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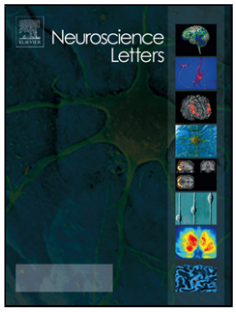

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What can we learn from brain autopsy in COVID-19?

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Highlights:

- Chronic neurological disease and acute abnormalities are present in COVID-19 brain autopsies
- Acute hypoxic-injury, hemorrhage, and minimal inflammation are frequently observed
- Low levels of viral SARS-CoV-2 RNA are present; cellular source remains unknown

Abstract:

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the causative agent of coronavirus disease 2019 (COVID-19) for which there have been over 50 million confirmed cases and 1.2 million deaths globally. While many SARS-CoV-2 infected individuals are asymptomatic or experience respiratory symptoms, extrapulmonary manifestations, including neurological symptoms and conditions, are increasingly recognized. There remains no clear understanding of the mechanisms that underlie neurological symptoms in COVID-19 and whether SARS-CoV-2 has the potential for neuroinvasion in humans. In this minireview, we discuss what is known from human autopsies in fatal COVID-19, including highlighting studies that investigate for the presence of SARS-CoV-2 in brain and olfactory tissue, and summarize the neuropathological consequences of infection. Incorporating microscopic and molecular findings from brain tissue into what we know about clinical disease will inform best practice management guidance and direct research priorities as it relates to neurological morbidity from COVID-19.

Key Words: COVID-19; SARS-CoV-2; brain autopsies; neuropathology; neuropathogenesis; immunohistochemistry; reverse transcriptase polymerase chain reaction

Manuscript:

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), an enveloped, single-stranded positive-sense RNA betacoronavirus, is the causative agent of coronavirus disease 2019 (COVID-19), for which there have been over 50 million confirmed cases and 1.2 millions deaths worldwide as of November 8, 2020 [1,2]. Morbidity and mortality are more common in older individuals and those with comorbidities, including cardiovascular disease, hypertension, obesity, and diabetes, although young people with no comorbidities are also at risk for critical illness [3–5]. While many SARS-CoV-2 infected individuals are asymptomatic or experience predominantly respiratory symptoms, extrapulmonary manifestations, including neurological symptoms and conditions, are increasingly recognized [6–8]. The majority of current studies on neurological manifestations are case reports or retrospective series focused on hospitalized patients through the extraction of medical record data, which have described disorders of consciousness, delirium, and neuromuscular and cerebrovascular complications [7–10]. Smell and taste disturbances in the absence of nasal obstruction are particularly characteristic of COVID-19, leading to speculation regarding the olfactory nerve as a possible route of central nervous system entry [11,12]. Other neurological findings include headache, myalgia, rhabdomyolysis, Guillain-Barre syndrome, encephalopathy, and myelopathy with rare cases of encephalitis based on imaging or cerebrospinal fluid [8,13–18]. SARS-CoV-2 has not been detected in cerebrospinal fluid in the majority of patients tested [8,19], highlighting the need for studies of autopsy brain tissue to understand COVID-19 neuropathogenesis and develop neurocognitive preserving treatment strategies.

Autopsies provide a wealth of information about the decedents, regardless of whether a likely cause of death was identified pre-mortem [20,21]. Due to initial uncertainties regarding the infectious properties of SARS-CoV-2 and limitations in personnel and personal protective equipment availability, autopsies for COVID-19 patients have been limited, although an increasing number of studies are now being published (reviewed in [22–24]). Reports of detailed neuropathological examinations have lagged behind general autopsy series, in part due to the initial focus on lung pathology combined with the longer (2-3 weeks) formalin fixation time preferred by most neuropathologists before cutting brains. Additional factors include the reluctance of some institutions to perform brain removal in COVID-19 cases due to concerns over electric bone saw generated aerosols, which can be effectively contained through the use of vacuum filters or hand saws [25,26]. Included in this review are peer-reviewed studies of autopsy findings published in English between January 1, 2020, and November 5, 2020. Two

different databases (PubMed, Google Scholar) were searched for key terms, including COVID-19, nCoV-2019, and SARS-CoV-2, crossed with autopsy, histology, histopathology, neuropathology, and post-mortem. This search was complemented with three review articles [22–24], text word searching and examining references in identified articles. A total of 24 studies were identified that included 149 individuals (range 1-43 subjects per series). Reported gross and microscopic findings and results of SARS-CoV-2 targeted studies are summarized in Table 1. Representative gross, microscopic, and ultrastructural findings are illustrated in Figure 1.

Gross brain autopsy findings were reported individually or in aggregate for 142 subjects. In keeping with the high prevalence of comorbidities in this patient population, evidence of prior brain disease was frequently identified, including neurodegeneration, prior strokes, tumor resection, demyelinating disease, and atherosclerosis. Acute gross abnormalities were much more limited, and a direct causal relationship with SARS-CoV-2 infection was not always straightforward to identify. A total of 92 (65%) of the gross brain examinations reported either no significant findings or no acute abnormalities. Of the remaining 50 cases, multiple findings were often described in individual brains. Hemorrhage was the most common abnormality reported, ranging from petechial bleedings and punctate subarachnoid hemorrhages (n=9) [14,27–31], to large cerebral/cerebellar hemorrhages (n=4) [27,32,33], hemorrhagic conversion of middle cerebral artery stroke (n=1) [34], and a recently drained subdural hematoma (n=1) [32]. Large acute and/or subacute infarcts (n=11) [29,31,33,35] as well as lacunar infarcts/microinfarcts and watershed infarcts (n=2) [29,30] were identified in several cases. Severe edema resulting in herniation (n=5) [27,31,33] as well as mild to moderate edema without herniation (n=34) [14,30,31,35,36] were also present.

Microscopic findings were reported for 146 of the cases in these studies. Similar to the gross examinations, histopathology identified correlates of pre-existing disease, including neurodegeneration, chronic/subacute strokes, hepatic encephalopathy, and arteriolosclerosis. No specific findings were reported for 25 (17%) of the cases. Mild to moderate acute hypoxic injury was the most common abnormality (n=58) [14,27,30,31,33,34,36–38], while severe hypoxic-ischemic injury (n=1) [36] and infarcts/focal ischemic necrosis (n=22) [14,29,31,32,35] were identified in several cases. Focal microhemorrhage or hemorrhagic suffusion was also frequently reported (n=23) [14,28–33], although intravascular microthrombi (n=12) [31,39] or neutrophilic plugs (n=3) [38] were less common. Mild focal perivascular, parenchymal, and leptomeningeal T-cell predominant lymphocytic infiltrates were identified in a large number of cases without clear evidence of vasculitis or meningoencephalitis (n=81) [27,29–31,33–39]. Moderate to intense microglial activation was noted, particularly in the brainstem (n=73), although similar results were also

reported in COVID-19-negative individuals with systemic inflammatory/septic clinical courses [31,33–36,38]. Axonal damage was identified in a few cases (n=5) [14,27,30]. Acute disseminated encephalomyelitis (ADEM)-like lesions were reported in a single case [14]. The olfactory system was examined to varying degrees, identifying prominent acute and chronic inflammation in the olfactory epithelium (n=14) [33,38,39], microglial activation (n=18) [36] and red neurons (n=1) [44] in the olfactory bulb, and only unremarkable age-related corpora amylacea in olfactory tracts.

Researchers across the globe have employed multiple strategies to directly assess for the presence of SARS-CoV-2 in brain tissue, including immunohistochemistry, in situ hybridization (ISH), targeted quantitative reverse transcriptase polymerase chain reaction (qRT-PCR), and transmission electron microscopy. At this time, immunohistochemistry, using antibodies that recognize the viral nucleocapsid (N) or spike (S) proteins, have been negative in most attempted human cases (n=58) [30,33,35,37,38], with the exception of a recent case series that reported positive staining in vagus and glossopharyngeal nerves and scattered cells in the medulla in a total of 16 cases [35]; in situ hybridization for viral RNA has been negative (n=1) [38]. Viral spike protein has been reported to be present in the olfactory epithelium in 5/6 patients; however, brain findings from these cases were not discussed [40]. A number of qRT-PCR assays have been employed targeting the N, S, envelope (E), open reading frame (ORF) 1/a, ORF1ab, or RNA-dependent reverse transcriptase (RdRp) genes, identifying low levels of virus in frozen or formalin-fixed paraffin-embedded brain tissue (34/84; 41%) [12,30–37,41] and olfactory bulb/tract (n=9/36; 25%) [31,33,36,37]. Viral subgenomic RNA, a marker of actively replicating virus, was positive in a single case (n=1/5; 20%) [43,44]. Transmission electron microscopy (TEM) without immunolabeling reported virus-like particles in the frontal lobe (n=1) [45].

While additional COVID-19 autopsy series continue to be published, the overall picture of acute hypoxic-injury, hemorrhage, and mild to moderate non-specific inflammation is unlikely to change significantly. Evidence of direct viral involvement in the brain or olfactory nerve is limited to the detection of low levels of viral RNA and rare viral antigen in cranial nerves and scattered brainstem cells. Diagnosis of coronavirus particles by electron microscopy is challenging due to similar appearing normal cellular structures, which has created significant controversy in the literature [42,43]. Due to the inherent bias of autopsy studies for severe, fatal disease, and additional institutional restrictions for which cases include brain evaluation, the frequency and extent of neuropathological findings are likely to be overestimated relative to the average COVID-19 patient. At the time of

this review, pediatric autopsies, including individuals with multisystem inflammatory syndrome in children (MIS-C), remain extremely limited. While the number of pediatric COVID-19 cases accounts for <2% of all cases [44], data obtained from brain tissue in this age-group can help address the unique pathophysiology of SARS-CoV-2 infection, including age-dependent immune-responses, hypercoagulability, and degree of hypoxic-ischemic injury.

Additional remaining areas of interest include characterizing the effects of remdesivir and other potential antiviral therapeutics, immunomodulatory medications including dexamethasone, anti-IL-6 or other monoclonal antibodies, and anticoagulants on brain tissue. Given that the therapeutic response to COVID-19 vastly differs between institutions, it remains a challenge to understand how therapeutic choices during acute hospitalization are responsible for the variability in observed neurological manifestations and neuropathological findings. Also, while not surprisingly this early in the pandemic, long-term neuropathological sequelae in COVID-19 survivors remain unstudied. There is evidence that neurological symptoms, including fatigue and headaches, linger for weeks to months in a subset of affected patients [45,46] and studies determining mechanisms for persistent neurological symptoms are needed.

There have been several efforts for sharing COVID-19 brain tissue, including the International Society of Neuropathology (ISN) Collaborative Efforts [47] and the COVID-19 Virtual Biobank at the University of Nebraska Medical Center [48]. To address many of the remaining unanswered questions regarding the neuropathological effects of COVID-19, large scale integrated studies from multiple institutions with relevant clinical metadata will be crucial. The ongoing collection of neurological tissue will be critical to inform best practice management guidance and to direct research priorities as it relates to neurological morbidity from COVID-19.

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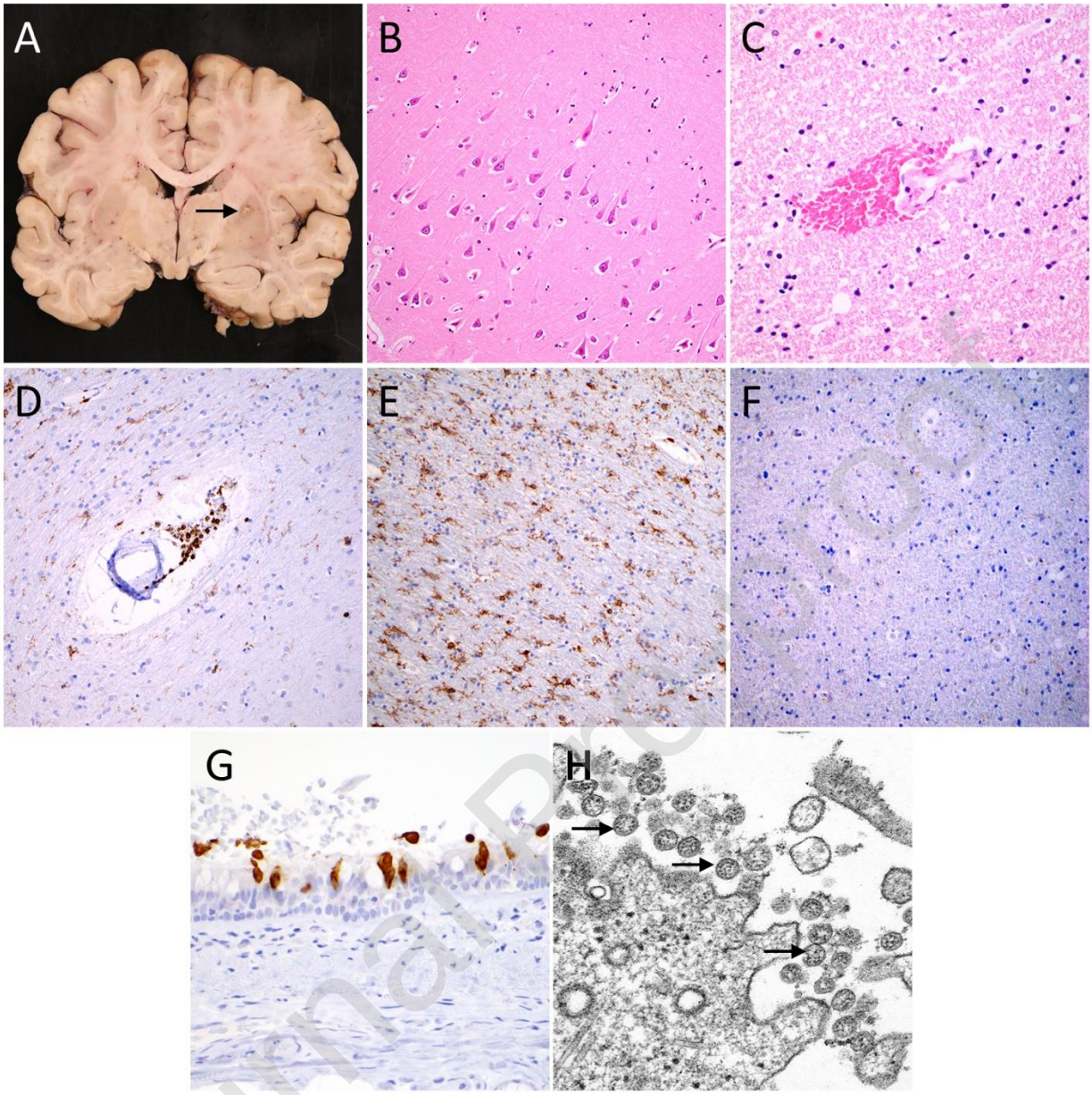

Figure 1 - Neuropathological findings of COVID-19. (A) Coronal brain slice from a 55 year old man who died from COVID-19 contains a calcified nodule (arrow) in the right globus pallidus, but is otherwise unremarkable. (B) Hematoxylin and eosin stained section of hippocampus shows scattered hypereosinophilic neurons indicative of acute hypoxic injury. (C) Hematoxylin and eosin stained section shows extravasated red blood cells suggestive of microhemorrhage (deep pink). (D) CD45 immunostaining (brown) highlights a small collection of perivascular immune cells. (E) CD45 immunostaining (brown) also highlights numerous resident immune cells of the brain parenchyma (microglia). (F) In comparison to panel E, a patient without COVID-19 shows minimal CD45 immunostaining (brown). (G) SARS-CoV-2 nucleocapsid immunohistochemistry (brown) shows a cytoplasmic staining pattern in respiratory epithelial cells of the trachea. (H) Transmission electron micrograph of SARS-CoV-2 from cultured cells shows spherical extracellular viral particles (arrows). Images B-F taken at 200x magnification, G at 400x magnification, and are each from a different patient. Image H is from the Centers for Disease Control and Prevents Public Health Image Library, courtesy of Courtesy Cynthia S. Goldsmith and A. Tamin.

Table 1: Summary of Published COVID-19 Reports with Autopsy Brain Findings

Reference	No. Cases Included; autopsy type	Macroscopic Evaluation	Microscopic Evaluation	SARS-CoV-2 RNA or Protein
Puelles et al. 2020 [41] Wichmann et. al. 2020 [49] Matschke et al. 2020 [35]	43; subset full autopsy with brain findings	Edema (n=23), fresh territorial infarct (n=6)	Fresh ischemic infarct (n=6), astrocytosis, microgliosis, perivascular, parenchymal, and leptomeningeal T cells (n=43)	qRT-PCR positive (13/27; median 4700 viral E gene copies/cell; range <1000 to 162,000) in frontal lobe and/or medulla Viral spike or nucleocapsid IHC positive in 16/40 cases (rare cells in medulla; 2 cases with vagus or glossopharyngeal nerves)
Solomon et al. 2020 [37]	18; brain-only findings	No specific findings	Mild to moderate acute hypoxic injury (n=18); rare foci of perivascular and leptomeningeal inflammation (n=3)	qRT-PCR positive (n=5; 5.0 to 59.4 N1/N2 copies/µL) Viral nucleocapsid IHC negative in all cases
Remmelink et al. 2020 [33]	11; full autopsy with brain findings	Recently drained subdural hematoma (n=1); cerebral hemorrhage (n=1)	Cerebral hemorrhage or hemorrhagic suffusion (n=8), focal ischemic necrosis (n=3), edema and/or vascular congestions (n=5), diffuse or focal spongiosis (n=10)	qRT-PCR positive (n=9; viral E gene; Ct: 28.67 to 35.11)
Schurink et al. 2020 [38]	11; full autopsy with brain findings	No specific findings	Hypoxic changes, activation/clusting of microglia, astrogliosis, perivascular cuffing of T cells most prominent in olfactory bulbs and medulla (n=11); neutrophilic plugs (n=3)	Viral nucleocapsid IHC negative in 11 cases
Fabbri et al. 2020 [31]	10; full autopsy with brain findings	Edema and meningeal congestion (n=10), cerebral infarction (n=3), uncal herniation (n=2), purulent leptomeninges (n=1), subarachnoid hemorrhage (n=1)	Global hypoxic-ischemic injury (n=10), acute hypoxic injury (all), intravascular microthrombi (n=10), macro and/or micro infarcts (n=10); perivascular microhemorrhage (n=10), microglial activation (n=5), perivascular/leptomeningeal lymphocytic inflammation (n=1)	qRT-PCR positive in olfactory nerve and brain tissue in (n=1; RdRp, E, and N genes)
Schaller et al.	10; full autopsy with	No specific findings	No specific findings	N.A.

2020 [50]	brain findings			
Hanley et al. 2020 [36]	9; full autopsy with brain findings	Hemorrhagic conversion of middle cerebral artery stroke (n=1)	Moderate to intense microglial activation; mild T-cell infiltrate around blood vessels and capillaries, and ischemic changes of variable extent in the neurons of the cortex and the white matter (n=5)	qRT-PCR positive (n=4; 10^1 to 10^4 viral E gene copies per µg total RNA) Subgenomic viral RNA positive (n=1; Ct ~32)
Deigendesch et al. 2020 [36] Menter et al. 2020 [49]*	7; full autopsy with brain findings	Moderate global brain edema without cerebral mass displacement (n=1)	Microglial activation in pons, medulla, and olfactory bulb; sparse perivascular and leptomeningeal infiltrates of lymphocytes; mild acute hypoxic-ischemic encephalopathy (n=3)	qRT-PCR positive in olfactory bulb (n=4), optic nerve (n=2); not detected in brainstem or cerebellum (ORFab1, S, and N genes)
von Weyhem et al. 2020 [38]	6; full autopsy with brain findings	Massive hemorrhage and herniation (n=2); petechial bleedings (n=4)	Hypoxic alterations (n=6); lymphocytic meningitis and encephalitis (n=6); brainstem neuronal cell loss in (n=4), axon degeneration (n=3)	N.A.
Bradley et al. 2020 [28]	5; full autopsy with brain findings	Scattered punctate subarachnoid hemorrhages (n=1)	Rare microhemorrhages in the brainstem (n=1)	N.A.
Kantonen et al. 2020 [50]	4; full autopsy with brain findings	Mild brain swelling, discoloration of watershed areas, lacunar infarcts, and microhemorrhages in cerebral and cerebellar white matter, deep gray matter, and brain stem (n=1)	High density acute microhemorrhages, severe hypoxic-ischemic injury, scattered T lymphocytes, and axonal spheroids (n=1); mild to moderate hypoxic-ischemic injury (n=3)	qRT-PCR negative in brain and olfactory mucosa (RdRp, N. and E genes) Viral spike IHC negative in brain, olfactory mucosa, and carotid body
Bussani et al. 2020 [51]	3; fill autopsy with brain findings	N.A.	Gliosis, neuronal loss, vascular rarefaction	N.A.
Barton et al. 2020 [52]	2; full autopsy with brain findings	No gross abnormalities	N.A.	N.A.
Jaunmuktane et al. 2020 [29]	2; brain-only findings	Large acute and subacute infarcts (n=1); white matter microhemorrhages and microinfarcts (n=1)	Hemorrhages and infarcts (n=2); mild leptomeningeal inflammation (n=1)	N.A.
Kirschenbaum et al. 2020 [39]	2; brain-only findings	N.A.	Perivascular leukocytic infiltrates in basal ganglia and intravascular	N.A.

			microthrombi (n=2); prominent leukocytic infiltrates in olfactory epithelium (n=2)	
Al-Dalahmah et al. 2020 [33]	1; full autopsy with brain findings	Cerebellar hemorrhage, acute infarcts in the dorsal pons and medulla, tonsillar herniation	Global hypoxia; numerous microglial nodules and neuronophagia in the inferior olives and cerebellar dentate nuclei; mild perivascular and sparse parenchymal and leptomeningeal lymphocytes; perivascular hemorrhages; chronic active inflammation in olfactory epithelium; red neurons in olfactory bulb and normal tract	qRT-PCR positive in nasal epithelium (Mean Ct 31.75, 278 copies/µL RNA), olfactory bulb (Ct 36.70, 11 copies/µL) cerebellar clot (Ct 33.0, 559 copies/µL), and cerebellum (Ct 37.17, 8 copies/µL) Viral nucleocapsid IHC negative Viral ISH negative
Craver et al. 2020 [53]	1; full autopsy with brain findings	No CNS lesions identified	No CNS lesions identified	N.A.
Dolhnikoff et al. 2020 [54]	1; full autopsy with brain findings	N.A.	Microglial reactivity	N.A.
Lax et al. 2020 [55]	1: full autopsy with brain findings	No acute alterations	No acute alterations	N.A.
Paniz-Mondolfi et al 2020 [12]	1; brain-only findings	N.A.	N.A.	qRT-PCR positive (four different assays targeting ORF1/a and E-gene, N1, N2, N3, N2 and E-gene, and ORF1ab and S genes) TEM showed viral like particles in frontal lobe sections
Reichard et al 2020 [14]	1; brain-only findings	Mild brain swelling and hemorrhagic white matter lesions	Focal hemorrhage, ADEM-like lesions, microinfarcts, damaged axons, hypoxic-ischemic injury	N.A.

* Provided data on angiotensin converting enzyme – 2 (ACE2) IHC in brain tissue and olfactory bulb.
Abbreviations: ADEM, Acute Disseminated Encephalomyelitis; Ct, cycle threshold; qRT-PCR, quantitative reverse transcriptase polymerase chain reaction; E gene, SARS-CoV-2 envelope gene; ORF1ab, open reading frame 1ab; IHC, immunohistochemistry; ISH, in-situ hybridization; RdRp, RNA-dependent RNA polymerase gene; N.A., not available or evaluated; TEM, transmission electron microscopy