

Hatchery Spray Cabinet Administration Does Not Damage Avian Coronavirus Infectious Bronchitis Virus Vaccine Based on Analysis by Electron Microscopy and Virus Titration

Author(s): Ha-Jung Roh, Brian J. Jordan, Deborah A. Hilt, Mary B. Ard, and Mark W. Jackwood

Source: Avian Diseases, 59(1):149-152.

Published By: American Association of Avian Pathologists

DOI: <http://dx.doi.org/10.1637/10918-081114-ResNote>

URL: <http://www.bioone.org/doi/full/10.1637/10918-081114-ResNote>

BioOne (www.bioone.org) is a nonprofit, online aggregation of core research in the biological, ecological, and environmental sciences. BioOne provides a sustainable online platform for over 170 journals and books published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Web site, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/page/terms_of_use.

Usage of BioOne content is strictly limited to personal, educational, and non-commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

Research Note

Hatchery Spray Cabinet Administration Does Not Damage Avian Coronavirus Infectious Bronchitis Virus Vaccine Based on Analysis by Electron Microscopy and Virus Titration

Ha-Jung Roh,^{AC} Brian J. Jordan,^A Deborah A. Hilt,^A Mary B. Ard,^B and Mark W. Jackwood^{AD}

^ADepartment of Population Health, Poultry Diagnostic and Research Center, 953 College Station Road, University of Georgia, Athens, GA 30602

^BDepartment of Pathology, Electron Microscopy Laboratory, 501 D. W. Brooks Drive, University of Georgia, Athens, GA, 30602

Received 27 November 2013; Accepted 1 July 2014; Published ahead of print 10 September 2014

SUMMARY. Previous studies in our laboratory showed that the Arkansas–Delmarva Poultry Industry (Ark-DPI) vaccine given to 1-day-old chickens by hatchery spray cabinet replicated poorly and failed to adequately protect broilers against homologous virus challenge, whereas the same vaccine given by eye-drop did replicate and the birds were protected following homologous virus challenge. To determine if mechanical damage following spray application plays a role in failure of the Ark-DPI vaccine, we examined the morphology of three Ark-DPI vaccines from different manufacturers using an electron microscope and included a Massachusetts (Mass) vaccine as control. One of the Ark-DPI vaccines (vaccine A) and the Mass vaccine had significantly ($P < 0.005$) fewer spikes than the other two Ark-DPI vaccines. We also found that the Ark-DPI and Mass vaccines had significantly ($P < 0.005$) fewer spike proteins per virus particle when compared to their respective challenge viruses. This observation is interesting and may provide some insight into the mechanism behind infectious bronchitis virus attenuation. No obvious differences were observed in virus morphology and no consistent trend in the number of spikes per virion was found in before- and after-spray samples. We also determined the vaccine titer before and after spray in embryonated eggs and found that both Ark-DPI and Mass vaccines had a similar drop in titer, $0.40 \log_{10}$ and $0.36 \log_{10}$, respectively. Based on these data, it appears that mechanical damage to the Ark-DPI vaccine is not occurring when delivered by a hatchery spray cabinet, suggesting that some other factor is contributing to the failure of that vaccine when given by that method.

RESUMEN. La administración de la vacuna contra bronquitis infecciosa por aerosol con un gabinete en la planta de incubación no daña al coronavirus aviar determinado por microscopía electrónica y por titulación del virus.

Estudios previos han demostrado que la vacuna Arkansas tipo Industria Avícola de Delmarva (Ark-DPI) aplicada a pollos de 1 día de edad por aspersión mediante un gabinete mostró una replicación pobre y no protegió adecuadamente a los pollos contra el desafío con un virus homólogo, mientras que la misma vacuna administrada por gota ocular, mostró replicación y las aves estuvieron protegidas después de la exposición al virus homólogo. Para determinar si ocurre daño mecánico después de la aplicación por aerosol y si este daño juega un papel en la falla de la vacuna Ark-DPI, se analizó la morfología de tres vacunas Ark-DPI de tres diferentes fabricantes utilizando un microscopio electrónico y se incluyó una vacuna serotipo Massachusetts como control. Una de las vacunas Ark-DPI (vacuna A) y la vacuna contra el serotipo Massachusetts mostraron significativamente ($P < 0.005$) menos espículas que las otras dos vacunas Ark-DPI. También se encontró que las vacunas Ark-DPI y Massachusetts tuvieron significativamente ($P < 0.005$) menos espículas por partícula viral en comparación con sus respectivos virus de desafío. Esta observación es interesante y puede proporcionar alguna información sobre el mecanismo de la atenuación del virus de la bronquitis infecciosa. No se observaron diferencias evidentes en la morfología del virus y no se encontró ninguna tendencia constante en el número de espigas por virión en muestras antes y después del aerosol. También se determinó el título de la vacuna por inoculación en huevos embrionados de pollo antes y después del aerosol y se encontró que ambas vacunas Ark-DPI y la vacuna Massachusetts mostraron una disminución similar en el título, $0.40 \log_{10}$ y $0.36 \log_{10}$, respectivamente. Con base en estos datos, parece que no ocurre daño mecánico a la vacuna Ark-DPI cuando se aplica mediante un gabinete de aspersión en la planta de incubación, lo que sugiere que algún otro factor está contribuyendo con la falla de la vacuna cuando se administra por este método.

Key words: Ark-DPI, electron microscope, infectious bronchitis virus, morphology, vaccine

Abbreviations: Ark-DPI = Arkansas–Delmarva Poultry Industry; EM = electron microscopy; IBV = infectious bronchitis virus; Mass = Massachusetts

Infectious bronchitis virus (IBV) is the causative agent of infectious bronchitis, a highly contagious upper respiratory tract disease of chickens. The disease affects productivity by reducing feed conversion rate in broilers and decreasing egg production and egg quality in layers. Vaccination with live attenuated IBV vaccines is routinely used to protect broilers against pathogenic IBV strains.

Replication of live attenuated vaccine strains in the upper respiratory tract induces a local mucosal immune response that protects birds from infection (7).

In the field, vaccines for broilers are often applied in two stages: a hatchery vaccination at 1 day of age for initial priming of the immune response and a field vaccination at 14–18 days of age, which is designed to boost the local immune response and protect the birds for the length of the grow-out. Currently, Arkansas–Delmarva Poultry Industry (Ark-DPI), Massachusetts (Mass), Delaware (DE072), and Georgia (GA98) are the most frequently used vaccine strains in the United States, and adequately vaccinated

^CPresent address: Division of Viral Hepatitis/Lab Branch, Centers for Disease Control and Prevention: 1600 Clifton Road, NE, Mail Stop A-33, Room 4-123, Atlanta, GA 30333

^DCorresponding author. E-mail: mjackwoo@uga.edu

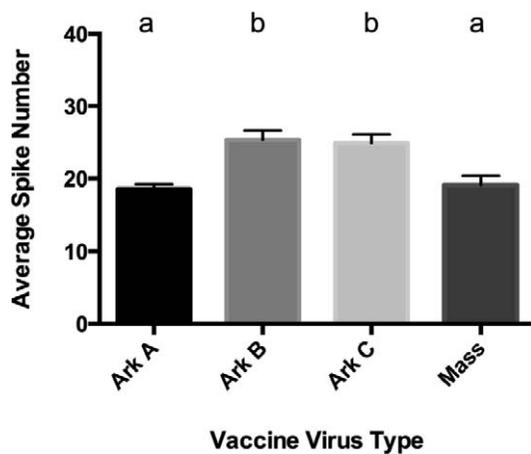


Fig. 1. Comparison of IBV Ark-DPI and Mass vaccine spike protein numbers before spray application (error bars = standard error of the mean). Different letters above the bars indicate statistical difference ($P < 0.005$).

chickens ought to be protected against the homologous pathogenic field strains. However, there have been reports indicating that the Arkansas-type virus can persist in vaccinated birds (1,5) and Ark-DPI-like viruses have been detected in the field (9). In addition, Ark-DPI-vaccinated birds brought from the field and challenged in an experimental setting with pathogenic Arkansas virus were not sufficiently protected (8). Experiments in our laboratory confirmed that the Ark-DPI vaccine failed to provide adequate protection against homologous challenge when the vaccine was applied using a hatchery spray cabinet, whereas Mass and GA98 type vaccines applied in the same manner successfully protected birds against homologous challenge (11). In that experiment, we also found that Ark-DPI vaccine applied by eye-drop replicated to relatively high levels and was efficacious, whereas the same Ark-DPI vaccines applied by hatchery spray cabinet replicated poorly in the vaccinated chickens.

The spikes on the surface of IBV have been reported to be relatively fragile and different strains may vary in their fragility (3,4). Thus, mechanical damage from spraying the Ark-DPI vaccine, which may potentially damage the viral host-cell attachment protein spike, could explain the failure of that vaccine to infect and induce

an immune response (4). To determine if Ark-DPI vaccines are damaged by sheering forces associated with spray vaccination, we used an electron microscope to examine the morphologic characteristics of that vaccine virus prior to and after spray and compared it to a Mass-type vaccine. In addition, because damage to the spikes preventing infection may not be overtly visible by electron microscopy (EM), the titer of the vaccines prior to and after spray was also examined using embryonated eggs.

MATERIALS AND METHODS

Vaccines. Commercially available monovalent live attenuated Ark-DPI vaccines from three different manufacturers (designated vaccines A, B, and C) and a Mass vaccine, as well as challenge viruses Ark/ArkDPI/81 and Mass/Mass41/41, were used in this study.

Transmission EM. All vaccine samples were examined using a transmission electron microscope to identify any virus particle morphologic differences between samples. Briefly, each sample was diluted 1:50 in phosphate-buffered saline and then centrifuged using an Airfuge (Beckman Coulter, Life Sciences, Indianapolis, IN) to concentrate the viruses. Samples were stained with 3% aqueous phosphotungstic acid, pH 7.0, for 90 sec on a formvar carbon-coated copper grid and viewed with a JEM-1210 transmission electron microscope (JEOL, Inc., Tokyo, Japan). Photomicrographs were taken of representative virus particles.

Spike protein count and statistical analysis. We counted spikes on the Ark-DPI and Mass vaccines from each manufacturer pre- and postspray and on the Ark/ArkDPI/81 and Mass/Mass41/41 challenge viruses. Prespray vaccine samples were collected immediately after diluting the vaccine to a working stock according to the manufacturer's specifications. Postspray samples of the working stock were collected in a 50-ml conical tube directly from the spray nozzle of the cabinet. Challenge virus samples were obtained from virus stocks immediately after they were thawed. For each sample, 30 virus particles in photomicrographs were assigned a number and spike proteins visible on the circumference were independently counted by three individuals without prior knowledge of their source. The number of spikes per virus particle for each sample was used in statistical analysis. Sample means were compared by one-way ANOVA and Tukey's multiple comparisons test using GraphPad statistical software (San Diego, CA).

Vaccine titration. To determine if a drop in vaccine titer occurred between the initial vaccine working solution and the vaccine after spray, the before- and after-spray samples for one Ark-DPI vaccine (vaccine C) and the Mass vaccine were titrated in embryonated eggs. Tenfold serial

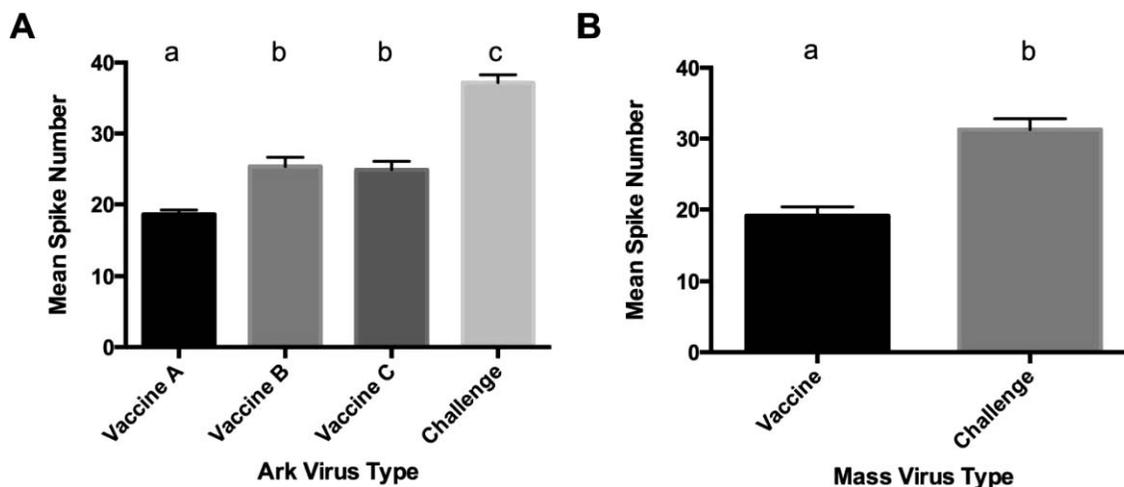


Fig. 2. Comparison of IBV Ark-DPI and Mass vaccine and challenge virus spike protein numbers before spray application (error bars = standard error of the mean). Different letters above the bars indicate statistical difference ($P < 0.005$). (A) Ark-DPI vaccine and challenge viruses. (B) Mass vaccine and challenge virus.

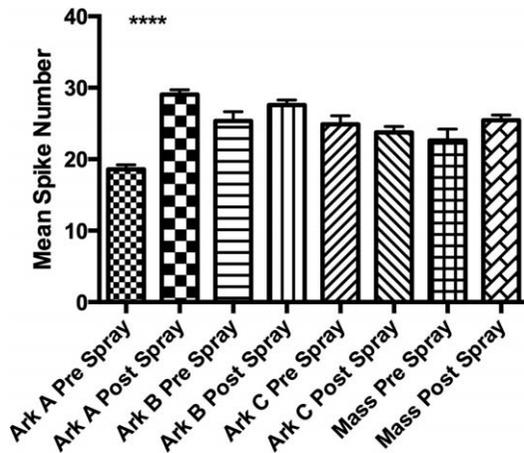


Fig. 3. Comparison of average number of spikes per virion before and after hatchery spray application (error bars = standard error of the mean). Asterisks indicate statistical difference between pre- and postspray samples for Ark-DPI vaccine A ($P < 0.005$).

dilutions of the samples were injected into 9- to 11-day of incubation embryonated specific-pathogen-free chicken eggs using standard procedures and the 50% embryo infectious dose (EID_{50}) was calculated by the method of Reed and Muench (6,10).

RESULTS AND DISCUSSION

Previously, we sprayed vaccinated broiler chicks with commercially available Ark-DPI and Mass-type vaccines and monitored replication of the vaccine in the birds by real time reverse transcriptase-PCR (11). In that study we observed inefficient replication of Ark-DPI vaccine viruses administered to 1-day-old chicks by a hatchery spray cabinet, and found that the vaccinated birds were not protected against Ark-DPI challenge (11).

To better understand IBV Ark-DPI vaccine virus failure when given by hatchery spray cabinet, we examined if sheering forces associated with spray vaccination damaged Ark-DPI vaccine virus particles. We analyzed the structure of virus particles and the average number of spikes associated with each virion by EM prior to spray and compared that to a Mass vaccine control (Fig. 1). Among the Ark-DPI-type vaccines from three different manufacturers, vaccine A had a statistically lower number of spikes compared to vaccines B and C, but the number of spikes on vaccine A was not statistically different from the Mass vaccine. We also compared the number of spikes on the vaccines with their respective challenge viruses, and found a statistically lower number of spikes on vaccine virus particles compared with the homologous challenge virus types (Fig. 2). This result suggests that fewer spike proteins may play a role in the attenuation of IBV.

Spike glycoproteins on the surface of the virus, which are involved in attachment and infection of the host cell, can be removed by high speed centrifugation ($100,000 \times g$), incubation at 37 C, or exposure to urea (2), making it plausible that they could be removed by the sheering forces associated with spray vaccination. However, we observed no morphologic differences after spray by EM, and with the exception of one vaccine, there were no differences in the number of spikes between pre- and postspray samples (Fig. 3). There was a significant difference in the number of spikes before and after spraying for Ark-DPI vaccine A, with the postspray sample having a higher number of spikes. We made every effort to accurately record the spikes on each virus particle but it seems

unlikely that more spikes would result from spraying the vaccine. It is possible that this result was due to the inherent difficulties of counting spikes in EM micrographs. No other differences in the number of spikes (Fig. 3) on virus particles were observed, indicating that the spraying process apparently did not damage the virus particles.

It is possible that aerosolization of vaccine virus using a hatchery spray cabinet could damage virus particles, rendering them unable to infect and replicate, but that damage may not be overtly visible by EM. Thus, we measured virus infectivity and replication by titration of samples in embryonated eggs collected from the vaccine working solution before and after spray application and found the working solution titers prespray to be $1 \times 10^{4.86}$ EID_{50}/ml for the Mass vaccine and $1 \times 10^{4.0}$ EID_{50}/ml for Ark-DPI vaccine. Postspray, there was a 0.36 \log_{10} decrease in the Mass vaccine titer ($1 \times 10^{4.5}$ EID_{50}/ml) and a 0.4 \log_{10} decrease in the Ark-DPI vaccine titer ($1 \times 10^{3.6}$ EID_{50}/ml). A similar decrease in titer for both vaccines indicates that the spraying process did not affect the Ark-DPI vaccine any more than the Mass vaccine, though we did observe that the initial Mass vaccine titer was almost a one full \log_{10} higher than the initial Ark-DPI vaccine titer. The difference in initial vaccine titer can be attributed to the manufacturers release titer for the vaccines since a full dose of each vaccine, as recommended by the manufacturer, was used in this study. These data indicate that both Mass-type and Ark-DPI-type vaccines are viable, as determined by infection and replication in embryonated eggs, following spray cabinet administration.

Since spikes on IBV are required for attachment and infection of the host cell, it is conceivable that fewer spikes on the virus surface could affect the overall ability of the virus to infect and thus replicate in the host. However, since vaccine viruses are adapted to grow in embryonated eggs, fewer spikes apparently does not affect the ability of those viruses to attach and enter embryonic cells, or a high rate of replication in the embryonic cells is sufficient to make up for a reduced infection rate. Fewer spikes on egg-adapted attenuated vaccine viruses compared with pathogenic viruses was an interesting finding in this study, and suggests that fewer spikes may contribute to the attenuated nature of the vaccine viruses in chicks. However, it is recognized that attenuation in IBV is likely related to many factors, including infection efficiencies and replication rates. And it is entirely possible that a fewer number of spikes on the surface of IBV vaccines has little or nothing to do with attenuation in chicks.

Based on these data, it appears that mechanical damage to the Ark-DPI vaccine is not occurring when that vaccine is given using a hatchery spray cabinet, suggesting that some other factor is contributing to the failure of that vaccine when it is sprayed. Since Ark-DPI vaccine following spray can replicate in embryonated eggs, and eye-drop administration of Ark-DPI vaccine is efficacious whereas spray application is not, it is possible that the infectious dose reaching the chicks is critical. Further studies are needed to determine if an infectious dose of Ark-DPI vaccine virus can be effectively delivered to chicks using a hatchery spray cabinet and if that correlates with replication of the vaccine and protection from challenge.

REFERENCES

- Alvarado, I. R., P. Villegas, N. Mossos, and M. W. Jackwood. Molecular characterization of avian infectious bronchitis virus strains isolated in Colombia during 2003. *Avian Dis.* 49:494-499. 2005.
- Britton, P., S. Evans, B. Dove, M. Davies, R. Casais, and D. Cavanagh. Generation of a recombinant avian coronavirus infectious

bronchitis virus using transient dominant selection. *J. Virol. Methods* 123:203–211. 2005.

3. Cavanagh, D., and P. J. Davis. Coronavirus IBV: removal of spike glycopolyptide S1 by urea abolishes infectivity and haemagglutination but not attachment to cells. *J. Gen. Virol.* 67:1443–1448. 1986.

4. Cavanagh, D., P. J. Davis, J. H. Darbyshire, and R. W. Peters. Coronavirus IBV: virus retaining spike glycopolyptide S2 but not S1 is unable to induce virus-neutralizing or haemagglutination-inhibiting antibody, or induce chicken tracheal protection. *J. Gen. Virol.* 67:1435–1442. 1986.

5. Gelb, J., Jr., and S. S. Cloud. Effect of serial embryo passage of an Arkansas-type avian infectious bronchitis virus isolate on clinical response, virus recovery, and immunity. *Avian Dis.* 27:679–687. 1983.

6. Gelb, J., Jr., and M. W. Jackwood. Infectious bronchitis. In: *A laboratory manual for the isolation, identification, and characterization of avian pathogens*, 5th ed. L. Dufour-Zavala, D. E. Swayne, J. R. Glisson, J. E. Pearson, W. M. Reed, M. W. Jackwood, and P. Woolcock, eds. American Association of Avian Pathologists, Kennett Square, PA. pp. 146–149. 2008.

7. Jackwood, M. W., and S. de Wit. Infectious bronchitis. In: *Diseases of poultry*, 13th ed. D. E. Swayne, J. R. Glisson, L. R. McDougald, L. K. Nolan, D. L. Suarez, and V. Nair, eds. John Wiley and Sons, Inc. Hoboken, NJ. pp. 139–159. 2013.

8. Jackwood, M. W., D. A. Hilt, A. W. McCall, C. N. Polizzi, E. T. McKinley, and S. M. Williams. Infectious bronchitis virus field vaccination coverage and persistence of Arkansas type viruses in commercial broilers. *Avian Dis.* 53:175–183. 2009.

9. Kingham, B. F., C. L. Keeler, Jr., W. A. Nix, B. S. Ladman, and J. Gelb Jr. Identification of avian infectious bronchitis virus by direct automated cycle sequencing of the S-1 gene. *Avian Dis.* 44:325–335. 2000.

10. Reed, L. J., and H. Muench. A simple method for estimating fifty percent endpoints. *Am. J. Hyg.* 27:493–497. 1938.

11. Roh, H. J., D. Hilt, S. M. Williams, and M. W. Jackwood. Evaluation of infectious bronchitis virus Arkansas-type vaccine failure in commercial broilers. *Avian Dis.* 57:248–259. 2013.