the first survey was made of laboratory infections (Kisskalt, 1915; Kruse, *et al*, 1991; Wedum, 1997). In 1915, Kisskalt published the first report from Germany listing cases of laboratory acquired Typhoid fever infections (Kisskalt, 1915; Kruse, *et al*, 1991; Pike, 1979). Interestingly, the first analyzed report of accidental laboratory acquired viral infection was published in 1935 (Francis & Magill, 1935; Pedrosa & Cardoso, 2011; Pike, 1979). This was also the first recorded case of Rift Valley Fever in the

Western hemisphere (Francis & Magill, 1935; Pedrosa & Cardoso, 2011; Pike, 1979; Schwentker & Rivers, 1934). In 1947 there was an outbreak of Q fever (caused by the rickettsial, *Coxiella burnetii*) affecting 47 people at the National Institutes of Health (NIH) (Huebner, 1947; Pike, 1969; Wedum, *et al*, 1957).

There are many case reports of LAIs in the literature; however, few studies have indicated the magnitude of the problem as it relates to the occupational health of laboratory workers (Sulkin & Pike, 1951; Pike, 1979; Wedum, *et al*, 1957). Before the 1950's, little consideration had been given to the need for adequate protection of personnel who came into daily contact with disease producing agents (Sulkin & Pike, 1951; Pike, 1979). In 1951, Sulkin & Pike summarized 1,342 infections, and 39 fatalities caused by a variety of disease producing agents that presumably were acquired as a result of laboratory work in the U.S. during 1930-1950 (Sulkin & Pike, 1951). A large proportion of the infections occurred among professional and technical workers, research assistants, and graduate students, including medical and veterinary students, animal caretakers, janitors, and dishwashers (Sulkin & Pike, 1951; Pike, 1976; Pike, 1979; Wedum, *et al*, 1957). Many variables make it virtually impossible to define risk of infection to laboratory personnel handling infectious material (Pike, 1979). However, researchers believe the number of recorded and documented infections that have occurred with various agents in laboratory settings may be the best indicator of risk (CDC, 2009a; Pike, 1976; Pike, 1979).

Pike and Sulkin, conducted a series of surveys during the middle of the 20th century which went to 4,000 laboratories of various types and received an approximate 50% response (Byers, 2009; Harding & Byers, 2006; CDC, 2009a; Pike, 1979; Pike & Sulkin, 1965). The surveys illustrated that 4,079 LAIs, due to 159 agents, were reported from 1935-1978; Fourteen

percent of these reports were from clinical laboratories and 168 of the cases resulted in fatalities (Byers, 2009; Harding & Byers, 2006; CDC, 2009a; Pike & Sulkin, 1965; Pike, 1976; Pike, 1978; Pike, 1979; Sulkin & Pike, 1951). Pike and Sulkin believed 4,079 cases was a lower limit, because it is likely not all infections were reported (Byers, 2009; Harding & Byers, 2006).

Harding and Byers conducted a literature survey of LAIs from 1979-2005 which indicated that 1,141 LAI with 24 fatalities were reported; 5 of these deaths were fetal deaths aborted as a consequence of maternal LAIs (Byers, 2009; Harding & Byers, 2000; Harding & Byers, 2006; Phillips, 2011). The findings of Harding and Byers indicated that 45% and 51% of clinical and research laboratories, respectively, accounted for the total LAIs reported (Byers, 2009; CDC, 2009a; Harding & Byers, 2000; Harding & Byers, 2006). This is considered significantly different from Pike and Sulkins findings prior to 1979 which indicated that 17% and 59% of clinical and research laboratories accounted for the total LAIs reported (CDC, 2009a; Hanson, *et al*, 1967; Sulkin & Pike, 1951)

It is believed the reports by Sulkin and Pike in the 1950s contributed to the decisions in the U.S. to modernize laboratories so they may more safely handle infectious agents (Phillips, 1961; Wedum, 1997). Many individuals have reported known infections, but the numbers do not include the many subclinical infections which likely occurred (Baron & Miller, 2008; Pike, 1979). These infections are impossible to calculate but some believe they may outnumber the clinically apparent diseases (Baron & Miller, 2008; Pike, 1979). The accurate quantification of risks associated with working in a biological laboratory is difficult to assess (Singh, 2009). Due to a lack of information on the actual numbers of infections and the population truly at risk, it is difficult to determine the true incidence of LAIs with any certainty (CDC, 2009a; Pedrosa & Cardoso, 2011). Fortunately, the recent Harding and Byers data indicates recent LAIs are

considered to be decreasing (Harding & Byers, 2006; Pedrosa & Cardoso, 2011; Phillips, 2011). This decrease is attributed to improvements in containment equipment, engineering controls, designation of biosafety levels (see below) and greater emphasis on safety training (see Appendix A) and equipment protocols (see Chapter 5) (Pedrosa & Cardoso, 2011; Phillips, 2011).

It is worth noting that aside from individuals becoming ill from poor biological safety, a research laboratory could lose its funding, especially federal funding (Phillips, 2011). An example of this was seen in 2007 when the CDC suspended Texas A&M's research on infectious diseases after the university failed to report two 2006 cases of human exposure to biological agents (Phillips, 2011). This example, as well as the aforementioned information, is a great reason why biosafety as well as occupational and public health are important in a laboratory setting.

Biosafety Levels

A biosafety level is the level of bio-containment precautions required to isolate dangerous biological agents in enclosed facilities. Bio-containment is classified by the danger to the surrounding environment as biological safety levels (BSL) (CDC, 2009b; Chosewood & Wilson, 2009; OSHA, 2006). The levels of containment range from the lowest biosafety level 1 (BSL-1) to the highest at level 4 (BSL-4), with higher numbers indicating a greater risk to the external environment (Chosewood & Wilson, 2009). In the United States (U.S.), the Centers for Disease Control and Prevention (CDC) specified these levels in 1984 via the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (BMBL) handbook (Chosewood & Wilson, 2009).

Biosafety Level (BSL-1)

This is the lowest level of bio-containment, where the containment zone may be only a chemical fume hood (OSHA, 2006). This level is considered suitable for work involving well-characterized agents not known to consistently cause disease in healthy adult humans and of no known or of minimal potential hazard to laboratory personnel and the environment (CDC, 2009b; OSHA, 2006). Work is performed with well-defined and characterized strains of viable organisms not known to cause disease in healthy individuals (OSHA, 2006). There are some agents in this level which are “opportunistic” and can cause disease in compromised individuals (OSHA, 2006). These laboratories are not necessarily separate from the general traffic patterns in a building (CDC, 2009b). Work is generally performed on open bench tops using standard microbiological practices (CDC, 2009b; OSHA, 2006). Special containment equipment and devices of facility design are not usually required, but can be adjusted based on appropriate risk assessment (CDC, 2009; OSHA, 2006). Laboratory personnel should have specific laboratory training in the procedures to be conducted in the laboratory and are supervised by personnel with general training in microbiology or related fields (CDC, 2009b; OSHA, 2006).

Biosafety Level (BSL-2)

This is the next level of bio-containment and builds upon the precautions of agent practices of BSL-1 (CDC, 2009b; OSHA, 2006). BSL-2 laboratory practices and procedures are suitable to work with agents that pose moderate risk to personnel and the environment (CDC, 2009b; Lord, *et al*, 1999; OSHA, 2006). The access to the laboratory is limited when work with these organisms is being conducted (CDC, 2009b; OSHA, 2006). Biological safety cabinets (BSC) or protective equipment is recommended for procedures which involve infectious aerosols or splashes (CDC, 2009b; Kruse, *et al*, 1991; Lord, *et al*, 1999; OSHA, 2006). Laboratory

personnel must have specific training in handling pathogenic agents, be familiar with hazards associated with the specific agents used, and be directed by scientists competent in handling infectious agents as well as familiar with good microbiological practices (CDC, 2009b; OSHA, 2006; Riley, *et al*, 1999).

Biosafety Level (BSL-3)

This level of bio-containment is applicable to clinical, diagnostic, teaching, research, or production facilities where work is conducted with dangerous or exotic agents and the potential for infection is real and may cause serious or potentially lethal disease through inhalation (CDC, 2009b; OSHA, 2006). Precautions for work with agents at this level require BSL-1 and BSL-2 practices plus work is performed in special containment facilities (OSHA, 2006; Riley, *et al*, 1999). Access to a BSL-3 laboratory is strictly limited to only individuals performing work (CDC, 2009b; OSHA, 2006). Laboratory personnel must have specialized training in handling pathogenic and potentially lethal agents and must be supervised by scientists competent in handling infectious agents (CDC, 2009b; OSHA, 2006; Riley, *et al*, 1999). All work involving the manipulation of infectious materials must be conducted within BSCs or other physical containment devices (CDC, 2009b; Riley, *et al*, 1999). Often a BSL-3 laboratory has special engineering and design features such as airlock entrance zones, sealed floors and wall penetrations, and directional airflow (negative pressure to the surrounding areas) to aid containment (CDC, 2009b; OSHA, 2006).

Biosafety Level (BSL-4)

This is the highest level of bio-containment (maximum containment), where the containment involves isolation of the organism by means of building systems, sealed rooms; with complex ventilation requirements, sealed containers, positive pressure personnel suits, and

procedures for entering and exiting the room (CDC, 2009b; OSHA, 2006). This level of containment requires practices and procedures for work with dangerous and exotic agents that pose a high individual risk of aerosol-transmitted laboratory infections and life-threatening disease (CDC, 2009b; OSHA, 2006). Frequently, the at-risk diseases may be fatal or be diseases where there are no available vaccines or treatments, or be due to a related agent with unknown risk of transmission (CDC, 2009b; OSHA, 2006). Agents with a related antigenic connection to agents requiring BSL-4 containment must be handled at this level until appropriate data is obtained to continue work at this level or to re-designate to another level (CDC, 2009b). Precautions for use of these agents include BSL-1, BSL-2, and BSL-3 practices plus specialized procedures for BSL-4 (CDC, 2009b; OSHA, 2006).

Laboratory workers must have specific and thorough training with strict adherence to appropriate specialized practices and procedures in handling extremely hazardous infectious agents (OSHA, 2006). Laboratory staff is trained in primary and secondary containment functions of standard and specialized practices and have shown to be proficient in the use of the agents at this level (CDC, 2009b; OSHA, 2006). High levels of security, in accordance with institutional policies, are used to obtain access to the facility to help assure only authorized personnel may be admitted to any area within the containment zone (CDC, 2009b).

The Centers for Disease Control and Prevention's Laboratory Biosafety Level Criteria offers a great review and explanation of Standard Microbiological Practices (SMP), Special Practices, Laboratory Facilities (Secondary Barriers), and Safety Equipment (Primary Barriers and Personal Protective Equipment (PPE)) for each level of biosafety.

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Chapter 4 - Field Experience

Student Learning Objectives

The learning objectives for the field experience included: Learning about the zoonotic disease Rift Valley Fever at the United States Department of Agriculture (USDA) Arthropod-Borne Animal Disease Research Unit (ABADRU) and working and gathering an understanding of procedures involved with zoonotic disease research and its application to a real world setting. Additional objectives included participation in occupational hazard guidelines and laboratory biosafety recommendations associated with agents in a BSL-2 laboratory. Comprehension was demonstrated via constructed educational presentations for future laboratory worker training (Appendix A & B).

Brief History of Organization

In 1862, President Abraham Lincoln signed into law the *Agricultural Act*, which established the USDA (USDA, 2012). This year marks the 150th anniversary of the USDA (USDA, 2012). During its time, the USDA has seen the *Hatch Act* of 1887, which provided funding for agricultural experimental stations in each state and the *Smith-Lever Act* of 1914, which funded cooperative extension services in each state to teach agriculture and related subjects to the general public (USDA, 2012). Additionally, the formation of the USDA has led to many provisions and units designed to meet the needs of farmers and ranchers, assure food safety, promote agricultural trade and production, protect natural resources, and foster communities for the general public (USDA, 2012).

One such unit is the Arthropod-Borne Animal Disease Research Unit (ABADRU), in Manhattan, Kansas, which is part of the Agricultural Research Service (ARS) and considered to

be the largest agricultural research organization in the world (USDA ARS, 2010a). Agricultural Research Service (ARS) is the principal scientific research agency of the USDA (USDA ARS, 2010a). The ABADRU recently relocated to Manhattan, Kansas from Laramie, Wyoming to enable researchers to work collaboratively with Kansas State University (KSU) researchers at the Biosecurity Research Institute (BRI) (USDA ARS, 2010a). The ABADRU studies animal – diseases transmitted by arthropods – primarily biting midges and mosquitoes. Furthermore, the ABADRU develops diagnostic tools, vaccines, and other technologies to protect human and animal health (USDA ARS, 2010a). Researchers study the molecular biology of these diseases, the insects that spread them, the epidemiology of these diseases, and how the disease affects humans and animals (USDA ARS, 2010a). Diseases of particular interest include exotic and domestic strains of Bluetongue virus (BTV), Vesicular Stomatitis virus (VSV), and Epizootic Hemorrhagic Disease virus (EHDV), as well as wild type and vaccine strains of Rift Valley fever virus (RVFV) (USDA ARS, 2010a). The mission of the ABADRU is to solve major endemic, emerging, and exotic arthropod-borne disease problems in U.S. livestock (USDA ARS, 2010a).

The ABADRU is housed in the Center for Grain and Animal Health Research (CGAHR), in Manhattan, Kansas just off the K-State University campus. The Center for Grain and Animal Health Research was established in 1919 and is the only USDA research laboratory in Kansas (USDA ARS, 2010b). The Center for Grain and Animal Health Research (CGAHR) is made up of five research units (USDA ARS, 2010b): The Engineering and Wind Erosion Research Unit (EWERU); which develops technology to measure and reduce wind erosion, the Grain Quality & Structure Research Unit (GQSRU) that investigates relationships between physical and chemical attributes and end-use quality for various products and develops rapid and precise predictive tests; the Hard Winter Wheat Genetics Research Unit (HWWGRU) which

finds and provides new genetic material to address hard winter wheat problems including insect pests, diseases and abiotic stresses; and, finally, the Stored Product Insect Research Unit (SPIRU), which develops new knowledge and methods for controlling insect pests in grain and food products (USDA ARS, 2010b).

Student Activities

A great deal of time was devoted to learning and creating educational materials to ensure laboratory biosafety. This was achieved through comprehension of the occupational risks and hazards associated with working in a BSL-2 laboratory, and the desired knowledge senior researchers want their workers to know. Each USDA worker needs to complete a series of tutorials prior to beginning work in any position at the CGAHR facility. These presentations covered general overviews of safety, use of personal protective equipment (PPE), after hour-laboratory use, and chemical hygiene. None of these presentations were specific to working in a BSL-2 laboratory, which is the type of laboratory in which the ABADRU conducts its research. Additionally, there was no presentation specifying the types of viral agents the unit works with. Both of these types of tutorials were deemed necessary for the unit. Dr Scott McVey and the CGAHR's safety officer, JoAnne Gresens, believed these would be beneficial, especially to student or temporary workers who may not have a biological background.

Through literature research of the Centers for Disease Control and Prevention (CDC), the USDA's compiled safety information, and the completion of PubMed searches, I was able to put together a PowerPoint presentation for new workers. Also, while working in the laboratory with senior researchers and laboratory technicians, I accumulated a list of important facts workers wished they would have been told prior to working in a laboratory and those things they would

like future workers to know in order to provide a safe and efficient work environment. This PowerPoint is designed specifically for the ABADRU laboratories and can be viewed with presenter's notes in Appendix A.

The presentation is a much needed educational tool and is designed to provide needed safety information as well as to provide a knowledge base for future workers to build on and utilize in order to be proactive during their training period in the laboratories. The biosafety presentation is designed to be viewed by an individual or a classroom of new employees. At the completion of the presentation, the participant(s) will take the accompanying quiz. Pending a score of 80% or better, the participant will receive a certificate of completion for their employee training records.

While working at the ABADRU, I learned about the zoonotic agent Rift Valley fever virus and the current research being conducted to help protect the health of both humans and animals alike (Chapters 1 & 2). This information was obtained through review of published literature, discussions with ABADRU staff, and presentations attended. During discussions with my mentors, it was decided an additional educational PowerPoint presentation providing an undergrad biology level overview of the two most commonly researched viruses at ABADRU, Bluetongue virus and Rift Valley fever virus, would also be a useful tool for new employees. The idea is to give employees with limited biology or virology background a basic understanding of what the viruses are and why the ABADRU's research on them is important. Additionally, it was thought if workers have a better understanding of the research being conducted, they may feel more a part of the unit and its success. Also, participants could then have a clear understanding of the health impact and risks associated with working with these viruses, thereby instilling a self-vigilance that would increase the safety and decrease possible health risks to

themselves and others in the laboratories. This educational PowerPoint presentation was constructed through the utilization of literature review, textbooks, ABADRU staff, and current publications. It can also be viewed with presenter's notes in Appendix B. By finishing the accompanying quiz, a certificate of completion will be added to each individual's employee records.

During the course of the field experience at the ABADRU, I was able to participate and observe in a series of research projects in one of the BSL-2 laboratories. Through these projects, I was able to see and experience laboratory protocols and procedures. Many of the projects involved mice and were focused on different strains of *Culicoides sonorensis*, which are raised at the CGHAR facility and utilize the mice for blood meals. The purpose of these projects were to gauge the immunologic response of the mice from the feedings by collecting blood, bone marrow, lymph tissue, spleens, and the skin from the feeding sites. These collected samples later underwent ELISA to detect the cells and antibodies produced as a response to the feeding. Throughout the course of these projects, I observed the study process and considered methods or procedures in the laboratory which could be addressed in order to decrease occupational hazards associated with infectious and zoonotic agents. These observations can be found in Appendix C.

In the late spring of 2012, the ABADRU received a P50 Microdose NeedleFree Injection System© to incorporate into their tool box. It was hypothesized that this device, or one like it, can be used to administer modified live vaccines (such as MP-12) or the live Rift Valley fever virus in a controlled research facility. The idea was that the device would benefit the ABADRU in three ways: 1) Increase worker safety by reducing the risk of accidental needle sticks from traditional needle and syringe administration, 2) Reduce the need to raise and maintain *Culicoides sonorensis* (midge) strains for research, and 3) Possibly improve the immunologic

response from vaccines. This thought process was sound, considering the risk of laboratory acquired infections (LAI) is always a concern when working with infectious (blood borne) materials and agents (Chapter 3). The use of a needle-free device may decrease unnecessary exposures in a biological laboratory. Additionally, the literature has demonstrated that the use of devices like the P50 Microdose NeedleFree Injection System© improves the immunologic response of some vaccines when given transdermally. This is believed to occur because of the antigen-presenting cells that can be found in this location. Moreover, this has also been shown to occur with smaller doses of vaccine antigen. Lastly, midges bite into the epidermis and superficial dermis to receive a blood meal and in so doing transmit virus; needle-free injection systems may be able to simulate the transmission method of these arthropod vectors.

Despite the hypothetical benefits, the safety of using such a device needs to be addressed with the goal of keeping the risk of LAIs low, especially if they can be avoided. Hence, another project was conducted during my field experience involving preliminary assessment of the biosafety of needle-free intradermal delivery of zoonotic vaccines and agents. One of the main concerns of using a needle-free device was the production of an aerosol or a splatter that could cause unwanted exposures to both administrator and animal. Therefore, Dr Mark Ruder, Dr McVey, and I collaborated to construct a project to evaluate the needle-free device's safety and set baseline data for future comparative evaluations at the ABADRU (see Chapter 5 for study report). While working with the ABADRU, I had an opportunity to participate in a field trip to the United States-Mexico Border Port of Entry (USPOE) in Santa Teresa, New Mexico with the Frontier program. The Frontier program is a joint program with Kansas State University (KSU) and New Mexico State University (NMSU), which includes interdisciplinary research for the historical studies of border security and trade policy. The trip involved students and

professionals from KSU, NMSU, Georgia Tech University, Minnesota, and companies such as Cargill. To give an overview of the trip, we first met with Dr Jason Ackleson to discuss the occurrence of border control interdiction and trade security. We then attended a round table discussion at the Immigration Policy and Human Rights: Perspective from Border Communities Conference in the Corbett Student Union, NMSU.

Following this session, we caravanned to the USPOE at Santa Teresa, New Mexico and met with the New Mexico Border Authority, U.S. Customs and Border Protection (USCBP), and a Food and Drug Administration (FDA) inspector. With these individuals, we discussed inspection of pedestrian traffic and commercial/non-commercial traffic. Additionally we discussed how freight trucks are selected, inspected, processed, and the protocol for passing/failing trucks in attempt to safe guard the U.S. Agriculture system as well as U.S. public health. We then met with the Division Director of New Mexico Department of Agriculture, at the Chihuahua Regional Cattle Union Livestock Facilities and discussed the logistics and procedures the USDA veterinarian and staff conducts to inspect and certify health of livestock, mainly cattle, wanting to be brought into the U.S. This trip underlined the real life activities, issues, and actions, which occur on a daily basis to protect and defend this nation's public health and food supply. More complete details of this trip are summarized in Appendix C.

All in all, this field experience resulted in numerous personal and academic gains. I was able to utilize knowledge gained from previously related classes. I gained exposure and understanding of the risks and hazards of zoonotic disease research, especially as it relates to attenuated Rift Valley fever virus (MP-12) in a Biosafety Level (BSL)-2 Research Facility. I achieved a working understanding of disciplines that directly protect public and animal health. Finally, I was able to achieve an understanding of the type of procedures that should be in place

to protect occupational biosafety and public health as well as decrease hazards to workers in the laboratory and research setting through practical application and my border experience. This was an invaluable experience and has deepened my views on the importance of public health and veterinary medicine.

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Chapter 5 - Preliminary Assessment of Biosafety for Needle-free Intradermal Delivery of Zoonotic Vaccines and Agents

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Abstract

This preliminary trial was designed to evaluate the biosafety for the P50 Microdose NeedleFree Injection System©. The purpose was to assess occupational biosafety and hazards to the administrator for the use of Rift Valley fever virus vaccine in a bioresearch laboratory. The assessment was conducted through the subjective use of visual analogue scales, overall impressions of pooling and aerosolization and the objective measurements of wetness, and surface splatters at the injection site. The objective of this study was to characterize potential risks to the user of the P50 Microdose NeedleFree Injection System© for intradermal injection of infectious agents or modified live vaccines in biosafety level (BSL) 2 and BSL-3 laboratories at the Arthropod-Borne Animal Disease Research Unit (ABADRU) as well as to evaluate differences in surface splatter and wetness for the test dose and pressure combinations.

Introduction

Occupational biosafety is an important consideration for laboratory workers and veterinarians working in bioresearch laboratories in general, but increased caution needs to be used when working with zoonotic agents (CDC, 2009; Singh, 2009; Sulkin & Pike, 1951). Vigilant safety is needed during handling, storage, and management of these agents in the laboratory setting (Miller, 2008; Pedrosa & Cardoso, 2011; Pike, 1979). Agents commonly studied at the Arthropod-Borne Animal Disease Research Unit (ABADRU) include domestic and

exotic strains of Bluetongue virus (BTV), Epizootic Hemorrhagic Disease virus (EHDV), Vesicular Stomatitis virus (VSV), and wild type and vaccine strains of the zoonotic Rift Valley fever virus (RVFV) (USDA, 2010). All of these viruses have an arthropod vector that transmits the virus during the blood feeding from mammals (CDC, 2009; Mullen & Durden, 2009).

Traditional arthropod research involves the raising and rearing of insect vectors for study purposes (CDC, 2009). This can be tedious and encompass unique containment challenges for researchers (CDC, 2009). Nevertheless, it is considered a necessary process because the arthropod vectors feed in a way that allows transmission of the virus and elicits an immunologic response by the host (NICD, 2011). For example, midges bite into the epidermis and superficial dermis to receive a blood meal and in so doing transmit virus (CDC, 2009; NICD, 2011). Recently, it has been hypothesized that the use of needle-free injection systems may be able to simulate the transmission method of arthropod vectors. If effective, this would allow research to be conducted without the need of arthropods. Furthermore, it would allow the delivery of vaccinations in a manner similar to infection method. It has already been established that needle-free intradermal administration of modified live vaccines elicits an appropriate immunologic response (Chen & Payne, 2002; Lambert & Laurent, 2008).

Vaccinations are typically given via injection of a vaccine formulation using a needle and syringe (Deng, *et al*, 2012). The skin is considered a sensitive immune organ and has been determined to be a practical site of vaccination due to antigen-presenting cells present at the dermis and epidermis (Chen & Payne, 2002; Lambert & Laurent, 2008). These data suggests that intradermal or transdermal delivery of vaccines, rather than standard intramuscular (IM) or subcutaneous (SQ) administration, may be more efficient and induce protective immune

responses with smaller amounts of vaccine antigen (Chen & Payne, 2002; Lambert & Laurent, 2008).

Unfortunately, intradermal injections using needle and syringe are technically challenging and safety concerns do exist (Deng, *et al*, 2012). Additionally, multiple injection sites are often required because the injection site volume is limited due to the strain on the cells at the site (Chen & Payne, 2002). There is also the risk of depositing more of the agent in the various non-target layers of dermis or the possibility of bypassing the dermis and injecting subcutaneously (Chen & Payne, 2002). These variables may impact consistency in delivery, resulting in variation in the host's immunological response to the administered agent.

Needle-based systems always present the risk of accidentally puncturing the administrator or assistant at the time of administration (Miller, & Pisani, 1999; Mitragotri, 2005; Ekwueme, *et al*, 2002). This poses a risk of directly injecting human workers with a modified live agent or blood-borne pathogens, which represents a major occupational health concern (Deng, *et al*, 2011; Ekwueme, *et al*, 2002). An alternative to the traditional needle and syringe that has been considered safer and possibly more efficient are needle-free devices, which delivers solutions with CO₂ pressure (Clements, *et al*, 2008; Deng, *et al*, 2011). This type of device is gaining popularity in the swine industry for its ease of use, effective delivery, and desired immunologic response (Chase, *et al*, 2008). Additionally, the device allows for the agent to be distributed through the dermal layer triggering a more efficient immunologic response in animals (Chase, *et al*, 2008; Ekwueme, *et al*, 2002).

Human medicine, there is a push to transition to needle-free devices in order to reduce the direct health-care and societal costs resulting from needlestick injuries, although the financial benefit is difficult to quantify (Miller, & Pisani, 1999; Ekwueme, *et al*, 2002). It is estimated

that there are more than 1.3 million human deaths that occur globally and a \$535 million loss annually due to current unsafe injection practices (Miller, & Pisani, 1999). Devices for needle-free techniques to deliver agents and vaccines are continually being developed (Mitragotri, 2005). As the market for needle-free delivery systems increases, it is highly likely more and more devices will be made available.

Other researchers have used skin models to evaluate cosmetic treatments, wound healing, and the evaluation of needle-free transcutaneous delivery of drugs and vaccines in both humans and animals (Schramm, *et al*, 2004; Deng, *et al*, 2012). Real animal skin likely provides the best model when evaluating the occupational risk associated with using needle-free administration of vaccines and agents in the field and in the laboratory. Needle-free devices may be a safer alternative to the needle and syringe in the research trials of BTV, EHDV, VSV, and RVFV. These devices may be well-suited to deliver vaccines or potentially live agents during experimental infections (Zehring, & Kristensen, 2009). Still, there are risks when working with these agents: One concern is the aerosolization of the agent at time of administration (Zehring, & Kristensen, 2009).

It is documented that humans can become infected with RVFV by mosquito bite or more commonly, by contact with virus contaminated blood or through inhalation of virus in aerosols originating from infected livestock (Mullen & Durden, 2002; Mullen & Durden, 2009). There have been reports of laboratory-acquired infection (LAI) cases of Rift Valley fever contracted through aerosols (Francis & Magill, 1935; Pedrosa & Cardoso, 2011; Pike, 1979; Schwentker & Rivers, 1934; Strauss & Strauss, 2008). Moreover, some researchers have suggested that the risk of aerosol generation by intradermal needle-free delivery devices needs to be examined (Zehring, & Kristensen, 2009). It is possible devices with needles might be more appropriate for

vaccines than alternative methods, which generate an aerosol (Clements, *et al*, 2008; Zehrung, & Kristensen, 2009). The primary objective of this study was to characterize potential risks to the user of the P50 Microdose NeedleFree Injection System© for intradermal injection of infectious agents or modified live vaccines in biosafety level (BSL) 2 and BSL-3 laboratories at the Arthropod-Borne Animal Disease Research Unit (ABADRU). An additional objective was to evaluate differences in surface splatter and wetness for the test dose and pressure combinations.

Materials and Methods

In this study, donated Boer goat's (*Capra hircus*) forelimbs with hide intact were received from the Animal Research Services (ARS) at Kansas State University (KSU). Limbs were stored in a sealed bag at -20°C. They were thawed at room temperature less than 24 hours prior to use. Hair was shaved at the injection sites hair clippers (#40 blade). The P50 Microdose NeedleFree Injection System©¹ uses compressed CO₂ as a power source (Figure 5.1). Adjustable dose settings for the P50 Microdose include 0.1-0.5 ml via a standardized 0.27 mm orifice with the ability to deliver transdermal to intramuscular injections based on the size of the animal, according to the manufacturer.

In order to determine the effectiveness of dose administration, we utilized two easily visualized compounds, a 5% Crystal Violet dye solution and a Germ Juice® solution (a fluorescent dye visualized with a black light). These solutions were drawn up in to 10 mL Luer-lock syringes to be attached to device. Doses and pressures for this preliminary evaluation were 50 µL/100 psi, 100 µL/90 psi, and 250 µL/90 psi for each respective combination. These dose/pressure combinations were based on early data in which various dose/pressure

¹ Pulse NeedleFree Systems, Inc. 8210 Marshal Drive, Lenexa, KS. 66214

combinations were tested on flank skin from the same goat hide. These combinations were chosen because higher pressures tested at these same selected doses resulted in intramuscular injections and when dose volumes were increased splatter was seen at all tested pressures. The device was primed by connecting a 10 mL syringe of the respective solution to the device. Next, the trigger was pulled, with the device's orifice being pressed into the open rubber stopper of a 500 mL Pyrex glass bottle, until a consistent stream was seen. Injection sites were marked on the goat limbs with a fine tipped marker to allow visualization of the injection site as well as to signify the appropriate marker for measuring observed wetness and surface splatter. The tip of the injection device was wiped with a paper towel in between injections to ensure no carry over wetness/ solution from the previous injection. To administer a dose, the device was held perpendicular to the skin with the trigger pulled and then pressed firmly against the skin (this ensured the same pressure for delivery of dose) (Figure 5.2).

Post injection, the sites were photographed. Next, any observed wetness or surface splatter was measured in millimeters (mm). Sites were observed for pooling and noted if aerosolization was seen during the injection. In half of the injections, a construction paper cone, white for the Crystal Violet and black for the Germ Juice® solutions [Figure 5.3] was modified to fit around the device. This was done in an attempt to collect and allow visualization of droplets that resulted from the delivery of the solution for each dose/pressure combination. Any droplets visualized indicated an affirmative for aerosolization. After initial data was collected, a #20 scalpel blade was used to cut into the injection site (marked site) at random to visually observe if the dye penetrated the dermis or further. A picture of the dissected site was taken. These steps were repeated for each of the 6 injection sites per dose/psi combination per limb. An additional comparison was conducted during this trial using a traditional intradermal syringe and

the 5% Crystal Violet solution. The syringe was used to administer six intradermal injections to objectively observe any difference between the methods. The injection sites were also dissected and photographed. The limbs were disposed via biohazard bags and taken to an incinerator at the Diagnostic Laboratory at Kansas State University (KSU). The work area was cleaned using 70% ethanol, paper towels, and Clorox wipes. The device was disassembled and cleaned with 70% ethanol to ensure complete cleaning.

Data for statistical analysis were entered in to Microsoft Excel, 2010. Means for the wetness and surface splatter measurements were calculated. The pooling and aerosolization data was ordinal and percentages were calculated for each, respectively.

Results

Results for the assessment of the P50 Microdose NeedleFree Injection System© with the Germ Juice® solution can be seen in Table 5.1. A mean wetness diameter of 23.2 mm was observed for the 50 µL/100 psi combination (Table 5.2). The presence of pooling was observed 0.0% of the time at this dose pressure combination. Surface splatter provided a mean radius of 16.7 mm (Table 5.2). The visualization of aerosolization was seen only 33.3% of the time. For the 100 µL/ 90 psi combination, a mean wetness diameter of 27.2 mm was observed (Table 5.2). Pooling was seen 66.7% of the time. The presence of surface splatter was seen at a mean radius of 24 mm (Table 5.2). Aerosolization was seen 100% of the time at this dose/pressure combination. The 250 µL/90 psi combination provided a mean wetness diameter of 38.5 mm. The presence of pooling was seen 83.3% of the time. The mean surface splatter observed at this combination provided a mean radius of 22.7 mm, which is lower compared to the 100 µL/90 psi combination; however, the droplets observed on the cone during the measurement of

aerosolization for the 250 μ L were much larger than the 100 μ L dose (Table 5.2). The aerosolization for the 250 μ L dose was also 100%. The wetness and surface splatter measurements can be seen for each dose/pressure combination in Figure 5.4 and Figure 5.5. Additionally, the pooling and aerosolization results can be seen for each dose/pressure combination in Figure 5.6 and Figure 5.7.

Table 5.1 summarizes the results for the assessment of the injection device with the Crystal Violet. The 50 μ L/100 psi provided a mean wetness diameter of 25.5 mm (Table 5.2). The presence of pooling was seen 50.0% of the time at this dose/pressure combination, while the mean surface splatter radius was measured at 0.0 mm (Table 5.2). The aerosolization was observed 0.0% of the time at this dose. The 100 μ L/90 psi combination demonstrated a mean wetness diameter of 25.8 mm. The pooling increased to 83.3% of the time. Surface splatter increased considerably from the first dose/pressure combination and provided a mean radius of 19.3 mm (Table 5.2). Aerosolization also increased considerable and was seen 100% of the time at this combination. At the 250 μ L/90 psi dose combination, a mean wetness diameter of 45.5 mm was seen; almost double the previous dose (Table 5.2). The observed pooling decreased slightly and was seen at 66.7% of the time. Like with the Germ Juice® solution, the surface splatter at the 250 μ L/90 psi combination provided a decreased mean radius splatter of 14.7 mm, when compared to the lesser dose (Table 5.2). Additionally, aerosolization was also observed 100% of the time, with greater droplet seen on the cone compared to the less dose of 100 μ L (Figure 5.8). The wetness and surface splatter results can be seen for each dose/pressure combination in Figure 5.9 and Figure 5.10. And the pooling and aerosolization results can be seen for each dose/pressure combination in Figure 5.11 and Figure 5.12.

Discussion

With advances in technology, it is important to evaluate the use and safety of devices as well as consider their use in practical and safe applications. The present investigation was conducted to subjectively and objectively characterize potential risks to the user of the P50 Microdose NeedleFree Injection System© for intradermal injection of infectious agents or modified live vaccines in a BSL-2 laboratory. The device has been successfully used for subcutaneous and intramuscular injections in livestock in many clinical and laboratory settings. Intradermal or transdermal applications are of interest for research purposes.

The ability of the device to produce surface splatter, wetness, pooling, and an aerosol during administration of an injection is evident in these results (Figures 5.8, 5.13, & 5.14). There was wetness and pooling of injection solution regardless of dose/pressure combination seen in this project (Figures 5.15 & 5.16). During the study trial, there was observed droplet formation on most of the cones when used, the tip of the injection device, and the skin adjacent to the injection site (Table 5.1 and Figures 5.7 & 5.12). The wetness and pooling indicates that either the entire dose did not get delivered at the pressure setting used, meaning a high pressure should be used, or that there was a “leaking” of the solution out through the injection site, which questions the accuracy of delivering a specific dose (Figure 5.13). Some of the injection sites increased the diameter of the wetness even minutes post injection, making the “leaking” hypothesis likely. It is doubtful the pressure should have been higher in this study due to the observed subcutaneous and submuscularis delivery seen with some of the injection sites. There are other groups that claim their human devices have low visible moisture at the injection site with little to no visible flow (Gutierrez, *et al*, 2009).

For a direct injection site comparison during the trial, a traditional intradermal syringe was used. As can be seen in Figure 5.17, the injection sites were much smaller with no surface splatter, wetness, or aerosolization; however, a small amount of pooling was seen at removal of the needle. Additionally, on dissection a small bleb in the transdermal space was observed with no evidence of subcutaneous delivery (Figure 5.18). In the original protocol, depth of injection was going to be another data point; however, during the study it became evident depth measurements grossly could not be done with any consistency. It is possible a histological review of the injection site post injection may offer a more consistent and useful means of measuring depth and spread in the dermal layers.

The Germ Juice® solution offered a unique visualization during injection. A UV light allowed for fine surface splatter to be observed, which was not seen with the 5% Crystal Violet solution, especially at the 50 µL/100 psi injection sites (Figures 5.19 & 5.20). The low number of data points and the questionable normal distribution of the data for the injection dose/pressure combinations indicate that more data points should have been acquired.

The needle-free injection device produces a high pressure, high speed, narrow stream of a liquid that penetrates the skin (Chase, 2008; Giudice, 2006). This results in transdermal, sometimes subcutaneous (SQ) to intramuscular (IM), penetration of the injected solution. As can be seen in Figures 5.21 and 5.22, there is clear evidence of transdermal delivery of Crystal Violet solution in this study. However, the use of the single orifice to deliver the injection solution begs the question: what if the use of a multi-orificed tip provided a more uniform intradermal distribution of solution without SQ or IM penetration.

It is possible that the use of dead and previous frozen tissue could have impacted the results seen here. The moisture content and tissue response was different from that of live, fresh

tissue. These variables could have increased or possibly decreased the amount of droplets and wetness observed. Therefore, it is recommended that future research be conducted on fresh or live tissues for better evaluation. Additionally, other devices as well as pressure and dose combinations should also be evaluated for their safety use and possible application in research settings.

Needle-free technology offers the benefits of fast injections compared with conventional needles and no needle disposal issues (Vishnu, *et al*, 2012; Zehrung & Kristensen, 2009). Due to data in this project, it is obvious there is a biosafety risk to the administrator when using this device as available for intradermal delivery of modified live vaccines or live zoonotic agents, such as Rift Valley fever virus (MP-12). At this point, it is difficult to precisely quantify the risk with certainty, but further method development should be conducted before this device is used for Rift Valley fever virus, even in a BSL-3 Ag laboratory.

Tables and Figures

Germ Juice® Solution								
Limb Location	Right Front	Data Points						Mean
Wetness (mm)	50 uL/100 psi	22	21	19	23*	27*	27*	23.2
Pooling (y/n)	50 uL/100 psi	0	0	0	0*	0*	0*	0.0%
Surface splatter (mm)	50 uL/100 psi	19	0	14	20*	22*	25*	16.7
Aerosolization (y/n)	50 uL/100 psi	.	.	.	0*	0*	1*	33.3%
Wetness (mm)	100 uL/90 psi	27	33	25	22*	28*	28*	27.2
Pooling (y/n)	100 uL/90 psi	1	0	1	1*	0*	1*	66.7%
Surface splatter (mm)	100 uL/90 psi	25	20	20	26*	24*	29*	24.0
Aerosolization (y/n)	100 uL/90 psi	.	.	.	1*	1*	1*	100.0%
Wetness (mm)	250 uL/90 psi	35	33	35	36*	40*	52*	38.5
Pooling (y/n)	250 uL/90 psi	1	0	1	1*	1*	1*	83.3%
Surface splatter (mm)	250 uL/90 psi	0	0	70	35*	31*	0*	22.7
Aerosolization (y/n)	250 uL/90 psi	.	.	.	1*	1*	1*	100.0%
Crystal Violet Solution								
Limb Location	Left Front	Data Points						Mean
Wetness (mm)	50 uL/100 psi	25	21	22	34*	25*	26*	25.5
Pooling (y/n)	50 uL/100 psi	0	1	0	1*	1*	0*	50.0%
Surface splatter (mm)	50 uL/100 psi	0	0	0	0*	0*	0*	0.0
Aerosolization (y/n)	50 uL/100 psi	.	.	.	0*	0*	0*	0.0%
Wetness (mm)	100 uL/90 psi	30	22	25	28*	24*	26*	26
Pooling (y/n)	100 uL/90 psi	0	1	1	1*	1*	1*	100.0%
Surface splatter (mm)	100 uL/90 psi	0	30	20	25*	20*	21*	25.8
Aerosolization (y/n)	100 uL/90 psi	.	.	.	1*	1*	1*	83.3%
Wetness (mm)	250 uL/90 psi	43	53	63	35*	28*	51*	45.5
Pooling (y/n)	250 uL/90 psi	1	0	1	1*	0*	1*	66.7%
Surface splatter (mm)	250 uL/90 psi	0	0	0	27*	24*	37*	14.7
Aerosolization (y/n)	250 uL/90 psi	.	.	.	1*	1*	1*	100.0%

*indicates cone use

Table 5-1: Intradermal P50 Device Evaluation of Germ Juice® and Crystal Violet solution

Germ Juice® Solution	
Wetness	x□
50 µL	23.2
100 µL	27.2
250 µL	38.5
Splatter	x□
50 µL	16.7
100 µL	24
250 µL	22.7
Crystal Violet Solution	
Wetness	x□
50 µL	25.5
100 µL	25.8
250 µL	45.5
Splatter	x□
50 µL	0.0
100 µL	19.3
250 µL	14.7

**Table 5-2: Means for the measured wetness and surface splatter for each dosing for both
Germ Juice® and Crystal Violet**



Figure 5-1: P50 Intradermal needle-free device with CO2 system



Figure 5-2: P50 Intradermal needle-free device being used to inject solution

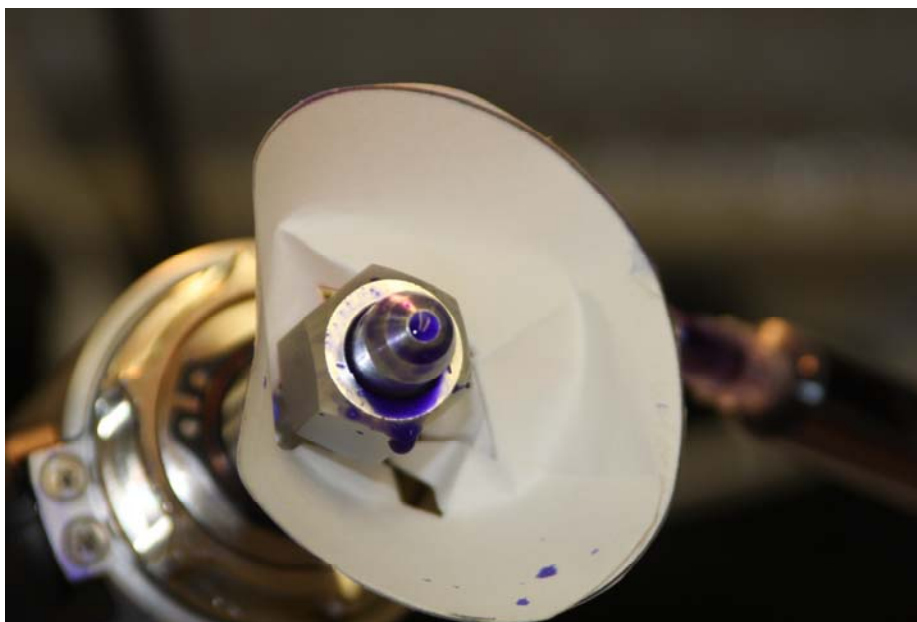


Figure 5-3: Cone attached to P50 Intradermal needle-free device

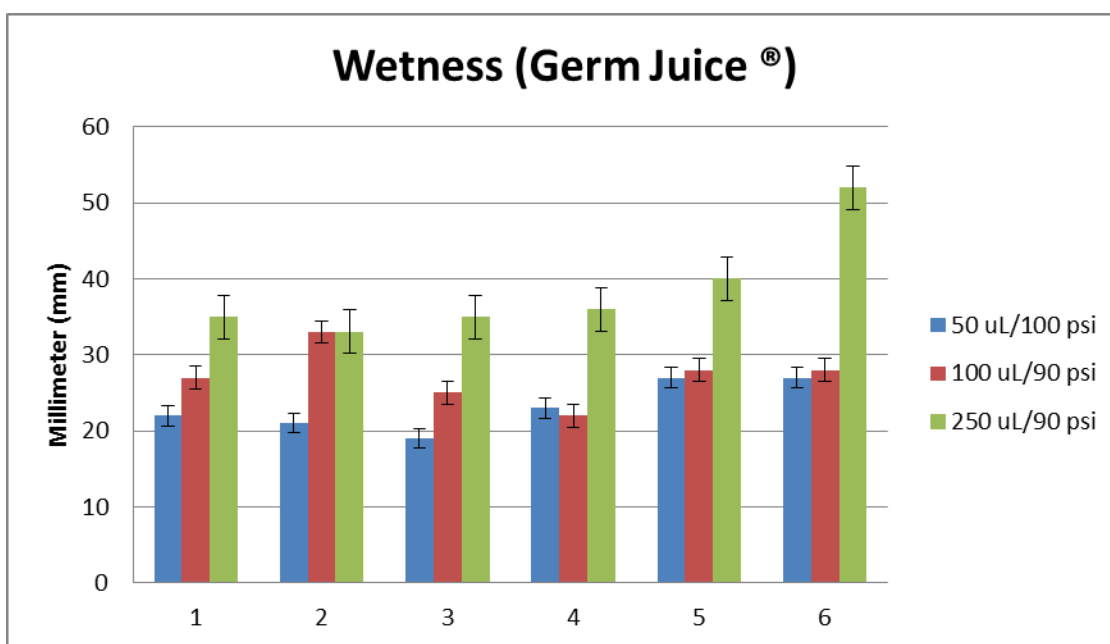


Figure 5-4: Graph illustrating the wetness observed for each dose/pressure combination for the Germ Juice[®] solution

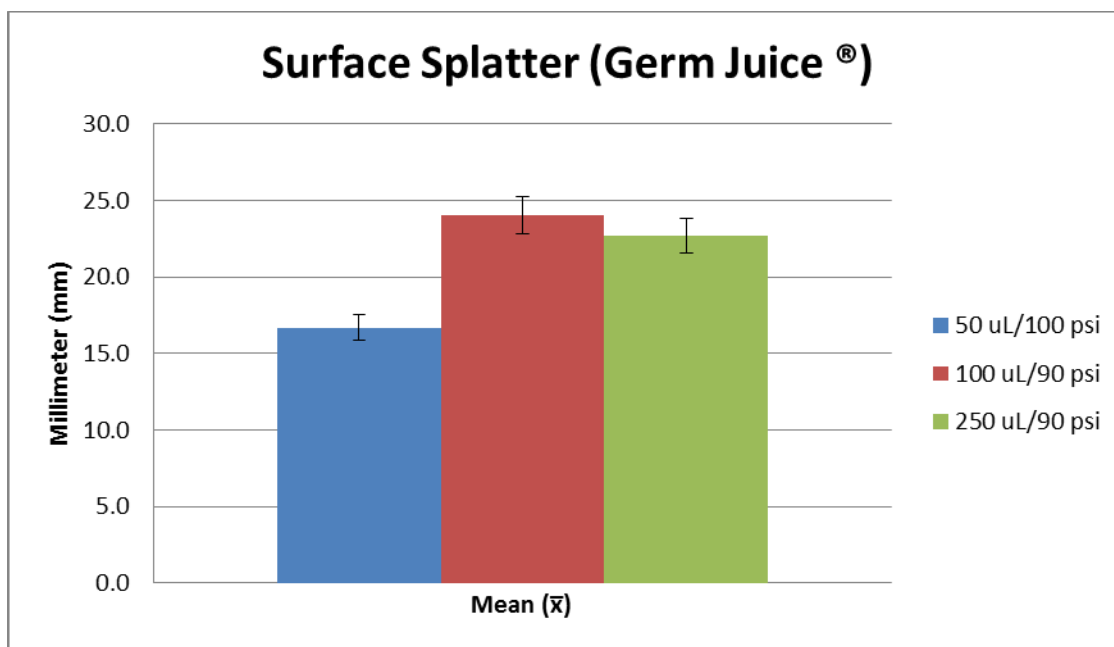


Figure 5-5: Graph illustrating the surface splatter for each dose/pressure combination for the Germ Juice® solution

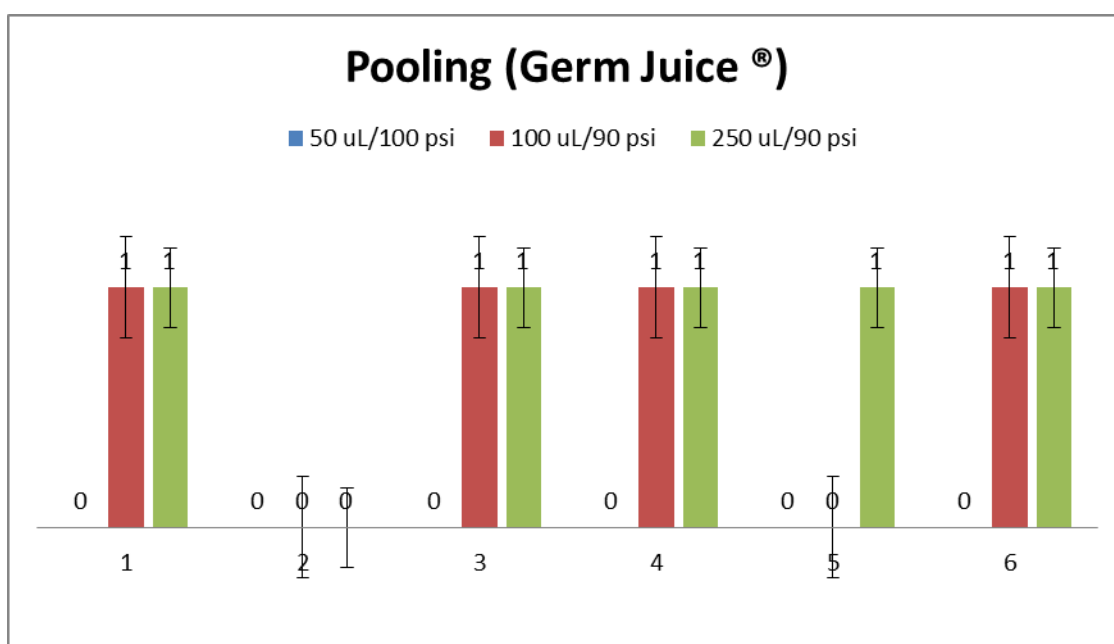


Figure 5-6: Graph demonstrating the observed pooling for each dose/pressure combination for the Germ Juice® solution

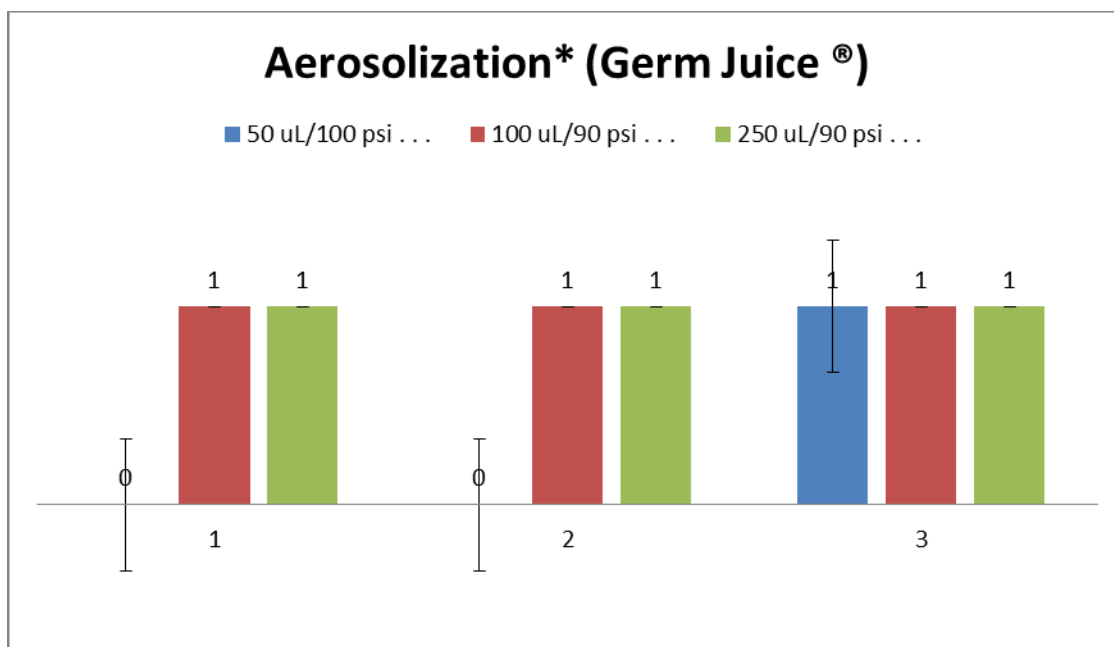


Figure 5-7: Graph demonstrating the aerosolization for each dose/pressure combination for the Germ Juice[®] solution (*indicates only three injections were evaluated)



Figure 5-8: Photographed cone during aerosolization observation at 250 μ L/90 psi

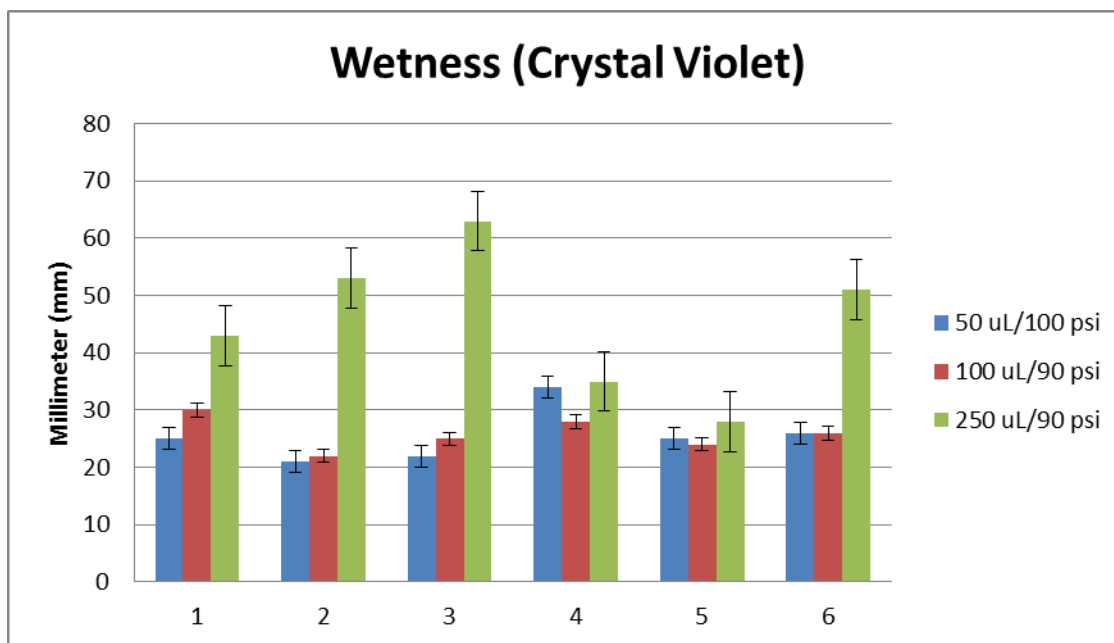


Figure 5-9: Graph illustrating the wetness observed for each dose/pressure combination for the Crystal Violet solution

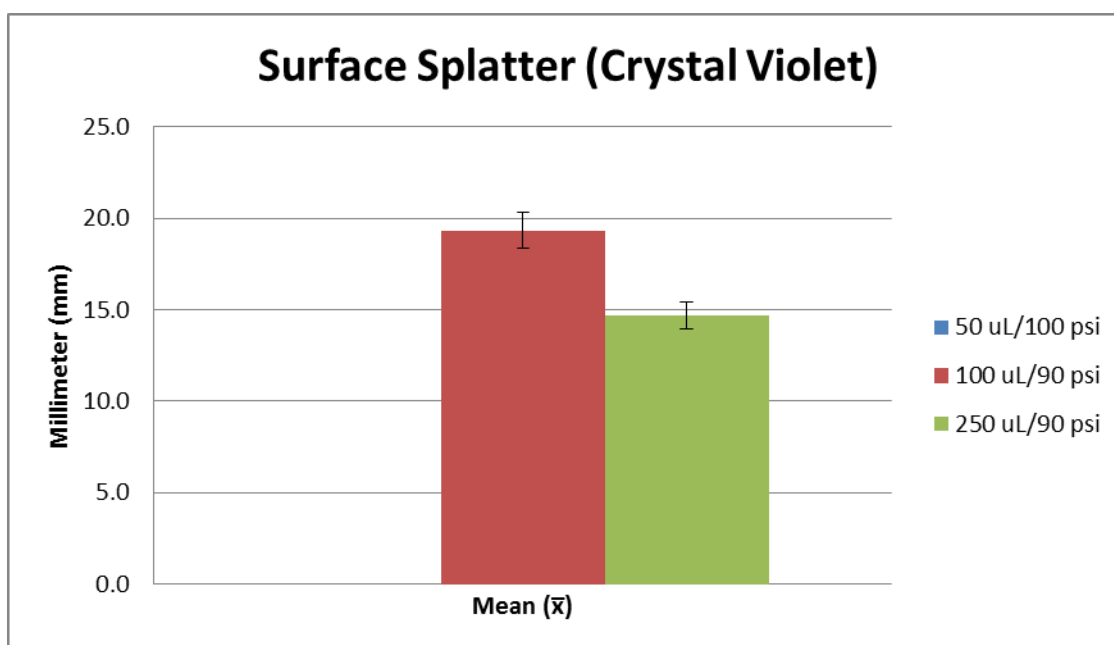


Figure 5-10: Graph illustrating the surface splatter for each dose/pressure combination for the Crystal Violet solution

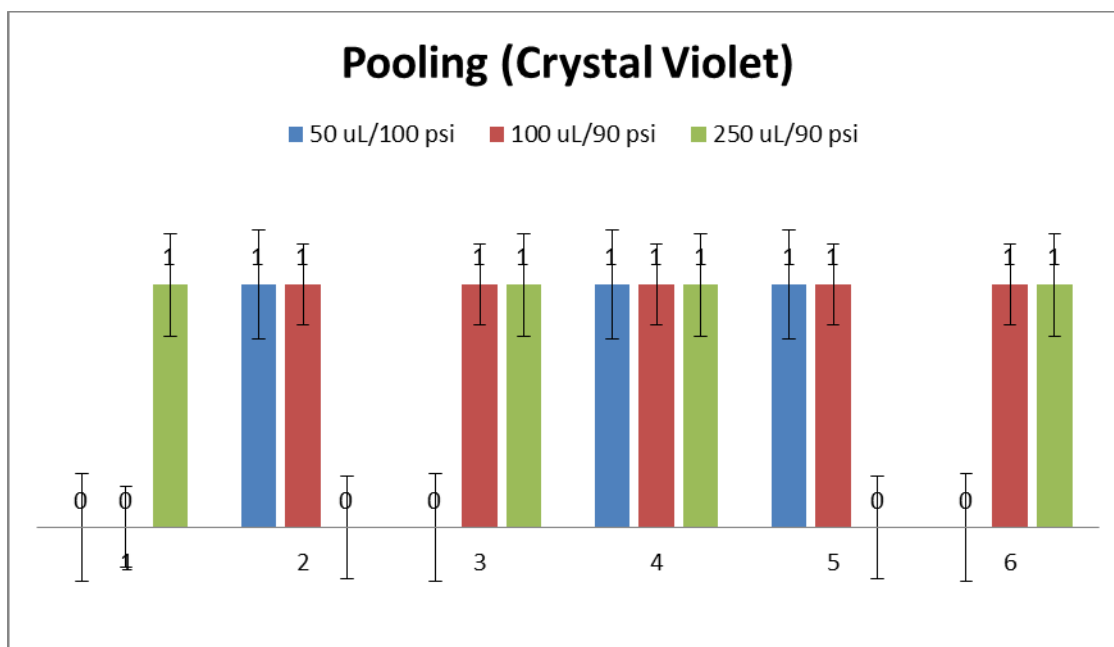


Figure 5-11: Graph demonstrating the observed pooling for each dose/pressure combination for the Crystal Violet

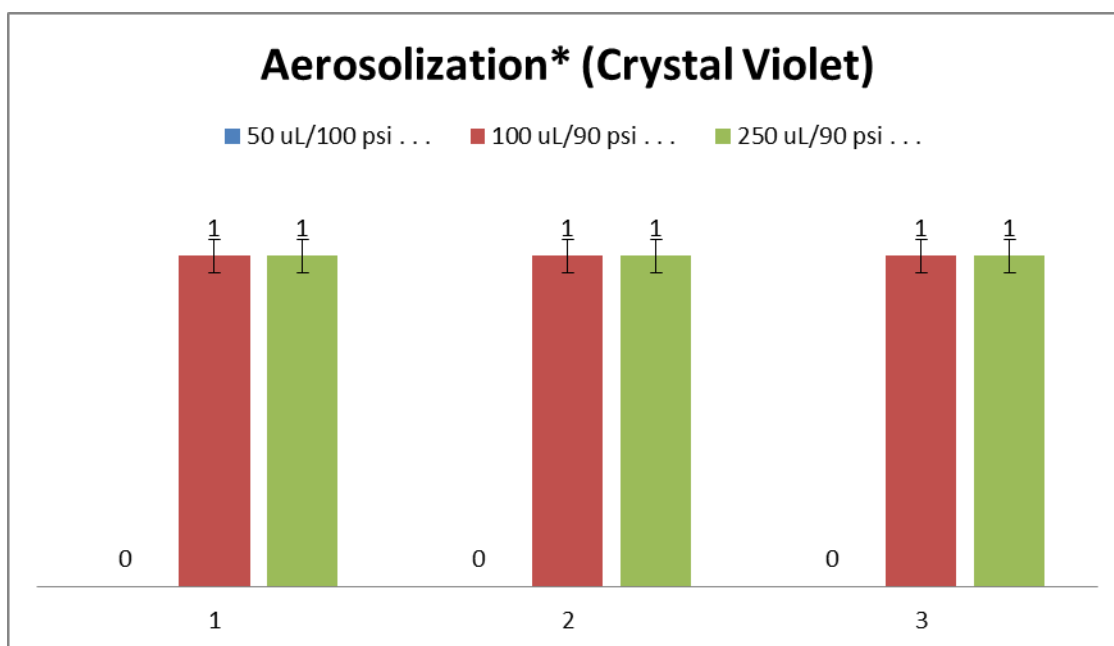


Figure 5-12: Graph demonstrating the aerosolization for each dose/pressure combination for the Crystal Violet solution (*indicates only three injections were evaluated)



Figure 5-13: Demonstration of wetness and surface splatter post 100 μ L injection of Crystal Violet



Figure 5-14: Demonstration of residue due to spray and aerosolization of Crystal Violet solution



Figure 5-15: Completed series of 50 µL injections of Crystal Violet

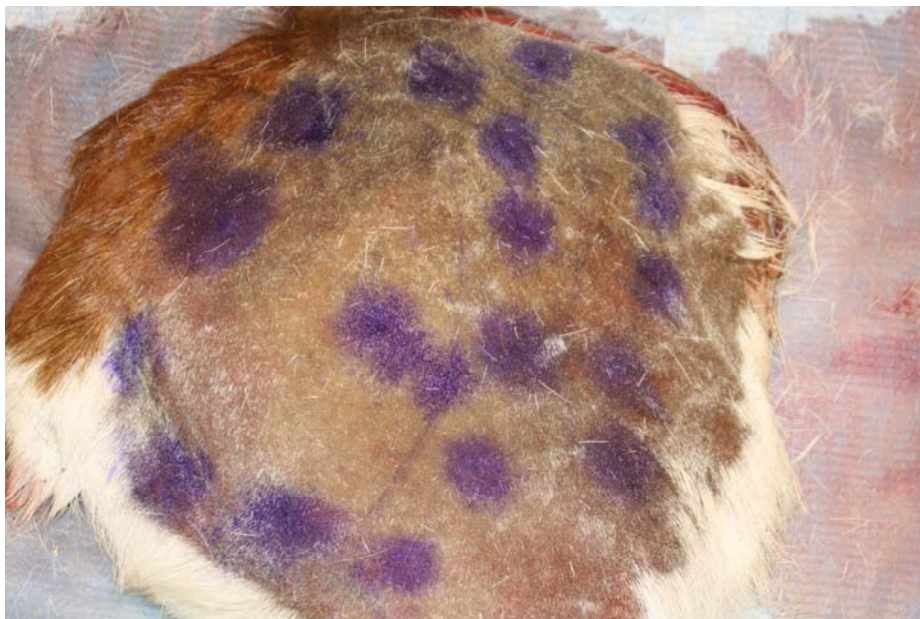


Figure 5-16: Completed injection series for Crystal Violet limb (similar pattern was conducted for Germ Juice® limb)

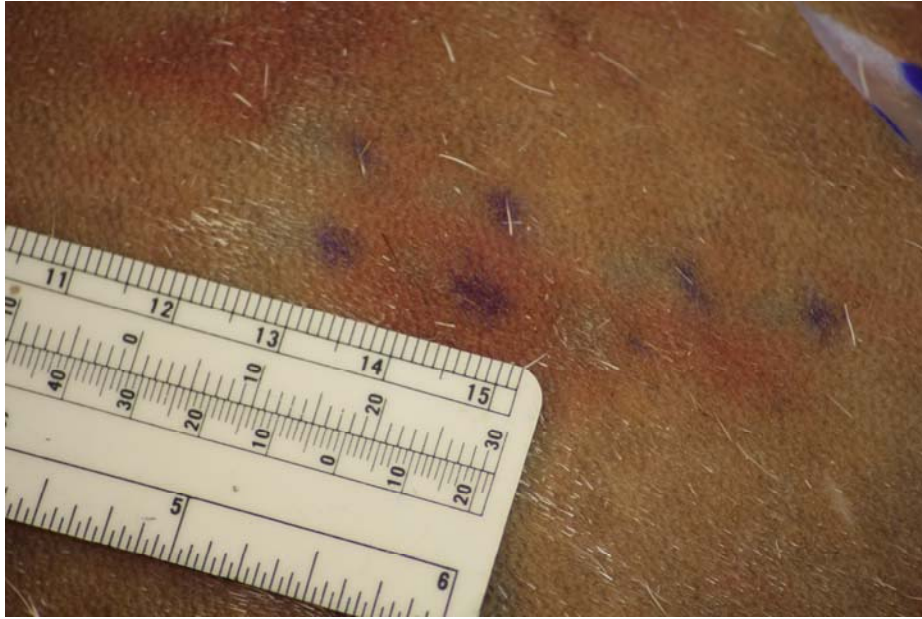


Figure 5-17: Example of traditional syringe and needle conducting six injections



Figure 5-18: Dissected injection sites for traditional needle and syringe



Figure 5-19: 50 μ L injection of Crystal Violet

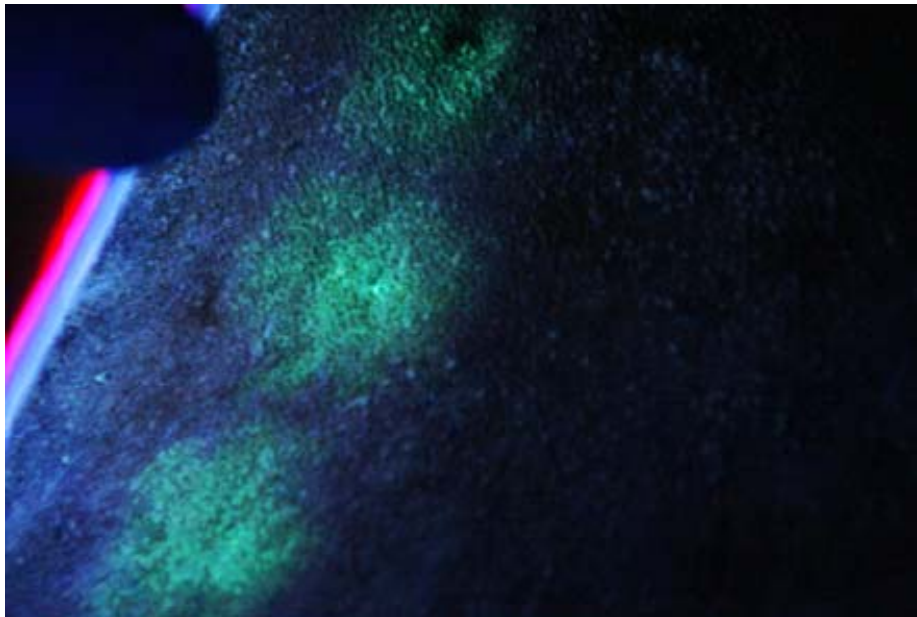


Figure 5-20: Surface splatter of a 50 μ L injection with Germ Juice®

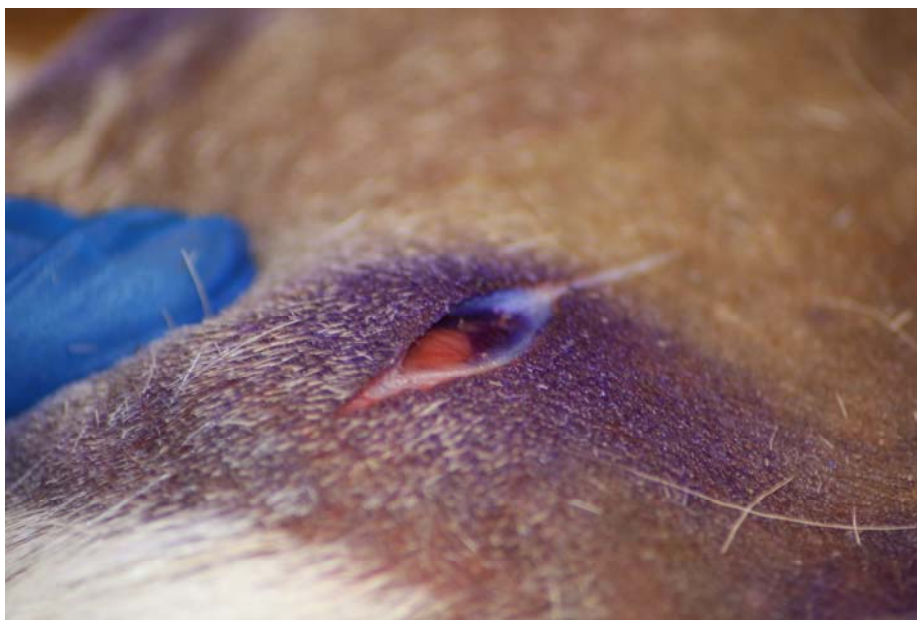


Figure 5-21: Dissected injection site for 100 μ L injection of Crystal Violet



Figure 5-22: Dissected injection site for 50 μ L injection of Crystal Violet demonstrating transdermal penetration

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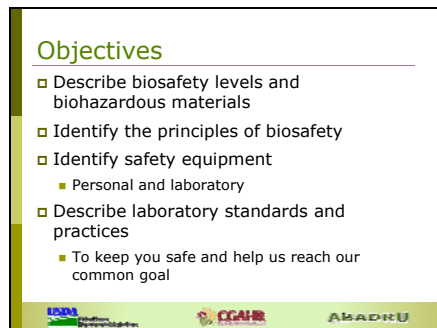
Appendix A – Arthropod-Borne Animal Disease Research Unit: BSL-2 Laboratory Biosafety Training Module

Slide 1



Hello and welcome to the Arthropod-Borne Animal Diseases Research Unit: BSL-2 Laboratory Biosafety Training Module here at the USDA Center for Grain and Animal Health Research (CGHAR).

Slide 2




The objectives of this presentation are to describe biosafety levels, and biohazardous materials, identify the principles of biosafety, identify safety equipment both personal and laboratory, and to describe laboratory standards and practices; to keep you safe and help us reach our common goal.

Slide 3

General Biosafety

- Who needs this training?

All individuals who work with or have access to biohazardous materials!



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I am sure you are asking yourself who needs this training?

And the answer is all individuals who work with or have access to biohazardous materials!! In other words, you.

Slide 4

Biosafety Levels (BSL)

- BSL-1: Student training/teaching labs
 - Agents are well-defined and characterized
 - Not known to cause disease
- BSL-2: Clinical/diagnostic labs
 - Agents are considered moderate-risk agents
 - Associated with human disease (varying severity)
 - Open bench
 - Standard microbiological techniques
 - Probability of splashes and aerosols is low

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Let's begin with a brief introduction to biosafety levels or BSLs....

BSL-1 laboratories are designed for student educational training and teaching labs. They are also used for working with well-defined and characterized agents not known to cause disease in normal healthy adults.

BSL-2 laboratories are designed for clinical, diagnostic purposes, and teaching laboratories working with indigenous moderate-risk agents present in the community and associated with human disease of varying severity. Work is conducted on an open bench with standard microbiological techniques, providing that the probability for splashes and aerosols is low. Lab personnel have specific training in handling pathogenic agents and are supervised by scientists competent in working with infectious agents and associated procedures.

Slide 5

Biosafety Levels (BSL)

- BSL-1: Student training/teaching labs
 - Agents are well-defined and characterized
 - Not known to cause disease
- BSL-2: Clinical/diagnostic labs
 - Agents are considered moderate-risk agents
 - Associated with human disease (varying severity)
 - Open bench
 - Standard microbiological techniques
 - Probability of splashes and aerosols is low

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Here at CGAHR we use BSL-1 and BSL-2 agents and this presentation will follow the standards set for those levels. We will focus on BSL-2 standards because BSL-2 builds on the protocols and standards set by BSL-1. However, to give you a big picture of the different biosafety levels we are going to define the BSL-3 and BSL-4.

Slide 6

Biosafety Levels (BSL)

- BSL-3: Research/production facilities
 - Agents may be indigenous or exotic
 - Associated with serious and potentially lethal infections
 - Potential for respiratory transmission
 - High transmissibility by aerosols
- BSL-4: Dangerous/exotic laboratories
 - Agents pose high individual risk of life-threatening disease
 - Vaccine or therapy may not be available

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BSL-3 laboratories are designed for clinical, teaching, research, or production facilities that work with indigenous or exotic agents, which may cause serious illness or potentially lethal infection. These agents may have the potential for respiratory transmission.

BSL-4 laboratories are designed for working with dangerous and exotic agents. These agents pose a high individual risk of life-threatening disease which may be transmitted via aerosol route and there is no available vaccine or therapy if there is accidental exposure.

Slide 7

Principles of Biosafety

- Fundamental objective:
 - Biological agent containment
- Purpose of the containment
 - Reduce/eliminate exposure
 - Laboratory & building personnel
 - Environment
 - Protect people from dangerous pathogens



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The fundamental objective of biosafety is to provide containment of potentially harmful biological agents. The purpose of containment is to reduce or eliminate exposure of laboratory personnel, other persons, as well as the outside environment.

Slide 8

Laboratory Practices

- Strict adherence
 - Standard microbiological practices and techniques
 - Laboratory biosafety manual
 - **READ** this manual prior to working
- Workers need to be...
 - Aware of potential hazards
 - Appropriately trained
 - Proficient in safe practices & techniques

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The laboratory biosafety manual is developed specifically for each independent laboratory in this facility. It identifies specific hazards in the laboratory, biosafety policies, and site specific standard operating procedures. The manual also identifies specific practices and procedures to minimize or eliminate these hazards.

All personnel in the laboratory are required to read the biosafety manual. Each person in the laboratory is responsible for the work being conducted in the laboratory. He/she should be trained and knowledgeable in specific techniques, safety procedures, and associated hazards. If ever you are uncertain, ask your supervising researcher.

Slide 9

Standard Operating Procedures

- Microbiological practices
 - Safe pipetting techniques
 - Procedures for handling sharps
 - Decontamination protocols
- Primary equipment barriers
 - Biological safety cabinet
 - Containment methods for safe centrifugation
 - Equipment certification




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Standard operating procedures or SOP includes the principal content of biosafety manuals. SOPs for microbiological practices include: safe pipetting techniques, procedures for handling sharps, and decontamination protocols. Standard operating procedures for use of primary equipment barriers include: working in a biological safety cabinet, containment methods for safe centrifugation, and procedures for equipment certification.

Slide 10

Laboratory Safety Standards

- Perform all procedures to minimize splashes and aerosols
 - Avoid working alone or after hours
 - Do **NOT** engage in "horseplay"
 - Avoid using cell phone in laboratory



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Some laboratory safety standards to keep in mind while working in ABADRU laboratories include performing all procedures in a manner to minimize splashes and aerosols. Additionally, avoid working alone or after hours in the laboratory. To aid in the prevention of accidents do not engage in horseplay. Lastly, for the sake of your attention span and the safety of those around you avoid using your cell phone in the lab this will reduce the risk of contamination and carelessness.

Slide 11

Common Sources of Exposure

- Accidental inoculation by needle/syringe
- Cuts/abrasions
 - Contaminated items & animal bites
- Inhalation of aerosols via work/accident
- Contact
 - Mucous membrane and contaminated surfaces
 - hands on surfaces
 - Oral ingestion
 - Inhalation/accident




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Some common sources of exposure in a BSL-2 laboratory include accidental inoculation by needle/syringe, cuts or abrasions through contaminated items or animal bites, inhalation of aerosols via work/accident, contact with mucous membrane and contaminated surface (ie hands or surfaces), oral ingestion and lastly through inhalation or accidental ingestion.

Slide 12

Safety Equipment

- Biological safety cabinet (BSC)
 - Containment of hazardous splashes, splatters, and/or aerosols
- Aerosols may be produced via
 - Pipetting, centrifuging, grinding, shaking, mixing, opening containers, harvesting infected tissues

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Safety equipment for all biosafety levels include biological safety cabinets or BSC, as well as enclosed containers, and engineering controls to minimize exposure to hazardous biological materials. The biological safety cabinet is the principle device to provide containment of hazardous splashes, splatters, and/or aerosols.

Aerosols can be generated from many different manipulations while working with infectious materials. You should always be working in the biological safety cabinet when working with infectious materials that can generate aerosols. Do your best to minimize aerosols.

Slide 13

Safety Equipment

- Primary barriers are designed to...
 - Protect laboratory personnel and environment
 - Prevent contamination of laboratory materials
- Primary barrier protectors

Primary barrier	Personnel	Product	Environment
Chemical Fumehood	X		
Class I Biosafety Cabinet	X		X
Class II Biosafety Cabinet	X	X	X
Class III Biosafety Cabinet	X	X	X


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Primary barriers are used to protect laboratory personnel, protect the environment, and prevent contamination of laboratory materials. There are several types of primary barrier protectors, these include as you can see on the left, chemical fume-hoods; which protect personnel, Class I biosafety cabinets which protects personnel and environment and Class II and III biosafety cabinets that protect personnel, products, and environment.

Slide 14

Safety Equipment Continued...

- Personal protective equipment (PPE)
 - Gloves
 - Change if contaminated or torn
 - Do **NOT** wash or reuse in the lab
 - Avoid** touching face or hair
 - Laboratory coats, gowns, sleeve covers, boots or shoe covers
 - Protect personal clothing
 - Reduce risk of self contamination/exposure
 - Wear closed-toe shoes and long pants



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Personal protective equipment or PPE is used to protect individuals. Use of PPE is required whenever there is risk of exposure to a hazardous material. PPE includes gloves, keep in mind if you have Latex allergies alternatives will be made available to you. When using gloves you need to change them if they become contaminated or torn and do NOT ever wash or reuse gloves in the

laboratory, there are plenty more for you to use. Additionally, while wearing gloves avoid touching your face or hair or other things that may be handled without gloves.

Other PPE includes laboratory coats, gowns, sleeve covers, boots or shoe covers which are worn to protect personal clothing and reduce self-contamination or exposure. Do not wear open toed shoes or shorts. If you plan to enter the laboratory you must wear closed-toe shoes and long pants.

Slide 15

Safety Equipment Continued...

- Personal protective equipment (PPE)
 - Safety glasses/goggles or face shields
 - When splashes with infectious material may occur
 - Wearing contact lenses
 - Eye, face, and respiratory protection
 - Manipulation of infectious materials
- Used with BSCs or as primary barrier



Protective eyewear, such as safety glasses, goggles, or face shields should be worn when splashes of infectious materials may occur or if laboratory personnel wear contact lenses. Eye, face, and respiratory protection should be worn if there is potential of aerosols or splashes generated when manipulating infectious materials.

All the PPE listed here can be used with biosafety cabinets or as the primary barriers. However, PPE will vary depending on the level of work conducted, amount being used, and type of biological materials used in the laboratory. This is because not all biohazardous agents are of the same level of virulence, pathogenicity, or transmissibility.

Slide 16

Safety Equipment Continued...

- Removal of protective clothing
 - Break room
 - Administrative offices
 - Restrooms
- Protective clothing disposal
 - Do **NOT** take home to wash
 - Laundered at CGAHR
 - Disposable coats



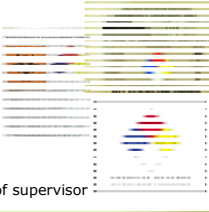
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Protective clothing worn in laboratory should be removed before leaving for non-laboratory areas. Examples of these areas include the break room, administrative offices, and restrooms. Protective clothing should also be disposed of properly. For example, laboratory coats are laundered by CGAHR; do not take them home to wash. All disposable lab coats should be disposed of with biological waste.

Slide 17

BSL-2 Standard Practices

- **READ** signs at the entrance to the laboratory
 - Restricted access
- Entry door contains
 - Authorized personnel
 - Biohazard signs
 - Agents present in lab
 - Contact information of supervisor



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Biohazard information is present on the entry door to the laboratory where infectious agents are present. Please read these signs.

The entry door: contains information pertaining to authorization (if you are uncertain of your authorization make sure to ask). The door signs also include biohazard signs, information of agents present in the lab, and the contact information of the laboratory supervisor. Other information that may be included is: chemical and/ or radioactive hazards, restricted access, safety information (PPE, respirator, immunization, and medical surveillance), high voltage area, magnetic field area, laser hazard, UV hazard.



In any area where door signs indicate that work is conducted with infectious organisms or other hazardous materials, equipment such as centrifuges, incubators, refrigerators, freezers, and analytical equipment should be viewed as "*potentially*" contaminated unless specifically known to be free of contamination. In contrast, if no signs are present identifying a hazard then the area is considered to be free of the

hazard.

Slide 18

BSL-2 Standard Practices

- Biohazardous materials include
 - Infectious organisms
 - Bacteria, viruses, fungi, parasites, prions, etc...
 - Diagnostic specimens
 - Recombinant DNA
 - Viral vectors, gene therapy, cloning
 - Cell cultures
 - Cells, tissues, blood, body fluids
 - Animal or plant cells which may contain pathogens





The biohazardous materials you may come in contact with at ABADRU include infectious organisms, such as bacteria, viruses, fungi, parasites, prions, etc..., diagnostic specimens, recombinant DNA, cell cultures; meaning cells, tissues, blood, or body fluids, and lastly animals or plant cells which may contain pathogens. As a rule of thumb treat, all research materials as if they are infectious, this will produce safe laboratory practices.

Slide 19

BSL-2 Standard Practices

- Hazard considerations
 - There are chemical hazards in the lab
 - These hazards can be defined and identified
 - Most chemical hazards are non-lethal, but they can cause injuries
 - Dose, route, toxicity, and interaction dependent
 - When in doubt **ASK** for assistance







The laboratories contain chemical hazards; these are all defined and identifiable in the lab. There is a spectrum of injury that can occur from chemical exposure ranging from none to lethal, often depending on dose (concentration), route of exposure, toxicity, and nature of the interaction with the body. When in doubt or if unfamiliar with a chemical or task ASK for assistance.

Slide 20

BSL-2 Standard Practices

- Wash hands frequently
- No eating, drinking, applying cosmetics or handling contact lenses
 - Do **NOT** deliberately smell or taste materials in lab
- Use mechanical pipetting devices
 - **NO** mouth pipetting



Make sure to washing your hands frequently in the laboratory. This includes when working with any agent or any material in the laboratory, after removing gloves, after entering, and before leaving the laboratory.



Laboratory personnel should not eat, drink, apply cosmetics or handle contact lenses in the laboratory either. Also, do not deliberately smell or taste materials in the lab.

One standard microbiological practice is the use of mechanical pipetting devices. No mouth pipetting is allowed. Mouth pipetting was once common in the 19th century and was strongly associated with typhoid infections. Pipette aids are recommended for your own protection.

Slide 21

BSL-2 Standard Practices

- Needles and sharps policy
 - Use sharps containers
 - Do **NOT** break, bend, sheath or reuse syringes or needles
- Do **NOT** handle broken glassware directly
 - Use forceps
- Avoid using damaged glassware


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Each lab has precautions for needles and sharps. Personnel should use sharps containers to dispose of needles and syringes and should not break, bend, re-sheath or reuse syringes or needles. Also, do not handle broken glassware directly; use appropriate, indirect methods to clean up broken glass. Most of the labs substitute plastic-ware when possible.

Slide 22

BSL-2 Standard Practices

- Use of chemical fumehood and biological safety cabinets (BSCs)
 - Work at least 6 inches into hood/BSC
 - Cover work surfaces with absorbent paper mat
 - Maintain uncluttered work area
 - Be careful not to block vents and air flow
 - Check operation before using




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When using the chemical fumehood and biological safety cabinets (BSC) make sure to work at least 6 inches into hood/cabinet, cover work surfaces with absorbent paper, maintain uncluttered work area, be careful not to block vents and air flow, and be sure to check operation before using equipment

Slide 23

BSL-2 Standard Practices

- Decontaminate lab equipment
 - After spills, splashes, other potential contamination
 - Before repair, maintenance, or removal from lab
- Spills of infectious materials
 - Must be contained, decontaminated, and cleaned up
 - Spill kits contain disinfectant, towels, biohazards bags, & forceps



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Decontaminated equipment should be tagged before repair, maintenance, or removal from lab. Decontamination of lab equipment should be done routinely, after spills, splashes, other potential contamination. As well as pre- and post-use.

Spills of infectious materials must be contained, decontaminated, and cleaned up immediately following incident. Procedures and guidelines are in the biosafety manual in each lab. IF you are in doubt of how to perform the procedure, ASK. We will cover this in more detail shortly.

Slide 24

BSL-2 Standard Practices

- Disinfection procedures pre-work in the biological safety cabinets
 - Pre-work
 - Squirt down surfaces in BSC with Roccal-D let stand for 3-5 minutes
 - Follow up with 70% ethanol and wipe down all surfaces
 - Important do **NOT** use ethanol first, it binds to proteins
 - Place absorbent paper mat down after wipe down



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When using a biological safety cabinet there are disinfection procedures that should be conducted pre and post work. Procedures for Pre-work involve squirting down surfaces in biological safety cabinets with Roccal-D. Let it stand for 3-5 minutes and wipe up area with paper towels. Follow up with 70% ethanol and then wipe down all surfaces. It is important not to use the 70% ethanol first because it will bind any present proteins to the work surface, making it difficult to impossible to remove. Remember Roccal-D first!

After everything is wiped down make sure to place an absorbent paper mat down in work area. If anything is spilled make sure to clean it up immediately.

Slide 25

BSL-2 Standard Practices

- Disinfection procedures post-work in the biological safety cabinets
 - Post-work
 - Use the Roccal-D to clean work area
 - Follow up again with the 70% ethanol
 - Make sure to clean the front and back of the glass on the BSC




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After you have completed your work in the biological safety cabinet, repeat the pre-work procedure, squirting down surfaces in cabinet with Roccal-D. Again let it stand for 3-5 minutes and wipe up area with paper towels. Follow up with 70% ethanol and then wipe down all surfaces. Make sure to clean the front and back of the glass on the BSC.

Slide 26

BSL-2 Standard Practices

- Decontaminate infectious materials before disposal
 - Secure materials in durable, leak-proof containers
 - Materials **need** to be autoclaved
- Access to lab is **restricted**
 - Especially when work is being conducted
 - You are responsible for waste generated



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In the laboratory, you should decontaminate potentially infectious materials before disposal. This includes securing materials in durable, leak-proof containers before transporting potentially infectious materials out of the laboratory for decontamination or autoclaving.

Access to BSL-2 laboratories is restricted especially when work is being conducted; therefore, you are responsible for waste generated in the laboratory.

Slide 27

BSL-2 Special Practices

- Trash vs. biological waste
 - Place material in a biohazard bag if...
 - Anything that came in contact with a sample of any source
 - Blood, bacteria, serum, virus, tissues, etc...
 - Was used in the chemical hood or BSCs
 - Or contact is questionable
 - Do **NOT** overstuff waste cans or biohazard bags
 - Only fill bags and cans only 1/2- 3/4 full so that the bags can be closed and handled easily

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To determine the difference between trash and biological waste, a good rule of thumb is to place anything that came into contact with a sample of any source be it blood, bacteria, serum, virus, vaccine, tissues, etc... or was used in the chemical hood or BSC, or if the contact history is questionable in to a biohazard bag. Everything else is considered trash and will go in its respective receptacle.


It is important not to overstuff waste cans or biohazard bags. Only fill bags




and can $\frac{1}{2}$ to $\frac{3}{4}$ full so that bags can be closed and handled easily.

Slide 28

BSL-2 Special Practices

- Biohazard bags go to room 127 for autoclaving
- Trash will be removed by custodian or yourself
- Routine cleaning of laboratory is your responsibility
 - Every Monday and Thursday counter tops and work areas are wiped down with 1% Clorox wipes, followed by damp paper towels
 - Once a month floors are swiffered








The biohazard bags go to room 127 for autoclaving. This room is shared by three units, make sure to be mindful of others.

The facility custodial staff will only come into the laboratory to remove trash. If the trash needs taken out sooner, it is your job to remove it. The custodial staff also will not clean the counter tops, floors, or work areas in the laboratory. The routine cleaning of the laboratory is your responsibility and that of your lab mates. Every Monday and Thursday counter tops and work areas are wiped down with 1% Clorox wipes and followed with a damp paper towel regardless of how recently the area was cleaned. And then once a month, depending on the traffic flow, the floors in the lab are swiffered.

Slide 29

BSL-2 Special Practices

- Cleaning spills
 - There are well-stocked spill response stations in each lab
 - Locate prior to working in the laboratory
 - Response materials for a spill clean-up must be appropriate for the item being cleaned
 - Material Safety Data (MSD) sheet
 - Found online via CGAHR chemical inventory system (CISPro)
 - Acid or base agent
 - Neutralize appropriately



In the event of a chemical spill, you should always first notify the personnel in the immediate area. If you are unsure, always err on the side of safety and have personnel evacuate the area. There are well-stocked spill response stations in each lab. Locate the station prior to working in the laboratory. The response material for a spill clean-up must be appropriate for the item being cleaned. For example, know if the material is acidic or basic and use the appropriate neutralizer respectively.

Slide 30

BSL-2 Special Practices

- Chemical Spill
 - Individual can handle <1 liter of liquid and <1 lb of solid - if larger need assistance
 - Notify others in area
 - Put on PPE (if not on)
 - Locate spill kit
 - Cover the area with chemical spill powder around the spill
 - Use scoop to ensure liquid is absorbed by powder
 - Place in polyethylene bag
 - Wipe up surrounding area
 - Place materials in bag and tag with hazardous wastes sticker
 - If you do **NOT** know what the hazard is or if substance is highly hazardous, call 9-911 – activate nearest fire alarm pull station to evacuate building

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To clean up a chemical spill, the general rule of thumb is an individual should be able to handle <1 liter of liquid and <1 lb of solid - if larger assistance should be gathered. Make sure to notify others in the area when a spill occurs. Put on the appropriate PPE and locate the laboratory spill kit. Make sure to cover the affected area and the area around the spill with chemical spill powder. Once the powder has absorbed the chemical, place the powder in a polyethylene bag. Make sure to wipe-up the surrounding area and place materials used in bag and tag with hazardous wastes sticker.

If you do **NOT** know what the hazard is or if substance is highly hazardous, call 9-911 – activate nearest fire alarm pull station to evacuate building

Slide 31

BSL-2 Special Practices

- Biological Spill
 - Notify others in area
 - Avoid inhaling airborne materials - leave area
 - Close door, post warning sign
 - Remove contaminated clothing
 - Turn inside out and place in biohazard bag
 - Notify lab supervisor and biosafety officer as soon as possible
 - Allow aerosols to disperse for 30 minutes
 - To clean spill:
 - Put on PPE
 - Locate spill kit
 - Cover the area with disinfectant-soaked towels and carefully pour disinfectant around spill
 - Allow thirty minutes of contact time
 - Wipe up surrounding area
 - Place materials and PPE in biocontainment bag and autoclave

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To clean up a biological spill follow procedures similar to cleaning a chemical spill. First, notify others in the area. Ensure to avoid inhaling airborne materials and leave the area. Close the door and post a warning sign to make sure others know (this is also an important reason to read all signs before entering a laboratory). Second, make sure to remove contaminated clothing, turning clothing inside out and placing them in a biohazard bag. Notify the lab supervisor and biosafety officer. Allow the aerosols from the agent to disperse for at least 30 minutes. Put on appropriate PPE and locate the laboratory spill kit. Third, cover the area with disinfectant-soaked towels and carefully pour disinfectant around spill, allow thirty minutes of contact time. Finally, make sure to wipe up the surrounding area, place the materials used and the PPE in a biocontainment

bag and autoclave the contents.

Slide 32

BSL-2 Special Practices

- Spill Clean-Up ...
 - If spill is inside the biosafety cabinet (BSC)
 - Continue running BSC during spill and clean up to contain aerosol
 - Place absorbent paper on spill and soak with disinfectant
 - Allow 20 minutes contact time. Wipe up spill, working from edges to center. Clean area with fresh paper towels soaked with disinfectant
 - Disinfect the interior of the BSC and other equipment inside the BSC
 - Discard contaminated disposable materials using biohazardous disposal procedures
 - Run the BSC 10 minutes after clean-up before resuming work or turning the BSC off
 - If you are working in a BSC and power goes off in the room or the BSC fan stops, leave the laboratory immediately

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If a spill occurs inside the biosafety cabinet (BSC), make sure to continue running the BSC during spill and cleanup to contain aerosol. First, place absorbent paper on spill and soak with disinfectant. Allow 20 minutes contact time. Second, wipe up spill, working from edges to center. Clean area with fresh paper towels soaked with disinfectant. Third, disinfect the interior of the BSC and other equipment inside the BSC. Fourth, discard contaminated disposable materials using biohazardous disposal procedures. Continue to run the BSC 10 minutes after clean-up before resuming work or turning the BSC off.

As a not: If you are working in a BSC and power goes off in the room or the BSC fan stops. Leave the laboratory immediately

Slide 33

BSL-2 Special Practices

- Spill Clean-Up ...
 - If the spill is outside the laboratory, in transit
 - In a public area, do NOT attempt clean up without appropriate PPE
 - Secure the area and keep people clear of spill
 - Call a trained coworker to assist in clean-up
 - Standby during the spill response and clean-up activity to provide information and assistance


UNMA CGAHR ABADRJ

If a spill occurs outside the laboratory or in transit between laboratories in a public area, and they sometimes do, do NOT attempt clean up without appropriate PPE. First, secure the area, keep people clear of spill. Second, call the biosafety officer to assist in the clean-up. Finally, stand by during the spill response and clean-up activity to provide information and assistance.

Slide 34

BSL-2 Special Practices

- To prevent spills, biohazardous materials must be placed in an unbreakable, leak-proof secondary container during transit
 - Materials must be stored in leak-proof containers during..
 - Collection
 - Handling
 - Processing
 - Storage
 - Transport within facility
 - Limited to Bluetongue virus, Vesicular Stomatitis virus, Epizootic Hemorrhagic disease virus, Rift Valley fever virus vaccine (MP-12)



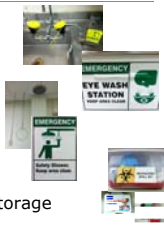
To prevent spills make sure to transport labeled biohazardous material in an unbreakable, leak-proof primary container, inside a second unbreakable, lidded container.


Potentially infectious materials must be placed in these types of containers during collection, handling, processing, storage and transport within a facility. Remember with in CGAHR transport of infectious materials is limited to blue tongue virus, vesicular stomatitis, epizootic hemorrhagic disease virus, and the MP-12 rift valley fever virus vaccine.

Slide 35

Laboratory Facilities

- Eyewash station
 - Located near sink
- Emergency shower
 - Locate in each lab
- Emergency equipment storage
 - Know where it is located





That ends the procedures for in the laboratory. Now on to facilities in each lab. There are eyewash stations available near sinks in each lab in case of accidental exposure to infectious materials. If exposed, flush open eyes for 15 minutes continually and notify help. There are also emergency showers in each lab. Locate shower in case of accidental exposure. If exposed, remove contaminated clothing, shoes, and jewelry, because time is critical to prevent serious skin burns. Know the location of emergency equipment, containing first aid kits, fire extinguishers, and biohazardous spill kits, in each laboratory and keep unobstructed at all times.

Slide 36

Laboratory Accidents

- Causes
 - Lack of training, knowledge, or experience
 - Carelessness (not vigilant)
 - Fatigue
 - Taking short cuts
 - Not enough time; working too fast
 - Deciding NOT to follow safe practices
 - Not believing it's hazardous

THINK SAFETY FIRST

SLOW DOWN

UNNA, CGAHR, ABADRU

Laboratory accidents can occur through many avoidable means. Lab accidents can be caused by a lack of training, knowledge, or experiences; if you are in doubt ask. They can also be caused by carelessness, fatigue, taking short cuts, working too fast, deciding not to follow safe practices, or an individual belief the material being worked with is not hazardous.

Slide 37

Potential Emergencies

- External events
 - Tornados and earthquakes
 - Power outages
 - Terrorism
- Internal events
 - Medical
 - Fires, explosions
 - Spills, splashes
 - Environmental releases

Know what to do *before* it happens

UNNA, CGAHR, ABADRU

Anticipate potential emergencies and learn what to do in case of external events such as tornados/earthquakes, power outages, or terrorism and internal events such as medical, fire, spills, or environmental releases.

The steps to these situations are in the biosafety manual. Also, ask your supervising researcher about his/her protocols in their lab.

Slide 38

Safety Committees

- Facility safety committee
 - Audits laboratories and recommends improvements/changes
- K-State's Institutional biosafety committee (IBC)
 - Reviews biosafety and rDNA protocols
- K-State's Institutional animal care and use committee (IACUC)
 - Oversight of animal care and use


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This facility works with a couple different safety committees. First, is the CGAHR safety committee, which audits laboratories and recommends improvements/changes. Second, is the Institutional biosafety committee (IBC), which reviews biosafety and rDNA protocols, and third is the Institutional animal care and Use Committee (IACUC) which conducts evaluations and oversight of animal care and use in the research setting.

Slide 39

Training and Injuries

- Supervisor ensures laboratory training
 - Duties to be performed
 - Procedures to prevent exposures
 - Exposure evaluation procedures
- Training updates
 - Policy/procedure changes
- Personnel report injuries/illness
 - Notify Supervisor and Safety Officer



Logos at the bottom: UNDA, CGAHR, ABADRU

Your laboratory supervisor will ensure that you receive specific training in the following areas: duties to be performed, procedures to prevent exposures, and exposure evaluation procedures. Your laboratory supervisor will update your training annually or when policies or procedures change in this facility.


If you become injured, ill, or more predisposed to infection please self-report to your supervisor and safety officer. Incidents that may result in exposure to infectious materials: Must be evaluated and treated according to biosafety manual.

Slide 40

Food For Thought

What could happen besides someone getting ill or hurt if these guidelines are not followed?

Could lose funding, collaborations, and/or research grants



Logos at the bottom: UNDA, CGAHR, ABADRU


So now that we have heard about all the things that need to be done and not done I'd like to present you with some food for thought. Does anyone know what could happen besides someone getting ill or hurt if these guidelines are not followed?

We could lose funding, collaborations, and/or research grants. Therefore it is important for your safety, health, and the future research of this unit to follow the presented procedures and guidelines.

Slide 41

Everyone's Responsibilities

- Practice safety consistently
- Be part of the unit (team)
- Seek knowledge and understanding
- ASK questions



Logos: USM, CGAHR, ABADRU

It is everyone is responsible for the consistent practice of safety in the laboratory, to do this be a part of the unit, seek knowledge and understanding through good science, and ASK questions.

Slide 42

Resources

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Logos: USM, CGAHR, ABADRU

Slide 43

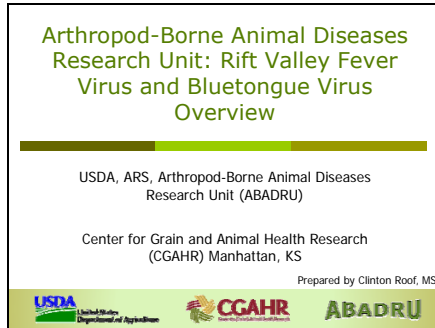
Quiz

- Separate Page

Logos: USM, CGAHR, ABADRU

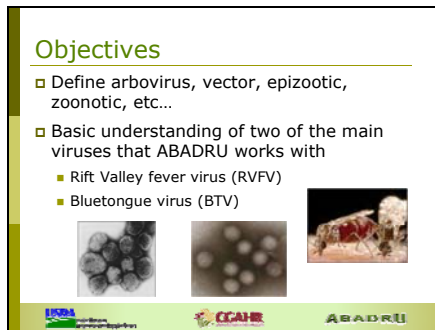
Appendix B – Arthropod-Borne Animal Diseases Research Unit: Rift Valley Fever Virus and Bluetongue Virus Overview

Slide 1



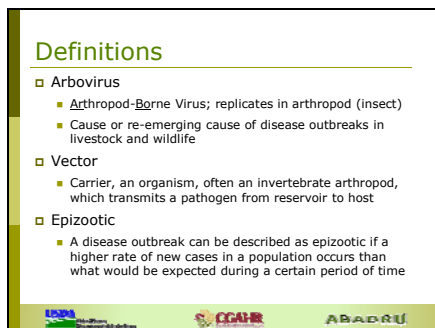
Hello and welcome to the Arthropod-Borne Animal Diseases Research Unit: Rift Valley fever virus and Bluetongue virus Overview here at the USDA Center for Grain and Animal Health Research.

Slide 2



The objectives of this presentation are to define some key terms associated with insect borne viruses as well as achieve a basic understanding of two of the main viruses that are worked with ABADRU. By the end, you should have an understanding of why research is conducted on Rift Valley fever virus (RVFV) and Bluetongue virus (BTV).

Slide 3

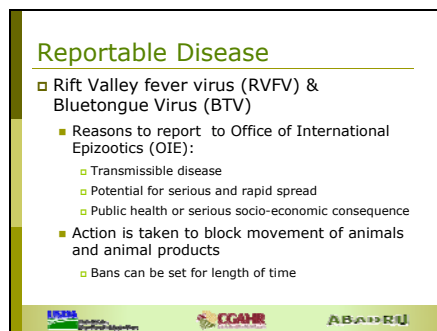


Let's begin with a definition of arbovirus. Arbovirus is an arthropod borne virus that replicates in insects. Often an arbovirus is the cause or the re-emerging cause of disease outbreaks in livestock and wildlife.

You will often hear that an insect that carries a virus is called a vector. A vector is a carrier or organism, often an invertebrate arthropod, which can transmit a pathogen from reservoir to host.

Additionally, you will hear that a vector carrying an arbovirus can result in an epizootic. This means that a disease outbreak can be described as epizootic if a higher rate of new cases in a population occurs than what would be expected during a certain period of time

Slide 4



Reportable Disease

- Rift Valley fever virus (RVFV) & Bluetongue Virus (BTV)
 - Reasons to report to Office of International Epizootics (OIE):
 - Transmissible disease
 - Potential for serious and rapid spread
 - Public health or serious socio-economic consequence
 - Action is taken to block movement of animals and animal products
 - Bans can be set for length of time

USDA CGAHR ABARRJ

Now that we have some basic definitions let's discuss a large commonality that RVFV and BTV have. Both of these viruses cause diseases that are considered reportable. The reporting involves notifying the World Organization of Animal Health/Office of International Epizootics (OIE) that the disease has been identified.


This is important because RVFV and BTV cause transmissible disease, which have a high potential for rapid and serious spread in a population and can also cause major public health or serious socio-economic consequences.

Once notified the OIE can take action to block or ban the movement of animals and animal products until the outbreak can be controlled or contained.

Slide 5

Rift Valley Fever Virus

- History
 - Scientists first isolated RVFV from blood of a lamb in Kenya, Africa's Rift Valley in 1931
 - Major outbreaks of RVFV have been noted throughout sub-Saharan Africa
 - Over 70 years



Logos: INRA, CGAR, ABAARI

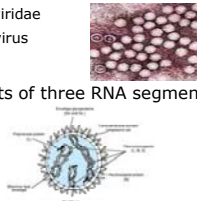
Let's cover Rift Valley fever virus first. In 1931, the virus was first isolated from the blood of a lamb in Kenya, Africa's Rift Valley hence the name. Since then, RVFV has been observed to be widespread throughout Africa, but has also been documented in Kenya, Somalia, Tanzania, Yemen, Egypt, Sudan, Madagascar, Senegal, and Saudi Arabia.

RVFV periodically causes outbreaks in these countries in ruminant species including sheep, cattle, and goat and has been reported in humans as well.

Slide 6

Rift Valley Fever Virus

- Enveloped single-stranded RNA virus
 - Family: Bunyaviridae
 - Genus: Phlebovirus
- Genome consists of three RNA segments
 - L, M, and S




Logos: INRA, CGAR, ABAARI

Rift Valley fever virus (RVFV) is an enveloped single-stranded RNA virus from the family Bunyaviridae of the genus Phlebovirus. The genome of the virus is made up of three RNA segments, L, M, & S. These segments are responsible for the function of the virus on the cellular level.

Slide 7

Rift Valley Fever Virus

- Mosquito-transmitted viral disease
 - Mosquito transovarial (vertical) transmission
 - Transmitted to offspring from mother
 - Zoonotic – Affects both humans and animals
 - Humans and Ruminants amplify the virus
 - Rapid short viremia



Logos: INRA, CGAR, ABAARI

RVFV is a mosquito (Arbo) transmitted viral disease. Infected mosquitoes transmit the virus when they feed as well as pass the virus to their offspring when they lay eggs. This is also called vertical transmission to offspring. So when the mosquitoes are hatched they are infected and have the ability to infect when they feed.

RVFV is a zoonotic virus; this means that both humans and animals may be affected. In the case of RVFV, ruminants and humans amplify the virus

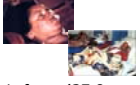
and are considered viremic (able to pass on the virus) in a short time period.




Humans, like animals, can become infected by mosquito bites; however, humans are more commonly infected through contact with virus contaminated blood or tissue, or through inhalation of virus in aerosols during slaughter of livestock.

Slide 8

Rift Valley Fever Virus

- Humans**
 - Incubation period 2-6 days
- Clinical Signs**
 - Flu-like syndrome, hemorrhagic fever (37.8-40°C), strong headaches, body pain, dizziness, nausea, epigastric discomfort, photophobia-retinitis, anorexia, and hemorrhage from body cavities
 - <2% can progress from illness to death
- Recovery within 4-7 days**












Once exposed, humans usually have an incubation period of 2-6 days. After this time, clinical signs appear and often include flu-like syndromes, hemorrhagic/bloody fever (37.8-40°C), strong headaches, body pain, dizziness, nausea, epigastric (stomach) discomfort, photophobia-retinitis (strong sensitivity to light), anorexia, and hemorrhage (blood) from body cavities. Typically, most people will recover from the disease in 4-7 days, but a little less than 2% will progress from illness to death.

Slide 9

Rift Valley Fever Virus

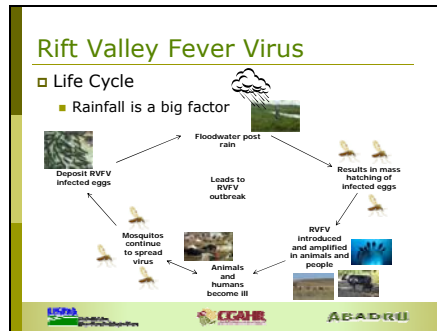
- Animals**
 - Incubation**
 - 12-36 hours in newborn lambs
 - Up to 72 hours in sheep, cattle, and dogs
- Clinical Signs**
 - Fever (40-42°C), anorexia, lymphadenopathy, weakness, nasal discharge, and usually death within 36 hrs
- Recovery**
 - Lambs: 90-100% mortality
 - Cattle: 10-70% mortality
 - Abortion is typical in 90-100% of affect ewes and cattle

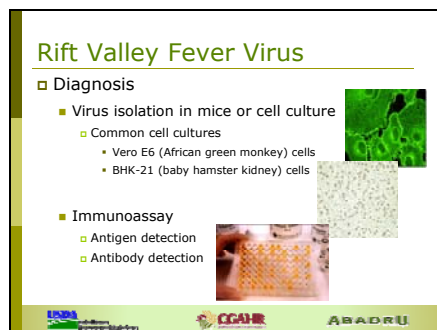
In animals, the incubation period is much shorter- ranging from 12-36 hrs in newborn lambs (who are considered the most susceptible) and up to 72 hrs in sheep, cattle, and dogs. After this time, clinical signs appear and often include fever (40-42°C), anorexia, lymphadenopathy, weakness, nasal discharge, and usually death within 36 hrs. Unlike humans, 90-100% of infected lambs and 10-70% of infected cattle will die from this disease. Additionally, 90-100% of pregnant ewes and cows will abort their fetus due to the virus.

Slide 10



The life cycle of a RVFV outbreak is highly correlated to rainfall. Once rain falls and causes floodwater, there is a massive surge of hatching of infected mosquito eggs. These new mosquitoes begin to feed and introduce the virus to humans and animals causing the virus to be further amplified. Affected humans and animals become ill. Mosquitoes continue to spread the virus over the population and then lay their RVFV infected eggs for future outbreaks.

Slide 11





If RVFV is suspected, the rapid diagnosis of the virus is key. Suspected individuals have blood or cells submitted to a laboratory where the virus, if present, is cultured and isolated in cells - typically in African green monkey or baby hamster kidney cells. Additionally, immunoassay can aid in diagnosis by detecting antigens or antibodies to the virus.




Slide 12

Rift Valley Fever Virus

- Turell, *et al*, 2008
 - Demonstrated the vector competence of North American mosquito species
 - Competent vector
 - *Culex tarsalis*
 - *Aedes vexans* from Louisiana and Florida

Culex tarsalis
Aedes vexans

You are probably asking why is a disease of Africa being researched in the U.S.? Well in 2008, a researcher named Turell, and associates demonstrated that there are mosquitos in North America capable of transmitting RVFV. These competent vectors include *Culex tarsalis* and *Aedes vexans* species. Therefore, it is important for us to better understand the virus and the associated disease as well as create effective vaccines and other preventative measures to decrease, if not eradicate, the virus.


Slide 13

Rift Valley Fever Virus

- Prevention of Infection
 - Attenuated and Killed Vaccines
 - Vector control
 - Insecticides
 - Repellants
 - Clothing
- Treatment
 - None – supportive care










With that said, our current prevention methods of RVFV include some attenuated and killed vaccines that have variable efficacy and some vaccines can actually cause abortion in pregnant animals. Other methods include the use of insecticides, repellants, and clothing, but these methods can be cost prohibitive or may result in other health side effects.

Additionally, there are no treatments available for individuals that become infected with RVFV. The best we can do right now is offer supportive care and wait as the disease runs its course.

Slide 14

Bluetongue Virus

- Midge-transmitted viral disease



- AKA - punkies, no-see-ums, and flying jaws
- Transmitted through insect bites of infected *Culicoides* sp. midge
- Unlike RVFV...
 - Animal transmission from mother to offspring is rare
 - Only midge to animal transmission can cause BTV to become endemic
- Animals (**NOT** humans) amplify the virus

Logos: OIE, FAO, WOAH, CCAR, ABADRI

Now let's discuss Bluetongue Virus or BTV. Bluetongue virus is a midge-transmitted viral disease. Midges are also called punkies, no-see-ums, and sometimes even flying jaws.

Bluetongue virus is transmitted through the insect bites of infected *Culicoides* sp. Unlike Rift Valley fever, animal transmission from mother to offspring is rare and only midge to animal transmission can cause BTV to become endemic.


It is not a zoonotic virus and only animals not humans can amplify the virus.

Slide 15

Bluetongue Virus

- History

- Identified in South Africa in early 1930s following the introduction of European breeds



- First isolated in the United States in 1952
 - Texas sheep
 - Now known in most of the US

Logos: OIE, FAO, WOAH, CCAR, ABADRI


BTV, like RVFV, was identified in South Africa in the early 1930s following the introduction of European breeds of cattle and sheep.

The first isolated Bluetongue virus in the United States, occurred in a Texas sheep in 1952. Now the virus can be found in most of the United States.

Slide 16

Bluetongue Virus

- Historically the virus has been limited between latitudes 40°N & 35°S
 - Excursions out of boundary are thought to be due to global change




USDA, OCAH, ASADRI

Historically, the Bluetongue virus has been limited between latitudes of 40°N & 35°S. However, Bluetongue infections have been reported outside of this boundary and researchers believe it may be due to global change. Meaning insects are able to survive and reproduce in areas once too cold for them.

Slide 17

Bluetongue Virus

- Enveloped single-stranded RNA virus
 - Family: Reoviridae
 - Genus: Orbivirus
- Genome consists of 10 dsRNA segments
 - Encodes for 10 proteins



USDA, OCAH, ASADRI

Bluetongue virus (BTV) is also an enveloped single-stranded RNA virus. It is of the family Reoviridae and the genus Orbivirus because it is a circular virus. Other viral agents that fall into this genus include African Horse Sickness virus (AHSV) and Epizootic Hemorrhagic Disease virus (EHDV).

The genome consists of 10 dsRNA segments which encodes for 10 proteins needed for the virus' survival.

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Bluetongue Virus

- Causes diseases in both domestic and wild ruminants, meaning cattle, sheep, goats, deer, antelope, and camels
- Cannot be transmitted to humans or horses
 - NOT Zoonotic
 - NOT Contagious




I'm not contagious!

USDA, OCAH, ASADRI

BTV causes disease in both domestic and wild ruminants, meaning cattle, especially sheep, goats, deer, antelope, and camels.

Bluetongue cannot be transmitted to humans or horses. This virus is not zoonotic and is not considered contagious, meaning if one animal is ill it cannot infect another animal. Only animals bitten by an infected culicoides can become ill.

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Bluetongue Virus

- Sheep
 - Clinical Signs can vary
 - High fever, depression, reddening of the mouth, nose and eyes, facial swelling, oral lesions, nasal discharge, and cyanotic (blue) tongue, coronitis-lameness, and death

- Morbidity (illness) ≈ 100%, mortality (death) > 50%
- Abortions & stillbirths seen

Logos: ISPA, CGAR, ABADRI

In sheep, clinical signs can vary, it is not uncommon to see fever, depression, reddening of the mouth, nose, and eyes, facial swelling, oral lesions, nasal discharge and cyanotic (blue) tongue, coronitis-lameness meaning inflamed/irritated feet), and death.

Approximately 100% of bitten sheep will become infected with >50% of the animals dying from the disease. Like with RVFV, it is not uncommon to see abortions and stillbirths from pregnant ewes.

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Bluetongue Virus

- Cattle
 - Clinical Signs
 - Disease is less severe, with reddening of the mouth, nose, and eyes, necrosis of the muzzle, and patchy dermatitis

- Decrease in milk production and causes abortions (early term)
- Can lead to secondary mastitis (udder) infections
- Can cause sub-clinical infection providing a source of virus for midges

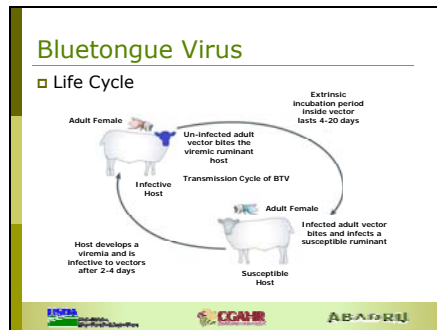
Logos: ISPA, CGAR, ABADRI

In cattle the clinical signs are often less severe, with a reddening of the mouth, nose and eyes seen, as well as necrosis of the muzzle and patchy dermatitis (meaning dying skin along the nose) and hair loss and skin lesions are seen on the body.

Ill cattle will have a decrease in milk production and the virus can cause early term abortions in pregnant cows.

Because cattle are usually less impacted by the disease, it is not uncommon to see sub-clinical infections which can provide a source for midges to continue to spread the disease without the animals looking sick.

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The life cycle of BTV outbreak begins with an uninfected adult vector biting a viremic ruminant host. After 4-20 days of incubation the vector can now infect a susceptible ruminant host. It takes the now bitten host 2-4 days to become viremic and be infective to other vectors. This then allows the virus to spread across a naïve population forming a circle of infected vectors infecting susceptible hosts.

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Bluetongue Virus

- Strains BTV- 1, 3, 5, 6, 9, 12, 14, 17, 19, 22, & 24
 - Introduced two ways
 - 1) infected vectors – carried by wind
 - 2) via infected animals
- Domestic and Exotic Strains/serotypes
 - Clinical signs and disease in the United States is considered moderate, however...
 - Europe currently has strains of virus that cause high death and illness in sheep and cattle alike

Logos: OIE, FAO, WOAH, CCAR, ABRU


There are several different strains or serotypes of BTV virus that have been reported. These include 1, 3, 5, 6, 9, 12, 14, 17, 19, 22, & 24. These strains are introduced to a naïve population in two ways, one) infected midge is carried or pushed by wind into an unaffected area or animals that are infected are introduced to an unaffected area with capable insects to spread the disease.


Though the clinical signs and disease in the United States is considered moderate, there are strains/serotypes in Europe that can cause high death and illness in both sheep and cattle alike.

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Bluetongue Virus

- Prevention of Infection
 - Attenuated Vaccine BTV-10
 - Sheep only
 - Only effective against BTV-10
 - California
 - Attenuated vaccine for serotypes 10, 11, 17 for use in CA only
 - Attenuated vaccine for serotypes 10, 11, 13, & 17 for wildlife species in CA
 - Vector control





With that said, our current prevention methods of BTV include some attenuated vaccines that have variable efficacy. Currently, across the United States there is an attenuated vaccine for the BTV-stain 10 in sheep only. This vaccine will not protect the sheep if another strain is carried in by an infected midge.

In California, there are attenuated vaccines for serotypes 10, 11, and 17, but they are approved for use in CA only and not the rest of the United States. Additionally, CA has some attenuated vaccines for serotypes 10, 11, 13, & 17 for wildlife species, also for us in CA only.

Like RVFV, there are no efficient treatments for animals that come down with BTV.

Currently, one of the best means of controlling BTV is to control the vectors. There are many forms of larvacides and adulticides, as well as, reduction of standing water that can help reduce the competent midge vectors.

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Conclusions

- Viruses
 - Are complicated with involvement of pathogens, vectors, animals, and sometimes humans
 - Can cause significant economic losses globally
 - Due to animal quarantines and trade restrictions
 - Control zones and movement restrictions
 - Loss of animal life and reproduction
 - Rift Valley fever can strongly impact human health

INM CGAHR ABADRU

In conclusion, both BTV and RVFV are complicated with the involvement of pathogens, vectors, animals, and sometimes humans. But, both are capable of causing significant economic losses globally due to animal quarantines and trade restrictions, not to mention the loss of animal life and reproduction. RVFV has the ability to strongly impact human health. And though RVFV is not in the U.S. It could significantly affect our healthcare and agriculture sectors if it did appear.

Because of the potential impacts of these two viruses and related viruses, it is important that groups such as the ABADRU continue to research them to develop, control, and protect public health as well as the U.S. livestock industries. These tools could benefit people the world over.

Slide 25

Resources

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Slide 26

Quiz

- Separate Page

INM CGAHR ABADRU

Appendix C – Field Experience Daily Log: At the USDA, CGAHR, and ABADRU

5/21/2012. 8:30 AM -10:30 AM & 1:00-4:00 PM: 5 Hours

I received a tour of the United States Department of Agriculture (USDA) Center for Grain and Animal Health Research (CGAHR) facility. I had my picture taken and received ID badge. I reviewed in house safety modules including use of personal protective equipment (PPE) (emailed results to USDA safety officer), after hour lab use, and chemical hygiene. I went over safety guidelines with Dr McVey. I completed the Institutional Animal Care and Use Committee (IACUC) module for Mouse/Rat research/handling (module 3), printed certificate.

5/22/2012 8:30 AM -12:00 PM & 1:00-4:00 PM: 6.5 Hours

I completed the Hazard communication safety module (emailed results to USDA safety officer). Completed the IACUC for Dog (module 2), Hamster (module 4), Guinea Pig (module 5), and Rodent Aseptic Surgery research/handling (module 7), printed certificates. I received email from Kansas State University (KSU) compliance office stating I had previously completed IACUC modules, 1,6,8,9,10 - 6/13/2010 & IRB Training Module: 1 - 7/15/2010. The training is good for 3 years from finish date; I have now completed all IACUC training modules. I attended a tutorial and mouse handling training at the KSU College of Veterinary Medicine (CVM) in Coles Hall, 10:30 AM-12:00 PM. I received certificate stating I am approved to handle rodents in the KSU Comparative Medicine Group (CMG) in Mosier Hall Animal Labs. These mice will be part of several studies at the Arthropod-Borne Animal Disease Research Unit (ABADRU) either for immunological response due to feeding *Culicoides sonorensis* on mice or vaccination purposes.

In the afternoon, I met with Dr McVey to discuss intradermal administration of both zoonotic agents and vaccines for infectious diseases. We discussed safety concerns and hazards associated administration of zoonotic agents, such as aerosolized blow back upon delivery of an injection. Meaning the zoonotic agent could be inhaled by the administrator. We talked about

conducting a literature search to find what research has been done with similar delivery devices and the safety methods and concerns associated with this method. I conducted a PubMed search and found twelve articles to be read. I attended a meeting with the lab technicians and veterinary medical supervisor to discuss a series of research and data collection projects that will begin next week. These projects will involve, feeding *Culicoides sonorensis* (midges) on mice and then conducting euthanasia on both fed and unfed mice. Upon necropsy heart blood, spleen, as well as inguinal and peritoneal lymph nodes will be collected for analysis. I will observe this process and consider methods or procedures in this lab that can be addressed to decrease occupational hazards associated with infectious and zoonotic agents.

5/23/2012 8:30 AM -12:00 PM & 1:00-4:00 PM: 6.5 Hours

I conducted literature search for occupational hazards with Rift Valley fever virus (RVFV) as well as the pathogenesis of the disease. I read the intradermal articles from yesterday attempting to find a suitable model for in house testing of agent delivery. I will be meeting at 10:30 AM to discuss this project with Dr McVey and Dr Ruder; both veterinary medical officers.

I discussed safety assessment of occupational hazards in BL-2 facilities, places/procedures to use care. I learned of a PowerPoint available which discusses needless-devices; the Pulse needle free devices (NFD). Additionally, I learned a NFD will be available to use in two weeks. During that time we can consider different dilutions (Evan's blue dye or crystal violet) to visualize the delivery of the device. We entertained thoughts on a high speed camera to attempt to view potential "blow back" droplets/Aerosolization of the delivery solution. I considered different volumes for delivery. I spoke about models for mimicking skin in calves and lambs that was found in the literature. We discussed the possibility of obtaining a lamb/sheep skin for testing purposes. We also talked about using a screen or some sort of filter on the device to see the misting or "blow back". We considered the testing of various distances, such as if an animal moved during the application of the agent. This raised questions such as, "are there different levels of wetness that can occur on the skin surface is there a chance to have more agent aerosolized?" We also addressed the primary concern of exploring this device's potential use is the safety and health of the worker/technician. The literature already shows that

administration of agents in this from work from an immunological standpoint. However, little to no literature talks about the safety of administration of a live agent or a modified live vaccine. I will continue to look for some examples as well as visit the PULSE NFD web page for their safety studies.

I conducted literature searches and requested books and articles from the KSU library system. I then read and summarized some documents. I completed safety modules including, Blood-borne pathogens, Institutional Biosafety Committee modules 1, 2, 3, & 4, and the Responsible conduct of Research module. I emailed all completed certificates to the house safety officer.

5/24/2012 8:30 AM -1:00 PM: 4.5 Hours

I arrived and filled out emergency contact information, signed and read IT use guidelines. I read documents to bring myself up to speed on current information regarding RVFV. I wrote and organized information in regards to the intradermal device project. I visited with Dr McVey about African Swine fever virus (ASFV) and its spread into Europe. This is another virus that the ABADRU will consider working with in the future.

5/25/2012 8:30 AM -12:00 PM: 3.5 Hours

I conducted literature research and reading of articles with note taking. The Secretary of Agriculture, Tom Vilsack, sent a directive for all USDA facilities to close early for the upcoming Memorial Day weekend. The office closed at noon.

5/28/2012

Closed for Memorial Day

5/29/2012 8:30 AM – 1:00 PM & 1:30 – 4:00 PM: 7.5 Hours

I continued to read and reflect on articles. I participated in a mouse study where the mice were anesthetized and then had their abdomens shaved. After this was completed containers

with “Stocking” lids (to allow *Culicoides sonorensis* to feed through the mesh) were placed on the shaved portion of the mouse. These containers were then velcroed for even surface area. The midges were allowed to feed for 30 minutes. After which the mice were removed, abdomens photographed, and were recovered and returned to their housing. The containers with the culicoides were stuck in a freezer so that the fed and unfed bugs could be separated and counted. In a few days the mice will have blood drawn and then analyzed for immunologic response from the feed, in particular from the culicoides saliva.

During the event I observed safety in the lab. One thing that caught my attention was the gloved hands that were used to touch the mice were also used to touch face, hair, pens, pants (exposed when sitting in lab coat or from coat being lifted to wipe, or rub gloves on pants) and other equipment that had been previously been handled without gloves or were handled without gloves following the study period where dirty gloves had been used to handle the same equipment.

5/30/2012 8:30 AM – 3:30 PM: 7 Hours

I observed the dissection and lymph node collection of previously frozen mice that had been euthanized for population control. This was to allow practice of collection of lymph nodes and femurs for a future study where lymph nodes will be necessary for data analysis. I discussed research being conducted in the laboratory. I observed plating of viruses for detection of interferon with the cultured cells.

From a safety stand point during my time in the lab today. It was observed that there is a large number of foot traffic in and out of the BSL-2 Lab. Including improperly clothed (open toe shoes) non-lab personnel, custodial staff, workers from other labs, and under-graduate workers with little to no lab safety training or training in proper lab procedures. Also I observed that all contaminated materials are placed in autoclave bags and are needed to be moved out of the lab down the hall to the autoclave. This raised the question “should an in lab autoclave be in use to reduce the risk?” Additionally, I noted that Rocal is used on contaminated materials and allowed the proper time to elapse to kill virus or virus components (24 hrs), prior to transport to autoclave. However, this raised another possible issue, “if an untrained worker (student or

custodial staff) were to pick up the dated material forgetting the date and begin to clean instruments that haven't had the proper time to set, what would be the risk?" I believe there is a need for new containment practices and improved training/awareness of the laboratory setting.

5/31/2012 8:30 AM -3:30 PM: 7 Hours

I continued reading collected documents, note taking, and organizing information. I spoke with Dr Chris Lehiy about sequencing proteins from the saliva of *Culicoides sonorensis*.

6/4/2012 8:30 AM – 4:00 PM: 6.5 Hours

I continued to read, research, and write about RVFV and occupational biosafety areas in the laboratory. I visited with Dr Ruder about Epizootic Hemorrhagic Disease virus (EHDV) in white-tail deer, antelope, and big horn sheep and its impact/rise in frequency of occurrence over the past thirty years across the United States.

I "played" with the needle free device, learning how to operate, clean, disassemble, and reassemble. I met with Dr Barb Drolet to discuss current laboratory research. She recently spent 3 weeks in Colorado conducting Blue Tongue Virus (BTV) research with sheep. During this time she used the NFD for inoculations. She observed that the device sprayed a lot of the inoculation around the injection site. Further raising the question, "if this is a device the ABADRU would want to use in house for RVFV research with either a modified live or live agent, what would be the risk?" Dr Mark Ruder and I have discussed Materials and Methods for experimenting with the NFD in house and discussed both efficiency of application and safety of the worker conducting the application.

6/5/2012 9:00 AM – 2:00 PM & 3:00 PM – 5:30 PM: 7.5 Hours

I attended an all staff meeting that all research groups were required to attend. The meeting discussed and explained ethics issues in research as well as ethic issues as a government employee. I observed and participated in another feeding of 30 laboratory mice with culicoides.

This was a continuation of mouse work from the week previous. I attended a planning meeting for 6/6/2012 research collection. Discussed statistics and the importance of appropriate n values with both control as well as treatment groups.

6/6/2012 8:30 AM – 2:30 PM & 3:00 PM - 5:30 PM: 8.5 Hours

We set up and prepped for today's data collection. 26 mice were anesthetized, had heart stuck for blood collection (CBC tubes), cervical dislocation to humanely euthanize, then the mice underwent necropsy where the inguinal and popliteal lymph nodes were collected. Abdominal skin was collected (where the culicoides fed the day previous) which was stretched and placed in formaldehyde. Additionally, I collected the spleens from all mice and placed in containers for further analysis. Areas were prepped and cleaned for continued study trials tomorrow. I continued conducting a literature review, reading text books, and supporting articles.

I considered questions from 5/30/2012. A sign was placed on the biolaboratory door today, stating "Emergencies only this week", to reduce the traffic flow in and out of the lab during study collection. This sign did not prevent personal from other labs, people with open toed shoes, or custodial staff from entering the lab without knocking or heeding to the sign. This makes me believe that training is not the only issue that needs to be addressed, but all staff needs to learn to read signs and abide to them. Because the reasons the individuals entered the lab were not of immediate importance and could have been addressed at a later time. People need to read signs for their own safety, regardless of their training level. I wonder what would occur if the sign stated authorized personnel only. I posted a sign with "authorized personnel only" to see how many people enter the lab during tomorrow's collection. I continued to work on the materials and methods for the in house intradermal study.

6/7/2012 9:00 AM – 12:30 PM & 2:30 PM – 5:00 PM: 6 Hours

We set up and prepped for today's data collection. 12 mice were anesthetized for this study; similar samples were collected from yesterday's study. I anesthetized all mice with a

xylazine/ketamine cocktail of 0.09 mL/k. I also collected the spleens from all mice and placed in containers for further analysis.

The sign added to the lab door the day previous, did little to prevent people from coming in to the laboratory. One individual did knock on the door; however, he was not part of CGAGR and was a biosafety cabinet (BSC) maintenance man. Other individuals entered at their leisure. This raises the issue of individuals not reading signs or paying attention to things around them... I believe this is an issue that needs to be addressed for safety of lab workers and supporting staff.

6/8/2012 9:00 AM – 12:00 PM & 1:30 PM – 4:00 PM: 5.5 Hours

I continued to put together the intradermal device study design. I spent time formulating appropriate materials and methods for the evaluation of occupational safety. I reviewed a PowerPoint that Dr Dick Hesse had put together on his evaluation of needle-free devices in swine. I continued writing and researching the intradermal device study design.

6/11/2012 8:30 AM – 1:00 PM & 2:00 PM – 5:00 PM: 7.5 Hours

I aided in the collection of femurs and humerus from 4 mice. The bones had the bone marrow removed and placed into culture dishes. The cells will be cultured and plated over the next 6 weeks to achieve mast cell plates for further research. I continued on my literature review, reading text books and supporting articles.

6/12/2012 9:00 AM – 12:30 PM & 1:30 PM – 5:00PM: 7 Hours

I attempted to go to the “gnat ranch” this morning to observe and learn how the *Culicoides sonorensis* are raised and kept for research colonies. However, no one was in the laboratory at this time.

I aided in anesthetizing and recovering of 12 mice for a culicoides feeding. The culicoides were allowed to feed for 30 minutes. After which the mice were removed, abdomens photographed, and were recovered and returned to their housing.

6/13/2012 8:30 AM – 1:30 PM & 2:15 PM – 5:00PM: 7.75 Hours

I arrived this morning at the cell culture lab that is housed behind the CGAHR. I met with Kruger Bryant, who is the microbiology laboratory technician for the CGAHR. He is responsible for all lines of cell cultures that are used in the CGAHR research labs. These cells range from lung, testis, kidney, heart, artery, of various wild and domestic animals. Some of these lines of cells have been going for over thirty years. This “library” of cells is housed in a nitrogen-jacketed refrigerator that keeps the samples at -180 for long term storage. I was told that this temperature will hold the cells for decades if necessary and when brought to the appropriate temperature will revive and begin dividing again. I received a full tour of the facility and was allowed to observe and question the processes involved with filling orders/requests for the various divisions within the CGAHR. These cell lines are very important not only with current ongoing research but for diagnostics involved with the zoonotic and epizootic viruses that affect various parts of the world. In fact these cell lines are sometimes supplied to other countries to aid with diagnostics when an outbreak occurs. This is crucial and is often an overlooked variable in the protection of public health and animals.

I attended an all staff meeting for ABADRU which is held monthly. The purpose is to bring everyone up to speed with projects and changes within the center. They are currently working on settling in to several new labs to serve this division and discussed the necessary steps that will need to occur in order for the transition to be done smoothly. Two presentations were given on Current Research Information System (CRIS) projects. These are 5 year projects with specific objectives and goals to be achieved or worked toward during that time period. The projects presented were one for BTV and the goals with vaccinations, serotyping, diagnostics and other collaborations. The second was with RVFV with similar goals and objectives. The purpose is to bring the unit up to snuff with what it will be working on and so that they wrap up any current projects that dealt with the previous CRIS project.

I continued to work on reading articles and writing. I spoke with Dr Ruder about refining the specifications for the intradermal project. Tomorrow Dr Hesse from the CVM KSU will be visiting to discuss his experiences with intradermal devices in swine.

6/14/2012 8:30 AM – 12:00 PM & 1:00 PM – 5:00PM: 6.5 Hours

Meet with the ABADRU unit and Dr Dick Hesse from the KSU CVM to discuss his experiences with intradermal devices and their efficiency/safety. These times provided us opportunity to brain storm and ask questions regarding previous work and consideration of use in Biolaboratory research or as an administration device of modified vaccines.

I met with Dr McVey after to further discuss the information. We decided the use of black or white construction paper would be helpful to place around the intradermal “gun” to subjectively observe either aerosolization of material, “blow back”, etc. With a means to also objectively evaluate the “splatter” with surface area. We planned to experiment with device tomorrow afternoon. I am planning to meet the USDA CGHAR safety officer, JoAnne Gresens, tomorrow with Dr McVey at 1:30PM to get the occupational biosafety guidelines underway to discuss goals.

I continued work on RVFV literature review. I believe I have completed it. I will send to Dr McVey and Dr Payne to see if I am on the correct tract.

6/15/2012 9:00 AM – 1:15 PM & 1:30 PM – 5:00 PM: 7.75 Hours

I practiced using the P-50 NFD using saline to test the various volumes and pressure combinations for administration. I also learned how to clean, disassemble, and reassemble the device post use. I discussed technique and process with Dr Ruder.

I set up and prepped for today’s data collection with the laboratory staff. The same 12 mice that were anesthetized from 6/11/2012 were used. I anesthetized all mice with a xylazine/ketamine cocktail of 0.1 mL/k. These mice had a heart stick performed to obtain up to

1 mL of blood from each mouse. Each mouse was then humanely euthanized according to IACUC protocol. The spleens were collected from all mice and placed in containers for further analysis. Photographs of mice from each group were conducted. I met with Dr McVey and Joanne Gresens; the safety officer for the USDA CGHAR. We discussed the necessity of producing a PowerPoint training module that could be used to teach new employees (especially students (high school and under-grad)) and temporary employees' basic biosafety procedures and guidelines in a BSL-2 laboratory. I will gather data and information from Joanne after my return from the FRONTIER field trip to the Mexico border this next week. My task is to assemble an appropriate PowerPoint to with accompanying quiz (to aid with certificate of completion) addressing the desired biosafety guidelines and tools. I spent the afternoon familiarizing and writing about the Biosafety levels (BSL), bio-containment, and the reasons for these designations and practices.

6/18-19/2012

I spent time in Las Cruces, NM.

6/20/2012 6:00 AM – 12:30 PM Location Las Cruces, NM at the NMSU campus: 6.5 Hours

FRONTIER group from KSU as well as students from K-State, New Mexico State University (NMSU), Georgia Tech, Minnesota, and professionals from companies such as Cargill were also in attendance. Dr Jason Ackleson (Ass. Prof. NMSU Dept. of Government) gave an overview of the border. He explained that the US-Mexico border is 1,951 miles long with 700 miles being fenced. Along this border there are 25 ports of entry (POE) that processes approx. 50 million people, 4 million trucks, and 90 million vehicles annually. He also stated that as of 2009 there are approx. 20, 119 agents that work to control the border. Dr Ackleson discussed the occurrence of border control interdiction and that trade security tension is rising. He stated that the desire is to have a secure and free flow of trade across the US-Mexico border.

After the overview the group attended a round table discussion at the Immigration Policy and Human Rights: Perspective from Border Communities Conference in the Corbett Student Union, NMSU. The individuals represented different groups, Dr Jason Ackleson, United States Citizenship and Immigration Service (USCIS); Sarah Nolan (PICO/Café) the local border communities; Viki Gaubeca American Civil Liberties Union (ACLU); and Dr Joe Heymen, an Anthropologist from University of Texas El Paso (UTEP). As a whole the committee discussed the lack of immigration reform at the national level, the increasing enforcement measures and the direct as well as social impact on communities in the US-Mexico border region, and the challenges community organizations face. I took additional notes on the individual speakers during their separate speaking times. Basically, all the speakers believe that there is disconnect from individuals in Washington D.C. and the border region. There is a belief, held by the ACLU, that border patrol officers use excessive force and need to be better trained. Additionally, there was a large push for allowing individuals who have lived in the US for a specific period of time, even if illegally, be granted US citizenship. These issues impact the border security and could directly affect trade and commerce between the US and Mexico.

12:30 PM – 7:00 PM Santa Teresa, NM United States Port of Entry (USPOE): 6.5 Hours

After the conference we caravanned to the USPOE at Santa Teresa. We first arrived at the NM Border Authority and were welcomed by Marco Herrera, International Logistics Manager for the POE. We then met with Officer Cooke, a US Customs and Border Protection (USCBP) agent. He is an entomologist by training and runs the inspection of pedestrian traffic and commercial/non-commercial traffic at the POE for agricultural related products and by-products. He provided a tour of their onsite laboratory, which is used to analyze suspected products. A power point presentation was given highlighting regulatory authorities, pests, disease, and the function of “safeguarding” of agriculture in the US. We then met with a Food and Drug Administration (FDA) inspector, who discussed how commercial/non-commercial freight trucks are selected, inspected, processed, and the protocol for passing/failing trucks.

The group then met with David Lucero, Division Director of New Mexico Department of Agriculture, at the Chihuahua Regional Cattle Union Livestock Facilities. David explained that the purpose of the facility is to process and pen cattle at the border he also illustrated the procedures for the livestock crossing. He explained that there are an average of 300,000 head of cattle processed at the facility and that they are destined for feedlots in Texas, Arizona, New Mexico, and the Mid-Western States. There are two facilities one on either side of the border. On the Mexico side the United States Department of Agriculture (USDA) veterinarian and staff inspects and certifies health of the animals. Upon approval of health and paperwork, the USCBP agents open the gate to the fence and the cattle are literally pushed across the border to the US facility pens.

7:00 PM – 9:00 PM La Posta restaurant, Mesilla, NM: 2 Hours

The caravan next traveled to Mesilla, NM to La Posta restaurant where individuals from the day as well as the NM Secretary of Agriculture, Jeff Witte. Here we discussed the events of the day and raised questions from things observed.

6/21-22/2012

I spent time in Las Cruces, NM.

6/25/2012 8:30 AM – 1:15 PM & 1:45 PM – 5:00 PM: 8 Hours

I arrived and met with Drs Ruder, McVey, and Drolet to receive a tour of the new vivarium lab that is being worked on and addressed things to be done to get the laboratory ready for US Animal and Plant Health Inspection Service (APHIS) to conduct their official inspection in the next few weeks. This laboratory will allow for the housing, handling, and use of mice, rats, hamsters, guinea pigs, and rabbits in research trials with the live RVFV in the (BSL-2) facility. We discussed the appropriate set up and safety procedures for such a lab as well as the required materials for this facility. Occupational safety with this type of lab is vital for the

workers/researchers. Dr Ruder will be working on the Standard Operating Procedures (SOP) that will be required for the Bio-Safety Laboratory Procedures Handbook.

I received a jump drive from Safety Officer, Joanne Gresens, containing the necessary materials and information necessary for producing a PowerPoint training module to teach new employees and temporary employees basic biosafety procedures and guidelines in a BSL-2 laboratory. I downloaded the material and began organizing the information.

I aided Dr Ruder with some organization of another lab that ABADRU is preparing for use. I visited with Dr Lehiy about epidemiology and viremia of RVFV and the possibility of an infected individual bringing the virus in to the US and causing the disease to spread a question raised by Kurkjian, *et al*, 2006. We also discussed mechanisms of delivering virus in research trials, such as those suggested with the NFD. The mechanism is not necessarily the desired goal, but rather the proteins that illicit the immune response without having to use the insect vector. Safety of the administrator is a high consideration in order to establish the correct proteins. Once the proteins are found then the need for a NFD, or traditional needle syringe will not be necessary. Additionally, the need to keep insects to be used as a vector will be completed.

6/26/2012 9:00 AM – 12:30 PM & 1:00 PM – 5:30PM: 8 Hours

I began sorting and reading through the occupational biosafety material, there is a lot of information. I began constructing PowerPoint presentation for ABADRU labs. I consulted with Dr McVey to ensure appropriate product was being produced. I discussed what Dr Lehiy would like students to know prior to beginning lab training. I took pictures of lab equipment and signs for PowerPoint presentation. I continued to work on the PowerPoint presentation.

6/27/2012 9:00 AM – 1:00 PM & 1:30 PM – 5:00 PM: 7.5 Hours

I continued sorting and collecting information on the occupational biosafety protocol for new employees. I also continued to produce the PowerPoint presentation, writing script for a

speaker and ensuring flow. I emailed the presentation to Dr Lehiy, Dr McVey, and Dr Ruder for feedback and suggestions.

6/28/2012 9:00 AM – 12:30 PM & 1:00 PM – 5:00 PM: 7.5 Hours

I arrived and aided the unit in moving and packing up an old lab and equipment to be set up in an available lab closer to the rest of the ABADRU labs. I received feedback on PowerPoint from Dr McVey suggested adding some slides on how to handle spills in a lab to the lab safety PowerPoint. He also requested an additional PowerPoint with a high school biology level explanation of RFVF and Blue Tongue virus (BTV) the two main arboviruses the unit works with. I worked on the second presentation, up to 23 slides.

I met with Dr Payne at 2:30 PM at the CVM, decided I need to reorganize my manuscript but that the information looked solid and that I was on a good path. I will have a revised version to her next Friday. I continued to work on presentations with critiques from the ABADRU staff.

6/29/2012 9:00 AM – 12:15 PM & 1:00 PM – 4:30 PM: 6.75 Hours

I arrived and met with Dr Ruder, discussed edits to the intradermal injection protocol as well as my rough draft. We later met in the lab and worked the device through a range of dose/pressure settings (0-500 μ L) and 60-120 psi to determine the best combination for the experiment next week. We decided on 50 μ L, 100 μ L, and 250 μ L to be the doses mostly likely to be applied in the laboratory setting. According to the device manual a psi of 60 is ideal for vaccinating fetal pigs and the devices max capacity is 120 psi. We decided to try 80-120 psi with a 20 psi increment for the experiment. We decided to get a section of donated Boer goat's (*Capra hircus*) hide out of the -20°C freezer for manipulation and practice, on Monday. We experimented with construction paper cones and Germ Juice® to visualize splatter from aerosolized injection material. We were able to visualize splatter with the device and decided it would be helpful for this experiment to use the cones. I cleaned the device and reassembled it for storage. I had lunch with the Unit staff today, treated by Dr McVey. I enjoyed getting to talk with and hear stories from the Unit's staff.

I discussed the probability of RVFV coming in to the US with Dr McVey as well as how other viruses have in the past and potentially could in the future. We discussed quarantine and eradication protocols and their impact on society, the public's health, and the economic infrastructure of the US. This discussion made me wonder what impact an epidemic would have on the health care system in the US. I continued to work on the PowerPoint presentations and brainstorm/come up with data sheet for the intradermal device experiment.

7/2/2012 9:00 AM – 5:00 PM: 8 Hours

I got the Boer goat's (*Capra hircus*) hide out of the -20°C freezer to defrost at room temperature. I prepped a work area in one of the ABADRU BSL-2 laboratories and using a #40 blade on hair clippers removed the hair off the goat hide. I met Dr Ruder in the laboratory to begin experimenting with the NFD pulse "gun". Using a 5% crystal violet solution, made from powdered crystal violet, we tested various dose and pressure combinations. With a dose of 100 µL we started with 80 psi and observed gross pooling of the injection solution at the subcutaneous (SQ) level; wetness was seen. At 100 psi with 100 µL the same observation was made, the dose went mostly SQ with staining seen in the dermis. At 120 psi with 100 µL no pooling was observed but a large 2 cm wetness was seen; the injection solution is going SQ. At a dose of 250 µL with 80 psi, pooling and wetness was seen at intradermal (ID) and SQ levels. At 100 psi and 250 µL a greater pooling and wetness was seen; there were some transdermal (TD) seen with some injections at this dose and pressure. At 110 psi and 250 µL, pooling and wetness was observed. There was also a distinct spray seen that caused injection solution to end up on the safety goggles, lab coats, and lab cabinets. At 120 psi and 250 µL, a greater pooling was seen with a >2.5 cm diameter wetness observed. At a dose of 500 µL and 80 psi there was lots of pooling seen with a good TD delivery with some superficial muscle delivery. At 100 psi and 500 µL there is lots of pooling and muscle penetration observed. At 120 psi and 500 µL there is no pooling but the injection solution is driven through the muscle in to the foam on the other side of the hide. Each pressure and dose combination was conducted several times followed by a dissection of the injection site with a #20 scalpel blade.

Due to these preliminary observations Dr Ruder and I decided that we should focus our efforts on small pressure/dose combinations for this first project. Therefore, with further experimentation we determined that for 50 μ L a 100 psi was appropriate, for 100 μ L a 90 psi was appropriate, and for 250 μ L a 90 psi was appropriated for this study. We decided to scrap the 500 μ L dosing due to the penetration of the muscle during delivery at all psi settings tested; assuming that this volume is too large for what is practical in the research laboratory.

7/3/2012 8:30 AM – 12:00 PM: 3.5 Hours

I worked on the power points, writing script for the presenter. The Sec of Ag sent a directive for all USDA facilities to close early for the upcoming Memorial Day weekend. The office closed at noon.

7/4/2012

Closed for Independence Day

7/5/2012 9:00 AM – 12:30 PM & 1:00 PM – 5:00 PM: 7.5 Hours

I worked on manuscript edits from my meeting with Dr Payne. I continued to do literature research focusing on epidemiology, healthcare impacts, and economic influence of zoonotic diseases. I also continued to work on the PowerPoint products for ABADRU. I prepared and set up for the intradermal injection experiment for tomorrow. I updated data sheets for collection and organization during the experiment. I pulled out the goat limbs from the -20°C to defrost overnight. I re-wrote and finalized the protocol with Dr Ruder.

7/6/2012 8:30 AM – 3:15 PM & 3:45 PM – 5:00 PM: 8 Hours

Dr Ruder and I prepped and set up for the intradermal device experiment. I shaved and cleaned the goat limbs. Using the device we followed a written protocol (see below), documenting, and photographing the results. After completion of the experiment we cleaned the

work site and removed waste from the laboratory. I began to document the results in excel to view in tables and graphs seen in Chapter 5.

P50 Needle Free Injection Device Evaluation Protocol:

- 1) Within 24 hours of experiment, thaw goat skin and limbs, which were stored in a sealed plastic bag at -20°C, to room-temperature.
- 2) Lay limbs on a work bench. Shave the injection site with a pair of hair clippers (#40 blade) and clean site.
- 3) Lube, assemble, and make ready to use the P50 Microdose injection system.
- 4) Attach 10 ml syringes containing either 5% crystal violet solution (crystal violet powder with deionized water) or Germ Juice ® (fluorescent dye) to the Luer-lock on the device. Utilize one data sheet for each solution type.
- 5) Wear protective eyewear, gloves, and lab coats during prep and throughout the evaluation.
- 6) To administer a dose, pull trigger while holding the Microdose perpendicular to the skin and press firmly (this will ensure the same pressure for delivery of dose).
- 7) Dose and pressurize for this evaluation with 50 µL/100 psi, 100 µL/90 psi, and 250 µL/90 psi for each respective combination.
- 8) Prime the gun using a 500 mL Pyrex glass bottle after each adjusted dose or psi this ensures proper amounts of both prior to use (gun is considered primed when a consistent stream is seen).
- 9) Mark injection sites with a fine tipped pen to allow visualization of the injection site as well as the appropriate marker for measuring observed wetness and surface splatter.
- 10) Wipe clean the tip of the injection device with a paper towel in between injections to ensure no carry over wetness/ solution from the previous injection.
- 11) Post injection, photograph the injection site. Measure any wetness or surface splatter in millimeters. Observe site for pooling and note if aerosolization is seen during the injection (record in table below)
 - a. If a cone is used, photograph the cone post injection. Any droplets visualized indicate an affirmative for aerosolization.
 - b. Record wetness if it is felt by the injection administrator during injection.
- 12) After initial data is collected, use a #20 scalpel blade to cut into the injection site (marked site). Obtain a picture of the dissected site and measure the injection depth.
- 13) Repeat instructions 6 – 10 for each of the 12 injection sites per dose/psi combo per limb.
- 14) Dispose of the goat limbs and hide via biohazard bags and take to an incinerator.
- 15) Clean work area using 70% ethanol, paper towels, and Clorox wipes.

16) Clean device with 70% ethanol and disassemble to ensure complete cleaning.

Intradermal P50 Device Evaluation													
	Limb Location Cone Used	Dose ul											
Pressure (PSI)	Data Points	50	50	50	50	50	50	50	50	50	50	50	50
100	Wetness												
	Pooling												
	Surface splatter												
	Aerosolization												
	Depth of Injection												
	Comments												
Pressure (PSI)	Data Points	100	100	100	100	100	100	100	100	100	100	100	100
90	Wetness												
	Pooling												
	Surface splatter												
	Aerosolization												
	Depth of Injection												
	Comments												
Pressure (PSI)	Data Points	250	250	250	250	250	250	250	250	250	250	250	250
90	Wetness												
	Pooling												
	Surface splatter												
	Aerosolization												
	Depth of Injection												
	Comments												

Key	0=no	1=yes			
Wetness	0mm=0	>0-5mm=1	>5-10mm=2		
Pooling	0 or 1				
Surface splatter	0mm=0	>0-5mm=1	>5-10mm=2	>10-15mm=3	>15-20mm=4
Aerosolization	0 or 1				
Depth of Injection	0mm=0	>0-2mm=1	>2-3mm=3	Muscle=4	

7/9/2012 10:00 AM – 5:00 PM: 7 Hours

I arrived and continued to edit and work on the intradermal/transdermal device manuscript, organizing collected data in table form. I also discussed results with Dr Ruder and what they could mean for future use of the device. I continued to edit my field experience report expanding the occupational biosafety section.

7/10/2012 9:00 AM – 1:30 PM & 2:30 – 5:00 PM: 7.5 Hours

I continued to work on my report. I worked on figures and graphs for the intradermal paper. Spoke with Dr Larson about statistical tools and appropriate tests for the data collected.

7/11/2012 10:00 AM – 5:15 PM: 7.25 Hours

I continued to work on my MPH report. I worked on figures and graphs for the intradermal paper. I began writing the results and discussion section of the intradermal paper.

7/12/2012 9:30 AM – 12:00 PM & 12:30 PM – 5:00 PM: 7 Hours

I continued to edit and work on the intradermal device manuscript. As well as finalize previous chapters in my report.

7/13/2012 9:30 AM – 12:00 PM & 12:30 PM – 5:00 PM: 7 Hours

I continued to work on the intradermal device manuscript. I met with Dr Larson at the KSU CVM to verify the stats in the paper. He made some suggestions and showed me how to use some other statistical tools in excel in the data attachment. Due to a time conflict rescheduled my defense date to 7/24/2012 at 10 AM. I made the necessary arrangements and phone calls. I continued to write about my field experience in the report.

7/16/2012 8:30 AM – 12:30 PM & 1:30 PM – 6:00 PM: 8.5 Hours

I continued to work on the intradermal device manuscript. I incorporated figures and tables in to the document, designating specific figures to match the text. I emailed both the biosafety and the virus overview PowerPoints to JoAnne Gresens, Dr McVey, and Dr Ruder for evaluation of product. I also emailed raw data from the 7/6/2012 experiment to Dr Ruder for his records. I formatted the tables and figures in my report and set up my table of contents. I continued working on the discussion for the intradermal paper.

I completed the discussion and edits to figures and tables for the intradermal paper. I plan to submit to Dr Ruder and Dr McVey this evening for their evaluation and feedback. I then continued to work on Chapter 4 of my report. I made edits and fielded ideas for the biosafety PowerPoint, it is almost completed.

7/17/2012 10:30 AM – 1:00 PM & 2:00 PM – 5:00 PM: 5.5 Hours

I continued to work on presentations. I met with Dr McVey and discussed presenting the PowerPoints to the ABADRU staff later this week. I continued to finish up the last chapter of my report, field experience.

7/18/2012 10:00 AM – 5:00 PM: 7 Hours

I continued to work on quizzes for PowerPoint presentations. I discussed the intradermal paper with Dr Ruder. We decided to send the paper to the other researchers. I worked on my final defense presentation.

7/19/2012 10:00 AM – 5:00 PM: 7 Hours

I worked on my final defense presentation.

7/20/2012 9:00 AM – 9:45 AM & 2:00 – 3:30 PM: 2.25 Hours

I met with Dr Ruder to discuss ongoing research. I left ABADRU mid-morning to attend a thesis defense at the KSU CVM. After, I met with Dr Payne to discuss preparations for my defense on Tuesday.

7/23/2012 9:30 AM- 4:15 PM: 6.75 Hours

I worked on my final defense presentation. I practiced my presentation in one of the ABADRU conference rooms to view the presentation and make sure I was within timing. I met with Dr McVey to discuss my defense.

7/24/2012:

I conducted my final defense for my MPH at 10:00 AM at the KSU CVM in Moiser N202.

7/26/2012 10:00 AM – 5:00 PM: 7 Hours

I worked on the edits that my committee recommended from my report. I worked on finalizing the presentations and put them in a file for the ABADRU to access. I returned text books and materials lent by the staff.

7/27/2012:

I attended a luncheon for myself and another intern that had completed her internship.

Total: 275.5 Hours of Field Experience