

1 **Title: Experimental infection of mink with SARS-COV-2 Omicron (BA.1) variant leads to**  
2 **symptomatic disease with lung pathology and transmission**

3 **Authors:** Jenni Virtanen<sup>1</sup>, Kirsi Aaltonen<sup>1</sup>, Kristel Kegler, Vinaya Venkat, Thanakorn Niamsap,  
4 Lauri Kareinen, Rasmus Malmgren, Olga Kivelä, Nina Atanasova, Pamela Österlund, Teemu  
5 Smura, Antti Sukura, Tomas Strandin, Lara Dutra, Olli Vapalahti, Heli Nordgren, Ravi Kant, Tarja  
6 Sironen

7 <sup>1</sup>These authors contributed equally to this article

8 **Author affiliations:** University of Helsinki, Helsinki, Finland (J. Virtanen, K. Aaltonen, K. Kegler,  
9 V. Venkat, T. Niamsap, L. Kareinen, R. Malmgren, O. Kivelä, N. Atanasova, T. Smura, A. Sukura,  
10 T. Strandin, L. Dutra, O. Vapalahti, H. Nordgren, R. Kant, T. Sironen); Finnish Meteorological  
11 Institute, Helsinki (N. Atanasova); Finnish Institute for Health and Welfare, Helsinki (P. Österlund);  
12 Helsinki University Hospital, Helsinki (O. Vapalahti)

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14 **Abstract**

15 We report an experimental infection of American mink with SARS-CoV-2 Omicron variant and  
16 show that minks remain virus RNA positive for days, develop clinical signs and histopathological  
17 changes, and transmit the virus to uninfected recipients warranting further studies and preparedness.

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19 **Text**

20 SARS-CoV-2 has been detected in farmed and feral American mink in multiple countries with  
21 evidence of extensive environmental contamination and human-to-mink and mink-to-human  
22 transmission (1-5). This has led to strict measures in mink farms and mink farming countries to  
23 prevent the spread of the disease. In late 2021, a new SARS-CoV-2 variant (Omicron),

24 characterized by possibly milder symptoms and more efficient human-to-human transmission, was  
25 detected, but its infectivity and spread in American mink is unknown (6, 7).

26 We tested the response of the American mink to the Omicron variant by infecting three male mink  
27 intranasally with  $4 \times 10^5$  PFU of the virus (see Appendix for methods). Infected minks were  
28 followed for seven days, sampled daily for saliva, and subjected to histopathological evaluation of  
29 upper and lower respiratory tracts on the last day of follow-up.

30 All experimentally infected mink showed mild to moderate signs of illness including lethargy,  
31 anorexia, diarrhea, nasal and lacrimal discharge, and sneezing. Consistent with earlier experiments  
32 with other variants (8, 9), saliva samples tested PCR positive 1-day post-infection (dpi) and  
33 remained that way throughout the follow-up (Table and Appendix). Even though some of the  
34 clinical signs may be due to other factors such as stress from the change of environment,  
35 consistency of symptoms to studies with other variants combined with PCR results, demonstrate  
36 that the Omicron variant also causes a symptomatic infection in mink.

37 To study if mink can transmit the virus, two minks were used as uninfected recipients in separate  
38 cages located 10-20 cm away from the cages of the infected mink and followed for ten days. Both  
39 recipients developed similar symptoms to the experimentally infected mink and were consistently  
40 PCR positive from day three onwards (Table), indicating mink-to-mink transmission. Even though  
41 there is currently no evidence of mink-to-human transmission of the Omicron variant, it seems  
42 likely based on our results and the information from other variants.

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47 **Table:** PCR results from saliva of three experimentally infected mink and two uninfected recipient mink

Mink Id	1 dpi	2 dpi	3 dpi	4 dpi	5 dpi	6 dpi	7 dpi	8 dpi	9 dpi	10 dpi
<b>Infected mink saliva</b>										
451	+	+	+	+	+	+	+			
453	+	+	+	+	+	+	+			
455	+	+	+	+	+	+	+			
<b>Recipient mink saliva</b>										
452	(+)	-	+	+	+	+	+	+	+	(+)
454	-	-	(+)	+	+	+	+	+	+	+

48 dpi, days post infection of experimentally infected mink; +, signal detected with both primers of Luna SARS-CoV-2  
49 RT-qPCR Multiplex Assay Kit; (+), signal detected only with one out of two primers; -, no signal with either of the  
50 primers

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52 Gross findings in the nasal cavity and lungs were subtle in both experimentally infected and  
53 recipient mink consisting of hyperemia of respiratory mucosa with small amounts of viscous  
54 exudate and non-collapsed, dark-red, and wet pulmonary lobes. All mink showed consistent  
55 histopathological changes in the upper and lower respiratory tracts. Multifocal degeneration and  
56 loss of respiratory epithelium with variable mucosal and submucosal neutrophilic infiltration was  
57 observed in the nose. The lumen contained sloughed epithelial cells, mucinous material, and  
58 degenerated neutrophils (Figure A, C). Viral nucleoprotein was widely distributed beyond intact  
59 cells, within sloughed cells, and mucosal respiratory epithelium (Figure B, D). The olfactory  
60 epithelium was inconsistent and mildly affected with only focal viral antigen detection. Unlike in  
61 some experimental infections reported in rodents, clear pathology was observed in the lungs (10). In  
62 two inoculated and both recipient mink, pulmonary lesions (Figure E, G) were associated with viral  
63 antigen expression (Figure F, H) and characterized by multifocal to coalescing alveolar damage  
64 with degeneration and/or necrosis of alveolar septa, infrequent hyalin membrane formation, and

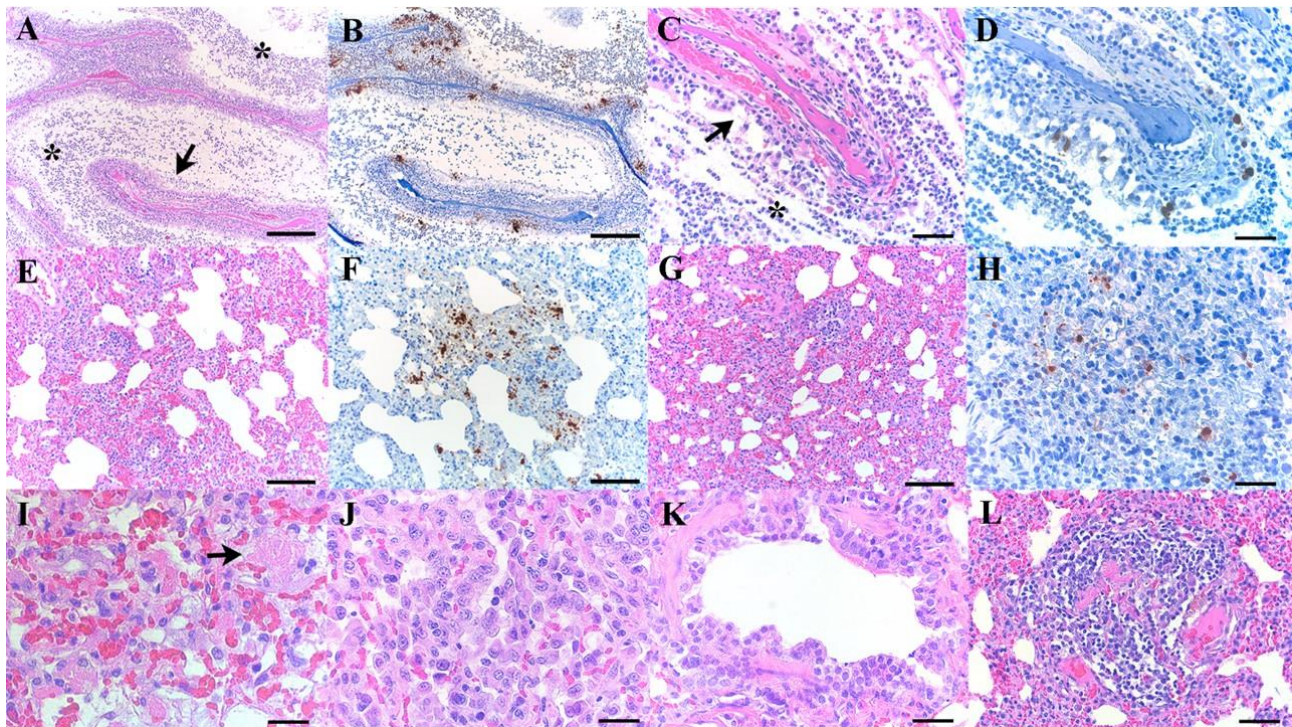
65 variable proliferation of type II pneumocytes (Figure I, J). Alveolar spaces contained macrophages,  
66 sloughed cells, edema, and hemorrhage. Bronchiolar epithelial degeneration and hyperplasia were  
67 variably present (Figure K), and the lumen filled with few sloughed cells and neutrophils. Bronchi  
68 were lined by hyperplastic epithelium with increased numbers of goblet cells. Other consistent  
69 findings were vasculitis (Figure L), perivasculitis, perivascular, and peri-bronchial edema. One  
70 inoculated mink had markedly thickened alveolar septa by mononuclear cells, marked proliferation  
71 of type II pneumocytes, intra-alveolar macrophages, few syncytial cells, bronchi and bronchiolar  
72 epithelial cell hyperplasia, vasculitis, and perivasculitis. Viral antigen could not be detected in this  
73 mink. Strikingly, all evaluated mink lacked viral antigen in the epithelium of bronchi and  
74 bronchioles.

75 The Omicron variant is different from other variants due to its more efficient spread, primarily  
76 attributable to immune escape and likely milder symptoms in humans (6, 7). This makes it more  
77 difficult to prevent virus introduction into the mink farms through asymptomatic humans, creating a  
78 more significant risk for the formation of virus reservoirs among farmed or feral mink. This study  
79 shows that mink can be infected by Omicron and importantly, efficiently transmit the virus to other  
80 mink. Despite the reports of lower virulence of Omicron, mink develop clinical disease and nasal  
81 and pulmonary microscopic lesions closely resemble infection with previously reported variants in  
82 mink and humans. A better understanding of the clinical symptoms helps detect the virus among  
83 mink earlier. Further studies are needed to determine the risk of transmission to humans, emergence  
84 of mink-specific mutations, the pathogenesis of pulmonary involvement, and prepare for this easily  
85 transmitted variant among farmed and feral mink.

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90 **Figure.** Histopathological changes and SARS-CoV-2 expression in the upper and lower respiratory tracts in  
91 experimentally infected mink with Omicron variant at 7 dpi and recipient mink after 10 days of follow up. A)  
92 Respiratory segment of the nose from an intranasally infected mink showing luminal accumulation of exudate (\*) and  
93 degeneration of mucosal epithelium (arrow, bar 500µm. B) Viral antigen is widely detected within nasal lumen and  
94 respiratory epithelium (bar 500µm). C) Respiratory epithelium from a recipient mink depicting marked degeneration  
95 and loss (arrow), intraluminal accumulation of sloughed cells and neutrophils (\*), and D) intraepithelial viral expression  
96 (C, D bar 50 µm). E-H) Lungs from intranasally infected (E, F) and recipient mink (H-I) showing alveolar damage with  
97 intralesional presence of viral nucleoprotein (E-G bar 200µm, H bar 50 µm). I, J) Marked degeneration and necrosis of  
98 alveolar septa and focal hyalin membrane (arrow, I), and prominent proliferation of type II pneumocytes (J) in an  
99 intranasally infected mink (I, J bar 25µm). K, L) Recipient mink showing bronchiolar epithelial degeneration and  
100 hyperplasia (K), and vasculitis (L) with complete destruction of blood vessel wall and mononuclear cell infiltration (K  
101 bar 50µm, L bar 100µm). Haematoxylin and eosin (HE) stain and immunohistochemistry, haematoxylin counterstain.

102

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143 Address for Correspondence: Jenni Virtanen, Department of Veterinary Biosciences, Faculty of  
144 Veterinary Medicine, P.O. Box 66, and Department of Virology, Faculty of Medicine, P.O. Box 21,  
145 00014 University of Helsinki, Helsinki, Finland; tel: +358407768832, email:  
146 [jenni.me.virtanen@helsinki.fi](mailto:jenni.me.virtanen@helsinki.fi)

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