JOURNAL OF ANIMAL SCIENCE
The Premier Journal and Leading Source of New Knowledge and Perspective in Animal Science

Technical Note: Comparison of traditional needle vaccination with pneumatic, needle-free vaccination for sheep

M. R. Mousel, T. D. Leeds, S. N. White and L. M. Herrmann-Hoesing

J Anim Sci 2008.86:1468-1471.
doi: 10.2527/jas.2007-0839 originally published online Feb 29, 2008;

Technical Note: Comparison of traditional needle vaccination with pneumatic, needle-free vaccination for sheep[1,2]

M. R. Mousel,*[3] T. D. Leeds,* S. N. White,† and L. M. Herrmann-Hoesing†

*USDA, ARS, US Sheep Experiment Station, Dubois, ID 83423; and †USDA, ARS, Animal Disease Research Unit, Pullman, WA 99164

ABSTRACT: Lateral transmission of blood-borne diseases can occur when a single needle is used repeatedly to vaccinate livestock. Needle-free technology to vaccinate sheep without damaging the carcass, causing lesions, or leaving needle fragments, and eliciting a similar antibody response as traditional needle vaccinations, has been hampered due to variable wool length. Vaccine delivery, injection time, and antibody response were evaluated for a prototype pneumatically powered, needle-free injector and for traditional needle injections. To determine optimal pressure for vaccine delivery with the pneumatic, needle-free injector, two 8-mo-old wethers were injected at pressures from 207 to 414 kPa in increments of 69 kPa. Injection time and antibody responses were evaluated using one hundred 8-mo-old wethers given primary and secondary inoculations of ovalbumin. Serum samples were collected before and after the inoculations on d 0, 14, 28, and 42. Optimal pressure to deliver a s.c. inoculation with the pneumatic, needle-free injector was 207 to 276 pKa. Inoculation of 100 wethers required 60% less time with the pneumatic, needle-free injector than with needle injections when a new needle was used on every animal. Antibody titers were the same ($P > 0.12$) for the pneumatic, needle-free and the needle injections on d 14, 28, and 42. In addition, antibody titers increased after primary and secondary inoculations, as expected. This study indicated that a pneumatic, needle-free injector can be used to elicit the same antibody response in sheep as a needle injection, and the pneumatic, needle-free injector was faster. The pneumatic, needle-free injector also would be expected to reduce lateral transmission of blood-borne diseases, and will save time, eliminate biohazard waste (e.g., used needles), and eliminate accidental needle sticks for livestock handlers when vaccinating sheep.

Key words: methodology, sheep, vaccination

J. Anim. Sci. 2008. 86:1468–1471
doi:10.2527/jas.2007-0839

INTRODUCTION

Vaccination of livestock is an important management tool to reduce disease and optimize production. However, repeatedly using one needle on multiple animals, which is a common practice, could lead to lateral transmission of disease (Otake et al., 2002). In addition, needle injections can damage the carcass (Morgan et al., 1993) and may leave broken needles in the carcass (Stier, 2003), reducing its value (George et al., 1996).

Pneumatically powered, needle-free injection devices are an alternative to needle injection. Needle-free systems use pressurized gas to drive vaccine through skin and into the subcutis or muscle (Vyrzhikovskaya and Bandakov, 1967; Jackson et al., 2001). Since the 1940s, these devices have been used to vaccinate humans (Hingson et al., 1963; Sarno et al., 2000). Immune responses are similar, and in some cases greater, when vaccines are injected with a needle-free device, compared with needles, in humans (Parent du Chatelet et al., 1997; Williams et al., 2000), swine (Houser et al., 2004; Jones et al., 2005), and cattle (Hollis et al., 2005).

Alternatives to needle vaccinations have been unavailable to sheep producers because pneumatic, needle-free injectors were not designed to cope with the fleece. Pneumatic, needle-free injectors on the market for livestock, primarily cattle and swine, are triggered to inject by mechanical pressure on the nozzle as it

[1]The use of trade, firm, or corporation names in this publication is for the information and convenience of the reader. Such use does not constitute an official endorsement or approval by the USDA or the ARS of any product or service to the exclusion of others that may be suitable.

[2]The authors wish to acknowledge T. Kellom, M. Williams, T. Northcutt, J. B. Taylor, and the US Sheep Experiment Station operations staff for animal handling, data collection, and laboratory procedures.

[3]Corresponding author: Michelle.Mousel@ars.usda.gov

Received December 31, 2007.
Accepted February 20, 2008.

touches skin, and are not hindered by thin coats of hair. In sheep, premature injections can occur when attempting to penetrate wool, thus resulting in unsuccessful delivery of the vaccine. Vaccines could be delivered via pneumatic, needle-free injection after shearing, but this may not fit with a producer's management plan.

The objective of this study was to evaluate a pneumatically powered, needle-free injector, modified for sheep, for vaccine delivery, throughput, and immune response, in comparison with traditional needle injections.

MATERIALS AND METHODS

The USDA, ARS, US Sheep Experiment Station Institutional Animal Care and Use Committee reviewed and approved all husbandry practices and animal procedures used in this study.

Injection Systems

Needle-free. A prototype Pulse250 was purchased from Pulse NeedleFree Systems (Lenexa, KS). The hand piece was modified such that the pressure required to mechanically trigger an injection was increased (the modification was done by Pulse NeedleFree Systems and is proprietary information) to enable wool penetration and skin contact before injection. The Pulse250 is a mobile unit capable of administering 0.5- to 2.5-mL doses in 0.5-mL increments and is pneumatically powered with gaseous CO_2 from the head space above the liquid CO_2 stored in a 0.7-L tank.

Needle. A Roux, pistol-grip, 50-mL syringe was purchased from Valley Vet (Marysville, KS). This model can administer 1- to 5-mL doses, in 1-mL increments, and was capable of needle changes between animals.

Injection Pressure

To determine the pneumatic pressure required to penetrate the skin and inject vaccines s.c. with the pneumatic, needle-free injector, two 8-mo-old wether lambs were anesthetized to a surgical plane with 16 mg/kg of pentobarbital sodium. Once anesthetized, the animals were injected in the neck through approximately 3.8 cm of wool, twice for each injection pressure evaluated. Pressure was increased with each 2-mL injection of Chinese ink (0.5 mL of ink diluted to 45 mL with sterile, isotonic saline; Sigma-Aldrich, St. Louis, MO), from 207 to 414 kPa in increments of 69 kPa. The lambs were also injected with a needle to compare the injection depth to that of the pneumatic, needle-free injections. After injection of the dye, the lambs were killed with 80 mg/kg of pentobarbital sodium. The injection sites were sheared and dissected to determine whether the injection penetrated the skin, was s.c., or was i.m.

Animals, Inoculation, and Sample Collection

Wether lambs (Rambouillet, Polypay, and Targhee) at 8 mo of age were used. Lambs were born in March and April, herded on sagebrush steppe (May to late June), subalpine range (late June to September), and sagebrush steppe (September to November), and then maintained together in a feedlot on a diet that met the NRC (1985) maintenance requirements from November to the end of the study in January 2007.

Before inoculation, 4 treatments, in a 2×2 factorial array, were randomly assigned to 100 wethers. Treatments were s.c. injection in the neck, with either the pneumatic, needle-free injector or a needle, of 6 mg of ovalbumin (Sigma-Aldrich, >90% pure) dissolved in 1 mL of sterile, isotonic saline and 1 mL of an aluminum hydroxide adjuvant (Alhydrogel, Accurate Chemical and Scientific Corp., Westbury, NY), or of 2 mL of a commercial vaccine, containing an aluminum hydroxide adjuvant, for a common sheep disease. Therefore, each lamb received a needle injection and a pneumatic, needle-free injection, with different antigens, on opposite sides of the neck. During inoculation, the lambs were sorted by injection type and antigen to minimize the frequency of cleaning and the switching of antigens in the injection systems. Primary inoculations were given on d 0 and secondary inoculations were given on d 28 of the experiment. Blood samples were collected via jugular venipuncture into uncoated vacuum tubes (Becton Dickinson, San Jose, CA) on d 0, 14, 28, and 42. Serum was collected within 2 h after blood collection and stored at −20°C until tested for antibodies.

Time Comparison

Injection time, time to change the needles between animals, and time to fill the needle injection system, or inject with the pneumatic, needle-free injector were recorded during administration of the secondary inoculation. The needle injection system was refilled 3 times. The time to replace bottles (2 total) for the pneumatic, needle-free injector (generally less than 10 s) was not recorded because, typically, large-volume vaccine bottles would be used and would not need to be changed when vaccinating only 100 sheep. Because each lamb received a needle and a pneumatic, needle-free injection, the time to inject 7 to 9 sheep (as many as would fit in the chute) was replicated 14 times (all 100 wethers timed) for each injection type.

Antibody Response

A commercial, synergistic, hemolytic inhibition test was used to test for antibodies to the commercial vaccine. Serum antibody titers to ovalbumin were determined using an ELISA, similar to the one described in Sevi et al. (2002). Ovalbumin, at 1 μg per well, was applied to each well, allowed to adhere overnight, washed with PBS (Sigma-Aldrich), the blocking buffer was applied for 1 h, and then serum was applied. Serum was titrated to 7 dilutions, the concentration dependent on what day the samples were collected, and assayed in triplicate. We deviated from the procedure of Sevi et al. (2002) so that 96-well, flat-bottomed microtiter plates (Nalge Nunc International, Rochester, NY) were incubated with BSA in PBS (blocking buffer; Sigma-Aldrich) for 1 h to reduce nonspecific binding, and the plates were allowed to develop color (darker color indicating a greater antibody concentration) for 25 min, and then the reaction was stopped with 1 *N* hydrochloric acid (Sigma-Aldrich). Positive controls were pooled from 20 serum samples collected on d 42 and assayed undiluted on each plate in quadruplicate. The inter- and intraassay CV were 6.0 and 4.6%, respectively, for the positive controls.

Statistical Analysis

To determine whether time differed with injection method, a paired-difference *t*-test was conducted using SAS (SAS Inst. Inc., Cary, NC). Serum dilutions (optical density, **OD**) had the background OD (without serum) subtracted and then were averaged for each dilution. An OD reading of less than 0.01 was considered negative for ovalbumin antibodies. Therefore, the adjacent dilution in the series, with an OD reading greater than 0.01, was considered the antibody titer. A logistic regression, proportional odds model (using PROC LOGISTIC of SAS) was used to test if the antibody titers were different for injection type, using a full model containing day of serum collection, breed, and injection type.

RESULTS

Optimal injection pressure was found to be 207 to 276 pKa (Figure 1). Pressures greater than this injected deeper than required for s.c. inoculations.

The pneumatic, needle-free injector was estimated to be 2.5 times faster than changing needles for every sheep. Time to inject 7 to 9 sheep with the pneumatic, needle-free injector averaged 60.6 s and was faster ($P < 0.001$) than inoculations with a needle, which averaged 155.3 s to inject 7 to 9 sheep.

The commercial synergistic hemolytic inhibition test did not detect antibodies to the commercial vaccine at d 14, 28, or 42, regardless of injection method. Thus, there were no antibody data from the commercial vaccine to analyze.

Antibody titers for ovalbumin for each week of serum collection and injection type are displayed in Figure 2. For d 0, nonspecific binding was detected for all sheep, with pneumatic, needle-free injected animals showing a titer of 1:1,000 and needle injected a titer of 1:500. After primary and secondary vaccination, antibody titers were not different ($P > 0.12$) regardless of injection method for d 14, 28, and 42. As expected, antibody titers were increased on d 14 and 42 after primary and secondary inoculations, respectively.

a)

b)

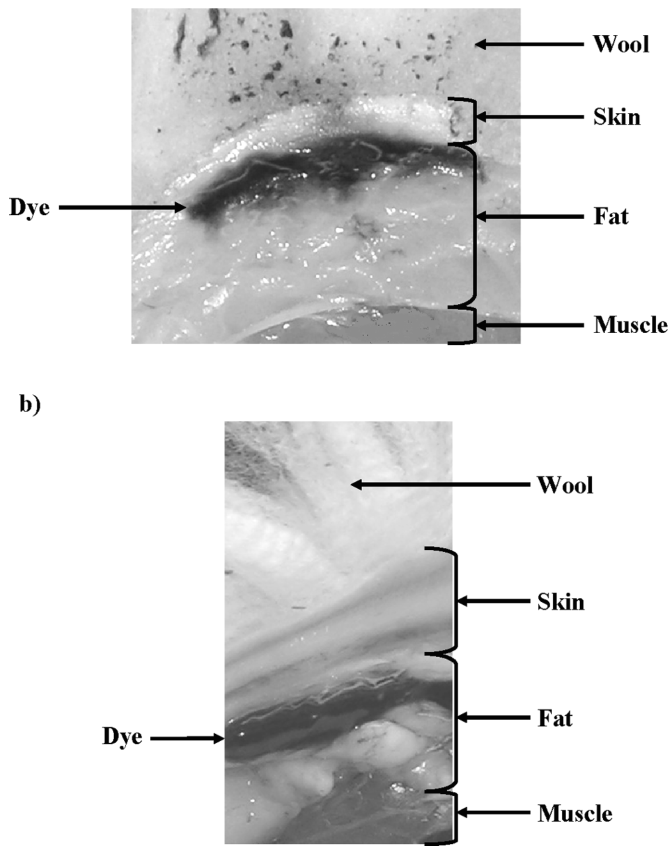

Figure 1. Demonstration of s.c. injections through wool with (a) the pneumatic, needle-free injector and (b) a needle injection. The injection pressure was 207 kPa for the pneumatic, needle-free injection. The dye is disseminated and a bubble within the fat in needle-free and needle injections, respectively. The skin is pulled back in both pictures to show that the dye is below the skin. The approximate depth of the skin is the width of the arrow in both pictures.

DISCUSSION

Pneumatic, needle-free and traditional needle injections elicited the same ovalbumin antibody response in our sheep on d 14, 28, and 42. Other studies have shown similar or greater immune response of vaccines delivered with needle-free injectors compared with needle injections (Williams et al., 2000; Houser et al., 2004; Hollis et al., 2005; Jones et al., 2005).

Modification of the pneumatic, needle-free injector to increase mechanical trigger pressure allowed for wool (approximately 3.8 to 5.1 cm staple length) penetration and skin contact before injection. Optimal injection pressure was found to be between 207 and 276 kPa for s.c. injections in 8-mo-old lambs, which is similar to that required for s.c. injection in swine with the Pulse250 (Pulse NeedleFree Systems).

One advantage of this technology over conventional needle vaccinations is speed of delivery (Giudice and

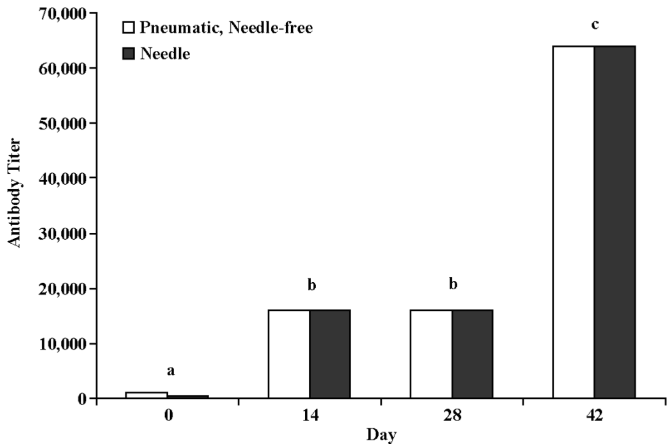

Figure 2. Antibody titers of 100 wethers injected with a single-use needle or a pneumatic, needle-free injector (at 207 kPA). Wethers were injected with 6 mg of ovalbumin after collection of serum on d 0 and with a secondary administration after collection of serum on d 28. Antibody titers within day did not differ ($P > 0.12$) by method of delivery. Among days, titers signified by different letters are different ($P < 0.01$).

Campbell, 2006). In this study, time to inject 100 sheep with the pneumatic, needle-free injector was 60% less than the time for needle injections when needles were changed between animals. At the US Sheep Experiment Station, we administer about 9,800 vaccinations per year. Thus we are able to administer about 5,970 more vaccinations in a specific timeframe with the pneumatic, needle-free injector than is possible when changing needles between animals. This represents a considerable time and labor savings. Additional advantages of the pneumatic, needle-free injector include elimination of the occupational hazard of accidental needle sticks and reduction of biohazard waste produced from discarded used or damaged needles. During this study, sheep appeared to be less stressed (less jumping, shying, vocalizing) when inoculated with the pneumatic, needle-free injector than when injected with a needle.

The results of this study indicate that a modified pneumatic, needle-free injector can be effective in administering antigens to sheep. We expect vaccines injected with the pneumatic, needle-free injector to produce similar or greater antibody responses as needle injections in sheep, as was demonstrated in swine (Houser et al., 2004) and cattle (Hollis et al., 2005); however, further studies are needed with sheep to evaluate this system with commercial vaccines.

LITERATURE CITED

George, M. H., G. L. Cowman, J. D. Tatum, and G. C. Smith. 1996. Incidence and sensory evaluation of injection-site lesions in beef top sirloin butts. J. Anim. Sci. 74:2095–2103.

Giudice, E. L., and J. D. Campbell. 2006. Needle-free vaccine delivery. Adv. Drug Deliv. Rev. 58:68–89.

Hingson, R. A., H. S. Davis, and M. Rosen. 1963. The historical development of jet injection and envisioned uses in mass immunization, and mass therapy based upon two decades' experience. Mil. Med. 128:516–524.

Hollis, L. C., J. F. Smith, B. J. Johnson, S. Kapil, and D. A. Mosier. 2005. A Comparison of serological responses when modified-live infectious bovine Rhinotracheitis virus vaccine, *Mannheimia haemolytica* bacterin-toxoid are administered with needle-free versus conventional needle-based injection in yearling feedlot steers. The Bovine Practitioner 39:106–109.

Houser, T. A., J. G. Sebranek, B. J. Thacker, J. T. Baas, D. Nilubol, E. L. Thacker, and F. Kruse. 2004. Effectiveness of transdermal, needle-free injections for reducing pork carcass defects. Meat Sci. 68:329–332.

Jackson, L. A., G. Austin, R. T. Chen, R. Stout, F. DeStefano, G. J. Gorse, F. K. Newman, O. Yu, and B. G. Weniger. 2001. Safety and immunogenicity of varying dosages of trivalent inactivated influenza vaccine administered by needle-free jet injectors. Vaccine 19:4703–4709.

Jones, G. F., V. Rapp-Gabrielson, R. Wilke, I. L. Thacker, B. J. Thacker, L. Gergen, D. Sweeney, and T. Wasmoen. 2005. Intradermal vaccination for *Mycoplasma hyopneumoniae*. J. Swine Health Prod. 13:19–27.

Morgan, J. B., J. B. Cannon, F. K. McKeith, D. Meeker, and G. C. Smith. 1993. National pork chain quality audit (packer-processor-distributor). Final report to the National Pork Producers Council. Des Moines, IA.

NRC. 1985. Nutrient Requirements of Sheep. 6th ed. Natl. Acad. Press, Washington, DC.

Otake, S., S. A. Dee, K. D. Rossow, H. S. Joo, J. Deen, T. W. Molitor, and C. Pijoan. 2002. Transmission of porcine reproductive and respiratory syndrome virus by needles. Vet. Rec. 150:114–115.

Parent du Chatelet, I., J. Lang, M. Schlumberger, E. Vidor, G. Soula, A. Genet, S. M. Stanaert, and P. Saliou. 1997. Clinical immunogenicity and tolerance studies of liquid vaccines delivered by jet-injector and a new single-use cartridge (Imule): Comparison with standard syringe injections. Vaccine 15:449–458.

Sarno, M. J., E. Blase, N. Galindo, R. Ramirez, C. L. Schirmer, and D. F. Truillo-Juarez. 2000. Clinical immunogenecity of measles, mumps and rubella vaccine delivered by the Injex jet injector: Comparison with standard syringe injection. J. Pediatric Infec. Dis. 19:839–842.

Sevi, A., M. Albenzio, G. Annicchiarico, M. Caroprese, R. Marino, and L. Taibi. 2002. Effects of ventilation regimen of the welfare and performance of lactating ewes in summer. J. Anim. Sci. 80:2349–2361.

Stier, R. F. 2003. The dirty dozen: Ways to reduce the 12 biggest foreign materials problems. Food Safety 9:44–50.

Vyrzhikovskaya, M. F., and L. F. Bandakov. 1967. Roentgenological examination of the subcutaneous injection of fluid form a needleless injector. Med. Tekh. 4:29–33.

Williams, J., L. Fox-Leyva, C. Christensen, D. Fisher, E. Schlicting, M. Snowball, S. Negus, J. Mayers, R. Koller, and R. Stout. 2000. Hepatitis A vaccine administration: Comparison between jet-injector and needle injection. Vaccine 18:1939–1943.