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Variant Infectious Bronchitis Virus Isolated From Indiana Chickens

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ABSTRACT Infectious bronchitis virus (IBV) associated with a catarrhal tracheitis, sudden decline in egg production, and reduced shell quality was isolated from an Indiana White Leghorn breeder flock. It was found to be serologically different from Massachusetts, Connecticut, Iowa 97, Iowa 609, Florida, Arkansas 99, JMK, Holte, Gray and SE 17 IBV serotypes. Two different Massachusetts vaccine strains protected chickens from respiratory signs but not against virus infection using the isolant for challenge in laboratory trials. The isolant was passed through a 0.22 μ filter. It was heat (56° C.), acid pH (3.0), ether and chloroform labile. In embryos it produced deaths or lesions of infectious bronchitis in one to five days after inoculation. It is suggested that this IBV isolant be designated Indiana-type.

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INTRODUCTION

Infectious bronchitis (IB) is a highly contagious, coronavirus-induced disease of chickens affecting the respiratory tract and reproductive performance. For many years IB vaccination has become standard practice using either a Massachusetts-type strain alone or combined with the Connecticut-type during the growing period of laying chickens. However, many variant strains have been isolated which are immunologically different from the vaccine virus used (Hofstad, 1958; Hopkins, 1969; Jungherr et al., 1956; Winterfield and Hitchner, 1962; Winterfield et al., 1964, 1971; Winterfield and Fadly, 1971). This paper describes the isolation of a variant IB virus from a flock which experienced production problems.

MATERIALS AND METHODS

Ten- to- 11-day-old susceptible chicken embryos were inoculated via the chorioallantoic (CA) cavity with suspensions of tracheal mucous or infective CA fluids in nutrient broth containing antibiotics. Titrations were performed by serially diluting (10-fold) CA fluids and inoculating five embryos per dilution. All embryos were candled daily. Deaths occurring within 48 hours after inoculation were considered non-specific except when using the isolant which caused embryo deaths as early as 24 hours. Between 2 and 7 days post-inoculation (PI), the embryos were examined for typical signs of (IBV) infection (National Academy of Science—National Research Council, 1971). CA fluids from stunted or dead embryos were examined for bacterial sterility and frozen at −65° C. After initial isolation, CA fluids were harvested 24 hours P.I., tested, and stored. EID₅₀ virus endpoints were calculated according to the method of Reed and Muench (1938). CA fluid containing the Indiana virus isolate was filtered through a 0.22 μ millipore filter. This material was then diluted 1:10 and injected into the CA cavity of 10-day-old SPF fertile embryos and observed for lesions or deaths twenty-four hours P.I.

Serums possessing high titers of virus neutralizing antibodies were obtained by infecting isolated groups of SPF chickens intratracheally with one serotype of IBV and bleeding them 4 weeks later. The following IB virus types were used: Massachusetts, Connecticut, Iowa 97, Iowa 609, JMK, Florida, Holte, Gray, SE17, and Indiana isolant. Antiserum derived from the Indiana virus was also tested against an Arkansas 99 serotype. The serums from one group were pooled, tested for steril-
ity, inactivated in a 56° C. water bath for 30 minutes, and frozen until needed.

Indiana virus and the respective serums were tested for neutralizing capabilities against homologous and heterologous immune serums and viruses. Homologous virus and immune serums of the other types were also tested for control purposes. Each trial consisted of a virus titration control and a titration of the virus with the dilutions mixed 1:1 with the respective serum. Serum virus mixtures were incubated at room temperature for 30 to 60 minutes prior to inoculation. EID\textsubscript{50} endpoints were calculated and log\textsubscript{10} neutralization indices (NIs) were determined from these endpoints seven days P.I.

In trial 1, sixty Hubbard broiler-type chickens were reared in isolation until four weeks of age and then were vaccinated with a commercial Massachusetts-type (M41) virus using a Root-Lowell sprayer at the low output. A virus titer of 4.17 log\textsubscript{10} EID\textsubscript{50} per ml. was used with the diluent being 80% distilled water and 20% glycerine. Exposure time to the spray was ten seconds. In the second trial, eighty, one-day-old Hubbard broiler-type chicks with Massachusetts-type parental immunity were vaccinated by eye-drop with 10\textsuperscript{4} EID\textsubscript{50} per chick of the Holland-72 Massachusetts virus. Nonvaccinated controls were maintained separately in adjacent, but isolated, quarters.

Low egg-passage virus types were used for challenge. Indiana and M41 IBV, were diluted with nutrient broth so as to provide each bird with 10\textsuperscript{3} EID\textsubscript{50}, given by eye-drop 4 weeks post-vaccination. Ninety-six hours post-challenge, the chickens were examined for rales and swabs of tracheas and clefts were taken for virus isolation. These swabs were placed in 3 ml. of nutrient broth containing antibiotics and frozen until isolation attempts were made.

In heat stability trials, vials of Indiana virus were immersed in a 56° C. water bath for varying fifteen minute intervals allowing for a two-minute temperature acclimation period. Titration of this fluid was then performed in embryos after cooling. The effect of acid pH on the agent was determined by addition of hydrochloric acid to infective CA fluid to attain a pH of approximately 3. After thirty minutes incubation at room temperature, the fluid was titrated. Ether susceptibility was tested by adding 0.2 ml. of pure diethyl ether to 1.0 ml. of CA fluid containing Indiana IBV. This was then mixed and placed in a refrigerator at 4° C. for 18 hours before being poured into a petri dish at room temperature to allow evaporation of the ether. The remaining fluid was then titrated. Analytical grade chloroform, 0.05 ml., was added to 1.0 ml. of CA fluid from an infective high titer pool of the isolated agent. This combination was shaken vigorously by hand for ten minutes; the chloroform allowed to settle and the fluid at the surface was then assayed. In each treatment above, untreated controls were maintained and titrated concurrently.

RESULTS

White Leghorn chickens from three different houses on an Indiana poultry farm were submitted to the diagnostic laboratory. A sharp decrease in egg production and increased mortality without apparent clinical signs was reported. Each house contained approximately 12,000 females and 1,000 males. All birds had been vaccinated with commercial IB vaccine virus strains (Massachusetts and Connecticut types) 3 times during the growing period. The older birds (53 weeks) in House A, showed a more drastic decline in production, 20% in two days, than either House B (40 weeks old), or House C (42 weeks old) which dropped five to ten percent in production in a week. Egg shell quality problems occurred two weeks after the initial production drop. The birds did not achieve normal production after this period of illness. Upon necropsy, approximately 1
week after the production drop, affected birds exhibited a catarrhal tracheitis, airsacculitis, and inactive ovaries. Virus isolation attempts from these birds were unyielding since the tracheal swab material was heavily contaminated with antibiotic resistant bacteria. However, swabs were then taken from several birds showing no respiratory signs and pooled for virus recovery attempts. Upon initial passage in embryos, only one of ten embryos showed stunting and dwarfing. CA fluid harvested from this embryo was then passed a second time in embryos, after a ten-fold dilution, and within 48 hours, a substantial number of embryos died.

Table 1 shows the log_{10} neutralization indices (NI's) of various pooled antiserums representing known IBV serotypes and the Indiana virus. Indiana antiserum did not significantly neutralize any of the heterologous viruses (NI ≤ 2.00) but did have an NI = 5.38 log_{10} EID_{50} with the homologous virus. Heterologous antiserums failed to significantly neutralize the Indiana virus but had high homologous titers.

Table 2 demonstrates the ability of M41 IBV vaccination by spray at four weeks of age to protect chickens from challenge by Indiana IBV. M41 vaccination did not produce satisfactory protection as shown by the criterion of virus shed-rate. Similarly, Table 3 indicates the lack of immunity against virus shed from Holland-72 (Massachusetts-type IBV) vaccination at one day of age and subsequent challenge with Indiana IBV.

Chemical and Physical Characteristics. This isolate was found to be a filterable agent since the filtrate (0.22 μ) when inoculated into embryos caused typical changes of IBV in the P.I. period. A reduction of 5.16 log_{10} EID_{50} was seen after thirty minutes of heat treatment at 56° C. and ≤10^1 EID_{50} was obtained by exposing infective CA fluid to acid pH (3.0) for thirty minutes. The virus was ether sensitive as a reduction in titer...
TABLE 2.—Protection of 8-week-old chickens against Indiana-type IBV when vaccinated with M41 strain of IBV at four weeks

| Vaccine virus | Challenge virus | M41 | Challenge virus | M41 |
|--------------|----------------|-----|----------------|-----|
| M41          | Indiana        | 0/10| 0/10           |     |
| Non-vaccinated | Indiana    | 10/10| 8/10           | 10/10| 3/10 |

1Number of chickens with rales or positive for virus/number of chickens examined or tested.

TABLE 3.—Protection from vaccination of day-old chicks by eye-drop with H72 strain of infectious bronchitis virus against Indiana-type IBV challenge at 4 weeks of age

| Vaccine | Challenge virus | M41 | Challenge virus | M41 |
|--------|----------------|-----|----------------|-----|
| H72    | Indiana        | 0/10| 0/10           |     |
| Non-vaccinated | Indiana    | 10/10| 10/10          | 10/10| 2/10 |

1Number of chickens with rales on positive for virus shed/number of chickens examined or tested.

The results reveal the isolation of a new serotype of IBV which has not been described previously. This isolate was associated with production problems even though an apparently satisfactory IB vaccination program was used. The immunity from Massachusetts-type IBV vaccination, however, apparently buffered the severity of adverse effects of infection as shown by the immunization trials. Whereas a reduction in respiratory rales was evident from cross-immunization and challenge, virus infection and shed was not significantly prevented. The latter criterion has been shown previously to be a sensitive criterion in evaluating the immune response from vaccination (Winterfield et al., 1971).

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