

INCIDENCE AND SEVERITY OF *ARCANOBACTERIUM PYOGENES* INJECTION
SITE ABSCESSSES WITH NEEDLE AND NEEDLE-FREE INJECTION METHODS

by

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Abstract

Nursery age pigs (n=198) were used to evaluate the difference in the occurrence of injection site abscesses between needle-free jet injection and conventional needle-and-syringe injection systems. Pigs were fed for 21 d prior to treatment administration to acclimate the pigs to the environment of the Kansas State University Segregated Early Weaning (SEW) unit. On d 21 each pig was injected with aluminum hydroxide adjuvant in the neck and ham with needle-free jet injection (Pulse Needle-Free Systems, Lenexa, KS) and conventional needle-and-syringe injection. Needle-free and conventional needle-and-syringe injections were randomly assigned to pig side yielding a total of 396 injections per treatment with a total of 792 injection sites. Immediately prior to injection, the external surface of the injection sites were contaminated with an inoculum of *Arcanobacterium pyogenes*, a bacterium commonly associated with livestock abscesses. The pigs were then fed for a period of 27 or 28 d. On d 27 or d 28 the pigs were humanely euthanized and sent to the Kansas State Veterinary Diagnostics Laboratory where necropsies were performed and the injection sites harvested for histopathological evaluation. The needle-free jet injection system was associated with more injection site abscesses than the conventional needle-and-syringe injection method for both neck ($P=0.0625$) and ham ($P=0.0313$) injection sites. Twelve abscesses were found at injection sites administered via needle-free jet injection method while only 1 abscess was found with the conventional needle-and-syringe injection method. 5 abscesses were found at the neck injection sites and 8 abscesses were found at ham injection sites. There were no significant differences seen in tissue granulation resulting from reaction to the adjuvant. In summary, the implementation of needle-free jet injection systems in market hog production will be beneficial to eliminate needles and needle fragments in meat products but, when in the presence of *Arcanobacterium pyogenes*, it may increase the occurrence of injection site abscesses in pork carcasses that will need to be trimmed in pork processing plants. Although more abscesses were associated with needle-free jet

injection, their occurrence was observed at a very low rate given that all injection sites were intentionally contaminated prior to injection.

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CHAPTER 1 - Review of Literature

History of Vaccination

Vaccination is a very important tool and management practice used by the livestock industry. Stern and Markel (2005) gave an account of the first instance of vaccination. In the 1790's Edward Jenner created the world's first vaccine for smallpox. Jenner's first vaccination was performed by inoculating an eight-year-old boy with pus from a cowpox lesion taken off of a milkmaid's hand. Six weeks after this inoculation, Jenner exposed two sites on the boy's arm with smallpox and the boy was unaffected. The next known vaccine was developed by Louis Pasteur in 1885 when he created a rabies antitoxin. This expanded the term "vaccination" beyond its Latin association with cows and cowpox to include all inoculating agents (Hansen, 1998). In the evolution of vaccines, many discoveries have developed from the exchange between human and animal medicine. One development was the creation of adjuvants by Gaston Ramon. Ramon discovered that the efficacy of an antitoxin for tetanus could be enhanced if substances known as adjuvants, such as aluminum hydroxide, were added to the vaccine (Lombard, Pastoret, & Moulin, 2007).

Today vaccination is a very common practice in livestock production. Medications are given as part of regular husbandry practices to improve health, control disease and increase productivity. These medications may be given by injection, orally or topically. Injections are commonly given into the muscle (intramuscularly), under the skin (subcutaneously) or into the bloodstream (intravenously) (Griffin, Smith & Grotulueschen, 1998).

Hazards Associated with Needle-and-Syringe Injection

Even though needle-and-syringe injection is very common in today's production systems, there are some drawbacks that may incline producers to look at alternative vaccine delivery methods. The use of needles for vaccination not only poses a safety hazard to workers and veterinarians in livestock production, but also to meat packing employees and possibly consumers.

Needlestick Injuries

One of the most apparent safety issues connected to needle-and-syringe injection is the likelihood of a needlestick injury to the person administering the injection. Accidental injection of veterinary medicines may result in ill health due to local damage at the site of injection, absorption of the medicine into the body, or infection transmitted from a previously used dirty needle (Skilton & Thompson, 2005). According to a survey conducted by Hafer, Langley, Morrow, and Tulis (1996), needlestick injury was the highest reported physical injury among swine veterinarians in the United States. In this survey, 73% of veterinarians reported they had incurred at least one needlestick injury during their career. In the two years prior to the survey, female veterinarians reported an average of 4.3 needlestick injuries while male veterinarians reported an average of 2.8 needlesticks. As a result of these injuries, veterinarians experience pain, local swelling, hematoma, infection, superficial abscess, and cellulitis. A similar study, where 304 large animal veterinarians were surveyed to estimate the major hazards they encountered in their day-to-day operations showed similar outcomes (Poole, Shane, Kearney, & McConnell, 1999). In a two-year time period, the highest reported injury was needlestick injuries. It was calculated that every practice surveyed had reported an average of 3.1 needlestick incidents.

Needle and Needle Fragments in Meat Products

Aside from production worker and veterinarian injuries, the use of hypodermic needles for injection can also create problems in the meat packing industry. This is of great concern because unknowing consumers can be put in a dangerous position. Physical hazards, such as broken hypodermic needles, are one of the concerns meat packers must address in their Hazard Analysis Critical Control Points (HACCP) plans (Hoff & Sundberg, 1999). Hoff and Sundberg (1999) evaluated the structural integrity of needles of two companies at two lengths (2.54 and 3.6 cm), 3 gauges (20, 18 and 16) and 2 hub materials (aluminum and plastic). Not all combinations were available from each company. These needles underwent a variety of tests investigating the needles resistance to compression, lateral bending, force needed to puncture swine skin and needle sharpness. A device was also constructed to simulate an animal moving while a needle

was in the skin. From these tests four different types of needle failure were observed: needle deformation (bending), needle/hub joint failure, hub failure, and needle breakage. Needles with plastic hubs were clearly inferior to needles with aluminum hubs in compression tests. Needles with a length of 2.54 cm showed more compression strength than the longer 3.6 cm needle. Needle strength was shown to increase with increased needle diameter or gauge. Bending the tips of needles required very small amounts of force and showed similar results to the compression tests in relation to needle length and gauge. Needles can be broken when injections are being administered and the animal begins moving. Other factors that may cause a needle to break such as the dulling of needles or using a needle that was bent and straightened were also investigated. Needle sharpness decreased the greatest within the first ten injections. Needles that were bent and straightened showed a decrease in force needed to fail and after a second straightening 96.7% of the needles broke. This study shows that there are numerous factors that can go into needle breakage or failure during injection.

Meat packing companies are very aware of the threat of metal fragments being hidden in carcasses. Meat products are commonly passed through metal detection systems to uncover metal physical hazards such as needles and needle fragments. However, Sundberg (2000) reported that needle fragment size and the different alloys used to construct needles can decrease needle fragment detection by metal detectors to less than 50% in fresh pork. The detectability of needles from different manufacturers ranged from 8-85%. To combat these problems, manufacturers have modified the alloys they use to increase the detectability of needles (Stier, 2003). Another aspect that alters the effectiveness of metal detection systems is the orientation of the needle relative to the detector. Sundberg and Hoff (2002) implemented the use of a state-of-the-art metal detector that is commonly used in packing plants to reveal the detectability of needle fragments imbedded in a 6.6 kg picnic roast. 1.27 and 2.54 cm needle fragments from 16 and 18-gauge needles were placed into picnics in 3 different positions; horizontally in the back of the roast perpendicular to the detector, horizontally in the side of the roast parallel to the detector as well as vertically in the center of the roast. The results revealed that needles that were located towards the back of a product and placed horizontal to the

detector were identified more frequently than needles placed parallel to the detector, in the side of the product, or needles placed vertically in the center of the product.

The most alarming aspect of the use of needles in livestock production is that needles could go undetected until the product reaches its absolute endpoint, the consumer. Incidences where needles are discovered in a fully-prepared product, or in the worst-cases scenario are unknowingly consumed, pose a very hazardous risk to that consumer and can come back to give a negative image to the meat and livestock production industries. In January of 2001 a Niagara Falls woman had a hypodermic needle lodged into her mouth after biting into a McDonald's chicken McNugget. Instances like this have resulted in large monetary lawsuits costing the industry millions of dollars (Murphy, 2001). Food safety is always a top priority in meat processing facilities. The fact that the detectability of needle fragments can vary should bring great concern to meat processors.

Pork Chain Quality Audit

The pork industry utilizes periodic audits to assess emerging and lingering problems that negatively impact the industry. In 1992, the National Pork Producers Council along with the National Pork Board conducted the Pork Chain Quality Audit which encompassed the entire pork industry from the farm to the plate (Meeker & Sonka, 1994). Several aspects of the industry ranging from swine genetics to economics were investigated. One problem that was discovered was the effect that abscesses and injection sites have on the value and marketability of pork carcasses. This audit revealed that meat purveyors listed injection-site blemishes as one of their top 8 pork quality concerns. Pork packers felt the incidence of abscesses in carcasses and cuts was too high and resulted in excessive amounts of necessary trimming required on carcasses. Pork processors listed the excessive amounts of abscesses in butts, hams, and bellies along with the high incidence of foreign materials, such as hypodermic needles, residing in raw pork as two of their primary concerns about pork quality.

When auditing slaughter floors across the country it was apparent that the presence of abscesses in pork carcasses can harm a pork packer's bottom line. Approximately 13% of all carcasses required some form of carcass trimming with 57% of

the trimming required to remove abscesses. Of all the carcasses audited, 8% had abscesses and 4.1% of carcasses revealed abscesses in the head/jowl region where injections are usually administered. In 1992, injection sites and abscesses cost the packing industry approximately \$0.47 per pig marketed and this number increased to \$0.57 per pig in 2002 (Stetzer & McKeith, 2003). Although only 0.2% of carcasses were condemned, 11.1% of those carcasses were condemned due to abscesses. During the fabrication portion of the audit, 3.7% of the meat cuts fabricated required trimming as a result of abscesses. More specifically, 21% of fabricated pork butts had small injection site blemishes and 6.7% contained moderate injection site blemishes.

The effects of injections and abscesses can also be seen at the meat processor level. Pork processors observed a loss on hams of \$0.05 per hog due to foreign objects, possibly needles, and \$0.03 per hog due to injection site blemishes or formed abscesses. In sausage production, a loss of \$0.02 per hog for both foreign objects and abscesses was observed.

In summary, the monetary loss associated with abscesses as well as injection site blemishes and lesions is a matter of concern for the pork industry. The economic losses associated with these defects undoubtedly warrant attempts to alter production systems to minimize their occurrence.

Needle-Free Jet Injection

Even though there are many forms of needle-free vaccination methods available, needle-and-syringe injection is still the most common form of vaccination. Instituting the use of needle-free devices would obviously eliminate the potential hazards of needles and needle fragments entering the food supply. Needle-free jet injection is the oldest method of needle-free immunization (Mitragotri, 2005). Jet injection dates back to the late 1800's when aquapuncture, a method that delivered jets of water was cited in medical literature (Weniger, 2004). The concept leading to modern jet injectors began in the 1930's when industrial workers reported cases of diesel being accidentally injected into their hands when small leaks formed in high pressure lines (Bremseth & Pass, 2001). Jet injectors use a high-speed jet to puncture the skin and deliver a substance. Insulin was one of the first substances to be administered via jet injection before being used to administer other

drugs such as penicillin (Baxter & Mitragotri, 2006). Multiuse nozzle jet injectors (MUNJI's) gained popularity in the 1950's when they were used for mass immunization against diseases like poliomyelitis, influenza, cholera and typhoid (Hingston, Davis & Rosen, 1963). However, the MUNJI devices were linked to a spread of hepatitis B and C virus in the 1980's (Mitragotri, 2005). New-generation needle-free technology for humans has implemented the use of single-dose cartridges. These needle-free jet injectors have been implemented slowly into livestock production, due to their high start-up cost versus the very low cost of needle-and-syringe injections, but have been gaining popularity in recent years (Chase, Daniels, Garcia, Milward & Nation, 2008).

Abscess Formation

As signified by the Pork Chain Quality Audit, abscesses are a significant problem for the industry. To properly prevent them, one must understand how these abscesses form and develop. Abscesses are localized sites of infection in the tissues of livestock. Abscesses can form near the surface of the body or in tissues deep within the body and when close to the skin surface can be observed as an extended or swollen mass of tissue which is filled with a fluid commonly known as "pus" (Harper, 2008). The abscess forms as an immune response to a foreign material or an infection. Injections may also lead to the formation of an abscess as they may cause a tissue reaction to medicines, vaccines, or dirty needles (Jones, 1996).

Arcanobacterium pyogenes

It has been shown that abscesses form in response to an infection. These infections could arise from a variety of bacteria such as *Arcanobacterium (Actinomyces) pyogenes*. *A. pyogenes* has been frequently isolated from pyogenic diseases mainly of cattle, sheep, goats and pigs (Lammler & Blobel, 1988). This bacterium has been shown to cause a variety of suppurative infections, such as liver abscesses and mastitis in cattle, as well as suppurative pneumonia and polyarthritis in pigs (Billington, Songer & Jost, 2002). *A. pyogenes* has also been seen to be a frequent bacterial cause of many types of abscesses in hogs ranging from foot to spinal abscesses (Merck, 2005).

Microbial Translocation of Needle-Free Jet Injectors

Knowing that bacteria located on the external surfaces of livestock can cause abscesses if introduced beneath the skin, Sutterfield, Crow, Dikeman, Phebus, Grobbel and Hollis (2009) examined the microbial translocation that takes place when beef longissimus muscles are injected with needle-free jet injection or standard needle injection. To assess this 15, beef longissimus muscles were inoculated with a nonpathogenic *Escherichia coli* strain before being injected via needle-free jet injection or a needle meat injector with a solution of water, salt (0.3%), phosphate (0.3%) and potassium lactate (1.5%) to a pump yield of 12%. After draining, two 5.1 cm diameter cross-sectional cores were taken aseptically from each loin. This procedure was replicated on 3 separated days. Results showed that *E. coli* counts were higher ($P < 0.001$) for needle-free injections than for needle injections. This suggests that needle-free jet injectors may be more likely to transmit microorganisms from a surface to an internal portion than regular needle injection.

Injection Site Lesions

It is well known that lesions and abscesses can develop at injection sites. Much research has been done to evaluate the development of lesions at injection sites where needle-and-syringe injection was used. However, there has been little research assessing the prevalence of lesions and abscesses developing at needle-free injection sites. Houser, Sebranek, Thacker, Baas, Nilubol, Thacker and Kruse (2004) compared the occurrence of abscesses at conventional needle-and-syringe and needle-free injection sites in swine. In this study, 4-5 week old pigs were given three injections of a commercial *Mycoplasma hyponeumoniae* vaccine in the neck by either a hypodermic needle or a needle-free jet injector; the control group received no injections. Once the hogs reached market weight (~118 kg) they were harvested and the injection sites were visually appraised for muscle and lymph node lesions before being excised to undergo histopathological evaluation to determine the presence of lesions. Upon gross evaluation of injection sites there were no differences ($P = 1.0$) in the occurrence of lesions as 2 lesions were found in each injection treatment group for both muscle and lymph nodes. Histological evaluation

yielded similar results as there were no significant differences for muscle lesions ($P = 0.768$) and lymph node lesions ($P = 1.0$).

The beef industry has also suffered economic loss due to injection site lesions. In response to the National Beef Quality Audit conducted in the early 1990's a project was conducted at Colorado State University to investigate the relationship between lesion development at injection sites during branding and weaning. George, Heinrich, Dexter, Morgan, Odde, Glock, Tatum, Cowman and Smith (1995) conducted this study. Injections of 4 different products (2-mL clostridial, 5-mL clostridial, Vitamin AD₃, and oxytetracycline (OTC)) were administered over the life of the study to a total of 84 calves. At branding (48.3 d of age) two injections were given, one in the right inside round and the other in the left inside round. The remaining 2 injections were administered at weaning time (199.3 d of age) with one given in the right top sirloin butt and the other into the left top sirloin butt. These calves were fed a normal finishing diet, with any further injections administered in the neck, and were harvested at 424 d. Within each injection time (branding and weaning) the 4 products used elicited significant differences ($P < 0.05$) in lesion incidence rates. One product (2-mL clostridial) showed a lower ($P < 0.05$) lesion incidence rate at the weaning injection site while the OTC injection at weaning produced a much higher ($P < 0.05$) incidence rate than when administered at branding. These results show that lesions created at an early age may persist all the way to harvest and injections later in the animal's life are more likely to be associated with lesions when the animal is harvested.

George, Morgan, Glock, Tatum, Schmidt, Sofos, Cowman and Smith (1995) also conducted an audit of beef packing plants to determine the incidence of injection site lesions and compare meat characteristics such as tenderness and collagen concentrations from cuts with and without lesions. This project audited three federally inspected beef plants where they fabricated steaks from the bottom-round subprimal and then removed and weighed any lesions that were found. From these bottom round steaks, normal (n=40) and lesioned (n=46) steaks were collected. Cores were taken from the middle of the lesion and at distances of 2.54, 5.08, and 7.62 cm from the center of the lesion to evaluate Warner-Bratzler shear force values. Normal (n=12) and lesioned (n=16) steaks were also retained to evaluate soluble and insoluble collagen as well as to confirm lesions by

histological evaluation. Bottom-rounds (n=15,456) were examined and showed that injection site lesions were found in $10.04 \pm 6.51\%$ of bottom-rounds with an average lesion weight of 191.59 ± 55.2 g. Warner-Bratzler shear-force measurements showed that lesioned steaks were significantly toughest in the center of the lesion and tougher at 2.54, 5.08, and 7.62 cm from the center of the lesions when compared to normal steaks. Soluble and insoluble collagen concentrations from the center of the lesion along with insoluble collagen concentration at 2.54 cm from the lesion center were significantly higher ($P < 0.001$) than concentrations seen in normal steaks. This study shows that lesions can be detrimental to the sensory characteristics of a meat product as tenderness is decreased in lesioned areas.

Abscesses and lesions have a propensity to form at injection sites. In addition, the time in the animal's life that the injection is administered also affects the occurrence and severity of lesions seen at slaughter. Injection later in life is associated with more carcass lesions. Lesions have also been shown to have negative effects on palatability traits such as tenderness. It is also important to note that no differences have been seen in lesion numbers at injection sites of needle-and-syringes or needle-free jet injectors which suggests neither method will increase or decrease the occurrence of lesions.

Serological Responses of Needle-Free Jet Injectors

With research showing no difference between lesion or abscess formation between needle-and-syringe injection and needle-free jet injection, it is important to investigate the effectiveness of these methods in terms of the serological response elicited by each injection type. Serological response is a commonly used trait to assess the effectiveness of a vaccine delivery system. To assess serological response antibody titer levels can be used to detect antibodies produced in response to the administration of a specific antigen. Hollis, Smith, Johnson, Kapil and Mosier (2005a) evaluated the effectiveness of needle-free jet injection systems by looking at the titer levels and serological response to 3 different types of vaccinations given by both a needle-free jet injector and needle-and-syringe method. To accomplish this, 104 Holstein heifers and steers ranging from 5 to 10 months of age, blocked by age and sex, were randomly assigned to receive a 5-way modified-live virus vaccine, *Mannheimia haemolytica* (MH)

bacterin-toxoid and 5-way *Leptospira* bacterin by either needle-free injection or a needle-and-syringe injection. Blood samples were taken at the time of injection and again 21 d later to evaluate the serum for antibody titers to infectious bovine rhinotracheitis (IBR), MH leukotoxin, and *Leptospira pomona* (LP). There were no significant differences in titer levels between injection type on d 0 and 21 for IBR, MH and LP in both the heifer and steer groups. Also, there were no differences in titer levels between injection types on d 0 for both groups. However, on d 21 the needle-free injection system produced higher blood titer levels for IBR ($P = 0.01$) and MH ($P = 0.02$) in the heifer group which infers a greater effectiveness.

Hollis, Smith, Johnson, Kapil and Mosier (2005b) also conducted a similar study with 111 yearling feedlot steers. A 5-way modified-live virus vaccine and MH bacterin-toxoid were administered by either needle-free or needle-and-syringe systems and blood samples were taken at the time of injection and again 21 d later. The blood serum was then analyzed for antibody titers for IBR virus and MH leukotoxin. Significantly higher titer levels were found at 21 d from animals receiving needle-free injections for both IBR ($P = 0.001$) and MH ($P = 0.06$). Titer levels were not significantly different at d 0 for both IBR and MH. This study also shows that needle-free jet injection can produce a higher response to vaccination of these two vaccines.

In conjunction with their study evaluating injection site lesions in swine, Houser, Sebranek, Thacker, Baas, Nilubol, Thacker and Kruse (2004) investigated the serological response produced by needle-free jet injectors and needle-and-syringe injections. Blood samples were collected prior to an initial injection of *Mycoplasma hyponeumoniae* vaccine, 11-13 d after a second *M. hyponeumoniae* vaccine injection and then again 23-25 d after a pseudorabies (PRV) vaccine was administered. Blood serum was tested for *M. hyponeumoniae* antibodies and PRV and values for needle-free injection and needle-and-syringe injection were not significantly different.

To further support the results stated in the previous studies, similar serological responses resulting from needle-free jet injection were seen in sheep. Wethers (n=100) were given subcutaneous injections in the neck with either a needle-free injector or a needle-and-syringe, and either 6 mg of ovalbumin dissolved in 1 mL of sterile, isotonic saline and 1 mL of aluminum hydroxide adjuvant, or 2 mL of a commercial vaccine

containing an aluminum hydroxide adjuvant, for a common sheep disease. Each wether received 1 injection by each type of injection device with a different vaccine on each side of the neck. Blood serum was evaluated for antibody titers to both vaccines by using an ELISA test. Antibody titer levels to ovalbumin resulting from either needle-free jet injection or needle-and-syringe injection were not difference (Mousel, Leeds, White & Herrmann-Hoesing, 2008). Therefore, these studies conclude that needle-free jet injection can produce antibody responses that are similar, if not higher than needle-and-syringe injection.

Lateral Transmission of Diseases

The use of a needle to administer drugs to numerous individuals has the potential to infect animals by transmitting disease from animal to animal. The capability of needles transmitting disease amongst livestock was investigated by Otake, Dee, Rossow, Joo, Deen, Molitor and Pijoan (2002). This study utilized 3 groups of pigs housed in separate rooms. The first group consisted of 7 pigs that were inoculated with porcine reproductive and respiratory syndrome virus (PRRSV) while another 3 served as a contact control. Groups 2 and 3 were composed of PRRSV-free pigs with group 2 having 3 pigs while group 3, the negative control group, consisted of 2 pigs. Vaccine injections were given by needle-and-syringe to all pigs in group 1 and 2 on d 5, 6 and 7. The same needle was used to vaccinate all of the pigs and the individual administering the injections showered and changed all clothing after injecting group 1 and prior to injecting group 2. Groups 1 and 2 had sera collected multiple times up to 28 days after inoculation. Sera were evaluated for the presence of viral nucleic acids and PRRSV antibodies. This experiment was replicated 4 times and each replicate showed a successful inoculation of group 1. Individuals in group 2 were infected in 2 of the replicates. The negative control group lacked any PRRSV positive individuals. This study shows that lateral transmission of disease due to needle-and-syringe is very plausible and should be a concern to producers.

There is a possibility that implementing the use of needle-free jet injectors could reduce or eliminate the risk of lateral transmission when vaccinating large numbers of animals. Reinbold, Coetzee, Hollis, Nickell, Reigel, Huff and Ganta (Accepted for publication 2009) investigated this argument by looking at transmission of *Anaplasma*

marginale disease in cattle. Their results imply that instituting the use of needle-free injection could decrease the amount of lateral transmission that occurs during vaccination.

Summary

The use of traditional needle-and-syringe injections is an effective mode of vaccination. However, with its benefits there are inherent dangers and hazards that are associated with its use. The presence of needles and needle fragments in meat products is a tremendous concern to the meat industry as it jeopardizes the safety of consumers which can come back to harm the profitability of meat companies. Worker safety is also affected by the use of needles to vaccinate livestock as they can incur a needlestick injury. These hazards are eliminated by the use of needle-free jet injection systems to vaccinate livestock. Additionally, the use of needle-free jet injectors has been shown to have equal or increased effectiveness in terms of serological responses in livestock while showing the potential to reduce the risk of lateral transmission of blood-borne diseases.

The swine industry has been hindered by the presence of abscesses in pork carcasses which have resulted in monetary losses due to trim loss and condemnations. Abscesses and lesions have been linked to injection sites and thus far there has been no evidence to support that needle-free jet injection devices significantly increase or reduce the number of abscesses and lesions at injection sites when compared to needle-and-syringe injections. Therefore, with the given advantages of needle-free jet injection it is warranted to further investigate its association with injection site abscesses in comparison to needle-and-syringe injection.

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CHAPTER 2 - Incidence and Severity of *Arcanobacterium pyogenes* Injection Site Abscesses with Needle or Needle-Free Injection

Abstract

Nursery age pigs (n=198) were used to evaluate the difference in the occurrence of injection site abscesses between needle-free jet injection and conventional needle-and-syringe injection systems. Pigs were fed for 21 d prior to treatment administration to acclimate the pigs to the environment of the Kansas State University Segregated Early Weaning unit. On d 21 each pig was injected with aluminum hydroxide adjuvant in the neck and ham with needle-free jet injection (Pulse Needle-Free Systems, Lenexa, KS) and conventional needle-and-syringe injection. Needle-free and conventional needle-and-syringe injections were randomly assigned to pig side yielding a total of 396 injections per treatment with a total of 792 injections sites. Immediately prior to injection, the external surface of the injection sites were contaminated with an inoculum of *Arcanobacterium pyogenes*, a bacterium commonly associated with livestock abscesses. The pigs were then fed for a period of 27 or 28 d. On d 27 or d 28 the pigs were humanely euthanized and sent to the Kansas State Veterinary Diagnostics Laboratory where necropsies were performed and the injection sites underwent histopathological evaluation. The needle-free jet injection system was associated with more injection site abscesses than the conventional needle-and-syringe injection method for both neck ($P=0.0625$) and ham ($P=0.0313$) injection sites. Twelve abscesses were found at injection sites administered via needle-free jet injection method while only 1 abscess was found where a conventional needle-and-syringe injection method was used. Five abscesses were found at the neck injection sites and 8 abscesses were found at the ham injection sites. Of the 13 abscesses that were found, 10 developed on the left side of the animal and only 3 were seen on the right side. There were no significant differences when evaluating tissue granulation, which occurs due to a reaction to the adjuvant. In summary, the

implementation of needle-free jet injection systems in market hog production will be beneficial to eliminate needles and needle fragments in meat products but, when in the presence of *Arcanobacterium pyogenes*, it may increase the occurrence of injection site abscesses in pork carcasses that will need to be trimmed in pork processing plants. Although more abscesses were associated with needle-free jet injection, their occurrence was still observed at a very low rate given that injection sites were intentionally contaminated prior to injection.

Key words: abscess, *Arcanobacterium pyogenes*, carcass trimming, needle fragments, needle-free injection

Introduction

Conventional needle injection systems are the most commonly used method to deliver vaccines and antibiotics in the swine industry. With the use of conventional needle systems comes the risk of breaking needles when administering injections. This creates a great concern for the meat packing industry as needles or needle fragments can be retained in the pork carcass and be passed into the food supply. Even though most meat packing facilities utilize a metal detection system to prevent metal pieces from entering the food supply they may not be able to detect some needles and needle fragments. Some of these metal pieces can go unnoticed due to small fragment sizes, different metal alloys used to make the needles, and the orientation of the needle in respect to the metal detector (Sundberg, 2000). If needles and needle fragments go undetected they become a serious safety hazard for consumers. There have been numerous lawsuits filed by consumers who have encountered needle fragments in meat products (Murphy, 2001). These lawsuits lower consumer confidence in their food supply and can cost packers and processors millions of dollars.

Aside from the potential food safety hazards that conventional needle systems may initiate, there are also worker safety issues in livestock production, specifically needlestick injuries. Accidental injection of veterinary medicines may result in ill health due to local damage at the site of injection, absorption of the medicine into the body, or infection transmitted from a previously used dirty needle (Skilton & Thompson, 2005). Hafer, Langley, Morrow, and Tulis (1996) stated that the highest reported physical injury among swine veterinarians was needlesticks with 73% of swine practitioners surveyed reporting that they experienced at least one needlestick injury in their career. In a two-year survey of large animal practitioners, the highest reported injury was needlestick injuries with a reported average of 3.1 needlesticks per veterinary practice (Poole, Shane, Kearney, & McConnell, 1999).

Another problem that has been linked with the use of needle-and-syringe injection is the spread of disease within a herd. When many animals are being vaccinated at one time it is not always possible or feasible to disinfect needles between injections of different animals. As a result, it has been reported that the utilization of one needle to

vaccinate multiple animals can result in lateral transmission of blood-borne illnesses (Otake, Dee, Rossow, Joo, Deen, Molitor & Pijoan, 2002).

The presence of abscesses in pork carcasses is a great problem that the pork industry is facing today. Pork carcasses that contain abscesses require trimming or even condemnation that costs packers both time and money. According to the Pork Chain Quality Audit (Meeker & Sonka, 1994), the presence of abscesses contributes to carcass trimming on 13% of pork carcasses. Moreover, the occurrence of abscesses in pork carcasses is the second leading cause of pork carcass condemnations in the U.S., accounting for 11% of condemned carcasses (Meeker, & Sonka, 1994). When abscesses are close to the skin surface, they may be observed as an extended or swollen mass of tissue which is filled with a fluid which is commonly referred to as “pus” that results from an immune reaction in response to an infectious bacteria or foreign matter (Harper, 2008). Abscesses and lesions have shown a tendency to form at injection sites. (George, Heinrich, Dexter, Morgan, Odde, Glock, Tatum, Cowman & Smith, 1995). Injection site lesions have been shown to have a negative effect on sensory characteristics, such as tenderness of meat products (George, Morgan, Glock, Tatum, Schmidt, Sofos, Cowman & Smith, 1995). One bacterium that is often associated with a myriad of different types of abscesses in swine is *Arcanobacterium pyogenes* (Merck, 2005). *A. pyogenes* is a common inhabitant of the mucousal surfaces of cattle and swine and is associated with a variety of infections (Trinh, Billington, Field, Songer, & Jost, 2001).

An alternative to conventional needle injection that has been adopted by some swine producers in the U.S. is needle-free jet injection. This system utilizes a compressed gas to properly deliver vaccines and antibiotics to livestock. Needle-free jet injection systems have been used since the 1940's to vaccinate humans (Mousel, Leeds, White, and Herrmann-Hoesing, 2008) and the concept has been around since the 1800's. Research has shown that needle-free jet injection systems can attain a serological response that is comparable and sometimes better than that of conventional needle injection methods in cattle (Hollis, Smith, Johnson, Kapil & Mosier, 2005) while producing similar numbers of injection-site lesions in pigs (Houser, Sebranek, Thacker, Baas, Nilubol, Thacker and Kruse, 2004). Additionally, research has suggested that the use of needle-free jet injectors to vaccinate large groups can reduce the lateral

transmission of diseases (Reinbold, Coetzee, Hollis, Nickell, Reigel, Huff & Ganta, Accepted for publication 2009).

The use of a needle-free jet injection system will eliminate the potential for broken needles being found in meat products, the hazard of needlestick injuries and should reduce the lateral transmission of diseases. There has been little research investigating the association of injection site abscesses with needle-free injection. Therefore, the objective of this study was to determine if the incidence of injection site abscesses in market pigs varies with the use of needle-free injection when compared to conventional needle injection when *Arcanobacterium pyogenes* is present.

Materials and Methods

Animal Background

The Kansas State University Institutional Animal Care and Use Committee approved protocols (protocol # 2741) used in this experiment. 200 nursery age pigs were transported to Kansas State University's segregated early weaning unit and were randomly assigned to pens with five pigs in each pen. The pigs then went through an initial conditioning period of 21 days to become acclimated to their environment prior to the start of the experiment.

Treatment Application

On day 0 of the trial each pig was weighed individually and feeders were weighed to monitor feed intake. Each pig (n=199) received a total of 4 intramuscular injections of a 2 mL dose of sterile aluminum hydroxide vaccine adjuvant. On one side of the animal a conventional needle-and-syringe injection method utilizing a disposable 18 gauge X 1.9 cm needle was used to administer an injection in the neck (n=199) and ham (n=199) with needles being changed after every 25 animals. On the opposite side of the animal a Pulse 250 needle-free injector (Pulse Needle-Free Systems, Lenexa, KS) set at 45 psi was used to administer injections in the neck (n=199) and ham (n=199). A random number generator was used to randomize the side of the animal receiving each type of injection. Immediately prior to injection, the skin over the injection site was contaminated by brushing the site with a standard inoculum of live *Arcanobacterium pyogenes* which was

prepared by the Kansas State Veterinary Diagnostic Laboratory (KSVDL). To simulate a common livestock production system, the injection devices were not decontaminated or disinfected between injections.

Animal Management

The pigs were housed in their originally assigned pens for 27 or 28 days while being monitored daily with feed additions weighed and recorded. 198 pigs were humanely euthanized on days 27 or 28 via jugular injection of 6 mL of Fatal Plus, 390 mg/mL pentobarbital (Vortech Pharmaceuticals, LTD, Dearborn, MI). The euthanized pigs were then sent to the KSVDL where necropsies were performed on all animals with tissue surrounding injection sites harvested for histopathological evaluation.

Histopathology

Representative portions of the reactive tissue surrounding the injection sites were placed in 10% neutral buffered formalin for histopathological evaluation. A score of “0” was given to tissue from injection sites that were normal when viewed under a microscope. A score of “1” was given to tissue that contained groups of swollen macrophages with some granulation surrounding them that were due to a reaction to the adjuvant. A score of “2” was given to tissue that had abscesses and granulation visible microscopically.

Statistical Analysis

The FREQ procedure of SAS (SAS Institute Inc., Cary, NC) was used, and injection site served as the experimental unit. The paired binary data were then analyzed using McNemar’s test.

Results and Discussion

Animal Management and Growth

A total of 200 pigs were delivered to the KSU SEW prior to the start of the trial. During the 21 day acclimation period, 1 pig was euthanized due to lameness. At the start of the trial, the average weight of the pigs was 11.22 kg. During the trial, one pig died

unexpectedly due to septicemia caused by *Haemophilus parasuis*. The average weight of the pigs at the conclusion of the trial was 31.5 kg. Pigs had an average daily gain of 0.71 kg/day. Average daily feed intake 1.17 kg/day and the feed to gain ratio was 1.64 kg feed/kg weight gain.

Occurrence of Abscesses

Out of 792 total injection sites, this study revealed a total of 13 abscesses or 1.6% of injection sites resulted in the formation of an abscess and 5.6% of the animals on trial had an abscess. Needle-free jet injection resulted in significantly more at both neck ($P = 0.0625$) and ham ($P = 0.0313$) injection locations (Table 2.1). Needle-free jet injection sites (n=396) resulted in 13 (3%) abscesses while needle-and-syringe injection sites (n=396) were associated with 1 (0.25%) abscess. When assessing injection location, there was no statistical difference ($P > 0.05$) between abscess formation as the neck location produced 5 abscesses and the ham location yielded 8 abscesses. There were also no differences between the occurrence of abscesses on the left and right side. Of the abscesses observed, 10 developed on the left sides of the animals while 3 developed on the right sides.

These results coincide with data from the Pork Chain Quality Audit which reported that abscesses occur at a very low rate as their data had an occurrence rate of 8% (Meeker & Sonka, 1994). This study also shows the difficulty to produce abscesses in a controlled experiment as the injection sites were intentionally contaminated to produce as many abscesses as possible. Results from this experiment contradict the findings of Houser, Sebranek, Thacker, Baas, Nilubol, Thacker and Kruse (2004) who found no differences in abscess formation between needle-free injection compared with conventional needle injections. The intentional contamination in this study may have assisted in the production of this difference as no inoculum was used in their study. These results would agree with results by Sutterfield, Crow, Dikeman, Phebus, Grobbel and Hollis (2009) which reported an increase in bacterial translocation using a needle-free injection system compared with a needle injection system for enhancing beef strip loins.

Table 2.1 Pigs with histological injection site abscesses after vaccination¹

Item	Needle-and-Syringe	Needle-Free ²	P-value
Neck			
Total	198	198	----
Positive	0	5	0.06
Negative	198	193	----
Ham			
Total	198	198	----
Positive	1	7	0.0313
Negative	197	191	----

¹ Nursery age pigs (n=198) were injected twice by needle-free injection on one side and twice by needle-and-syringe injection on the opposite side before being euthanized after 27 or 28 d and having injections sites evaluated for abscess formation.

² Pulse Needle-Free Systems, Lenexa, KS

Injection Site Tissue Reactions

Aside from abscesses, injection site tissues that yields a reaction to the adjuvant were also assessed (Table 2.2). There were no significant difference between needle-free injection and needle-and-syringe injection in injection site tissues that showed granulation, resulting in a histopathological score of 1, for neck ($P = 0.51$) and ham ($P = 0.29$) injection sites. This data suggests that the immune systems of the pigs was reacting similarly to each of the injection types which would agree with Houser, Sebranek, Thacker, Baas, Nilubol, Thacker and Kruse (2004) who observed similar serological responses when evaluating needle-free and needle-and-syringe injection systems.

Table 2.2 Pigs with histological injection site granulation after vaccination¹

Item	Needle-and-Syringe	Needle-Free ²	P-value
Neck			
Total	198	198	----
Granulation	20	24	0.51
No granulation	178	174	----
Ham			
Total	198	198	----
Granulation	24	32	0.29
No granulation	173	166	----

¹ Nursery age pigs (n=198) were injected twice by needle-free injection on one side and twice by needle-and-syringe injection on the opposite side before being euthanized after 27 and 28 d and having injection sites evaluated for abscess formation.

² Pulse Needle-Free Systems, Lenexa, KS

Conclusion

There is no doubt that the utilization of needle-free jet injectors would eliminate the possibility of needles and needle fragments entering the food supply. Other research indicates that the use of needle-free jet injection systems will not lower the serological responses produced during vaccination. However, the results from this study suggest that implementing the use of needle-free jet injectors into swine production may lead to an increase in injection site abscesses when *Arcanobacterium pyogenes* is present on the skin surface. These abscesses will require trimming at pork harvesting facilities. To readily understand the effect that different injection systems have on injection site abscess formation under commercial operating conditions, further research is needed.

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