




Distribution of equine coronavirus RNA in the intestinal and respiratory tracts of experimentally infected horses

Yoshinori Kambayashi¹ · Daiki Kishi¹ · Takanori Ueno¹ · Minoru Ohta¹ · Hiroshi Bannai¹ · Koji Tsujimura¹ · Yuta Kinoshita¹ · Manabu Nemoto¹ 

Received: 2 February 2022 / Accepted: 15 April 2022 / Published online: 31 May 2022
© The Author(s), under exclusive licence to Springer-Verlag GmbH Austria, part of Springer Nature 2022

Abstract

Equine coronavirus (ECoV) causes pyrexia, anorexia, lethargy, and sometimes diarrhoea. Infected horses excrete the virus in their faeces, and ECoV is also detected in nasal samples from febrile horses. However, details about ECoV infection sites in the intestinal and respiratory tracts are lacking. To identify the ECoV infection sites in the intestinal and respiratory tracts, we performed an experimental infection study and analysed intestinal and respiratory samples collected from four infected horses at 3, 5, 7, and 14 days post-inoculation (dpi) by real-time reverse transcription polymerase chain reaction (real-time RT-PCR) and *in situ* hybridization (ISH). Two horses became febrile, but the other two did not. None of the horses had diarrhoea or respiratory signs, and severe cases were not observed in this study. None of the horses showed obvious abnormalities in their intestinal or respiratory tracts. Real-time RT-PCR and ISH showed that ECoV RNA was present throughout the intestinal tract, and ECoV-positive cells were mainly detected on the surface of the intestine. In one horse showing viremia at 3 dpi, ECoV RNA was detected in the lung by real-time RT-PCR, but not by ISH. This suggests that the lung cells themselves were not infected with ECoV and that real-time RT-PCR detected viremia in the lung. The other three horses were positive for ECoV RNA in nasal swabs but were negative in the trachea and lung by real-time RT-PCR and ISH. This study suggests that ECoV broadly infects the intestinal tract and is less likely to infect the respiratory tract.

Introduction

Equine coronavirus (ECoV) belongs to the species *Beta-coronavirus 1* in the genus *Betacoronavirus*, which also includes bovine coronavirus (BCoV) [1]. There have been a number of outbreaks of ECoV infection in Japan [2–4] and the United States [5, 6]. Infected horses show pyrexia, anorexia, lethargy, and sometimes diarrhoea [5]. Experimental infection studies have shown that infected horses excrete a

large amount of ECoV in their faeces regardless of clinical signs, and ECoV is considered to be mainly transmitted by the faecal–oral route [7, 8]. In two clinical studies on horses and a donkey that had died, it was reported that ECoV was detected in the jejunum and colon, using immunohistochemistry with antibodies against BCoV [9, 10]. These clinical signs and observations suggest that ECoV has intestinal tropism, but it is unknown whether ECoV infects intestinal tissues other than the jejunum and colon. BCoV, which is genetically related to ECoV, infects both the intestinal and respiratory tracts of cattle and causes enteric and respiratory diseases [11]. ECoV has also been detected in nasal swabs from febrile horses [7, 12–14], but it is unclear whether ECoV infects other parts of the respiratory tract as BCoV does. In this study, to identify infection sites of ECoV in the intestinal and respiratory tracts, we performed an experimental infection study and analysed intestinal and respiratory samples collected from infected horses over time by real-time reverse transcription polymerase chain reaction (real-time RT-PCR) and *in situ* hybridization (ISH).

Handling Editor: Diego G. Diel.

Yoshinori Kambayashi and Daiki Kishi contributed equally to this work.

These data were presented, in part, at the 11th International Equine Infectious Diseases Conference in 2021, the proceedings of which are published in *Equine Veterinary Journal* 2021;53(Suppl. S56):66.

✉ Manabu Nemoto
nemoto_manabu@equinst.go.jp

¹ Equine Research Institute, Japan Racing Association, 1400-4 Shiba, Shimotsuke, Tochigi, Japan

Materials and methods

Horses and inoculum

Four 1-year-old Thoroughbred horses were used in this study. All horses were healthy and did not have virus-neutralizing antibodies against ECoV (strain NC99), i.e., the antibody titres were less than 1:8, at 0 days post-inoculation (dpi). Neutralization tests with strain NC99 were performed as described previously [2]. An ECoV-positive faecal sample was used as the inoculum; the inoculum was watery diarrheic faeces collected from a 4-year-old female draft horse during an ECoV outbreak in 2012 [4]. Using bacterial culture testing, the inoculum was confirmed to be negative for *Clostridium perfringens*, *Clostridioides difficile*, and *Salmonella* species potentially related to intestinal diseases in horses [15]. The ECoV-positive sample was stored at -80°C until inoculum preparation. The sample was diluted 1:10 in phosphate-buffered saline (PBS), and 500 ml of this 10% faecal suspension was administered via a transnasal catheter into the oesophagus of each experimental horse under sedation [7]. The suspension contained 3.6×10^{10} copies of the ECoV nucleocapsid gene. Viral copy numbers were determined as described below.

Clinical observation and sampling

Clinical signs and rectal temperatures were recorded daily. Rectal temperatures exceeding 38.6°C were defined as fever. Faecal samples, nasal swabs, serum, and EDTA blood samples were collected daily. Faecal samples were homogenized in a disposable homogenizer (BioMasher Standard, Takara bio, Kusatsu, Japan) at a ratio of 1:10 (w/v) in homogenization buffer comprising Dulbecco's modified Eagle's medium (Sigma-Aldrich, St. Louis, MO, USA) supplemented with 200 units of penicillin, 200 μg of streptomycin, and 0.5 μg of amphotericin B per millilitre (Antibiotic-Antimycotic; Thermo Fisher Scientific, Waltham, MA, USA). Nasal swabs were collected and immersed in PBS supplemented with 0.6% tryptose phosphate broth (Sigma-Aldrich) with 500 units of penicillin, 500 μg of streptomycin, and 1.25 μg of amphotericin B per millilitre (Antibiotic-Antimycotic; Thermo Fisher Scientific). Faecal suspensions and nasal swabs were centrifuged at 21,500 g for 5 minutes and 860 g for 15 minutes, respectively, and the supernatant was used for RNA extraction. EDTA blood samples were diluted 1:1 (v/v) in PBS for extraction of viral RNA. Horses were euthanized at 3 dpi (horse #1), 5 dpi (horse #2), 7 dpi (horse #3), and 14 dpi (horse #4), and the intestinal tract (duodenum, jejunum,

ileum, caecum, colon, and rectum), trachea, lungs, and lymph nodes (cranial mesenteric, caecal, colonic, caudal mesenteric, and pulmonary lymph nodes) were collected for real-time RT-PCR and histopathological analysis. Tissue samples were homogenized at a ratio of 1:10 (w/v) in homogenization buffer and centrifuged in the same manner as faecal samples for real-time RT-PCR. For histopathological analysis, tissue samples were fixed in 10% neutral buffered formalin and then embedded in paraffin.

Real-time RT-PCR

Viral RNA was extracted from the faecal samples, nasal swabs, diluted EDTA blood samples, and tissue samples (400 μl for each sample), using an automated nucleic acid isolation machine (MagLEAD; Precision System Science, Matsudo, Japan), and eluted into 100 μl of elution buffer. Real-time RT-PCR was performed using specific primers (ECoV-380f 5'-TGGGAACAGGCCCGC-3' and ECoV-522r 5'-CCTAGTCGGAATAGCCTCATCAC-3') and a TaqMan MGB probe (ECoV-436p 5'-6-FAM-TGGGTCGCTAAC AAG-MGB-3') (Thermo Fisher Scientific) to detect the nucleocapsid gene [16], using TaqPath 1-Step RT-qPCR Master Mix, CG (Thermo Fisher Scientific) according to the manufacturer's instructions. Thermal cycling conditions were as follows: an initial hold at 25°C for 2 minutes, 50°C for 15 minutes and 95°C for 2 minutes, and then 40 cycles of 95°C for 3 seconds and 60°C for 30 seconds. To produce a standard curve, control ECoV RNA was synthesized for us by Fasmac (Atsugi, Japan). This corresponded to a portion of the nucleocapsid gene including the target region of the real-time RT-PCR assay. Samples were judged to be positive if they contained more than 10 copies per reaction. Ten copies per reaction corresponded to 6.3×10^3 ($10^{3.8}$) copies per gram of faecal and tissue samples, 6.3×10^2 ($10^{2.8}$) copies per millilitre of nasal swab suspension, and 1.3×10^3 ($10^{3.1}$) copies per millilitre of EDTA blood samples. Real-time RT-PCR reactions were performed in triplicate, and average copy numbers were determined.

To confirm that the extraction from samples was properly performed, a housekeeping gene (beta-2-microglobulin) was used as an internal control, using specific primers and a probe kit (Assay ID: Ec03468699_m1, TaqMan Gene Expression Assays; Thermo Fisher Scientific).

Haematoxylin and eosin (HE) staining and *in situ* hybridization (ISH) for ECoV

HE staining was performed using routine procedures as described previously [17]. ISH to detect ECoV RNA in

formalin-fixed, paraffin-embedded tissues was performed using RNAscope technology (Advanced Cell Diagnostics Inc., Newark, CA, USA) according to the manufacturer’s instructions. The probe (Advanced Cell Diagnostics) targeted the nucleoprotein gene of strain NC99 (accession number EF446615) [18]. After staining, ECoV-positive cells in 20 randomly selected fields at 400× magnification were counted using PathoCount ver. 1.2.0 (Mitani Corporation, Tokyo, Japan). The average number of ECoV-positive cells per field was calculated. As negative controls, intestinal samples collected from a 1-year-old horse that had not been inoculated with ECoV were also used for HE staining and ISH.

Results

Two out of four horses had temperatures above 38.6 °C, one at 2 dpi (39.5 °C, horse #1) and one at 3 dpi (38.9 °C, horse #2) (Fig. 1). The other two (horses #3 and #4) did not show a fever during the study period. None of the four horses showed clinical signs other than fever, such as diarrhoea, colic, or respiratory disease.

The ECoV RNA copy numbers in faecal samples, nasal swabs, and EDTA blood samples are shown in Figure 2. ECoV RNA was detected in faecal samples collected from horses #1, #2, and #3 from 3 dpi to the day of euthanasia (at 3, 5, and 7 dpi, respectively), and in horse #4 at 2–11 and 13 dpi. ECoV RNA was detected in nasal swabs from horse #2 at 4–5 dpi, horse #3 at 6–7 dpi, and horse #4 at 3–7, 9, 11, and 13 dpi. ECoV RNA was detected in EDTA blood samples from horse #1 at 2–3 dpi, and horse #4 at 2–9 and 11 dpi.

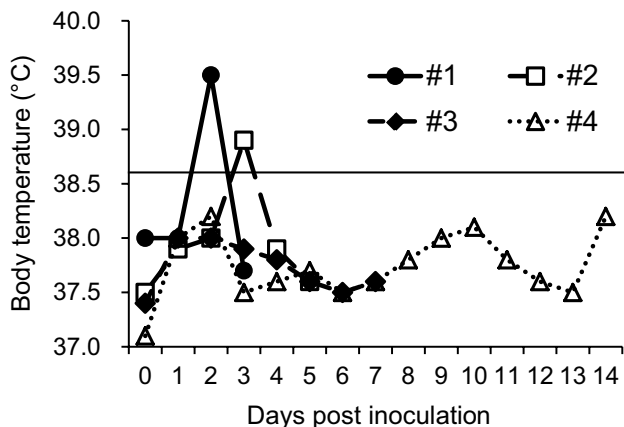


Fig. 1 Body temperatures of four experimentally infected horses. Body temperatures of horses #1, #2, #3, and #4 were recorded until 3, 5, 7, and 14 days post-inoculation, respectively. The horizontal line indicates 38.6 °C

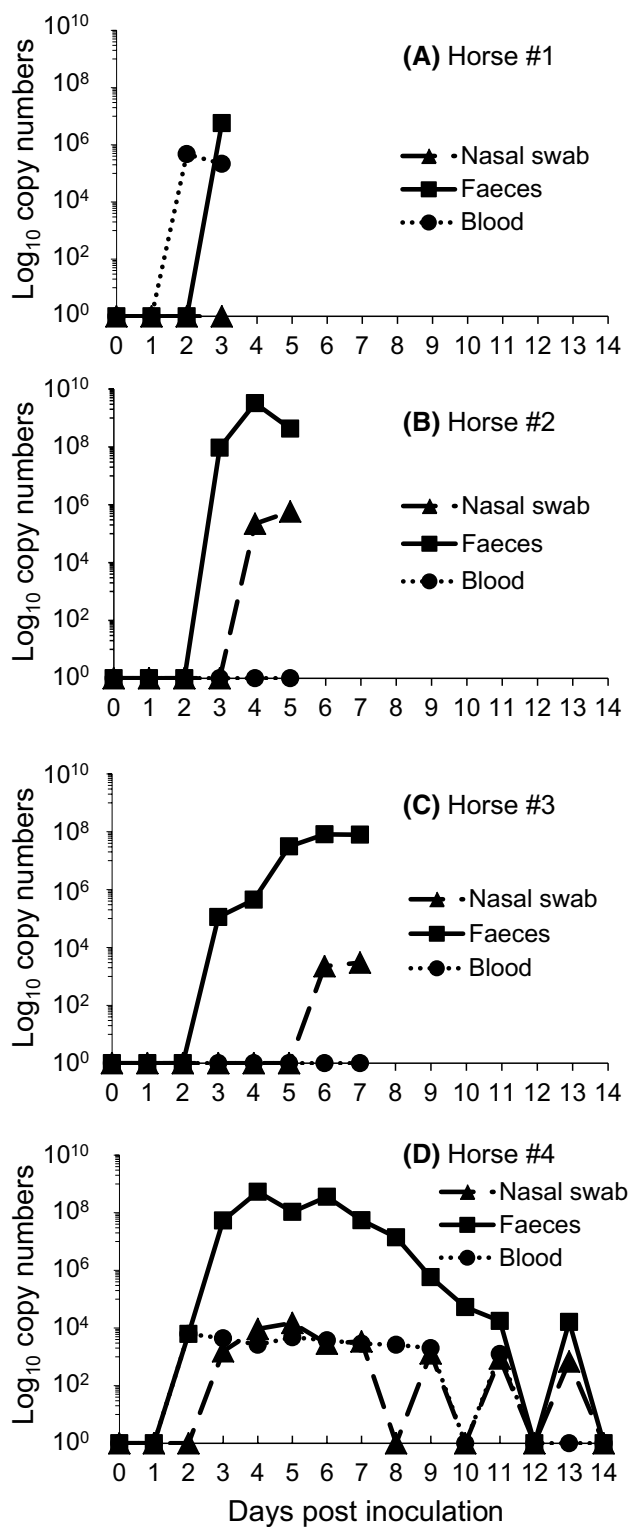


Fig. 2 Detection of equine coronavirus RNA in nasal swabs, faecal samples, and EDTA blood samples of infected horses by real-time reverse transcription polymerase chain reaction. The copy numbers of equine coronavirus RNA per gram (faecal samples) or millilitre (nasal swabs and EDTA blood samples) are shown as the logarithm to the base 10. Samples that had more than 6.3 × 10³ (10^{3.8}) copies per gram in faeces, 6.3 × 10² (10^{2.8}) copies per millilitre in nasal swabs, and 1.3 × 10³ (10^{3.1}) copies per millilitre in EDTA blood samples were considered positive for ECoV

Table 1 Copy numbers of equine coronavirus RNA in each tissue collected from experimentally infected horses

Tissue	Horse no. (collection day)			
	#1 (3 dpi)	#2 (5 dpi)	#3 (7 dpi)	#4 (14 dpi)
Duodenum	–	8.5	8.2	–
Jejunum	9.8 ^a	8.6	7.0	4.2
Ileum	10.3	9.5	6.6	4.4
Caecum	9.0	11.6	10.1	5.9
Colon	6.0	10.7	7.8	6.0
Rectum	–	11.0	9.0	–
Trachea	–	–	–	–
Lung	6.2	–	–	–
Cranial mesenteric LN	9.7	6.2	6.7	6.2
Caecal LN	5.4	7.3	6.4	5.1
Colonic LN	–	4.0	7.9	5.3
Caudal mesenteric LN	5.2	–	4.1	–
Pulmonary LN	–	–	–	–

–, negative; LN, lymph node

^aThe copy numbers of equine coronavirus RNA per gram are shown as the logarithm to the base 10. Samples that had more than 6.3×10^3 ($10^{3.8}$) copies per gram in tissues were considered positive for ECoV

The ECoV RNA copy numbers in each tissue are shown in Table 1. Real-time RT-PCR showed that, at 3 dpi, ECoV RNA was detected from the jejunum to the colon and in the cranial mesenteric, caecal, and caudal mesenteric lymph nodes. Notably, a large quantity of ECoV RNA was observed from the jejunum to the caecum and in the cranial mesenteric lymph nodes. At 5 and 7 dpi, ECoV RNA was broadly detected from the duodenum to the rectum and was observed in the largest quantities in the caecum. At 14 dpi, ECoV RNA was detected from the jejunum to the colon. ECoV RNA was also detected in the cranial mesenteric, caecal, and colonic lymph nodes at 5, 7, and 14 dpi, as well as in the caudal mesenteric lymph nodes at 7 dpi. The quantities of ECoV RNA in intestinal tissues other than the colon were lower at 14 dpi than at 3, 5, and 7 dpi. In the respiratory tract, only lung tissue collected at 3 dpi was positive for ECoV RNA, and lung tissue collected at 5, 7, and 14 dpi was negative. ECoV RNA was not detected in the trachea in this study.

Gross pathology revealed no obvious abnormalities in the intestinal or respiratory tracts in any of the horses, but all horses had swelling of the cranial mesenteric, caecal, and colonic lymph nodes. Microscopic investigation of the jejunum at 3 dpi showed atrophy of villi, detachment of epithelial cells, and macrophage accumulation at the tips of the villi and in the lamina propria (Fig. 3A, B). Similar

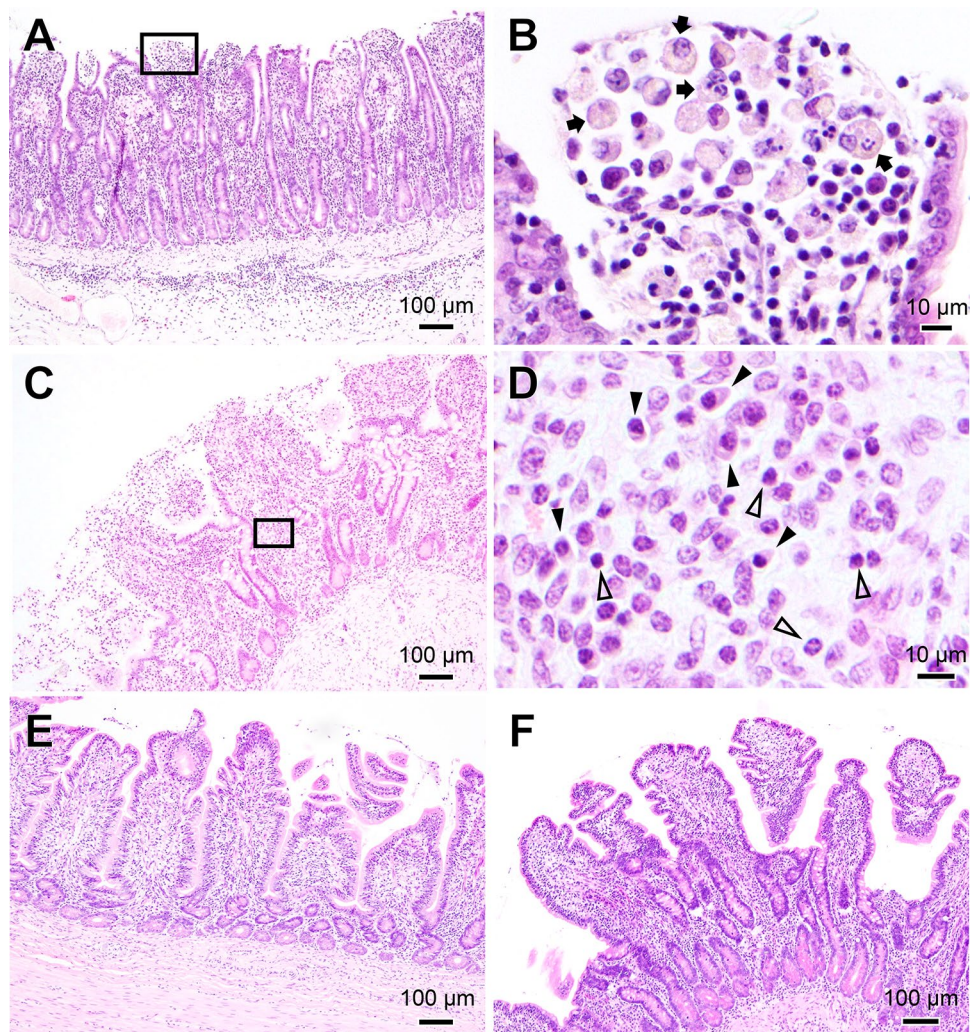
findings were also observed in the jejunum at 5 and 7 dpi and in the ileum at 3, 5, and 7 dpi. At 14 dpi, lymphocytes and plasmacytes were accumulated in the lamina propria in the ileum (Fig. 3C, D). Accumulation of lymphocytes and plasmacytes was also observed in the jejunum at 7 and 14 dpi and in the ileum at 7 dpi. Follicular hyperplasia was observed in the cranial mesenteric, caecal, and colonic lymph nodes at 3, 5, 7, and 14 dpi; in addition, follicular hyperplasia was also observed in caudal mesenteric lymph nodes at 5, 7, and 14 dpi. No abnormalities in the respiratory tract were revealed by microscopic examination. Additionally, no abnormalities were found in the jejunum (Fig. 3E) and ileum (Fig. 3F) collected from a horse that had not been inoculated with ECoV.

The results of ISH are shown in Table 2 and Figure 4. At 3 dpi, ECoV-positive cells were detected in the tips of villi from the jejunum (Fig. 4A, B) to the caecum and in the cranial mesenteric lymph nodes (Table 2), and epithelial cells in the jejunum were detached in lesions infected with ECoV (Fig. 4A, B). ECoV-positive cells were detected from the duodenum to the rectum at 5 dpi (Fig. 4C, D) and were detected in all parts of the intestinal tract other than the jejunum at 7 dpi (Table 2). At 3, 5, and 7 dpi, there were ECoV-positive signals in the epithelial cells and macrophages of the lamina propria (Fig. 4B, D), but there were no ECoV-positive cells in lymph nodules related to the gastrointestinal tract. In contrast, at 14 dpi, ECoV RNA was detected in the lymph nodules from the jejunum (Fig. 4E) to the colon (Table 2). Although there were fewer ECoV-positive cells at 14 dpi than at 3, 5, and 7 dpi, ECoV-positive cells were also observed at the tips of the villi in the jejunum. No ECoV-positive cells were observed in the trachea or lungs of any of the horses (Fig. 4F and Table 2). There were no ECoV-positive cells in the jejunum (Fig. 4G) and rectum (Fig. 4H) of the horse that was not inoculated with ECoV.

Discussion

In this study, intestinal and respiratory tract samples were collected at different time points from horses that were experimentally inoculated with ECoV to investigate the tissue tropism of this virus. Real-time RT-PCR and ISH showed that infected lesions were distributed from the small intestine to the large intestine and enteric lymph nodes following a time course. Corresponding to the detection of ECoV RNA by real-time RT-PCR, swelling of enteric lymph nodes was observed in gross pathological analysis. Histological analysis showed that lymphocytes and plasmacytes had accumulated in the lamina propria and that macrophages had

Fig. 3 Haematoxylin and eosin staining of the jejunum at 3 days post-inoculation (dpi) (panels A and B) and the ileum at 14 dpi (panels C and D). The boxes in panels A and C indicate the areas shown in panels B and D, respectively. Atrophy of villi, detachment of epithelial cells, and macrophage accumulation (arrows) at the tips of the villi were observed in the jejunum at 3 dpi (A and B). Lymphocytes (open arrowheads) and plasmacytes (arrowheads) had accumulated in the lamina propria in the ileum at 14 dpi (C and D). The jejunum (E) and ileum (F) of a horse that was not inoculated with ECoV were used as negative controls



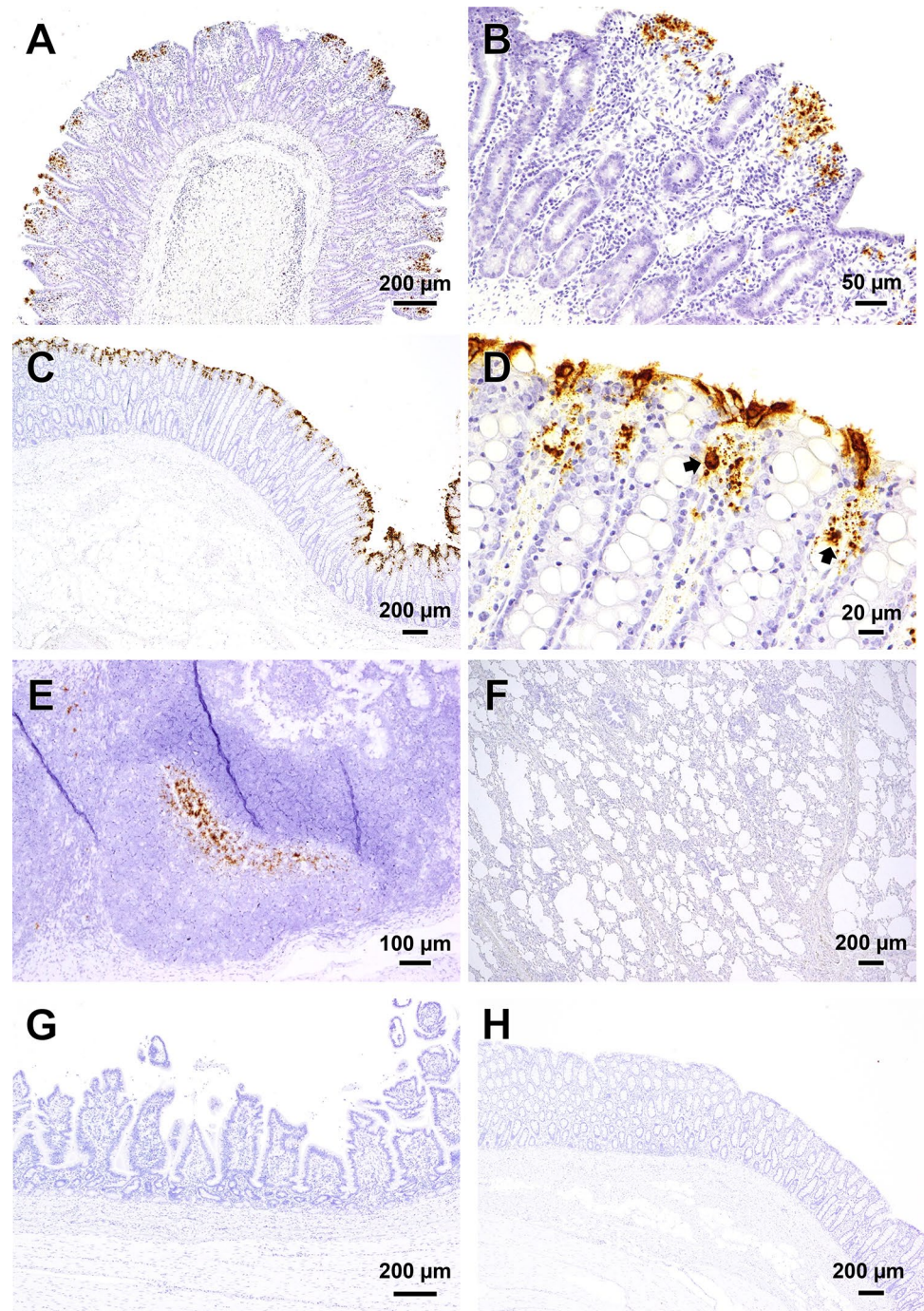
phagocytized ECoV. These results show that ECoV broadly infects the intestinal tract.

Gross pathological analysis revealed no major abnormalities in the intestinal tract. The histopathological analysis also showed that ECoV was localized on luminal surfaces, and significant damage was not observed within intestinal tissues. This would be one of the reasons why the infected horses in this study showed only mild clinical signs. Most horses that are naturally infected with ECoV in the field have only mild clinical signs, and this study is likely to reproduce such cases. This suggests that, in the majority of infected horses, although ECoV infects intestinal surfaces throughout the intestinal tract, it does not cause significant damage to the intestinal tract. Nevertheless, although rare, severe cases can occur, and there has been a report of ECoV infecting the cytoplasm of deep gland enterocytes in the jejunum and causing hyperammonemia in severe cases of necrotizing

enterocolitis and encephalitis [9]. Such differences in the distribution of ECoV within the intestinal tissues between the current study and previous studies may be associated with the severity of clinical signs. Other additional factors may be necessary for the worsening of clinical signs. Our current study could not reproduce severe cases, and further studies will be required to address unknown factors causing the difference in viral distribution within intestinal tissues and in disease severity.

In this study, none of the four horses showed respiratory signs, such as cough or nasal discharge, and none showed any abnormalities by gross pathology and microscopic investigation. At 3 dpi, ECoV RNA was detected in the lung by real-time RT-PCR but was not detected by ISH. Horse #1 showed viremia at 3 dpi, and the lung was found during post-mortem examination to contain a large amount of blood. These observations suggest that the lung

Fig. 4 Analysis of *in situ* hybridization using the jejunum collected from the infected horse at 3 days post-inoculation (dpi) (A and B), the rectum at 5 dpi (C and D), a lymph node in the jejunum at 14 dpi (E), and the lung at 3 dpi (F). Panels B and D are higher magnifications of panels A and C, respectively. Macrophages are indicated by arrows (D). The jejunum (G) and rectum (H) of a horse that was not inoculated with ECoV were used as negative controls



cells were not infected with ECoV and that ECoV RNA was detected in the lung by real-time RT-PCR because of viremia. In the other three horses, ECoV RNA was detected from nasal swab samples from 3 dpi onward, but not in the trachea or the lung by either real-time RT-PCR or ISH at 5, 7, and 14 dpi. The highest copy number of ECoV RNA in nasal swabs was 5.8×10^5 copies at 5 dpi in horse #2, and that in faecal samples was 3.2×10^9 copies at 4 dpi in horse #2. The copy numbers in nasal swabs were

all at least one order of magnitude lower than the copy numbers in faecal samples collected on the same days, and this is consistent with our previous experimental infection study [7]. It is likely that horses' noses touched ECoV-positive faeces on the floor, introducing ECoV into their nasal cavity. An experimental infection study using calves inoculated orally with BCoV showed that BCoV infected the respiratory tract and induced epithelial damage in the nasal turbinates, trachea, and lungs, and interstitial

Table 2 Distribution of equine coronavirus positive cells in intestinal and respiratory tissues analysed by *in situ* hybridization

Tissue	Horse no. (collection day)			
	#1 (3 dpi)	#2 (5 dpi)	#3 (7 dpi)	#4 (14 dpi)
Duodenum	– ^a	+ ^b	+	–
Jejunum	++ ^c	+	–	+
Ileum	++	+	+	+
Caecum	+	++	++	+
Colon	–	++	++	+
Rectum	–	++	+	–
Trachea	–	–	–	–
Lung	–	–	–	–
Cranial mesenteric LN	+	–	–	–
Caecal LN	–	–	–	–
Colonic LN	–	–	–	–
Caudal mesenteric LN	–	–	–	–
Pulmonary LN	–	–	–	–

dpi, days post-inoculation; LN, lymph node

^a–, The average number of ECoV-positive cells per field was < 1

^b+, The average number of ECoV-positive cells per field was ≥ 1

^c++, The average number of ECoV-positive cells per field was > 10

pneumonia, even though the respiratory tract was not inoculated directly with BCoV [19]. In another study, involving experimental exposure by direct contact with infected calves, it was reported that the level of BCoV RNA in nasal swabs was more than 1×10^9 copies at the maximum and was sometimes higher than in faecal samples collected on the same days [20]. In contrast, the experimental ECoV infection of horses via the oral route in this study did not result in damage to the respiratory tract or massive viral replication, as described above, suggesting that ECoV is less likely to infect the respiratory tract.

In conclusion, this experimental infection study suggests that ECoV broadly infects the intestinal tract and is less likely to infect the respiratory tract in horses.

Acknowledgements We thank Akiko Kasagawa, Miwa Tanaka, Akira Kokubun, Kaoru Watanabe, Kayo Iino, Sayoko Shibata, and Nobuko Takada (Equine Research Institute, Japan Racing Association) for their invaluable technical assistance.

Author contributions Conceptualization: Minoru Ohta, Manabu Nemoto. Investigation: Yoshinori Kambayashi, Daiki Kishi, Takanori Ueno, Minoru Ohta, Hiroshi Bannai, Koji Tsujimura, Yuta Kinoshita, Manabu Nemoto. Methodology: Daiki Kishi, Takanori Ueno. Project administration: Takanori Ueno, Minoru Ohta, Manabu Nemoto. Supervision: Manabu Nemoto. Visualization: Yoshinori Kambayashi, Daiki Kishi, Takanori Ueno, Manabu Nemoto. Writing – original draft: Yoshinori Kambayashi, Daiki Kishi, Manabu Nemoto. Writing

– review & editing: Takanori Ueno, Minoru Ohta, Hiroshi Bannai, Koji Tsujimura, Yuta Kinoshita

Funding This study was funded by the Japan Racing Association (Tokyo, Japan).

Declarations

Conflict of interest The authors declare no conflicts of interest.

Ethical approval The experimental protocol and all animal procedures were approved by the Animal Care Committee of the Equine Research Institute of the Japan Racing Association.

References

- Decaro N, Lorusso A (2020) Novel human coronavirus (SARS-CoV-2): a lesson from animal coronaviruses. *Vet Microbiol* 244:108693
- Kambayashi Y, Bannai H, Tsujimura K, Hiramata A, Ohta M, Nemoto M (2021) Outbreak of equine coronavirus infection among riding horses in Tokyo, Japan. *Comp Immunol Microbiol Infect Dis* 77:101668
- Oue Y, Ishihara R, Edamatsu H, Morita Y, Yoshida M, Yoshima M, Hatama S, Murakami K, Kanno T (2011) Isolation of an equine coronavirus from adult horses with pyrogenic and enteric disease and its antigenic and genomic characterization in comparison with the NC99 strain. *Vet Microbiol* 150:41–48
- Oue Y, Morita Y, Kondo T, Nemoto M (2013) Epidemic of equine coronavirus at Obihiro racecourse, Hokkaido, Japan in 2012. *J Vet Med Sci* 75:1261–1265
- Pusterla N, Vin R, Leutenegger C, Mittel LD, Divers TJ (2016) Equine coronavirus: an emerging enteric virus of adult horses. *Equine Vet Educ* 28:216–223
- Pusterla N, Vin R, Leutenegger CM, Mittel LD, Divers TJ (2018) Enteric coronavirus infection in adult horses. *Vet J* 231:13–18
- Nemoto M, Oue Y, Morita Y, Kanno T, Kinoshita Y, Niwa H, Ueno T, Katayama Y, Bannai H, Tsujimura K, Yamanaka T, Kondo T (2014) Experimental inoculation of equine coronavirus into Japanese draft horses. *Arch Virol* 159:3329–3334
- Schaefer E, Harms C, Viner M, Barnum S, Pusterla N (2018) Investigation of an experimental infection model of equine coronavirus in adult horses. *J Vet Intern Med* 32:2099–2104
- Giannitti F, Diab S, Mete A, Stanton JB, Fielding L, Crossley B, Sverlow K, Fish S, Mapes S, Scott L, Pusterla N (2015) Necrotizing enteritis and hyperammonemic encephalopathy associated with equine coronavirus infection in equids. *Vet Pathol* 52:1148–1156
- Davis E, Rush BR, Cox J, DeBey B, Kapil S (2000) Neonatal enterocolitis associated with coronavirus infection in a foal: a case report. *J Vet Diagn Investig* 12:153–156
- Vlasova AN, Saif LJ (2021) Bovine coronavirus and the associated diseases. *Front Vet Sci* 8:643220
- Miszczak F, Tesson V, Kin N, Dina J, Balasuriya UB, Pronost S, Vabret A (2014) First detection of equine coronavirus (ECoV) in Europe. *Vet Microbiol* 171:206–209
- Pusterla N, Holzenkaempfer N, Mapes S, Kass P (2015) Prevalence of equine coronavirus in nasal secretions from horses with fever and upper respiratory tract infection. *Vet Rec* 177:289

14. Pusterla N, James K, Mapes S, Bain F (2019) Frequency of molecular detection of equine coronavirus in faeces and nasal secretions in 277 horses with acute onset of fever. *Vet Rec* 184:385
15. Nomura M, Kuroda T, Tamura N, Muranaka M, Niwa H (2020) Mortality, clinical findings, predisposing factors and treatment of *Clostridioides difficile* colitis in Japanese thoroughbred racehorses. *Vet Rec* 187:e14
16. Pusterla N, Mapes S, Wademan C, White A, Ball R, Sapp K, Burns P, Ormond C, Butterworth K, Bartol J, Magdesian KG (2013) Emerging outbreaks associated with equine coronavirus in adult horses. *Vet Microbiol* 162:228–231
17. Ochi A, Sekiguchi M, Tsujimura K, Kinoshita T, Ueno T, Katayama Y (2019) Two cases of equine multinodular pulmonary fibrosis in Japan. *J Comp Pathol* 170:46–52
18. Zhang J, Guy JS, Snijder EJ, Denniston DA, Timoney PJ, Balasuriya UB (2007) Genomic characterization of equine coronavirus. *Virology* 369:92–104
19. Park SJ, Kim GY, Choy HE, Hong YJ, Saif LJ, Jeong JH, Park SI, Kim HH, Kim SK, Shin SS, Kang MI, Cho KO (2007) Dual enteric and respiratory tropisms of winter dysentery bovine coronavirus in calves. *Arch Virol* 152:1885–1900
20. Oma VS, Traven M, Alenius S, Myrmet M, Stokstad M (2016) Bovine coronavirus in naturally and experimentally exposed calves; viral shedding and the potential for transmission. *Virology* 13:100

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.