

# **Completed Research**

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## **Project #F023**

### **Significance of Minor Viral Subpopulations within Ark-type Infectious Bronchitis Vaccines**

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#### **"Possible Explanation for Frequent Ark-like IBV Isolations from Chickens"**

In spite of intensive vaccination programs, IBV continues to cause losses in commercial chicken flocks. Approximately 60 percent of IBV isolates from broiler respiratory disease cases in the Southeast U.S. are Ark-like. Our earlier characterization of spike (S) genes of live-attenuated Ark-type IBV vaccines indicated that the predicted amino acid sequence of the S1 portion of the S protein of one Ark-type vaccine exhibited marked heterogeneity, while another appeared much more homogeneous. Surprisingly, during a single passage of either vaccine in chickens, a single IBV S1 coding sequence, differing from the major vaccine sequence, was found between 3 and 14 days after vaccination in all samples from individual chickens examined. This sequence is similar, but not identical, to the non-attenuated ArkDPI sequence. Our results suggested that a minor component of the vaccine replicates more efficiently than the major attenuated vaccine population in the upper respiratory tract of chickens. Our project extended and examined the significance of these findings.

#### **Objectives:**

- Determine whether live-attenuated IBV vaccines in addition to those already examined contain minor subpopulations that replicate more efficiently in the upper respiratory tract of chickens.
- Determine whether the different subpopulation structures of Ark vaccines result in differences in vaccine reaction, vaccine virus persistence or immune response.
- Characterize Ark vaccine virus subpopulations persisting longer than 14 days in vaccinated chickens.
- Characterize S1 gene sequences of Ark-type field isolates obtained from respiratory disease cases submitted to Alabama State Diagnostic Laboratories and conduct phylogenetic analyses to determine their relationship to minor populations of Ark-type vaccines replicating in

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chickens.

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### **Results:**

1. All four Ark-type IBV vaccines examined contained one or more minor subpopulations that were selected for in chickens during a single passage in chickens. With vaccines A and C, the selected subpopulations were readily apparent in the S1 sequence of the vaccine present in the vaccine vial. With vaccines B and D, the selected subpopulations were very minor in the vaccine vial. More than one minor subpopulation was frequently selected from vaccine B. In contrast to Ark-type vaccines, DE072 and Mass IBV vaccines did not contain minor vaccine subpopulations that were selected for in chickens.
2. Study 1 - Groups of one day old SPF chickens were vaccinated by eye drop with IBV Ark vaccine A, B, C, or D. We demonstrated that, in general, IBV vaccines containing predominant viral subpopulations more efficiently replicating in the upper respiratory tract of chickens (vaccines A and C) induced more severe respiratory signs and tracheal lesions and produced higher virus levels than one of the vaccines (D) containing only very minor viral subpopulation selected for in chickens. Vaccine B produced unexpected results, which might be explained by the selection of different subpopulations of vaccine B in different chickens. Study 2 — Groups of 12-wk-old chickens were vaccinated by eye drop with IBV Ark vaccine A or B. Virus levels and IBV-specific IgA antibodies in tears were higher in chickens vaccinated with vaccine B than those vaccinated with vaccine A.
3. Sequences of S1 genes of IBV in trachea in Study 2 (above) showed that at 20 days post vaccination, most of the chickens vaccinated with vaccine B had the same subpopulation in trachea, suggesting that this subpopulation persists longer in trachea than the other vaccine B subpopulations.
4. Although Ark-type IBV field isolates frequently contain one or more of the changes identical to those in subpopulations selected in chickens from Ark-type vaccines, in the over 600 S1 gene sequences we have acquired from IBV-Ark vaccines replicating in individual chickens, we have not yet identified any sequence that is identical to any field isolate. All differ in at least one position.

**Impact :** We have shown that IBV-Ark vaccines, unlike other serotype IBV vaccines, contain minor subpopulations that are rapidly selected for in chickens. This might help explain the frequent isolation of Ark-type IBV from vaccinated flocks. The different relative proportions of the minor subpopulations able to efficiently replicate in chickens and thus be selected for had an effect on vaccine virus levels, vaccine reaction, and immune response. This information will be useful in future development and testing of live-attenuated vaccines as the optimal balance is sought between the ability of an attenuated IBV vaccine to adequately replicate in the respiratory tract of chickens to stimulate a protective immune response and its ability to cause unwanted damage to the respiratory epithelium. One approach suggested by our results might be to generate and evaluate defined mixtures of homogeneous, highly-attenuated IBV and specific less-attenuated IBV populations.