On-farm evaluation of a needle-free injection device to vaccinate beef calves under Western Canadian conditions

By

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ABSTRACT

This study was conducted to compare animal performance, presence of skin reactions and immune response following vaccination of beef calves via needle-free (NF) and needle-syringe (NS) vaccination techniques. Spring-born (Study A) and fall-born (Study B) calves were vaccinated against bovine viral diarrhea virus (BVDV) and *Clostridium chauvoei* (*C. chauvoei*) via NF and NS vaccination techniques. The parameters measured in this study included body weight (BW), skin reactions and serum antibodies. Animal performance and antibody levels against BVDV and *C. chauvoei* did not differ between vaccination techniques. However, NF vaccinated calves had a greater frequency of skin reactions when compared to NS vaccinated calves, except for day 42 of Study B. It can be concluded that a needle-free injection device (NFID) can be used effectively to stimulate an immune response without impacting animal performance, but may cause a greater frequency of skin reactions.

Keywords: bovine viral diarrhea virus, *Clostridium chauvoei*, needle-free vaccination, needle-syringe vaccination, immune response, antibody level, animal performance, skin reactions, beef calves

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ABBREVIATIONS

A. marginale	=	*Anaplasma marginale*
BHV-1	=	Bovine herpes virus-1
BRD	=	Bovine respiratory disease
BRSV	=	Bovine respiratory syncytial virus
BVDV	=	Bovine viral diarrhea virus
BW	=	Body weight
C. chauvoei	=	*Clostridium chauvoei*
C. haemolyticum	=	*Clostridium haemolyticum*
C. novyi	=	*Clostridium novyi*
C. perfringens	=	*Clostridium perfringens*
C. septicum	=	*Clostridium septicum*
C. sordellii	=	*Clostridium sordellii*
C. tetani	=	*Clostridium tetani*
CD	=	Cluster of differentiation
CFU	=	Colony forming unit
CO_2	=	Carbon dioxide
CP	=	Cytopathic
CTL	=	Cytotoxic T lymphocytes
d	=	Days
E. coli	=	*Escherichia coli*
ELISA	=	Enzyme-linked immunosorbent assay
FAT	=	Fluorescent antibody tests
gC	=	Glycoprotein C
HACCP	=	Hazard Analysis and Critical Control Points
IBR	=	Infectious bovine rhinotracheitis
ID	=	Intradermal
IF	=	Immunofluorescence

IFN	=	Interferon
IFN-γ	=	Interferon-gamma
IHC	=	Immunohistochemistry
IIF	=	Indirect immunofluorescence
IL	=	Interleukin
IM	=	Intramuscular
LCs	=	Langerhans cells
LP	=	*Leptospira pomona*
MH	=	*Mannheimia haemolyitca*
MHC	=	Major histocompatibility complex
MLV	=	Modified-live virus
N_2	=	Nitrogen gas
NCP	=	Noncytopathic
NF	=	Needle-free
NFID	=	Needle-free injection device
NK	=	Natural killer
NS	=	Needle-syringe
OD	=	Optical density
PAMPs	=	Pathogen-associated molecular patterns
PBS	=	Phosphate buffered saline
PCR	=	Polymerase chain reaction
PI	=	Persistently infected
PI-3	=	Parainfluenza-3 virus
PRRs	=	Pattern-recognition receptors
PRRSV	=	Porcine reproductive and respiratory syndrome virus
PSI	=	Pounds per square inch
RNA	=	Ribonucleic acid
RT-PCR	=	Reverse transcription polymerase chain reaction
S/P RATIO	=	Sample to positive ratio

SC	=	Subcutaneous
TCR	=	T cell receptor
Th	=	T helper
VI	=	Virus isolation
VN	=	Virus neutralization
WBC	=	White blood cell

1. INTRODUCTION

Cattle are often exposed to a number of different pathogens, including viruses and bacteria, which can result in disease. In order to protect cattle from becoming infected, vaccination against potential pathogens is a common practice in beef production systems and is given as part of regular husbandry practices to improve animal health and increase animal productivity.

Traditionally, vaccines are administered to cattle using a needle-syringe (NS) vaccination technique. This technique has been used extensively in cow-calf beef production systems because it is simple to operate, relatively inexpensive and easily adaptable to different production environments. However, potential disadvantages of the technique, such as broken needle fragments in meat cuts (Stier, 2003; van Drunen Littel-van den Hurk, 2006), needlestick injuries (Weese and Jack, 2008) and disease transmission (Otake et al., 2002; Reinbold et al., 2010), have become a growing concern to producers, consumers and the beef industry. These disadvantages have led to the investigation of alternative vaccination techniques to vaccinate cattle, such as the use of needle-free injection devices (NFID). Needle-free (NF) vaccination techniques may offer advantages over conventional NS vaccine delivery methods, including elimination of broken needles (Chase et al., 2008a; Stier, 2003), reduced disease transfer (Reinbold et al., 2010), reduced vaccination time (Mousel et al., 2008), greater antigen dispersion (Bennett et al., 1971; Chase et al., 2008a; Parent du Chatelet et al., 1997; Reis et al., 1998), elimination of needle disposal (Chase et al., 2008a) and elimination of needle-sticks to individuals giving injections. Needle-free injection devices are triggered by

mechanical compression as the nozzle touches the skin, which produces a high pressure stream that can penetrate the skin and deposit the vaccine into the desired tissue (Jackson et al., 2001; Mousel et al., 2008).

Previous research in cattle (Hollis et al., 2005a; Hollis et al., 2005b; Pires et al., 2007; van Drunen Littel-van den Hurk,S., 2006) has demonstrated a comparable and sometimes enhanced immune response when vaccines are delivered with NF compared to NS vaccination techniques, depending on the sex of the animals and the vaccine antigen measured. However, little research has been conducted on evaluating a NFID when it is used to vaccinate cattle in a temperate climate, such as Western Canada.

This study was conducted to examine animal performance, presence of skin reactions and immune response following vaccination of beef calves against bovine viral diarrhea virus (BVDV) and *Clostridium chauvoei* (*C. chauvoei*) using NF and NS vaccination techniques.

2. LITERATURE REVIEW

2.1. PROTECTON AGAINST DISEASE

Protection of an animal's body against disease comes from a complex system of overlapping and interlinked defense mechanisms that together can destroy or control invading pathogens (Tizzard, 1992). This complex system is defined as the immune system, which can be further divided into two branches, innate and adaptive immunity, which collaborate to protect the body against disease (Kindt et al., 2007).

2.1.1. Innate immunity

The innate immune system is found in all organisms and is considered the first line of host defense against pathogens (Akira et al., 2006; Medzhitov and Janeway, 1997). When microorganisms evade the physical and chemical barriers of the body, which include skin, mucous membranes and acid components, various chemical and cellular defenses of the innate immune system take over (Kindt et al., 2007; Tizzard, 1992). The cells and tissues of the innate immune system are capable of recognizing microorganisms via germline-encoded pattern-recognition receptors (PRRs), which are located on the surface of cells of the innate immune system (Akira et al., 2006). There are numerous PRRs, including Toll-like receptors, which recognize and bind to distinct microbial components known as pathogen-associated molecular patterns (PAMPs) that are located on the surface of pathogens (Akira et al., 2006; Kindt et al., 2007; Medzhitov and Janeway, 1997). Binding of PRRs to PAMPs, activates intracellular signaling cascades, which induce the expression of a variety of unique genes involved in pro-

inflammatory immune responses (Akira et al., 2006). Macrophages, neutrophils, mast cells, eosinophils, natural killer (NK) cells and dendritic cells are the key cells involved in innate immunity, all of which bear PRRs and have distinct roles in responding to the presence of foreign organisms (Janeway and Medzhitov, 2002).

The activation of the innate immune response is extremely important as it has been reported as a prerequisite for triggering the adaptive immune response (Akira et al., 2001). In fact, in the absence of an innate immune system, the response of the adaptive immune system may be quite feeble (Kimbrell and Beutler, 2001; Tizzard, 1992).

2.1.2. Adaptive immunity

Contrary to innate immunity, which is found in all organisms, the adaptive immune system is only found in vertebrates and is capable of self-nonself discrimination, indicating that the immune system only responds to foreign antigens (Bendelac et al., 2001; Medzhitov and Janeway, 1997). The adaptive immune system can recognize and initiate a protective immune response against potentially lethal pathogens, but is also able to remember previous pathogen encounters (Cooper and Alder, 2006). This ability to remember pathogens allows for the immune system to either repel a second invasion or quickly eliminate the recurrent pathogen as a result of a faster and more efficient immune response (Cooper and Alder, 2006). Vaccination of livestock targets the adaptive immune system to predispose animals to antigens that they may encounter in the future, so that the animals can elicit a quick and effective memory immune response against the antigen in order to prevent infection.

Adaptive immunity is mediated by both T and B lymphocytes (cells), which express clonally distributed antigen receptors (Bendelac et al., 2001; Medzhitov and Janeway, 1997). Both T and B lymphocytes use their cell-surface antigen receptors to recognize and bind to antigenic configurations of specific pathogens through cell membrane proteins called major histocompatibility complex (MHC) molecules, which can be divided into two major types: MHC I and MHC II (Kindt et al., 2007; Tizzard, 1992). There are two major branches of adaptive immunity: cell-mediated immunity, which is mediated by T lymphocytes, and humoral immunity, which is mediated by B lymphocytes and the production of antibodies (Jiang and Chess, 2009).

2.1.2.1. Cell-mediated immunity

The principle role of cell-mediated immunity is to detect and eliminate intracellular pathogens, such as viruses and intracellular bacteria (Kindt et al., 2007). T lymphocytes are produced in the bone marrow and then migrate to the thymus for maturation, and once mature, express a unique cell surface antigen-binding molecule, called the T cell receptor (TCR) (Andersen et al., 2006). These TCRs have the ability to recognize and bind to antigens, in the form of short peptides, which are bound to an MHC molecule (Moss et al., 1992). Mature T lymphocytes express on their TCRs two types of cluster of differentiation (CD) molecules, $CD4^+$ and $CD8^+$, and as a consequence are categorized as T helper (Th) cells and cytotoxic T lymphocytes (CTL), respectively (Tizzard, 1992). Cluster of differentiation 8^+ CTL bind only to MHC I complex molecules, while $CD4^+$ Th cells bind only to MHC II complex molecules (Moss et al., 1992; Tizzard, 1992). Cluster of differentiation 4^+ Th lymphocytes can be further

classified into two different types, Th1 or Th2, based on their cytokine profile (Romagnani, 1996). T helper 1 cells are able to produce interferon-gamma (IFN-γ), interleukin (IL)-2, and tumor necrosis factor-α, which promotes macrophage activation, antibody-dependent cell-mediated cytotoxicity, phagocytosis, intracellular killing and delayed type hypersensitivity (Mosmann and Coffman, 1989; Romagnani, 1994). Contrary to Th1 cells, Th2 cells produce IL-4, IL-5, IL-6, IL-9, IL-10 and IL-13 in response to pathogens, which provides optimal help for humoral immune responses and the production of antibodies (Romagnani, 1996).

In addition to antigen-specific T lymphocytes, the cell-mediated immune system also consists of antigen non-specific cells such as NK cells, macrophages, neutrophils and eosinophils (Kindt et al., 2007).

2.1.2.2. Humoral immunity

Before a humoral immune response is initiated, B lymphocytes, which mature in the bone marrow or Peyer's patches, must bind to antigens via a membrane-bound immunoglobulin B cell receptor (Pape et al., 2007; Tizzard, 1992; Vos et al., 2000). Thereafter, B lymphocytes present antigen peptide-MHC II complexes to $CD4^+$ Th cells (Garside et al., 1998; Okada et al., 2005; Pape et al., 2003). During B and Th cell interaction, B cells receive signals from $CD4^+$ Th cells that lead to B cell clonal expansion, somatic mutation, immunoglobulin class switching, and plasma cell differentiation (Mills and Cambier, 2003; Okada et al., 2005). Activated B lymphocytes will then differentiate into either high-affinity memory B cells or antibody-secreting plasma cells that are specific for foreign antigens (Vinuesa et al., 2005). Antibodies

secreted from these plasma cells are capable of binding to foreign antigens, neutralizing them and facilitating their elimination (Kindt et al., 2007).

A humoral immune response to a pathogen can develop following infection, vaccination or by passively transferring antibodies from the colostrum of dams. Antibodies present in the colostrum of cows are ingested by newborn calves, giving them the ability to fight off infectious disease (Chase et al., 2008b). Neonatal and young calves depend on passive immunity transferred from dams as the primary basis for immunological protection during the first two-four weeks of life, because newborns are immunologically naïve at birth (Chase et al., 2008b). The ability of the newborn calf to absorb colostral antibodies begins to decrease six-twelve hours after birth and ends by 48 hours after birth due to a process known as intestinal closure (Chase et al., 2008b; Sangild, 2003). Although maternally-derived antibodies can protect calves from infection, they can also neutralize the antigen present in a vaccine, thereby interfering with the immune response generated via vaccination (Chase et al., 2008b; Tizzard, 1992).

2.1.3. Vaccination

Vaccination has been reported as a cost-effective measure to promote animal health and has been primarily developed to prevent disease as a result of infection (van Oirschot et al., 1999). In 1796, the world's first vaccination was performed by Edward Jenner, who inoculated an eight-year-old boy with pus from a cowpox lesion (Stern and Markel, 2005). Six weeks after inoculation, Jenner exposed two sites of the boy's arm to smallpox and the boy was unaffected and protected from subsequent exposures.

2.1.3.1. Routes of vaccine administration

The most common and simplistic methods of administrating vaccines to cattle are via the subcutaneous (SC) or intramuscular (IM) routes, both of which are capable of stimulating systemic immunity (Kahn et al., 2011a; Buddle et al., 2008; Itzchak et al., 1992; Jericho and Babiuk, 1983). Subcutaneous injections are administered into the SC space directly below the skin, while IM injections are administered into the muscle. However, vaccines may also be successfully administered to cattle via intranasal and oral routes (Conroy, 2008; Buddle et al., 2008; Jericho and Babiuk, 1983). Furthermore, recent advances in vaccination technology, such as NF vaccination, has allowed for vaccines to be administered into and across different skin layers, also known as intradermal (ID) or transdermal routes (Conroy, 2008; Kahn et al., 2011a; Itzchak et al., 1992).

In order to reduce economic losses and injection site lesions from occurring in high quality and high priced beef cuts, programs such as “Beef Quality Assurance” and the “Canadian Cattleman’s Quality Starts Here Program” have been initiated in the beef industry to encourage that injections be given in the neck and SC when possible. Van Donkersgoed et al. (2000) determined that products administered SC in the neck produced minimal tissue damage and economic losses.

As intradermal vaccinations are placed within or between the layers of the skin , Langerhans cells (LCs) present at the skin may be able to enhance the immune response stimulated following ID vaccination. These cells have high migratory mobility and are some of the most effective antigen presentation cells in primary immune responses

(Bodey et al., 1997). Furthermore, it has been reported that effective targeting of LCs during vaccination will open up novel applications in disease control (Chen et al. 2002).

2.1.3.2. Types of vaccines

2.1.3.2.1. Modified-live virus and inactivated vaccines

Vaccines commonly used in the vaccination of beef cattle may contain either modified-live or inactivated organisms. Modified-live virus (MLV) vaccines contain an attenuated strain of the pathogen, which although weakened or altered in some manner to minimize the virulence of the pathogen, can still replicate in the host, inducing an immune response (Kahn et al., 2011a; Mitragotri, 2005). An inactivated vaccine is most often created by inactivating the pathogen in some manner, usually by using heat or chemicals (Kahn et al., 2011a). However, the inactivated organism can still be recognized by the host's immune system in order to stimulate an immune response (Kahn et al., 2011a). Inactivated organisms are commonly less immunogenic than MLV vaccines and as a result, commonly require the use of adjuvants to increase their immunogenicity (Kahn et al., 2011a; Mitragotri, 2005). These adjuvants may, however, cause local inflammation and hypersensitivity reactions, leading to skin reactions or lesions arising at or below the skin surface (Kahn et al., 2011a; Mitragotri, 2005).

The advantages and disadvantages to both inactivated and MLV vaccines are described in Table 1.

Table 1 Advantages and disadvantages of modified-live virus and inactivated vaccines (Kahn et al., 2011a; Mitragotri, 2005; Radostits et al., 2000).

	ADVANTAGES	DISADVANTAGES
INACTIVATED VACCINE	- No possibility of pathogen returning to a virulent state - Safe for use in pregnant animals - No induction of immunosuppression - No contamination with foreign agents	- High cost - Two initial doses required - Larger vaccine volumes required - Adjuvant that may cause potential adverse reactions required - Short duration of immunity
MLV VACCINE	- Single dose vaccination - Stimulates a strong humoral and cellular immune response - Long duration of immunity due to replication of virus - No adjuvant needed	- Pathogen may return to a virulent state - Possible immunosuppression - Not safe for use in pregnant animals - Considerable care required in vaccine preparation, storage and handling

2.1.3.3. Injection site reactions

Reactions can develop at injection sites following administration of vaccines and antimicrobials. Inflammation and skin reactions indicate that immune defenses of the body have been stimulated (Tizzard, 1992). However, lesions and abscesses can develop at injection sites (George et al., 1995a; Van Donkersgoed et al., 1997; Van Donkersgoed et al., 1999; Van Donkersgoed et al., 2000) costing the beef industry millions of dollars annually from losses in trim (Van Donkersgoed et al., 1997) , devaluation of cuts and consumer dissatisfaction from eating tough beef (George et al., 1995b) .

A survey was conducted in 1996 and 1997 at five Canadian packers to measure the prevalence of injection site lesions in the top butt, boneless blade, outside round,

inside round, and eye of the round (Van Donkersgoed et al., 1997). The estimated prevalence of injection site lesions were 18.8% in top butts, 22.2% in boneless blades, 4.9% in the eye of the round, 1.8% in the inside round and 7.6% in the outside round. George et al. (1995a) conducted a similar study to examine injection site lesions in carcasses of cattle receiving injections at branding and weaning. Four different products, a 2-mL clostridial vaccine, 5-mL clostridial vaccine, vitamin AD_3 and an oxytetracycline antibiotic, were administered IM in inside rounds at branding and were found to cause injection site lesions in 72.5%, 92.7%, 5.3% and 54.2% of the carcasses, respectively. Furthermore, injection site lesions and scarring have been observed in calves slaughtered 10-15 months following administration of numerous types of vaccines and antimicrobials, indicating that reactions may persist for prolonged periods of time (George et al., 1995a; Van Donkersgoed et al., 1999).

In 1997, losses associated with injection site lesions have been estimated at $8.95 per fed animal processed or $19 million dollars annually (Van Donkersgoed et al., 1997). In addition to trim losses, George et al. (1995b) reported that concentrations of insoluble and soluble collagen were much higher ($P<0.001$) in lesion-afflicted vs. control steaks, implying that lesions can cause severe tissue changes that can dramatically affect tenderness and consequently affect consumer satisfaction and retail price.

2.2. VACCINE ADMINISTRATION TECHNIQUES

2.2.1. Conventional needle-syringe vaccination

2.2.1.1. Advantages of needle-syringe vaccinations

Traditionally, vaccines are administered to cattle using a NS vaccination technique and this approach is currently being used in the industry. This technique is relatively inexpensive, easily adaptable to different production environments and is simplistic to operate. Another advantage of the NS vaccination technique is that most syringes are highly portable and can be readily transported. Syringes and needles are also available in a variety of sizes, giving producers and veterinarians the flexibility of injecting one or multiple animals, while obtaining the correct depth of administration.

2.2.1.2. Hazards associated with needle-syringe vaccinations

Despite the simplicity associated with the conventional NS vaccination technique, there are potential risks to producers and veterinarians, as well as consumers.

Inadvertent punctures or needlestick injuries can occur during NS vaccinations and needle disposals (Weese and Jack, 2008). Hafer et al. (1996) reported that 73% of surveyed swine veterinarians had incurred at least one needlestick throughout their career. A similar study determined that needlestick injuries were the most common injury reported by large animal veterinarians, as they occurred an average of 3.06 needlesticks/person/three years (Poole et al., 1999). The physical trauma of needlestick injuries are usually minor, but nonetheless are still a concern because of the plausible infections that could occur from inoculation of bloodborne pathogens, organisms from the animal's skin, organisms from fine-needle aspirates and organisms in vaccines

(Weese and Jack, 2008). Substances in vaccines and antimicrobials can also pose other potential risks to humans, ranging from local irritation to systemic reactions (O'Neill et al., 2005; Weese and Jack, 2008).

Broken needle fragments may also be detrimental as a consequence of its impact on consumer perception (Stier, 2003; van Drunen Littel-van den Hurk, 2006). Physical hazards, such as broken needles, are one of the concerns producers and meat packers should address in their Hazard Analysis and Critical Control Points (HACCP) plans (Hoff and Sundberg, 1999). Hoff and Sundberg (1999) characterized the strength and limitations of hypodermic needles in order to determine the risk of leaving broken needles in the flesh of animals. They investigated the structural integrity of needles from two manufactures at two lengths (1.0 and 1.5 inches), three gauges (20, 18, 16) and two hub materials (aluminum and plastic). The authors reported four basic types of failure, including needle failure, needle/hub joint failure, hub failure and needle breakage, all of which could leave broken needles in animal flesh. Furthermore, it was observed that straightening an already bent needle increases needle failure.

Use of a single needle to inject multiple animals may result in blood-borne infectious diseases, such as bovine leukosis and anaplasmosis, to be transmitted from one animal to another (Coetzee et al., 2010; Hollis et al., 2005a; Reinbold et al., 2010). Vaccinating susceptible steers with a needle previously used to inject a steer infected with *Anaplasma marginale* (*A. marginale*), resulted in six of ten susceptible steers becoming infected with *A. marginale* through iatrogenic transmission (Reinbold et al., 2010). Similarly, Otake et al. (2002) also evaluated the mechanical transmission of porcine reproductive and respiratory syndrome virus (PRRSV) from infected to

susceptible pigs by needles. It was determined that the transmission of PRRSV through contaminated needles occurred in six out of twelve susceptible pigs.

2.2.2. Needle-free vaccination

The previously mentioned hazards and risks associated with NS vaccinations have led to the investigation of alternative vaccination techniques, such as NFID. Needle-free injection devices were developed in the 1940's and have been used over the years to vaccinate humans (Hingston et al., 1963) and swine (Chase et al., 2008a). Since the 1950's, NFIDs have been used in humans to administer vaccines in military induction centers, epidemic situations, and mass immunization campaigns around the world (Reis et al., 1998).

2.2.2.1. Mechanics of needle-free vaccinations

Needle-free injection devices utilize mechanical compression, which occurs as the nozzle touches the skin, to power vaccine injections through a small orifice (Jackson et al., 2001; Mousel et al., 2008). A high pressure stream then penetrates the skin and deposits the vaccine into the desired tissue (Jackson et al., 2001). A typical NFID generally consists of an energy source, amplifying system, high-pressure hose and handpiece (Grant, 2010). Vaccines injected via NF techniques penetrate through different skin layers and are deposited in either the ID, SC or IM tissue layers, depending on the pressure or force at which the vaccine leaves the orifice (Stuart Webb, personal communication; Mike Agar, personal communication; Mitragotri, 2005).

Needle-free injection devices can be categorized according to the source of power used to attain the mechanical compression to power injections: spring-powered, battery-powered and compressed-gas-powered (Chase et al., 2008a). Spring-powered devices have been primarily used for SC administration and are considered to be compact, highly durable and low cost (Chase et al., 2008a; Mitragotri, 2006). Both spring-powered and battery-powered devices have limited range of force and versatility, while gas-powered devices have sustained force generation, more flexibility, and the ability to administer larger vaccine volumes (Chase et al., 2008a; Mitragotri, 2006). Disadvantages of gas-powered NFIDs include the cumbersome size and need for multiple components, as well as an exhaustible energy source (Chase et al., 2008a).

Currently, two models of NFIDs are distributed in Canada for animal use: AcuShot (AcuShot Needle-Free) and Pulse (Pulse NeedleFree Systems).

2.2.2.1.1. AcuShot Needle-Free

Injection devices manufactured by AcuShot Needle-Free use a rechargeable lithium polymer battery which powers an inert nitrogen (N_2) power cylinder (Grant, 2010; Mike Agar, personal communication). There are five colour-coded power cylinders available which provide orifice pressures ranging from 6,000 pounds per square inch (PSI) to 30,000 PSI (Mike Agar, personal communication). The lifespan of a cylinder is approximately 500,000 injections (Mike Agar, personal communication) and vaccines are administered from a handpiece equipped with a 0.3 mm diameter orifice (Grant, 2010; Mike Agar, personal communication).

2.2.2.1.2. Pulse NeedleFree Systems

Injection devices manufactured by Pulse NeedleFree Systems are powered by compressed carbon dioxide (CO_2), compressed air or N_2 gas (Grant, 2010). Orifice pressures range from 1,000 PSI to 11,000 PSI and vaccines are administered through a high-pressure hose towards a handpiece with either a 0.27 mm or 0.35 mm diameter orifice, depending on the size of the animal (Stuart Webb, personal communication). Three different models of Pulse NFIDs are available depending on the dose of vaccine delivered: Pulse 50 (capable of injecting 0.1-0.5 mL), Pulse 250 (capable of injection 0.5-2.5 mL) and Pulse 500 (capable of injection 1.0-5.0 mL).

2.2.2.2. Advantages of needle-free vaccinations

Needle-free vaccination techniques offer a number of advantages compared to conventional NS vaccine delivery methods. Firstly, many of the potential concerns of NS vaccination techniques associated with the presence of needles are eliminated, including the risk of broken needles in meat cuts and the risk of needlestick injuries.

Secondly, Reinbold et al. (2010) demonstrated that in ten susceptible steers NF vaccination did not transfer *A. marginale* from one animal to another, whereas the conventional NS vaccination technique resulted in six of ten steers becoming infected through iatrogenic transmission. Contrary to these findings, Sweat et al. (2000) demonstrated that NFIDs can transfer volumes of blood that are sufficient to contain pathogenic agents, which can subsequently cause infection. Therefore, due to the contradictions seen in the two studies mentioned above, future research may be required

before it can be concluded that NFIDs are capable of reducing transmission of disease from one animal to another.

Thirdly, NFIDs may be able to enhance the immune response stimulated following NF vaccination, due to the mechanics of the injections. Bennett et al. (1971) injected adult Spraugue-Dawley rats with trypan blue and radiopaque dyes using a NFID (Syrijet Mark II), and determined that immediately after the dyes penetrated the dermis, they spread laterally in all directions and showed a noticeable SC spread. Contrary to NS injections which bypass the skin when depositing vaccines and travel along SC fat resulting in little vaccine dispersion due to low velocity, vaccines delivered via NF injection are dispersed more widely in the tissues (Fig. 1) and penetrate through different layers of the skin (Bennett et al., 1971; Grant, 2010). This results in increased inflammation, which typically causes recruitment of immune-competent inflammatory cells and allows for a larger contact volume between the vaccine antigen and immune cells (Giudice and Campbell, 2006; Parent du Chatelet et al., 1997). Furthermore, the skin acts as a physical barrier and first line of immune surveillance against a pathogen, and is therefore a particularly attractive target for vaccines to travel through en route to SC or IM injections (Mitragotri, 2005; Peachman et al., 2003). As mentioned previously, LCs present in the epidermis of the skin may enhance the immune response stimulated following NF vaccination as they have high migratory mobility and are some of the most effective antigen presentation cells in primary immune responses (Bodey et al., 1997).

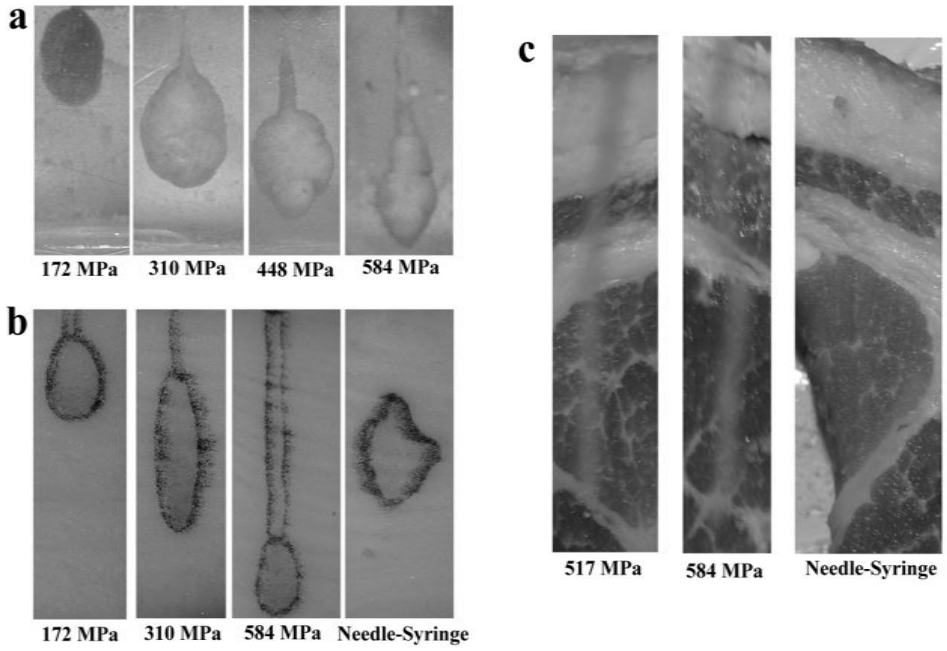

Fig. 1. Dispersion of dye into: (a) gel showing an increasing depth of penetration and elongation of the elliptical distribution with pressure; (b) poly foam circled in permanent marker for clarity; (c) swine tissue with a needle-free and needle-syringe device (used with permission from Grant, 2010)

Lastly, Mousel et al. (2008) compared the injection time of a NFID to a NS vaccination technique and demonstrated that the time to inject 7-9 lambs with a NFID was faster (60.6 seconds) than vaccination with a NS technique in which a new needle was used on every lamb (155.3 seconds).

2.2.2.3. Disadvantages of needle-free vaccinations

Although NF vaccination techniques may offer many advantages over conventional NS vaccination, there are several disadvantages that could hinder or delay the acceptance of the technology.

Initially, the start-up cost of the NF injection equipment, as well as the additional maintenance costs, is probably the largest disadvantages of the system. The cost of the Pulse NFID is approximately $2,500 to $3,000 for the device only (Stuart Webb, personal communication), while the cost of the AcuShot NFID is approximately $4,000 to $5,000 for the device and replacement parts (Mike Agar, personal communication).

In addition, NF vaccination techniques not only require additional training for vaccine administration and maintenance, but also require additional maintenance as a consequence of the complexity of the equipment compared to NS vaccination (Chase et al., 2008a; Reis et al., 1998; Stuart Webb, personal communication).

Use of NF vaccination techniques in humans has also been associated with higher levels of pain when compared to NS vaccinations (Jackson et al., 2001). However, comparable research has not been conducted in livestock. Furthermore, NF vaccination in humans has been associated with more frequent injection site reactions, such as soreness, redness and swelling (Mathei et al., 1997; Williams et al., 2000). In addition, Gerlach et al. (2009) determined that swine injected with an aluminum hydroxide adjuvant via a NF method as compared to an NS injection method at injections sites previously contaminated with *Arcanobacterium pyogenes*, had a greater number of abscesses in both the neck (P=0.0625) and ham (P=0.0313). Similarly, Sutterfield et al. (2009) observed that NF injections slightly increased the microbial translocation of *Escherichia coli* (*E. coli*) into the muscle interior by as much as 0.5 $\log_{10}$ colony forming unit (CFU)/g compared to needle injections when injected into the surface of beef loins contaminated with *E. coli*. Therefore, it is apparent that vaccination via NF techniques may inject contaminants on the surface of the skin/hair into the animals, such as *E. coli* and

Arcanobacterium pyogenes, resulting in injection site reactions and abscesses. Contrary to the above findings, Houser et al. (2004) reported that there was no significant difference in the number of gross or histological lesions observed at the injection sites of swine carcasses following vaccination using a NF or NS vaccination technique.

Finally, NFIDs have been reported to have a delivery error which results in small amounts of vaccine residue, ranging from 0.0004 mL (Chase et al., 2008a) to 0.0026 mL (Schloesser et al., 2008), left on the surface of the skin/hair. Visually, residues may appear substantial and may hinder or delay the acceptance of the technology (Jones et al., 2005), as a consequence of concerns regarding its ability to deliver the necessary vaccine dose.

2.2.3. Immune responses of needle-free vs. needle-syringe vaccination techniques

Immune response, as measured by antibody response, following vaccination of 4-6 month old dairy and beef heifers with 1010 CFU of Brucella abortus strain RB51 was comparable between NF and NS vaccination techniques (Pires et al., 2007). Similarly, studies conducted in rabbits (Aguiar et al., 2001), sheep (Mousel et al., 2008) and swine (Houser et al., 2004) have also demonstrated a comparable immune response between NF and NS techniques.

Van Drunen Little-van den Hurk (2006) demonstrated that calves vaccinated with a bovine herpes virus-1 (BHV-1) glycoprotein-C (gC) vaccine using a NF injection system (Biojector 2000, Bioject Inc.), were able to induce significantly enhanced gC-specific antibody titers, lymphoproliferative responses and numbers of IFN-γ secreting

cells in the peripheral blood when compared to those vaccinated via NS. Similarly, an enhanced immune response has also been observed in rabbits (Aguiar et al., 2001).

There are also studies that have found both a comparable and enhanced immune response between NF and NS techniques depending on sex of the animal (Hollis et al., 2005a), antigen measured (Hollis et al., 2005b) and NFID used (Jackson et al. 2001). For example, Hollis et al. (2005a) determined that the immune response, as measured by antibody level, of 5-10 month old Holstein heifers to infectious bovine rhinotracheitis (IBR), *Mannheimia haemolyitca* (MH) and *Leptospira pomona* (LP), were not (P=0.11, P=0.51 and P=0.51, respectively) different between NF and NS vaccination techniques. However, when measured in 5-10 month old Holstein steers, antibody response to IBR (P=0.01) and MH (P=0.02) were higher in NF steers, while the response to LP (P=0.38) was not different between techniques. Furthermore, immune response, as measured by antibody level, of yearling feedlot steers (Hollis et al., 2005b) to IBR was found to higher (P=0.001) following vaccination with a NF technique compared to an NS technique, but the response to MH was not different (P=0.06) between techniques. Jackson et al. (2001) also reported that the antibody response elicited in humans to a trivalent inactivated influenza vaccine was higher (P=<0.01) in patients who received the vaccine via a NFID manufactured by Bioject (Biojector 2000, Bioject Inc.) compared to the NS technique or a NFID system manufactured by VitaJet (VitaJet, Bioject Inc.), which were comparable in the immune response elicited.

2.3. BOVINE VIRAL DIAHRREA VIRUS

Bovine viral diarrhea virus is a positive-stranded and enveloped ribonucleic acid (RNA) virus in the genus *Pestivirus*, belonging to the Flaviviridae family (Sandvik, 2005). Bovine viral diarrhea virus is classified into cytopathic (CP) or noncytopathic (NCP) biotypes according to the presence or absence of observable cytopathologic effects in infected cell cultures (Baker, 1995). The virus has been divided into two genotypes, BVDV1 and BVDV2 (Ridpath et al., 1994), and more recently has been further divided into four subgenotypes in North America: BVDV1a, BVDV1b, BVDV2a and BVDV2b (Flores et al., 2002; Fulton et al., 2005; Ridpath et al., 2000). The heterogeneity of the isolates results in differences in neutralizing epitopes, cytopathology and virulence, which hampers the ability to detect and control BVDV (Ridpath et al., 1994).

Clinical signs of BVDV infection may include fever, diarrhea, rapid respiration, pneumonia, erosive stomatitis, inappetance, lymphopenia and thrombocytopenia (Baker, 1995; Campbell, 2004; Carman et al., 1998; Houe, 1995). Infection with BVDV also causes reproductive failure characterized by decreased conception rate, abortion, stillbirth, weak neonates, congenital defects and the birth of persistently infected (PI) calves (Hansen et al., 2010; Radostits et al., 2000).

2.3.1. Persistently infected cattle

Infection of a naïve pregnant cow with a NCP BVDV strain in the first 150 days (d) of gestation causes transplacental infection of the fetus, resulting in the induction of immunotolerance to the infecting BVDV strain and persistent infection in the fetus (Hansen et al., 2010). Bovine viral diarrhea virus results in PI calves because the immune

system of the fetus, when exposed in the first 150 days of gestation, has not yet developed sufficient immunocompetence to recognize the infecting BVDV virus as foreign (Brownlie et al. 1998). As a result, the BVDV antigen is recognized as part of the host antigen repertoire. The ability of BVDV to interfere with the induction of a type 1 interferon (IFN) response in the host is another mechanism employed by the virus to develop persistent infections (Baigent et al., 2004; Charleston et al., 2001). The presence of a PI fetus in the womb of a pregnant cow causes the downregulation of chemokine receptor 4 and TCR signalling, thereby impairing fetal development and the fetal immune response (Smirnova et al., 2009).

Persistently infected cattle serve as a continuous and major source of BVDV in the environment due to life-long shedding of the virus (Houe, 1995). Persistently infected calves can be normal at birth, but are often unthrifty and usually succumb to fatal mucosal disease after superinfection with a homologous strain of CP BVDV (Brownlie, 1991; Radostits et al., 2000; Sentsui et al., 2001).

2.3.2. Immunosuppression caused by BVDV

Bovine viral diarrhea virus tends to have an affinity for infecting cells of the immune system, including B cells, T cells, monocytes, macrophages, and dendritic cells, which may cause immunosuppression (Sopp et al., 1994).

As it is an immunosuppressive agent, BVDV plays a pivotal role in the pathogenesis of bovine respiratory disease (BRD) by allowing opportunistic micro-organisms to induce respiratory and mucosal disease (Campbell, 2004; Potgieter, 1997; van Oirschot et al., 1999). Bovine respiratory disease is one of the most economically

important cattle diseases, which negatively impacts health, performance and carcass quality (Campbell, 2004; Gardner et al., 1999). Richer et al. (1988) investigated BRD outbreaks with multiple virus infections and demonstrated that BVDV was isolated from pneumonic cattle more frequently than any other virus. Furthermore, it has also been reported that the combined infection of BVDV with bovine respiratory syncytial virus (BRSV) or IBR causes increased severity of enteric and respiratory diseases (Brodersen and Kelling, 1999; Potgieter et al., 1984).

2.3.3. Detecting BVDV

The availability of rapid, economical, highly sensitive and simple diagnostic methods to detect persistent or acute BVDV infections is an important part of control programs designed to eradicate BVDV (Goyal and Ridpath, 2005).

2.3.3.1 Virus isolation

Virus isolation (VI) in cell cultures, followed by fluorescent antibody staining with BVDV-specific antibodies, is the gold standard for detecting persistent and acute BVDV infection (Hilbe et al., 2007; Ridpath et al., 2002; Saliki et al., 1997). Bovine viral diarrhea virus can be isolated from a number of animal specimens, including serum, whole blood, semen, nasal swabs, feces and fetuses (Goyal and Ridpath, 2005). However, the presence of antibodies against BVDV or low levels of virus may interfere with BVDV isolation from serum samples (Brock et al., 1998). As this technique is laborious and time consuming, it is not practical for use with large sample sizes (Cornish et al., 2005; Saliki et al., 1997).

2.3.3.2 Reverse transcription polymerase chain reaction assays

Reverse transcription polymerase chain reaction (RT-PCR) assays have become a popular diagnostic method because they are not affected by the presence of antibodies, have high sensitivity and specificity and have the ability to make use of pooled samples (Hilbe et al., 2007; Sandvik, 2005; Zimmer et al., 2004). False negatives are a concern with RT-PCR as the BVDV nucleotide sequence is quite variable, therefore, careful selection and testing of primers is important to maintain high sensitivity and specificity (Hamel et al., 1995; Hilbe et al., 2007; Ridpath et al., 1993). Hamel et al. (1995) has described a relatively quick and simple one-tube RT-PCR method using RNA extracted directly from a variety of bovine specimens, including whole blood and tissues.

2.3.3.3 Immunohistochemistry

Immunohistochemistry (IHC) assays based on tissue samples, such as ear notches, are popular as it can be used to analyze large sample sizes in a short period of time, there is no potential interference with maternal antibodies and sample collection is convenient (Goyal and Ridpath, 2005; Hilbe et al., 2007; Njaa et al., 2000). Disadvantages include intensive labour and time requirements, multistep processes prone to technical error, and finally, interpretation of results is subjective (Cornish et al., 2005).

2.3.3.4 Enzyme-linked immunosorbent assay

A number of antigen-capture enzyme-linked immunosorbent assays (ELISAs) have been proven to yield comparable results to VI, IHC and RT-PCR in detection of BVDV (Cornish et al., 2005; Fulton et al., 2006; Hilbe et al., 2007). There are a number

of commercially available ELISA kits, which are able to detect the BVDV antigen in nasal swabs, serum, plasma, whole blood and tissue samples. Antigen-capture ELISAs are rapid, cost effective, and simple to operate (Cornish et al., 2005; Fulton et al., 2006). However, these assays can result in false negatives due to the interference of maternal BVDV antibodies (Zimmer et al., 2004).

2.3.4. Measuring antibody response to BVDV

Serological techniques are used to detect and measure BVDV-specific antibodies, therefore making them effective tools to detect BVDV infection and measure immune response following vaccination (Radostits et al., 2000).

2.3.4.1 Virus neutralization

Virus neutralization (VN) is able to quantify the amount of antibodies present in cattle sera by determining the highest serum dilution required to neutralize a standard amount of virus infectivity in cell culture (Franco Mahecha et al., 2011). This technique is considered the gold standard and has been used by many researchers to measure the immune response following vaccination and/or challenge of cattle with BVDV (Kirkpatrick et al., 2008; Munoz-Zanzi et al., 2002; Zimmerman et al., 2006). Limitations of VN include time and labour requirements, as well as the cost (Franco Mahecha et al., 2011).

2.3.4.2. Enzyme-linked immunosorbent assay

Indirect, blocking and sandwich ELISA techniques to detect and measure BVDV antibodies have been shown to have a similar specificity, sensitivity and correlation when compared to VN (Graham et al., 1997; Graham et al., 1998; Howard et al., 1985; Kramps et al., 1999). Similar to VN, many research studies have effectively used ELISA to measure the immune response induced against BVDV following vaccination (Alvarez et al., 2012; Kurcubic et al., 2011; Pecora et al., 2009; Raue et al., 2011). Antibody detection/quantification ELISAs have been reported as being rapid, less labor intensive than VN, relatively inexpensive and suitable for automation (Franco Mahecha et al., 2011; Graham et al., 1997). However, factors including non-specific binding, quality of antigen coating the well, quality of test samples (ex: serum quality) and quality of test reagents may influence the results of ELISA tests (Schrijver and Kramps, 1998).

2.3.5. Vaccination

In order to reduce the economic impact of BVDV in feedlot cattle and reproductive females, vaccination has become an important component of BVDV prevention and control programs (Campbell, 2004; Chase et al., 2004; Radostits et al., 2000; van Oirschot et al., 1999). Failures to vaccinate or use vaccines properly have been associated with BVDV outbreaks in Quebec and Ontario (Bolin and Ridpath, 1992; Pellerin et al., 1994). In addition, the antigenic diversity and antigenic cross-reactivity among BVDV strains makes controlling BVDV by vaccination somewhat difficult (Edwards and Paton, 1995).

2.3.5.1. Types of vaccines

There are a wide variety of both MLV and inactivated BVDV vaccines currently licensed for use in Canada. Furthermore, multivalent vaccines in which BVDV is incorporated into vaccines with other respiratory viruses including IBR, parainfluenza-3 virus (PI-3) and BRSV are often used in Western Canada to protect against BRD (Van Donkersgoed et al., 1991).

2.3.5.2. Vaccination protocols

2.3.5.2.1. Vaccinating for fetal protection

Breeding females are vaccinated against BVDV to provide protection of the developing fetus, thereby preventing embryonic mortality, abortions, congenital defects and the birth of weak or PI calves (Platt et al., 2008; Radostits et al., 2000; van Oirschot et al., 1999). For optimal protection, breeding females should be vaccinated at least three weeks prior to breeding (Radostits et al., 2000). Vaccination of breeding females with MLV vaccines containing type 1 BVDV has been demonstrated to provide fetal protection that varied from 83% to 92% (Cortese et al., 1998; Dean et al., 2003; Kovacs et al., 2003; Leyh et al., 2011). Furthermore, cross-protection between type 1 and type 2 BVDV strains can be obtained in breeding females vaccinated with MLV vaccines (Brock and Cortese, 2001; Dean and Leyh, 1999; Ficken et al., 2006). In addition to MLV vaccines, inactivated BVDV vaccines have also been shown to provide fetal protection, however, the quality of protection is quite variable (Brownlie et al., 1995; Patel et al., 2002; Zimmer et al., 2002).

Neutralizing antibodies present in the blood and colostrum of pregnant females, produced as a consequence of vaccination, will protect the fetus from infection during gestation and newborn calves following birth (Potgieter, 1995). The length of protection against BVDV that unvaccinated calves receive from the colostrum of vaccinated dams is quite variable and is dependent on the amount of colostral antibodies ingested. Bolin and Ridpath (1995) demonstrated in calves receiving various amounts of colostrum, that the severity and duration of clinical signs following challenge with BVDV, decreased as the level of passively acquired neutralizing antibodies increased. Colostral BVDV antibodies may offer prolonged protection against disease as they have been reported to have a half-life of 21-23 d and can still be detected in 4-6 month old calves (Bolin, 1995; Fulton et al., 2004; Radostits et al., 2000). Fulton et al. (2004) also demonstrated that the time to seronegative status for unvaccinated calves receiving only colostrum was between 192.2 d, 179.1 d and 157.8 d for BVDV types 1a, 1b and 2, respectively. Furthermore, Bolin and Ridpath (2005) demonstrated that following the ingestion of colostrum, calves with passively acquired neutralizing BVDV antibody levels lower than a titer of 256 were not sufficiently protected from clinical disease following challenge with virulent BVDV.

2.3.5.2.2. Vaccinating calves to protect against acute infection

The vaccination of calves, especially upon entrance into feedlots, is one of the goals of BVDV control programs (Campbell, 2004). Vaccinating calves against BVDV slows the rate of antibody catabolism or depletion, which may increase the length of protection against clinical or acute BVDV infection. Fulton et al. (2004) reported a half-life in vaccinated calves of 33.5 d, 30.3 d and 44.9 d compared to 23.1 d, 22.8 d and 22.9

d in non-vaccinated calves for BVDV types 1a, 1b and 2, respectively. In addition, the time to seronegative status in vaccinated calves was 274.9 d, 238.0 d and 332.4 d compared to 192.2 d, 179.1 d and 157.8 d in non-vaccinated calves for BVDV types 1a, 1b and 2 (Fulton et al. 2004).

It is common practice to vaccinate beef calves at a young age when maternally derived antibodies are still present, which may interfere with the immune response generated to vaccination (Tizzard, 1992). Ellis et al. (2001) determined that high concentrations of BVDV-specific maternal antibodies can interfere with vaccination of 10-14 d old calves, resulting in susceptibility to BVDV challenge four months later. However, Zimmerman et al. (2006) demonstrated that vaccination of five-week old crossbred dairy calves with a MLV vaccine containing BVDV, when plasma concentrations of maternal BVDV antibodies were high, was able to protect calves when challenged. Kaeberle et al. (1998) also reported that vaccinating beef calves at 28-67 d of age, when maternal antibodies are present, can still induce a significant humoral immune response to BVDV. Furthermore, colostral antibody titers (levels) ≤ 32 were demonstrated not to interfere with the immune response generated to vaccination (Radostits et al., 2000; Zimmerman et al., 2006). Endsley et al. (2003) demonstrated that vaccination of seven-week old calves with either MLV or inactivated vaccines, when maternal antibodies were still present, did not generate any detectable increase in serum neutralizing BVDV antibodies. However, BVDV-specific memory B cells were still generated as the same calves were able to develop an anamnestic (memory) antibody response following revaccination. Similarly, Kirkpatrick et al. (2008) observed an

anamnestic response in beef calves vaccinated against BVDV at 67 d of age and revaccinated at 190 d of age (Kirkpatrick et al., 2008).

Upon entrance into feedlots, newly weaned calves are exposed to many stressors, including recent transportation, changes in nutrition, changes in environment, and changes in social interaction as a result of commingling calves from different sources, which can depress their ability to mount an immune response against pathogens. In order to combat this depressed immune response upon entrance into feedlots, preconditioning or backgrounding programs have been introduced to reduce stress and build immunity in calves prior to anticipated exposure to pathogens like BVDV (Step et al., 2009). Step et al. (2008) reported that weaning calves 45 d prior to entrance into feedlot operations and vaccinating them with a viral vaccine containing BVDV, resulted in improved health and performance in the receiving and feeding period. Furthermore, calves that arrived at feedlots with positive antibody titers against BVDV were associated with decreased risks of obtaining BRD (Booker et al., 1999; Martin et al., 1999) and with higher weight gains (Martin et al. 1999) when compared to calves with no antibody titers against BVDV.

2.4. *CLOSTRIDIUM CHAUVOEI*

2.4.1. Pathogenesis of infection

Clostridium chauvoei, a soil-borne anaerobic bacterium belonging to the *Clostridium* genus, causes a fatal disease of cattle and other ruminants known as blackleg (Kijima-Tanaka et al., 1997). Clostridial diseases, such as blackleg, can affect beef cattle of all ages, but are a primary concern in cattle between six months and one to two years of age (Sojka et al., 1992; Troxel et al., 2001). Infection with *C. chauvoei* often causes

serious toxemia and sudden death when activated latent spores are ingested or colonize muscle tissue following injury (Araujo et al., 2010; Kijima-Tanaka et al., 1997; Miyashiro et al., 2007; Sojka et al., 1992; Useh et al., 2003). *Clostridium chauvoei* can survive for as long as 11 years in the soil but soil disturbances, such as excavation, may activate latent spores and create conditions necessary for bacteria proliferation (Barnes et al., 1975). Animals infected with *C. chauvoei* often exhibit symptoms such as anorexia, depression, weakness and lameness (Kijima-Tanaka et al., 1997; Sojka et al., 1992). Hot, painful swelling is usually present in the limb that is presenting lameness (Kijima-Tanaka et al., 1997; Sojka et al., 1992). The clinical course of disease is acute and animals are often found dead 12 to 48 hours post infection (Kijima-Tanaka et al., 1997; Sojka et al., 1992).

2.4.2. Detecting *C. chauvoei*

The symptoms and course of blackleg are similar to malignant edema caused by *C. novyi*, anthrax caused by *Bacillus anthraces* and infection with other *Clostridium* species, including *C. septicum*, *C. perfringens* and *C. sordellii* (Kuhnert et al., 1997). Therefore, to diagnose blackleg and differentiate it from other diseases, the *C. chauvoei* bacteria or antigen must be identified in clinical specimens. Similarly, there are also strategies which may be used to detect if there has been an immune response initiated to *C. chauvoei*, either via infection or vaccination.

2.4.2.1. Culturing

Clinical specimens potentially infected with *C. chauvoei* may be cultured and grown in laboratories to isolate and identify the bacterium. The genus *Clostridium* contains anaerobic, gram-positive and catalase negative spore-forming bacteria (Osbaldiston and Stowe, 1971). The majority of *C. chauvoei* colonies seen on the surface of agar plates are classified as umbonate with a raised lip, but occasionally smooth colonies are seen (Batty and Walker, 1965). *Clostridium chauvoei* colonies closely resemble those of *C. septicum*, making it difficult to distinguish the two organisms and make a proper diagnosis (Batty and Walker, 1965). Culturing *C. chauvoei* is laborious and requires well-trained personnel (Miyashiro et al., 2007). Furthermore, as *C. chauvoei* is a fastidious organism, it requires an enriched medium (Batty and Walker, 1965) and an anaerobic environment (Sojka et al., 1992) to ensure growth in cultures. Therefore, culturing to detect *C. chauvoei* infection is being replaced by simpler and faster techniques.

2.4.2.2. Immunofluorescence

Immunofluorescence (IF), also known as fluorescent antibody tests (FAT), is an IHC staining technique that allows for the detection of antigens using specific fluorescein-labelled antibodies, which are visible when viewed under a fluorescent microscope (Haines and Clark, 1991). Two methods of IF staining exist: direct IF, where the primary antibody specific for the antigen is labelled, and indirect, where the secondary antibody specific for the primary antibody is labelled (Haines and Clark, 1991).

Immunofluorescence has been successfully used by researchers and diagnostic laboratories to detect *C. chauvoei* in cattle. The IF or FAT technique has been used to confirm diagnoses of *C. chauvoei* outbreaks in cattle by detecting the bacterium in infected tissues, which included tongue (Malone et al., 1986, forestomach (Sojka et al., 1992) and intestine tissues (Harwood et al., 2007). Hamaoka and Terakado (1994) also demonstrated that the *C. chauvoei* bacterium could be detected and viewed under a microscope by conducting an indirect immunofluorescence (IIF) assay. Indirect immunofluorescence assays may be utilized in the future to quantify antibodies against *C. chauvoei* that are present in the serum of vaccinated cattle.

2.4.2.3. Polymerase chain reaction

Polymerase chain reaction (PCR) assays are gaining interest as a diagnostic tool because *C. chauvoei* and *C. septicum* share phenotypic properties, which makes it difficult to differentiate the organisms using traditional methods such as culturing and IF (Batty and Walker, 1965). Kuhnert et al. (1997) investigated a PCR technique based on the 16S ribosomal RNA gene sequence using specific oligonucleotide primers, and demonstrated that the technique was able to detect *C. chauvoei* in an outbreak with the same sensitivity as culture morphology and FAT. Similarly, Kojima et al. (2001) developed a highly sensitive and simple one-step PCR system for the rapid and accurate identification of *C. chauvoei* by targeting the flagellin gene. More recently, Garofolo et al. (2011) developed a real time PCR taqman assay to identify *C. chauvoei*.

2.4.2.4. Agglutination and indirect hemagglutination

The interaction between antibodies and a particular antigen may result in visible clumping called agglutination, which can be used to detect and quantify antibody levels (Kindt et al., 2007). Agglutination tests have been used by many researchers to detect and quantify antibodies against *C. chauvoei* (Chandler and Gulasekharam, 1974; Chandler, 1975; Macheak et al., 1972; Schipper et al., 1978; Troxel et al., 1997; Troxel et al., 2001). These agglutination tests require the use of a specially prepared *C. chauvoei* agglutination antigen that is able to interact with antibodies causing agglutination (Macheak et al., 1972). However, in indirect hemagglutination tests, the *C. chauvoei* antigen is adsorbed to the surface of treated erythrocytes, allowing antibodies in the serum to bind with the antigen-erythrocyte combination, thereby creating visible agglutination (Bhatia, 2009; Tamura et al., 1985).

2.4.2.5. Enzyme-linked immunosorbent assay

Enzyme-linked immunosorbent assays have been effectively used to measure the antibody response of cattle following vaccination against *C. chauvoei* (Araujo et al., 2010; Srinivasan et al., 2001). Similarly, previous studies in rabbits (Crichton et al., 1990) and mice (Mattar et al., 2002), have also applied ELISA techniques to measure the antibody response to *C. chauvoei*.

2.4.3. Vaccination

Vaccination against *C. chauvoei* is a reliable prophylactic approach to protect cattle against infection (Araujo et al., 2010; Kahn et al., 2011b; Kijima-Tanaka et al., 1997; Useh et al., 2003).

2.4.3.1. Types of vaccines

A wide variety of clostridial vaccines against blackleg are available, which consist of inactivated cultures of *C. chauvoei* (Kahn et al., 2011b). Most clostridial vaccines are presented as multivalent vaccines, which consist of *C. chauvoei* along with one or more of the following: *C. septicum*, *C. novyi*, *C. sordelli*, *C. haemolyticum*, *C. perfringens* types B, C and D and *C. tetani* (Crichton et al., 1990; Kahn et al., 2011b). To enhance protective immunity, clostridial vaccines often contain adjuvants such as aluminum compounds (Lindblad, 2004). These adjuvants can often cause tissue reactions, lesions and swelling at the injection site (Kahn et al., 2011b; Willson et al., 1995).

2.4.3.2. Immune response to vaccination against C. chauvoei

The immune response stimulated by vaccination of cattle against *C. chauvoei* has been investigated in numerous studies (Araujo et al., 2010; Schipper et al., 1978; Srinivasan et al., 2001; Troxel et al., 1997; Troxel et al., 2001). However, one of the challenges associated with the quantification of the immune response elicited following vaccination against *C. chauvoei* is the similarity of the bacterium to *C. septicum*. Hamaoka and Terakado (1994) demonstrated via an IIF assay that *C. chauvoei* and *C. septicum* possess common antigens on their cell surface. Therefore, the quantification of

an immune response to *C. chauvoei* may be affected by the presence of antibodies against *C. septicum*.

Vaccination of cattle against *C. chauvoei* stimulates the production of antibodies specific to the bacterium, which may offer protection against blackleg disease. Araujo et al. (2010) demonstrated that three different vaccination regimens significantly increased the serum antibody levels against *C. chauvoei* when evaluated 30 d post-primary and 30 d post-booster vaccinations. However, it is not known whether the increase in antibody levels was able to protect the calves, as they were not challenged with *C. chauvoei*.

Timely booster vaccinations according to vaccine protocols are important to obtain optimal protection, however, on some occasions producers fail to revaccinate at the proper time (Troxel et al., 2001). Araujo et al (2010) demonstrated that calves receiving a primary vaccination at four months of age and a booster at eight months of age (Group 1) or a primary vaccination at eight months of age and booster at nine months of age (Group 2) had similar immune responses, while the calves that were only vaccinated once at eight months of age (Group 3) had a response similar to that of the unvaccinated control group. Therefore, it was concluded that vaccinating calves against *C. chauvoei* twice (primary and a booster vaccination) is able to stimulate an effective immune response, independent of when the primary vaccination is given. However, because the calves were not challenged against *C. chauvoei* it is not known whether the increase in antibody levels was able to offer protection against disease. Similarly, Troxel et al. (1997) concluded that 120 d between primary and booster vaccinations against *C. chauvoei* seems to be too long for adequate protection, as agglutination antibody titers for

C. chauvoei were not significantly increased in calves when measured 21 d following revaccination.

Clostridium chauvoei specific maternal antibodies present in the colostrum of dams are ingested by young calves and are important in protecting naïve calves against blackleg. Troxel et al. (1997) demonstrated that vaccination of pregnant dams against clostridial diseases approximately four months prior to calving can enhance passive immunity, thereby increasing antibody titers in calves and possibly offering prolonged protection of newborn calves against *C. chauvoei* infection. However, these maternal antibodies may also affect the immune response elicited following the vaccination of calves against *C. chauvoei*. Schipper et al. (1978) demonstrated that vaccination of 1-6 week old calves (Group 1) against *C. chauvoei* only resulted in 20% of vaccinated calves with an increase in antibody titers following vaccination, while the other 80% had no change or a decrease in antibody titers following vaccination. However, 66.7% of calves vaccinated at 4-12 months of age (Group 2) had an increase in antibody titers in response to vaccination against *C. chauvoei*. Similarly, Troxel et al. (2001) investigated the ability of a clostridial vaccine to elicit an antibody response in newborn calves and reported that vaccination of three d old newborn calves with the same clostridial vaccine as their dams, did not elicit an increased antibody response when compared to unvaccinated controls. This may indicate that younger animals (three d to six weeks of age) will not respond as readily to vaccination against *C. chauvoei* as older animals (4-12 months of age), due to the possibility of interference by maternal antibodies or immunoincompetence.

Although injection site reactions and lesions may affect carcass quality, they may also have a positive effect on the immune response acquired following vaccination.

Troxel et al. (2001) reported that 64.9% of vaccinated heifers developed injection site lesions, but also demonstrated that heifers with lesions had elevated antibody titers against *C. chauvoei* on days 28 ($P<0.08$) and 84 ($P<0.07$) when compared to heifers with no lesions.

3. RESEARCH HYPOTHESES AND OBJECTVIES

3.1. HYPOTHESES

Spring and fall-born crossbred beef calves vaccinated against BVDV and *C. chauvoei* using a NF vaccination technique will have a comparable animal performance, as measured by body weight (BW), and immune response, as measured by antibody level, to calves vaccinated against BVDV and *C. chauvoei* using a conventional NS vaccination technique. In addition, the presence of local skin reactions after vaccinating beef calves against BVDV and *C. chauvoei* will be comparable when using a NF or NS vaccination technique. It is expected that the differences in mechanics and vaccine dispersion patterns between NF and NS vaccination techniques will not result in differences in animal performance, immune response or presence of skin reactions between the two techniques.

The season (warm vs. cold temperature) in which beef calves are vaccinated in will impact the ability of a NFID to effectively vaccinate cattle as a consequence of device malfunction in sub-zero temperatures.

3.2. OBJECTIVES

The overall objective of this study was to provide an on-farm evaluation of a commercially available NFID to vaccinate beef calves under Western Canadian conditions and to compare the NF vaccination technique to a conventional NS vaccination technique. Specific objectives were to: (i) compare BW, antibody response and presence of local reactions in calves after vaccination against BVDV and *C. chauvoei*

using NF or NS vaccination techniques; (ii) determine if season of vaccination has an effect on the ability of a NFID to effectively vaccinate cattle.

4. MANUSCRIPT I

Effect of a needle-free vaccination technique on antibody response in spring-born and fall-born beef calves after vaccination against bovine viral diarrhea virus

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4.1. ABSTRACT

Antibody production was measured in both spring-born (Study A) and fall-born (Study B) beef calves following vaccination against BVDV using either NF or NS vaccination techniques. At two months of age (day 0), calves were vaccinated with a commercially available MLV combination vaccine, containing IBR, BRSV, PI-3 and BVDV types 1 and 2, either via a NF or a NS vaccination technique. On day 119 of the studies, calves received a booster vaccination using the same vaccination technique and vaccine product. Ten calves were left unvaccinated (Control) against BVDV, serving as controls. Body weight was recorded on days 0, 21, 42, 119, and 140 and post-vaccination skin reactions were recorded on days 21, 42, 119 and 140. Presence of visible vaccine residue at the surface of the skin/hair following NF vaccinations was recorded on days 0 and 119. Blood samples were collected on days 0, 21, 42, 119, and 140. Antibody levels in serum, as measured by sample to positive ratio (S/P Ratio), were analyzed by a semi-quantitative BVDV ELISA. Furthermore, the trends in the immune response stimulated against BVDV in the two seasons of vaccination (Study A and B) were similar. Animal performance in either study, as measured by BW, did not differ ($P>0.05$) between vaccination techniques and was observed not to differ ($P>0.05$) between vaccinated (NF and NS) or unvaccinated (Control) calves. Needle-free vaccination was observed to cause a greater frequency ($P<0.05$) of skin reactions after vaccination compared to NS vaccination. Throughout both Study A and B, antibody levels against BVDV were not significantly different ($P>0.05$) between vaccination techniques. In the presence of circulating BVDV maternal antibodies, the primary antibody response against BVDV after NF and NS vaccination was observed to be limited. However, this did not to affect

the memory immune response as both NF and NS vaccinated calves showed an anamnestic antibody response following booster vaccination. Vaccinated (NF and NS) calves in both studies exhibited a negative correlation between the antibody level observed on day 0 (maternal antibody level) and day 140. Therefore, it may be evident that circulating maternal antibodies present prior to vaccination of two-month old calves against BVDV, may interfere with the ability of the immune system to respond to vaccination. Visible vaccine residue occurred for 21.6% of NF vaccinations, but did not compromise antibody response. In summary, a NF vaccination technique is effective in stimulating an immune response against BVDV.

Keywords: bovine viral diarrhea virus, antibody response, NF vaccination, NS vaccination, beef calves, ELISA, animal performance, skin reactions

4.2. INTRODUCTION

Bovine viral diarrhea virus is a positive-stranded and enveloped RNA virus in the genus *Pestivirus*, belonging to the Flaviviridae family (Sandvik, 2005). The economic impact of BVDV infection is significant as it is an immunosuppressive agent, which has been reported to leave cattle susceptible to invasion by other micro-organisms that may induce BRD and mucosal disease in feedlot cattle (Campbell, 2004; Potgieter, 1997; van Oirschot et al., 1999). Infection of pregnant females with BVDV can also result in decreased conception rates, abortion, stillbirth, weak neonates, congenital defects and the birth of PI calves (Hansen et al., 2010; Radostits et al., 2000). Therefore, vaccinating cattle for BVDV, especially at the cow-calf producer level, is an important component of BVDV prevention and control (Chase et al., 2004; van Oirschot et al., 1999).

Inactivated and MLV monovalent or multivalent vaccines are widely used to protect cattle against BVDV (Van Donkersgoed et al., 1991). Traditionally, vaccines are administered to cattle using a NS vaccination technique. Injections administered via a NS vaccination technique may result in producers or veterinarians accidently puncturing themselves when handling the needle (Weese and Jack, 2008). In addition, broken needle fragments may be found in the meat, which could be detrimental to consumer perception (van Drunen Littel-van den Hurk, 2006). Furthermore, blood-borne infectious diseases such as bovine leukosis (Hollis et al., 2005a) and anaplasmosis (Reinbold et al., 2010) can also be transmitted from animal to animal when a single needle is used to inject multiple animals. The disadvantages associated with NS vaccinations have led to the development of alternative vaccination techniques, including the use of NFIDs. Needle-free injection devices were first developed in the 1940's and have been used previously to

vaccinate humans (Hingston et al., 1963) and swine (Chase et al., 2008a). These devices utilize mechanical compression, triggered when the nozzle touches the skin, to power vaccine injections through a small orifice, producing a high pressure stream that can penetrate the skin and deposit the vaccine into the desired tissue (Jackson et al., 2001; Mousel et al., 2008). Although NS vaccination techniques are inexpensive and easily adaptable to different production environments, NF vaccination techniques may offer advantages, including elimination of broken needles (Chase et al., 2008a; Stier, 2003), reduced disease transfer (Reinbold et al., 2010), reduced vaccination time (Mousel et al., 2008), greater antigen dispersion (Bennett et al., 1971; Chase et al., 2008a; Parent du Chatelet et al., 1997; Reis et al., 1998), elimination of needle disposal (Chase et al., 2008a) and elimination of needle-sticks to individuals giving injections.

Previous studies in humans (Jackson et al., 2001), rabbits (Aguiar et al., 2001), sheep (Mousel et al., 2008), swine (Houser et al., 2004) and cattle (Hollis et al., 2005a; Hollis et al., 2005b; Pires et al., 2007; van Drunen Littel-van den Hurk,S., 2006) have shown no significant decrease and an occasional increase in immune response when vaccines are delivered with NF versus conventional NS vaccination techniques. Previous research investigating the use of NFIDs in cattle has focused on evaluating the immune response of cattle after vaccination against MH (Hollis et al., 2005a; Hollis et al., 2005b), LP (Hollis et al., 2005a), *Brucella abortus* (Pires et al., 2007) and BHV-1 (Hollis et al., 2005a; Hollis et al., 2005b; van Drunen Littel-van den Hurk,S., 2006), also known as IBR. To the author's knowledge, there is little information available regarding the efficacy of a NF vaccination technique in administering a MLV combination vaccine, containing BVDV (types 1 and 2).

The purpose of this study was to compare animal performance, as measured by BW, presence of skin reactions, and the antibody response of calves following vaccination against BVDV using a NF or NS vaccination technique.

4.3. MATERIALS AND METHODS

4.3.1. Experimental animals

Two replicated studies (Study A and B) were performed in two commercial cow-calf beef herds located in the Province of Manitoba, Canada. The herd used for Study A consisted of 96 spring-born crossbred beef calves (106.55 ± 16.79 kg), while the herd used for Study B consisted of 98 fall-born crossbred beef calves (100.73 ± 16.22 kg). In both herds, animal feed and handling management followed standard industry practices.

Prior to the start of Study A and B, blood samples from all calves were collected via jugular venipuncture into sodium heparin vacutainer tubes (BD Vacutainer). Blood samples were tested for the presence of BVDV by a one-tube RT-PCR using total RNA extracted from whole blood (Hamel et al., 1995) at the Veterinary Diagnostic Services Laboratory (Manitoba Agriculture, Food and Rural Initiatives). All animals tested in Study A and B were determined to be negative for BVDV, therefore no animals were excluded from the experiment.

4.3.2. Vaccination protocol

As described in Table 2, calves in Study A were randomly assigned to one of three vaccination groups: NF vaccination (n=47), NS vaccination (n=39), and an unvaccinated control (n=10). In Study B, calves were also randomly assigned to the same

three vaccination groups: NF vaccination (n=48), NS vaccination (n=40), and an unvaccinated control (n=10).

All calves were weighed (kg) prior to vaccination or data collection on days 0, 21, 42, 119 and 140 of Study A and B. According to vaccine manufacturer's recommendations, calves in the NF and NS groups were vaccinated at approximately two months of age (day 0) and revaccinated at six months of age (day 119) with a commercially available MLV combination vaccine (Express 5, Boehringer Inhelheim (Canada) Ltd.) containing IBR, BVDV (types 1 and 2), PI-3, and BRSV. Needle-free vaccinations were administered with a NFID (Pulse 250 NeedleFree Injection System, Pulse NeedleFree Systems) on the left side of the neck using a skin-tenting technique (See Appendix I). In both studies, the NF vaccinations were administered to calves by one individual who had been previously trained in the use and maintenance of the NFID. Carbon dioxide was used as the energy source for the NF vaccinations on day 0 and 119 of Study A and day 0 of Study B. However, the booster NF vaccination on day 119 required the use of compressed N_2 as the power source instead of liquid CO_2, as per the direction of Pulse NeedleFree Systems, to ensure that the injection was not compromised as a consequence of the subzero temperatures. Using guidelines from Pulse NeedleFree Systems, the NFID was set at pressures of 45-50 pounds per square inch (PSI) and 85 PSI to administer the vaccine on days 0 and 119, respectively, in order to perform a SC injection. Immediately following each NF vaccination on day 0 and 119, the presence of visible vaccine residue on the surface of the skin/hair was recorded. The amount of vaccine residue present was not quantified, however, any visible fluid seen on the surface of the skin/hair was noted. In the NS group, vaccinations were administered SC with a

multi-dose pistol-grip syringe (Kane Veterinary Supplies Ltd), fitted with an 18G by 1 inch detectable needle (Partnar Animal Health Inc.), on the left side of the neck using the same skin-tenting technique that was used to administer the NF vaccinations. A sufficient quantity of vaccine (20 mL) was drawn into the syringe to vaccinate ten calves. To simulate standard industry practices, following vaccination of ten calves, the used needle was replaced with a new sterile needle and a sufficient quantity of vaccine (20 mL) was drawn into the syringe to vaccinate an additional ten calves. If a needle became bent or burred, it was immediately replaced. Furthermore, on days 21, 42, 119 and 140 of both studies, vaccinated calves (NF and NS) were visually inspected, palpated and scored for the presence of skin reactions, as in Troxel et al., (1997). Any apparent raised surfaces observed at the injection sites were considered skin reactions occurring as a result of vaccination.

In addition to vaccination with a MLV combination vaccine, all calves in Study A and B were vaccinated with Clostri Shield 7 (Novartis Animal Health Canada Inc.) to protect calves from diseases caused by *Clostridium* bacteria.

Animal handling and care procedures in this study were approved by the University of Manitoba Animal Care Committee in compliance with the guidelines of the Canadian Council of Animal Care (1993).

4.3.3. Antibody testing

Blood samples from all calves were collected via jugular venipuncture into serum

Table 2 Time schedule for administration of a MLV combination vaccine and subsequent data collection.

Study	Group	*N*	Vaccinations (days)	Blood collection[a] (days)	BW[a] (days)	Injection site assessment[a] (days)
Study A	Control	10	None	0, 21, 42, 119, 140	0, 21, 42, 119, 140	None
	NF	47	0, 119	0, 21, 42, 119, 140	0, 21, 42, 119, 140	21, 42, 119, 140
	NS	39 (38[b])	0, 119	0, 21, 42, 119, 140	0, 21, 42, 119, 140	21, 42, 119, 140
Study B	Control	10	None	0, 21, 42, 119, 140	0, 21, 42, 119, 140	None
	NF	48	0, 119	0, 21, 42, 119, 140	0, 21, 42, 119, 140	21, 42, 119, 140
	NS	40 (39[c]) (38[d])	0, 119	0, 21, 42, 119, 140	0, 21, 42, 119, 140	21, 42, 119, 140

n, Number of animals; NF, Needle-free vaccinated calves; NS, Needle-syringe vaccinated calves; Control, Unvaccinated calves
[a] Prior to vaccination, where applicable.
[b] After day 21 of Study A, one calf was removed from the trial for reasons unrelated to the study
[c] After day 21 of Study B, one calf was removed from the trial for reasons unrelated to the study
[d] After day 42 Study B, another calf was removed from the trial for reasons unrelated to the study

vacutainer tubes (BD Vacutainer) on days 0, 21, 42, 119 and 140 of Study A and B (Table 2). Following the collection of blood, samples were allowed to clot and then centrifuged at 1100 *g* for 10 min. Aliquots of serum were stored at -20°C until required for antibody testing. Serum samples collected from all calves in both studies were tested for the level of antibodies against BVDV using a commercial semi-quantitative indirect ELISA test kit (IDEXX BVDV Total Ab Test Kit, IDEXX Laboratories, Inc.). Analysis was performed according to the manufactures' recommendations using reagents from the ELISA kit, which included negative and positive controls. Absorbance was determined at a wavelength of 450 nm using an absorbance microplate reader (Spectra Max 340PC, Molecular Devices).

Antibody levels were expressed as S/P Ratio calculated as: S/P Ratio = (Sample optical density (OD) – Negative control mean OD) / (Positive control mean OD – Negative control mean OD). Prior to being subjected to statistical analysis, the antibody levels (S/P Ratio) were transformed on a $\log_{10}$ (S/P Ratio +1) scale. The indirect ELISA test kit used for the determination of BVDV antibodies states that a calf with an S/P Ratio of 0.20 or less, which corresponds to 0.079 on a $\log_{10}$(S/P Ratio + 1) scale, is considered seronegative.

4.3.4. Statistical analysis

Data for Study A and B were analyzed separately and significance for all tests were determined by a two-tailed test using a 5% significance level ($P<0.05$).

Analysis of BW and antibody level was completed in SAS 9.2 (SAS Institute Inc.) using a linear mixed model for repeated measures with fixed effects of group, days post vaccination and their interaction and random effects of animal within group. Individual animal was regarded as the experimental unit in the three vaccination groups (Control, NF and NS). In the model for BW, the covariance of performance across times was accounted for using the unstructured covariance method (Wang and Goonewardene, 2004). For antibody level analysis, the unstructured covariance method was also used and in addition, unequal covariance matrices were modelled for each group. Correlations between the antibody levels at the start and end of both studies for all groups (Control, NF and NS) were obtained from the mixed procedure. Significance of the correlations (H_0: r=0 vs. H_a: r≠0) was evaluated using a two tailed t-test (Steel et al., 1997).

Data for presence of visible vaccine residue was analyzed using the same linear mixed model for repeated measures with individual animal regarded as the experimental unit but with six treatment groups (Control, NS, NF no vaccine residue (NFnoR), NF primary vaccination vaccine residue (NFpR), NF booster vaccination vaccine residue (NFsR) and NF primary and booster vaccinations vaccine residue (NFpsR). The covariance of performance across times was accounted for using the unstructured covariance method (Wang and Goonewardene, 2004). Contrasts were completed in order to determine if NF vaccinated calves with visible vaccine residue (NFpR, NFsR and NFpsR) noted on the skin/hair surface following primary and/or booster vaccination differed in antibody levels compared to NF vaccinated calves with no vaccine residue visible (NFnoR).

Confidence limits (95%) of the estimated frequencies of NF and NS animals having at least one skin reaction at the site of vaccine administration on days 21, 42, 119 and 140 were calculated and used to determine significant differences between groups (Steel et al., 1997). However, an error in data collection regarding the presence of skin reactions was noted on days 21 and 42 of Study A. Therefore, data collected on those two sampling days was removed and analysis of skin reactions in Study A was only completed on data collected on days 119 and 140.

4.4. RESULTS

4.4.1. Body weight

No difference ($P>0.05$) between Control, NF and NS groups was observed for BW throughout either study (Table 3 and Figs. 2 & 3), with calves in Study A averaging 1.19 ± 0.04 kg/day, 1.09 ± 0.02 kg/day and 1.14 ± 0.02 kg/day, respectively, and calves in Study B averaging 0.81 ± 0.05 kg/day, 0.90 ± 0.02 kg/day and 0.92 ± 0.03 kg/day, respectively.

Table 3 Factors affecting body weight (BW)

		P Values		
Item	Study	Trt	DaysPV	Interaction
Body Weight	A	0.1374	<0.0001*	0.4078
	B	0.2366	<0.0001*	0.4696

* = Significant ($P<0.05$)

4.4.2. Skin reactions

In both studies, the NF vaccinated calves had a greater (P<0.05) frequency of skin reactions at the site of vaccination than did NS vaccinated calves (Figs. 4 and 5).

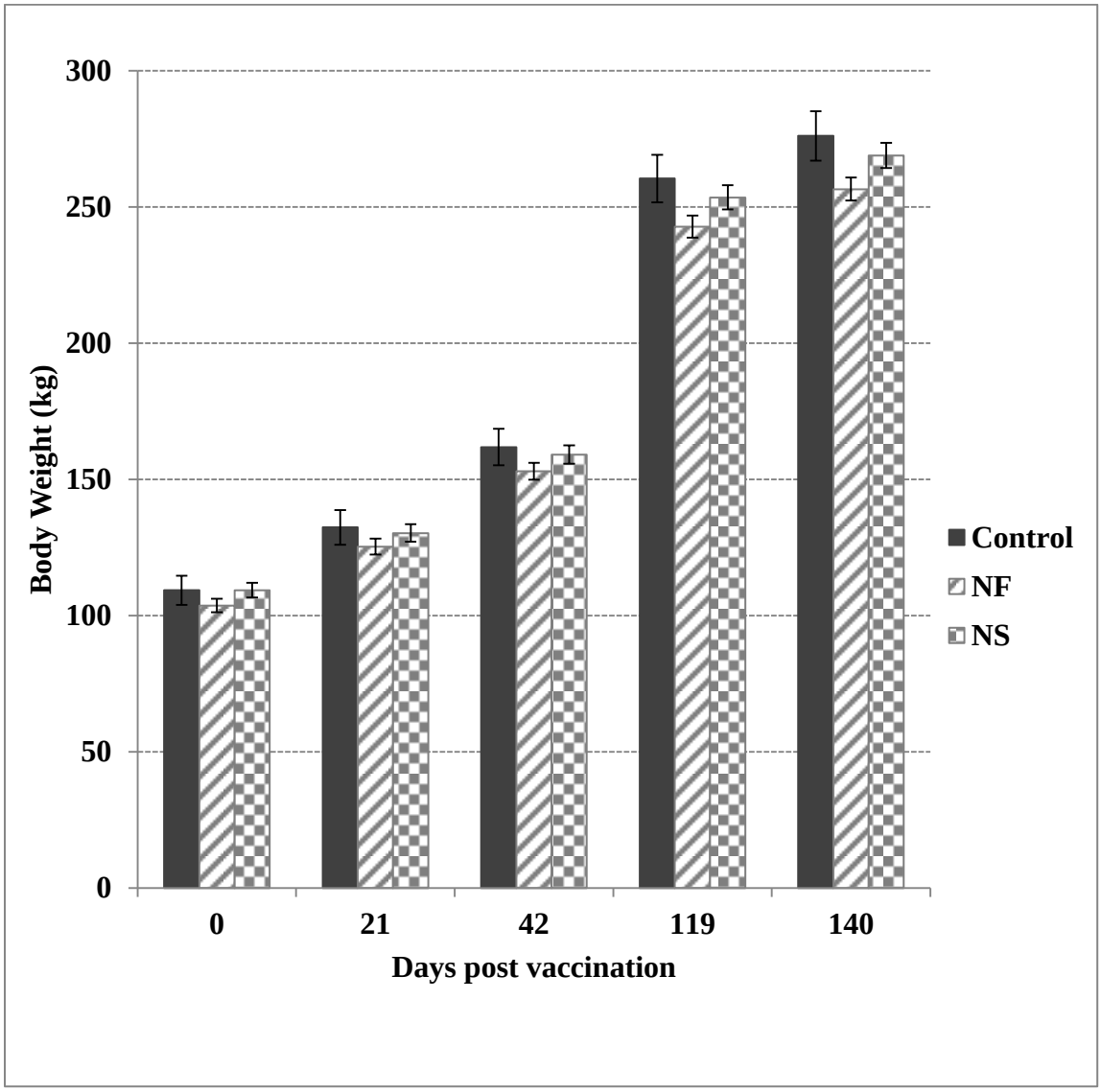

Fig. 2. Body weight of spring-born calves (Study A) unvaccinated (Control) or vaccinated against bovine viral diarrhea virus using needle-free (NF) or needle-syringe (NS) techniques (standard errors shown as bars).

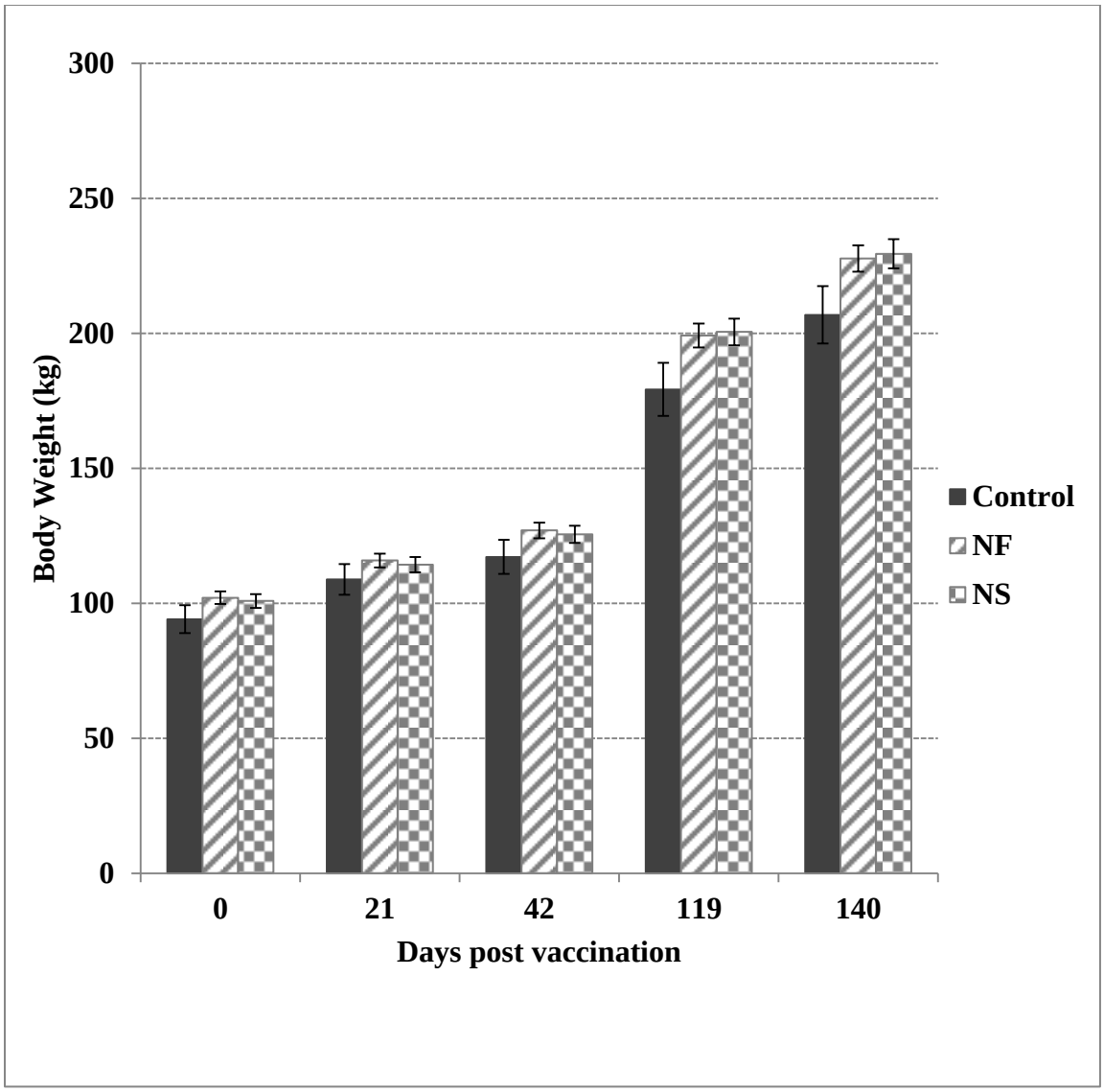

Fig. 3. Body weight of fall-born calves (Study B) unvaccinated (Control) or vaccinated against bovine viral diarrhea virus using needle-free (NF) or needle-syringe (NS) techniques (standard errors shown as bars).

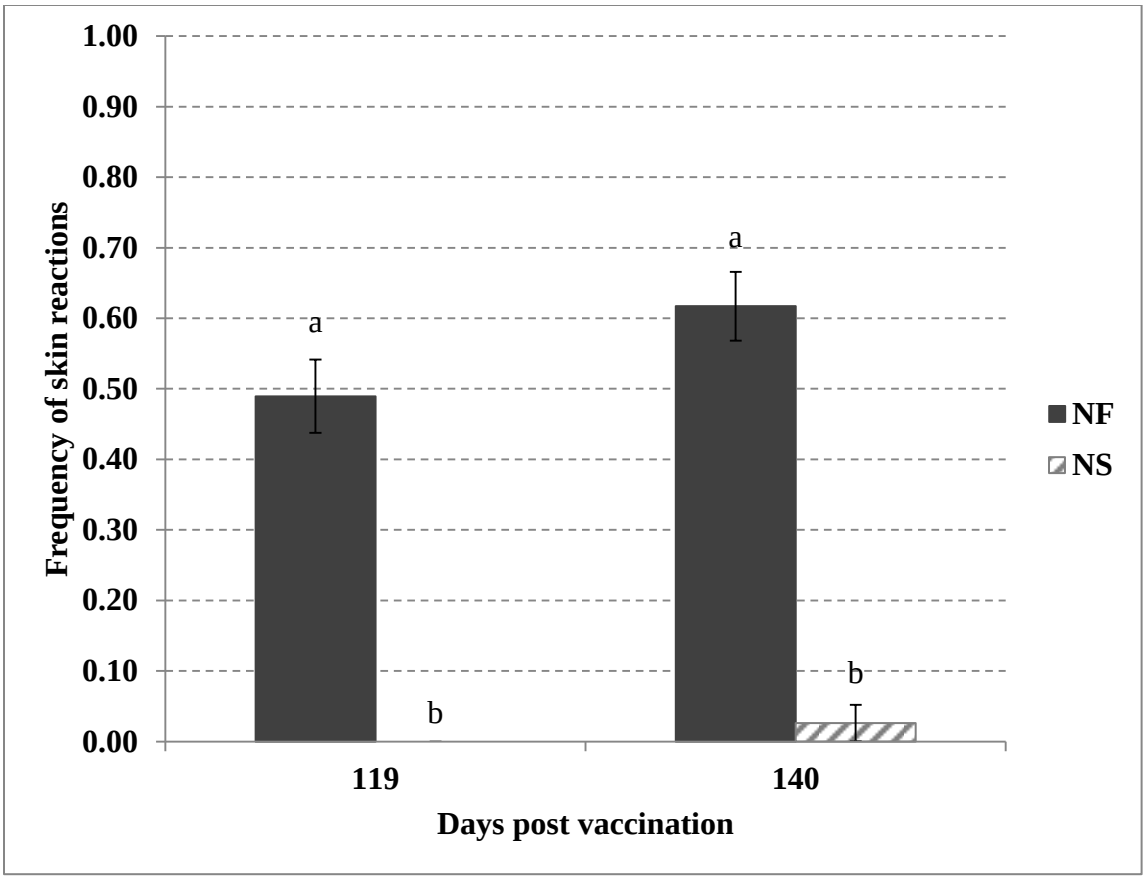

Fig. 4. Frequency of skin reactions after vaccination of spring-born calves (Study A) against bovine viral diarrhea virus using needle-free (NF) or needle-syringe (NS) techniques (95% confidence limits shown as bars).

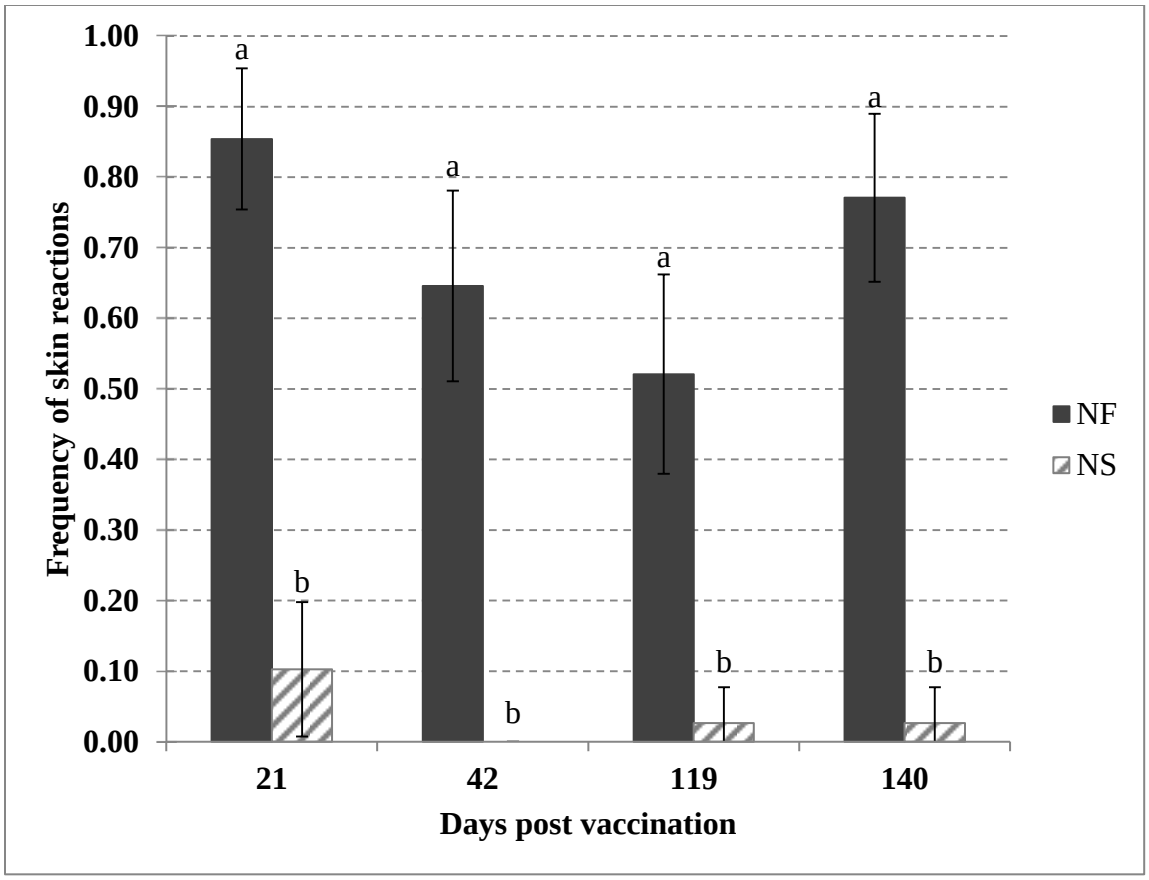

Fig. 5. Frequency of skin reactions after vaccination of fall-born calves (Study B) against bovine viral diarrhea virus using needle-free (NF) or needle-syringe (NS) techniques (95% confidence limits shown as bars).

4.4.3. Antibody level (Study A)

Throughout the study, antibody levels against BVDV were not different ($P>0.05$) between NF and NS vaccination techniques (Fig. 6). Higher ($P<0.05$) antibody levels in vaccinated calves (NF & NS) relative to unvaccinated (Control) calves were found on days 42, 119 and 140 of the study.

As indicated in Table 4, on day 21 of Study A, NF vaccinated calves had a lower ($P=0.0002$) antibody level compared to the antibody level at the start of the study, whereas the NS vaccinated calves were not different ($P>0.05$). The antibody level of NF vaccinated calves on day 42 of Study A did not differ ($P>0.05$) from the antibody level on day 21. However, NS vaccinated calves had a higher ($P=0.0138$) antibody level on day 42 compared to day 21.

Calves in both vaccination groups (NF and NS) had a lower ($P<0.0001$) antibody level on day 119 compared to the antibody level on day 42. Following the booster vaccination, the antibody level on day 140 had recovered and was found to be higher than the antibody level on day 119 for both NF ($P<0.0001$) and NS ($P=0.0033$) vaccinated calves.

The antibody level of unvaccinated (Control) calves on day 119 was below 0.079, suggesting that unvaccinated calves were seronegative by day 119.

Table 4 Within treatment antibody levels [$\log_{10}$ (S/P Ratio + 1)] in spring-born (Study A) and fall-born calves unvaccinated (Control) or vaccinated against bovine viral diarrhea virus using needle-free (NF) or needle-syringe (NS) techniques.

Study	Trt	DaysPV	Antibody Level ($\log_{10}$(S/P Ratio + 1)	DaysPV	Antibody Level ($\log_{10}$(S/P Ratio + 1)	P-value
Study A	Control	0	0.3085[a]	21	0.2932[a]	0.2854
		21	0.2932[a]	42	0.2379[b]	<0.0001
		42	0.2379[a]	119	0.0450[b]	<0.0001
		119	0.0450[a]	140	0.0348[a]	0.3189
	NF	0	0.3267[a]	21	0.3140[b]	0.0002
		21	0.3140[a]	42	0.3211[a]	0.4568
		42	0.3211[a]	119	0.2141[b]	<0.0001
		119	0.2141[a]	140	0.2874[b]	<0.0001
	NS	0	0.2944[a]	21	0.2952[a]	0.9402
		21	0.2952[a]	42	0.3300[b]	0.0138
		42	0.3300[a]	119	0.2332[b]	<0.0001
		119	0.2332[a]	140	0.2872[b]	0.0033
Study B	Control	0	0.3328[a]	21	0.3103[b]	0.0026
		21	0.3103[a]	42	0.2892[b]	0.0068
		42	0.2892[a]	119	0.1289[b]	<0.0001
		119	0.1289[a]	140	0.0768[b]	0.0002
	NF	0	0.2856[a]	21	0.2871[a]	0.8039
		21	0.2871[a]	42	0.3131[b]	0.0012
		42	0.3131[a]	119	0.2366[b]	<0.0001
		119	0.2366[a]	140	0.2849[b]	<0.0001
	NS	0	0.2843[a]	21	0.2709[b]	0.0058
		21	0.2709[a]	42	0.3059[b]	<0.0001
		42	0.3059[a]	119	0.2272[b]	<0.0001
		119	0.2272[a]	140	0.2714[b]	<0.0001

Trt, Treatment; DaysPV, Days post vaccination

[a,b] Antibody levels in the same row with different superscripts differ significantly ($P<0.05$)

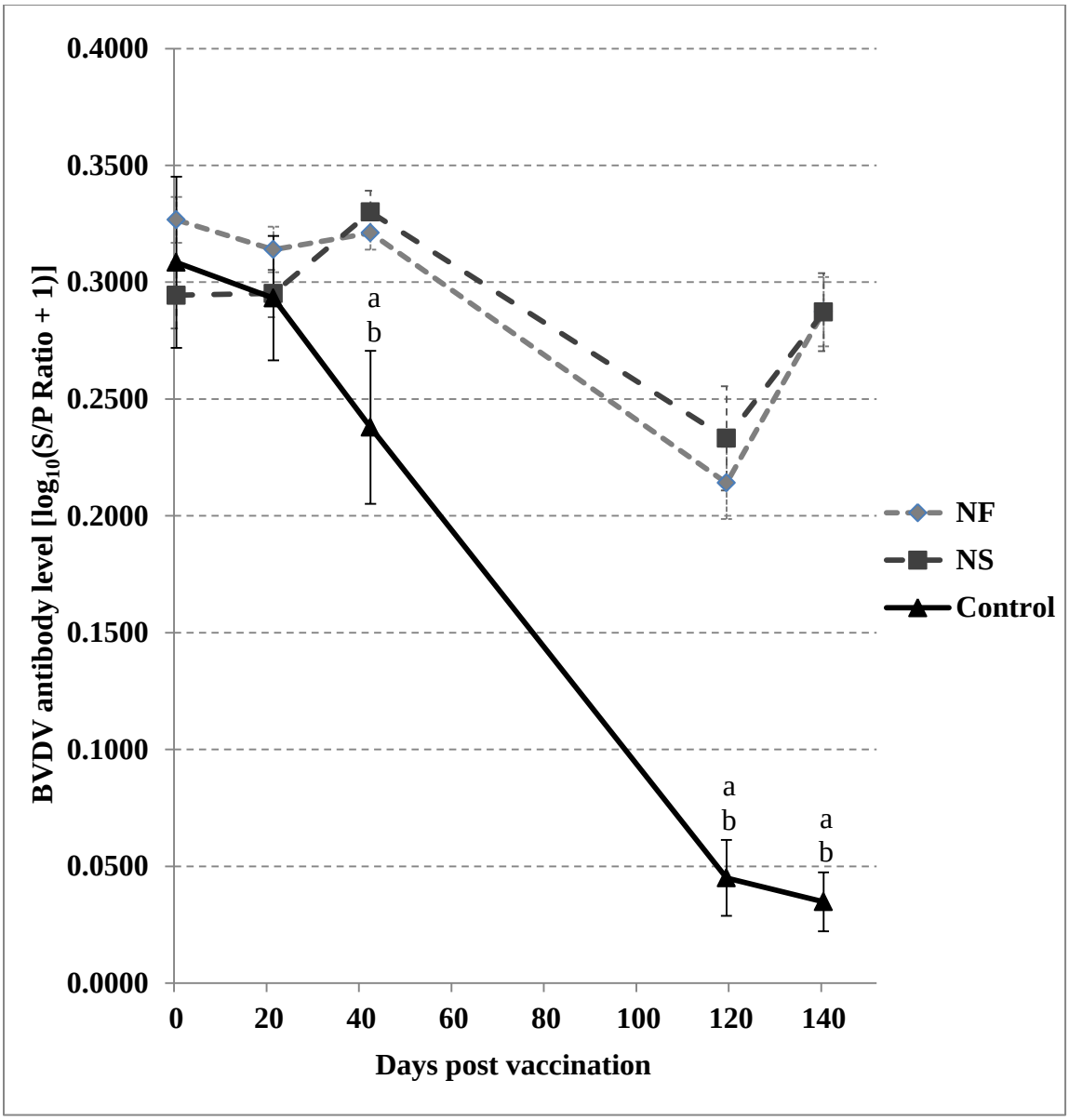

Fig. 6. Antibody levels [$\log_{10}$ (S/P Ratio + 1)] of spring-born calves (Study A) unvaccinated (Control) or vaccinated against bovine viral diarrhea virus using needle-free (NF) or needle-syringe (NS) techniques [a] $P<0.05$ NF vs. Control; [b] $P<0.05$ NS vs. Control (standard errors shown as bars).

4.4.4. Antibody level (Study B)

During Study B, antibody levels against BVDV were not different ($P>0.05$) between NF and NS vaccination techniques (Fig. 7). Higher ($P<0.05$) antibody levels in

vaccinated calves (NF & NS) relative to unvaccinated (Control) calves were only found on days 119 and 140 of the study.

The trend in immune response stimulated following vaccination of spring-born (Study A) and fall-born calves (Study B) was comparable between the two seasons of vaccine administration.

As indicated in Table 4, on day 21 of Study B, NS vaccinated calves had a lower (P=0.0058) antibody level compared to the antibody level at the start of the study, however, the antibody level of NF vaccinated calves on day 21 did not differ (P>0.05) from the antibody level at the start of the study. Both NF (P=0.0012) and NS (P<0.0001) vaccinated calves in Study B had a higher antibody level on day 42 compared to the antibody level on day 21.

Calves in both vaccination groups (NF and NS) had a lower (P<0.0001) antibody level on day 119 compared to the antibody level on day 42. Following the booster vaccination, the antibody level on day 140 was higher than the antibody level on day 119 for both NF (P<0.0001) and NS (P<0.0001) vaccinated calves.

The antibody level of unvaccinated (Control) calves on day 140 was below 0.079, suggesting that unvaccinated calves were seronegative by day 140.

4.4.5. Correlation between the antibody levels at the start (day 0) and end of the study (day 140)

As indicated in Table 5, the correlation (r = 0.6562) between antibody levels at the start and end of Study A for unvaccinated (Control) calves was significantly different from zero (r = 0). The correlation between the antibody levels at the start and end of the

study were also found to be significantly different from zero (r = 0) for NF (r = -0.5673) and NS (r = -0.5023) vaccinated calves.

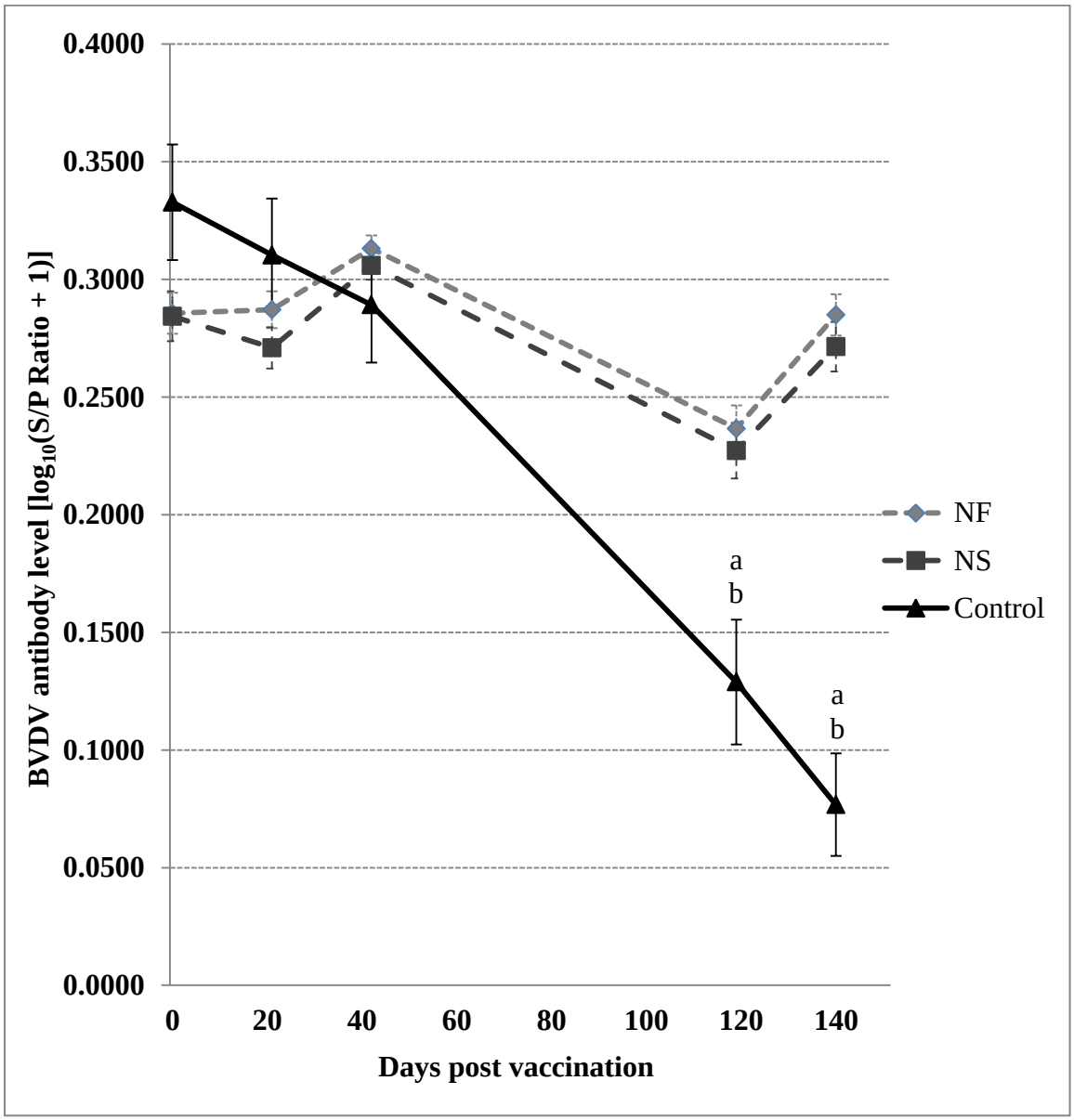

Fig. 7. Antibody levels [log10 (S/P Ratio + 1)] of fall-born calves (Study B) unvaccinated (Control) or vaccinated against bovine viral diarrhea virus using needle-free (NF) or needle-syringe (NS) techniques [a]P<0.05 NF vs. Control; [b]P<0.05 NS vs. Control (standard errors shown as bars).

Conversely, in Study B, the correlation between antibody levels at the start and end of the study for Control (r = 0.5864), NF (r = -0.1896) and NS (r = -0.0602) calves were not significantly different from zero (r = 0).

Table 5 Correlation of antibody levels on days 0 and 140 for spring (Study A) and fall-born (Study B) calves unvaccinated (Control) or vaccinated against bovine viral diarrhea virus using needle-free (NF) or needle-syringe (NS) techniques.

Study	Group	Correlation
Study A	Control	0.6562*
	NF	-0.5673*
	NS	-0.5023*
Study B	Control	0.5864
	NF	-0.1896
	NS	-0.0602

* = Significant (P<0.05)

4.4.6. Vaccine residue

Varying amounts of visible vaccine residue occurred for 21.6% of NF vaccinations in Study A and B combined, indicating possible failure of the injection in the animal.

On day 0 of Study A, 29.8% of calves in the NF group had visible vaccine residue on the surface of the skin/hair following primary vaccination at a pressure of 45-50 PSI. Subsequently, when the booster vaccination was administered on day 119, 29.8% of calves in the NF group had visible vaccine residue following vaccination at a pressure of 85 PSI. Similarly on day 0 of Study B, 18.8% of calves in the NF group had visible vaccine residue following primary vaccination at a pressure of 45-50 PSI. Following the

booster vaccination (85 PSI) administered on day 119, it was found that 8.3% of calves in the NF group had visible vaccine residue.

The presence of visible vaccine residue on the surface of the skin/hair after the primary and booster vaccinations in both studies did not compromise ($P>0.05$) antibody response in NF vaccinated calves.

4.5. DISCUSSION

Previous studies using NFIDs have not examined the impact of this technique on animal performance. As BW did not differ among treatment groups, it can be concluded that primary vaccination of two-month old calves and subsequent booster vaccinations with a MLV combination vaccine using a NFID does not affect animal performance.

There is little published literature investigating the skin reactions observed after vaccinating cattle via a NFID. In the current study, NF vaccinated calves had a greater frequency of skin reactions at the site of vaccine administration than did NS vaccinated cattle. However, in Study A and B the impact of skin reactions of both NF and NS vaccinated calves on carcass quality were not explored. When used in swine, Houser et al. (2004) demonstrated that there was no significant difference in the number of gross or histological lesions observed at the injection sites of carcasses following vaccination using a NF or NS technique. In swine, the frequency of injection site abscesses observed in the presence of bacterial contamination, has been examined using NF and NS vaccination techniques (Gerlach et al. 2009). Prior to the vaccination of nursery-aged pigs with an aluminum hydroxide adjuvant, injection sites were contaminated with *Arcanobacterium pyogenes*. Contrary to Houser et al. (2004), use of a NFID resulted in a

greater number of abscesses, in both neck (P=0.0625) and ham (P=0.0313) injection locations, than did the NS vaccination method. Similarly, an increase in the frequency of skin reactions has been reported in humans administered type A (Williams et al., 2000) and B (Mathei et al., 1997) hepatitis vaccines. In addition, administration of a trivalent inactivated influenza vaccine to humans using a NFID also led to an increase in local skin reactions when compared to NS vaccination (Jackson et al., 2001). However, it is difficult to compare the results seen in the current study to those in previous studies completed in swine and humans due to the significant differences between species.

The observed increase in skin reactions following NF vaccinations in both Study A and B, may have been caused by many factors, including pressure trauma associated with vaccination or contamination by transmitting microorganisms present on the skin/hair surface into the tissue surrounding the injection site (Gerlach et al., 2009; Sutterfield et al., 2009). It does not appear that the skin reactions caused by NF and NS vaccination techniques were severe enough to affect the ability of calves to suckle or graze, as BW did not differ between groups.

In the current study, NF and NS vaccinated calves had a comparable immune response following vaccination against BVDV. This is in agreement with previous research that has demonstrated that vaccination of cattle (Hollis et al., 2005a; Hollis et al., 2005b; Pires et al., 2007; van Drunen Littel-van den Hurk,S., 2006) via a NF vaccination technique results in a comparable and sometimes enhanced immune response when compared to NS vaccination, depending on the sex of the animals and the vaccine antigen measured. Pires et al. (2007) demonstrated that vaccine administration method, NF or NS

techniques, did not have an effect on the immune response, as measured by antibodies, of 4-6 month old dairy and beef heifers vaccinated with 1010 CFU of *Brucella abortus* strain RB51. Hollis et al. (2005a) also demonstrated that the antibody response of 5-10 month old Holstein heifers to IBR (P=0.11), MH (P=0.51) and LP (P=0.08) were not different between NF and NS vaccination techniques. However, the same authors also reported that the response of 5-10 month old Holstein steers to IBR (P=0.01) and MH (P=0.02) was higher in NF vaccinated calves, while the response to LP (P=0.38) was not different between administration techniques. The authors of the study have no knowledge as to why the antibody response differed between heifers and steers, however, in Study A and B, the effect of gender was analyzed and was demonstrated not to affect antibody response and was therefore removed from the statistical model. Moreover, Hollis et al. (2005b) reported that the antibody response of yearling feedlot steers to vaccination against IBR was higher (P=0.001) in NF compared to NS vaccinated calves, but the response to vaccination against MH did not differ (P=0.06) between administration techniques. Furthermore, Van Drunen Little-van den Hurk (2006) discovered that calves vaccinated with a BHV-1 gC vaccine using a NFID (Biojector 2000, Bioject Inc.) were able to induce significantly enhanced gC-specific antibody titers, lymphoproliferative responses and numbers of IFN-γ secreting cells in the peripheral blood compared to those vaccinated via NS. Similar studies conducted in humans (Jackson et al. 2001), rabbits (Aguiar et al., 2001), sheep (Mousel et al., 2008) and swine (Houser et al., 2004) have also determined that NF vaccination stimulates a comparable and sometimes enhanced immune response compared to NS vaccination.

Needle-free injection devices are able to sometimes enhance the immune response stimulated following vaccination when compared to NS vaccination because vaccines delivered via NF injection are dispersed more widely in the tissues and penetrate through different layers of the skin (Bennett et al., 1971; Grant, 2010). This results in increased inflammation, which causes recruitment of immune-competent inflammatory cells and allows for increased contact between the vaccine antigen and immune cells (Giudice and Campbell, 2006; Parent du Chatelet et al., 1997). Furthermore because NF injections travel through different layers of the skin, LCs present in the epidermis may enhance the immune response as they have high migratory mobility and are effective antigen presentation cells (Bodey et al., 1997).

A direct comparison of the immune response stimulated between the two seasons of vaccine administration (Study A and B) could not be completed because of the differences between the two herds, such as animal genetics, feed and environmental conditions. However, the trends in the immune response stimulated in the two seasons of vaccination were similar, suggesting that season of vaccination does not impact the immune response stimulated following vaccination by either NF or NS techniques.

Higher antibody levels in vaccinated (NF & NS) relative to unvaccinated (Control) calves on days 42, 119 and 140 of Study A and days 119 and 140 of study B, demonstrated that vaccinated calves did elicit an antibody response against BVDV and, further, that animal exposure to BVDV did not occur during the study.

In both studies, vaccination of two-month old beef calves with a commercially available MLV combination vaccine did not elicit a detectable increase in antibody level

at 21 d post primary vaccination (day 21). Similarly, Endsley et al. (2003) reported that vaccination of seven-week old calves did not generate any detectable increase in serum neutralizing BVDV antibodies when vaccinated with either MLV or inactivated vaccines. Furthermore, it has been suggested that circulating maternal BVDV antibodies can interfere with vaccination of calves against BVDV at 10-14 d of age, which may result in susceptibility to BVDV challenge four months later (Ellis et al., 2001). The failure to detect an increase in antibody response following primary vaccination does not imply that calves were not protected. In a study in which crossbred dairy calves were vaccinated with a MLV vaccine containing BVDV at five weeks of age when plasma concentration of maternal BVDV antibodies were still high, calves produced a strong and protective immune response after being challenged with BVDV three and a half months following vaccination (Zimmerman et al., 2006). Therefore, when circulating maternal antibodies are present, it is not possible to determine if calves are protected following primary vaccination based solely on observed antibody response (Platt et al. 2009). It may be necessary to challenge calves with virulent BVDV to be able to assess protection (Platt et al. 2009). When only antibody response is recorded as an outcome, it is difficult to differentiate between a failure of a calf to mount an antibody response from an antibody response that is obscured by the presence of maternal antibodies (Robert Tremblay, personal communication).

Although a detectable primary antibody response was not apparent until day 42, a quick anamnestic (memory) response following the booster vaccination was observed. The antibody level on day 140 of both studies, 21 d post the booster vaccination, was significantly higher than the antibody level present prior to the booster vaccination (day

119) for both NF and NS vaccinated calves. Similarly, Endsley et al. (2003) reported that vaccination of seven-week old calves against BVDV, while maternal antibodies are present, can generate BVDV-specific memory B cells and develop an anamnestic antibody response following revaccination at 14 weeks. This phenomenon has also been reported by Kirkpatrick et al. (2008) who observed an anamnestic response in beef calves which were vaccinated for BVDV types 1 and 2 at approximately 67 d of age and revaccinated at approximately 190 d of age.

Unvaccinated (Control) calves were observed to be seronegative by day 119 and day 140 in Study A and B, respectively. It has been previously reported that maternally derived antibodies against BVDV have a half-life of approximately 21-23 d and can still be detected in calves that are 4-6 months of age (Bolin, 1995; Fulton et al., 2004; Radostits et al., 2000). Time to seronegative status for non-vaccinated calves has been estimated to be between 192.2 d, 179.1 d and 157.8 for BVDV types 1a, 1b and 2, respectively (Fulton et al., 2004). In the current study, unvaccinated (Control) calves were approximately six months of age in Study A and B when they were determined to be seronegative. It was also observed that vaccination of calves at two months of age slowed the rate of antibody depletion, as vaccinated calves (NF & NS) in both studies had a significantly higher antibody level on day 140 than did unvaccinated calves (Control). Fulton et al. (2004) reported a half-life for BVDV types 1a, 1b and 2 in vaccinated calves of 33.5 d, 30.3 d and 44.9 d compared to 23.1 d, 22.8 d and 22.9 d in non-vaccinated calves, respectively. These same authors also reported that time to seronegative status in calves vaccinated for BVDV types 1a, 1b and 2 was 274.9 d, 238.0 d and 332.4 d compared to 192.2 d, 179.1 d and 157.8 d in non-vaccinated calves, respectively. These

studies suggest that the rate of BVDV antibody depletion is slowed when animals are vaccinated.

In Study A and B, it was observed that vaccinated calves (NF and NS) had a negative correlation between the antibody levels at the start (day 0) and end of the studies (day 140). However, only those in Study A, -0.5673 (NF) and -0.5023 (NS), were determined to be significantly different from zero. Nonetheless, the negative correlations seen in NF and NS vaccinated calves may further indicate that the circulating BVDV maternal antibodies present prior to vaccination may have interfered with the ability of calves to stimulate an immune response following vaccination. The negative correlation implies that calves with high levels of circulating BVDV maternal antibodies at the start of the study, had a lower antibody level at the end of the study and vice versa for calves with low levels of circulating BVDV maternal antibodies at the start of the study. This resulted in all calves still having a reasonable antibody level on day 140 and results in a more uniform antibody level and possible protection against BVDV infection in the herd (Robert Tremblay, personal communication; Juan-Carlos Rodriguez, personal communication).

Needle-free injection devices have been reported to leave residual vaccine on the surface of the skin/hair, which may be unavoidable and may hinder or delay the acceptance of the technology (Jones et al., 2005). Chase et al. (2008a) proposed that the residual vaccine volume seen at injection sites following NF vaccination is usually quite small (0.0004 mL). Although vaccine residue was not quantified in Study A and B, it was visually apparent on the skin/hair of some of the NF vaccinated calves after vaccination

on day 0 and 119, presumably due to differences in hide thickness and hair coat among calves. Human error may have also contributed to the vaccine residue, as during some injections the device may have not been positioned exactly perpendicular (90°) to the skin, resulting in some of the fluid stream to be left at the skin/hair surface. However, the presence of residue did not appear to compromise the antibody response stimulated following NF vaccination against BVDV.

4.6. CONCLUSIONS

In summary, NF vaccination was observed not to affect animal performance, but was found to cause a greater frequency of skin reactions after vaccination than did the conventional NS vaccination technique. Therefore, further research should be conducted in cattle to determine the impact of skin reactions on carcass quality and if trimming losses are increased when calves are vaccinated using a NF vaccination technique. Previous studies in cattle and the present research shows that NFIDs are capable of stimulating a comparable and sometimes enhanced immune response to a number of vaccines when compared to NS vaccination. It was observed that vaccination of beef calves at two months of age with a commercially available MLV combination vaccine when maternal BVDV antibodies are still present, using either a NF or NS vaccination techniques, may mask the primary antibody response elicited after primary vaccination. However, this did not affect the memory immune response stimulated, as both NF and NS vaccinated calves showed an anamnestic response following the booster vaccination. Due to the negative correlation observed in NF and NS vaccinated calves between antibody levels at the start and end of Study A and B, it may be evident that circulating

maternal antibodies present prior to vaccination of two-month old calves, may interfere with the ability of the immune system to respond to vaccination. Despite the fact that vaccine residue was apparent on some NF vaccinated calves, residue was found not to compromise the antibody response stimulated following vaccination of calves against BVDV using a NFID. This research has demonstrated that vaccination of beef calves against BVDV at a young age using either a NF or NS vaccination technique is effective in stimulating an immune response. However, it is also evident that producers must ensure to booster the animals as described in vaccine protocols, in order to overcome the issue of maternal antibodies affecting immune response and to obtain optimal protection against BVDV.

5. MANUSCRIPT II

Effect of a needle-free vaccination technique on antibody response in spring-born and fall-born beef calves after vaccination against *Clostridium chauvoei*

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5.1. ABSTRACT

Antibody production was measured in both spring-born (Study A) and fall-born (Study B) beef calves following vaccination at two months of age (day 0) with a commercially available clostridial vaccine containing *C. chauvoei*, *C. septicum*, *C. novyi*, *C. sordelli*, *C. perfringens* types B, C and D using either NF or NS vaccination techniques. On day 21 of both studies, calves received a booster vaccination using the same vaccination technique and vaccine product. Body weight was recorded on days 0, 21, 42, 119, and 140 and post-vaccination skin reactions were recorded on days 21, 42, 119 and 140. Presence of visible vaccine residue at the surface of the skin/hair following NF vaccinations was recorded on days 0 and 21. Blood samples were collected on days 0, 21, 42, 119, and 140. Serum antibody levels against *C. chauvoei* were analyzed in 30 calves per vaccination group by an IIF technique. Furthermore, the trends in the immune response stimulated against *C. chauvoei* in the two seasons of vaccination (Study A and B) were similar. Animal performance, as measured by BW, did not differ ($P>0.05$) between vaccination techniques in either study. Needle-free vaccination was observed to cause a greater frequency ($P<0.05$) of skin reactions after vaccination than did NS vaccination, except for day 42 of Study B. Throughout Study A and B, antibody levels against *C. chauvoei* were not significantly different ($P>0.05$) between vaccination techniques. Visible vaccine residue occurred for 24.7% of NF vaccinations, but did not compromise antibody response. In summary, NF vaccination is effective in stimulating an immune response against *C. chauvoei*.

Keywords: *Clostridium chauvoei*, antibody response, NF vaccination, NS vaccination, beef calves, indirect immunofluorescence, animal performance, skin reactions

5.2. INTRODUCTION

C. chauvoei is a soil-borne anaerobic bacterium belonging to the *Clostridium* genus, which causes a fatal disease of cattle and other ruminants known as blackleg (Kijima-Tanaka et al., 1997). Diseases caused by *Clostridium* organisms can affect beef cattle of all ages, but are a primary concern in cattle between six months and two years of age (Sojka et al., 1992; Troxel et al., 2001). *C. chauvoei* infection often causes serious toxemia and sudden death when activated latent spores are ingested by the animal or colonize the muscle of an animal after injury (Araujo et al., 2010; Kijima-Tanaka et al., 1997; Miyashiro et al., 2007; Sojka et al., 1992; Useh et al., 2003). The clinical course of blackleg tends to be acute and animals are often found dead 12-48 hours post infection (Kijima-Tanaka et al., 1997; Sojka et al., 1992).

Vaccination against *C. chauvoei* is one of the most reliable prophylactic approaches to protect cattle against infection (Araujo et al., 2010; Kijima-Tanaka et al., 1997; Useh et al., 2003). A wide variety of vaccines are available, which consist of inactivated cultures of *C. chauvoei* (Kahn et al. 2011b). Furthermore, most commercial vaccines against blackleg used in the cattle industry are generally presented as multivalent vaccines, which consist of *C. chauvoei* and other organisms in the *Clostridium* genus (Crichton et al., 1990). Clostridial vaccines are routinely administered to cattle using a NS vaccination technique. However, NFIDs, which were developed in the 1940's and have been previously used to vaccinate humans (Hingston et al., 1963) and swine (Chase et al., 2008a), are currently being investigated as an alternative to NS vaccination. Needle-free injection devices utilize mechanical compression, triggered when the nozzle touches the skin, to power vaccine injections through a small orifice, producing a high pressure

stream that can penetrate the skin and deposit the vaccine into the desired tissue (Jackson et al., 2001; Mousel et al., 2008). Needle-free vaccination techniques offer advantages over conventional vaccine delivery methods, including elimination of broken needles (Chase et al., 2008a; Stier, 2003), reduced disease transfer (Reinbold et al., 2010), reduced vaccination time (Mousel et al., 2008), greater antigen dispersion (Bennett et al., 1971; Chase et al., 2008a; Parent du Chatelet et al., 1997; Reis et al., 1998), elimination of needle disposal (Chase et al., 2008a) and elimination of needle-sticks to individuals giving injections.

Previous research demonstrated that NF vaccination stimulated a comparable and sometimes enhanced immune response compared to NS vaccination after vaccination against MH (Hollis et al., 2005a; Hollis et al., 2005b), LP (Hollis et al., 2005a), *Brucella abortus* (Pires et al., 2007), BVDV (Manuscript I) and BHV-1, commonly known as IBR (Hollis et al., 2005a; Hollis et al., 2005b; van Drunen Littel-van den Hurk, 2006). Similarly, studies in humans (Jackson et al., 2001), rabbits (Aguiar et al., 2001), sheep (Mousel et al., 2008), swine (Houser et al., 2004) have shown the same results. However, to the author's knowledge, there is no information available on the efficacy of a NF vaccination technique in administering a commercially available clostridial vaccine, containing *C. chauvoei*, using a NF or NS vaccination technique.

The purpose of this study was to compare animal performance, as measured by BW, presence of skin reactions, and the antibody response of calves following vaccination against *C. chauvoei* using a NF or NS vaccination technique.

5.3. MATERIALS AND METHODS

5.3.1. Experimental animals

Two separate studies (Study A and B) were performed in two commercial cow-calf beef herds located in the Province of Manitoba, Canada. The herd used for Study A consisted of 86 spring-born crossbred beef calves (106.24 ± 16.68 kg), while the herd used for Study B consisted of 88 fall-born crossbred beef calves (101.46 ± 16.96 kg). In both herds, animal feed and handling management followed standard industry practices.

5.3.2. Vaccination protocol

As indicated in Table 6, calves in Study A, were randomly assigned to one of two vaccination groups: NF vaccination (n=47) and NS vaccination (n=39). In Study B, calves were also randomly assigned to one of two vaccination groups: NF vaccination (n=48) and NS vaccination (n=40). The treatments did not include unvaccinated calves as the risk of infection with *Clostridium* bacteria in these commercial herds was high.

All calves were weighed (kg) prior to vaccination or data collection on days 0, 21, 42, 119 and 140 of Study A and B. According to vaccine manufacturer's recommendations, calves in the NF and NS groups were vaccinated at approximately two months of age (day 0) and revaccinated on day 21 with a commercially available clostridial vaccine (Clostri Shield 7, Novartis Animal Health Canada Inc.) containing

Table 6 Time schedule for administration of a clostridial vaccine and subsequent data collection.

Study	Group	*N*	Vaccinations (days)	Blood collection and BW (days)	Injection site assessment (days)
Study A	NF	47	0, 21	0, 21, 42, 119, 140	21, 42, 119, 140
	NS	39 (38[a])	0, 21	0, 21, 42, 119, 140	21, 42, 119, 140
Study B	NF	48	0, 21	0, 21, 42, 119, 140	21, 42, 119, 140
	NS	40 (39[a]) (38[b])	0, 21	0, 21, 42, 119, 140	21, 42, 119, 140

n, Number of animals; NF, Needle-free vaccination; NS, Needle-syringe vaccination
[a] After day 21 of Study A and B, one calf in each study was removed from the trial
[b] After day 42 Study B, another calf was removed from the trial

C. chauvoei, *C. septicum*, *C. novyi*, *C. sordelli*, *C. perfringens* types B, C and D. Needle-free vaccinations were administered with a NFID (Pulse 250 NeedleFree Injection System, Pulse NeedleFree Systems) on the right side of the neck using a skin-tenting technique (See Appendix I). In both studies, the NF vaccinations were administered to calves by one individual who had been previously trained in the use and maintenance of the NFID. Carbon dioxide was used as the energy source for the NF vaccinations on day 0 and 21 of Study A and day 0 of Study B. However, the booster NF vaccinations on day 21 of Study B required the use of compressed N_2 as the power source instead of liquid CO_2 as per the direction of Pulse NeedleFree Systems, to ensure that the injection was not compromised as a consequence of the subzero temperatures. Using guidelines from Pulse NeedleFree Systems, the NFID was set at pressures of 45-55 PSI and 60-65 PSI to administer the vaccine on days 0 and 21, respectively, in order to perform a SC injection. Immediately following each NF vaccination on day 0 and 21, the presence of visible

vaccine residue on the surface of the skin/hair, if seen, was recorded. The amount of vaccine residue present was not quantified, however, any visible fluid seen on the surface of the skin/hair was noted. In the NS group, vaccinations were administered SC with a multi-dose pistol-grip syringe (Kane Veterinary Supplies Ltd), fitted with an 18G by 1 inch detectable needle (Partnar Animal Health Inc.), on the right side of the neck using the same skin-tenting that was used to administer the NF vaccinations. Enough vaccine (20 mL) was drawn into the syringe to vaccinate ten calves. To simulate standard industry practices, following vaccination of ten calves, the used needle was replaced with a new sterile needle and enough vaccine (20 mL) was drawn into the syringe to vaccinate an additional ten calves. If a needle became bent or burred, it was immediately replaced. Furthermore, on days 21, 42, 119 and 140 of both studies, calves were visually inspected, palpated and scored for the presence of skin reactions, as in Troxel et al., (1997). Any apparent raised surfaces observed at the injection sites were considered skin reactions occurring as a result of vaccination.

In addition to vaccination with a clostridial vaccine, all calves in Study A and B were vaccinated with Express 5 (Boehringer Inhelheim (Canada) Ltd.) to protect calves from infection of viruses which cause respiratory disease.

All experimental procedures were conducted in compliance with the Canadian Council of Animal Care as approved by the University of Manitoba Animal Care Committee (1993).

5.3.3 Indirect immunofluorescent antibody testing

Blood samples from all calves were collected via jugular venipuncture into serum vacutainer tubes (BD Vacutainer) on days 0, 21, 42, 119 and 140 of Study A and B (Table 6). Following the collection of blood, samples were allowed to clot and then centrifuged at 1100 *g* for 10 min. Aliquots of serum were then stored at -20°C until analyzed. Antibody testing was only completed on serum samples collected on days 0, 21, 42 and 119. Furthermore, in Study A and B only serum from 30 calves in the NF vaccination group and 30 calves in the NS vaccination group were evaluated for the level of antibodies against *C. chauvoei*.

To evaluate the level of antibodies against *C. chauvoei*, an IIF technique modified from Hamaoka and Terakado (1994), Rahimi et al. (2011) and Weintraub et al. (1979) was used. *Clostridium chauvoei* ATCC 10092 bacteria was cultured in Shaedler Anaerobe Broth (CM0497, Oxoid Ltd.) and incubated anaerobically at 37°C for 24 hours, using a BBL GasPak 100 Anaerobic Jar System (Becton Dickinson). Following 24-hour incubation, the cultures were transferred into conical flasks and centrifuged at 751 *g* for 5 minutes. The supernatant was then removed and the remaining bacterial pellet was washed by re-suspending it with approximately 15 mL of 1x phosphate buffered saline (PBS) (0.0067M, Hyclone), followed by centrifugation at 751 *g* for 5 minutes and removal of the supernatant. This wash procedure was repeated a second time. The two wash procedures were performed on the bacterial cells to avoid non-specific binding and background noise (Figure 8). Thereafter, the resulting bacterial pellet was re-suspended in 1mL of 1x PBS. Following re-suspension, the antigen (*C. chauvoei* + 1x PBS) was further diluted 1:2 in order to contain a *C. chauvoei* bacterial concentration of

approximately 10^2. As shown in Fig. 9b, the further dilution, (1:2) of the *C. chauvoei* antigen was performed in order to visualize the fluorescence of individual bacterial cells. The 1:2 dilution was determined to be optimal when compared to undiluted antigen (Fig. 9a) or antigen diluted 1:4 (Fig. 9c). After diluting the antigen 1:2, 5*µl* of the *C. chauvoei* antigen was added to each well of 10-well IF microscope slides (Polysciences, Inc.). Slides were air dried in a biosafety cabinet and stored at -20°C until required for antibody testing.

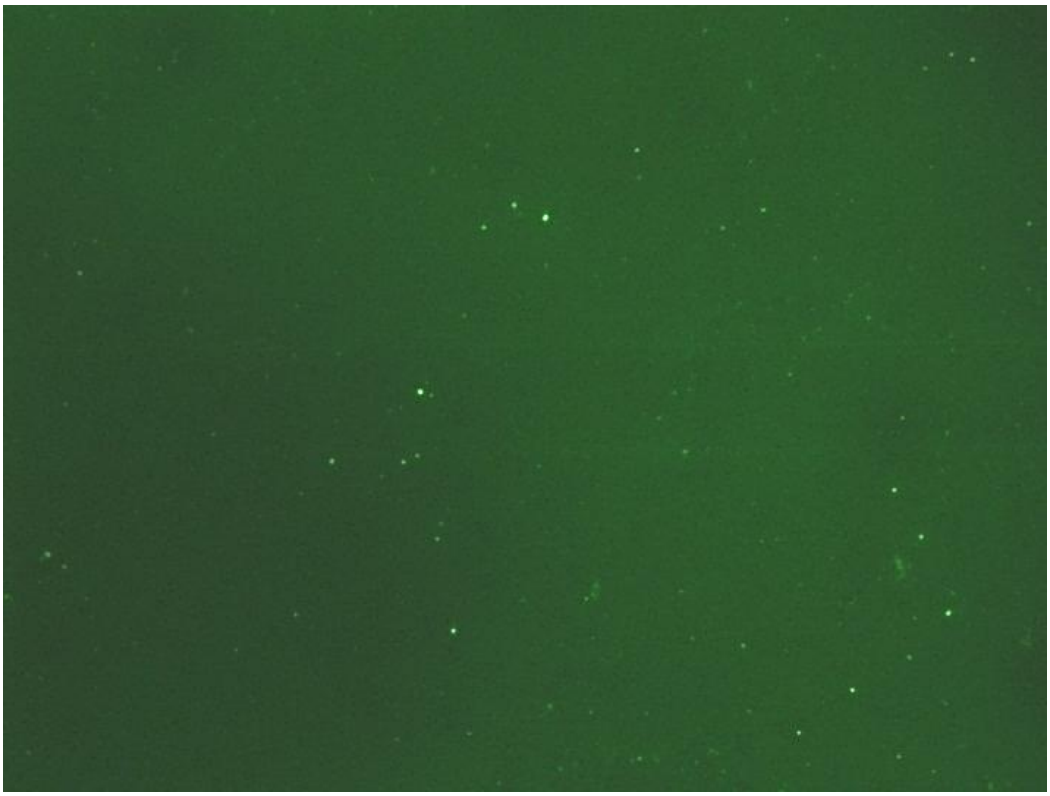

Fig. 8. Indirect immunofluorescence of negative control (1x PBS) added to *C. chauvoei* antigen.

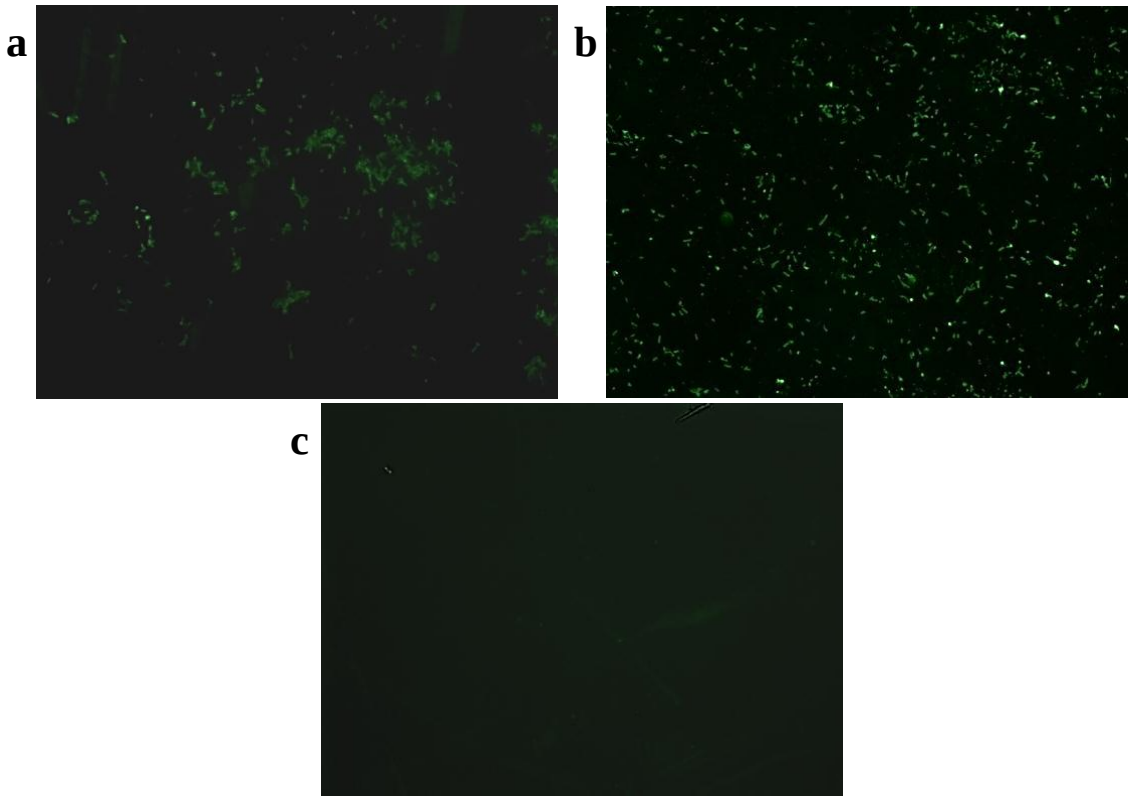

Fig. 9. Indirect immunofluorescence of undiluted positive control antiserum added to *C. chauvoei* antigen: a) undiluted; b) diluted 1:2; c) diluted 1:4.

Serum samples collected from the 30 NF and 30 NS vaccinated calves on days 0, 21, 42 and 119 of Study A and B were serially diluted (1:8 – 1:4,096) with 1x PBS. Thereafter, 5*µl* of each serial dilution was added to separate wells of the previously frozen 10-well IF microscope slides fixed with *C. chauvoei*. The IIF test was standardized by adding 5*µl* of positive control serum to each well of a previously frozen *C. chauvoei* fixed slide. Serum was obtained from a Holstein dairy heifer that had been immunized with two doses of a commercially available clostridial vaccine, with a 21 d interval between vaccinations. Serum was collected from this heifer 15 d post the second immunization, to ensure an optimal antibody response against *C. chauvoei*. A negative

control was examined simultaneously by adding 5μl of 1x PBS to each well of a second *C. chauvoei* fixed slide. Thereafter, all slides were incubated at 37°C in a moist chamber for 30 minutes.

Following removal from the incubator, slides were rinsed with 1x PBS and 5μl of anti-bovine IgG (H&L, GOAT) antibody fluorescein conjugate (Rockland) was added to all wells. The anti-bovine IgG (H&L, GOAT) antibody fluorescein conjugate was diluted 1:50 in order to visualize fluorescence of individual bacterial cells. A 1:50 dilution (Fig. 10a) of the anti-bovine IgG (H&L, GOAT) antibody fluorescein conjugate was determined to be the optimal dilution for visual observation compared to a dilution of 1:100 (Fig. 10b) or a dilution 1:1,000 (Fig. 10c). Thereafter, slides were again incubated at 37°C in a moist chamber for 30 minutes.

Following incubation the slides were removed, rinsed a second time with 1x PBS and mounted using one drop of fluorescent antibody mounting fluid (50% glycerol/50% buffer, pH=9.0-9.6, VMRD, Inc.). All slides were read on an inverted fluorescent microscope (EVOS) using the 40x objective lens. The endpoint antibody level was determined as the highest serum dilution (well) on each slide to show fluorescence. The *C. chauvoei* antibody levels (titers) were then expressed as the $\log_2$ of the reciprocal of the dilution factor as described in Brugh (1977).

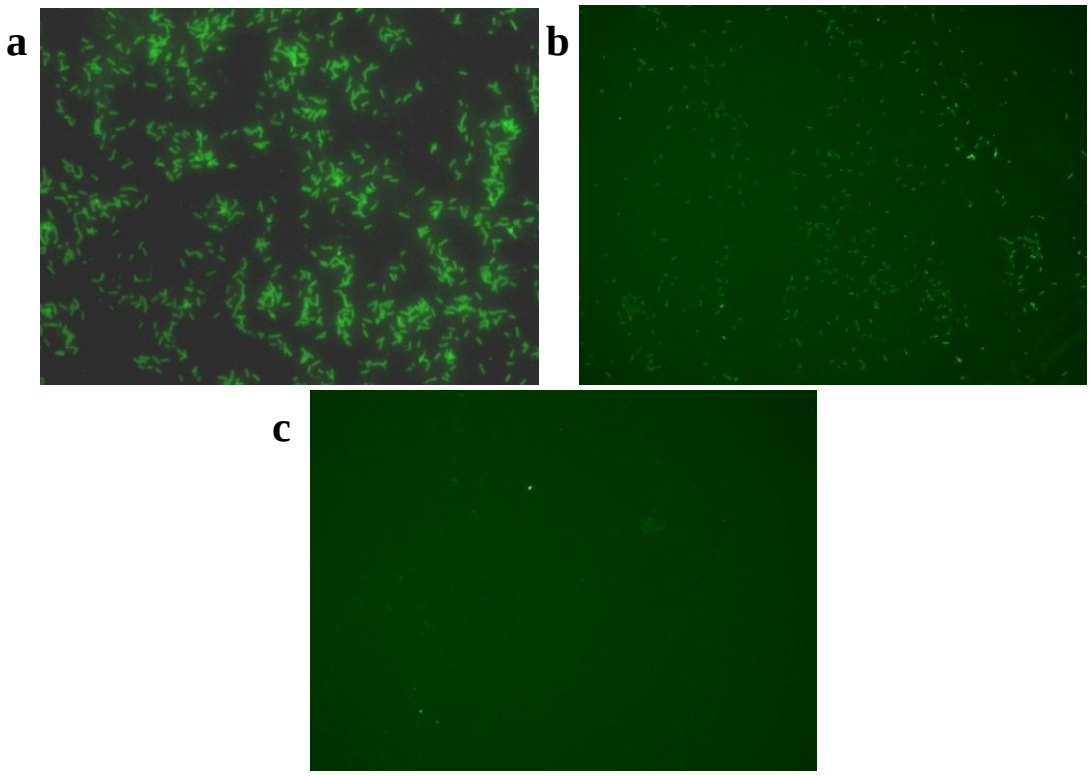

Fig. 10. Indirect immunofluorescence of undiluted positive control antiserum using the anti-bovine IgG antibody fluorescein conjugate diluted: a) 1:50; b) 1:100; c) 1:1,000.

5.3.4. Statistical analysis

Data for Study A and B were analyzed separately and significance for all tests were determined by a two-tailed test using a 5% significance level (P<0.05).

Analysis of BW and antibody level was completed in SAS 9.2 (SAS Institute Inc.) using a linear mixed model for repeated measures with fixed effects of group, days post vaccination and their interaction and random effects of animal within group. Individual animal was regarded as the experimental unit in the two vaccination groups (NF and NS). Covariance of performance across times was accounted for using the unstructured covariance method (Wang and Goonewardene, 2004).

Data for presence of visible vaccine residue was analyzed using the same linear mixed model for repeated measures with the individual animal regarded as the experimental unit but with five treatment groups (NS, NF no vaccine residue (NFnoR), NF primary vaccination vaccine residue (NFpR), NF booster vaccination vaccine residue (NFsR) and NF primary and booster vaccinations vaccine residue (NFpsR). The covariance of performance across times was accounted for using the unstructured covariance method (Wang and Goonewardene, 2004). Contrasts were completed in order to determine if NF vaccinated calves with visible vaccine residue (NFpR, NFsR and NFpsR) noted on the skin surface after primary and/or booster vaccination differed in antibody levels compared to NF vaccinated calves with no vaccine residue visible (NFnoR).

Confidence limits (95%) of the estimated frequencies of NF and NS animals having at least one skin reaction at the site of vaccine administration were calculated and used to determine significant differences between groups (Steel et al., 1997).

5.4. RESULTS

5.4.1. Body weight

Body weight (P>0.05) did not differ between NF and NS vaccination groups throughout either study (Figs. 11 & 12 and Table 7), with calves in Study A averaging 1.19 ± 0.04 kg/day, 1.09 ± 0.02 kg/day and 1.14 ± 0.02 kg/day, respectively, and calves in Study B averaging 0.81 ± 0.05 kg/day, 0.90 ± 0.02 kg/day and 0.92 ± 0.03 kg/day, respectively.

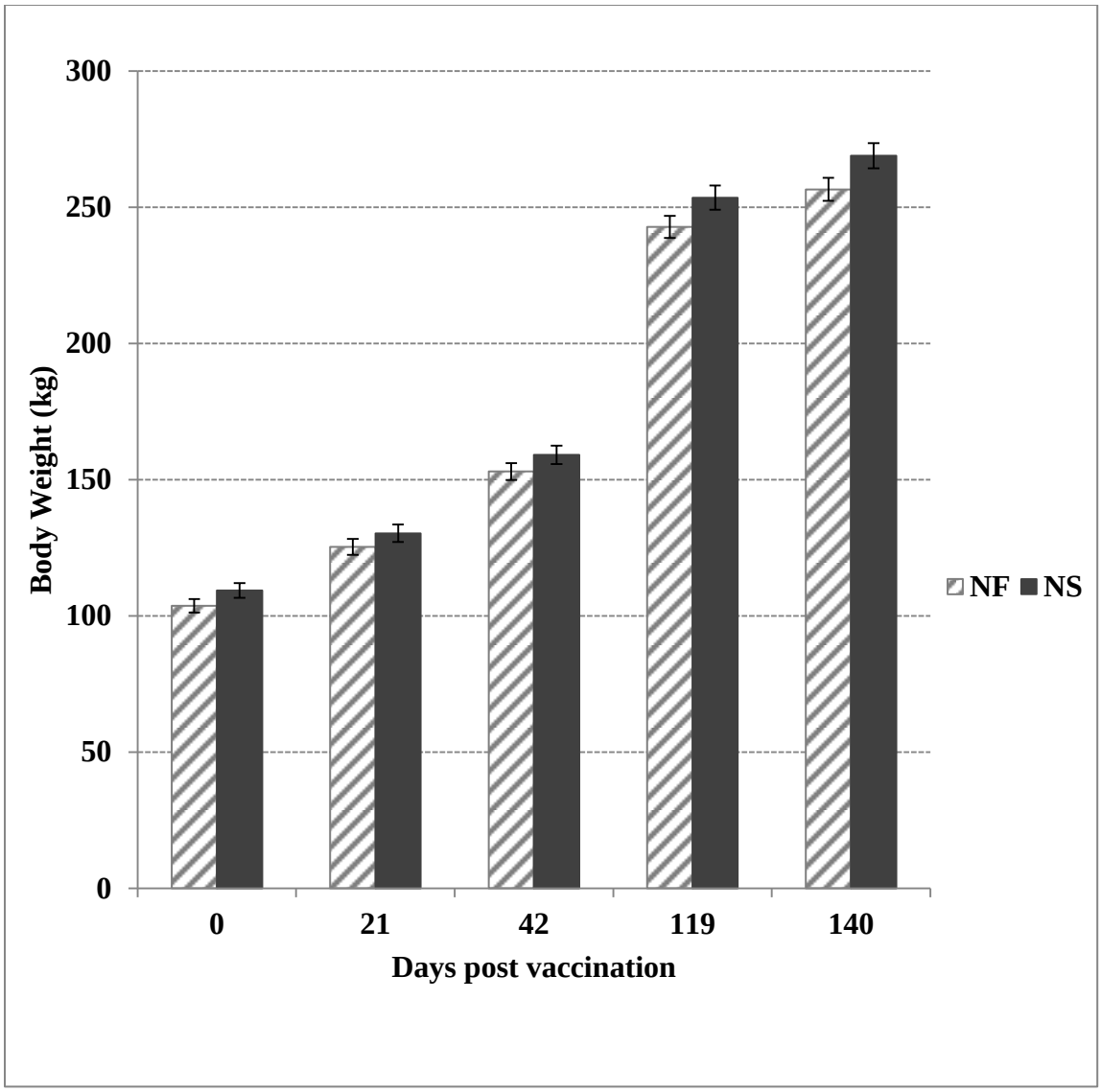

Fig. 11. Body weight of spring-born calves (Study A) vaccinated against *Clostridium chauvoei* using needle-free (NF) or needle-syringe (NS) techniques (standard errors shown as bars).

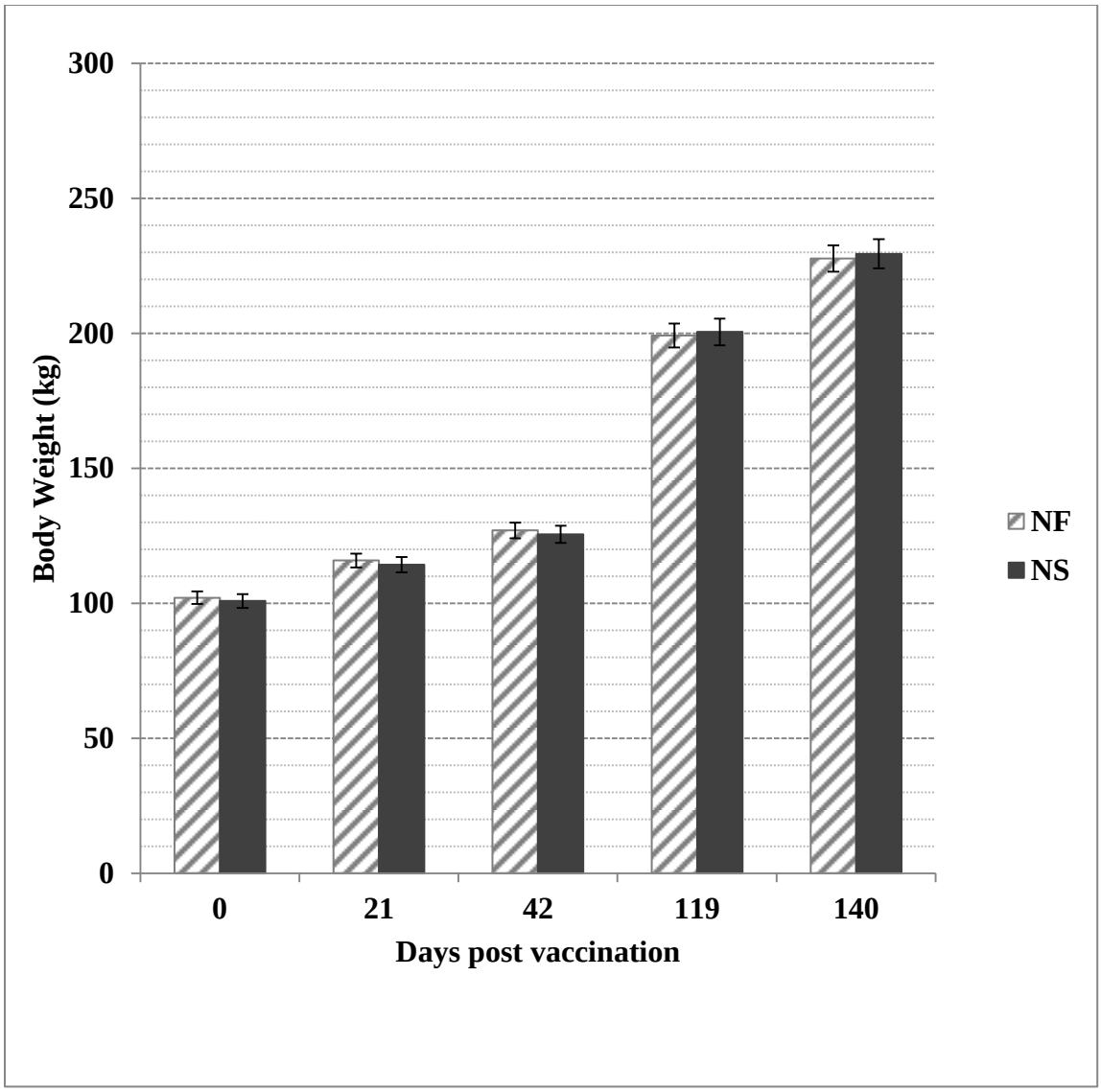

Fig. 12. Body weight of fall-born calves (Study B) vaccinated against *Clostridium chauvoei* using needle-free (NF) or needle-syringe (NS) techniques (standard errors shown as bars).

Table 7 Factors affecting body weight (BW)

		P Values		
Item	Study	Trt	DaysPV	Interaction
Body Weight	A	0.0882	<0.0001*	0.3174
	B	0.9677	<0.0001*	0.9046

* = Significant (*P*<0.05)

5.4.2. Skin reactions

Throughout Study A, the NF vaccinated calves had a higher (*P*<0.05) frequency of skin reactions at the site of vaccination compared to NS vaccinated calves as shown in Fig. 13.

On days 21, 119 and 140 of Study B, the NF vaccinated calves had a higher (*P*<0.05) frequency of skin reactions at the site of vaccination than NS vaccinated calves as shown in Fig. 14. However, the frequency of skin reactions observed on day 42 of the study was found to be similar (*P*>0.05) in both NF and NS vaccinated calves.

5.4.3. Antibody level (Study A & B)

As shown in Figs. 15 and 16, throughout both Study A and B, the antibody levels against *C. chauvoei* were not different (*P*>0.05) between NF and NS vaccination techniques.

The trend in immune response stimulated following vaccination of spring-born (Study A) and fall-born calves (Study B) was comparable between the two seasons of vaccine administration.

As indicated in Table 8, following primary vaccination in both studies, the antibody level against *C. chauvoei* on day 21 for both NS ($P<0.0001$) and NF ($P<0.0001$) vaccinated calves was higher when compared to the antibody level observed at the start of the study (day 0). Similarly, on day 42 of both studies, 21 days post the booster vaccination, the antibody level against *C. chauvoei* in both NS ($P=0.0006$ and $P<0.0001$ for Study A and B, respectively) and NF ($P<0.0001$) vaccinated calves was higher than the antibody level observed on day 21. Furthermore in Study A and B, calves in both vaccination groups (NF and NS) had a lower ($P<0.0001$) antibody level against *C. chauvoei* on day 119 when compared to the antibody level on day 42.

5.4.4. Vaccine residue

Vaccine residue was visually apparent on the skin/hair surface of 24.7% of NF vaccinations in Study A and B combined, indicating possible failure of the injection in the animal.

On day 0 of Study A, 34% of calves in the NF group had visible vaccine residue on the surface of the skin/hair following primary vaccination at a pressure of 45-50 PSI. Subsequently, when the booster vaccination was administered on day 119, 19.1% of calves in the NF group had visible vaccine residue following vaccination at a pressure of 85 PSI. Similarly on day 0 of Study B, 27.1% of calves in the NF group had visible vaccine residue following primary vaccination at a pressure of 45-50 PSI. Following the booster vaccination (85 PSI) administered on day 119, 18.8% of calves in the NF group had visible vaccine residue.

The presence of visible vaccine residue on the surface of the skin/hair after the primary and booster vaccinations in both studies did not compromise (P>0.05) antibody response in NF vaccinated calves.

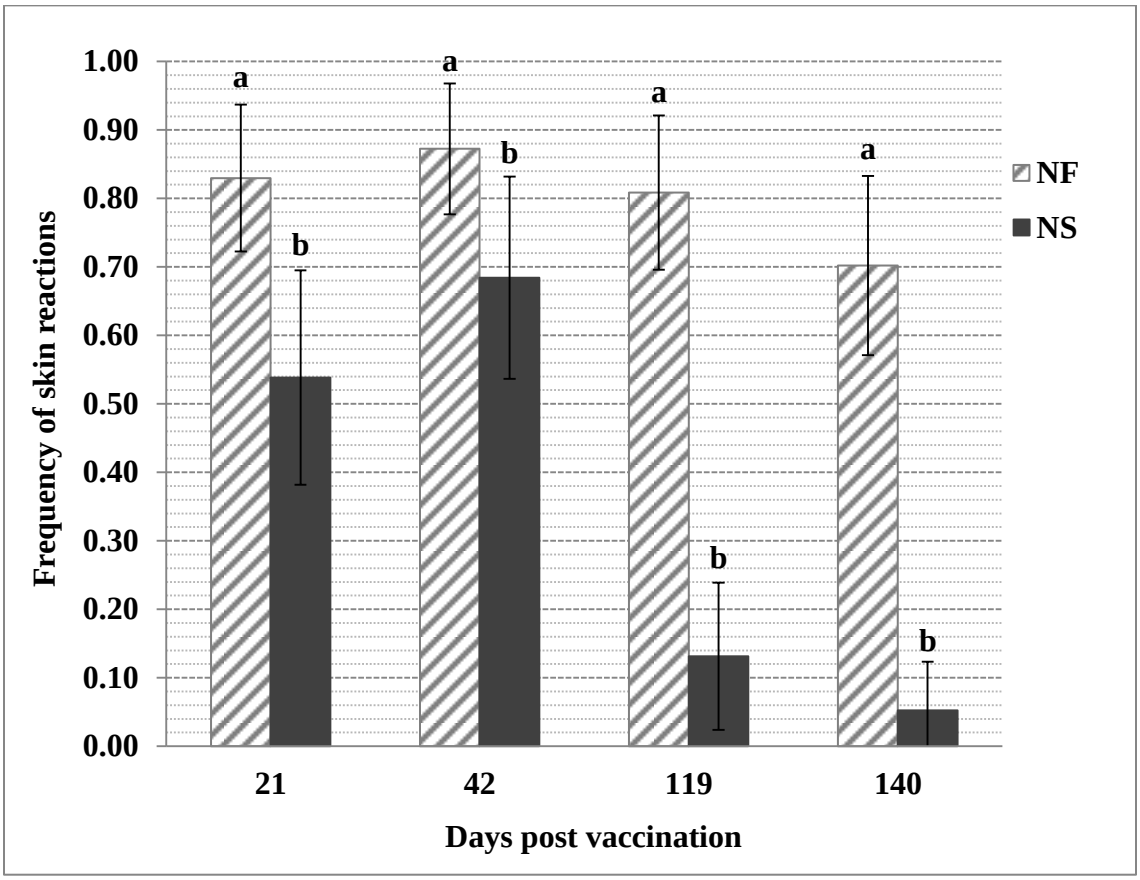

Fig. 13. Frequency of skin reactions after vaccination of spring-born calves (Study A) against *Clostridium chauvoei* using needle-free (NF) or needle-syringe (NS) techniques (95% confidence limits shown as bars).

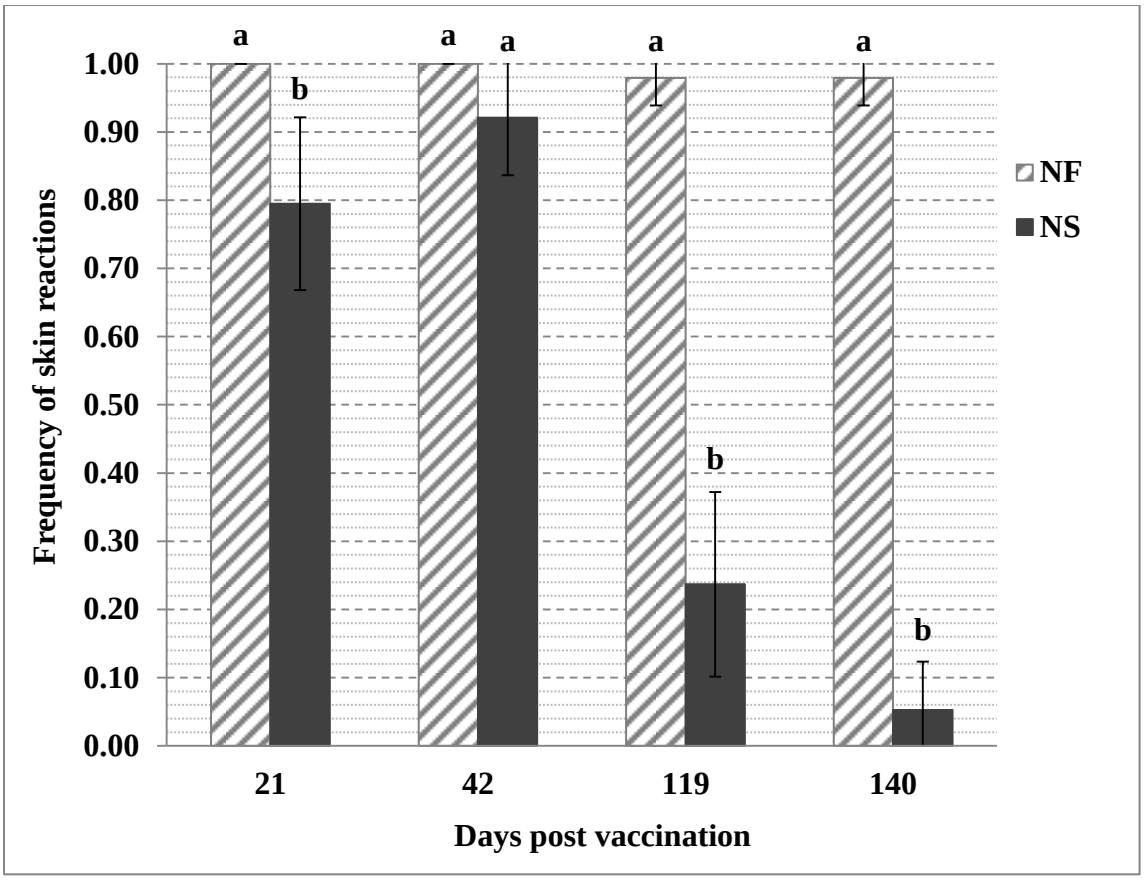

Fig. 14. Frequency of skin reactions after vaccination of fall-born calves (Study B) against *Clostridium chauvoei* using needle-free (NF) or needle-syringe (NS) techniques (95% confidence limits shown as bars).

Table 8 Within treatments antibody levels in spring-born (Study A) and fall-born calves vaccinated against *C. chauvoei* virus using needle-free (NF) or needle-syringe (NS) techniques.

Study	Trt	DaysPV	Antibody Level	DaysPV	Antibody Level	P-value
Study A	NF	0	7.6000[a]	21	8.9667[b]	<0.0001
		21	8.9667[a]	42	9.7333[b]	<0.0001
		42	9.7333[a]	119	7.1667[b]	<0.0001
	NS	0	7.7333[a]	21	9.0000[b]	<0.0001
		21	9.0000[a]	42	9.5000[b]	0.0006
		42	9.5000[a]	119	6.9333[b]	<0.0001
Study B	NF	0	7.6000[a]	21	9.0000[b]	<0.0001
		21	9.0000[a]	42	10.5333[b]	<0.0001
		42	10.5333[a]	119	8.5667[b]	<0.0001
	NS	0	7.9333[a]	21	9.1667[b]	<0.0001
		21	9.1667[a]	42	10.3667[b]	<0.0001
		42	10.3667[a]	119	8.1667[b]	<0.0001

Trt, Treatment; DaysPV, Days post vaccination

[a,b] Antibody levels in the same row with different superscripts differ significantly ($P<0.05$)

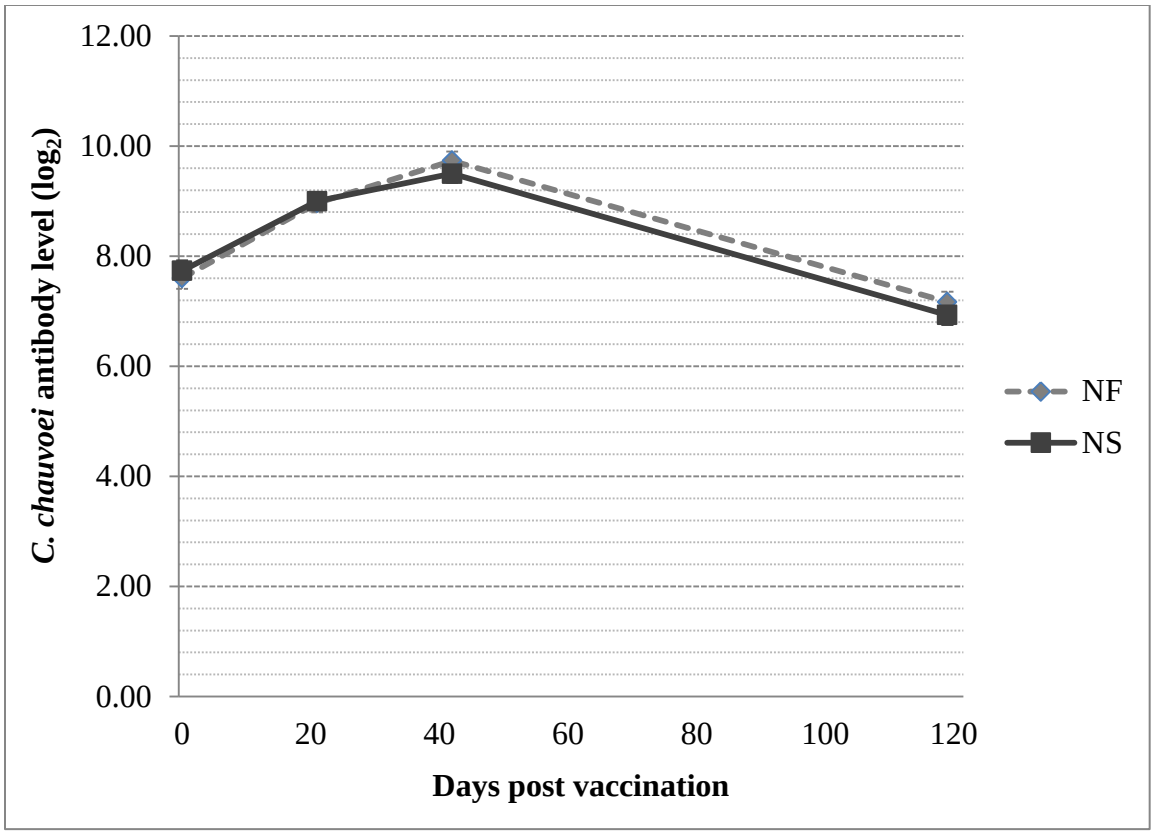

Fig. 15. Antibody levels of spring-born calves (Study A) after vaccination against *Clostridium chauvoei* using needle-free (NF) or needle-syringe (NS) techniques (standard errors shown as bars).

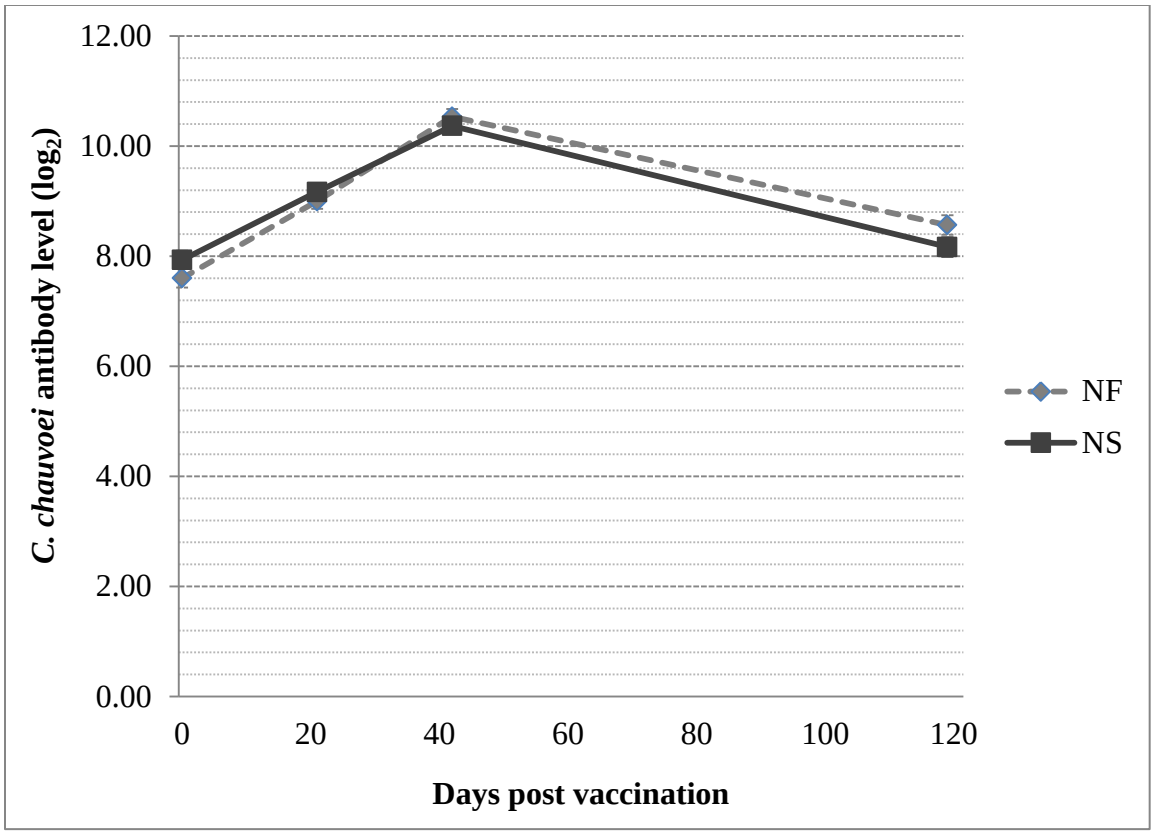

Fig. 16. Antibody levels of fall-born calves (Study B) after vaccination against *Clostridium chauvoei* using needle-free (NF) or needle-syringe (NS) techniques (standard errors shown as bars).

5.5. DISCUSSION

The use of a NFID to vaccinate two-month old beef calves with a multivalent clostridial vaccine containing *C. chauvoei* did not affect animal performance, as BW was observed to be similar between NF and NS vaccination techniques. This is similar to the results obtained following the vaccination of two-month old beef calves with a MLV combination vaccine containing BVDV (Manuscript I).

In the current study, NF vaccinated calves had a greater frequency of skin reactions than did NS vaccinated calves, except on day 42 of Study B. The authors have no explanation as to why the frequency of skin reactions on day 42 of Study B were

found to be comparable between NF and NS vaccination techniques. In both Study A and B, the impact of skin reactions on carcass quality were not explored. Unpublished research (Manuscript I) in two-month old beef calves vaccinated with a MLV combination vaccine containing BVDV using a NFID resulted in a greater frequency of skin reactions than did NS vaccination. Similarly, Gerlach et al. (2009) observed that injection sites in swine previously contaminated with *Arcanobacterium pyogenes* had a greater number of abscesses, in both the neck (P=0.0625) and ham (P=0.0313), when injected with an aluminum hydroxide adjuvant using a NF compared to a NS vaccination technique. Contrary to Gerlach et al. (2009), Houser et al. (2004) reported in swine that there was no significant difference in the number of gross or histological reactions observed between NF and NS vaccination groups. Finally, previous research conducted in humans revealed that NF vaccinations resulted in more frequent local injection site reactions than did NS vaccination (Jackson et al., 2001; Mathei et al., 1997; Williams et al., 2000). It is important to note that it is difficult to compare the results seen in cattle to those observed in swine and humans, due to the differences between species. Although skin reactions and lesions caused by vaccination may affect carcass quality, they have also been reported to have a positive effect on the immune response acquired following vaccination. Troxel et al. (2001) demonstrated that heifers with lesions following NS vaccination had elevated antibody titers against *C. chauvoei* on days 28 (P<0.08) and 84 (P<0.07) when compared to heifers with no lesions. However, in Study A and B, the impact of skin reactions on immune response were not explored.

As mentioned in Manuscript I, the observed increase in skin reactions in both Study A and B following NF vaccination of beef calves with a clostridial vaccine could

have been caused by many factors such as injury caused by the vaccination (i.e. pressure trauma), contamination by transmitting microorganisms from the skin/hair surface into the tissue surrounding the injection site (Gerlach et al., 2009; Sutterfield et al., 2009) or by irritation from the oil-based adjuvant used to enhance the immunogenicity of the vaccine. Furthermore, it can be speculated that because BW did not differ ($P>0.05$) between vaccination techniques, the skin reactions caused by both vaccination techniques were not severe enough to affect animal performance.

In the current study, NF and NS vaccinated calves had a comparable immune response following vaccination against *C. chauvoei*. As mentioned in Manuscript I, previous research demonstrated that vaccination of cattle (Hollis et al., 2005a; Hollis et al., 2005b; Pires et al., 2007; van Drunen Littel-van den Hurk,S., 2006) via a NF vaccination technique results in a comparable and sometimes enhanced immune response when compared to NS vaccination, depending on the sex of the animals and the vaccine antigen measured. Similarly, unpublished research (Manuscript I) in two-month old beef calves vaccinated against BVDV also demonstrated a comparable immune response between NF and NS vaccination techniques. Studies conducted in humans (Jackson et al. 2001), rabbits (Aguiar et al., 2001), sheep (Mousel et al., 2008) and swine (Houser et al., 2004) have also demonstrated the same results. The possible reason for NFIDs being able to sometimes enhance the immune response stimulated following vaccination when compared to NS vaccination, may be due to the capability of NF vaccination to penetrate vaccines through different layers of the skin and disperse the vaccines more widely in the tissues (Bennett et al., 1971; Grant, 2010). This results in increased inflammation, which causes recruitment of immune-competent inflammatory cells and allows for increased

contact between the vaccine antigen and immune cells (Giudice and Campbell, 2006; Parent du Chatelet et al., 1997). Furthermore because NF injections travel through different layers of the skin, LCs present in the epidermis may enhance the immune response as they have high migratory mobility and are effective antigen presentation cells (Bodey et al., 1997).

A direct comparison of the immune response stimulated between the two seasons of vaccine administration (Study A and B) could not be completed because of the differences between the two herds, such as animal genetics, feed and environmental conditions. However, the trends in the immune response stimulated in the two seasons of vaccination were similar, suggesting that season of vaccination does not impact the immune response stimulated following vaccination by either NF or NS techniques.

Vaccination of cattle against *C. chauvoei* is able to stimulate the production of antibodies specific to the bacterium, which may offer protection against disease. In Study A and B, NF and NS vaccinated calves exhibited an increased antibody level against *C. chauvoei* when evaluated 21 d post-primary (day 21 vs. day 0) and booster (day 42 vs. day 21) vaccinations. Similarly, Araujo et al. (2010) demonstrated that three different vaccination regimens were able to significantly increase the serum antibody levels against *C. chauvoei* when evaluated 30 d post-primary and 30 d post-booster vaccinations. However, it is not known in both studies whether the observed increase in antibody levels was able to protect the calves, as they were not challenged with *C. chauvoei*. Contrary to the findings above, Schipper et al. (1978) demonstrated that vaccination of 1-6 week old calves against *C. chauvoei* only resulted in 20% of vaccinated calves showing an increase in antibody titers following vaccination, while the

other 80% showed no change or a decrease in antibody titers following vaccination. Troxel et al. (2001) also reported that vaccination of three d-old newborn calves with the same clostridial vaccine as their dams, did not elicit an increased antibody response compared to unvaccinated controls. Therefore, an increased antibody level against *C. chauvoei* is not always seen following the vaccination of young calves, possibly due to the interference of maternal antibodies or immunoincompetence.

Quantification of the antibody response against *C. chauvoei* performed in this research may have been affected by the presence of antibodies against *C. septicum*. Hamaoka and Terakado (1994) demonstrated via an IIF assay that *C. chauvoei* and *C. septicum* possess common antigens on their cell surface. Therefore, because NF and NS vaccinated calves were vaccinated with a clostridial vaccine (Clostri Shield 7, Novartis Animal Health Canada Inc.) which contained both *C. chauvoei* and *C. septicum*, antibodies against *C. septicum* may have bound to the *C. chauvoei* antigen fixed to the IIF slides and may have incorrectly evaluated the antibody level against *C. chauvoei*.

As mentioned in Manuscript I, NFIDs have been reported to leave residual vaccine on the surface of the skin/hair, which may be unavoidable and may hinder or delay the acceptance of the technology (Jones et al., 2005). Despite the fact that vaccine residue was apparent on some NF vaccinated calves in the current study, vaccine residue was shown not to compromise antibody response. The visible vaccine residue observed on day 0 and 21 of Study A and B, may be due to differences in hide thickness and hair coat between calves. Human error may have also contributed to the vaccine residue, as during some injections the device may have not been positioned exactly perpendicular (90°) to the skin, resulting in some of the fluid stream to be left at the skin/hair surface.

5.6. CONCLUSIONS

In summary, previous studies in cattle and the present research demonstrate that NFIDs are capable of stimulating a comparable and sometimes enhanced immune response to a number of vaccines when compared to the conventional NS vaccination technique. Despite the fact that visible vaccine residue was apparent on some NF vaccinated calves, residue was found not to compromise the antibody response acquired against *C. chauvoei* after vaccination using a NFID. Needle-free vaccination did not affect animal performance, as measured by BW, but was observed in Study A to cause a greater frequency of skin reactions following vaccination against *C. chauvoei* than did the NS technique. Similarly, in Study B, the frequency of skin reactions following vaccination was observed to be greater in NF compared to NS vaccinated calves on days 21, 119 and 140, but was found to be similar between administration techniques on day 42. Therefore, further research should be conducted in cattle to determine if skin reactions affect carcass quality and if trimming losses are increased when calves are vaccinated using a NF vaccination technique.

6. GENERAL DISCUSSION

6.1. Animal performance, immune response and vaccine residue

Use of a conventional NS vaccination technique presents the risk of broken needle fragments in beef products, which could be detrimental to consumer perception (van Drunen Littel-van den Hurk, 2006). Needle-free injection devices eliminate the needle and the risk of broken needle fragments in meat products, which increases food safety. Therefore, on-farm food safety programs for beef, such as Verified Beef Production and the Manitoba Food Safety Program For Farms, are interested in NFIDs as an alternative to NS vaccination. However, in order for NF vaccination to be accepted as an alternative, beef producers must be ensured that the technique will be as effective as the conventional NS technique and that it will not cause adverse effects that may diminish profits.

Following the vaccination of spring and fall-born crossbred beef calves against BVDV and *C. chauvoei*, the immune response and animal performance of calves did not differ between NF and NS vaccination techniques. This supports the hypothesis that immune response, as measured by antibody level against both pathogens, and animal performance, as measured by BW, following vaccination of calves against BVDV and *C. chauvoei* is comparable between the two vaccination techniques (NF and NS).

To the author's knowledge, this is the first study that attempted to compare the animal performance of calves following vaccination via NF and NS techniques. Unlike in human studies in which NF vaccination has been associated with increased pain following vaccination when compared to NS vaccination (Jackson et al. 2001), pain response has not been quantified in livestock. Increased pain and stress following

vaccination of cattle may cause them to reduce feed intake, thereby affecting animal gain. Even though pain and stress were not monitored in the current study, we can speculate that NF vaccination did not appear to cause any adverse effects in calves that would reduce their feed intake and animal gain. This was supported by the observation that animal performance was not impacted by vaccination technique in either spring-born or fall-born calves following vaccination against BVDV and *C. chauvoei*.

Previous research investigating NF vaccination in cattle (Hollis et al., 2005a; Hollis et al., 2005b; Pires et al., 2007; van Drunen Littel-van den Hurk,S., 2006) has also demonstrated a comparable and sometimes enhanced immune response when vaccines are delivered with NF compared to NS vaccination techniques, depending on the sex of the animals and the vaccine antigen measured. Likewise, studies conducted in humans (Jackson et al., 2001), rabbits (Aguiar et al., 2001), sheep (Mousel et al., 2008) and swine (Houser et al., 2004) have also demonstrated the same results.

It is also important to note that vaccination of calves at a very young age, with either NF or NS vaccination techniques, may result in an impaired or reduced immune response. In Table 5 of Manuscript I, we observed a negative correlation between the maternal BVDV antibody level on day 0 and the BVDV antibody level on day 140. This implies that calves with high levels of maternal BVDV antibodies present prior to vaccination did not respond as well to vaccination compared to those with low maternal BVDV antibodies prior to vaccination. Maternal antibodies may be neutralizing some of the BVDV antigen in the vaccine, resulting in the catabolism or depletion of antibodies. Therefore, if a producer has some calves that are quite young (less than 2-3 months of

age) at the time of vaccination, it may be wise to wait until these calves are older to vaccinate them in order to obtain an optimal immune response.

Despite the fact that varying amounts of vaccine residue occurred in 21.6% of NF vaccinations in Manuscript I (BVDV) and 24.7% of NF vaccinations in Manuscript II (*C. chauvoei*), residue did not compromise the antibody response against either BVDV or *C. chauvoei*. Even though the amounts of vaccine residue were not quantified in the current study, it may be speculated that residue was minimal, thereby providing enough vaccine to stimulate an immune response. Nonetheless, the vaccine residue observed following NF vaccinations may hinder or delay the acceptance of the technology (Jones et al., 2005) as a consequence of concerns regarding its ability to deliver the necessary vaccine dose. Furthermore, producers are paying a significant price to vaccinate their animals and will not want waste vaccine associated with the small amounts of residue at the surface of the skin/hair.

6.2. Needle-free vaccination, vaccine type and commercial application

In the current study two different types of commercially available vaccines were used: a MLV vaccine containing pathogenic viruses (Express 5, Boehringer Inhelheim (Canada) Ltd.) and a bacterial vaccine containing inactivated *Clostridium* organisms along with an oil-based adjuvant (Clostri Shield 7, Novartis Animal Health Canada Inc.). The results obtained demonstrated that the Pulse 250 NFID (Pulse NeedleFree Systems) used in the study was able to effectively stimulate an immune response, as measured by antibody level, against both BVDV and *C. chauvoei*. This implies that a NFID is capable of effectively vaccinating calves against more than one disease and that vaccine type

(MLV and inactivated vaccines) does not affect the NFIDs ability to stimulate an immune response.

It is common practice to vaccinate animals with more than one vaccine at a given time, in order to protect them against numerous infectious diseases. Therefore, in most occasions more than one NFID would be required by producers, or cattle must be handled more than once, which causes increased stress on animals and requires additional labour and time. Furthermore, the need to purchase two NFIDs increases the cost associated with the use of a NF vaccination system. The commercially available lightweight Pulse 250 NFID model used in the current research study only contained one pneumatic amplifier, one vaccine bottle attachment and one N_2 or CO_2 canister. Therefore, two separate NFID`s were required in order to vaccinate the calves at two months of age (day 0). Needle-free injections devices are quite costly, as a Pulse NFID is approximately $2,500 to $3,000 for the device only (Stuart Webb, personal communication), while the cost of an AcuShot NFID is approximately $4,000 to $5,000 for the device and replacement parts (Mike Agar, personal communication). However, in November 2009, as part of the On-Farm Food Safety Program offered under the federal-provincial Growing Forward Program, Manitoba Agriculture Food and Rural Initiatives agreed to provide up to $2,000.00 for Manitoba swine producers to apply toward the purchase of a needle-free injector (Peter Veldhuis, personal communication). This funding is currently available for swine producers to implement the needle-free injection technology. In the future, the On-Farm Food Safety Program may provide a similar funding opportunity to Manitoba beef producers in order to offset the cost of implementing needle-free injection technology in their operations.

Needle-free vaccination systems may be best suited for feedlot cattle operations that will be vaccinating a large number of animals on a frequent basis, opposed to cow-calf operations that will be vaccinating three or four times a year. Due to the added technology and complexity of NFIDs, the more times the device is used, the more effectively an operation can implement a NF vaccination system. Furthermore, because NFIDs are costly to purchase and maintain, only cattle operations that will be using the system on a large number of animals on a frequent basis will be able to take full advantage of the NF vaccination technology.

6.3. Needle-free vaccination and season of vaccine administration

Needle-syringe vaccinations are inexpensive and easily adaptable to different production environments, whereas NF vaccinations require more training and maintenance as a consequence of the complexity of the equipment. Therefore, additional precautions must be taken to ensure that a NFID is capable of effectively vaccinating cattle in sub-zero temperatures, which may hinder the acceptance of the NF vaccination system by producers.

During the booster NF vaccinations against BVDV performed in fall-born calves (Study B) on February 14, 2012 (average temperature = -8.9 °C), which corresponds to day 119, the vaccine froze in the line when the device was not used for long periods of time. However, it was observed that if the NFID was being used continuously, the vaccine did not freeze. Therefore, producers must be aware that if they let the device sit for extended periods of time in sub-zero temperatures, the vaccine may freeze in the line of the NFID. In addition, if unforeseen circumstances that may prolong the period of time

between vaccinations occur in cold weather, the NFID must be kept warm in order to reduce the risk of the vaccine freezing. Furthermore, booster NF vaccinations against BVDV and *C. chauvoei* performed in fall-born calves (Study B) on day 21 and day 119 required the use of compressed N_2 as the power source instead of liquid CO_2 as per the direction of Pulse NeedleFree Systems. Liquid CO_2 must first expand into a gas before it can be used as a power source, which requires heat (Jason Morrison, personal communication). This expansion may be hindered in sub-zero temperatures (Jason Morrison, personal communication) and freezing of valves and regulators may occur (Med-Tech Gases, Inc.), therefore affecting the ability of CO_2 to cause enough pressure to trigger the injections. Therefore, compressed N_2 should be used in cold weather to ensure the appropriate pressure is produced to trigger NF injections. In addition to changing the pressure source in sub-zero temperatures, the NFID should be equipped into a backpack so that it can be worn on the shoulders of the individual vaccinating the cattle. Thereafter, the NFID can be covered with a poncho to keep it out of the wind and cold weather. Furthermore, the individual performing the vaccinations should position the vaccine line of the NFID through his/her sleeve to reduce the chance of vaccine freezing in the line.

A direct comparison of the immune response stimulated between the two seasons of vaccine administration could not be completed because of the differences between the two herds, such as animal genetics, feed and environmental conditions. However, it was observed that the trends in the immune response stimulated following vaccination against BVDV and *C. chauvoei* were similar in the two seasons of vaccine administration (spring-born vs. fall-born calves). Therefore, with the implementation of the necessary precautions explained above, it is possible to stimulate an effective immune response

with a NFID regardless of the season of vaccination. These findings are contrary to the hypothesis that season (warm vs. cold temperature) in which beef calves are vaccinated will impact the ability of a NFID to effectively vaccinate cattle.

6.4. Skin reactions

With exception of day 42 in Study B (Manuscript II), the NF vaccination technique resulted in a greater frequency of skin reactions when compared to NS vaccination. These findings are contrary to the hypothesis that the presence of local skin reactions following vaccination of beef calves against BVDV and *C. chauvoei* would be comparable when using a NF or NS vaccination technique. However, the impact of skin reactions of both NF and NS vaccinated calves on carcass quality was not explored in this research. Therefore, from the results obtained in this research it can only be implied that NF vaccination causes an increased frequency of skin reactions when observed at the skin/hair surface of the animal and that the effects of these reactions on carcass quality are unknown. The skin reactions seen in this study could have been caused by many factors such as injury caused by the vaccination (i.e. pressure trauma), irritation from the oil-based adjuvant used to enhance the immunogenicity of the clostridial vaccine (Clostri Shield 7) or by contamination of transmitting microorganisms from the skin/hair surface into the tissue surrounding the injection site (Gerlach et al., 2009; Sutterfield et al., 2009).

The presence of skin reactions following NF vaccination may hinder the acceptance of NFIDs as an alternative to NS vaccination because beef producers may believe that the NF system causes adverse effects on the carcass, thereby diminishing profits. If in future research NFIDs are shown to cause skin reactions or injection site

lesions that reduce carcass quality and cause increased trimming losses, on-farm food safety programs for beef may be unwilling to provide funding for beef producers to adopt NF vaccination systems. Nonetheless, skin reactions observed at the site of vaccination are not always a negative observation as inflammation and skin reactions indicate that immune defenses of the body have been stimulated (Tizzard, 1992). A certain level of reactions and inflammation are required at vaccination sites in order to optimize immune response, however, vaccine contents (vaccine antigens and/or adjuvants) may also cause local inflammation and hypersensitivity reactions, leading to skin reactions or lesions arising at or below the skin surface that may impact carcass quality. Therefore, it is important to note that while skin reactions may be observed as a negative effect of vaccination, as long as they do not impact carcass quality, they are required to optimize immune response.

6.5. Future research

The high incidence of skin reactions seen following vaccination of cattle using a NF vaccination technique warrants further exploration. Future research should evaluate the impact of skin reactions on carcass quality, as it is of particular interest in the cattle industry because lesions and abscesses can develop at injection sites (George et al., 1995a; Van Donkersgoed et al., 1997; Van Donkersgoed et al., 1999; Van Donkersgoed et al., 2000) resulting in millions of dollars of loss on an annual basis as a consequence of the need to trim damaged tissue (Van Donkersgoed et al., 1997), as wells as devaluation of cuts and consumer dissatisfaction from eating tough beef (George et al., 1995b) .

Needle-free injection devices have been previously reported and further demonstrated in this study to leave residual vaccine on the surface of the skin/hair following vaccination, which may be unavoidable and may hinder or delay the acceptance of the technology (Jones et al., 2005). In the current research it was observed that vaccine residue did not impact the ability of the NFID to stimulate an immune response. It is also important to note that zero residue can be achieved by using high pressures to ensure 100% penetration of the vaccine through the skin. However, this is not without consequence as vaccines will then penetrate deep into the animal tissue resulting in potential damage to the carcass (Larry Hollis, personal communication). Therefore, future research should be conducted to determine the correct pressure ranges which should be used in cattle. This would provide producers with more accurate ranges of pressure settings necessary to reduce vaccine residue following NF vaccination. However, practical limitations, including breed differences, hair coat, hide thickness and temperature, may impact the ability of researchers to determine more accurate ranges of pressure settings necessary to reduce vaccine residue. In the beef industry crossbreeding is the norm, therefore within a herd we often have animals with different genetics, resulting in different hair coat and hide thicknesses.

Furthermore, future research investigating the use of NF vaccination in all ages and stages of cattle, including mature cows, mature bulls and feedlot cattle, should be conducted to assure cow-calf beef operations that NFID will be able to effectively vaccinate animals ranging from ~50-1,000 kg.

7. CONCLUSIONS

This research has demonstrated that a NFID (Pulse 250, Pulse NeedleFree Systems) can be effectively used to vaccinate beef calves under Western Canadian conditions. The NF vaccination technique was able to stimulate an immune response in beef calves, after vaccination against BVDV and *C. chauvoei*, which was comparable to that obtained via conventional NS vaccination. In addition, we can speculate that a NFID can be used to effectively vaccinate different types of cattle vaccines, and that because the trends in antibody response acquired against BVDV and *C. chauvoei* were similar in spring-born and fall-born calves, season of vaccine administration does not appear to impact the ability of a NFID to effectively vaccinate cattle. Furthermore, even though vaccine residue was apparent on some NF vaccinated calves following vaccination, this residue was found not to compromise the immune response stimulated against both BVDV and *C. chauvoei*.

Animal performance did not differ between vaccination techniques, implying that NF vaccination does not cause any adverse effects that will affect animal gain. In addition, the NF vaccination technique caused a greater frequency of skin reactions when compared to the NS vaccination technique. However, the impact of these skin reactions on carcass quality was not investigated and therefore it can only be concluded that the NF vaccination technique caused a greater frequency of skin reactions at the surface of the skin.

In conclusion, NF vaccination is a relatively new technique to the cattle industry requiring future research to be conducted before it becomes accepted as an alternative to

the conventional NS technique by producers and on-farm food safety programs for beef, such as Verified Beef Production and the Manitoba Food Safety Program For Farms.

8. REFERENCES

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9. APPENDICIES

The information in this section represents preliminary trials (Appendix I) completed to determine the proper NF vaccination method to be used in the research study. As a result of the preliminary trials conducted we determined that the beef calves approved for use in this research study would be vaccinated via a NFID using a skin tenting injection method and the NFID would be set at an initial pressure of 45 PSI.

9.1. Appendix I

9.1.1. Preliminary trial investigating skin reactions after vaccinating calves using a needle-free injection device

The preliminary trial was conducted in order to examine the skin reactions that could develop on calves that are vaccinated with Clostri Shield 7® (Novartis Animal Health Canada Inc.) using a NFID. Two Holstein bull dairy calves were vaccinated with 2 ml of Clostri Shield 7® in the left side of the neck using the Pulse 250 NFID. As per the direction of Stuart Webb (Pulse NF Systems), the device was set at a pressure of 55 PSI.

Injection sites were examined 24 hours post-vaccination for the presence of skin reactions. It was determined that NF vaccination at 55 PSI caused a significant amount of swelling at the injection site. Furthermore, when the injection sites were palpated, calves exhibited significant pain. To reduce the swelling and pain caused by vaccination, the two calves were given Anafen® (MERIAL Canada, Inc.), a non-steroidal anti-inflammatory drug. Therefore, setting the NFID at 55 PSI was deemed to be too high of a

pressure to administer this vaccine into the SC tissue of calves, as the swelling at the injection sites was located in the IM tissue

It was concluded that more preliminary work would be required to determine the proper NF vaccination method and pressure setting that would be required to administer a complete dose of vaccine into the SC tissue of a calf without causing unacceptable lesions.

9.1.2. Preliminary trial investigating the skin-tent vaccination technique with a needle-free injection device to administer a subcutaneous injection and limit skin reactions

Following the results obtained in the previous section, a second preliminary trial was conducted to examine if skin tenting could be used to administer a cattle vaccine in the SC tissue using a NFID. In addition, this trial was also conducted to determine if skin reactions would develop when calves are vaccinated via a NFID using the skin tenting technique and if these reactions would differ when compared to a calf vaccinated using a NS technique.

Four Holstein dairy calves were used in this trial. Three calves were vaccinated with 2 ml of Clostri Shield 7® in the right side of the neck using a NFID set at three different pressures (40, 45 and 50 PSI), while the fourth calf was vaccinated with 2 ml of Clostri Shield 7® in the right side of the neck using a NS technique.

Rectal temperatures and blood samples were taken from the four calves prior to vaccination and 24 hours post-vaccination. As described in Table 9, the rectal temperatures and white blood cell (WBC) counts of all calves increased 24 hours post-

vaccination, indicating that an immune response to the vaccine was generated in both NF and NS techniques. Furthermore, the rectal temperatures and WBC counts of the three calves vaccinated via a NFID were similar to that of the calf vaccinated via the NS vaccination technique, indicating that the skin tenting can be used to effectively administer a cattle vaccine using a NFID.

Table 9 Rectal temperature (°C) of calves vaccinated with Clostri Shield 7 using a needle-syringe (NS) vaccination technique and a needle-free (NF) vaccination technique set at different pressures (40, 45 and 50 PSI).

Calf ID	Treatment	Rectal temp. prior to vaccination	Rectal temp. 24 hours post-vaccination	WBC counts prior to vaccination	WBC counts 24 hours post-vaccination
1	NF @ 40 PSI	39.4°C	40.5°C	9.9×10^9	15.0×10^9
2	NF @ 45 PSI	38.7°C	40.8°C	N/A	23.4×10^9
3	NF @ 50 PSI	38.6°C	40°C	10.4×10^9	18.0×10^9
4	NS	39.1°C	40.4°C	8.1×10^9	15.6×10^9

On days one, two, three and eight post vaccination, calves were visually inspected and palpated for skin reactions. As described in Table 10, all calves had visible skin reactions when examined 24 hours post-vaccination, however the skin reactions of the three calves vaccinated via NF injection were similar to the calf vaccinated via NS injection. In addition, the skin reactions of all calves had subsided when examined eight days post-vaccination and were deemed to be administered in the SC tissue.

The results of this preliminary trial indicated that skin tenting could be used in order to administer a cattle vaccine SC via a NFID. Furthermore, it was determined that the skin reactions seen following vaccination did not differ between NF or NS vaccination techniques.

9.1.3. Preliminary trial investigating the appropriate needle-free vaccination method (skin tent vs. perpendicular injections) and pressure (40, 45 and 50 PSI) required to administer a complete dose of colored vaccine product into the subcutaneous tissue.

This preliminary trial was designed to finalize the appropriate vaccination method and adequate pressure required for a NFID to administer a complete dose of colored vaccine product into the SC tissue of two-month old calves, without leaving visible vaccine residue on the skin surface.

Six Holstein dairy calves (average age 60 ± 9 days) were utilized in the preliminary trial and were randomly assigned to three treatments (two calves per treatment) as mentioned below:

1. Two NF injections of a colored vaccine product in the left side of the neck (one at 40 PSI and the other at 45 PSI) administered using the skin tenting technique and two other NF injections of a colored vaccine product in the right side of the neck (one at 40 PSI and the other at 45 PSI) administered perpendicular to the skin surface.

Table 10 Skin reactions of calves vaccinated with Clostri Shield 7 using a needle-syringe (NS) vaccination technique and a needle-free (NF) vaccination technique set at different pressures (40, 45 and 50 PSI).

Calf ID	Treatment	Reaction measurement 24 hours post-vaccination (W x L x D)	Reaction measurement 48 hours post-vaccination (W x L x D)	Reaction measurement 72 hours post-vaccination (W x L x D)	Reaction measurement eight days post-vaccination (W x L x D)
1	NF @ 40 PSI	54 mm x 140 mm x 30 mm	53 mm x 106 mm x 30 mm	50 mm x 90 mm x 25 mm	pea-sized (hard) injection bleb
2	NF @ 45 PSI	18 mm x 75 mm x 18 mm	25 mm x 80 mm x 18 mm	22 mm x 76 mm x 16 mm	12 mm x 50 mm x 9 mm
3	NF @ 50 PSI	32 mm x N/A mm x 32 mm	32 mm x N/A x 25 mm	N/A, N/A, N/A	pea-sized (hard) injection bleb
4	NS	68 mm x 94 mm x 25 mm	60 mm x 90 mm x 17 mm	53 mm x 67 mm x 17 mm	nickel-sized (hard) injection bleb

2. Two NF injections of a colored vaccine product in the left side of the neck (one at 45 PSI and the other at 50 PSI) administered using the skin tenting technique and two other NF injections of a colored vaccine product in the right side of the neck (one at 45 PSI and the other at 50 PSI) administered perpendicular to the skin surface.
3. Two NF injections of a colored vaccine product in the left side of the neck (one at 50 PSI and the other at 40 PSI) administered using the skin tenting technique and two other NF injections of a colored vaccine product in the right side of the neck (one at 50 PSI and the other at 40 PSI) administered perpendicular to the skin surface.

Each calf was subjected to four NF vaccinations of a colored vaccine product (two NF vaccinations administered into a skin tent on the left side of the neck & two NF vaccinations administered perpendicular to the skin surface). The presence or absence of visible vaccine residue on the skin surface following each injection was recorded. In addition, all calves were humanely euthanized following injections and neck tissues were harvested in order to examine the dispersion of the colored vaccine product.

It was determined that only a 45 PSI skin tent injection, a 50 PSI perpendicular injection and a 50 PSI skin tent injection were able to administer a complete vaccine dose after four injections (Table 11). Furthermore, after examining the harvested neck tissues for the dispersion of the colored vaccine product, it was evident that of the injection method and pressure combinations, only a 45 PSI skin tent injection dispersed the colored vaccine product into the SC tissue (Fig. 17). Therefore, the results of this trial

demonstrated that a SC injection of a complete dose of colored vaccine product can be achieved by administering a colored vaccine product via a NFID set at an initial pressure of 45 PSI using the skin tenting method.

Table 11 Presence or absence of visible vaccine residue on the skin surface following vaccine administration.

Pressure	Injection Method	Visible Vaccine Residue
40 PSI	Perpendicular	50% of injections
	Skin Tent	25% of injections
45 PSI	Perpendicular	75% of injections
	Skin Tent	NONE
50 PSI	Perpendicular	NONE
	Skin Tent	NONE

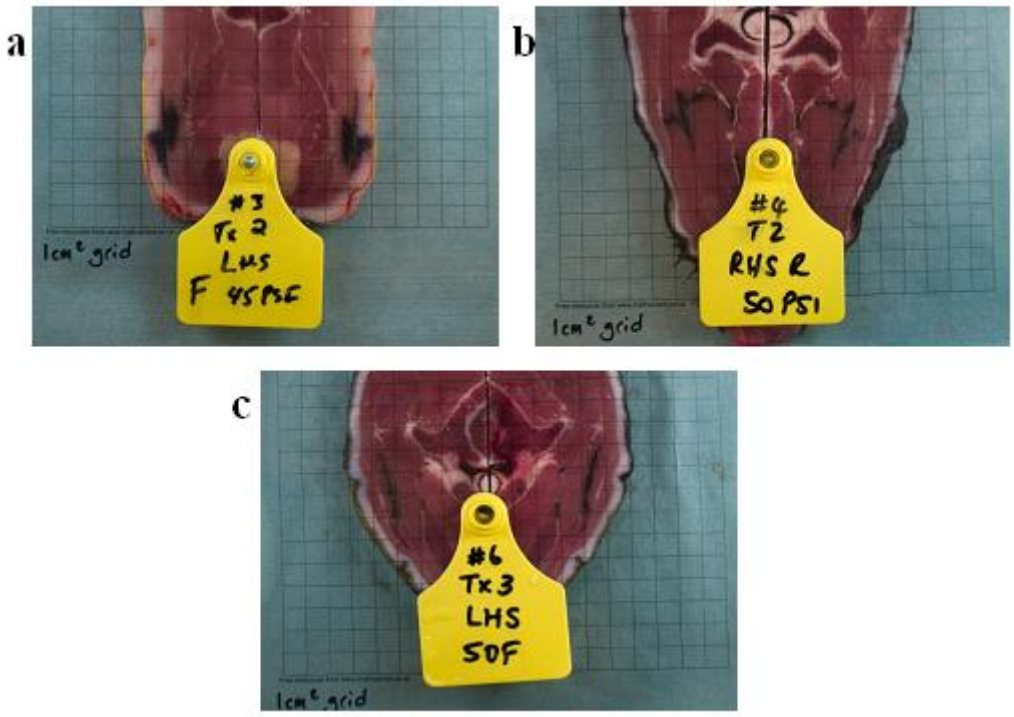

Fig. 17. Dispersion of a colored vaccine product in the neck tissues of calves injected: a) using a skin tenting technique with a needle-free injection device set at 45 PSI; b) using a perpendicular to the skin surface technique with a needle-free injection device set at 50 PSI; c) using a skin tenting technique with a needle-free injection device set at 50 PSI.