**Table S2.** Molecular comparison between Astrakhan viruses and other H5N8 viruses during 2014-2020.

| | **Virus** | **Ak/3212/2020** | **Ck/Ak/321-01/2020** | **Ck/Ak/2171-1/2020** | **Ck/RoD/308-02/2020** | **Ck/V/1488/2018** | **Ck/RoD/44/2017** | **Ck/V/18/2017** | **Ck/Ak/3131/2016** | **Ck/NL/14015526/2014** |
|---|---|---|---|---|---|---|---|---|---|---|
| **Protein** | **\Host Residue** | human | chicken | chicken | chicken | chicken | chicken | chicken | chicken | chicken |
| **PB2** | 4 | I | I | I | I | I | T | I | I | I |
| | 66 | M | M | M | M | M | M | M | I | M |
| | 67 | I | I | I | I | I | I | I | I | V |
| | 82 | N | N | N | N | S | N | N | N | N |
| | 89 | V | V | V | V | V | V | V | V | V |
| | 106 | T | T | T | T | T | T | T | T | A |
| | 109 | V | V | V | A | V | V | V | V | V |
| | 139 | V | V | V | V | V | V | V | V | I |
| | 176 | I | I | I | I | V | I | I | I | I |
| | 187 | R | R | R | R | K | K | K | R | K |
| | 199 | A | A | A | A | T | A | A | A | A |
| | 261 | I | I | I | I | I | V | I | I | I |
| | 292 | V | V | V | V | V | V | V | V | T |
| | 338 | V | V | V | V | V | V | V | V | I |
| | 339 | K | K | K | K | K | K | K | K | R |
| | 340 | R | R | R | R | R | R | R | K | K |
| | 344 | I | I | I | I | I | V | V | V | V |
| | 354 | V | V | V | V | I | I | I | I | I |
| | 356 | I | I | I | I | V | V | V | V | V |
| | 453 | P | P | P | P | L | P | P | P | P |
| | 508 | R | R | R | K | K | R | R | R | Q |
| | 522 | H | H | H | H | Q | Q | Q | Q | L |
| | 556 | S | S | S | N | N | N | N | N | N |
| | 559 | T | T | T | T | T | T | T | T | I |
| | 575 | M | M | M | M | M | M | M | M | V |
| | 611 | D | D | D | D | D | D | D | N | D |
| | 613 | V | V | V | V | V | V | V | V | I |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 617 | K | K | K | K | K | K | K | R | K |
| | 627 | E | E | E | E | E | E | E | E | E |
| | 628 | Q | Q | Q | Q | Q | Q | Q | Q | H |
| | 630 | R | R | R | R | R | R | R | K | R |
| | 666 | T | T | T | T | T | T | T | T | A |
| | 667 | V | V | V | V | V | V | V | I | V |
| | 677 | G | G | G | E | E | E | E | E | E |
| | 678 | Y | Y | Y | Y | Y | Y | Y | D | D |
| | 684 | A | A | A | A | A | A | A | S | T |
| | 699 | K | K | K | K | K | K | K | K | R |
| | 701 | D | D | D | D | D | D | D | D | D |
| | 702 | K | K | K | K | K | K | K | K | R |
| | 757 | A | A | A | A | A | A | A | A | V |
| **PB1** | 57 | T | T | T | T | T | T | T | T | K |
| | 99 | H | H | H | H | H | H | H | H | H |
| | 110 | A | A | A | A | A | A | A | A | T |
| | 113 | I | I | I | I | I | V | V | V | I |
| | 142 | S | S | S | S | A | A | A | A | A |
| | 168 | R | R | R | R | R | R | R | R | K |
| | 181 | V | V | V | V | I | I | I | I | I |
| | 215 | R | R | R | R | R | R | R | R | K |
| | 216 | N | N | N | S | S | S | S | S | N |
| | 219 | I | I | I | I | I | I | I | I | V |
| | 302 | I | I | I | V | I | I | I | I | I |
| | 317 | M | M | M | M | M | M | M | M | I |
| | 361 | S | S | S | S | S | N | S | S | S |
| | 368 | I | I | I | I | I | I | I | I | I |
| | 374 | T | T | T | T | T | T | T | T | A |
| | 375 | N | N | N | N | N | N | N | N | T |
| | 384 | P | P | P | P | S | S | S | S | S |
| | 398 | D | D | D | D | D | D | D | D | E |
| | 569 | Q | Q | Q | Q | Q | Q | H | Q | Q |
| | 571 | R | R | R | R | K | R | R | R | R |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 591 | I | I | I | I | I | V | V | V | V |
| | 614 | D | D | D | E | E | E | E | E | E |
| | 633 | S | S | S | S | S | N | N | S | S |
| | 645 | V | V | V | V | I | V | V | V | V |
| | 655 | M | M | M | M | M | M | I | M | M |
| | 667 | I | I | I | I | I | I | I | I | V |
| | 694 | S | S | S | S | S | S | S | S | N |
| | 740 | F | F | F | F | L | F | F | F | F |
| | 744 | M | M | M | M | M | M | L | M | M |
| | 753 | L | L | L | L | L | L | L | L | I |
| **PB1-F2** | del. | 1-38 aa | 1-38 aa | 1-38 aa | 1-38 aa | 1-38 aa | 1-38 aa | 1-38 aa | 1-38 aa | No |
| | 43 | Q | Q | Q | Q | Q | Q | Q | Q | L |
| | 45 | T | T | T | T | T | T | T | T | I |
| | 46 | M | M | M | M | M | M | M | T | T |
| | 54 | L | L | L | L | L | L | L | L | R |
| | 56 | A | A | A | A | A | A | A | A | V |
| | 66 | N | N | N | N | N | N | N | N | S |
| | 71 | Y | Y | Y | Y | Y | Y | Y | Y | S |
| | 75 | L | L | L | L | L | L | L | L | H |
| | 79 | R | R | R | R | R | R | R | R | Q |
| | 82 | S | S | S | S | S | S | S | S | L |
| | 84 | S | S | S | S | S | S | S | S | N |
| **PA** | 20 | A | A | A | A | A | A | A | A | T |
| | 27 | D | D | D | D | D | D | D | D | N |
| | 36 | A | A | A | A | T | A | A | A | A |
| | 59 | E | E | E | E | E | E | E | G | K |
| | 96 | N | N | N | N | N | N | N | H | N |
| | 100 | V | V | V | V | V | V | V | V | V |
| | 101 | E | E | E | E | E | E | E | E | D |
| | 113 | R | R | R | R | R | R | R | K | K |
| | 115 | D | D | D | N | N | N | N | N | N |
| | 127 | V | V | V | V | A | V | V | V | V |
| | 129 | I | I | I | I | I | I | I | I | T |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 162 | T | T | T | T | T | T | T | T | I |
| | 184 | N | N | N | N | N | S | S | N | N |
| | 216 | D | D | D | D | D | Y | D | D | D |
| | 224 | A | A | A | A | S | S | S | S | S |
| | 237 | E | E | E | E | E | E | E | E | K |
| | 242 | I | I | I | I | I | I | I | I | L |
| | 261 | L | L | L | L | L | L | L | L | M |
| | 268 | L | L | L | L | I | L | L | L | L |
| | 308 | T | T | T | T | T | I | I | I | I |
| | 312 | K | K | K | R | K | K | K | K | K |
| | 327 | E | E | E | E | E | E | E | E | V |
| | 343 | S | S | S | S | A | A | A | A | V |
| | 356 | K | K | K | K | K | K | K | K | K |
| | 386 | D | D | D | D | E | D | D | D | D |
| | 400 | S | S | S | S | S | S | S | S | P |
| | 404 | A | A | A | A | A | A | A | A | S |
| | 409 | S | S | S | S | S | S | S | S | S |
| | 472 | A | A | A | A | A | A | A | A | S |
| | 492 | K | K | K | K | K | R | K | K | K |
| | 545 | V | V | V | V | I | I | I | I | I |
| | 561 | M | M | M | M | V | M | M | M | M |
| | 598 | T | A | A | A | A | A | A | A | A |
| | 615 | R | R | R | R | R | R | R | K | K |
| | 618 | T | T | T | T | A | T | T | T | T |
| | 631 | G | G | G | G | G | G | G | G | S |
| | 672 | L | L | L | L | L | L | L | L | F |
| | 682 | D | D | D | D | N | D | D | D | D |
| | 683 | L | L | L | L | I | L | L | L | L |
| | 700 | V | V | V | V | V | V | V | A | V |
| **HA** | 14 | E | E | E | E | E | E | E | E | K |
| | 38 | N | N | N | N | N | N | N | N | T |
| | 72 | R | R | R | R | R | R | S | S | R |
| | 94 | S | S | S | S | S | S | S | S | T |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 97 | D | D | D | D | N | D | D | D | D |
| | 114 | I | I | I | I | I | I | I | I | T |
| | 138 | Q | Q | Q | Q | L | Q | Q | Q | Q |
| | 140 | A | A | A | A | T | T | T | T | A |
| | 141 | P | P | P | P | P | P | P | P | S |
| | 181 | S | S | S | S | S | S | S | S | P |
| | 195 | T | T | T | T | T | T | T | T | D |
| | 198 | I | I | I | I | I | I | I | I | V |
| | 236 | D | D | D | D | N | N | N | N | N |
| | 268 | G | G | G | G | G | G | G | G | E |
| | 282 | V | V | V | V | V | V | V | V | I |
| | 374 | I | I | I | I | I | I | I | I | V |
| | 490 | Q | Q | Q | Q | Q | Q | Q | Q | K |
| | 522 | A | A | A | A | V | V | V | V | V |
| | 531 | M | M | M | M | M | M | M | M | I |
| | 532 | M | M | M | M | M | V | V | V | V |
| | 549 | I | I | I | I | V | I | I | I | I |
| | CS | REKRRKR↓GLF | REKRRKR↓GLF | REKRRKR↓GLF | REKRRKR↓GLF | REKRRKR↓GLF | REKRRKR↓GLF | REKRRKR↓GLF | REKRRKR↓GLF | RERRRKR↓GLF |
| | RBS | QRG | QRG | QRG | QRG | QRG | QRG | QRG | QRG | QRG |
| **NP** | 16 | S | S | S | G | G | G | G | G | G |
| | 50 | S | S | S | S | S | S | S | N | S |
| | 51 | D | D | D | D | D | D | D | D | N |
| | 105 | V | V | V | V | V | V | V | I | M |
| | 129 | S | S | S | A | A | A | A | T | A |
| | 201 | V | V | V | V | I | I | I | I | I |
| | 219 | F | F | F | F | Y | Y | Y | Y | Y |
| | 371 | I | I | I | I | M | M | M | M | M |
| | 374 | M | M | I | M | M | M | M | M | M |
| | 452 | R | R | R | K | R | R | R | K | K |
| | 460 | G | G | G | G | S | G | G | G | G |
| | 492 | N | N | N | N | N | N | N | N | S |
| **NA** | Stalk del. | No | No | No | No | No | No | No | No | No |
| | 9 | A | A | A | A | A | A | A | A | V |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 28 | L | L | L | L | V | V | V | V | V |
| | 29 | N | S | S | S | S | S | S | S | S |
| | 33 | M | M | M | M | M | M | M | M | T |
| | 37 | L | L | L | L | L | P | L | L | L |
| | 47 | K | K | K | K | K | K | K | K | N |
| | 50 | I | I | I | I | I | I | I | I | V |
| | 51 | I | I | I | V | V | V | V | V | V |
| | 70 | S | S | S | S | S | S | N | N | S |
| | 71 | V | V | V | V | A | V | V | V | V |
| | 73 | E | E | E | E | K | E | E | E | E |
| | 82 | A | A | A | A | A | A | A | A | T |
| | 107 | I | I | I | I | V | V | V | V | V |
| | 114 | F | F | F | F | F | Y | F | F | F |
| | 137 | A | A | A | A | A | A | A | A | S |
| | 181 | T | T | T | T | A | T | T | T | T |
| | 202 | I | I | I | I | V | V | V | V | V |
| | 205 | V | V | V | V | I | V | V | V | V |
| | 214 | I | I | I | I | V | V | V | V | V |
| | 246 | S | S | S | S | A | A | A | A | A |
| | 264 | D | D | D | D | D | D | D | D | G |
| | 265 | Q | Q | Q | Q | Q | Q | Q | Q | R |
| | 266 | A | A | A | A | T | T | T | T | T |
| | 274 | H | H | H | H | H | H | H | H | H |
| | 292 | R | R | R | R | R | R | R | R | R |
| | 296 | I | I | I | M | T | T | T | T | T |
| | 304 | V | V | V | V | V | V | V | V | I |
| | 330 | A | A | A | A | A | A | A | A | T |
| | 360 | M | M | M | M | V | V | V | V | V |
| | 398 | L | L | L | L | L | L | L | L | S |
| | 451 | H | H | H | H | H | H | H | H | Y |
| | 470 | G | G | G | G | K | K | K | K | K |
| | 471 | M | M | M | M | M | M | M | M | T |
| **M1** | 15 | V | V | V | V | V | V | V | V | I |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 30 | D | D | D | D | D | D | D | D | D |
| | 33 | A | A | A | A | V | V | V | V | A |
| | 41 | A | A | A | A | A | A | A | T | A |
| | 42 | L | L | L | L | I | L | L | L | L |
| | 83 | A | A | A | A | S | A | A | A | A |
| | 95 | R | R | R | R | R | R | R | R | K |
| | 101 | R | R | R | R | R | R | R | R | K |
| | 120 | T | T | T | T | T | T | T | A | T |
| | 133 | K | K | K | K | K | K | K | R | R |
| | 139 | A | A | A | A | T | T | T | T | T |
| | 143 | L | L | L | L | L | L | L | L | F |
| | 165 | I | I | I | I | I | I | I | I | M |
| | 168 | T | T | T | T | T | I | I | T | I |
| | 200 | A | A | A | A | A | A | A | A | S |
| | 205 | V | V | V | V | I | V | V | V | V |
| | 207 | S | S | S | S | S | S | S | S | N |
| | 214 | Q | Q | Q | Q | H | Q | Q | Q | Q |
| | 218 | T | T | T | T | T | T | T | T | T |
| | 224 | S | S | S | S | S | S | S | S | N |
| | 232 | D | D | D | D | D | D | D | D | N |
| | 242 | K | K | K | K | K | K | K | K | N |
| | 248 | L | L | L | L | M | M | M | M | M |
| **M2** | 12 | K | K | K | K | R | R | R | R | R |
| | 13 | N | N | N | N | N | N | N | N | T |
| | 14 | G | G | G | G | G | E | G | G | E |
| | 18 | N | N | N | N | K | K | K | K | R |
| | 23 | S | S | S | S | N | S | S | S | S |
| | 28 | I | I | I | I | I | I | I | I | V |
| | 31 | S | S | S | S | S | S | S | S | N |
| | 35 | I | I | I | I | V | I | I | I | I |
| | 51 | V | V | V | V | V | V | V | V | I |
| | 54 | R | R | R | R | R | C | R | R | R |
| | 61 | R | R | R | R | R | R | R | R | I |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 65 | T | T | T | T | T | A | T | T | T |
| **NS1** | del. | 218-237 aa | 218-237 aa | 218-237 aa | 218-237 aa | 218-237 aa | 218-237 aa | 218-237 aa | 218-237 aa | No |
| | 3 | P | P | P | P | S | S | S | S | S |
| | 6 | M | M | M | M | M | M | M | M | V |
| | 7 | L | L | L | L | L | L | L | L | S |
| | 17 | Y | Y | Y | Y | Y | Y | Y | Y | H |
| | 20 | K | K | K | K | R | K | K | K | K |
| | 42 | S | S | S | S | S | S | S | S | S |
| | 47 | G | G | G | G | G | S | G | G | G |
| | 48 | T | T | T | T | N | N | N | N | S |
| | 53 | S | S | S | S | G | G | G | G | D |
| | 56 | A | A | A | A | T | T | T | T | T |
| | 60 | E | E | E | E | A | A | A | A | A |
| | 64 | I | I | I | I | I | I | I | I | T |
| | 65 | V | V | V | V | V | V | V | V | L |
| | 66 | K | K | K | K | E | E | E | E | E |
| | 70 | K | K | K | K | E | E | E | E | G |
| | 80 | T | T | T | T | T | T | T | T | N |
| | 81 | V | V | V | V | V | V | V | V | I |
| | 84 | G | G | G | G | S | S | S | S | V |
| | 86 | S | S | S | S | S | S | S | S | T |
| | 92 | D | D | D | D | D | D | D | D | D |
| | 103 | F | F | F | F | F | F | F | F | F |
| | 106 | M | M | M | M | M | M | M | M | M |
| | 109 | R | R | R | R | Q | Q | Q | Q | Q |
| | 114 | P | P | P | P | S | S | S | S | S |
| | 127 | N | N | N | N | N | N | N | N | T |
| | 139 | N | N | N | N | N | N | N | N | D |
| | 143 | A | A | A | A | A | A | A | A | T |
| | 166 | F | F | F | F | F | F | F | F | L |
| | 176 | N | N | N | N | N | N | N | N | I |
| | 189 | N | N | N | N | D | D | D | D | D |
| | 202 | A | A | A | A | A | A | A | A | T |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 205 | N | N | N | N | N | N | N | N | S |
| | 207 | D | D | D | D | N | N | N | N | N |
| | 216 | P | P | P | P | P | P | P | P | S |
| **NS2** | 3 | P | P | P | P | S | S | S | S | S |
| | 6 | M | M | M | M | M | M | M | M | V |
| | 7 | L | L | L | L | L | L | L | L | S |
| | 19 | M | M | M | M | M | M | M | M | L |
| | 27 | G | G | G | G | D | D | D | D | G |
| | 31 | I | I | I | I | M | M | M | M | M |
| | 36 | G | G | G | G | G | G | G | G | E |
| | 48 | T | T | T | T | T | T | T | T | A |
| | 60 | S | S | S | S | S | S | S | S | N |
| | 70 | G | G | G | G | G | G | G | G | S |
| | 83 | V | V | V | M | V | V | V | V | M |

Abbreviations: aa: amino acids; Ak: Astrakhan; Ck: chicken; CS: cleavage site; del.: deletion; NL: Netherlands; RBS: receptor-binding site (residues 222~224 in the H5 numbering or 226~228 in the H3 numbering); RoD: Rostov-on-Don; V: Voronezh.

Any residue differences in the nine viruses are presented. The residues are marked in red, if they are mutated (1) only in the human virus or (2) in three Astrakhan viruses in 2020 or (3) in all four viruses in 2020.