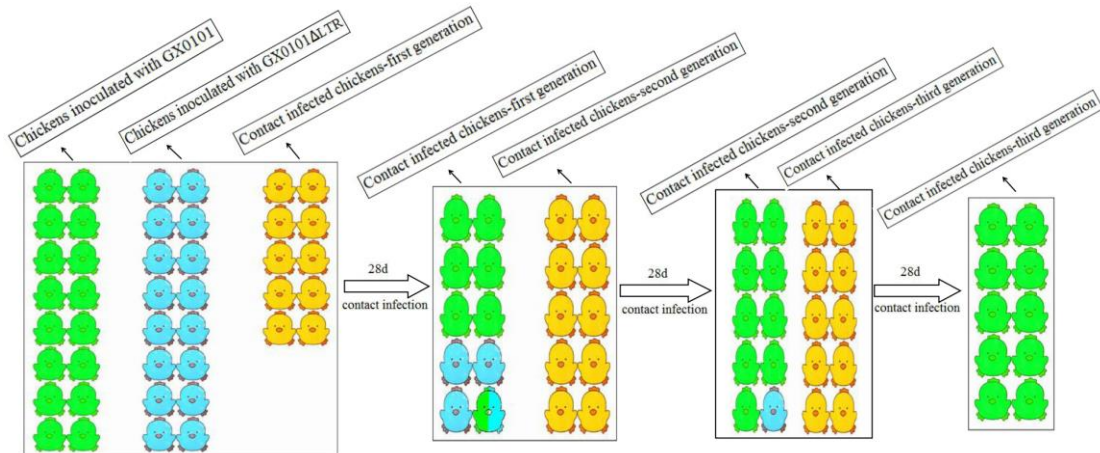
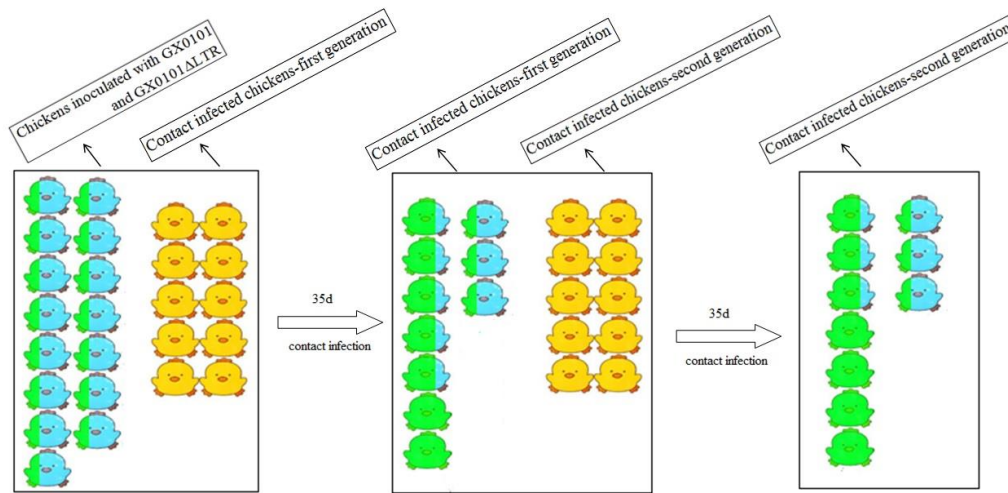


## Supplementary Material



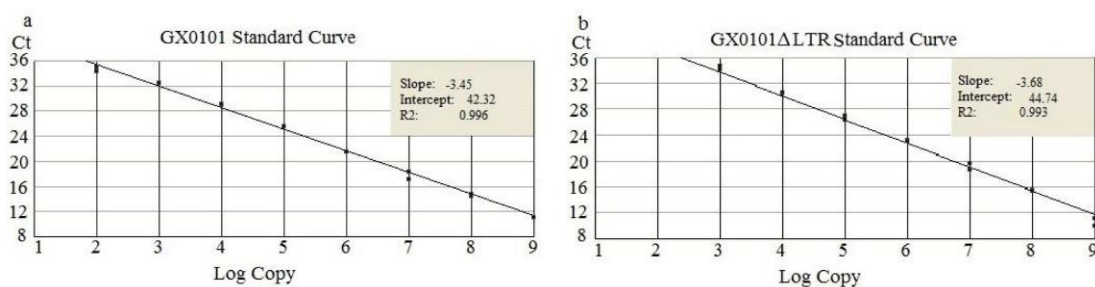
**Figure 1 Sketch map of simulating MDV natural transmission in birds when the same dose of GX0101 or GX0101ΔLTR was inoculated.**

Horizontal transmission capacity of GX0101 and GX0101ΔLTR were compared via successive contact infections by simulating MDV natural transmission in SPF chicks. Green birds were infected with GX0101; blue birds were infected with GX0101ΔLTR; yellow ones were uninfected birds.



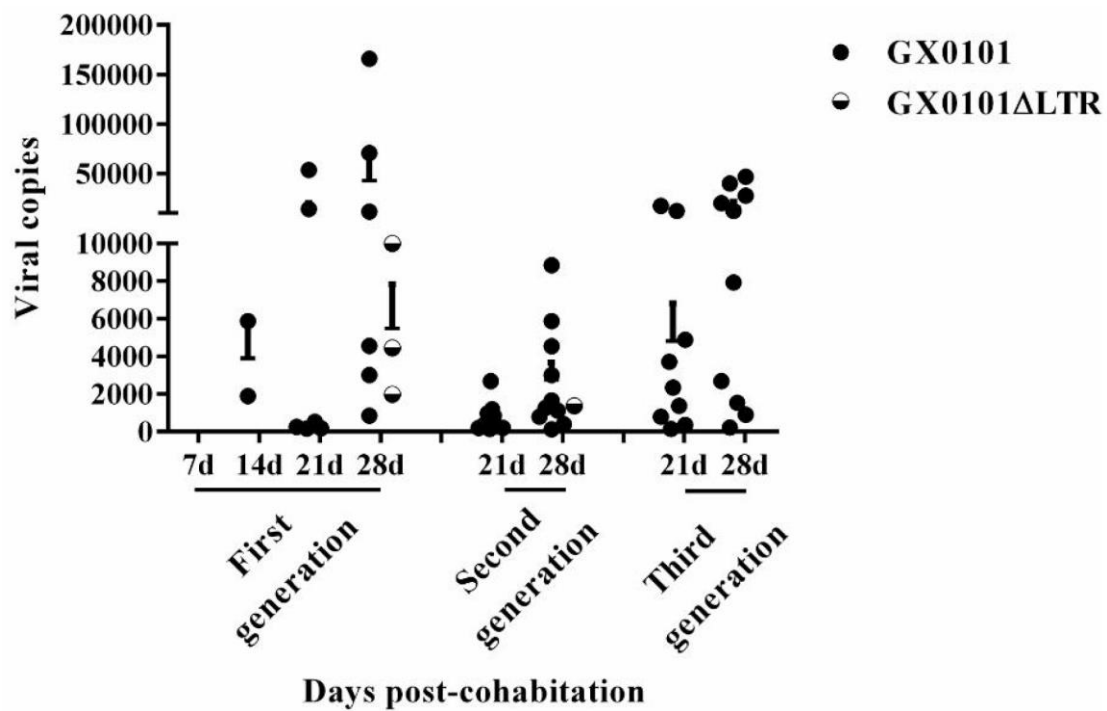
**Figure 2 Sketch map of simulating MDV natural transmission in birds when different dose of GX0101 or GX0101 $\Delta$ LTR was inoculated.**

Fifteen SPF birds were inoculated with 100 PFU of GX0101 or 2000 PFU of GX0101 $\Delta$ LTR by the IA route. Horizontal transmission capacity of GX0101 and GX0101 $\Delta$ LTR in birds was compared via successive contact infections. Green birds were infected with GX0101; blue birds were infected with GX0101 $\Delta$ LTR; yellow ones were uninfected birds.



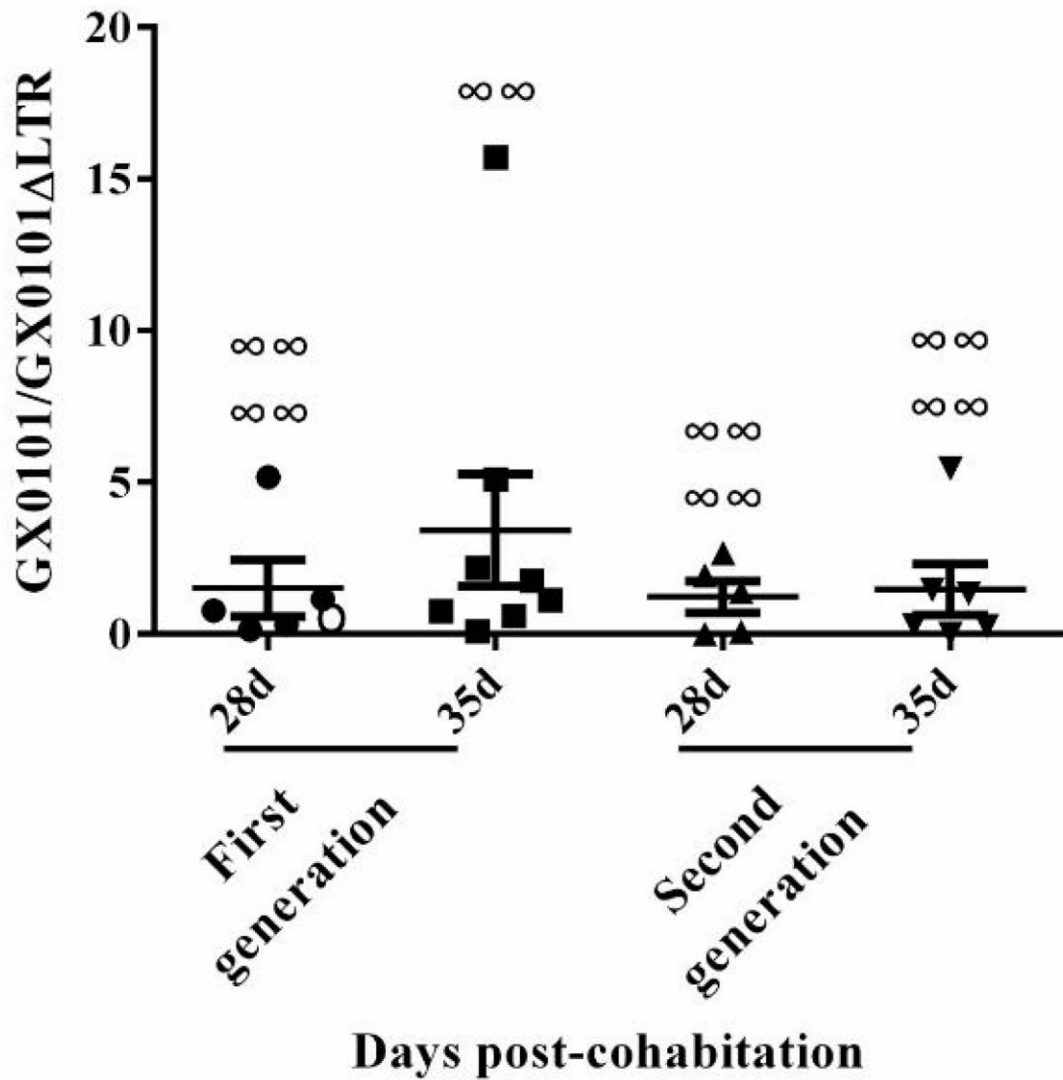
**Figure 3 Standard curves of GX0101 (a) and GX0101 $\Delta$ LTR (b)**

The Ct values of GX0101 (a) and GX0101 $\Delta$ LTR (b) from  $10^9$  to  $10^1$  copies were detected. Log Copy: Log (Copy number of the virus).



**Figure 4 GX0101 has significant advantage of horizontal transmission over GX0101ΔLTR when inoculating with the same dose**

SPF chicks were inoculated with 1000 PFU of GX0101 or GX0101ΔLTR. Horizontal transmission capacity of GX0101 and GX0101ΔLTR were compared via successive contact infections by simulating MDV natural transmission. Viral copies of GX0101 and GX0101ΔLTR in the first round of contact chicks were determined by detecting the DNA of the feather tips using DF-qPCR at 7, 14, 21 and 28 days post-inoculation, respectively. Viral copies in the second and the third rounds of contact chicks were determined after 21 and 28 days cohabitation, respectively.



**Figure 5 GX0101 still has significant advantage of horizontal transmission over GX0101ΔLTR when a lower dose was inoculated**

SPF chicks were inoculated with 100 PFU of GX0101 or 2000 PFU of GX0101ΔLTR. Viral copies of the two viruses in the first and second rounds of contact chicks were determined after 28 and 35 days cohabitation, respectively. The ratio of viral copies (GX0101/GX0101ΔLTR) in feather follicles was calculated. ∞: GX0101 was only detectable; ○: GX0101ΔLTR was only detectable; ●, ■▲▼: The ratio of viral copies (GX0101/GX0101ΔLTR) in feather follicles was calculated at different time points.

