***This information product has been peer reviewed and approved for publication as a preprint by the U.S. Geological Survey.***

**An optimized rabies vaccine vehicle for orotopical administration to wild vampire bats**

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**Supplemental Information**

**A graph of different colored lines

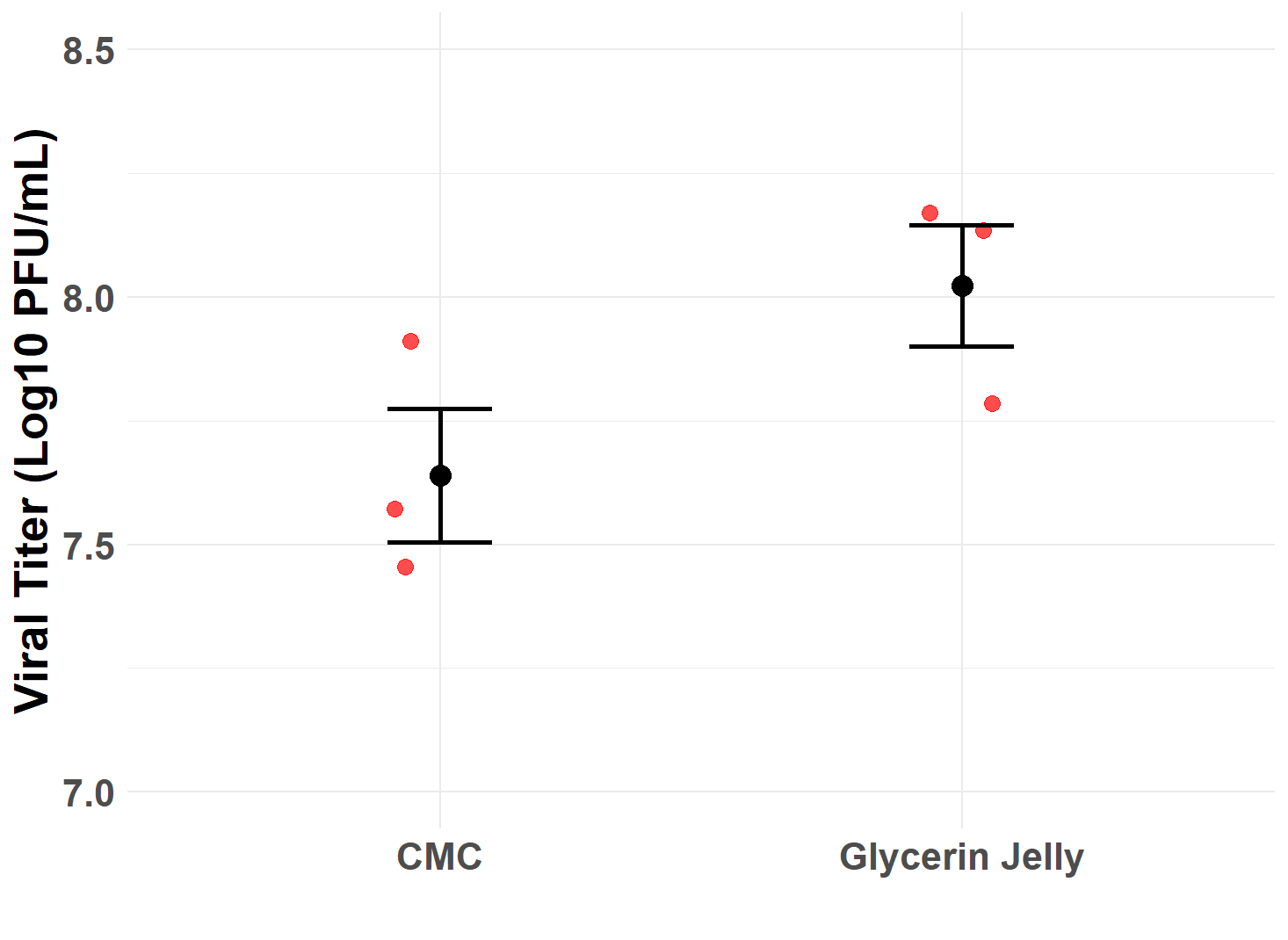
AI-generated content may be incorrect.**

**Figure S1. Effect of carboxymethyl cellulose (CMC) concentration on viscosity at 0** °**C and 20** °**C**. Steady rate sweep test was performed with an ARES LS2 Advanced research grade rheometer (TA Instruments, New Castle, Delaware, USA) to measure viscosity of 10%, 12.5%, and 15% CMC at **A)** 0 °C and **B)** 20 °C. Error bars represent standard error of three experimental replicates.

**A graph of a train and a train

AI-generated content may be incorrect.**

**Figure S2. Dynamic strain sweep test of 12.5% carboxymethyl cellulose (CMC) gel and glycerin jelly (GJ) at a frequency of 1 Hz at 0** °**C and 20** °**C.** Viscoelastic properties of CMC and GJ were measured by a dynamic strain sweep test at **A)** 0 °C and **B)** 20 °C. G’ represents the elastic modulus – energy stored during deformation, while G” represents the loss modulus – energy dissipated during deformation. All rheological measurements were recorded with an ARES LS2 Advanced research grade rheometer (TA Instruments, New Castle, Delaware, USA). Error bars represent standard error of three experimental replicates exceptfor GJ at 0 °C where only one full replicate was accurately captured.



**Figure S3. Recovery of a recombinant raccoonpox vaccine expressing a mosaic lyssavirus glycoprotein (RCN-MoG) from carboxymethyl cellulose (CMC) gel and glycerin jelly (GJ) at T=0.** An initial titer of 108 plaque forming units (PFU)/mL was mixed into CMC and GJ and frozen at -80 °C. The gels were thawed, dissolved, and titered by plaque assay on Vero cells (ATCC CCL-81). Red dots represent experimental replicates and error bars represent standard error.

**Real Time qPCR of stability samples with SYBR Green**

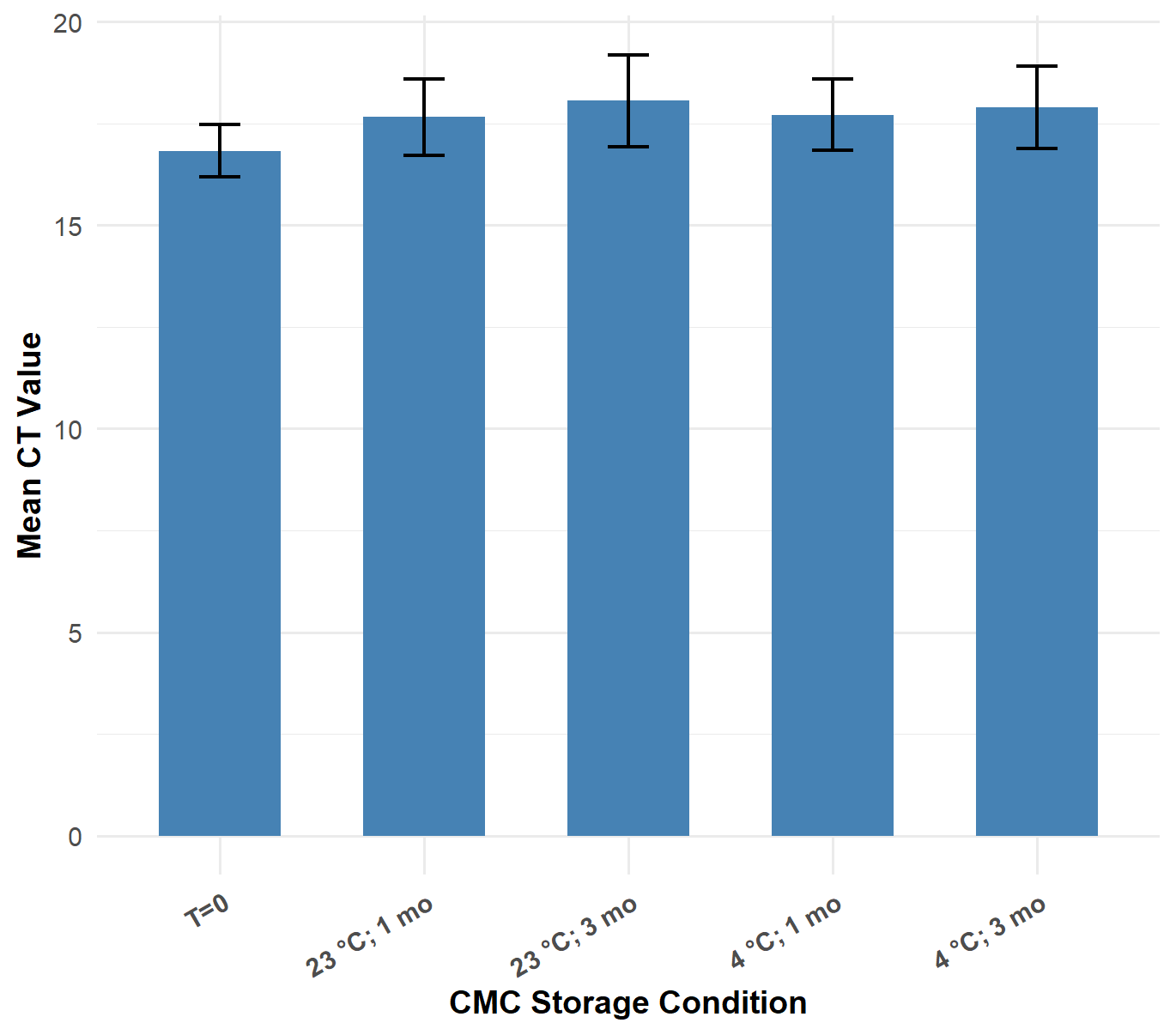
DNA was extracted from our recombinant raccoonpox vaccine expressing a mosaic lyssavirus glycoprotein (RCN-MoG) stored in CMC gel at T=0, and one month and three months at both 4 °C and 23 °C using Zymo Quick DNA Mini Prep kit (Zymo Research, Tustin, California, USA). Tables S1 and S2 were used for preparing the master mix and programming thermocycle conditions. The primer MoG-F targets the MoG protein, while RCN-R targets the raccoonpox vector.

**Table S1. SYBR green quantitative reverse transcription polymerase chain reaction (qPCR) master mix**

|  |  |
| --- | --- |
| **Reagent** | **Volume per sample (μL)** |
| Sso Advanced Universal SYBR Green Master Mix (Bio-Rad, Hercules, California, USA) | 10 |
| MoG-F primer (5’-GGAAGAGTAATATCTTCTTGGGAATC-3’) | 0.5 |
| RCN-R primer (5’CTATAACTATTTTTCCATTGTTTGCCATG-3’) | 0.5 |
| Nuclease free water | 7 |
| Sample DNA | 2 |
| **Total** | **20** |

**Table S2. SYBR green quantitative reverse transcription polymerase chain reaction (qPCR) thermocycle conditions**

|  |  |  |  |
| --- | --- | --- | --- |
| **Step** | **Temperature (**°**C)** | **Time** | **# of Cycles** |
| **Initial Denaturation** | 98 | 3 min | 1 |
| **Denaturation** | 98 | 30 s | 40 |
| **Annealing and Extension** | 60 | 20 s |



**Figure S4. Ct values from SYBR Green quantitative PCR measuring DNA stability of our recombinant raccoonpox vaccine expressing a mosaic lyssavirus glycoprotein (RCN-MoG) within carboxymethyl cellulose (CMC) gel in samples stored at 4** °**C and 23** °**C for one and three months.** Ct values were averaged across results of DNA extracted from all three experimental replicates of the RCN-MoG stability assay. Error bars represent the standard error. No statistical comparisons were significant by t-test.