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Research Article
In vivo and *In vitro* Inoculations of Live Viruses Alter Parthenogenesis in Chinese Painted Quail[1]

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Abstract

Background and Objectives: Avian parthenogenesis is embryonic development that occurs without fertilization. Virgin birds exhibiting parthenogenesis have reduced reproductive performance following mating. Previously in the 1960s, *in vivo* exposure of chickens and turkeys to certain live viruses was shown to increase the incidence of parthenogenesis as well as parthenogen size. However, no modern information is available on the effect of current virus vaccine strains or their mode of action on parthenogenesis in poultry. Hence, the objectives of this study were to determine the *in vivo* and *in vitro* effects of live pigeon pox (PP) virus as well as the *in vitro* effect of live Newcastle disease (ND) virus on parthenogenesis. **Materials and Methods:** Two experiments were conducted using virgin Chinese painted quail hens. The *in vivo* effect of live PP virus following vaccination and the *in vitro* effects of live PP and ND viruses following direct administration over the germinal disc of cultured quail eggs on parthenogenesis were determined. **Results:** It appears that vaccination of virgin hens with live PP virus has the potential to increase parthenogenesis as well as parthenogen size by the direct action of the virus on the embryo. Moreover, under *in vitro* conditions, live ND virus was found to exert similar effects as live PP virus. **Conclusion:** As vaccination for pox and ND is a routine practice in the modern poultry industry, it is possible that vaccination of birds carrying the parthenogenetic trait could impact their overall fertility and hatchability.

Key words: *In vitro* culture, ND virus, parthenogenesis, pox virus, quail, vaccination

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

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INTRODUCTION

Poultry viruses, such as avian pox and Newcastle disease (ND), are known to have a negative impact on poultry reproductive performance[1-4]. For instance, avian pox viruses, DNA viruses, infect a wide range of domestic poultry causing a drop in egg production and impaired reproductive efficiency[3,4]. Likewise, ND virus, an RNA virus, produces severe lesions in the poultry reproductive tract, thus impairing egg production and altering egg characteristics[1,2].

Apart from poultry viruses, a natural phenomenon that negatively impacts poultry reproductive performance is parthenogenesis[5,6]. Parthenogenesis is the spontaneous development of an embryo from an avian egg without going through the usual process of fertilization[7,8]. The phenomenon has been observed in a variety of avian species, including chickens[9], turkeys[10] and quail[11]. It is a heritable trait[6,12] and hinders the normal process of fertilization, thus resulting in reduced fertility and hatchability in birds exhibiting parthenogenesis[6,5,13,14]. Moreover, birds carrying the trait exhibit decreased egg production[6,12] as well as altered egg characteristics[14-16]. Hence, if overlooked, parthenogenesis could result in significant economic losses to the poultry industry.

Studies conducted in the 1960s found that certain live poultry viruses, like fowl pox[17,18], ND[8], Rous sarcoma[19] and avian leukosis[20], following either natural infection or vaccination, increased the occurrence of parthenogenesis in chickens[19] and turkeys[17,18] . In fact, the DNA viruses increased the incidence of parthenogenesis as well as embryo size and number[21,18] , whereas the RNA viruses increased only the incidence[20,8]. Further, these effects on parthenogenesis were shown only by the live viruses and not by their killed counterparts[22]. However, it is unknown how these live viruses exert their effect to stimulate parthenogenesis. Within the bird's body it is possible that the viruses may either act directly on the embryo or indirectly on the physiological systems such as the immune system and/or reproductive system to enhance parthenogenesis[23].

Sarvella and Gehman[24], reported that live fowl pox virus had no stimulating effect on parthenogenetic development following *in ovo* injection into chicken eggs. However, thereafter no further studies were conducted to better understand the role of viruses on parthenogenesis. As a result, no information is available on the effect of current virus vaccine strains on parthenogenesis or their mechanism of action. Therefore, 2 experiments were conducted using virgin Chinese painted quail (*Coturnix chinensis*) hens. Chinese painted quail belong to the same family, Phasianidae, as chickens and turkeys; this along with their small size, rapid sexual maturity and short incubation length make them an excellent animal model for avian reproduction studies[25,26]. Moreover, currently, these quail are extensively used for avian parthenogenesis research[11,23]. The objective of Experiment 1 was to determine the *in vivo* effect of live pigeon pox (PP) virus on parthenogenesis following vaccination. The objective of Experiment 2 was to gain a better understanding of the mechanism of action of DNA and RNA viruses on parthenogenesis. Hence, the *in vitro* effects of live PP and ND viruses on parthenogenesis were determined following the direct administration of the live viruses over the germinal disc area. Of all the different avian pox viruses, live pigeon pox virus was chosen for the current study because it shares a common antigenic relationship with fowl pox virus. Moreover, pigeon pox is highly virulent and is routinely employed as a vaccine in both chickens and turkeys[27].

MATERIALS AND METHODS

Experiment 1: *In vivo* PP virus exposure

Housing and egg collection: Two lines of Chinese painted quail hens were used in this study: P-line, birds genetically selected for parthenogenesis for over 10 generations[12] and C-line, a random group of birds not selected for parthenogenesis. For both lines of quail, males and females were brooded together until 4 week of age and were fed a commercial starter diet. At 4 week of age females were separated from males and were placed on a commercial layer diet. Hens were fed ad libitum and were exposed to 17 h of light per day. At 5-6 week of age, a total of 19 hens (10 C-line hens and 9 P-line hens) were vaccinated using a commercially available live PP virus vaccine at the dose rate of 10^3 EID_{50}/dose (10^5 EID_{50} mL^{-1}; Hygieia Biological Laboratories, P.O. Box 8300,Woodland,CA 95776,USA) via the wing web route and were housed separately from 18 non-vaccinated hens (9 C-line hens and 9 P-line hens). Hence, the 4 treatments utilized in the study were: C-line non-vaccinated (CNV), C-line vaccinated (CV), P-line non-vaccinated (PNV) and P-line vaccinated (PV) birds. At 6 wk of age, hens were individually caged for subsequent egg collection. Daily, eggs were collected from 37 virgin hens, labeled and were weighed individually prior to incubation, then incubated for 10 days at 37.5°C and 50% relative humidity. After the incubation period, eggs were weighed again to determine egg weight loss and were broken open to determine the incidence of parthenogenesis and albumen pH as well as yolk, albumen and shell weights[11,15,28]. To determine the incidence of parthenogenesis, the germinal discs were examined using a 2 x magnifying lamp; and if detected, parthenogen size was measured across the greatest width of the germinal disc

area[11]. For parthenogen size, absolute as well as relative to egg, yolk and albumen weights were calculated. As most parthenogens appeared as unorganized, undifferentiated cells, embryos were not staged using Hamburger and Hamilton staging of normal embryos[29]. All the birds used were treated in accordance with the Guide for Care and Use of Laboratory Animals[30].

Experiment 2: *In vitro* virus exposure

Housing and egg collection: Virgin Chinese painted quail hens, intensely selected for parthenogenesis[12], were used. A total of 18 and 20 virgin hens were used for Experiments 2a and 2b, respectively. All the birds were placed in individual cages, fed a commercial layer diet ad libitum and received 17h light/day. Birds used were treated in accordance with the Guide for Care and Use of Laboratory Animals[30]. Daily, eggs were collected, labeled and stored for 0-3 days at 20°C. Prior to *in vitro* culture, eggs were broken out and albumen pH, germinal disc diameter and presence of parthenogenetic development (PD) were determined. Based on the macroscopic examination of eggs for PD, they were classified as exhibiting no initial PD or with initial PD[11]. Then, albumen surrounding the egg yolks were removed and the resulting naked egg yolks were subjected to *in vitro* culture[31,32,25].

***In vitro* culture:** The culture technique used was a modification of quail embryo culture originally described by Ono [31] and Ono *et al.*[32,25] (Fig. 1). Surrogate plastic containers were thoroughly wiped with 70% ethanol and filled with chicken thin albumen (pH 8.2-8.6) which served as the culture medium. Chicken thin albumen was used because it was shown to yield embryo viability of >90 % for *in vitro* cultured quail eggs[25]. Further, albumen pH 8.2-8.6 is the optimum pH range for early embryo development[33]. Naked quail egg yolks were transferred to the surrogate containers and virus treatments were directly administered over the germinal disc area to study the direct effect of live viruses on PD. Surrogate containers were tightly sealed with polyethylene wrap and were secured using paraffin film. The culture was then incubated, with the sealed end facing up, for 48 h under standard egg incubation conditions. After 48 h of incubation, chicken thin albumen pH[28] and germinal disc diameter were measured[11] to determine the albumen pH change and germinal disc diameter change over incubation. Also, incidence of parthenogenesis was determined (Fig. 1).

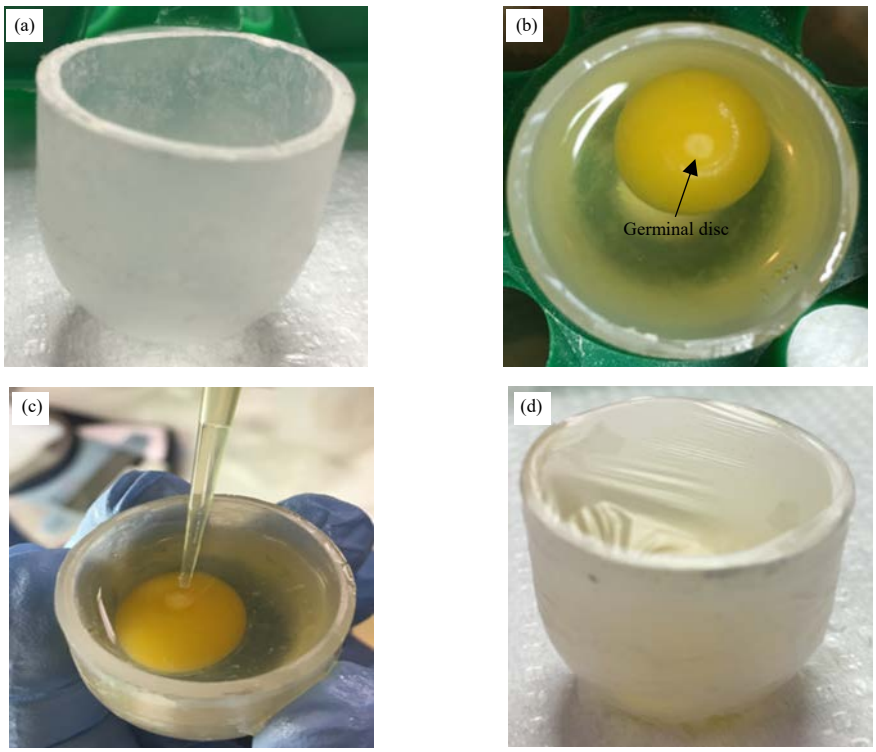

Fig. 1(a-d): *In vitro* culture and virus treatment of quail eggs. (a) Surrogate plastic container, (b) Naked quail egg yolk in the surrogate container filled with chicken thin albumen as the culture medium, (c) Direct inoculation of 50 µL live virus treatments (either live Pigeon Pox virus or live Newcastle Disease virus) over the germinal disc area of quail egg and (d) Surrogate container sealed with polyethylene wrap

Experiment 2a: *In vitro* PP virus exposure: A total of 151 eggs were used. A commercially available live PP vaccine (Hygieia Biological Laboratories, P.O. Box 8300, Woodland, CA 95776, USA; Dose: $10^6 EID_{50}$ mL^{-1}) diluted in 1mL normal saline served as the source of live PP virus. To determine the direct effect of live PP virus on embryo development, 50 µL of live virus vaccine was directly administered over the germinal disc area and was compared against no virus treatment (control). As per preliminary data, direct administration of 50 µL normal saline was shown to have no significant effect on PD, hence normal saline treatment was not incorporated in this study. The 4 treatments used in the study were: No initial PD+Control, No initial PD+PP virus, Initial PD+Control and Initial PD+PP virus.

Experiment 2b: *In vitro* ND virus exposure: A commercially available live ND vaccine (B1 type, LaSota strain; Dose: 10^6 EID_{50} mL^{-1}) was the source of live ND virus. Again, the vaccine was diluted in 1 mL normal saline and 50 µL was directly inoculated over the germinal disc area. The 4 treatments were: No initial PD+Control, No initial PD+ND virus, Initial PD+Control and Initial PD+ND virus. Control represents no virus treatment and a total of 128 eggs were used in the study.

Statistical analysis: In Experiment 1, a 2 line (C-line and P-line) x2 vaccination (Non-vaccinated and Vaccinated) factorial arrangement of treatments was utilized. Data were analyzed as a completely randomized design with hen serving as the experimental unit. In Experiment 2, a 2 (No initial PD and Initial PD) x2 (Control and virus treated) factorial arrangement of treatments was used and data were analyzed as a randomized complete block design with hen as block. When global $p<0.10$, means were separated using Fisher's protected LSD with α set at 0.05[34].

RESULTS

Experiment 1: *In vivo* PP virus exposure: Line and PP vaccination effects for absolute and relative parthenogen size, albumen pH, egg weight loss and incidence of parthenogenesis after 10 days of incubation are presented in Table 1. There were line and vaccination main effects for absolute as well as relative parthenogen size. P-line and vaccinated birds exhibited a larger absolute parthenogen size ($p = 0.02$ and 0.08, respectively) as well as a greater parthenogen size relative to egg ($p = 0.012$ and 0.06, respectively), yolk ($p = 0.009$ and 0.032, respectively) and albumen ($p = 0.023$ and 0.08, respectively) weights as compared to C-line and non-vaccinated birds, respectively. However, there was only a line main effect for albumen pH and incidence of parthenogenesis. Hens from the P-line had a lower albumen pH ($p = 0.009$), yet a higher incidence of parthenogenesis ($p = 0.02$) compared to C-line birds. Likewise, for percentage egg weight loss there was only a vaccination main effect with PP vaccinated birds having a lower percentage egg weight loss ($p = 0.001$) versus non-vaccinated

Table 1: Line and PP[1] vaccination effects for absolute and relative parthenogen size, albumen pH, egg weight loss and incidence of parthenogenesis after 10 days of incubation in Experiment 1: *In vivo* PP[1] virus exposure (mean±SEM)[2]

	Parthenogen size at 10 days						
	Absolute (mm)	Relative to egg weight (mm g^{-1})	Relative to yolk weight (mm g^{-1})	Relative to albumen weight (mm g^{-1})	Albumen pH at 10 days	Egg weight loss at 10 days (%)	Incidence of parthenogenesis at 10 days (%)
Lines[3]							
C-line	0.67±0.30[b]	0.14±0.06[b]	0.36±0.18[b]	0.27±0.13[b]	9.74±0.13[a]	7.30±0.52	13.49±5.73[b]
P-line	1.66±0.30[a]	0.34±0.06[a]	1.06±0.19[a]	0.68±0.12[a]	9.26±0.13[b]	6.67±0.53	33.35±5.89[a]
PP vaccinations							
Non-vaccinated	0.79±0.30[b]	0.16±0.06[b]	0.41±0.19[b]	0.31±0.13[b]	9.64±0.13	8.36±0.53[a]	16.89±5.89
Vaccinated	1.49±0.30[a]	0.31±0.06[a]	0.97±0.18[a]	0.62±0.12[a]	9.38±0.13	5.69±0.52[b]	29.09±5.73
Interaction[3]							
CNV	0.20±0.41	0.04±0.08	0.10±0.26	0.10±0.19	9.91±0.18	9.30±0.75	3.72±8.33
CV	1.09±0.39	0.22±0.07	0.58±0.25	0.41±0.17	9.60±0.17	5.50±0.71	22.28±7.90
PNV	1.38±0.41	0.27±0.08	0.72±0.26	0.52±0.18	9.38±0.18	7.43±0.75	30.05±8.33
PV	1.94±0.41	0.41±0.08	1.41±0.26	0.84±0.18	9.14±0.18	5.91±0.75	36.65±8.33
p-values							
Lines	0.02	0.012	0.009	0.023	0.009	0.330	0.02
PP vaccinations	0.08	0.060	0.032	0.080	0.130	0.001	0.14
Interaction	0.68	0.780	0.700	0.980	0.830	0.130	0.47

[a-b] For each main effect or interaction, means within a column with different superscripts are significantly different at $p<0.10$, [1]PP: Pigeon Pox, [2]37 virgin hens laid 553 eggs that were incubated for 10 days. SEM: Standard error of the mean. [3]C-line: Control line, P-line: Parthenogenetic line, CNV: C-line non-vaccinated, CV: C-line vaccinated, PNV: P-line non-vaccinated and PV: P-line vaccinated birds

birds. There was no line by PP vaccination interaction for any of these parameters presented in Table 1. Also, there was no PP vaccination effects for egg production (p = 0.28).

Experiment 2a: *In vitro* PP virus exposure: The effects of initial embryonic development and PP virus treatment on albumen pH change, germinal disc diameter and incidence of parthenogenesis after 48 h of incubation are presented in Table 2. For albumen pH change over 48 h of incubation, there were initial embryonic development (p = 0.03) and virus treatment (p = 0.02) main effects as well as an interaction (p = 0.09). Eggs with initial PD or PP virus treatment, had a lower albumen pH change as opposed to eggs with no initial PD or controls, respectively. Further, eggs with initial PD exposed to PP virus had the least change in albumen pH over incubation as compared to all the other treatments. For germinal disc diameter at 48h of incubation (p = 0.002) as well as diameter change over incubation (p = 0.006), eggs with PP virus treatment showed a greater diameter as well as germinal disc growth as opposed to control eggs. Moreover, eggs with initial PD after exposure to PP virus had the greatest germinal disc diameter after incubation (p = 0.05; Table 2, Fig. 2). For germinal disc diameter change over incubation, eggs with initial PD exposed to PP virus showed some growth in germinal disc size as opposed to eggs with initial PD not exposed to PP virus (p = 0.03). In addition, for incidence of parthenogenesis at 48 h of incubation, eggs with initial PD (p = 0.0007) had a greater incidence as compared to eggs with no initial development. Also, eggs with initial PD exposed to PP virus (p = 0.03) had a greater incidence as compared to untreated control eggs.

Experiment 2b: *In vitro* ND virus exposure: The effects of initial embryonic development and ND virus treatment on albumen pH, germinal disc diameter and incidence of parthenogenesis after 48 h of incubation are presented in Table 3. A ND virus treatment main effect was observed for albumen pH, pH change over incubation, germinal disc diameter, diameter change over incubation and incidence of parthenogenesis. Eggs exposed to ND virus had the lowest albumen pH at 48h of incubation (p = 0.02) as well as the least change in pH over incubation (p = 0.001) as opposed to control eggs. Also, eggs exposed to ND virus had the greatest germinal disc diameter at 48 h of incubation (p = 0.0002) and

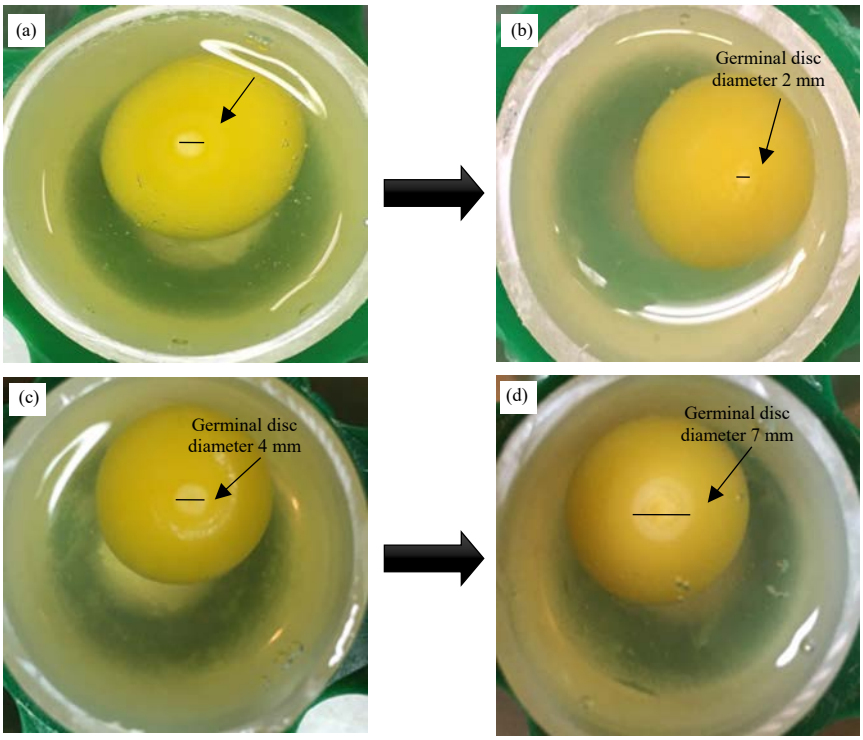

Fig. 2(a-d): *In vitro* culture of quail eggs with initial parthenogenetic development and live Pigeon Pox virus treatment. (a-b) Control egg (no virus treatment) with initial parthenogenetic development (a) following 48 h of incubation showing a decrease in germinal disc size (b). (c-d) Egg with initial parthenogenetic development (c) exposed to live Pigeon Pox virus following 48 h of incubation showing an increase in germinal disc size (d)

Table 2: Initial embryonic development and PP[1] virus treatment effects on albumen pH, germinal disc diameter and incidence of parthenogenesis observed after 48 h of incubation in Experiment 2a: *In vitro* PP[1] virus exposure (Mean±SEM)[2]

	Albumen pH at 48 h	Albumen pH change (48-0h)	Germinal disc diameter at 48 h (mm)	Germinal disc diameter change (mm, 48h-0 h)	Incidence of parthenogenesis at 48 h (%)
Initial embryonic development[3]					
No initial PD	8.94±0.03	0.50±0.04[a]	4.65±0.09	0.003±0.10	30.04±5.83[b]
Initial PD	8.82±0.05	0.30±0.06[b]	4.88±0.12	0.04±0.14	70.73±9.22[a]
PP virus treatments[1]					
Control	8.92±0.04	0.52±0.05[a]	4.58±0.11[b]	-0.17±0.13[b]	45.52±7.55
PP virus	8.88±0.03	0.34±0.05[b]	4.90±0.10[a]	0.18±0.11[a]	48.86±6.10
Interactions[3]					
No initial PD+control	8.94±0.04	0.56±0.06[a]	4.60±0.14[b]	-0.05±0.16[ab]	35.07±9.25[b]
No initial PD+PP virus	8.94±0.04	0.45±0.05[a]	4.70±0.11[b]	0.05±0.13[a]	26.00±7.40[b]
Initial PD+control	8.87±0.07	0.50±0.09[a]	4.54±0.20[b]	-0.36±0.22[b]	63.00±12.75[b]
Initial PD+PP virus	8.88±0.06	0.14±0.08[b]	5.18±0.16[a]	0.40±0.20[a]	90.30±10.72[a]
p-values					
Initial embryonic development	0.12	0.02	0.300	0.800	0.0007
PP virus treatments	0.73	0.03	0.002	0.006	0.1500
Interactions	0.34	0.09	0.050	0.030	0.0300

[a-b]For each main effect or interaction, means within a column with different superscripts are significantly different at p<0.10, [1]PP: Pigeon pox. [2]18 virgin hens laid 151 eggs that were cultured *in vitro* and incubated for 48 h. SEM: Standard error of the mean., [3]PD: Parthenogenetic development, PP virus: Pigeon Pox virus

Table 3: Initial embryonic development and ND[1] virus treatment effects on albumen pH, germinal disc diameter and incidence of parthenogenesis observed after 48 h of incubation in Experiment 2b: *In vitro* ND[1] virus exposure (mean±SEM)[2]

	Albumen pH at 48 h	Albumen pH change (48-0 h)	Germinal disc diameter at 48 h (mm)	Germinal disc diameter change (mm, 48-0 h)	Incidence of parthenogenesis at 48 h (%)
Initial embryonic development[3]					
No initial PD	8.65±0.05	0.28±0.05	4.37±0.13	-0.39±0.12	11.84±4.75[b]
Initial PD	8.88±0.05	0.38±0.05	4.47±0.15	-0.38±0.14	22.46±5.51[a]
ND virus treatments[1]					
Control	8.88±0.05[a]	0.45±0.05[a]	4.02±0.15[b]	-0.65±0.13[b]	10.27±5.29[b]
ND virus	8.65±0.05[b]	0.22±0.05[b]	4.75±0.14[a]	-0.16±0.12[a]	21.62±4.91[a]
Interactions[3]					
No initial PD+control	8.76±0.07	0.43±0.07	4.16±0.20[b]	-0.41±0.17[bc]	7.70±7.06
No initial PD+ND virus	8.57±0.06	0.15±0.06	4.54±0.18[b]	-0.37±0.16[b]	15.31±6.41
Initial PD+control	8.80±0.07	0.47±0.08	3.83±0.22[b]	-0.94±0.20[c]	13.64±7.97
Initial PD+ND virus	8.75±0.07	0.31±0.08	5.10±0.21[a]	0.14±0.20[a]	30.56±7.63
p-values					
Initial embryonic development	0.14	0.220	0.2300	0.6600	0.050
ND virus treatments	0.02	0.001	0.0002	0.0007	0.022
Interactions	0.67	0.570	0.0500	0.0140	0.340

[a-b]For each main effect or interaction, means within a column with different superscripts are significantly different at p<0.10, [1]ND: Newcastle disease. [2]20 virgin hens laid 128 eggs that were cultured *in vitro* and incubated for 48 h. SEM: Standard error of the mean. [3]PD: Parthenogenetic development, ND virus: Newcastle disease virus

the least decrease in size over incubation (p = 0.0007). In fact, for germinal disc diameter and diameter change over incubation there was an interaction between initial embryonic development and ND virus treatment. For germinal disc size at 48 h of incubation, eggs with initial PD exposed to ND virus had the greatest size (p = 0.05). Further, for germinal disc size change over incubation, eggs with initial PD exposed to ND virus were the only eggs that showed any growth in embryo size (p = 0.014). For incidence of parthenogenesis at 48 h of incubation, eggs treated with ND virus (p = 0.022) and eggs with initial PD (p = 0.05) had a significantly greater incidence compared to control and no initial PD eggs, respectively.

DISCUSSION

In the current study, it appears that live poultry viruses, PP and ND from current vaccine strains, have the potential to increase the incidence of parthenogenesis as well as parthenogen size in quail. For instance in the current study, live PP virus vaccination significantly increased absolute and

relative parthenogen size. In fact, a similar effect was observed in chickens[35] and turkeys[35,17] following live fowl pox virus vaccination. In turkeys, even twin, triplet and quadruplet parthenogenetic embryos were observed following vaccination[21]. Also, the effect of vaccination of parent stock was reported to increase the occurrence of parthenogenesis in the non-vaccinated progenies[17]. Further in the current study, eggs laid by live PP virus vaccinated birds had a lower percentage egg weight loss as opposed to non-vaccinated birds. Wells *et al.*[16] reported that percentage egg weight loss was negatively correlated with incidence of parthenogenesis and parthenogen size. Therefore, as eggs laid by vaccinated birds in the current study had a greater parthenogen size, this likely lead to less moisture loss from these eggs during incubation as compared to eggs from non-vaccinated birds. In the present study, the effects of live PP virus following the *in vivo* treatment could be through indirect or direct viral actions on the embryo.

Additionally, based on our *in vitro* study, it appears that one possible mechanism of action for the *in vivo* effect of live PP virus on quail parthenogenesis is the direct action of the virus on the embryo. In the current study, direct administration of live PP virus onto the germinal disc was found to increase parthenogen size by 8.70 percent (0.41/4.73) in the eggs initially exhibiting PD. As pox virus has the ability to cause fusion of cells[36], it might serve as an organizer in the eggs where PD has already been initiated resulting in more advanced embryos. Moreover, in the absence of live PP virus, eggs with initial PD were shown to lose embryo size over incubation. This in turn clearly demonstrates the direct effect of live PP virus on enhancing parthenogen size. Additionally, live PP virus treatment significantly increased the incidence of parthenogenesis at 48 h in eggs with initial PD as opposed to all other treatments. In fact, for eggs that initially exhibited 100% PD before incubation, virus treatment maintained embryo detectability after incubation for 48 h at 90% as opposed to 63% for eggs not exposed to virus. It is possible that virus treatment enhanced parthenogen livability. Further, an interaction for albumen pH change over incubation also revealed that virus treated eggs with initial PD exhibited the least increase in pH over time. This low albumen pH was likely due to CO_2 production by viable parthenogens[28,37]. In fact, Rosa *et al.*[28,37] reported that parthenogenetic embryos alter albumen characteristics similar to that of a normal fertilized egg embryo. For instance, parthenogenetic embryos utilize albumen O_2 and produce CO_2, mostly present as bicarbonate, thus lowering albumen pH[38]. This indicates that parthenogens observed in the current study were viable embryos and further strengthens the finding that parthenogen livability was enhanced by live PP virus treatment. Therefore, it is likely that one of the modes of action of live PP virus on parthenogenesis is by directly acting on the embryo and enhancing parthenogen size as well as livability.

On the other hand, live fowl pox virus was reported to have no effect on parthenogenesis following *in ovo* injection into chicken eggs[24]. However, prior to their *in ovo* injection, eggs were not classified based on the presence of initial PD, unlike the current study. Probably most of the eggs *in ovo* injected with the virus were the ones with no initial PD and the virus was not able to induce PD in those unfertilized eggs with no initial PD. In fact, this is in agreement with our current *in vitro* findings, where eggs with no initial PD following virus exposure showed no significant change in the incidence of parthenogenesis or germinal disc size as opposed to eggs not exposed to virus. In general, this indicates that live pox viruses, under *in vitro* conditions, cannot induce PD in eggs with no initial embryonic development.

Previously, Olsen[8,20] reported that under *in vivo* conditions the effect of viruses on avian parthenogenesis varied with DNA and RNA viruses. RNA viruses, ND and leukosis viruses, were found to have an effect only on the incidence of parthenogenesis but not on the embryo size[8,20]. In contrast, in the present study, even though the degree of growth was about 50% less (0.14 vs 0.40 mm) compared to live PP virus treated eggs, live ND virus was found to increase parthenogen size 3 percent (0.14/4.92) in the eggs with initial PD. Possibly this is due to the ability of ND virus, like PP virus, to cause cell fusion thus, resulting in more advanced embryos[1]. Moreover, live ND virus, similar to live PP virus, enhanced parthenogen livability as the ND virus treated eggs had a higher incidence of parthenogenesis and lower albumen pH[28,37] over incubation as opposed to eggs not treated with virus. Therefore, based on the current study, it appears that the RNA virus, live ND virus, can exert its effect directly on the embryo to enhance parthenogenesis and embryo size similar to the DNA virus, live PP virus. However, as mentioned before, these *in vitro* findings cannot be directly extrapolated to *in vivo* conditions where multiple physiological systems may interact to alter embryo formation. In fact, these *in vivo* vs *in vitro* differences may explain why the current *in vitro* findings of increased parthenogen size due to ND exposure are contradictory to that of Olsen's *in vivo* findings[8,20].

CONCLUSION AND FUTURE RECOMMENDATION

It appears that vaccination of virgin hens with live PP virus has the potential to increase parthenogenesis as well as

parthenogen size and livability by the direct action of the virus on the embryo. However, further *in vitro* and *in vivo* studies are required to have a better understanding of other modes of viral action, including interactions of live PP virus with various physiological systems such as the immune system which may indirectly enhance parthenogenesis. Under *in vitro* conditions, live ND virus was found to exert similar effects as live PP virus by directly acting on the embryo. Additional research is needed to determine if the *in vitro* effect of live ND virus is the same under *in vivo* conditions. Further, effects of killed viruses used as current vaccine strains should be studied and compared to their live counterparts. As vaccination for pox and ND is a routine practice in the modern poultry industry, it is possible that vaccination of birds that carry the parthenogenetic trait will reduce fertility and hatchability due to enhanced parthenogenesis.

SIGNIFICANCE STATEMENT

This study is the first to discover the effects of current virus vaccine strains, pigeon pox and Newcastle disease, on parthenogenesis and their mechanism of action using Chinese painted quail as the model. The results of this study should further assist poultry scientists and reproductive biologists uncover the critical area of reproductive loss due to parthenogenesis that has eluded researchers for years. Thus, with this current research serving as foundational data, a new theory on live virus vaccinations and their effects on reproductive performance may be attained.

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