Drop of Egg Production in Chickens by Experimental Infection with an Avian Metapneumovirus Strain PLE8T1 Derived from Swollen Head Syndrome and the Application to Evaluate Vaccine

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ABSTRACT. Decreases in egg production and increased incidence of abnormal eggs due to malformation of egg shells were observed in specific pathogen free (SPF) 173-day-old laying hens inoculated intravenously with an avian metapneumovirus (aMPV) strain PLE8T1. This strain was derived from an isolate from broiler birds exhibiting swollen head syndrome (SHS). Some SPF birds inoculated with the virus showed, slight diarrhea without any respiratory symptoms. Thus, the PLE8T1 strain was used as a challenge virus to evaluate efficacy of aMPV vaccines. SPF chickens which received a live attenuated aMPV vaccine (NEMOVAC; Merial) at 7 or 77 days old and an inactivated aMPV vaccine (OVO-4; Merial) at 105 days old were protected against poor egg production caused by the challenge with the PLE8T1 strain. Thus, aMPV, the PLE8T1 strain passaged 22 times after isolation, from birds exhibiting SHS, could induce a drop in egg production in laying hens accompanied by malformation of egg shells. It was suggested that this challenge system could be applied to evaluate the efficacy of aMPV vaccine.

KEY WORDS: avian metapneumovirus, egg drop, laying hens, swollen head syndrome, vaccine.

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Avian metapneumoviruses (aMPV) known as avian pneumoviruses belong to the subfamily *Pneumovirinae* within the *Paramyxoviridae*. aMPVs are currently separated into four different subgroups (A to D) [1].

The first case of aMPV infection was reported in turkeys in the late 1970s in South Africa [2]. It has been known as the causative agent of turkey rhinotracheitis (TRT) characterized by various respiratory syndromes including nasal discharge and coughing. Experimental infection with aMPV induced a drop in egg production in turkeys [5, 7].

In chickens, swollen head syndrome (SHS) was first reported in South Africa [11], and later it was shown to be associated with aMPV infection [8, 15–17]. Although SHS was primarily reported in broiler chickens, it was also present in laying hens. In laying chickens, clinical symptoms associated with aMPV infection include respiratory symptoms and a drop in egg production as reported in broiler breeding hens [13, 14].

Experimental infection of laying hens by intravenous route with a virulent aMPV strain isolated from chickens exhibiting a drop in egg production could successfully induce a drop in egg production in the challenged birds [6]. Similar experiments with other aMPV strains have not been reported so far.

The objective of the present work in this paper is to investigate the pathogenicity of a strain of aMPV which was isolated from broiler chickens affected with SHS in laying hens and to evaluate the efficacy of live attenuated and inactivated aMPV vaccines using this strain as a challenge virus.

MATERIALS AND METHODS

Virus: The challenge strain used, designated strain PLE8T1 (subgroup B), originates from strain PR86020 which was isolated from tracheal and nasal materials of 28-day-old broiler chickens exhibiting SHS [15]. Strain PLE8T1 was established by 8 passages in chicken tracheal organ cultures (TOC) and 14 passages in specific pathogen free (SPF) chickens. It was passed another six times in TOC to be used. It has been confirmed to induce nasal discharge in 5-week-old SPF chickens when inoculated with strain PLE8T1 by the nasal and ocular routes.

aMPV vaccines: A live attenuated aMPV vaccine used in this study was a commercially available vaccine, based on subgroup B strain (NEMOVAC, Merial). An inactivated aMPV vaccine based on subgroup B strain (OVO-4, Merial) which also contained Newcastle disease, infectious bronchitis and egg drop syndrome 1976 antigens was used.

Serology: Anti-aMPV antibodies in sera were detected by a commercial ELISA kit (SVANOVIR™ APV-Ab; SVANOVA Biotech AB, Uppsala, Sweden) as described previously [10]. Antibody response was expressed in terms of percent inhibition (PI). Samples with a PI greater than 40% were considered positive for antibodies to aMPV.

Virus titration: Virus was titrated by the same method as described previously [4].

Experimental design Experiment 1: attempt to induce a drop in egg production and egg quality following aMPV inoculation. Two groups of 14 White Leghorn SPF hens were used. Group 1 was inoculated intravenously (i.v.) with 10^{5.7} median ciliostatic doses (CD₅₀) of strain PLE8T1 at

Table 1. Experimental design for efficacy of aMPV vaccines against aMPV strain PLE8T1 infection in laying hens (Experiment 2)

Group	No. in group	Live priming (7 days old)	Live priming (77 days old)	Inactivated vaccine (105 days old)	Intravenous challenge (168 days old)
A	13	+	ND	+	+
AC	12	+	ND	+	- ^{c)}
B	11	ND ^{b)}	+	+	+
C	11	ND	ND	ND	+

a) Vaccinated or challenged.

b) Not done.

c) Saline was inoculated instead of challenge virus.

173 days of age. Group 2 received an equivalent volume of saline i.v. as a negative control.

Hens were observed for the presence of clinical signs and deleterious effects on egg production for a period of two weeks before inoculation through four weeks post inoculation (p.i.). As decrease in egg production and/or abnormality of egg shell quality was observed in Group 1 on 17 days p.i., two birds from both groups were selected at random and necropsied for gross pathology. A blood sample was collected from each hen on 0 and 8 days p.i. and when the experiment was terminated.

Experiment 2: Efficacy of aMPV vaccines against aMPV infection in laying hens. The experimental design is summarized in Table 1. One-hundred and twenty-six White Leghorn SPF chickens were allocated into two groups at 7 days of age. A group of 70 birds was inoculated intranasally with live attenuated aMPV vaccine at one dose ($10^{3.3}$ TCID₅₀/dose). A second group of 56 birds did not receive any treatment at this age. The birds were housed under negative pressure in isolators. Male birds were removed from the experiment at 35 days of age.

At 77 days of age, unvaccinated female birds were randomly placed into two groups, Groups B and C. Group B was vaccinated intranasally with live attenuated aMPV vaccine with one dose. At 105 days of age, all birds except for Group C were vaccinated intramuscularly in the leg with inactivated aMPV vaccine with one dose. At 168 days of age, female birds vaccinated at 7 days of age were divided into two groups, Groups A and AC. All groups except Group AC were challenged i.v. with $10^{5.7}$ CD₅₀ of aMPV strain PLE8T1 at 168 days of age. Group AC received the same volume of saline i.v. as negative control.

The hens were examined for clinical signs and egg production for two weeks before inoculation and four weeks p.i. A blood sample was collected from each hen of Groups A, AC and C at 35 days of age, from Groups B and AC at 77 days of age, and from all groups at 105, 133, 168 and 196 days of age.

Egg production and statistical analysis: The percentage egg production for each group was calculated on a weekly basis. The incidence of poor egg shell quality was calculated as the percentage of all eggs laid/group/week. The egg production data were analyzed by the analysis of variance

method. Ratio of the eggshell quality data were analyzed using a chi-squared test.

RESULTS

Experiment 1: Slight diarrhea was observed in 11 of 14 birds in strain PLE8T1-infected group (Group 1) on 7 through 13 days p.i. The most severe diarrhea was observed on 8 days p.i. No clinical abnormality other than diarrhea was observed in Group 1.

Egg production curve is shown in Fig. 1(a). Some hens in Group 1 started to lay abnormal eggs as early as 2 days p.i. The number of hens which stopped laying increased from 7 days p.i. Egg production of Group 1 had dropped to 36% by 2 weeks p.i. and then gradually recovered. One hen in Group 1 did not lay any eggs until 24 days p.i. Egg production of Group 1 was significantly lower than that of negative control group (Group 2) during 2 and 3 weeks p.i. ($p < 0.01$).

Abnormal eggs observed in Group 1 were malformation of egg shells, including soft or thin shells. These abnormal eggs were laid predominantly before the stop of egg production and in the convalescent phase. Percentage of abnormal eggs produced in Group 1 during 1, 2, 3 and 4 weeks p.i. was 17, 20, 13 and 1%, respectively, whereas that in Group 2 was 0–1% (Fig. 1b). Percentage of abnormal eggs of Group 1 was significantly higher than that of Group 2 during 1, 2 and 3 weeks p.i. ($p < 0.01$).

Eleven of 14 hens in Group 1 had decreased egg production and/or produced abnormal eggs, whereas only one of 14 hens in Group 2 produced abnormal eggs. One of two birds in Group 1 necropsied on 17 days p.i. had edematous changes in the mucous membrane of uterus and the other bird in this group had congestion of uterus. No other clinical abnormality was observed in any of the birds in Group 2.

No antibody to aMPV was detected in any pre-inoculation chicken sera or in Group 2 throughout this investigation. Chickens in Group 1 showed strong antibody response to aMPV challenge by 8 days p.i., and high PI was maintained until the end of the experiment (Fig. 2).

Experiment 2: No bird showed any abnormality after challenge with PLE8T1 strain other than poor egg production. Two of 12 hens in Group AC laid abnormal eggs. One showed excessive laying and produced soft and thin shell

Fig. 1. (a) Egg production following challenge with strain PLE8T1. Each point represents the mean of 14 birds up to 17 days p.i., and the mean of 12 birds (excluding 2 birds necropsied) are shown on 18 days p.i. and thereafter. Egg production (%) = $\{(\text{Total number of eggs produced during one week of observation}/(\text{number of days observed} \times \text{number of birds})) \times 100\}$. (b) Abnormal egg production following challenge with strain PLE8T1. Abnormal egg production (%) = $(\text{Total number of abnormal eggs produced during one week of observation}/\text{Total number of eggs produced during one week of observation}) \times 100$. Birds were challenged with the virus at 173 days of age (arrow). ** Statistical differences ($p < 0.01$).

eggs. The other laid small eggs. The percentage egg production of Group AC was maintained at more than 85% until the experiment was finished. Seven of 11 hens in Group C either stopped production or laid abnormal eggs characterized by malformation of egg shells. Egg production dropped to the lowest value (51%) at 2 weeks p.i. No abnormality was observed in Group B which maintained high percentage of egg production (approximately 90%). Two of 13 birds in Group A laid abnormal eggs and the production decreased to 77% at 2 weeks p.i. but showed gradual recovery thereafter. Percentage egg production of Groups AC and B were significantly higher than that of Group C during 2 weeks p.i. ($p < 0.05$, and $p < 0.01$, respectively) (Fig. 3).

Mean PI of Groups A and AC at 4 weeks after vaccination with the live attenuated vaccine were 51.3 and 56.0, respectively. Group B remained seronegative for aMPV at the same time. Mean PI of Groups A and AC prior to vaccination with the inactivated vaccine were approximately 73,

Fig. 2. Antibody response against aMPV post intravenous inoculation with strain PLE8T1 (Experiment 1). Antibody response was expressed in terms of percent inhibition (PI) by the commercial blocking ELISA kit. Sample with a PI greater than 40% were considered positive for antibodies to aMPV.

Fig. 3. Egg production before and after challenge with aMPV strain PLE8T1 (Experiment 2). Egg production (%) = $\{(\text{Total number of eggs produced during one week of observation}/(\text{number of days observed} \times \text{number of birds})) \times 100\}$. Since Group A encountered a problem in watering equipment, only the results up to 20 days p.i. are shown. There were significant differences between Groups AC, B and C (* $p < 0.05$ and ** $p < 0.01$, respectively). Birds were challenged with aMPV PLE8T1 at 168 days of age (arrow).

whereas the mean titer of Group B was 57.7. Pre-inoculation PI was above 90 for all groups except Group C which was seronegative. Group C also became seropositive at 4 weeks p.i. (Fig. 4).

DISCUSSION

Picault *et al.* [15] isolated the strain PR86020, which is the original strain of PLE8T1 used in the present experiment from broiler chickens exhibiting SHS. They confirmed that strain PR86020 induced respiratory symptoms in 4-week-old chickens and turkeys in a challenge experiment. However, they did not investigate whether or not this strain

Fig. 4. aMPV antibody response of each group to aMPV vaccination followed by challenge (Experiment 2). Birds were inoculated with live vaccine at 7 or 77 days of age (blank arrow), with inactivated vaccine at 105 days of age (dotted arrow) and were challenged with aMPV strain PLE8T1 at 168 days of age (solid arrow).

causes a drop in egg production in laying hens. PLE8T1 strain used in the present study was obtained through passages of PR86020 strain in TOC and chickens. We evaluated the effect of this strain on egg production in laying hens and observed that it induced a clear drop in egg production in chickens. Our observation suggested that aMPV that is known to have respiratory virulence also could have virulence to reproductive organs. A drop in egg production induced by experimental challenge of chickens with aMPV was reported [6]; however, the aMPV strain used in the challenge was the one isolated from chickens showing an egg drop [3]. Therefore, this is the first report on reproduction of an egg drop in chickens by an aMPV isolated from chickens showing only respiratory symptoms.

The strain used in the present experiment is known to induce respiratory symptoms by the intranasal inoculation, and therefore it seems to have a tropism to upper respiratory tracts like other aMPV strains [9]. Nevertheless, this strain, when inoculated intravenously, did not induce any respiratory symptoms but only the effect on egg production with gross lesions in the uterus was observed in the present experiments. It was thought that intravenous inoculation may provide a useful system in evaluating the effect on egg production as previously described [6].

An aMPV-associated drop in egg production has been reported in chickens affected with systemic symptoms of SHS in a field outbreak [13, 14]. An aMPV is known to cause SHS by complex infection with multiple respiratory pathogens, and an attempt to induce SHS by inoculation of aMPV alone has not been successful [12]. In Experiment 1, however, only transient diarrhea was observed at the peak of abnormal egg production and no respiratory symptoms was observed. In Experiment 2, no other symptoms than a drop in egg production was observed in challenged control birds. Although it was unclear why the diarrhea was not reproduced in common with both trials, it was easier to reproduce drop in egg production than diarrhea in this challenge sys-

tem. These findings suggest the possibility of aMPV causing poor egg production in hens without exhibiting any overt clinical symptoms when the level of exposure is high and that the hens exhibiting only poor egg production in the field may have involvement of aMPV infection.

In the present experiment, the efficacy of aMPV vaccines was evaluated in two vaccination programs by administration of a live attenuated vaccine at 7 or 77 days of age and an inactivated vaccine at 105 days of age. Both programs induced high serological response prior to challenge at 168 days of age and protected the birds from a drop in egg production compared to the unvaccinated challenge controls. Cook *et al.* [6] reported the necessity of live priming followed by administration of inactivated vaccine to provide complete protection of laying chickens against aMPV challenge. Our study examined the timing of priming vaccine. When the two programs were compared, the hens receiving priming at 77 days of age showed better protection than the hens receiving priming at 7 days of age. This finding may suggest that the interval between priming and administration of inactivated vaccine may affect the vaccine efficacy. It is suggested that aMPV vaccines have efficacy not only against respiratory symptoms but also against an egg drop caused by aMPV infection.

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