

1 **Age-dependent progression of SARS-CoV-2 infection in Syrian hamsters**

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21

22 **Abstract**

23 In late 2019, an outbreak of a severe respiratory disease caused by an emerging
24 coronavirus, SARS-CoV-2, resulted in high morbidity and mortality in infected humans¹.
25 Complete understanding of COVID-19, the multi-faceted disease caused by SARS-CoV-
26 2, requires suitable small animal models, as does the development and evaluation of
27 vaccines and antivirals². Because age-dependent differences of COVID-19 were identified
28 in humans³, we compared the course of SARS-CoV-2 infection in young and aged Syrian
29 hamsters. We show that virus replication in the upper and lower respiratory tract was
30 independent of the age of the animals. However, older hamsters exhibited more
31 pronounced and consistent weight loss. *In situ* hybridization in the lungs identified viral
32 RNA in bronchial epithelium, alveolar epithelial cells type I and II, and macrophages.
33 Histopathology revealed clear age-dependent differences, with young hamsters launching
34 earlier and stronger immune cell influx than aged hamsters. The latter developed
35 conspicuous alveolar and perivascular edema, indicating vascular leakage. In contrast,
36 we observed rapid lung recovery at day 14 after infection only in young hamsters. We
37 propose that comparative assessment in young versus aged hamsters of SARS-CoV-2
38 vaccines and treatments may yield valuable information as this small-animal model
39 appears to mirror age-dependent differences in human patients.

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41 **Main Text**

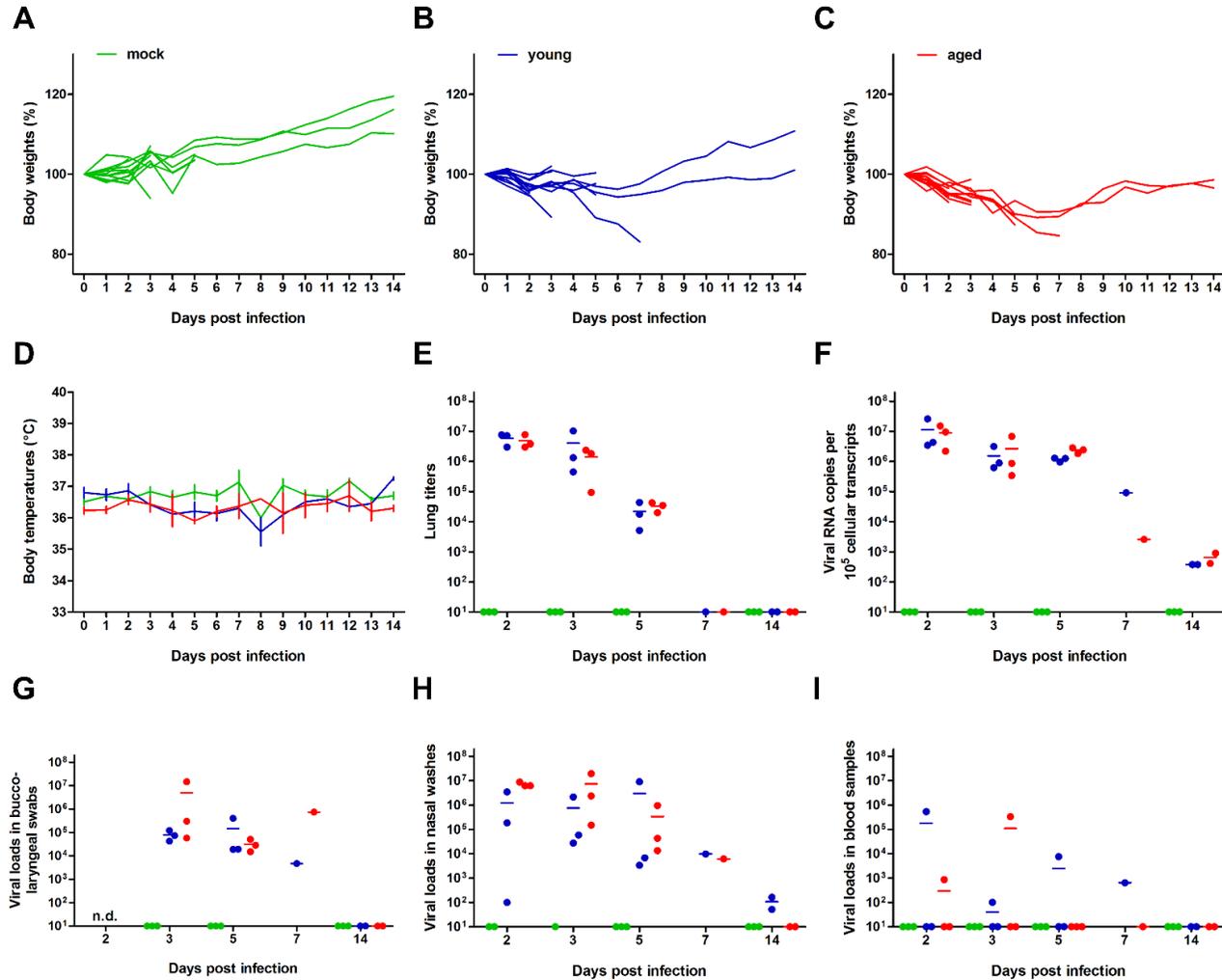
42 Emerging coronaviruses⁴ have caused serious global public health concerns in the past
43 two decades and cause infections that lead to severe respiratory and partly systemic
44 disease. These include severe acute respiratory syndrome (SARS)-CoV as well as Middle
45 East respiratory syndrome (MERS)-CoV, both of which resulted in high morbidity and
46 mortality in infected humans⁵. Similar to other emerging CoV, the novel SARS-CoV-2 likely
47 arose from an ancestor in bats and amplified in an as yet unknown animal reservoir before
48 making its jump into the human population⁶. SARS-CoV-2 has pushed global health
49 systems to the brink of breakdown. The remarkably fast and unexpected spread of SARS-
50 CoV-2 can be attributed to efficient replication in the upper respiratory tract and robust
51 human-to-human transmission, a characteristic that clearly distinguishes SARS-CoV-2
52 from SARS- and MERS-CoV. While COVID-19 is primarily a respiratory syndrome, it can
53 induce quite variable clinical signs. It has become evident that differences in type and
54 severity of SARS-CoV-2-induced disease seems to be correlated with the age of patients
55 and is exacerbated by pre-existing conditions^{4,7}.

56 The availability of reliable animal models is of critical importance for pathogenesis studies
57 as well as the development and preclinical evaluation of vaccines and therapeutics. For
58 SARS-CoV-2, the susceptibility of several animal species was predicted by *in silico*
59 analysis based on comparisons of the entry receptor for SARS-CoV and SARS-CoV-2,
60 human angiotensin converting enzyme 2 (hACE2). More specifically, the interaction of
61 the viral spike (S) glycoprotein receptor binding domain with its hACE2 counterpart was
62 examined^{8,9}, and in some cases examined *in vivo*¹⁰. Productive SARS-CoV-2 infection
63 was shown in non-human primates, which developed respiratory disease recapitulating
64 moderate disease as observed in humans¹¹⁻¹⁴. Mice are not naturally susceptible to SARS-
65 CoV-2, but mouse-adapted virus strains have been developed and used in BALB/c

66 mice^{15,16}. Moreover, transgenic mice expressing hACE2 represent a lethal SARS-CoV-2
67 infection model resulting in significant weight loss and permitting robust virus replication
68 in the respiratory tract including the lungs¹⁷. Ferrets have provided valuable data in the
69 case of SARS-CoV^{18,19}, and two studies describe the infection of ferrets with SARS-CoV-
70 2 and successful transmission to in-contact animals without clinical signs^{20,21}.

71 First and preliminary studies also focused on the assessment of a Syrian hamster model
72 that had previously been used successfully in SARS and MERS research^{18,19,22,23}. It was
73 suggested that hamsters are highly susceptible although they were reported to show no
74 or only moderate respiratory signs and body weight losses. It is important to note,
75 however, that only young male hamsters of 4 to 5 weeks of age were used in these
76 studies^{24,25}.

77 We sought to explore age-related differences in the course of SARS-CoV-2 infection in
78 Syrian hamsters, and to establish a small-animal model that resembles the more severe
79 SARS-CoV-2-infection observed particularly in elderly patients. For our experiments, we
80 used a total of 36 female and male Syrian hamsters (*Mesocricetus auratus*), which were
81 either 6- (n=24) or 32- to 34-week-old (n=12). Hamsters were kept in individually ventilated
82 cages (IVCs), and randomly assigned to three groups: mock (n=12, 6-week-old), young
83 infected (n=12, 6-week-old) and aged infected (n=12, 32- to 34-week-old). Animals were
84 mock-infected with supernatants of cell culture medium taken from uninfected Vero E6
85 cells or infected with 1×10^5 plaque-forming units of SARS-CoV-2 München (SARS-CoV-
86 2M; BetaCoV/Germany/BavPat1/2020)²⁶. During the 14-day experiment, body
87 temperatures, body weights and clinical signs were recorded daily. Animals were
88 euthanized and sampled at different time points after infection to assess virus titers in
89 various organs and to examine pathological changes in the lungs (Fig. 1 and 2).



90

91 **Figure 1: Body weight changes, body temperatures, and viral loads of young versus**
92 **aged Syrian hamsters infected intranasally with SARS-CoV-2.** Individual relative body
93 weights of (A) mock-infected, (B) young and (C) aged hamsters over the course of 14
94 days after infection are given. (D) Temperature changes (as means with SEM). Viral loads
95 were determined from homogenized right cranial lung lobes. (E) Virus titers of 25 mg of
96 tissue determined by plaque assay in Vero E6 cells, and (F) corresponding virus genome
97 copy numbers as determined by RT-qPCR. Viral loads were also determined by RT-qPCR
98 in (H) bucco-laryngeal swabs, (I) nasal washes and (G) 25 μ l of whole blood samples. The
99 color codes represent mock-infected, infected young (6-week-old, blue) and aged
100 hamsters (33- to 35-week-old, red).

101

102 First, we observed age-dependent SARS-CoV-2-induced body weight losses, with more
103 pronounced weight reductions in aged compared to young hamsters (Fig. 1A-C). Mean
104 body weight losses peaked at 6 to 7 days post-infection (dpi), with partial recovery until 14
105 dpi in both infected groups. There were no differences in body temperatures between the
106 infected groups or between infected and mock-infected animals (Fig. 1D).

107 Next, we determined viral titers and SARS-CoV-2 RNA copy numbers in various tissues
108 by RT-qPCR^{27,28}, and performed virus titrations from lung homogenates using Vero E6
109 cells (Fig. 1E-H). Results were similar between age groups and confirmed high viral loads
110 in respiratory samples at early time points after infection, but relatively rapid clearance of
111 infection. It seems important to note that RT-qPCR of blood samples revealed viremia in
112 two male individuals from both age groups with viral RNA copy numbers of $>10^5$ at 5 and
113 7 dpi, respectively (Fig. 1I). In these individuals, we also detected relatively high levels of
114 viral RNA in the spleen, kidneys and duodenum indicating systemic spread of SARS-CoV-
115 2 in some cases (Table S1). To further investigate potential dissemination of infection, we
116 tested the aforementioned organs from all animals sacrificed at 5 dpi and found them to
117 be either negative for SARS-CoV-2 RNA or to contain only low levels of viral RNA (Tab.
118 S1).

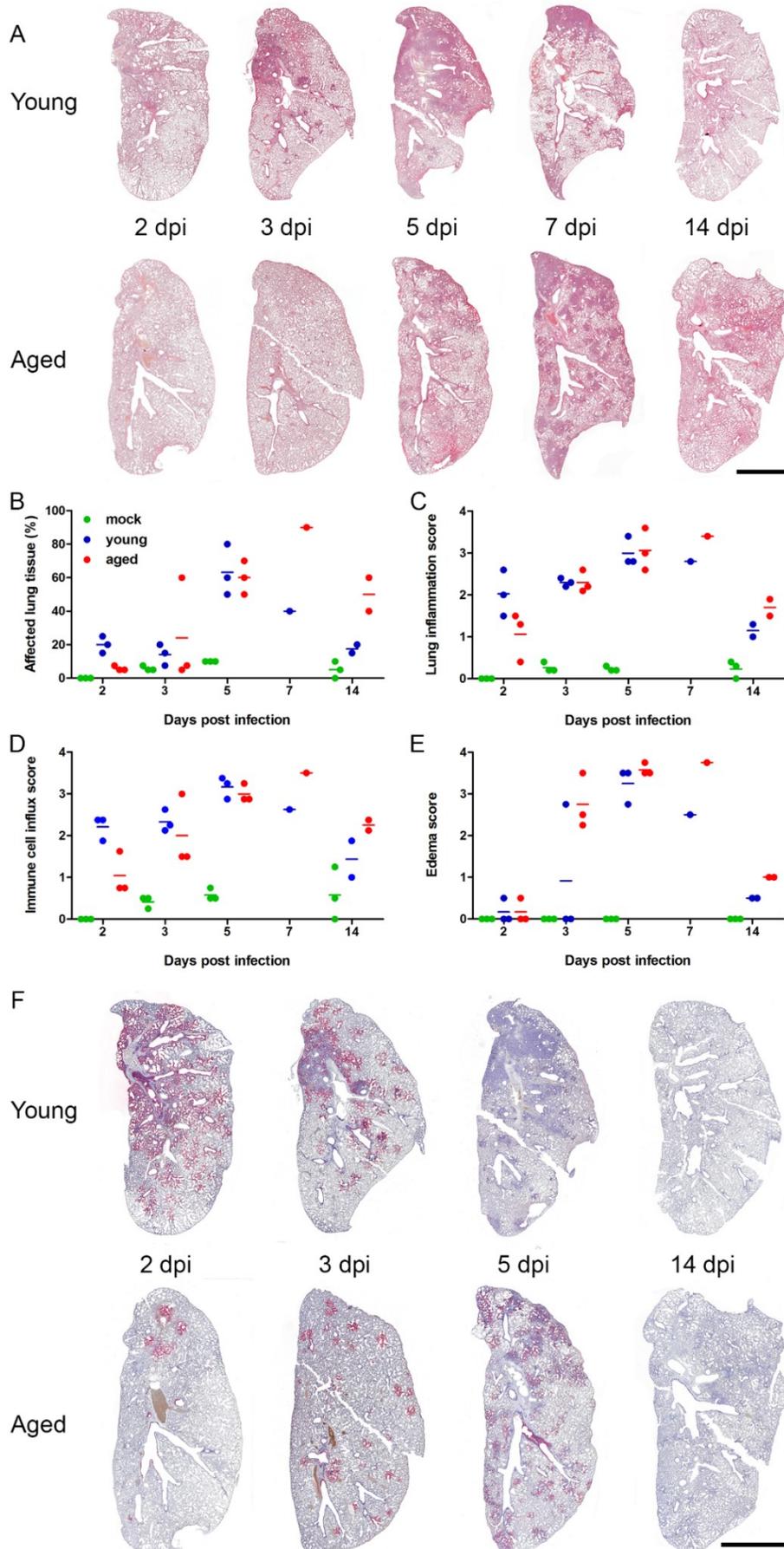
119 Viral loads in bucco-laryngeal swabs and nasal washes appeared to be a reliable
120 surrogate of viral loads in the lungs in both age groups, as the average loads ranged
121 between 10^4 and 10^7 copies, respectively, at early times after infection, indicating that
122 these sampling techniques can be used to monitor SARS-CoV-2 replication in Syrian
123 hamsters (Fig. 1G-H).

124 At 14 dpi, hamsters had mounted a robust humoral immune response as evidenced by
125 relatively high titers of neutralizing antibodies. It is worth noting that antibody titers were
126 higher in young when compared to aged hamsters (Table S1).

127 Histopathology revealed clear age-dependent differences, with young hamsters launching
128 an earlier and stronger immune cell influx into the lungs associated with a faster recovery
129 than their aged counterparts (Fig. 2A-E). At 2 dpi, young hamsters developed a marked
130 necro-suppurative bronchointerstitial pneumonia with strong alveolar and interstitial influx
131 of neutrophils and macrophages as well as perivascular lymphocytic cuffing, which was
132 much milder or absent in the aged group (Fig. S1A, right panel). In contrast, only aged
133 animals developed pronounced alveolar and perivascular edema indicating vascular
134 leakage at 3 dpi (Fig. S2C). A more diffuse, severe bronchointerstitial pneumonia was
135 similarly present in both groups at 5 dpi with onset of tissue regeneration, including
136 hyperplasia of bronchial epithelium (Fig. S1C, arrowhead) and type II alveolar epithelial
137 cells.

138 Only at that time point, the arterial and venous endothelium of animals in both groups was
139 swollen and vacuolated, with necrotic endothelial cells separated from the underlying
140 basement membrane by the presence of subendothelial lymphocytes and neutrophils (Fig.
141 S2C, left), consistent with what has been described as endothelialitis in human SARS-
142 CoV-2 infection²⁹. At 7 dpi, recovery as indicated by marked hyperplasia of bronchial
143 epithelial cells and type II alveolar epithelial cells were seen in both groups (Fig. S2A).
144 Interestingly, lung tissues had almost recovered in young hamsters at day 14, while the
145 aged animals still had persistent tissue damage and active inflammation (Fig. S2B).

146 From 2 dpi onwards, SARS-CoV-2 RNA was detected by *in situ* hybridization in bronchial
147 epithelial cells, debris in the bronchial lumen, alveolar epithelial cells type I and type II as
148 well as macrophages in both groups, again with clear age-dependent differences over
149 time (Fig. 2F). Young animals had high amounts of viral RNA in numerous bronchial
150 epithelial cells and within the bronchial lumen that was accompanied by marked spreading
151 through the lung parenchyma on 2 and 3 dpi. In contrast, aged animals, had less virus



153 **Figure 2: Lung histopathology (A-E) and detection of SARS-CoV-2 RNA (F) at**
154 **different time points after infection. (A)** Time-dependent course of pneumonia in young
155 and adult hamster (Bar = 0.5 cm) **(B)** Semiquantitation of lung lesions as assessed by
156 histopathology scores from 0 (absent) to 4 (severe changes) for the following parameters:
157 **(C)** Lung inflammation score taking into account i) severity of pulmonary inflammation; ii)
158 bronchitis (iii) bronchial and alveolar necrosis; iv) hyperplasia of alveolar epithelial cells
159 type II. **(D)** Immune cell influx score taking into account the infiltration of lung tissue with
160 i) neutrophils; ii) macrophages; iii) lymphocytes; iv) perivascular lymphocytic cuffing; and
161 **(E)** edema score including i) alveolar and ii) perivascular edema. **(F)** Time-dependent
162 distribution of SARS-CoV-2 RNA signals in young and adult hamsters as detected by *in*
163 *situ*-hybridization. (Bar = 0.5 cm)

164

165 RNA present in the bronchi. We detected only a scattered pattern of infected bronchial
166 epithelial cells and sporadic areas of parenchymal infection at 2 and 3 dpi. At 5 dpi, viral
167 RNA was undetectable in the bronchi of young hamsters, and only small infected areas
168 containing low levels of RNA with a patchy distribution were detected. It is noteworthy that
169 aged animals, at the same time after infection, had increased numbers of infected areas
170 with a similarly patchy distribution throughout the lungs as well as copious amounts of viral
171 RNA associated with cellular debris in the bronchial lumen. Using this technique, no viral
172 RNA was detected at 14 dpi in either group.

173 In summary, our study examined the suitability of a small animal model to study SARS-
174 CoV-2 infections. Intranasal infection of Syrian hamsters resulted in weight loss and robust
175 virus replication in the upper and lower respiratory tract. We further demonstrate that aged
176 32- to 34-week-old hamsters experienced higher and more consistent weight loss after
177 intranasal infection, while body temperatures and virus replication in upper airways and
178 lungs were similar between both age groups. Furthermore, we show that, using *in situ*
179 hybridization, viral RNA was detectable in bronchial epithelial cells, type I and type II
180 alveolar epithelial cells, and macrophages. All these cell types are potential targets of

181 SARS-CoV-2 in human lung tissue; hence, infection of hamsters of different ages seems
182 to closely reflect what has been reported for human patients^{25,30}. In contrast to SARS-
183 CoV-2 titers, histopathological changes differed markedly between young and aged Syrian
184 hamsters over time: younger animals launched more severe reactions at early time points
185 after infection, while lesions and inflammation in the lungs became more pronounced and
186 widespread at later time points in the elderly.

187 Based on the data presented here, we propose that comparative preclinical assessments
188 of SARS-CoV-2 vaccines and other treatment options in young versus aged hamsters may
189 yield valuable and relevant results as this small animal model appears to mimic age-
190 dependent differences in humans. The development of a substantial humoral immune
191 response emphasizes that hamsters are likely suitable for vaccination trials. Our
192 observations also confirm that body weight loss appears to be the only robust clinical
193 parameter in SARS-CoV-2 infection of Syrian hamsters. This makes the difference in body
194 weight loss between age groups with more consistent losses in aged hamsters only more
195 important as it provides an objective way to judge clinical efficacy of antiviral therapy or
196 vaccination.

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205

206 **Author contributions**

207 Conceptualization: NO, LDB, ADG and JT

208 Investigation: NO, LDB, KD, AA, DV, DH, DK and JT

209 Writing: NO, LDB, KD, MB, ADG, and JT

210 Editing: all authors had the opportunity to comment on the draft manuscript.

211

212 **Conflict of interests**

213 The authors declare no competing interests.

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215 **References**

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