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## Protection of Chickens After Live and Inactivated Virus Vaccination Against Challenge with Nephropathogenic Infectious Bronchitis Virus PA/Wolgemuth/98

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**SUMMARY.** Protection provided by live and inactivated virus vaccination against challenge with the virulent nephropathogenic infectious bronchitis virus (NIBV) strain PA/Wolgemuth/98 was assessed. Vaccinations with combinations of live attenuated strains Massachusetts (Mass) + Connecticut (Conn) or Mass + Arkansas (Ark) were given by eyedrop to 2-wk-old specific-pathogen-free leghorn chickens. After live infectious bronchitis virus (IBV) vaccination, some chickens at 6 wk of age received an injection of either an oil emulsion vaccine containing inactivated IBV strains Mass + Ark or an autogenous vaccine prepared from NIBV PA/Wolgemuth/98. Challenge with PA/Wolgemuth/98 was given via eyedrop at 10 wk of age.

Serum IBV enzyme-linked immunosorbent assay antibody geometric mean titers (GMTs) after vaccination with the combinations of live attenuated strains were low, ranging from 184 to 1354, prior to NIBV challenge at 10 wk of age. Both inactivated vaccines induced an anamnestic response of similar magnitudes with serum GMTs of 6232–12,241.

Assessment of protection following NIBV challenge was based on several criteria: virus reisolation from trachea and kidney and renal microscopic pathology and IBV-specific antigen immunohistochemistry (IHC). Live attenuated virus vaccination alone with combinations of strains Mass + Conn or Mass + Ark did not protect the respiratory tract and kidney of chickens after PA/Wolgemuth/98 challenge. Chickens given a live combination vaccination of Mass + Conn and boosted with an inactivated Mass + Ark vaccine were also susceptible to NIBV challenge on the basis of virus isolation from trachea and kidney but showed protection on the basis of renal microscopic pathology and IHC. Live IBV-primed chickens vaccinated with an autogenous inactivated PA/Wolgemuth/98 vaccine had the highest protection against homologous virulent NIBV challenge on the basis of virus isolation.

**RESUMEN.** Niveles de protección conferidos por vacunas a virus vivo e inactivado contra el desafío con la cepa nefropatogénica del virus de la bronquitis infecciosa aviar PA/Wolgemuth/98.

Se estudiaron los niveles de protección conferidos por vacunas a virus vivo e inactivado frente al desafío con la cepa nefropatogénica del virus de la bronquitis infecciosa aviar PA/Wolgemuth/98. La vacunación con vacunas combinadas con virus de las cepas Massachusetts (Mass) y Connecticut (Conn), o Mass y Arkansas (Ark), fueron aplicadas por la vía ocular a aves del tipo Leghorn, libre de patógenos específicos de 2 semanas de edad. Después de la aplicación de las vacunas vivas del virus de la bronquitis infecciosa aviar, algunas aves recibieron una inyección de vacuna oleosa con virus inactivados de las cepas Mass y Ark, o una vacuna autógena preparada con la cepa nefropatogénica PA/Wolgemuth/98 del virus de la bronquitis infecciosa. Se desafiaron las aves con la cepa PA/Wolgemuth/98 por la vía ocular

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a las 10 semanas de edad. La media geométrica (GMT) de los niveles de anticuerpos séricos, medidos mediante la prueba de inmunoabsorción con enzimas asociadas (ELISA), fueron bajos después de la vacunación con las cepas vivas (de 184 a 1354) y antes del desafío a las 10 semanas de edad. Las dos vacunas inactivadas indujeron una respuesta anamnésica de producción de anticuerpos similar, con niveles promedios de 6232 a 12,241. La determinación de los niveles de protección obtenidos después del desafío con la cepa PA/Wolgemuth/98 fue basada en varios criterios: el reisolamiento viral a partir de muestras de tráquea y riñón, presencia de lesiones microscópicas renales y presencia de antígenos específicos del virus de bronquitis en los tejidos examinados mediante la técnica de inmunohistoquímica (IHC). La sola vacunación con cepas vivas atenuadas combinadas del tipo Mass y Ark o Mass y Conn no confirió protección contra el desafío con la cepa PA/Wolgemuth/98 en el tejido respiratorio y riñones de las gallinas. Las aves vacunadas con la combinación de vacunas vivas Mass y Conn más una dosis de vacuna inactivada con las cepas Mass y Ark fueron susceptibles al desafío con base en el reisolamiento del virus a partir de la tráquea y riñón, pero mostraron protección en base a los cambios microscópicos del tejido renal y la prueba de IHC. En base al reisolamiento viral, las aves primovacunadas con las cepas vivas atenuadas combinadas y reforzadas con la vacuna autógena de la cepa PA/Wolgemuth/98 presentaron los niveles más altos de protección contra el desafío virulento homólogo.

**Key words:** chicken, coronavirus, inactivated autogenous vaccination, infectious bronchitis, IBV, kidney, nephropathogenic, Pennsylvania, PA/Wolgemuth/98

**Abbreviations:** Ark = Arkansas; Conn = Connecticut; DE = Delaware; ELISA = enzyme-linked immunosorbent assay; GMT = geometric mean titer; IB = infectious bronchitis; IBV = infectious bronchitis virus; IHC = immunohistochemistry; Mass = Massachusetts; NIB = nephropathogenic infectious bronchitis; NIBV = nephropathogenic infectious bronchitis virus; PA = Pennsylvania; SPF = specific-pathogen free; TPB = tryptose phosphate broth

Nephropathogenic infectious bronchitis (NIB) occurred in Pennsylvania from 1997 to 2000 (14). Twenty-eight cases were reported in commercial broiler- and layer-type chicken flocks, with mortality as high as 20%. Gross and microscopic lesions of the kidney were consistent with NIB. Isolation of nephropathogenic infectious bronchitis virus (NIBV) was successful from 21 cases, with the remaining cases confirmed by infectious bronchitis virus (IBV)-specific immunohistochemistry (IHC) staining. The NIBV isolates were characterized by polymerase chain reaction product cycle sequencing of the S1 gene (7, 8). The S1 sequence analysis indicated the isolates from the NIB outbreak were two unique genotypes, Pennsylvania (PA)/Wolgemuth/98 and PA/171/99. Furthermore, both NIBV genotypes were unrelated by S1 sequencing to previously recognized endemic strains Massachusetts (Mass), Connecticut (Conn), JMK, Arkansas (Ark), and Delaware (DE)/072/92 in Pennsylvania as well as to each other. Genotype PA/Wolgemuth/98 was isolated almost exclusively during the first 14 mo of the outbreak, whereas PA/171/99 was recovered during the final 18 mo (14).

In Pennsylvania, NIB occurred in commercial broiler, pullet, and layer flocks in spite of

IBV vaccination. Initial laboratory challenge studies demonstrated that immunization of broilers with combinations of live Mass + Ark strains afforded 60% protection of the trachea against challenge with PA/Wolgemuth/98, whereas both Mass + Conn and Mass + DE/072/92 live virus combinations provided only 9% protection (3,9). The purpose of this research was twofold: to re-evaluate the immunity provided by live virus vaccination and to determine the potential of inactivated IBV vaccination to enhance protection against NIBV PA/Wolgemuth/98.

## MATERIALS AND METHODS

**Chicken embryos and chickens.** Specific-pathogen-free (SPF) white leghorn embryonated chicken eggs and chickens were purchased from SPAFAS, Inc., Norwich, CT. Chickens for all experiments were housed in glove port isolation units in the Allen Laboratory, University of Delaware, Newark, and provided feed and water *ad libitum*.

**Virus and vaccines.** NIBV strain PA/Wolgemuth/98 was recovered from the kidneys of 39-day-old commercial broiler chickens in Lancaster County, Pennsylvania, in 1998 (14). Total mortality in the flock was about 20%. A seed stock of PA/Wolgemuth/98 was prepared from the second chorioallan-

toic sac passage in SPF embryonated eggs and was titrated as described (2). Reference strains Mass 41, Conn, and Ark DPI are maintained as low-embryo-passage challenge viruses at the University of Delaware.

Attenuated live IBV vaccine strains Mass BB60, Conn L2, and Ark DPI were supplied as single-entity allantoic fluid stocks by Schering-Plough Animal Health, Millsboro, DE. The stocks were titrated in SPF embryonated eggs.

Two commercial inactivated IBV water in oil emulsion vaccines were supplied by Lohmann Animal Health International, Waterville, ME. One was a commercial vaccine containing the Mass + Ark strains and the other was an experimental autogenous vaccine containing the PA/Wolgemuth/98 strain.

**Virus reisolation from tracheal swabbings and kidney.** Tracheal swabbings were placed in 3 ml tryptose phosphate broth (TPB) with antibiotics (10,000 IU penicillin G and 10,000 µg streptomycin/ml) and stored at -70 C until used for virus isolation.

Kidney homogenates were made with tissue collected from the cranial division. Approximately 1 g of tissue was placed in 3 ml TPB with antibiotics. Homogenates were frozen and thawed three times and clarified by centrifugation at  $2000 \times g$  for 5 min. The supernatant was collected and stored at -70 C until used for virus isolation.

Tracheal swabbings and kidney homogenates were inoculated into 10-to-11-day-old SPF embryos via the chorioallantoic sac (2). Seven days postinoculation, the embryos were evaluated for IBV lesions such as stunting, curling, and kidney urates.

**Microscopic pathology.** Kidney from the cranial division was fixed in 10% neutral buffered formalin, processed routinely, sectioned into 3-to-5-µm sections, and stained with hematoxylin and eosin. Microscopic lesions attributed to infection of the kidney with PA/Wolgemuth/98 centered on the medullary cones. Infection consistently produced minimal to severe lymphoplasmacytic infiltration of the medullary cones, often accompanied by edema, heterophilic infiltration, and tubular degeneration/necrosis. Heterophilic tubular casts often accompanied the tubular degenerative changes present in the medullary cones. Lymphoplasmacytic infiltration of the intrarenal branches of the ureter accompanied by tubular degeneration was sometimes noted. Less prominent focal interstitial lesions in the medulla and cortex were also observed. These lymphocytic lesions tended to be most severe and numerous in the lower medulla compared with the outer cortex.

**IHC.** IHC was performed on renal tissues from chickens. Formalin-fixed, paraffin-embedded, unstained 3-to-5-µm sections were used for IHC staining as described (14).

**Efficacy of live and inactivated IBV vaccination vs. NIBV challenge.** One hundred twenty eight 2-wk-old SPF leghorn chickens were assigned to 10 treatment groups of 12 or 13 birds each (Table 1). Groups 1, 3, 5, 6, and 7 were vaccinated ocularly with a combination of commercial live vaccine strains Mass + Conn, and groups 2, 4, 8, 9, and 10 received vaccine strains Mass + Ark DPI. Vaccinated chickens received approximately  $10^4$  mean embryo infective dose per bird of each strain.

Four weeks post live IBV vaccination, treatment groups 1 and 2 received the commercial Mass + Ark inactivated vaccine and groups 3 and 4 were given the experimental autogenous PA/Wolgemuth/98 inactivated vaccine. Each chicken was injected subcutaneously in the back of the neck with 0.5 ml of the appropriate inactivated vaccine. Twenty-four additional IBV-susceptible 6-wk-old chickens were obtained from SPAFAS for use as challenge controls for treatment groups 11–14. The challenge control chickens, six per group, were banded and placed in the appropriate isolators. Five chicks in each of groups 1, 3, 5, 6, and 7, given the Mass + Conn live IBV vaccination, were bled and challenged intranasally and intratracheally with strain Mass 41, Conn, Ark DPI, or PA/Wolgemuth/98 of IBV. Five chicks in each of groups 2, 4, 8, 9, and 10, given Mass + Ark live virus vaccination, were bled and challenged. Serum samples were evaluated by enzyme-linked immunosorbent assay (ELISA) (Idexx Laboratories, Inc., Westbrook, ME) for IBV antibodies to evaluate the ability of the inactivated vaccinations to induce an anamnestic response after the live IBV priming vaccination.

On day 5 after challenge, tracheal swabs were taken from all chickens for virus reisolation attempts, and the birds were returned to their isolators. Kidneys for virus isolation and renal microscopic pathology and IHC were obtained on day 9 or 10 after challenge. Given the large numbers of samples to be collected, kidneys were obtained from the Mass + Conn live IBV-vaccinated groups 1, 3, 5, 6, and 7 on day 9 after challenge, whereas the Mass + Ark-vaccinated groups 2, 4, 8, 9, and 10 were sampled on day 10 after challenge. Three of six nonvaccinated challenge control birds of groups given Mass 41, Conn, Ark DPI, and PA/Wolgemuth/98 (groups 11–14) were sampled for kidney on day 9, and the remaining three control birds from the same groups were sampled on day 10. Results of a previous pathogenesis experiment indicated susceptible chickens were uniformly positive for renal microscopic lesions and virus isolation on days 6–14 after PA/Wolgemuth/98 strain challenge (3,9).

**Statistics.** Statistical analysis was performed on prechallenge IBV serum antibody titers by ANOVA. After PA/Wolgemuth/98 challenge, the incidences of IBV reisolation from trachea and kidney, renal mi-

Table 1. Protection of chickens after vaccination with live IBV strains Mass + Conn or Mass + Ark and an oil emulsion booster of inactivated Mass + Ark strains or the PA/Wolgemuth/98 strain *vs.* PA/Wolgemuth/98 challenge.

Treatment group	Vaccine treatment (live/inactivated) <sup>A</sup>	Challenge IBV <sup>B</sup>	IBV serum antibody (ELISA) <sup>C</sup>	Assessment of protection <sup>CD</sup>			
				Trachea virus isolation	Kidney virus isolation	Kidney microscopic pathology	Kidney IHC
1	M+C/M+A <sup>A</sup>	PA/Wolg/98	12,241 <sup>a</sup>	13/13 (100) <sup>a</sup>	8/13 (62) <sup>a</sup>	4/13 (31) <sup>a</sup>	3/13 (23) <sup>a</sup>
2	M+A/M+A	PA/Wolg/98	8300 <sup>a</sup>	11/13 (85) <sup>a</sup>	10/13 (77) <sup>a</sup>	10/13 (77) <sup>b</sup>	6/12 (50) <sup>ab</sup>
3	M+C/PA/Wolg/98	PA/Wolg/98	6232 <sup>b</sup>	5/13 (38) <sup>b</sup>	3/13 (23) <sup>b</sup>	6/13 (46) <sup>ab</sup>	5/13 (38) <sup>ab</sup>
4	M+A/PA/Wolg/98	PA/Wolg/98	7819 <sup>ab</sup>	4/13 (31) <sup>b</sup>	1/13 (8) <sup>b</sup>	8/13 (61) <sup>ab</sup>	6/13 (46) <sup>ab</sup>
5	M+C live only	PA/Wolg/98	243 <sup>c</sup>	12/12 (100) <sup>a</sup>	8/11 (73) <sup>a</sup>	10/12 (83) <sup>b</sup>	7/12 (58) <sup>ab</sup>
6	M+C live only	Mass 41	184	2/13 (15)	0/13 (0)	0/13 (0)	2/13 (15)
7	M+C live only	Connecticut	222	0/13 (0)	0/13 (0)	0/13 (0)	0/13 (0)
8	M+A live only	Mass 41	895	1/13 (8)	0/13 (0)	0/13 (0)	0/13 (0)
9	M+A live only	Arkansas DPI	1354	0/13 (0)	0/13 (0)	0/13 (0)	0/13 (0)
10	M+A live only	PA/Wolg/98	891 <sup>d</sup>	10/12 (83) <sup>a</sup>	9/12 (75) <sup>a</sup>	9/12 (75) <sup>b</sup>	8/12 (67) <sup>b</sup>
11	None	Mass 41	Not tested	6/6 (100)	0/6 (0)	0/6 (0)	0/6 (0)
12	None	Connecticut	Not tested	6/6 (100)	2/6 (33)	2/6 (33)	2/6 (33)
13	None	Arkansas DPI	Not tested	5/5 (100)	2/6 (33)	0/6 (0)	0/6 (0)
14	None	PA/Wolg/98	Not tested	6/6 (100) <sup>a</sup>	6/6 (100) <sup>a</sup>	5/6 (83) <sup>b</sup>	5/6 (83) <sup>b</sup>

<sup>A</sup>M+C = Mass + Conn; M+A = Mass + Ark. Two-week-old SPF white leghorns vaccinated ocularly with live vaccine strains M+C or M+A. Inactivated oil emulsion vaccines containing M+A or PA/Wolgemuth/98 given by subcutaneous injection at 6-wk-old.

<sup>B</sup>PA/Wolgemuth/98 challenge given ocularly at 10 wk of age. Serum geometric mean antibody titers were compared by ANOVA. The incidence of trachea and kidney virus isolation, kidney microscopic pathology (interstitial nephritis), and renal IBV-specific IHC staining of chickens challenged with PA/Wolgemuth/98 (groups 1–5, 10, and 14) was analyzed by chi-square.

<sup>C</sup>Different lowercase superscripts denote significant differences ( $P < 0.05$ ).

<sup>D</sup>No. positive/total (percentage positive in parentheses).

croscopic pathology, and kidney IHC in chickens in groups 1, 2, 3, 4, 5, 10, and 14 were analyzed by chi-square.

## RESULTS

Vaccination of chickens with combinations of live IBV Mass + Conn or Mass + Ark offered little or no protection of the trachea and kidney *vs.* NIBV challenge with the PA/Wolgemuth/98 strain (Table 1). However, live IBV vaccination was highly protective on the basis of virus isolation from the trachea after challenge with homologous virulent virus strains Mass 41, Conn, and Ark DPI. Low serum IBV antibody titers (184–1354) were observed in live virus–vaccinated chickens. Mortality after IBV challenge was not observed in any of the treatment groups.

Booster vaccination with the oil emulsion vaccines containing inactivated Mass + Ark strains and inactivated PA/Wolgemuth/98 induced anamnestic IBV serum antibody responses ranging from 6232 to 12,241 as measured by ELISA. On the basis of virus isolation, inactivated vaccination with PA/Wolgemuth/98 provided statistically greater protection than the heterologous Mass + Ark inactivated vaccine *vs.* virulent PA/Wolgemuth/98 challenge. Priming immunization for inactivated PA/Wolgemuth/98 vaccination was successful with either of the combinations of live IBV strains, Mass + Ark or Mass + Conn. Chickens vaccinated with inactivated PA/Wolgemuth/98 had significantly fewer isolations of the challenge NIBV recovered from the trachea and kidney compared with nonvaccinated challenge control chickens. Chickens given live Mass + Conn followed by inactivated Mass + Ark vaccination were susceptible to NIBV challenge on the basis of virus isolation from trachea and kidney but showed numerically greater protection than inactivated PA/Wolgemuth/98-vaccinated birds on the basis of renal microscopic pathology and IHC. Fewer chickens ( $P < 0.05$ ) vaccinated with live Mass + Conn and inactivated Mass + Ark had microscopic interstitial nephritis and were positive for IHC staining after NIBV challenge compared with nonvaccinated challenge control chickens.

## DISCUSSION

Vaccination with combinations of live IBV attenuated strains Mass + Conn or Mass + Ark

did not provide protection against challenge with the virulent NIBV strain PA/Wolgemuth/98 by several criteria: virus reisolation from the trachea and kidney and renal microscopic lesions, and IBV-specific IHC staining. In a previous trial performed in broiler chickens (3,9), Mass + Ark vaccination afforded greater protection of the trachea (60%) and the kidney (38%) on the basis of virus isolation. Mass + Conn vaccination resulted in poor protection of the trachea (9%) but induced 46% protection of the kidney upon PA/Wolgemuth/98 challenge. Broilers were challenged 4 wk after vaccination. In contrast, in the current study, the SPF leghorns were challenged 8 wk after vaccination. Cross-protection to NIBV PA/Wolgemuth/98 challenge after Mass + Ark vaccination may have waned even though the SPF leghorns were highly immune to the homologous Mass 41 and Ark DPI challenge viruses. Live virus immunization with Mass + Ark was reported to produce cross-protection against selected variants (4). However, PA/Wolgemuth/98 is a different serotype (3,9,14), and, as with many IBV serotypes, little or no cross-protection after vaccination may be elicited upon challenge with a heterologous serotype.

As is commonly the case with strains demonstrating a respiratory tropism, the best protection *vs.* virulent NIBV challenge is achieved with a live strain of the homologous serotype (6,10,11). However, the option of using a PA/Wolgemuth/98-type live vaccine to control the disease in commercial flocks is not feasible at the present time because a safe attenuated virus is not available. Although vaccination with live Mass + Conn and Mass + Ark strains did not reduce PA/Wolgemuth/98 virus reisolations from the kidney or the incidence of microscopic renal lesions, attenuated vaccines may still offer partial protection as measured by criteria other than those used in this study. Immunization with heterologous vaccine strains H120, H52, and D274, given singly, decreased the number of days NIBV strain B1648 replicated in the trachea and reduced mortality after challenge (10). These findings suggest that live IBV vaccination may offer partial protection even against virulent heterologous NIBV serotypes. In broilers and young pullets, live IBV vaccination is the primary practical approach to controlling infectious bronchitis (IB) in the field.

Inactivated vaccination of live IBV-primed chickens is an option for controlling NIBV PA/Wolgemuth/98 in layers and breeders by offering systemic immunity to prevent or reduce renal disease. Other studies have also demonstrated renal protection (5) afforded by inactivated IBV vaccination as well as improved egg production and shell quality (1). Moreover, on the basis of virus reisolation results after NIBV challenge, chickens receiving autogenous strain inactivated vaccination were better protected compared with those given an inactivated vaccination containing heterologous IBV strains. Chickens injected with inactivated PA/Wolgemuth/98 yielded statistically fewer ( $P < 0.05$ ) virus isolations from trachea and kidney than chickens receiving the vaccination with the inactivated Mass + Ark combination. However, chickens given inactivated Mass + Ark after live Mass + Conn priming vaccinations demonstrated significantly increased NIBV protection compared with other inactivated IBV vaccination treatments as assessed by microscopic renal pathology and IBV-specific IHC staining. The reasons for this finding are not apparent.

As seen in this study and others (6,12), inactivated IBV vaccination stimulates increased local respiratory as well as systemic immunity upon challenge with a homologous strain. NIBV reisolation from the tracheas of chickens given inactivated PA/Wolgemuth/98 was reduced ( $P < 0.05$ ) compared with nonvaccinated challenge control chickens. Because vaccination with live Mass + Conn or Mass + Ark alone did not protect the trachea *vs.* virulent PA/Wolgemuth/98 challenge, the reduction in respiratory virus isolations is due to mucosal immunity produced by the combination of the live IBV priming and inactivated PA/Wolgemuth/98 vaccinations. Furthermore, the inability of chickens receiving live IBV priming in conjunction with inactivated Mass + Ark vaccination to exhibit respiratory immunity demonstrates the importance of selecting a strain with a high degree of antigen similarity to the challenge virus for inclusion in the inactivated vaccine. Respiratory immunity is critical in controlling IB by reducing the potential spread of the virus to noninfected flocks.

The PA/Wolgemuth/98 strain was demonstrated to be nephropathogenic in nonvaccinated challenge control chickens as evidenced by its consistently high rate of isolation from the

kidney (6/6), incidence of interstitial nephritis (5/6), and IBV-specific antigen staining of the kidney (5/6). Nonnephropathogenic challenge strains Mass 41, Conn, and Ark DPI exhibited little or no renal tropism. Of chickens challenged with Mass 41, virus was not reisolated from the kidney. Furthermore, renal lesions and IBV antigen staining were not evident. Few chickens challenged with Conn (2/6) and Ark DPI (2/6) had virus reisolated from the kidney. However, the two birds that yielded Conn virus upon reisolation displayed interstitial nephritis and IBV antigen staining. Although the Conn strain is considered to have primarily a respiratory tropism, its potential to cause renal disease has been reported (13).

Virus isolation, microscopic pathology, and IHC were used to evaluate protection of the kidney after NIBV challenge. Chickens vaccinated with live IBV strains were highly susceptible to PA/Wolgemuth/98 challenge by each of the renal protection parameters. However, for chickens that received the inactivated PA/Wolgemuth/98 vaccination (groups 3 and 4; Table 1), virus isolation attempts from kidney often proved negative whereas microscopic pathology (interstitial nephritis) and IBV-specific antigen staining were positive. We speculate that inactivation of infectious challenge virus by immune responses in the kidney may occur prior to the resolution of microscopic lesions and clearance of noninfectious viral antigens detected by IHC. Because kidney sampling in our study was limited to days 9 or 10 after NIBV challenge, additional research with an inactivated vaccination model is needed to establish the relationship of the rate of infectious virus clearance and the resolution of renal lesions and IBV antigen persistence.

NIB occurred in Pennsylvania from January 1997 to July 2000 (14). The present study evaluated the protection of live and inactivated IBV vaccination *vs.* NIBV PA/Wolgemuth/98, an isolate recovered from nine cases almost exclusively during the first 14 mo of the outbreak. However, another S1 genotype, PA/171/99, was isolated from 11 cases during the final 18 mo. The efficacy of live and inactivated IBV vaccination *vs.* PA/171/99 has not been evaluated. In the event of the emergence of a new NIBV genotype in Pennsylvania, further research will be needed to establish the cross-pro-

tection potential of various vaccine combinations.

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