Effect of Vac-Pac Plus on the viability of a live infectious bronchitis vaccine

Reprinted from J. Appl. Poult. Res. 19:152–156

N. A. Kamau ,* D. W. Lee ,† C. K. Kheong ,‡ J. E. Park ,* and H. J. Shin *§1
** Laboratory of Infectious Disease, College of Veterinary Medicine, Chungnam National University, Daejeon, Korea 305-764; and † Merial Korea, ‡ Merial Singapore, and § Research Institute of Veterinary Medicine, 220 Gungdong, Yuseong, Daejeon, 305-764, Korea*

INTRODUCTION

R.S. Izard[a], Animal Science Products, Inc, Nacogdoches, Texas, USA.

Vac-Pac Plus…The proper tool for the job

You probably know the phrase, "use the proper tool for the job". It is an axiom reminding us that good tools make for good work, because short cuts compromise safety and quality. If you stop and think about it, the saying is true in poultry farming, especially vaccination programs. For a live vaccine to safely protect your birds, it must be potent enough to create immunity and it must remain alive long enough to hold that potency until the very last bird has been dosed. Vaccine manufacturers and regulators ensure vaccine vials contain the proper initial potency; it's up to you and me to use the best tools in our box to preserve that vaccine, keeping it safe and stable until every bird is immunized.

Asian researchers at Korea's Laboratory of Infectious Disease and Research Institute of Veterinary Medicine teamed up to study infectious bronchitis (IB) vaccine stability, using the industry's leading vaccine stabilizer, Vac-Pac Plus. Their quote, *"…better preservation qualities…*," is taken directly from their report, reprinted here. It describes some of the problems encountered when trying to build fully-protective titers, whether from IB, Newcastle disease virus, or any live vaccine. Stabilizing the vaccine to ensure that birds receive uniformly strong protection is a key concern. Uniform, full protection can only be assured through drinking water vaccinations when each bird receives a full dose of active vaccine. During a typical vaccination, destructive elements in the water will inactivate much of the vaccine, causing some birds to receive less than a full dose. In the past, products of animal origin, like milk powder, were recommended in large amounts in the drinking water (2Kg per 1000 liters) to improve the vaccine's survival.

More recently, biosecurity concerns over materials of animal origin have prompted regulators and poultry companies to avoid stabilizers containing milk powders. The researchers also noted the very weak stabilization from slow-dissolving milk powder, requiring 20-fold more to stabilize the IB vaccine than Vac-Pac Plus. The Vac-Pac Plus used in this research is an instantly soluble granular powder that activates immediately, contains no products of animal origin, and, with much more protecting power, requires only 100 grams to stabilize a full 1000 liters of water.

Vac-Pac Plus is the second-to-none, internationally-recognized leader in vaccine stability. It's the proper tool for the job. Safer for your vaccine, safer for your birds.

[a]Corresponding author: rsizard@asp-inc.com

Effect of Vac-Pac Plus on the viability of a live infectious bronchitis vaccine

N. A. Kamau,* D. W. Lee,† C. K. Kheong,‡ J. E. Park,* and H. J. Shin*§[1]

**Laboratory of Infectious Disease, College of Veterinary Medicine, Chungnam National University, Daejeon, Korea 305-764; and †Merial Korea, ‡Merial Singapore, and §Research Institute of Veterinary Medicine, 220 Gungdong, Yuseong, Daejeon, 305-764, Korea*

Primary Audience: Flock Supervisors, Quality Assurance Personnel, Researchers, Veterinarians

SUMMARY

In the absence of stabilizers, vaccines administered in water are likely to be inactivated by free chlorine or other metals. Vac-Pac Plus, developed by Animal Science Products Inc. (Nacogdoches, TX), is a powdered stabilizer for vaccines administered via drinking water. The efficacy of this product was demonstrated in this study by its ability to preserve the viability of live infectious bronchitis vaccine reconstituted in water containing free chlorine. Maintenance of viable vaccine during administration is requisite for effective immune stimulation and response after vaccination.

Key words: infectious bronchitis virus, Vac-Pac Plus, Bioral H120

2010 J. Appl. Poult. Res. 19:152–156
doi:10.3382/japr.2009-00096

DESCRIPTION OF PROBLEM

Infectious bronchitis (**IB**) virus causes IB, an acute, highly contagious viral respiratory disease in chickens, characterized by tracheal rales, coughing, and sneezing [1–3]. Several vaccines against IB virus are available commercially [4, 5]; however, the routes of administration pose various distinct challenges. The conjunctival, intranasal, and in-contact routes induce better resistance to challenge than the drinking water route [6]. The latter, however, has the advantages of easier management and cost effectiveness. Low immune responses in the water route are sometimes a result of inactivation of vaccines by chlorine in the water [7, 8].

The manufacturers of Nobilis IB H120 and Nobilis IB MA5 vaccines [9] recommend the use of iron-free, chlorine-free water in addition to 2 g/L of skim milk to protect the vaccine. Even though chlorine-free water is used, the vaccine may be rendered unviable by other factors, such as metals in, high temperatures of, and osmotic pressure of the water [8, 10]. There is therefore a need for vaccines to be stabilized. Stabilizers ensure the vaccines are protected from chlorine and most sanitizing chemicals, which are usually chlorine based [10, 7]. Vacci-Guard [11] is said to neutralize chlorine in water, whereas second-generation Spray-Vac, {from Animal Science Products Inc. [12], has also been reported to stabilize vaccines [7]. The objective of this

[1]Corresponding author: shin0089@cnu.ac.kr

study was to determine whether Vac-Pac Plus [12] would preserve viable live IB vaccine in a manner comparable with skim milk.

MATERIALS AND METHODS

Chemicals, Vaccine, and Eggs

For testing before use, the Vac-Pac Plus [12] was dissolved according to the manufacturer's instructions. Commercial skim milk was obtained for the comparison test. Live attenuated vaccine against IB, mass-type Bioral H120 [13], was used to inoculate 225 washed, 9- to 11-d-old specific pathogen-free embryonated eggs for the titration of IB vaccine virus [14].

Diluent Systems

Four diluent systems for the vaccine were designed using laboratory-distilled water: 1) distilled water only; 2) chlorinated water, which was made by the addition of sodium hypochlorite to a concentration of 4 ppm; 3) chlorinated water with Vac-Pac Plus; and 4) chlorinated water with a 0.2% suspension of skim milk.

Preparation of the Vaccine Suspension and Mixture

Live IB virus vaccine was reconstituted in 3 mL of distilled water. A 0.5-mL quantity of the rehydrated vaccine suspension was diluted 100× in distilled water, followed by a 10-fold dilution of 1 mL of the diluted viral suspension in 0.85× PBS solution. A further 10-fold dilution was made, followed by four 6-fold serial dilutions. This range of 5 dilutions was then selected for inoculation at time 0 ($\mathbf{T_0}$) in embryonated eggs as described previously [15], with 5 replicates for each dilution.

Preparation of the Vaccine Suspension + Diluent Systems for Titration at 1 h Postinoculation and 2 h Postincubation

The rehydrated vaccine suspension was inoculated into each of the 4 diluents at a ratio of 1:99 mL and incubated at 30°C. At 1 h postinoculation ($\mathbf{T_1}$), 1 mL of the virus mixture was removed from each of the diluent systems and diluted 10 times in 0.85× PBS.

A serial dilution ensued for the titration of the viral mixture at T_1 in embryonated eggs. The remaining viral mixture at T_1 for each tube was promptly returned to incubation for another 1 h. At 2 h postincubation ($\mathbf{T_2}$), 1 mL of viral mixture was taken and treated the same as for T_1.

Dilutions of the Vaccine Suspension + Diluent Systems as Inocula for Titration at T_0, T_1, and T_2

The rehydrated vaccine was diluted 100-fold in each of the 4 different diluents in replicates of 5. From this, two 10-fold dilutions (A and B) were made, followed by 4 more dilutions with a dilution factor of 0.8 (i.e., C, D, and E; Table 1).

Selection of Dilutions from the 4 Different Diluent Systems for Titration at T_0, T_1, and T_2

Suspensions B, C, D, E, and F were used for the following diluent systems: 1) distilled water + IB vaccine, 2) chlorinated water + IB vaccine

Table 1. Serial dilution of viral suspension + diluent systems for times T_0, T_1, and T_2 for inoculation of eggs[1]

Suspension	Dilution factor, $\log_{10}$	Viral suspension used for dilution, µL	PBS,[2] µL	Inoculum
A	1	1,000 (T_0, T_1, T_2)	9,000	
B	2	220 (A)	1,980	Inoculate in 5 eggs
C	2.8	375 (B)	2,000	Inoculate in 5 eggs
D	3.6	375 (C)	2,000	Inoculate in 5 eggs
E	4.4	375 (D)	2,000	Inoculate in 5 eggs
F	5.2	375 (E)	2,000	Inoculate in 5 eggs

[1]T_0 = inoculation at time 0; T_1 = 1 h postinoculation; T_2 = 2 h postincubation.
[2]0.85× PBS solution.

Table 2. Vaccine virus titers at times T_0, T_1, and T_2 in 4 different diluent systems[1]

Time	Titer, $\log_{10}$ EID_{50}/vial			
	Distilled water	Chlorinated water	Chlorinated water + Vac-Pac Plus[2]	Chlorinated water + skim milk
T_0	7.34	7.34[3]	7.34[3]	7.34[3]
T_1	7.34	6.86	7.5	7.34
T_2	7.34	6.7	7.02	7.02

[1]T_0 = inoculation at time 0; T_1 = 1 h postinoculation; T_2 = 2 h postincubation; EID_{50} = 50% embryo infectious dose.
[2]Animal Science Products Inc. (Nacogdoches, TX).
[3]Vaccine virus titer in distilled water at T_0 is the titer reading for the other 3 diluent systems.

+ Vac-Pac Plus, and 3) chlorinated water + IB vaccine + skim milk. Suspensions A, B, C, D, and E were used for chlorinated water + IB vaccine. A 0.2-mL quantity of each suspension of A, B, C, D, and E or B, C, D, E, and F from each of the 4 diluent systems was inoculated into embryonated hen eggs (9 to 11 d old) through the allantoic route in replicates of 5. The eggs were incubated at 37°C for 7 d.

Reading and Interpretation of Results

Reading of results was done by aseptically removing the embryos to record their deaths. Embryos that died during the first 24 h were eliminated as nonspecific mortalities.

At 7 d postincubation or observation, the live embryonated eggs were opened and examined for stunted growth, hemorrhage, and necrotic livers to interpret the titer of these viruses in vaccines. The endpoint virus titer was that which caused 50% embryo infection, given as the 50% embryo infectious dose (**EID_{50}**). A reduction of 1.3 $\log_{10}$ EID_{50} or more in the virus titer of Bioral H120 was considered significant according to the manufacturer [12].

RESULTS AND DISCUSSION

Vaccine with Distilled Water

This diluent system was intended to act as a control as well as to determine the viability of the vaccine in the absence of inactivating chemicals. There was no decrease in the titer values of the vaccine treated with distilled water from T_0 to T_1 and from T_0 to T_2 (Table 2 and Figure 1). This showed the good preservation quality of the stabilizer in the vaccine itself.

Vaccine with Chlorinated Water

The values of titers declined in the vaccine treated with chlorinated water alone from 7.34 at T_0 to 6.86 at T_1 and eventually to 6.7 at T_2 (Table 2 and Figure 1), registering a decrease of 0.48 and 0.64 $\log_{10}$ EID_{50}, respectively (Table 3).

Vaccine with Chlorinated Water + Vac-Pac Plus

The addition of the putative stabilizer, recommended for administration of vaccines in water, was meant to protect the Bioral H120 vaccine from free chlorine. The results reflected a stable vaccine in this diluent system so that the titer at T_0 (7.34) increased to 7.5 at T_1 (Table 2 and Figure 1). After 2 h, however, the titer decreased to 7.02, which was equivalent to 0.32 $\log_{10}$ EID_{50} (Table 3).

Vaccine with Chlorinated Water + Skim Milk

The same level of titer (7.34) was observed at T_0 and T_1 (Table 2 and Figure 1). There was an equal decrease in this system at T_2 to a titer of 7.02 (Table 2 and Figure 1), equivalent to a decrease of 0.32 $\log_{10}$ EID_{50} (Table 3). The result obtained from the diluent system using skim milk was similar to that using Vac-Pac Plus.

Maintenance of viable vaccines in water should translate to higher titers in the chicken. These, in turn, are able to replicate to substantial amounts to stimulate immunity in the body. Vaccine administration is therefore an important

Table 3. Reduction in the vaccine virus titer[1]

Time	Titer, $\log_{10}$ EID_{50}/vial			
	Distilled water	Chlorinated water	Chlorinated water + Vac-Pac Plus[2]	Chlorinated water + skim milk
T_0 to T_1	0	0.48	−0.16	0
T_0 to T_2	0	0.64	0.32	0.32

[1]T_0 = inoculation at time 0; T_1 = 1 h postinoculation; T_2 = 2 h postincubation; EID_{50} = 50% embryo infectious dose.
[2]Animal Science Products Inc. (Nacogdoches, TX).

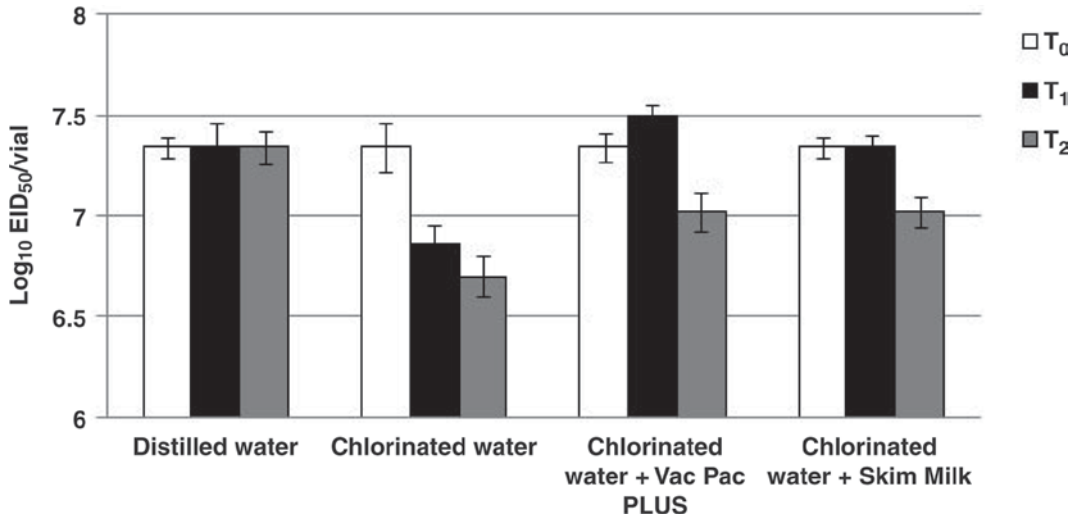

Figure 1. Titers of Bioral H120 (Merial, Lyon, France) in $\log_{10}$ 50% embryo infectious dose per vial. The virus remained viable in distilled water. High titers were observed for Vac-Pac Plus (Animal Science Products Inc., Nacogdoches, TX) and skim milk after 1 h, and although the titers decreased after 2 h, the levels were much higher than in the absence of any stabilizer (i.e., chlorinated water alone).

step in the immunization protocol because there are chances of losing effective titers. The chlorinated water with vaccine also demonstrated that the virus could not withstand the detrimental effect of 4 ppm of chlorine in the water, as reported previously [7]. It has been advised that vaccines should be diluted in distilled or nonchlorinated water [16, 9]. This may prove impractical on poultry farms because chlorine is used as the major sanitizer in tap water systems. Therefore, the need arises to stabilize vaccines in chlorinated water. Skim milk has been found to be essential in stabilizing vaccines [8, 10], whereas 1× PBS has been shown to protect viable vaccine in water [16]. Vac-Pac Plus was able to preserve a viable vaccine for 1 h, resulting in a virus titer at T_1 that was the highest among the 4 diluent systems. Compared with skim milk, Vac-Pac Plus had better preservation qualities, and when the titer decreased, the value was equal to that of the skim milk diluent system.

The decrease in titers (0.32 $\log_{10}$ EID_{50}) at T_2 for both of these systems was considered significant because it reflects a reduction in the actual number of original virus particles by 2-fold, or one-half [10]. For the purposes of our evaluation, high concentrations of chlorine were used (4 ppm). This condition compares with that of other products tested, namely, Spray-Vac (4 ppm) [7] and Vacci-Guard (3 ppm) [11].

Generally, the chlorine in water may not be as high, and the vaccine could possibly be protected by Vac-Pac Plus for up to 2 h or more. However, the manufacturers of Vacci-Guard recommend that the vaccination time should not extend for more than 2 h because of the possibility of viable vaccine loss. Because the virus titer when using Vac-Pac Plus was comparable with that of skim milk, which is known to preserve adequate amounts of vaccine [8], we conclude that Vac-Pac Plus successfully preserved the vaccine.

CONCLUSIONS AND APPLICATIONS

1. Vac-Pac Plus is able to preserve viable vaccine in chlorinated water.
2. The product preserved Bioral H120, and the titers were comparable with those of skim milk.
3. Vac-Pac Plus can therefore be used in vaccines administered through drinking water.

REFERENCES AND NOTES

1. Abdel-Monei, A. S., M. F. El-Kady, B. S. Ladman, and J. Gelb Jr. 2006. S1 gene sequence analysis of a nephropathogenic strain of avian infectious bronchitis virus in Egypt. Virol. J. 3:78.

2. Cavanagh, D., and S. A. Naqi. 2003. Infectious bronchitis. Pages 101–119 in Diseases of Poultry. 11th ed. Y. M. Saif, ed. Iowa State Univer Press, Ames.

3. Zhang, D. Y., J. Y. Zhau, W. Q. Chen, and J. G. Chen. 2009. Co-expression of IBV structural proteins and chicken interleukin-2 for DNA immunization. Vet. Med. (Praha) 54:169–171.

4. Roussan, D. A., G. Y. Khawaldeh, and I. A. Shaheen. 2009. Infectious bronchitis virus in Jordanian chickens: Seroprevalence and detection. Can. Vet. J. 50:77–80.

5. Wang, L., D. Junker, and E. W. Gollisson. 1993. Evidence of natural recombination within the S1 gene of infectious bronchitis virus. Virology 192:710–716.

6. Ratanasethakul, C., and R. B. Cumming. 1983. Immune response of chickens to various routes of administration of Australian infectious bronchitis vaccine. Aust. Vet. J. 60:214–216.

7. Leigh, S. A., S. L. Branton, and S. D. Collier. 2008. Stabilization of live *Mycoplasma gallsectum* vaccines during vaccination with second-generation Spray-Vac vaccine stabilizer. J. Appl. Poult. Res. 17:278–282.

8. Woodward, H. L., and D. C. Tudor. 1975. A skim milk stabilized vaccine for Newcastle disease (B1-type La-Sota): Its effectiveness under modern commercial cage layer methods of delivery. Poult. Sci. 54:866–871.

9. Schering-Plough Animal Health, Omaha, NE.

10. Bermudez, A. J., and B. S. Brown. 2003. Disease prevention and diagnosis. Pages 17–83 in Diseases of Poultry. 11th ed. Y. M. Saif, ed. Iowa State University Press, Ames.

11. Merrick's Inc., Middleton, WI.

12. Animal Science Products Inc., Nacogdoches, TX.

13. Merial, Lyon, France.

14. Lohmann Animal Health, Cuxhaven, Germany.

15. Payment, P., and M. Trudel. 1993. Methods and Techniques in Virology. Marcel Dekker, New York, NY.

16. Leigh, S. A., J. D. Evans, S. L. Branton, and S. D. Collier. 2008. The effects of increasing sodium chloride concentration on *Mycoplasma gallisepticum* vaccine survival in solution. Avian Dis. 52:136–138.