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SPRAY-VAC®

IMPROVES NEWCASTLE DISEASE VACCINE STABILITY

Spray-Vac® Improves Newcastle Disease (ND) Vaccine Stability

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Vaccinating poultry via mass spray systems has become the predominant way to immunize birds in many poultry production units. Spraying gives poultry integrators more positive control over the timing and administration of ND virus vaccine than vaccinating in drinking water. One spray-vaccination drawback is the lack of convenient, safe and inexpensive water to dilute the vaccine for delivery. Readily available public and farm water sources are obvious choices; however, these water supplies routinely contain oxidizers, including chlorine, nitrates and mineral elements which rapidly inactivate ND vaccines. To avoid the risk associated with using tap water as the spray solution, vaccine manufacturers typically recommend distilled water as the diluent.

Distilled water is free of potentially harmful oxidizers, but it is neither convenient or inexpensive compared to public or farm water. Distilled water is also more acidic than many people realize which is not ideal for vaccines. Supplying a large enough volume of distilled water to spray typical commercial poultry farms is a significant logistical challenge. Previous work with infectious bronchitis vaccine showed Spray-Vac[2], a vaccine-stabilizing water additive, helps poultry vaccinators overcome both the logistical challenges of distilled water and concerns over vaccine inactivation in tap water. Davis and Lasher (2000) added Spray-Vac to water containing chlorine at concentrations similar to those typically found in tap water. Spray-Vac stabilizer protected the vaccine, permitting the use of convenient, low-cost farm water while guarding the vaccine against oxidation. The present series of experiments sought to answer if Spray-Vac exerts the same stabilizing influence on ND vaccine.

ND Prevalence and Risk

As an acute viral infection, ND poses a significant risk to global poultry production, with the potential to result in trade restrictions. Also termed avian pneumoencephalitis, it is seen primarily as a mild to severe respiratory disease. However, it can also manifest itself as a more serious disease, inducing diarrhea, nervous symptoms and variable mortality. The virus spreads within flocks via air, respiratory fluids, feces, and contaminated food. People and equipment inadvertently carry the virus between flocks. Virulent strains are found among the poultry populations of Asia, Africa, and some Central and South American countries. The U.S. and Canada maintain import restrictions and eradication programs to prevent infection by virulent strains. Live vaccines are mass-administered to flocks in the drinking water, by spray, or as nasal or eye drops.

Research Objective

Scientists conducted research at two laboratories to determine if Spray-Vac exerts a stabilizing influence on a live ND vaccine rehydrated in chlorinated water. Study locations were Charles River Avian Products and Services (SPAFAS) Laboratory, Storrs, CT, and Lasher Associates, Inc., Millsboro, DE.

1 Lasher Associates, Inc. Millsboro, DE provides consulting services worldwide to manufacturers of poultry vaccines.
2 Animal Science Products, Inc. Nacogdoches, TX manufactures a variety of stabilizing additives designed to improve the successful application of poultry vaccines.

Materials and Methods

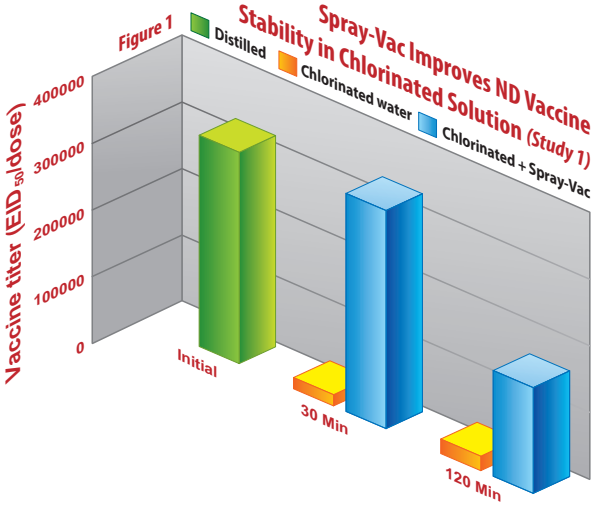
In the first study conducted by SPAFAS, one vial of a live commercially available ND vaccine was reconstituted and then further diluted in sterile distilled (DI) water to obtain an initial titer; DI water containing chlorine (negative control) and DI water containing chlorine plus Spray-Vac. The vaccine in water was titered immediately. The vaccine in the other two preparations was titered after 30 and 120 min. In the second study conducted by Lasher Associates, the basic protocol used at SPAFAS was repeated for the vaccine in water only and in water containing chlorine[3].

Titrations

Titrations were conducted using specific-pathogen-free (SPF) embryonated eggs. The method was one commonly used by vaccine manufacturers described in Title 9, Code of Federal Regulations §113.329. Embryo deaths occurring during the first 24 hours after inoculation were disregarded. After 7 days incubation, surviving embryos were tested for HA activity. A titer was considered satisfactory when at least one dilution had 50 to 100 percent positives, and at least one dilution had 0 to 50 percent positives. Embryos were counted as positive if they had died, or if they were HA-positive. The method of Reed and Muench was used to calculate the EID^{50} per dose.

Results

Study 1: Effect of Spray-Vac stabilizer on the titer of ND vaccine in water chlorinated at 4 ppm (figure 1). The titer of the ND vaccine at time zero with no exposure to chlorine was $10^{5.5}$ EID^{50}/dose. Exposing the virus to water containing 4 ppm chlorine and no Spray-Vac stabilizer reduced titers to less than or equal to $10^{4.5}$ EID^{50}/dose, which is a loss of 90% or more of the vaccine titer. Stabilizing the vaccine with Spray-Vac prevented reduction in titer at 30 minutes, with titers of $10^{5.5}$ EID^{50}/dose equaling those found in the initial inoculation. After 120 minutes incubation, the stabilized ND vaccine maintained titers ($10^{5.2}$ EID^{50}/dose) more than five-times greater than non-stabilized vaccine. Although Spray-Vac greatly improved ND vaccine performance, the range of dilutions used in the trial allowed nonstabilized vaccine titers to fall below the lowest quantification limit. Because this prevented the final titer-destroying effect of chlorine from being calculated, a second study was conducted.



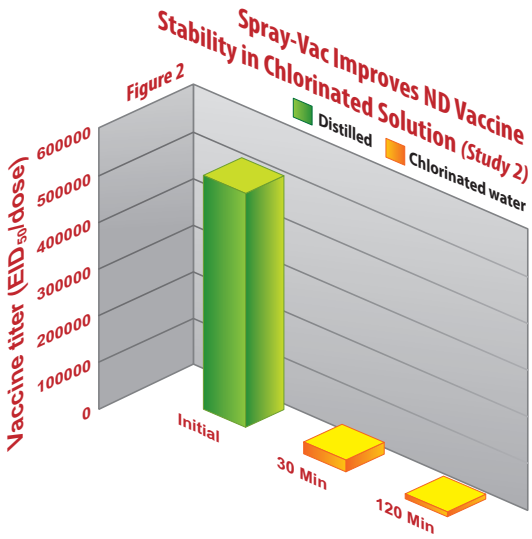
Study 2: Effect of chlorinated (4 ppm) water on the titer of ND vaccine (figure 2). In the second study, the titer of the vaccine with no exposure to chlorine at time zero was $10^{5.7}$ EID^{50}/dose. After 30 minutes exposure to chlorine at 4 ppm, the titer dropped to $10^{4.2}$ EID^{50}/dose. Then, after 120 minutes, the titer had fallen further to $10^{3.0}$ EID^{50}/dose.

Discussion

Water on poultry farms is commonly chlorinated to aid in reducing numbers of potentially harmful microorganisms. In general, a chlorine level of 4 ppm is considered the practical upper limit, as higher levels have been found to adversely influence water intake. Because the chlorine in drinking water systems is also virucidal, it inactivates live vaccines, such as ND vaccine. To avoid inactivating vaccines in spray solutions, many poultry growers resort to the inconvenient use of relatively expensive distilled water as the spray solution. As seen in these studies, Spray-Vac protects ND vaccine from the harmful effects of tap water and eliminates the

3 In the first study, the full effect of chlorinated water on the vaccine was not determined. The second study was conducted to determine such by using lower virus dilutions to inoculate the embryos.

need for distilled water in the spray solution. Adding 4 ounces of Spray-Vac to each gallon of tap water (30 ml/liter) shields vaccines and rescues them from inactivation for longer than typical spray application times.



In the first study for thirty minutes incubation, Spray-Vac completely preserved the vaccine's effectiveness, providing at least ten times more live ND vaccine per dose than the non-stabilized solution. At 120 minutes, Spray-Vac still bolstered virus survivability by at least five-fold when compared to non-stabilized chlorinated water. Ultimately, the severe titer-destroying effect of chlorine was below the lower quantification limit of the first study. It was only possible to calculate that Spray-Vac preserved at least 5 or 10 times more vaccine, but not precisely how much more.

The second study was designed to determine precisely how much vaccine was inactivated, so the stabilizer's true improvement could be calculated. The second study demonstrated that exposure to chlorine at 4 ppm for 30 minutes induced a 30-fold reduction in ND vaccine titer as compared to the initial titer at time zero. Then after 120 minutes, the titer had been reduced a full 500-fold from its initial value. Applying these more precisely determined minimums to data from both studies. It is anticipated that at 30 minutes, chlorinated water stabilized with Spray-Vac would have 30 times more live virus than chlorinated water alone. In fact, no titer loss at all would be expected at this time interval. Furthermore, at 120 minutes, use of Spray-Vac is projected to yield approximately 250 times more live vaccine virus.

Spray-Vac was previously shown to completely rescue a fragile infectious bronchitis vaccine held in spray solutions for 30 and 120 minutes. The non-stabilized IB vaccine in the previous research lost 60 to 80% of its original titer. The non-stabilized ND vaccine used in the present study lost a much greater percentage of its initial titer, suggesting more sensitivity to oxidation and emphasizing the importance of proper stabilization.