

Review of Coronavirus in Rats: A Potential Zoonotic Threat (Ulasan Virus Korona pada Tikus: Suatu Potensi Ancaman Zoonosis)

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ABSTRACT

The current COVID-19 pandemic has highlighted the importance of the Coronaviridae family as a threat to public health in the emergence of a deadly zoonotic disease. Rats are the possible primary host of the infection as they are highly populated in urban areas, creating a significant epidemic risk. The tendency of coronaviruses (CoVs) to overcome species barriers and adapt to hosts typically found close to humans emphasised the need for further study on coronavirus infection. Sialodacryoadenitis virus (SDAV) and Parker's rat coronavirus (PRC) are the most commonly isolated pathogens for coronavirus infections in the laboratory and wild rats. They are contagious and could be transmitted to susceptible rats by direct contact, fomites, or aerosol. Coronavirus genera include *Alphacoronavirus* and *Betacoronavirus*, which are restricted to bats and other mammalian hosts, while the *Gammacoronavirus* and *Deltacoronavirus* are restricted to birds. All known rat coronaviruses are members of the beta genus. *Betacoronavirus* are divided into five subgenera, i.e., *Embecovirus*, *Hibecovirus*, *Merbecovirus*, *Nobecovirus*, and *Sarbecovirus*. All rat coronaviruses are categorised as the *Embecovirus* subgenus. Most studies have proven that rat coronaviruses are responsible for hepatitis, enteritis, reproductive problems, and respiratory and salivary gland infections, including episcleritis, and dacryoadenitis. The scant literature data, mostly comprising publications from the last century, does not adequately explain the etiopathology of SDAV and PRC infections. This review provides an overview of the knowledge on the characteristics, transmission, clinical signs, pathology, and diagnosis of rat coronaviruses, besides better understanding their zoonotic potentials.

Keywords: Coronavirus; Parker's rat coronavirus; rats; sialodacryoadenitis virus; zoonosis

ABSTRAK

Pandemik COVID-19 semasa telah menekankan kepentingan famili Coronaviridae sebagai ancaman kepada kesihatan awam dalam kemunculan penyakit zoonosis yang boleh membawa maut. Tikus adalah hos utama jangkitan yang mungkin kerana mereka mempunyai populasi yang tinggi di kawasan bandar, mewujudkan risiko wabak yang ketara. Kecenderungan coronavirus untuk mengatasi halangan spesies dan menyesuaikan diri dengan perumah yang biasanya ditemui berdekatan dengan manusia menekankan keperluan untuk kajian lanjut mengenai jangkitan coronavirus. Virus sialodacryoadenitis (SDAV) dan coronavirus tikus Parker (PRC) adalah patogen yang paling biasa dipencilkan bagi jangkitan coronavirus pada tikus makmal dan liar. Mereka berjangkit dan boleh menjangkiti tikus yang mudah terdedah melalui sentuhan langsung, fomit atau aerosol. Genus coronavirus termasuklah *Alphacoronavirus* dan *Betacoronavirus* adalah terhad kepada kelawar dan perumah mamalia lain, manakala *Gammacoronavirus* dan *Deltacoronavirus* adalah terhad kepada burung. Semua coronavirus tikus yang diketahui adalah ahli genus beta. *Betacoronavirus* dibahagikan kepada lima subgenus, iaitu, *Embecovirus*, *Hibecovirus*, *Merbecovirus*, *Nobecovirus* dan *Sarbecovirus*. Semua coronavirus tikus dikategorikan sebagai subgenus *Embecovirus*. Kebanyakan kajian telah

membuktikan bahwa coronavirus tikus bertanggungjawab untuk radang hati, radang usus, masalah pembiakan, jangkitan saluran pernafasan dan jangkitan kelenjar air liur, termasuklah episkleritis dan dacryoadenitis. Data kepustakaan yang sedikit, yang kebanyakannya terdiri daripada penerbitan dari abad yang lalu, tidak menjelaskan dengan secukupnya etiopatologi jangkitan SDAV dan PRC. Kajian ini memberikan gambaran pengetahuan secara keseluruhan tentang ciri, penularan, tanda klinikal, patologi dan diagnosis coronavirus tikus, selain memberi pemahaman lebih baik tentang potensi zoonosis mereka.

Kata kunci: Coronavirus; coronavirus tikus Parker; tikus; virus sialodacryoadenitis; zoonosis

INTRODUCTION

Emerging zoonotic diseases are a major concern worldwide, most likely due to spillover from wildlife to human populations. It significantly impacts public health and socioeconomic, as evidenced by the recent SARS-CoV-2 pandemic (WHO 2021). Rodents become an important evolutionary host for the transmission of zoonotic pathogens such as coronavirus (CoV). Coronaviruses can infect humans and animals, causing respiratory, enteric, hepatic, and neurological problems (Lau et al. 2014). Coronaviruses are classified in the subfamily *Orthocoronavirinae*, part of the *Coronaviridae* family under the *Nidovirales* order. They are spherical virions with a diameter of 125 nm and enveloped RNA viruses with club-shaped spikes protruding from their surface, evoking the solar corona. They have a positive-sense single-stranded RNA genome and a helical-symmetric nucleocapsid (Malik 2020). Due to the enzymatic error during the RNA replication process, RNA viruses will likely undergo rapid mutation. As a result, there is an urgent need to raise public awareness about coronavirus in rats. Although the rat coronavirus (RCV) has not been proven transmissible to humans, it is most likely a strain mutation. Therefore, this study aims to better understand rat coronaviruses and assess the risk of their zoonosis to prepare for a potential public health threat.

To date, murine coronavirus is the only coronavirus discovered in rodents, involving the subgenus *Embecovirus* and the genus *Betacoronavirus*. It was discovered in mice, called the mouse hepatitis virus or murine coronavirus (MHV). In 1970, an MHV variant was found in rats. Coronaviruses such as sialodacryoadenitis virus (SDAV) and Parker's rat coronavirus (PRC) are prevalent in rats (Decaro & Lorusso 2020). Rat coronavirus antibodies were prevalent in the laboratory and wild rats before the outbreaks occurred in the laboratory and pet rats. PRC was found in the lungs of infected rats. Both SDAV and PRC cause necrosis of the

salivary and lacrimal glands. SDAV is associated with a high morbidity rate but a low mortality rate. However, some rats may sustain irreversible eye damage. Atypical oestrous cycles and mortality of neonates have been related to disease outbreaks (Barthold, Griffey & Percy 2016). The development of interstitial pneumonia in the lower respiratory tract is associated with an increased mortality rate among young rats (Maclachlan, Dubovi & Fenner 2016). Coronaviruses in rats are extremely contagious and can be transmitted to naive rats via aerosol and fomites.

The importance of rodents in the coronavirus evolution is associated with embecoviruses, including a novel *Betacoronavirus* discovery in China (Lau et al. 2014). Embecoviruses infect humans to cause respiratory disease (Zappulli 2020). Coronaviruses, on the other hand, are not restricted to the *Betacoronavirus* genus in rats. The viruses were also detected in ricefield rats (*Rattus losea*), Norway rats (*Rattus norvegicus*), Asian house rats (*Rattus tanezumi*), and Chinese white-bellied rats (*Niviventer confucianus*). They sequenced the virus's entire genome and identified a divergent alphacoronavirus known as the species of Lucheng Rn rat coronavirus (LRNV) and *Betacoronavirus* (Wang et al. 2015).

METHODS

A literature search on coronaviruses in rats was conducted using databases such as Google Scholar, PubMed, Science Direct, and Scopus. Text word searches using keywords of the desired topic, including 'coronavirus', 'Parker's rat coronavirus', 'rats', 'sialodacryoadenitis virus', and 'zoonosis', were performed to retrieve the articles. Articles that were duplicated or irrelevant to the topic were excluded from the review. After the screening process, which considered the inclusion and exclusion criteria, 47 published studies on rat coronaviruses have been revised and summarised in this review.

IMPORTANCE OF CORONAVIRUS IN RATS AS ZOOONOTIC POTENTIAL

Coronaviruses of known and novel RNA viruses with unknown zoonotic potential were discovered in rodents in Ghana (Suu-Ire et al. 2022). Throughout the last two decades, emerging coronavirus outbreaks, which is the current pandemic of a novel coronavirus (SARS-CoV-2) in China, significantly affected healthcare systems and economies in all continents, causing radical alterations in common habits and lifestyles. These findings demonstrate the importance of the viral family as a zoonotic public health threat. SARS-CoV-2 has been reported recently across the species barrier (Dhama et al. 2020). Most coronavirus-related diseases that affect humans originate from animals, particularly wildlife (Huong et al. 2020). Despite its widespread distribution, the study and data on coronavirus infection in animals, particularly rats, in Malaysia is lacking. Rats and other rodents have been found in high densities in close proximity to humans, contributing to a significant zoonotic threat (Meerburg, Singleton & Kijlstra 2009; Mohd-Qawiem et al. 2022a). Annisa (2020) reported

evidence of contact between humans and rats, bats, and other agricultural animals due to regular exposure to coronavirus-positive materials such as manure and urine, increasing the zoonotic risk.

DISCOVERY OF RAT CORONAVIRUSES

The first report of the newly discovered coronavirus family member appeared in the 1960s when laboratory rats developed severe sialadenitis, dacryoadenitis, and lower respiratory tract transmission disease (Jonas et al. 1969). Meanwhile, the other study confirmed the finding of a pathogenic agent, which was then identified via electron microscopy as virus-like particles of infected rat salivary glands (Jonas et al. 1969). Parker, Cross and Rowe (1970) continued their work by isolating PRC from the lungs of asymptomatic rats. A second novel strain, SDAV, was discovered to be antigenically related to MHV and the PRC (Bhatt, Percy & Jonas 1972). Further studies have been conducted on this subject, emphasising other strains. Figure 1 shows the phylogenetic relationships among members of the *Coronavirinae* subfamily, as reported by King et al. (2011).

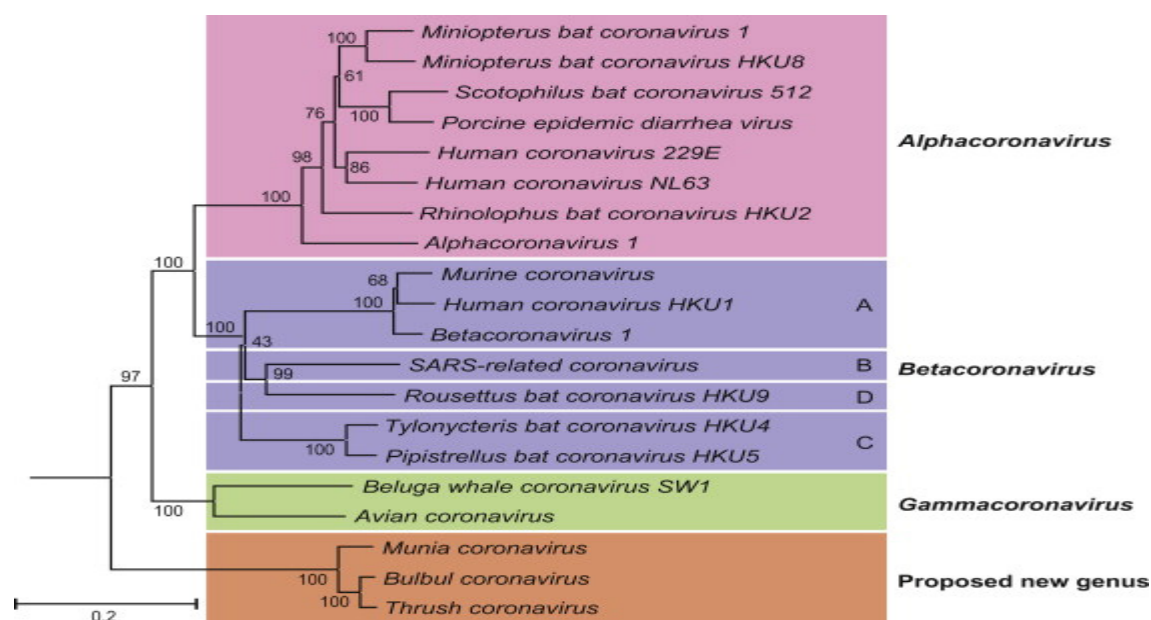


FIGURE 1. Phylogenetic relationships among members of the *Coronavirinae* subfamily. The tree identifies four major monophyletic clusters (colour-coded) corresponding to the genera *Alpha-*, *Beta-*, and *Gammacoronavirus* and an envisaged new genus. It identifies the distinct *Betacoronavirus* lineages A through D

CLASSIFICATION OF CORONAVIRUSES

Coronaviruses, along with toroviruses, roniviruses, arteriviruses, and mesoniviruses, are members of the *Nidovirales* order (nido = nest; Gorbalenya et al. 2006). Positive-sense RNA viruses (*Nidovirales*) are non-segmented enveloped viruses. Among the most distinguished characteristics are the highly conserved genomic organisation, the ribosomal frameshifting expression of non-structural proteins genes, and several unique non-conventional enzymatic activities encoded by the large replicate. *Cornidovirineae* viruses are the most susceptible to epidemics and pandemics of the eight *Nidovirales* suborders. Two subfamilies within the *Coronaviridae* family are *Letovirinae* and *Orthocoronavirinae* (Bukhari et al. 2018).

Coronaviridae is the largest family with the longest genome (30,000 nucleotides) and the largest virion (spherical, 80 to 180 nm in diameter). Both birds and mammals facilitate the spread of diseases, affecting the respiratory, nervous, and gastrointestinal systems. Alphacoronaviruses (14 subgenera and 19 species), betacoronaviruses (5 subgenera and 14 species), and deltacoronaviruses (3 subgenera and 7 species) are the four orthocoronaviruses genera (Walker et al. 2020). Human-infecting alpha and beta viruses are the most remarkable for medical research (alphacoronaviruses: HCoV-229E and HCoV-NL63, and betacoronaviruses: HCoV-OC43, HCoV-HKU1, and SARS-CoV, MERS-CoV, and SARS-CoV-2), as shown by Wartecki and Rzymiski (2020) (Figure 2).

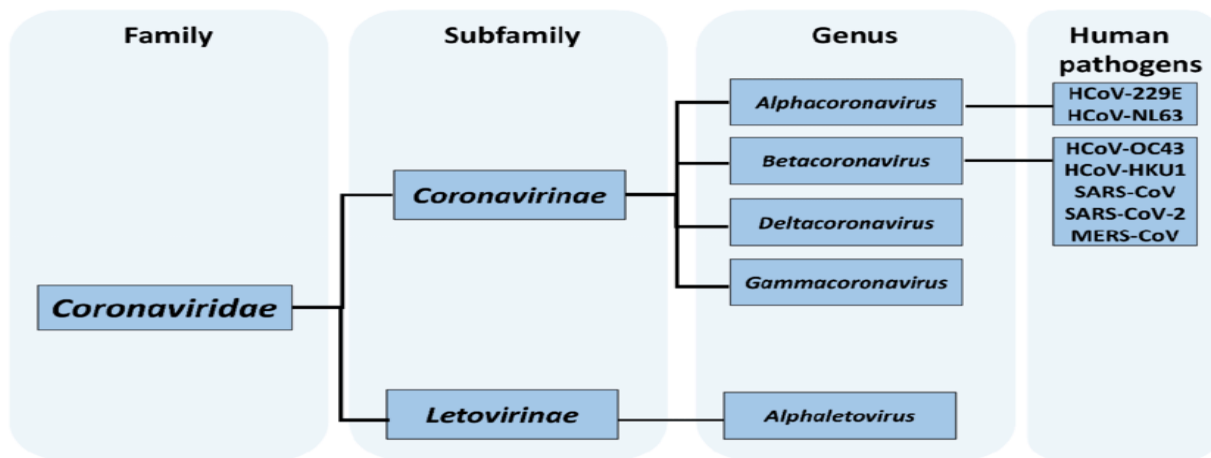


FIGURE 2. *Coronaviridae* family taxonomy with a record of species that are known to be pathogenic to humans and cause respiratory diseases

Currently, seven human coronaviruses are known (Table 1), almost all of which are zoonotic in origin and capable of crossing species boundaries. While alpha- and betacoronaviruses are found in bats and other mammalian hosts, gamma- and deltacoronaviruses are discovered exclusively in birds, with some strains infecting marine mammals (Zhou, Qiu & Ge 2021). Porcine epidemic diarrhoea virus (PEDV), transmissible gastroenteritis coronavirus (TGEV), feline coronavirus (FCoV), mouse hepatitis virus (MHV), and infectious bronchitis virus (IBV) are examples of common viruses in chickens. The rapid spread of companion animal

coronaviruses among livestock and companion animals sparked extensive research in the latter half of the twentieth century (Cui, Li & Shi 2018).

SPECIES OF CORONAVIRUSES

Coronavirus is a pathogen that affects a wide variety of birds and mammals. Coronavirus species are classified into three groups based on the non-essential gene count and arrangement, as well as the presence or absence of a hemagglutinin-esterase (HE) protein in the virion (Mahy 2008). The species of coronavirus are listed in Table 2.

TABLE 1. Coronaviruses in humans

| CoV genus | CoV subgenus | CoV species | Related risk disease | Reference(s) |
|-------------------------|----------------------|--|--|--|
| <i>Alphacoronavirus</i> | <i>Setracovirus</i> | Human coronavirus NL63 (HCoV-NL63) | Mild respiratory disease | Van der Hoek et al. (2004), Fouchier et al. (2004) |
| <i>Alphacoronavirus</i> | <i>Duvinacovirus</i> | Human coronavirus 229E (HCoV-229E) | Acute respiratory disease | Sun et al. (2021), Lvov et al. (2020) |
| <i>Betacoronavirus</i> | <i>Embecovirus</i> | Betacoronavirus 1 (HCoV-OC43) | Acute respiratory disease | Walsh, Shin & Falsey (2013), Decaro & Lorusso (2020) |
| <i>Betacoronavirus</i> | <i>Embecovirus</i> | Human coronavirus HKU1 (HCoV-HKU1) | Mild respiratory disease | Woo et al. (2005) |
| <i>Betacoronavirus</i> | <i>Sarbecovirus</i> | Severe acute respiratory syndrome-related coronavirus (SARS-CoV-1) | Severe respiratory disease, diarrhoea | Ksiazek et al. (2003) |
| <i>Betacoronavirus</i> | <i>Marbecovirus</i> | Middle East respiratory syndrome-related coronavirus (MERS-CoV) | Severe respiratory disease, diarrhoea, vomit | Zaki et al. (2012) |
| <i>Betacoronavirus</i> | <i>Sarbecovirus</i> | Severe acute respiratory syndrome-related coronavirus (SARS-CoV-2) | Severe respiratory disease, diarrhoea | Zhou, Qiu & Ge (2020), Wu et al. (2020) |

CoV= Coronavirus; CoVs= Coronaviruses

TABLE 2. Species of coronavirus

| Group 1 | Group 2 | Group 3 |
|---|---|---|
| Canine coronavirus Feline coronavirus Human coronavirus 229E Porcine epidemic diarrhoea virus Transmissible gastroenteritis virus | Bovine coronavirus Human coronavirus OC43 Human enteric coronavirus Murine hepatitis virus Porcine hemagglutinating encephalomyelitis virus Puffinosis coronavirus Rat coronavirus Severe acute respiratory syndrome coronavirus | Infectious bronchitis virus Pheasant coronavirus Turkey coronavirus |

RAT CORONAVIRUSES

All known rat coronaviruses are beta genus members. Betacoronaviruses are classified into five subgenera: *Embecovirus*, *Hibecovirus*, *Merbecovirus*, *Nobecovirus*, and *Sarbecovirus*. The subgenus *Embecovirus* includes SDAV, PRCV, and the novel China *Rattus* Coronavirus (ChRCoV) HKU24 (Walker et al. 2020). The ChRCoV, isolated in China from Norway rats, is believed to be the source of *Betacoronavirus 1*. This discovery is significant because it is a distinct species not derived from wild birds or bat hosts, and it establishes a new lineage of A Beta-CoV (A CoVs) from rodents as the primary host. ChRCoV HKU24 is a *Betacoronavirus 1* member belonging to the murine lineage, and it can potentially spread from rodents to other mammals via interspecies transmission. Interestingly, this transmission occurred prior to the late nineteenth century of the human Coronavirus OC43 appearance (Lau et al. 2014). Additionally, it is critical to note that the SDAV and other species of rat coronaviruses belong to the same genus as the most pathogenic and highly epidemic CoVs for

humans, including the SARS-CoV, SARS-CoV-2, and MERS-CoV (Zhou, Qiu & Ge 2021).

VIRION STRUCTURE AND BIOLOGICAL FUNCTIONS OF PROTEINS

SDAV virion has a spherical architecture with an average diameter of 80 to 180 nm, as demonstrated by tomography and cryo-electron microscopy (Jonas et al. 1969). It contains a genomic core of non-segmented, positive-sense ssRNA balanced by a nucleocapsid protein surrounded by a viral membrane envelope. Tentacle-shaped spikes on the virion's surface give it the appearance of a corona, the most distinguishing feature of all coronaviruses. CoVs have a helically symmetric nucleocapsid, which is unusual in positive-sense RNA viruses but prevalent in negative-sense RNA viruses (Wang, Grunewald & Perlman 2020). SDAV is composed of coronaviral structural proteins, including the spike (S), nucleocapsid (N), membrane (M), envelope (E), and hemagglutinin esterase (HE), which are all encoded at the 3'-end of the viral genome (Bartak et al. 2021), as shown in Figure 3.

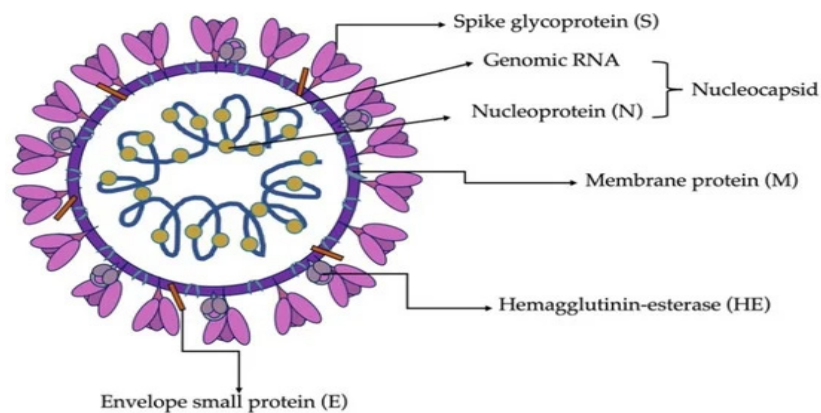


FIGURE 3. SDAV virion structure diagram

The homotrimer protein S is involved in the fusion of the virus membrane and the entry of virus genetic material into the host cell. It is the causative agent of SDAV infection due to the high virulence and tissue tropism variability. S protein is a 149.6 kD N-linked glycosylated type I transmembrane protein with a 1357 amino acid sequence. S1 and S2 are cleaved into two subunits by host proteases (Raamsman et al. 2000). The

low similarity to MHV-A59 and MHV-JHM (76.6%) is due to additional sequences in the SDAV virus's N-terminal half of the S-protein gene (Yoo et al. 2000). Several studies have shown that mutations in the S protein gene or other associated factors can affect the CoV virulence, tissue tropism, host range, or immune response in mammals (V'kovski et al. 2021). Protein M, a structural protein of 228 amino acids with a molecular weight

of 26 kD (Yoo et al. 2000), is a monomer comprising three hydrophobic domains tightly coupled to the viral envelope (Hogue & Machamer 2007). It contains an N-terminal glycosylated ectodomain (extracellular domain) and a C-terminal glycosylated endodomain (cytoplasmic site of the intracellular membrane). Protein M evolves the membrane region necessary for virion assembly and the uptake of structural protein residues by modifying the membrane structure, however, it is required for a virus-like particle (VLP) folding (Neuman et al. 2011). Additionally, the M protein interacts with RNA by acting as a carrier of the genomic packaging signal (Narayanan et al. 2003).

The homopentamer (E) has a molecular weight of 10.1 kD and contains 88 amino acids. It is present in minimal amounts in the virion. It interacts with protein M during virion morphogenesis and assembly. Additionally, it functions as a viroporin by forming pentameric protein-lipid pores that facilitate ion transport (Wilson et al. 2004). The helical nucleocapsid protein (N) contains 454 amino acids with a molecular mass of 49.4 kD, almost identical to the MHV N protein. It contributes to replication by forming homodimers and homooligomers, binding and processing genomic RNA, and synthesising nucleocapsids. Additionally, it can impede translation in the host cell's ribosomes. Besides interacting with proteins M and E, protein N folds and initiates the replication of newly assembled viruses. Other murine coronaviruses, such as MHV-JHM, contain a nucleocapsid protein that acts as an enhancer and determinant of neurovirulence (Bartak et al. 2021).

The haemagglutinin esterase (HE) of SDAV, the virus's fifth major structural protein, binds to sialic acid residues on glycoproteins and glycolipids on the surface. It is a homodimer of 439 amino acids with a molecular weight of 49 kD (Yoo et al. 2000). The HE lectin domain promotes virion attachment while increasing the activity of sialate-O-acetyl esterase toward clustered sialoglycotopes. In comparison to MHVs, the 14 HE conserves a great deal of energy. It is found only in a few embecoviruses (MHV and other rodent CoVs, BCoV, HCoV-OC43, and HKU1). However, it is unknown if HE is required for MHV-JHM, MHV-S, or MHV-DVIM infection, as it is an auxiliary binding molecule for the S protein (Bartak et al. 2021).

POTENTIAL ZOOSES

A majority of Vietnamese and Cambodians consume wild-caught animals (Annisa 2020). Since the early

2000s, the demand for rat meat has increased due to favourable public opinion, particularly among wealthy diners. It has become a great concern due to the recent COVID-19 outbreak in China, caused by a coronavirus-infected bat that was consumed. Rats are found in almost every city, and humans are more likely to come into contact with them than other animals (Han et al. 2015). With the increasing consumption of rats, early detection of coronavirus in rats is critical to prevent a new disease outbreak. The detection of coronaviruses in restaurants increased between 2013 and 2014, and their apparent amplification of high-risk infection to the end-users most likely explains the zoonotic spillover to humans (Huong et al. 2020). In rats, some viruses like paramyxoviruses often spread infections between species, manifesting disease in spillover hosts, including humans (Mohd Qawiem et al. 2022b).

Coronaviruses infect all rats regardless of the wild or laboratory origin. Hence, we must first acknowledge our understanding of the virus replication cycle to consider SDAV as a zoonotic threat rather than an enzootic laboratory threat. It becomes incomplete once it enters a host due to the hypervariable region of the family's main structural glycoprotein, S-spike protein, being susceptible to transient mutations, another inter-species barrier crossing event similar to the one that occurred with SARS-CoV-2 in Wuhan in 2019 (Cui, Li & Shi 2018). Parker, Cross and Rowe (1970) and Bhatt, Percy and Jonas (1972) were among the first researchers in the 1970s to report SDAV infection in mice. Due to the close relationship between coronaviruses and distantly related animals, the recent discovery of new coronaviruses became a great concern. Quesenberry and Carpenter (2011) alerted the risk of zoonotic disease, particularly if the rodent is kept as a pet and housed with immunocompromised individuals. It is discouraged due to the risk of infections from pathogens, including coronavirus that may spread to humans.

TRANSMISSION AND CLINICAL SIGNS OF CORONAVIRUS IN RATS

The virus spreads rapidly following infection of a susceptible rat population, primarily through saliva or nasal secretions (Barthold, Griffey & Percy 2016). Prior SDAV infection may help protect the rat from reinfection for up to 15 months. Epizootics have a low mortality rate but a moderate to severe morbidity rate. Morbidity frequently reaches 100% in rats housed conventionally. Two types of infection exist. It occurs in

endemic virus-infected breeding colonies. In young non-immune rats, this infection causes conjunctivitis that can last up to seven days. The second type is characterised by acute episcleritis and photophobia in naive rats ranging from weaning to adulthood (Bartak et al. 2021).

The common clinical signs of coronavirus infection in rats are sniffing, epiphora, blepharospasm, and cervical enlargement. It is possible to develop dark red encrustations around the external nares and eyes. These porphyrin-containing compounds are synthesised by injured Harderian glands and fluoresce pink under ultraviolet (UV) light exposure. During the convalescent period, ocular lesions can occur unilaterally or bilaterally. It caused reproductive problems in rats, including infant mortality and irregular menstrual cycles, but no signs of intrauterine transmission. It also caused emaciation, oedema, and anorexia (Sato et al. 2001).

SDAV infection with symptoms is currently manageable in the laboratory. Infected animals can be controlled by quarantined or eliminated once PCR tests confirm the infection. Mild and asymptomatic infections can worsen into a problem as the virus evolves and develops resistance to treatment. Sanitising mouse or rat cages and properly disposing of waste help prevent SDAV transmission. Soiled bedding can effectively transmit MHV, mouse parvovirus (MPV), and Theiler's mouse encephalomyelitis virus (TMEV).

PATHOLOGY OF CORONAVIRUS IN RATS

The pathological findings of coronavirus in rats have been illustrated in Figures 4, 5 and 6 as described previously

by Barthold, Griffey and Percy (2016). Excessive lacrimation or red encrustations around the eyelids and external nares are observed in clinically affected rats. Oedema of the parotid and/or submandibular (submaxillary) salivary glands may be visible when the skin of the ventral neck is reflected. The affected glands are swollen and pale compared to normal glands (Figure 4(a)-4(b)). Keratitis sicca, inadequate intraocular drainage, hyphema, and megaloglobus are all symptoms of lacrimal gland dysfunction (Figure 4(c)). Infraorbital lacrimal glands share many of the same difficulties as Harderian lacrimal glands. The glands are typically blotchy brown. In the acute stage, these salivary glands exhibit coagulation of ductal epithelium necrosis, inconsistent adjacent acini involvement, and dilation of the normal morphology. Oedema of the interstitial space and infiltration of mononuclear and polymorphonuclear cells are frequently seen.

Non-keratinising squamous metaplasia of salivary ductal and acinar structures (Figure 5(a)) and lacrimal glands began at 7 to 10 days after exposure, along with responsive cervical lymph node hyperplasia. At this stage, the primary cellular infiltrates are lymphocytes, plasma cells, mast cells, and macrophages. Salivary and lacrimal glands, particularly the Harderian glands, exhibit squamous metaplasia (Figure 5(b)). After 3 to 4 weeks of exposure, normal acinar and ductal epithelial cell regeneration occurs. Despite the presence of poorly differentiated epithelial cells and aggregations of the mononuclear cell, including mast cells, the salivary glands are typically histologically normal at this stage (Figure 6(a)). For a few weeks, the Harderian glands



FIGURE 4. Submandibular salivary gland of a rat. a) Healthy rat. b) The acute stage of the sialodacryoadenitis virus (SDAV) of an infected rat appeared pale and enlarged (arrow), with periglandular oedema. c) Megaloglobus along with hyphema in SDAV infection

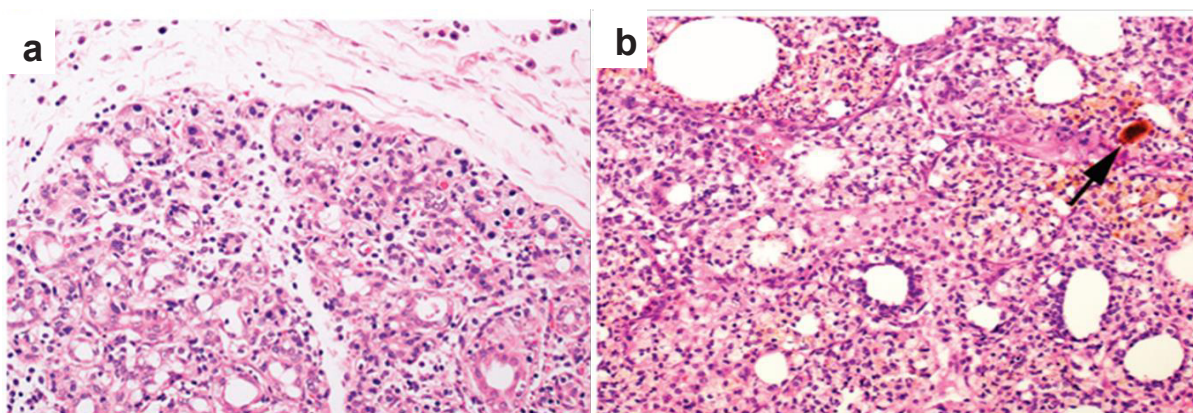


FIGURE 5. Photomicrograph of submandibular salivary gland and Harderian gland of severe SDAV infection in rats. a) Submandibular salivary gland: Interlobular and periglandular swelling, along with epithelial necrosis and leukocytic infiltration. b) Harderian gland

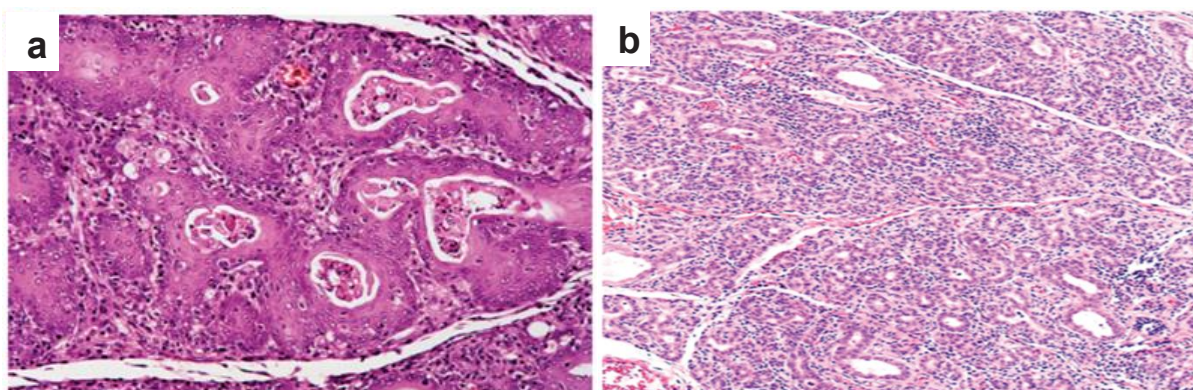


FIGURE 6. Photomicrograph of parotid salivary gland and Harderian gland of recovering rat from SDAV infection. a) Parotid salivary gland: The interstitium is filled with mononuclear leukocytes, while the ducts and acini are surrounded by undifferentiated hyperplastic epithelium. b) Harderian gland: The ducts and acini show much squamous metaplasia

may develop prolonged inflammatory lesions with pigmented interstitial deposition (Figure 6(b)).

Acute necrotising rhinitis is characterised by the infiltration of mononuclear and polymorphonuclear cells. Epithelia of the respiratory and olfactory systems become damaged and inflamed. For example, the vomeronasal organ may require more than 14 days to heal. This disease is characterised by leukocytic infiltration, epithelial hyperplasia, and flattening and

loss of ciliated cells. Focal alveolitis results in alveolar macrophage hypercellularity and activation. Lower respiratory tract lesions typically resolve within 8 to 10 days of exposure. In athymic nude rats, SDAV causes chronic infections and wasting syndrome. Inflammatory lesions in the salivary and lacrimal glands, as well as chronic suppurative rhinitis, are also seen. Antigens from the virus were detected in infected tissues, including the urinary epithelium (Barthold, Griffey & Percy 2016).

DIAGNOSIS OF CORONAVIRUS IN RATS

Despite the virus's respiratory tract-specific nature, the pathogenesis includes entry into systemic blood vessels and the gastrointestinal tract. This explains why clinical signs such as viremia and gastrointestinal symptoms also serve as diagnostic indicators for this disease. On microscopic examination, the presence of common lesions of the salivary and lacrimal glands is sufficient to make the diagnosis. The Harderian and the submaxillary and parotid salivary glands should be histologically examined. Virus isolation is an inconvenient method to diagnose the disease in most cases. The serological method is the preferred test before the SDAV exposure. Immunohistochemical techniques and serological tests include the multiplex fluorescent immunoassay (MFI) with IFA confirmation and the enzyme-linked immunosorbent assay (ELISA) could be used for disease diagnosis.

Several differential diagnoses in coronavirus-infected rats include mycoplasma, Sendai virus, or pneumonia virus of mice (PVM) infections with clinical signs of nasal and ocular discharge, with *Pseudomonas aeruginosa* causing subcutaneous oedema of the head, increased levels of ambient ammonia inducing ocular and nasal irritation, and stress-associated chromodacryorrhea. Chromodacryorrhea, known as red porphyrin encrustations surrounding the eyes and nose, is an uncommon indication of SDAV infection. Porphyrins may also be released from the Harderian glands in response to stressful conditions and chronic illnesses, such as chronic respiratory disease (CRD), as mentioned by Barthold, Griffey and Percy (2016).

After exposure to coronavirus for 4 to 6 days, the viral antigen can be detected in the respiratory tract and infected salivary and lacrimal glands. PCR has been used to confirm the diagnosis (Annisa 2020). According to Besselsen, Wagner and Loganbill (2002), a specimen of infected tissue, faeces, and oral or cage swabs is used to perform the molecular diagnostic RT-PCR for the detection of the M gene (membrane glycoprotein gene), the N gene (nucleocapsid gene), and the pool gene to confirm the initial diagnosis. The positive rats should be quarantined or eliminated. Animal rooms should be avoided for at least 5 to 8 weeks throughout the quarantine period. Due to the high degree of SDAV contagiousness, personnel who handle the infected animals may become a major source of infection, as the virus particles could be transmitted through protective clothing (Bartak et al. 2021).

CONCLUSION

Detection of RCV enables strategy for the possibility of mutations and cross-species barriers for preparedness for zoonotic threats. The unknown zoonotic potential exposes the public to virus spillover risk through direct and indirect contact with rats. This virus subgenus was discovered in rodents and shown to be pathogenic in rats. Although a virus mutation is inevitable, detection of the virus can significantly slow its spread, thereby decreasing the likelihood of mutation. Consequently, it is possible to avert a future pandemic. Individuals involved in the food supply chain who expose to the risk of rats must be screened for viruses to ensure the biosecurity of food production systems and improve global health. Understanding coronavirus infections in rats is critical to facilitate early detection, prevention, and future outbreak prevention of the possible zoonotic risk. The spread of the virus by limiting the population by rising cleanliness and waste management for rat population control should be prioritised. Standard precautions, such as thorough hand washing, should be done when handling pet rodents. As a recommendation, early detection or screening should be conducted, especially in urban areas such as wholesale markets where the rat population is high. There is a possibility of more strains could be found if more screenings are done. Other than that, further research on the virus entry, receptor and how it interacts on the coronavirus surface in the host cell of rats is important to provide a better understanding to prevent a further zoonotic outbreak.

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