Supplementary Materials

Title
A Single Dose Investigational Subunit Vaccine for Human Use against Nipah virus and Hendra virus

Running title
Single dose Nipah human vaccine efficacy

Authors
Thomas W. Geisbert,1,2 Kathryn Bobb,3 Viktoriya Borisevich,1,2 Joan B. Geisbert,1,2 Krystle N. Agans,1,2 Robert W. Cross,1,2 Abhishek N. Prasad,1,2 Karla A. Fenton,1,2 Hao Yu,3 Timothy R. Fouts,3 Christopher C. Broder,4 and Antony S. Dimitrov3,4,*

Affiliations
1Galveston National Laboratory, University of Texas Medical Branch, Galveston, TX 77550, USA. 2Department of Microbiology and Immunology, University of Texas Medical Branch, Galveston, TX 77550, USA. 3Profectus BioSciences, Inc., Baltimore, MD 21224, USA. 4Department of Microbiology and Immunology, Uniformed Services University, Bethesda, MD 20814, USA.
*To whom correspondence should be addressed: antony.dimitrov.ctr@usuhs.edu

Supplementary Figures 1 to 3
Supplementary Tables 1 to 9
Supplementary Fig. 1. Study 1 viral RNA in $\log_{10}$ average genome equivalents (GEq)/mL:
(A) Blood samples RNA peak readings for all animals, controls and immunized. The filled square (■) corresponds to the survived control peak reading. None of the vaccinated AGM showed any detectable viral RNA reading; (B) Blood RNA loads for control AGMs. The survived AGM-1-C1-2 was able to clear viral RNA in the blood to undetectable at the study end.
Supplementary Fig. 2. Study 1 tissue viral RNA loads in $\log_{10}$ average genome equivalents (GEq)/g. Viral RNA in tissue for the survived control, AGM-1-C1-2 denoted with (■), trended lower than the tissue viral load for the controls succumbed to disease. There was one AGM, 09113, denoted with (●), from the prime/boost group that had viral RNA in the lung and spleen but not in other tested tissues. All other immunized AGMs were sterilely protected.
Supplementary Fig. 3. Immunogenicity study to test HeV-sG/Alum ratio effect. Two groups of three rabbits were immunized with formulation at 1/10 ratio of HeV-sG to Al$^{3+}$ (rabbits 1A, 1B, and 1C) and at 1/25 ratio of HeV-sG to Al$^{3+}$ (rabbits 2A, 2B, and 2C). Sera were tittered for anti-HeV-sG antibodies. Results are grouped for each rabbit – titers from left to right for days 14, 28 (the day of boost dosing), 42, and 56 (study end day). Both formulations showed indistinguishable immune responses, measured in serum titers.
| NHP     | Sex | Virus | Regimen  | Clinical illness | Clinical pathology |
|---------|-----|-------|----------|------------------|--------------------|
| AGM-1-1-1 | F   | HeV   | Single Dose | None. Subject survived to study endpoint (d28). | Lymphocytopenia (d10, 14); granulocytopenia (d6, 8, 10, 14, 28); erythrocytopenia (d14); thrombocytopenia (d14); anemia (d14) |
| AGM-1-1-2 | M   | HeV   | Single Dose | None. Subject survived to study endpoint (d28). | Granulocytopenia (d14) |
| AGM-1-1-3 | F   | HeV   | Single Dose | None. Subject survived to study endpoint (d28). | Lymphocytopenia (d10); erythrocytopenia (d10); monocytopenia (d14); monocytosis (d28) |
| AGM-1-2-1 | M   | NiV-B | Single Dose | None. Subject survived to study endpoint (d28). | Monocytopenia (d28) |
| AGM-1-2-2 | F   | NiV-B | Single Dose | None. Subject survived to study endpoint (d28). | Thrombocytopenia (d6); granulocytopenia (d8, 14); erythrocytopenia (d14); anemia (d14); monocytosis (d6, 14, 28); lymphocytosis (d28); hypoglycemia (d6, 21) |
| AGM-1-2-3 | F   | NiV-B | Single Dose | None. Subject survived to study endpoint (d28). | Lymphocytopenia (d3, 6, 8, 10, 14, 28); monocytopenia (d14); erythrocytopenia (d14); anemia (d14); hypoglycemia (d3, 8) |
| AGM-1-3-1 | M   | HeV   | Prime/Boost | None. Subject survived to study endpoint (d28). | Lymphocytopenia (d10); monocytopenia (d10); granulocytopenia (d14) |
| AGM-1-3-2 | M   | HeV   | Prime/Boost | None. Subject survived to study endpoint (d28). | Lymphocytopenia (d6); thrombocytopenia (d3, 14); monocytosis (d3); erythrocytopenia (d14); anemia (d14) |
| AGM-1-3-3 | F   | HeV   | Prime/Boost | None. Subject survived to study endpoint (d28). | Lymphocytopenia (d10); ALT ↑ (d21) |
| AGM-1-3-4 | M   | HeV   | Prime/Boost | None. Subject survived to study endpoint (d28). | Lymphocytopenia (d6, 10, 14); monocytopenia (d6, 10); erythrocytopenia (d10, 14); thrombocytopenia (d14); anemia (d14); granulocytopenia (d14) |
| AGM-1-3-5 | F   | HeV   | Prime/Boost | None. Subject survived to study endpoint (d28). | Lymphocytopenia (d10); monocytopenia (d6, 14); granulocytopenia (d14); erythrocytopenia (d14); anemia (d14); hypoglycemia (d8); ALT ↑ (d21) |
| AGM-1-3-6 | F   | HeV   | Prime/Boost | None. Subject survived to study endpoint (d28). | Lymphocytopenia (d10); monocytopenia (d6, 14); granulocytopenia (d8) |
| AGM-1-4-1 | M   | NiV-B | Prime/Boost | None. Subject survived to study endpoint (d28). | Monocytopenia (d6) |
| AGM-1-4-2 | M   | NiV-B | Prime/Boost | None. Subject survived to study endpoint (d28). | Granulocytopenia (d3, 14, 28); monocytosis (d3, 6, 8, 14, 21, 28); hypoglycemia (d28) |
| Code     | Gender | Prime/Boost | Treatment | Clinical Observations                                                                 | Laboratory Observations                                      |
|----------|--------|-------------|-----------|---------------------------------------------------------------------------------------|-----------------------------------------------------------------|
| AGM-1-4-3 | M      | NiV-B       | None      | Subject survived to study endpoint (d28).                                               | Lymphocytopenia (d6, 14); erythrocytopenia (d14); anemia (14)    |
| AGM-1-4-4 | F      | NiV-B       | None      | Subject survived to study endpoint (d28).                                               | Monocytosis (d21)                                               |
| AGM-1-4-5 | F      | NiV-B       | None      | Subject survived to study endpoint (d28).                                               | Lymphocytosis (d8); monocytosis (d8, 28); granulocytosis (d6, 14, 21) |
| AGM-1-4-6 | F      | NiV-B       | None      | Subject survived to study endpoint (d28).                                               | Granulocytopenia (d3); thrombocytopenia (d14); monocytopenia (d14); erythrocytopenia (d14); granulocytosis (d6); monocytosis (d21); lymphocytosis (d21) |
| AGM-1-C1-1 | M      | HeV         | Control   | Depression (d7); weakness (d7); recumbency (d7); abdominal breathing (d7). Subject succumbed to disease (d7). | Lymphocytopenia (d6); granulocytopenia (d3, 6); thrombocytopenia (d6, 7); monocytosis (d3, 6, 7); granulocytosis (d7); hyperglycemia (d7); CRE ↑ (d7); hypoalbuminemia (d7); hypoproteinemia (d7); CRP ↑ (d7) |
| AGM-1-C1-2 | M      | HeV         | Control   | Anorexia (d9, 10); tachypnea (d9-11); depression (d10). Subject survived to study endpoint (d28). | Lymphocytopenia (d6, 8); monocytopenia (d6); thrombocytopenia (d8, 10); monocytosis (d14); BUN ↑ (d10); hypoalbuminemia (d10); hypoamylasemia (d3); CRP ↑ (d8, 10) |
| AGM-1-C1-3 | F      | HeV         | Control   | Anorexia (d8); depression (d8); weakness (d8); hypothermia (d8); recumbency (d8); unresponsiveness (d8); bradypnea (d8). Subject succumbed to disease (d8). | Lymphocytopenia (d3, 6); monocytopenia (d6); thrombocytopenia (d8); granulocytosis (d8); hypoglycemia (d8); CRE ↑ (d8); hypoalbuminemia (d8); hypoproteinemia (d8); hypoamylasemia (d6); CRP ↑ (d8) |
| AGM-1-C2-1 | M      | NiV-B       | Control   | Anorexia (d6); hypothermia (d6); depression (d6); weakness (d6); recumbency (d6); unresponsiveness (d6); bradypnea (d6); nasal exudate (d6). Subject succumbed to disease (d6). | Lymphocytopenia (d3, 6); monocytosis (d6); granulocytopenia (d3); thrombocytopenia (d6); granulocytosis (d6); BUN ↑ (d6); CRE ↑ (d6); hypoalbuminemia (d6); hypoproteinemia (d6); hypoamylasemia (d6); AST ↑↑↑↑ (d6); ALP ↓ (d6) |
| AGM-1-C2-2 | M      | NiV-B       | Control   | Pleural effusion (d6); abdominal breathing (d7); depression (d7); weakness (d7); recumbency (d7); unresponsiveness (d7); bradypnea (d7). Subject succumbed to disease (d7). | Lymphocytopenia (d6); monocytopenia (d7); granulocytopenia (d3, 6); thrombocytopenia (d7); BUN ↑ (d7); CRE ↑ (d7); hypoalbuminemia (d7); AST ↑ (d7); CRP ↑ (d6); ↑↑↑ (d7) |
| AGM-1-C2-3 | F      | NiV-B       | Control   | Anorexia (d8); depression (d8); weakness (d8); hypothermia (d8); recumbency (d8); unresponsiveness (d8); abdominal breathing (d8). Subject succumbed to disease (d8). | Lymphocytopenia (d8); thrombocytopenia (d8); monocytopenia (d6, 8); granulocytosis (d8); hypoglycemia (d3); hyperglycemia (d8); BUN ↑ (d8); CRE ↑ (d8); hypoalbuminemia (d8); CRP ↑ (d8) |
Supplementary Table 1. Clinical description and outcome of HeV-sG-vaccinated African green monkeys following henipavirus challenge.

Days after henipavirus challenge are in parentheses. All reported findings are in comparison to baseline (d0) values. Anorexia is defined as no food consumed from the previous day. Fever is defined as a temperature more than 2.5 °F over baseline, or at least 1.5 °F over baseline and ≥ 103.5 °F. Hypothermia is defined as a temperature ≤3.5°F below baseline. Lymphocytopenia, monocytopenia, erythrocytopenia, thrombocytopenia, and granulocytopenia are defined by a ≥35% drop in numbers of lymphocytes, monocytes, erythrocytes, platelets, or granulocytes, respectively. Lymphocytosis, monocytesis, and granulocytosis are defined by a 100% or greater increase in numbers of lymphocytes, monocytes, or granulocytes, respectively. Hyperglycemia is defined as a 100% or greater increase in levels of glucose. Hypoglycemia is defined by a ≥25% decrease in levels of glucose. Anemia is defined as a concurrent ≥25% decrease in erythrocyte count, Hct, and Hgb. Hypoalbuminemia is defined by a ≥25% decrease in levels of albumin. Hypoproteinemia is defined by a ≥25% decrease in levels of total protein. Hypoamylasemia is defined by a ≥25% decrease in levels of serum amylase. Hyperamylasemia is defined as a ≥ 100% increase in levels of serum amylase. Hypocalcemia is defined by a ≥25% decrease in levels of serum calcium. Increases in ALT, AST, ALP, CRE, CRP, Hct, and Hgb were graded on the following scale: ↑ = 1-5 fold (2-5 fold for ALT/AST), ↑↑ = >5-10 fold, ↑↑↑ = >10-20 fold, ↑↑↑↑ = >20-fold, ↓ = ≥50% decrease. (BUN) blood urea nitrogen, (ALT) alanine aminotransferase, (AST) aspartate aminotransferase, (ALP) alkaline phosphatase, (CRE) Creatinine, (CRP) C-reactive protein, (Hct) hematocrit, (Hgb) hemoglobin.
| NHP     | Sex | Regimen   | Clinical illness                                                                 | Clinical pathology                                                                 |
|---------|-----|-----------|-----------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|
| AGM-2-1-1 | F   | 100 µg, 7dbc | Anorexia (d5). Subject survived to study endpoint (d28).                           | Monocytopenia (d4, 21, 28); granulocytopenia (d4, 7, 28); lymphocytosis (d10, 14) |
| AGM-2-1-2 | M   | 100 µg, 7dbc | None. Subject survived to study endpoint (d28).                                    | Monocytopenia (d4, 7, 21); granulocytopenia (d4, 7); lymphocytosis (d14); monocytosis (d10, 14) |
| AGM-2-1-3 | M   | 100 µg, 7dbc | Anorexia (d9); abdominal breathing (d9, 10); open-mouth breathing (d9); rhinorrhea (d9); tachypnea (d11, 12); hypothermia (d21). Subject survived to study endpoint (d28). | Granulocytopenia (d4, 10, 28); thrombocytopenia (d10); lymphocytosis (d14, 21); monocytosis (d7, 10, 14, 21, 28); CRP ↑ (d7, 10) |
| AGM-2-2-1 | F   | 100 µg, 14dbc | Abdominal breathing (d8). Subject survived to study endpoint (d28).                | Granulocytopenia (d4, 10, 21, 28); monocytosis (d7, 10, 21, 14, 28); hypoglycemia (d10) |
| AGM-2-2-2 | F   | 100 µg, 14dbc | None. Subject survived to study endpoint (d28).                                    | Monocytopenia (d4, 10, 14, 21, 28); granulocytopenia (d4, 14, 21, 28) |
| AGM-2-2-3 | M   | 100 µg, 14dbc | None. Subject survived to study endpoint (d28).                                    | Thrombocytopenia (d10); lymphocytosis (d10); monocytosis (d10) |
| AGM-2-2-4 | M   | 100 µg, 14dbc | None. Subject survived to study endpoint (d28).                                    | Granulocytopenia (d4, 7); thrombocytopenia (d7); monocytosis (d7, 10, 14, 28); lymphocytosis (d10, 14, 28) |
| AGM-2-3-1 | F   | 300 µg, 14dbc | Anorexia (d6, 20, 21, 24, 25); depression (d20-25); weakness (d20-25); ptosis (d20-25); facial palsy (d20-23); hunched posture (d22-24); tremor (d22, 24); recumbency (d25); bradypnea (d25); hypothermia (d21, 25). Subject succumbed to disease (d25). | Monocytosis (d4, 7, 10, 14); lymphocytopenia (d25); monocytopenia (d25); granulocytopenia (d4, 7, 25); granulocytosis (d10); hyperglycemia (d21, 25); BUN ↑ (d21), ↑↑ (d25); CRE ↑↑ (d25); hyperamylasemia (d25); ALP ↑ (d25); CRP ↑↑ (d25) |
| AGM-2-3-2 | F   | 300 µg, 14dbc | None. Subject survived to study endpoint (d28).                                    | Monocytopenia (d4); granulocytopenia (d7) |
| AGM-2-3-3 | M   | 300 µg, 14dbc | None. Subject survived to study endpoint (d28).                                    | Granulocytopenia (d10); monocytosis (d14) |
| AGM-2-3-4 | M   | 300 µg, 14dbc | None. Subject survived to study endpoint (d28).                                    | Monocytopenia (d7); granulocytosis (d14); CRE ↑ (d28); CRP ↑ (d7) |
| AGM-2-C-1 | M   | Control    | Fever (d7); abdominal breathing (d7). Subject succumbed to disease (d8).           | Lymphocytopenia (d7); granulocytopenia (d4); monocytosis (d7); granulocytosis (d7); hypoglycemia (d7); hypoamylasemia (d7); CRP ↑ (d7) |
| AGM-2-C-2 | F   | Control    | Anorexia (d6); depression (d6); weakness (d6); hunched posture (d6); hypothermia (d6); recumbency (d6); tachypnea (d6); abdominal breathing (d6). Subject succumbed to disease (d6). | Thrombocytopenia (d6); monocytosis (d6); granulocytosis (d6); BUN ↑ (d6); CRE ↑ (d6); hypoalbuminemia (d6); hypoproteinemia (d6); hyperamylasemia (d6); CRP ↑ (d6) |
Supplementary Table 2. Clinical description and outcome of HeV-sG-vaccinated African green monkeys following NiV-B challenge. Abreviation “dbc” stands for “days before challenge”. Days after NiV-B challenge are in parentheses. All reported findings are in comparison to baseline (d0) values. Anorexia is defined as no food consumed from the previous day. Fever is defined as a temperature more than 2.5 °F over baseline, or at least 1.5 °F over baseline and ≥ 103.5 °F. Hypothermia is defined as a temperature ≤3.5°F below baseline. Lymphocytopenia, monocytopenia, erythrocytopenia, thrombocytopenia, and granulocytopenia are defined by a ≥35% drop in numbers of lymphocytes, monocytes, erythrocytes, platelets, or granulocytes, respectively. Lymphocytosis, monocytosis, and granulocytosis are defined by a 100% or greater increase in numbers of lymphocytes, monocytes, or granulocytes, respectively. Hyperglycemia is defined as a 100% or greater increase in levels of glucose. Hypoglycemia is defined by a ≥25% decrease in levels of glucose. Anemia is defined as a concurrent ≥25% decrease in erythrocyte count, Hct, and Hgb. Hypoalbuminemia is defined by a ≥25% decrease in levels of albumin. Hypoproteinemia is defined by a ≥25% decrease in levels of total protein. Hypoamylasemia is defined by a ≥25% decrease in levels of serum amylase. Hyperamylasemia is defined as a ≥ 100% increase in levels of serum amylase. Hypocalcemia is defined by a ≥25% decrease in levels of serum calcium. Increases in ALT, AST, ALP, CRE, CRP, Hct, and Hgb were graded on the following scale: ↑ = 1-5 fold (2-5 fold for ALT/AST), ↑↑ = >5-10 fold, ↑↑↑ = >10-20 fold, ↑↑↑↑ = >20-fold, ↓ = ≥50% decrease. (BUN) blood urea nitrogen, (ALT) alanine aminotransferase, (AST) aspartate aminotransferase, (ALP) alkaline phosphatase, (CRE) Creatinine, (CRP) C-reactive protein, (Hct) hematocrit, (Hgb) hemoglobin.
### Supplementary Table 3. Viral RNA in blood samples in Study 2 measured by qRT-PCR.

All data for samples with RNA copy reading below LOD (Limit of Detection, $1.00 \times 10^3$ GEq/mL) are skipped and only data for samples with positive readings are shown in the table. Data for both controls AGM-2-C-1 and AGM-2-C-2 blood samples at the day of challenge, D0, are shown for reference.

| AGM / Group | Day  | RNA copies (GEq/ml) | $\log_{10}$ RNA copies / ml |
|-------------|------|---------------------|----------------------------|
| AGM-2-C-1 / Control | D0   | <LOD                | <LOD                       |
|              |      |                     |                            |
|              | D7   | $1.24 \times 10^7$  | $7.09$                     |
| AGM-2-C-2 / Control | D0   | <LOD                | <LOD                       |
|              |      |                     |                            |
|              | D4   | $6.04 \times 10^5$  | $5.78$                     |
|              | D6   | $5.60 \times 10^7$  | $7.75$                     |
| AGM-2-1-1 / Group 1 | D4   | $1.54 \times 10^5$  | $5.19$                     |
|              | D7   | $2.88 \times 10^5$  | $5.46$                     |
| AGM-2-1-2 / Group 1 | D7   | $1.34 \times 10^6$  | $6.13$                     |
| AGM-2-1-3 / Group 1 | D7   | $4.74 \times 10^6$  | $6.68$                     |
|              | D10  | $3.33 \times 10^6$  | $6.52$                     |
| Tissue sample from: | LOG$_{10}$ (RNA copies (GEq/gram)) |
|--------------------|-----------------------------------|
|                    | AGM-2-C-2 Control | AGM-2-C-1 Control | AGM-2-1-2 Group 1 | AGM-2-1-1 Group 1 | AGM-2-3-4 Group 3 | AGM-2-3-1 Group 3 |
| Axillary LN         | 7.91               | 7.58               | 5.22               |                   |                   |                   |
| Inguinal LN         | 8.02               | 7.96               | 5.36               |                   |                   |                   |
| Liver               | 7.85               | 7.01               |                   |                   | 5.31               |                   |
| Spleen              | 5.45               | 8.16               | 5.59               | 5.75               | 6.30               |                   |
| Kidney              | 7.91               | 7.83               | 5.04               |                   |                   |                   |
| Adrenal             | 7.68               | 7.26               |                   |                   |                   |                   |
| Lung RU             | 9.72               | 8.57               |                   |                   |                   |                   |
| Lung RM             | 9.66               | 9.34               |                   |                   |                   |                   |
| Lung RL             | 9.41               | 9.13               |                   |                   |                   |                   |
| Lung LU             | 9.74               | 9.02               |                   |                   |                   |                   |
| Lung LM             | 9.29               | 9.30               |                   |                   |                   |                   |
| Lung LL             | 9.54               | 9.28               |                   |                   |                   |                   |
| Brain Front         | 6.69               | 6.95               |                   |                   |                   |                   |
| Brain Stem          | 7.60               | 7.60               |                   | 5.23               | 6.12               |                   |
| CSC                 | 6.80               | 5.77               | 4.85               |                   |                   | 4.92               |
| Pancreas            | 6.44               | 6.44               |                   |                   |                   |                   |
| Urinary bladder     | 6.30               | 6.98               |                   |                   |                   |                   |
| Gonad               | 7.71               | 6.83               |                   |                   |                   |                   |
| Uterus or Prostate  | 7.60               | 7.41               |                   |                   |                   |                   |
| Conjunctiva         | 6.76               | 7.36               |                   |                   |                   |                   |
| Eye                 | 6.30               | 6.96               |                   |                   |                   |                   |

**Supplementary Table 4. Study 2 tissue samples viral RNA measured by qRT-PCR.** Only samples with RNA copy reading above LOD (Limit of Detection, $1.00 \times 10^3$ GEq/gram) are shown. Missing number in the table means measured value is below LOD. The AGM that died on D25 had NiV RNA in brain stem and CSC, however, no virus could be isolated from these tissues.
| AGM ID  | Challenge Day | End of Study Day |
|---------|---------------|------------------|
| Control |               |                  |
| AGM-2-C-2| < LOD        | n/a              |
| AGM-2-C-1| < LOD        | n/a              |
| Group 1  |               |                  |
| AGM-2-1-1| < LOD        | 1280             |
| AGM-2-1-2| < LOD        | 160              |
| AGM-2-1-3| < LOD        | 640              |
| Group 2  |               |                  |
| AGM-2-2-1| 20           | 320              |
| AGM-2-2-2| 10           | 320              |
| AGM-2-2-3| < LOD        | 320              |
| AGM-2-2-4| < LOD        | 320              |
| Group 3  |               |                  |
| AGM-2-3-1| < LOD        | 320*             |
| AGM-2-3-2| < LOD        | 160              |
| AGM-2-3-3| 10           | 160              |
| AGM-2-3-4| < LOD        | 640              |

**Supplementary Table 5. Study 2 Serum Neutralization Titers (SNT)** from samples at the challenge day and termination day. Asterisk (*) refers to the AGM that died on D25, which day was the end of study day for this animal. The limit of detection (LOD) for this assay is 10.
| ID number   | Gender | Body weight (kg) | Treatment Group |
|------------|--------|-----------------|-----------------|
| AGM-1-C1-1 | M      | 5.17            | Control, HeV (C1). |
| AGM-1-C1-2 | M      | 5.50            |                 |
| AGM-1-C1-3 | F      | 4.32            |                 |
| AGM-1-C2-1 | M      | 5.24            | Control, NiV (C2). |
| AGM-1-C2-2 | M      | 5.68            |                 |
| AGM-1-C2-3 | F      | 4.68            |                 |
| AGM-1-1-1  | M      | 3.56            | Single dose, HeV (1). |
| AGM-1-1-2  | M      | 4.67            |                 |
| AGM-1-1-3  | F      | 3.62            |                 |
| AGM-1-2-1  | M      | 4.62            | Single dose, NiV (2). |
| AGM-1-2-2  | F      | 3.52            |                 |
| AGM-1-2-3  | F      | 3.90            |                 |
| AGM-1-3-1  | F      | 4.34            |                 |
| AGM-1-3-2  | M      | 4.25            |                 |
| AGM-1-3-3  | F      | 3.38            | Prime/Boost, HeV (3). |
| AGM-1-3-4  | M      | 5.00            |                 |
| AGM-1-3-5  | F      | 3.70            |                 |
| AGM-1-3-6  | F      | 3.70            |                 |
| AGM-1-4-1  | M      | 4.56            |                 |
| AGM-1-4-2  | M      | 3.94            |                 |
| AGM-1-4-3  | M      | 5.14            | Prime/Boost, NiV (4). |
| AGM-1-4-4  | F      | 3.44            |                 |
| AGM-1-4-5  | F      | 3.68            |                 |
| AGM-1-4-6  | F      | 3.68            |                 |

**Supplementary Table 6. Animal Identification Number and Group Assignment in Study 1.**

Body weights measured at the initial physical exam are shown in this table.
| ID number | Gender | Body weight (kg) | Treatment Group |
|-----------|--------|-----------------|-----------------|
| AGM-2-1-1 | F      | 4.00            | Group 1         |
| AGM-2-1-2 | M      | 5.12            | 100-mg dose     |
| AGM-2-1-3 | M      | 5.45            | challenge on day 7 post immunization |
| AGM-2-2-1 | F      | 3.44            | Group 2         |
| AGM-2-2-2 | F      | 3.76            | 100-mg dose     |
| AGM-2-2-3 | M      | 5.54            | challenge on day 14 |
| AGM-2-2-4 | M      | 5.09            | post immunization |
| AGM-2-3-1 | F      | 4.04            | Group 3         |
| AGM-2-3-2 | F      | 3.16            | 300-mg dose     |
| AGM-2-3-3 | M      | 4.66            | challenge on day 14 |
| AGM-2-3-4 | M      | 5.92            | post immunization |
| AGM-2-C-1 | M      | 5.81            | Control challenged day 14 |
| AGM-2-C-2 | F      | 3.30            | Control challenged day 7 post immunization |

**Supplementary Table 7. Animal Identification Number and Group Assignment in Study 2.**

Body weights measured at the initial physical exam are shown in this table.
| Study Day | Post Challenge Day | Whole Blood | Sera |
|-----------|-------------------|-------------|------|
|           |                   | Hematology  | Viral RNA qRT-PCR | Serum Chemistry | Anti-HeV-sG Antibodies / SNT |
| 0         |                   | X           | X               |                | - / X |
| 28        |                   | X           | X               | - / X          |      |
| 56        | 0                 | X           | X               | X              | X / X |
| 59        | 3                 | X           | X               | X              | X / - |
| 62        | 6                 | X           | X               | X              | X / - |
| 64        | 8                 | X           | X               | X              | X / - |
| 66        | 10                | X           | X               | X              | X / - |
| 70        | 14                | X           | X               | X              | X / - |
| 77        | 21                | X           | X               | X              | X / - |
| 84        | 28                | X           | X               | X              | X / X |

**Supplementary Table 8.** Schedule for clinical and analytical evaluation samples collection schedule in Study 1. Quantification for antibody binding titers and serum neutralization titers (SNT) is shown in Figs. 2 and 3.
| Study Day | Post Challenge Day | Whole Blood | Sera |
|-----------|--------------------|-------------|------|
|           |                    | Hematology  | Viral RNA qRT-PCR | Serum Chemistry | Neutralization Titers (50%) |
| 0         | -14                | X           | X             | X             | X#                        |
| 7         | -7                 | X           | X             | X             | X#                        |
| 14        | 0                  | X           | X             | X             | X                         |
| 18        | 4                  | X           | X             | X             |                           |
| 20        | 6                  | X           | X             | X             |                           |
| 21        | 7                  | X           | X             | X             |                           |
| 24        | 10                 | X           | X             | X             |                           |
| 28        | 14                 | X           | X             | X             |                           |
| 35        | 21                 | X           | X             | X             |                           |
| 39        | 25                 | X           | X             | X             | X                         |
| 42        | 28                 | X           | X             | X             | X                         |

**Supplementary Table 9. Schedule for clinical and analytical evaluation samples collection in Study 2.** The superscript # denotes control samples taken to measure base pre-immunity (days -7 and -14). AGMs from groups 2 and 3 were sampled at day -14, but not at day -7.