Effects of Reticuloendotheliosis Virus on the Viability and Reproductive Performance of Japanese Quail

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Primary Audience: Veterinarians, Researchers, Flock Supervisors

SUMMARY

The effects of reticuloendotheliosis virus (REV) infection were studied using an experimental model in Japanese quail during 2 consecutive generations. The REV used in this study (APC-566) was isolated from Attwater’s prairie chickens (Tympanuchus cupido attwateri). Tumors were induced by APC-566 as early as 6 wk of age. Mortality was significantly higher in groups of quail with a higher frequency of REV infection. Egg production, hatchability, and fertility rates decreased in infected quail as compared with uninfected control quail. The BW of infected quail were significantly reduced at 8 wk of age in the first generation of infected quail (breeders) and at 3 and 6 wk of age in the second generation (quail broilers) compared with uninfected quail.

Key words: reticuloendotheliosis virus, Japanese quail, performance data

DESCRIPTION OF PROBLEM

Reticuloendotheliosis virus (REV) is an avian gammaretrovirus closely related to mammalian type-C retroviruses. Reticuloendotheliosis virus is immunologically, morphologically, and structurally distinct from the avian leucosis-sarcoma viruses. Reticuloendotheliosis virus causes various syndromes in multiple avian species, including severe runting, an acute nonneoplastic syndrome with high mortality, severe immune suppression, and T-, B-, or both T- and B-cell lymphomas. Nondefective REV strains have been isolated from various avian species including chickens, turkeys, ducks, pheasants, geese, Japanese quail, peafowl, and prairie chickens [1]. Reticuloendotheliosis virus infection can cause dramatic economic losses from a runting syndrome or chronic neoplasia with mortality. Although these manifestations are not common, exposure to REV appears to be widespread in the field [2]. Significant losses can occur when REV-contaminated vaccines are administered to very young chickens. Weights of infected chickens may be 20 to 50% lower than uninfected controls by 3 to 5 wk after infection [3]. Weight gain reduction has also been reported in infected ducks [4].

The Attwater’s prairie chicken (APC; Tympanuchus cupido attwateri) is a wild species of grouse on the verge of extinction. The APC was previously known to be susceptible to REV [5], and presently they are endangered partly due to enzootic REV infection. We isolated REV from

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several APC specimens. One of these viruses was named APC-566 and was randomly selected for these studies.

Japanese quail (*Coturnix coturnix japonica*) may be used as a model for studying vertically transmissible avian diseases [6] because of their rapid development and short generation interval and because they are biologically related to domestic chickens (*Gallus gallus*) [7]. Japanese quail become sexually mature at approximately 6 to 7 wk of age as compared with 24 to 28 wk for chickens, and they require much less space, labor, and maintenance cost. In addition, Japanese quail are prolific layers for most of the year [7]. To date, the effects of REV infection on the performance of commercial Japanese quail have not been examined and reported. This study was performed using Japanese quail as a model to better understand the effects of REV on the viability and performance of quail breeders and broilers.

**MATERIALS AND METHODS**

**Quail**

Commercially produced fertile eggs of Japanese quail (*Coturnix coturnix japonica*) were obtained from a local commercial hatchery after testing the breeders to insure absence of infectious REV and antibodies in the breeding population used to produce the eggs.

**Viruses, Virus Isolation, Virus Titration, and Antibody Detection**

A field strain of REV named REV APC-566 was isolated in our laboratory from whole blood of an adult specimen of APC (*Tympanuchus cupido attwateri*) held at the Fossil Rim Wildlife Center [8]. Reticuloendotheliosis virus was isolated in DF-1 cells (ATCC UMNSAH/DF-1, CRL-12203) and confirmed to be REV by indirect immunofluorescence, PCR, and sequencing of the entire virus genome [9]. A virus stock was prepared by passaging 3 times the REV-infected DF-1 cells. After the third passage in DF-1 cells, the virus stock was titrated and frozen at −80°C until used for this research. The virus titer in the stock was calculated by the Reed and Muench method [10] using indirect immunofluorescence to identify REV-positive virus cultures. Briefly, DF-1 cells were plated at a concentration of approximately 150,000 cells/mL, inoculated with serial 10-fold dilutions of REV APC-566, and incubated at 39°C for 7 d. The inoculated cells and control cultures were then trypsinized, replated, and incubated for approximately 2 to 4 d until they reached approximately 80% confluence, and they were then fixed with a cold acetone-alcohol solution (60:40 vol/vol). Indirect immunofluorescence was performed following a modified preestablished procedure [11]. Skim milk in PBS (5%) was used as a blocking agent to reduce nonspecific fluorescence, followed by incubation with polyclonal REV antibody [12]. The cells were washed in PBS and incubated with fluorescein isothiocyanate-labeled goat-anti-chicken IgG [13]. A final wash in PBS preceded examination with a fluorescence microscope. The REV-positive and REV-negative control cell cultures were included in all virological assays. Virus isolation from experimentally infected quail was done from plasma or serum following a modified procedure involving inoculations in DF-1 cells and immunofluorescence on infected cells trypsinized and passaged only once. Reticuloendotheliosis virus antibodies were detected using a commercially available enzyme-linked immunosorbent assay (ELISA) as per the manufacturer’s instructions [14].

**Experimental Design**

**First Quail Generation (F1).** One hundred microliters of REV APC-566 stock was inoculated into Japanese quail embryos via the yolk sac at 4 d of embryonation. The titer of the inoculum was 10^3.4 tissue culture infectious doses 50% in 0.1 mL. Six experimental groups of quail chicks were produced to contain various proportions of inoculated quail chicks. The experimental groups were made to include 0, 5, 10, 25, 50, and 100% inoculated embryos. Success of infection was verified by PCR on the spleens of naturally dead quail, but the actual rate of REV infection at hatch was not determined due to the small size of the quail chicks, which made sampling impossible without causing death. The control group (G1) contained 120 chicks from noninoculated embryos. Groups 2, 3, 4, 5, and 6 contained 60 chicks each at hatch. The experimental groups were raised in separate floor pens until 6 wk of age. At this age, cloacal
Table 1. Mean BW of growing quail breeders and quail broilers (g)

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>8 wk</th>
<th>n</th>
<th>3 wk</th>
<th>6 wk</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>60</td>
<td>234.57a</td>
<td>60</td>
<td>120.97a</td>
<td>227.21a</td>
</tr>
<tr>
<td>G2</td>
<td>30</td>
<td>242.34a</td>
<td>60</td>
<td>111.53bc</td>
<td>226.92a</td>
</tr>
<tr>
<td>G3</td>
<td>30</td>
<td>238.10a</td>
<td>60</td>
<td>112.35b</td>
<td>226.92a</td>
</tr>
<tr>
<td>G4</td>
<td>30</td>
<td>236.08a</td>
<td>60</td>
<td>104.26cd</td>
<td>217.02ab</td>
</tr>
<tr>
<td>G5</td>
<td>30</td>
<td>225.38ab</td>
<td>52</td>
<td>99.04d</td>
<td>216.06ab</td>
</tr>
<tr>
<td>G6</td>
<td>18</td>
<td>214.29b</td>
<td>29</td>
<td>98.73d</td>
<td>208.89b</td>
</tr>
</tbody>
</table>

a–dDifferent superscripts in the same column indicate significant differences at \( P < 0.05 \).

and esophageal swab samples were collected from 18 females and 12 males per group. All birds were placed in standard quail egg production cages at a 1:3 male-female ratio, and each cage contained 1 male and 3 hens. Group 1 (uninoculated) contained 36 hens and 12 males. Groups 2 to 5 contained 18 hens and 6 males each. Group 6 contained only 13 hens and 5 males, because mortality during growing in this group was higher than expected. Any extra birds not needed for the reproductive phase of the experiments were bled, euthanized, and examined by necropsy. Daily egg production was recorded, and all eggs produced were collected daily and either stored at 18°C for weekly incubation or used for albumen sample collection. All experimental breeder quail were kept until 20 wk of age, at which time they were bled, swabbed, euthanized by cervical dislocation, and examined postmortem. Any quail exhibiting clinical signs was immediately euthanized to avoid unnecessary distress. Tissues collected for PCR, histopathology, or both included spleen, liver, kidney, heart, brain, nerve, esophagus, gizzard, proventriculus, small intestine, pancreas, gonads, oviduct, skeletal muscle, and thymus.

**Second Quail Generation (F2).** All eggs produced during the 12th and 13th week of age were pooled in 2 groups to produce 2 hatches, 1 hatch for eggs from wk 12 (F2a) and another hatch for eggs from wk 13 (F2b). Thirty chicks produced per breeder group (or less if 30 chicks were not available) were raised in floor pens and kept until 6 wk of age. The combined number of F2 chicks hatched and raised per group is summarized in Table 1. In total, groups G1 to G4 contained 60 chicks each (30 for F2a and 30 for F2b). Group 5 contained 22 chicks in F2a and 30 chicks in F2b, for a combined total of 52. Group 6 contained 10 chicks in F2a and 19 chicks in F2b, for a combined total of 29. All second generation quail (F2) were weighed at 3 and 6 wk of age, and their average weights were statistically compared. At the termination of the experiment (6 wk of age), all quail were bled for virus isolation and antibody ELISA, euthanized by cervical dislocation, and examined on necropsy. Spleens, livers, and suspect tissues were collected for microscopic examination.

**Egg Production, Hatchability, and Embryodiagnosis.** All eggs produced from the 6th to 19th wk of age were collected, and the hen-housed egg production was calculated per group. All eggs produced during the 9th and 18th wk of age were incubated for chick production. Embryodiagnosis was done in all the eggs not hatched, and the causes of lack of viability were divided into 5 categories: a) infertile; b) early mortality (1 to 4 d of incubation); c) late mortality (14 to 17 d of incubation, i.e., after transfer), cull chicks, or both; d) pipped but not hatched; and e) broken eggshell.

**Statistical Analysis**

The statistical significance of differences in quail BW and embryodiagnosis was evaluated using the ANOVA of Duncan and Scheffe using the SAS software package [15]. The \( \chi^2 \) test was applied to analyze differences between mortalities. A \( P \)-value of \( \leq 0.05 \) was used to define statistical significance.

**RESULTS AND DISCUSSION**

This study concentrated on the viability and reproductive performance of REV-infected quail. Detailed results of viremia, antibody production, pathogenicity and transmission, and characterization of the neoplastic lesions in-
produced by REV will be discussed in a separate article. Briefly, uninoculated F1 and F2 quail remained negative for REV by virus isolation and REV antibody ELISA for the duration of the study. Reticuloendotheliosis virus was reisolated only from quail belonging to inoculated groups of quail. At 20 wk of age, REV was reisolated from 12.5, 5.5, and 64.5% of surviving F1 quail in groups with 25, 50, and 100% inoculated embryos, respectively. Reticuloendotheliosis virus was not reisolated from F1 groups with 0, 5, or 10% infected embryos. Also, at 20 wk of age, anti-REV antibodies were detected using ELISA in 0.0, 0.0, 16.0, 16.6, 55.5, and 92.8% of surviving F1 quail in groups with 0, 5, 10, 25, 50, and 100% inoculated embryos, respectively. Also, at 20 wk of age, anti-REV antibodies were detected using ELISA in 0.0, 0.0, 16.0, 16.6, 55.5, and 92.8% of surviving F1 quail in groups with 0, 5, 10, 25, 50, and 100% inoculated embryos, respectively.

At 6 wk of age, all samples collected from all the groups of F2 quail broilers (335 samples in total) were REV-negative on virus isolation and REV antibody-negative by ELISA.

Reticuloendotheliosis virus APC-566 proved to be oncogenic in Japanese quail, producing lymphosarcomas and other tumors in up to 4.6% of birds from infected groups in F1 (results not shown).

The mortality rate on the F1 generation was significantly higher in groups of quail with a higher frequency of REV infection (50 and 100% inoculated embryos). Most of the mortality in the remaining groups resulted from aggression and possibly overall unthriftiness (Table 2). Mortality caused by REV has been rarely reported in chickens [16, 17]. This study did not include a sham-inoculated group and thus the actual effect of inoculating embryos on early mortality could not be determined. However, overall mortality was significantly higher in the groups containing a larger proportion of inoculated embryos and a higher percentage of REV-infected quail.

Affected birds in commercial flocks have been commonly culled before natural death, and a culling loss of >50% from 5 to 8 wk has been described [18]. The present experiment showed that REV can cause high mortality rates in Japanese quail. Such mortality can be high, especially when the quail are kept for prolonged periods of time, as is the case in quail breeders.

The group with 100% infection as embryos showed the lowest average BW at 8 wk, being significantly different from all remaining groups, except those with 50% infection (Table 1). The uninfected F1 control quail did not attain the highest BW by 8 wk of age, but average BW in this group was significantly higher than in the group with 100% inoculated embryos. However, such birds were raised at a higher density, which may have contributed to their slower growth in the first generation of quail (F1), albeit it cannot be concluded that density was indeed the reason for lower BW in this group. Early BW in the second generation (3 and 6 wk of age) was significantly reduced in the group in which 100% of quail were infected, suggesting that REV infection may reduce growth significantly in the progeny of infected quail breeders (Table 1). Motha [19] showed that chickens infected as embryos had significantly reduced BW at 6, 25, and 51 d of age when compared with uninfected birds. Weights of infected chickens were reduced 20 to 50% compared with uninfected controls [20, 21, 22].

Hen-housed egg production was significantly reduced in the groups in which 50 and 100% of quail were infected (Table 3). Embryodiagnosis of unhatched eggs revealed a numeri-

### Table 2. Experimental design and mortality of Japanese quail breeders (first generation)

<table>
<thead>
<tr>
<th>Group</th>
<th>Inoculated</th>
<th>Uninoculated</th>
<th>Infected (%)</th>
<th>0 to 6 wk</th>
<th>Total at 20 wk</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>0</td>
<td>60</td>
<td>0</td>
<td>2.5</td>
<td>10.8</td>
</tr>
<tr>
<td>G2</td>
<td>3</td>
<td>57</td>
<td>5</td>
<td>16.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>18.3</td>
</tr>
<tr>
<td>G3</td>
<td>6</td>
<td>54</td>
<td>10</td>
<td>11.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20.0</td>
</tr>
<tr>
<td>G4</td>
<td>15</td>
<td>45</td>
<td>25</td>
<td>16.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>26.6&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>G5</td>
<td>30</td>
<td>30</td>
<td>50</td>
<td>18.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>33.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>G6</td>
<td>60</td>
<td>0</td>
<td>100</td>
<td>38.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>61.6&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Denotes significant differences at \( P < 0.05 \) from the control group (G1).

<sup>b</sup>Denotes significant differences at \( P < 0.01 \) from the control group (G1).
Table 3. Mean hen-housed egg production and embryodiagnosis of incubated eggs from reticuloendotheliosis virus-inoculated Japanese quail

<table>
<thead>
<tr>
<th>Group</th>
<th>Embryodiagnosis¹</th>
<th>Egg production²</th>
<th>Incubated eggs</th>
<th>Hatchability</th>
<th>Infertile</th>
<th>Early dead</th>
<th>Late dead/cull</th>
<th>Pipped</th>
<th>Broken egg</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td></td>
<td>64.91 a</td>
<td>1,955</td>
<td>60.97 ab</td>
<td>29.46 ab</td>
<td>0.91</td>
<td>1.87</td>
<td>4.78</td>
<td>2.26</td>
</tr>
<tr>
<td>G2</td>
<td></td>
<td>80.22 a</td>
<td>1,167</td>
<td>82.69 a</td>
<td>8.45 a</td>
<td>0.92</td>
<td>2.72</td>
<td>2.37</td>
<td>1.50</td>
</tr>
<tr>
<td>G3</td>
<td></td>
<td>68.54 a</td>
<td>973</td>
<td>73.47 ab</td>
<td>19.99 ab</td>
<td>0.86</td>
<td>2.34</td>
<td>1.92</td>
<td>0.86</td>
</tr>
<tr>
<td>G4</td>
<td></td>
<td>72.68 a</td>
<td>1,064</td>
<td>59.47 ab</td>
<td>30.81 ab</td>
<td>0.87</td>
<td>3.15</td>
<td>2.87</td>
<td>1.67</td>
</tr>
<tr>
<td>G5</td>
<td></td>
<td>40.76 b</td>
<td>572</td>
<td>59.44 ab</td>
<td>29.22 ab</td>
<td>1.51</td>
<td>4.01</td>
<td>2.94</td>
<td>1.70</td>
</tr>
<tr>
<td>G6</td>
<td></td>
<td>42.70 b</td>
<td>483</td>
<td>49.43 b</td>
<td>36.92 b</td>
<td>1.71</td>
<td>4.17</td>
<td>3.50</td>
<td>2.57</td>
</tr>
</tbody>
</table>

¹Different superscripts in the same column indicate significant differences at $P < 0.05$.

²Embryodiagnosis for 10 wk of egg production and incubation (wk 9 through 18).

³Average hen-housed egg production for 14 wk of egg production (wk 6 through 19).

CONCLUSIONS AND APPLICATIONS

1. Japanese quail may be used as a model for studying detrimental effects of field strains of REV on viability and performance of commercial poultry in a relatively short period of time.
2. Japanese quail embryos infected with REV had higher mortality caused by stunting, tumor development, or both.
3. Reticuloendotheliosis virus infection was detrimental for BW in 2 consecutive generations and was also associated with decreased egg production, hatchability, and fertility in Japanese quail.

REFERENCES AND NOTES

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14. Idexx Laboratories, Westbrook, ME.

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