ANIMAL SCIENCE PRODUCTS® INCORPORATED

Hatchery Managers Turn the Tide

Looking out the hatchery door you feel a pressure moving toward you. There is a steady force pushing global poultry vaccination to earlier and earlier ages. Like an ocean tide, this flow persistently and irresistibly shifts a higher proportion of vaccinations toward hatcheries. Newcastle Disease (ND) vaccination is one being impacted.

ND virus, with its highly-contagious nature, affects all of the important commercial poultry species. It is endemic in many countries and is being battled worldwide. International trade barriers resulting from ND prevalence is a threat. Guarding birds from airborne ND virus, direct contact, and even contaminated hatchery shells is important; and the earlier the better. Some key reasons for earlier spray vaccination ages are:

- Birds with faster-growing genetics do not have as much time to develop immunity under traditional vaccination programs.
- Live ND vaccine in day-old chicks builds defenses very quickly. Local respiratory immunity rapidly appears within 4 hours following vaccination in day-old chicks, offering crucial early protection while priming antibody production for longer-lasting defense.
- Simple, inexpensive and accurate spraying equipment for hatcheries makes vaccination more convenient and dependable, compared to time-consuming and inconsistently-applied grower vaccinations.
- Fast-growing poultry companies find it easier and safer to train and manage hatchery vaccinations than growing-house vaccinations.
- Independent growers that buy day-old-chicks press their commercial hatcheries to supply birds with their immune systems already stimulated and their defenses against prevalent diseases like ND under development.

Live antigens in spray vaccines must completely survive in order to provide the fullest immunity. Early vaccine manufacturers recommended that, if hatcheries planned to spray the live vaccine, they use distilled water. This recommendation came about because the vaccine manufacturers lacked the ability to manage tap water quality throughout their global customer base. Vaccines perform best when they are shielded against tap water containing natural or added oxidizing mineral

elements, inappropriate pH and unbalanced electrolyte concentrations. Modern, new-generation vaccine stabilizers are designed to counteract these risks, rescuing vaccines so hatcheries can conveniently and safely vaccinate with local water sources instead of costly and cumbersome bottled water. New stabilizer technologies nurture vaccines in local

tap water, make spraying more convenient and efficient, and reduce the logistics of sourcing and storing bottled water. They also reduce the plastic containers that must be disposed or recycled. Carbon-footprint-conscious poultry companies are taking note, eliminating distilled water as part of their "green initiatives."

Earlier research in Lasher Associates' lab confirmed the protective effect Spray-Vac® had on a fragile bronchitis vaccine. Now a more recent report describes research to learn if the new-generation vaccine stabilizer also rescues a live ND vaccine in water with high oxidative potential.

Research Summary

A live freeze-dried ND vaccine rehydrated in water was introduced into (a) more water alone, (b) water containing free available chlorine at 4 ppm, or (c) water containing free available chlorine at 4 ppm plus Spray-Vac® Stabilizer at the point-of-use concentration. Vaccine virus was titrated in specific-pathogen-free (SPF) embryos at 0 and 60 minutes for the water-alone treatment, and at 30 and 60 minutes for both the chlorine and chlorine plus Spray-Vac treatments. It was determined that Spray-Vac® fully protected the vaccine virus from the deleterious effect of free chlorine at 4 ppm.

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Location:
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Materials and Methods

The researchers chose the same long-standing experimental model used by ND vaccine manufacturers to validate the initial potency of each production run of their vaccines. The method is prescribed by USDA regulators, published in Title 9, U.S. Code of Federal Regulations §113.329, and is used world-wide by regulatory authorities for vaccine potency assessment. Although this research discusses ND vaccine stability in the context of hatchery spray, the results apply equally to field boost sprays in grower facilities.

Spray vaccine preparation. In this ND vaccine stability study, a representative vial of commercial freeze-dried vaccine containing 1000 doses was rehydrated according to label directions as a hatchery normally would with 10 ml of sterile water. The vaccine vial was gently agitated to uniformly disperse solid particles. To prepare for titrations, the vaccine was then further diluted to 1 label dose per ml in each of 3 prepared spray solutions and held at room temperature throughout the experiment:

1. Positive control- Distilled water common to hatcheries.
2. Negative control- The same distilled water with added oxidizer standardized at 4 ppm chlorine to replicate hatchery tap water.
3. Stabilized- The same water as the negative control with Spray-Vac[1] liquid stabilizer concentrate added at the recommended concentration of 32 ml per liter of spray.

[1] Spray-Vac® Stabilizer. The stabilizer was supplied by Animal Science Products, Inc., Nacogdoches, TX 75963.

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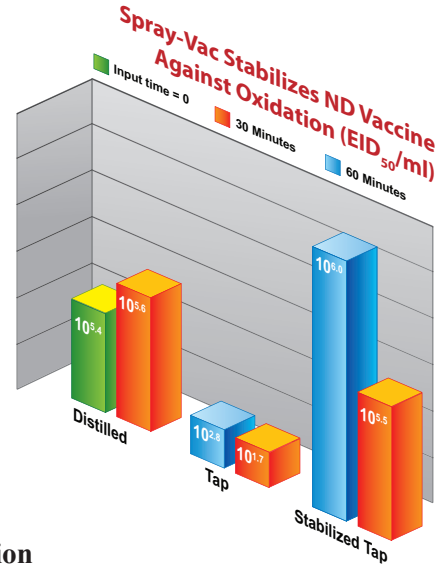
EID_{50} vaccine titrations. Each of these 3 initial spray solutions served as the 10^0 dilution, and was then 10-fold serially diluted with separate transfer pipettes for embryo inoculations. Titrations were conducted using specific-pathogen-free (SPF) embryonated eggs. Each titration was replicated 3 times, inoculating 6 10-day SPF eggs at each dilution.

The distilled water positive control was titered immediately to measure the amount of live vaccine virus input at time 0, then again at the 60-minute endpoint. The negative-control-replicating tap water and its stabilized counterpart were both titered after 30 and 60 minutes in the spray solutions.

All incubating embryos were candled daily. Embryos that died within the first 24 hours following inoculation were considered unrelated to virus effects and were disregarded. Thereafter dead embryos were refrigerated immediately after candling. After 7 days incubation, surviving embryos and dead ones in the highest dilution that also contained live embryos were tested for HA activity using a 5% suspension of red blood cells, comingled from 3 adult SPF chickens, in phosphate buffered saline. Embryos were counted as positive if they had died, or if they were HA-positive. A titer was considered valid when the most concentrated dilution had 50 to 100 percent positives, and the least concentrated dilution had 0 to 50 percent positives. The method of Reed and Muench was used to calculate the EID_{50} per dose.

Results

The effect of Spray-Vac® Stabilizer on the titer of ND vaccine in chlorinated water is illustrated graphically in the figure. Geometric mean titers (GMT) of the ND vaccine in distilled water (positive control with no exposure to chlorine) at the input time zero and after 60 min were $10^{5.4}$ and $10^{5.6}$ EID_{50}/ml, respectively. This is the baseline titer a hatchery would expect under normal conditions when spraying with distilled water. The GMT of the replicated-tap-water negative control (vaccine exposed to water containing chlorine and no stabilizer) were $10^{2.8}$ and $10^{1.7}$ EID_{50}/ml at 30 and 60 minutes, respectively. The 2.8-log reduction after only 30 minutes means the non-stabilized vaccine had already lost over 99.7% of its value. In contrast, the titers of the stabilized solution (vaccine diluted in water containing 4 ppm chlorine and Spray-Vac) increased at the 30-minute interval to $10^{6.0}$ (+74%) and $10^{5.5}$ EID_{50}/ml at 60 minutes (+26%).



Discussion

In order to successfully immunize chickens via spray, one of the most important considerations centers on delivery of live virus at the recommended dosage. To accomplish adequate delivery, spray equipment must be fully functional, the operator must uniformly deliver the spray over the chickens, the virus must be rehydrated to the proper dilution and the virus must remain viable in the diluent, which is commonly drawn from a distilled water bottle or a water tap. The steadily increasing number of hatchery vaccinations places a greater burden on sourcing and storing distilled water, and disposing of the bottles.

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Spraying with tap water that is readily available, convenient and inexpensive is naturally a better choice; however, water quality always poses some risk to the vaccine unless it is first stabilized. Water can challenge a vaccine's immunizing potential if harmful oxidizing elements are present. Sometimes it is easy to see the impact of oxidation, like steel being rusted by the tide. But vaccines are not steel. Very small concentrations of oxidizing elements can inactivate fragile vaccine, eroding the foundation of a poultry health and food safety program, unless the vaccine is stabilized.

This trial was conducted to assess both (1) the degree of ND virus inactivation induced by water containing a common amount of oxidizer and (2) the rescue effect of Spray-Vac® Stabilizer on ND virus in the same water. ND vaccine virus was chosen as the specimen virus because of its wide-spread use in chickens by the spray route. The chlorine concentration of 4 ppm was chosen because it is an oxidative element within a range normally encountered in municipal or well water systems. A pilot study previously conducted in this laboratory had confirmed the ND vaccine's sensitivity and helped establish the appropriate titration dilutions for the present experiment.

Spray-Vac was very effective in preserving the viability of ND vaccine virus in the presence of 4 ppm free chlorine, even up to one hour. At 30 minutes, the stabilized chlorine solution had over 1500 times more live virus than the same solution without stabilizer. At 60 minutes, Spray-Vac bolstered virus survivability by over 6000-fold when compared to the effect of chlorine alone.

On the other hand, the viability of the virus degraded rapidly in non-stabilized spray water containing normal concentrations of oxidizer, and continued to do so through the 60-minute incubation. In addition, Spray-Vac itself had no adverse effect on the virus when compared to the effect of distilled water alone.

Although the difference between the two time points for both the distilled water and stabilized water treatments varied three-fold to four-fold, this variation is within the biological variation, or "noise," of expected titer results when only a few titers are compared. In general, biological variation becomes significant in terms of virus titer as it approaches 10-fold (one-log) differences. Similarly, of the four titers for these groups, the titer in distilled water alone at time zero was the lowest. Although precautions were taken to avoid vaccine aggregates, one possible explanation is that upon rehydration of the freeze-dried vaccine cake, inconspicuous small clumps may remain in suspension, containing more viral particles than expected. Testing this explanation will require many more titration studies and thus, remain a continuing focus of investigation.

Hatchery managers feel the increasing tide of vaccinations pressing them. Spray-Vac relieves some of the pressure by safely permitting vaccines to be sprayed with local tap water, nurturing vaccines better than distilled water. In addition to shielding against oxidizers, Spray-Vac buffers the spray to a more perfect pH and balances electrolytes better than distilled water. Adding Spray-Vac to local hatchery tap water makes an ideal solution.