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Rhodanine derivative LJ001 inhibits TGEV and PDCoV replication in vitro

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Artificial info

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Abstract

Transmissible gastroenteritis virus (TGEV) and porcine deltacoronavirus (PDCoV) are members of the family Coronaviridae and mainly cause acute diarrhea/vomiting, dehydration and mortality in piglets, which lead to huge economic losses to the swine industry. Rhodanine derivative LJ001 has been verified to be effective against some enveloped virus infections in vitro. In this study, we evaluated the antiviral activity of LJ001 towards TGEV and PDCoV replication on swine testicular (ST) cells. Our results showed the 50% cellular cytotoxicity (CC_{50}) value of LJ001 was 146.4 µM on ST cell. The virus titers of TGEV and PDCoV were obviously decreased in the presence of LJ001 with the concentrations of 3.125 and 12.5 µM, and LJ001 potently inhibited TGEV and PDCoV infection at the replication stages of viral life cycle. Further study indicated that LJ001 inhibited TGEV and PDCoV replication by inhibition of viral RNA and protein synthesis, and reducing virus yields at 12 and 24 h post-inoculation. These data indicated that LJ001 had antiviral activities on TGEV and PDCoV replications in vitro, which may serve as a new candidate for treatment of coronaviruses infections.

1. Introduction

Coronaviruses are enveloped, single-stranded and positive-sense RNA viruses that can infect and cause diseases in avian and mammalian species (Xu et al., 2018). According to the genome and antigenicity features, coronaviridae is divided into four genera: Alphacoronavirus (α-CoV), Betacoronavirus (β-CoV), Gammacoronavirus (γ-CoV) and Deltacoronavirus (δ-CoV) (King et al., 2012; Lu et al., 2015). To date, there are five different species of CoVs that cause diseases in pigs (Wang et al., 2019): α-CoV, including porcine epidemic diarrhea virus (PEDV), transmissible gastroenteritis virus (TGEV), swine enteric alphacoronavirus (SeACoV) and porcine respiratory coronaviruses (PRCV); β-CoV, including porcine hemagglutinating encephalomyelitis virus (PHEV); and δ-CoV, including porcine deltacoronavirus (PDCoV). TGEV, PEDV, PDCoV and SeACoV can cause swine enteric coronavirus disease and lead to significant economic losses for the pork industry (Jung et al., 2016; Yang et al., 2010; Huang et al., 2013).

TGEV, a member of the genus Alphacoronavirus in the family coronaviridae, was first identified as an etiological agent of transmissible gastroenteritis in swine in 1946 in the United States, and since then, it has become one of the most common viral causes of diarrhea in pig herds (Niederwerder and Hesse, 2018). TGEV causes severe watery diarrhea, vomiting, and dehydration in suckling piglets less than 2 weeks old, resulting in a high rate of mortality (up to 100%) (Niederwerder and Hesse, 2018). Inactivated and live-attenuated vaccines against TGEV have been proven effective in preventing TGEV infections in some extent, however, protection of TGEV infections is not complete in clinic (Jin et al., 2018). To solve this problem, enhancing the immune effects of TGEV vaccines and development of new effective antiviral agents are the effective ways.

PDCoV, belonging to the Deltacoronavirus genus of the Coronaviridae family, is a novel swine enteropathogenic coronavirus with worldwide distribution (Hu et al., 2016). PDCoV was first identified in pigs in Hong Kong in 2012 (Woo et al., 2012), then the detection of PDCoV in swine herds were reported in the US, Canada, China, South Korea, and Thailand (Wang et al., 2014b; Ma et al., 2015; Janetanakit et al., 2016; Lee et al., 2016). Similar to TGEV, PDCoV causes severe diarrhea, vomiting, and dehydration in piglets (Jung et al., 2016). There are currently no approved treatments or vaccines available for PDCoV. Therefore, screening drugs that are effectively against PDCoV infections are needed.

LJ001, a novel small-molecule rhodanine derivative, has been shown to have broad-spectrum antiviral activities in vitro (Balmer et al., 2017). LJ001 can inhibit the entry and spread of some enveloped viruses,
including human immunodeficiency virus (HIV), hepatitis C virus (HCV), influenza, Ebola, arenaviruses and poxviruses (Balmer et al., 2017). LJ001 could inhibit enveloped virus infection at the stage of virus entry, for it mainly acts on viruses, and not the cell (Wolf et al., 2010). Further research showed the antiviral activity of LJ001 was light-dependent, and required the presence of molecular oxygen. The exact mechanism was that the LJ001-generated singlet oxygen (\(^1\text{O}_2\)) mediated lipid oxidation, which then negatively affects on the biophysical properties of viral membranes (membrane curvature and fluidity), and then result in the virus-cell membrane fusion (Vigant et al., 2013). The virion structures and the viral envelope functions remain intact during LJ001 treatment, so the virions treated with LJ001 are able to bind to their receptors (Wolf et al., 2010). Previous research confirms that even though LJ001 was lipophilic, it still could bind to both viral and cellular membranes, and inhibit virus-cell but not cell-cell fusion (Balmer et al., 2017; Wolf et al., 2010). Although LJ001 has shown the antiviral activities on many viruses, the effects of LJ001 on coronaviruses replication have yet to be demonstrated. So in current study we conducted experiments about LJ001 antiviral activity on TGEV and PDCoV replication on swine testicular (ST) cells. The following report described the LJ001 antiviral activities on TGEV and PDCoV replication.

2. Materials and methods

2.1. Cell line, viruses and drug

ST cells were purchased from the Institute of China Veterinary Medicine Inspection. Cells were cultured in Dulbecco’s Modified Eagle’s Medium (DMEM, Gibco) containing 10 % fetal bovine serum (FBS, Gibco). TGEV strain HN-2012 and PDCoV strain CH-01 were isolated and identified by our laboratory. TGEV were propagated in ST cell with maintenance medium (DMEM supplemented with 1% antibiotic-antimycotic) (Jin et al., 2018). PDCoV were propagated in ST cell with maintenance medium (DMEM supplemented with 1% antibiotic-antimycotic and 1% pancreatin). (Zhang et al., 2019).

LJ001, kindly provided by Professor Guoyu Yang of Henan Agricultural University, was dissolved in DMEM containing 0.1 % (v/v) dimethylsulfoxide (DMSO, Solarbio), and stored at 4 °C and protected from light.

2.2. Cell viability assay (cytotoxicity)

Cell viability assay was performed with ST cells in 96-well plates. Briefly, cells were seeded into 96-well plates and grown to 100 % confluence after 24 h. Eight wells containing a monolayer of cells were added with the different concentrations of LJ001 (0.782, 1.563, 3.125, 6.25, 12.5, 25, 50, 100 and 200 μM), and the cells that treated with DMEM containing 0.1 % DMSO were served as mock controls. After 24 h, cells were washed twice with D-Hanks, and then incubated with 100 μl of 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (5 mg/mL) solarbio for 4 h. The reaction was stopped by adding 150 μl of DMSO, and the absorbance was measured at 570 nm. The CC\(_{50}\) was calculated using GraphPad Prism software.

2.3. Assays the antiviral activity of LJ001

To investigate the inhibitory effect of LJ001 on TGEV or PDCoV infection, ST cells were incubated with different concentrations of LJ001 (0.782, 3.125 and 12.5 μM) at various stages of viral infection (MOI = 0.01) (Fig. 2A). Briefly, ST cells were seeded into 24-well plate, and were subjected to the following three experiments: (1) for pre-treatment groups, cells were incubated with LJ001 (0.782, 3.125 and 12.5 μM) for 1 h prior to viral inoculation. After that, cells were washed with D-Hanks and infected with virus at 37 °C in 5% CO\(_2\) for 1 h. The maintenance medium (DMEM without FBS) was added to the plates and incubated for 24 h. (2) For co-treatment groups, cells were infected with virus in the presence of different concentration of LJ001 (0.782, 3.125 and 12.5 μM) for 1 h. After washed, maintenance medium for supplemented with the corresponding concentrations of LJ001 (0.782, 3.125 and 12.5 μM) were added and cell were incubated for 24 h. (3) For post-treatment groups, cells were firstly infected with virus for 1 h at 37 °C. Cells were washed with D-Hanks, maintenance medium supplemented with different concentration of LJ001 (0.782, 3.125 and 12.5 μM) was added and incubated for 24 h. The cell culture supernatants were collected at 24 h post-inoculation (hpi). For all the experiments, ST cells incubated with 0.1 % DMSO or virus only were used as controls. All groups were performed in three independent experiments. Virus titer was determined by TCID\(_{50}\) assay as previously described (Hu et al., 2015).

2.4. RNA extraction and quantitative real-time reverse-transcription PCR (RT-qPCR)

RNA was extracted from ST cells using TRIzol reagent (TaKaRa, China) and cDNA was synthesized by using SuperQuick RT MasterMix (TaKaRa, China) according to the manufacturer’s instructions. RT-qPCR was performed using SYBR Green PCR Master (TaKaRa, China) and the specific primers were shown in Table 1. Data were normalized against β-actin expression and are expressed as fold differences between control and treated cells using the 2-ΔΔCT method.

2.5. Western blot analysis

To determine whether LJ001 could influence the viral proteins synthesis, the expression levels of viral N proteins of TGEV and PDCoV were tested by western blot at 12 and 24 hour post-infection (hpi). Protein lysates were obtained from ST cells using ice-cold lysis RIPA buffer containing 10 Mm phenylmethylsulfon fluoride (PMSF). Total protein concentration was determined by SDS-PAGE analysis and transferred onto polyvinylidene fluoride (PVDF) membrane (Millipore, USA). Membranes were blocked with 5% nonfat milk for 3 h at room temperature, and then incubated with primary antibody at 4 °C overnight. The following primary antibodies were used: β-actin (Abcam, USA), TGEV N and PDCoV N polyclonal antibody (prepared in our laboratory using standard methods). After incubated with the primary antibody, HRP-conjugated secondary antibody were added and incubated for 2 h. Protein bands were detected by enhanced chemiluminescence reagents (Thermo, USA), and analyzed by ImageJ software.

2.6. Statistical analyses

Results were expressed as the means ± standard deviation (SD) from three independent experiments. Statistical analyses were performed using Student’s t-test. Differences were considered significant at p < 0.05. Statistical significance is indicated in figures as follows: * p < 0.05, ** p < 0.01.

| Genes | Sequences (5’-3’) | Size (bp) |
|-------|-------------------|----------|
| β-actin | F: GGGGCATCCAGAAGACTAC\nR: GATCTCCCTTCTGATCCTGC | 137 |
| TGEV M | F: ATGGTAGAACTGGTGTTGGTATT\nR: CACATGGCGTTACAGAGTAGAT | 120 |
| PDCoV M | F: GACCAATGCGCTCAATTCT\nR: TGGCGGATTTCTGACTGATATG | 99 |
3. Results

3.1. Minimal cytotoxicity of LJ001

The chemical structure of LJ001 is (S)-3-Allyl-5-[5-(phényl-2-furyl)méthylene]-2-thioxo-1,3-thiazolidin-4-one (Fig. 1A). LJ001 cytotoxicity in the range of 0.782–200 μM was evaluated in three independent experiments by MTT assay. The results showed that LJ001 exhibited no significant cytotoxicity at the concentrations from 0.782 to 25 μM (Fig. 1B). It showed slight cytotoxicity on ST cells at the concentration of 25 μM but without statistical significance (p > 0.05). The CC50 value of LJ001 on ST cells was about 146.4 μM (Fig. 1C).

3.2. LJ001 possesses antiviral activity against TGEV and PDCoV infection

To determine whether LJ001 has the negative effect on the production of TGEV and PDCoV, ST cells were treated with various concentrations of LJ001 (ranging from 0.782 μM to 12.5 μM) before (pre-treatment), during (co-treatment), and after (post-treatment) the virus inoculation (Fig. 2A), all experiments were performed 24 h after infection. In the pre-treatment group, LJ001 had only marginal effect on TGEV infection with slight reduction of viral titers (Fig. 2B). In the co-treatment and post-treatment groups, no obvious antiviral effects were observed on the TGEV replication when 0.782 μM of LJ001 was added. While LJ001 at the concentrations of 3.125 and 12.5 μM obviously suppressed TGEV replication, and the virus titers (TCID50) decreased approximately by 1.35–2.0 log10 respectively (Fig. 2C and D).

The effect of LJ001 on PDCoV infection was also evaluated, the result showed only the 12.5 μM of LJ001 pre-treatment group induced a slight decrease in the PDCoV virus titer (Fig. 3A). In the co-treatment group, exceeding 24 % and 36 % of decrease of the viral titers were observed in the 3.125 and 12.5 μM of LJ001 treated-groups as compared to the virus only group, respectively (Fig. 3B). The similar phenomena were also observed in the case of LJ001 post-treated groups (Fig. 3C), which resulted in 1.3 and 2.1-log10 decrease of PDCoV viral titers in the 3.125 and 12.5 μM of LJ001-treated groups, respectively.

3.3. LJ001 inhibits the life cycle of viral replication

To further investigate which steps of the viral life cycle were affected by LJ001, LJ001 were added to ST cells during virus infection. According to the cytotoxicity test and antiviral activity, 12.5 μM of LJ001 was selected to the following experiments. Briefly, ST cells were co-incubated with virus (TGEV or PDCoV, MOI = 0.01) and LJ001 (12.5 μM) for 1 h. After washing, maintenance medium supplemented with 12.5 μM of LJ001 was added to the cells. The intracellular viral RNA levels, extracellular viral titers, and expression levels of viral N protein were examined at 1 (the time point for virus entry), 6, 12 and 24 hpi after treatment and post-treatment groups, no obvious antiviral effects were observed on the TGEV replication when 0.782 μM of LJ001 was added. While LJ001 at the concentrations of 3.125 and 12.5 μM obviously suppressed TGEV replication, and the virus titers (TCID50) decreased approximately by 1.35–2.0 log10 respectively (Fig. 2C and D).

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4. Discussion

The threat of emerging and re-emerging coronaviruses highlights the need to develop broad-spectrum antivirals. Rhodamine derivative LJ001 has previously reported to inhibit the entry of numerous lipid-enveloped viruses at non-cytotoxic concentrations. LJ001 exerts antiviral activities by binding to both viral and cellular membranes and inhibits virus–cell fusion (Wolf et al., 2010). In this study, LJ001 was shown to have antiviral activities against two swine enteropathogenic coronaviruses (TGEV and PDCoV) infection in vitro, which can serve as a new candidate for treatment of swine enteric coronaviruses infections. Moreover, there is an urgent need for targeted and effective COVID-19 treatments during the COVID-19 pandemic, we deduced the SARS-CoV-2 also needs to be tested under similar in vitro conditions with or without LJ001 treatment, especially in comparison to those positive control groups treated with known antivirals, such as remdesivir.

Our cellular toxicity assays revealed that the cytotoxicity of LJ001 to ST cells was as high as 146.4 μM, and the LJ001 of 12.5 μM has no obvious influence on ST cell viability and morphology (Fig. 1). Wolf et al. also demonstrated there was no effect on active cell metabolism on LJ001-treated Vero cells (Wolf et al., 2010). In the pre-treatment group (Figs. 2B and 3A), different concentration of LJ001 (12.5, 3.125 and 0.782 μM) was added to ST cells and incubated for 1 h, then cells were infected with TGEV or PDCoV, the miscible liquids were detected at 24 hpi. The results showed that LJ001 had no obvious effect on cells. Wolf et al. demonstrated that LJ001 does not act on virus infection, there is no obvious inhibitory effects for VSV infection after the cell pretreated with LJ001 (Wolf et al., 2010).

Present research demonstrated that LJ001 could inhibit the infection for Influenza A, HIV, Rift Valley Fever Virus (RVFV) and HCV (Wolf et al., 2010).
Fig. 2. Antiviral effect of LJ001 against TGEV infection. (A) Schematics of LJ001 addition experiments. ST cells were incubated with virus (MOI = 0.05) for 1 h. Different concentrations of LJ001 were added at prior to infection (-1 h) as well as at 0 h or 1 h post-infection. All experiments were performed 24 h after infection. (B) pre-treatment group, LJ001 was used for pretreatment of ST cells at 37 °C for 1 h. (C) co-treatment group, cells were infected with virus in the presence of different concentration of LJ001 for 1 h, (D) post-treatment group, cells were firstly infected with virus for 1 h at 37 °C, after washed, different concentration of LJ001 was added and incubated for 24 h. All experiments were performed 24 h after infection, and the TGEV viral titers in the culture supernatant were measured by TCID$_{50}$ assay. The experiment was performed three times independently. Differences were considered significant at (*) 0.01 < $p$ < 0.05, (**) $p$ < 0.01.

Fig. 3. Antiviral effect of LJ001 against PDCoV (MOI = 0.5) infection. ST cells were treated with LJ001 and PDCoV as described above. (A) pre-treatment group, LJ001 was used for pretreatment of ST cells at 37 °C for 1 h. (B) co-treatment group, cells were infected with virus in the presence of different concentration of LJ001 for 1 h, (C) post-treatment group, cells were firstly infected with virus for 1 h at 37 °C, after washed, different concentration of LJ001 was added and incubated for 24 h. All experiments were performed 24 h after infection, and the PDCoV viral titers in the culture supernatant were measured by TCID$_{50}$ assay. The experiment was performed three times independently. Differences were considered significant at (*) 0.01 < $p$ < 0.05, (**) $p$ < 0.01.
et al., 2010; Vigant et al., 2013). In our study, the cells were infected with virus in the presence of different concentration of LJ001 (0.782, 3.125 and 12.5 μM) for 1 h, then the presence of different concentration of LJ001 (0.782, 3.125 and 12.5 μM) were added, the miscible liquids were detected at 24 hpi in the co-treatment group. The results showed that the LJ001 have obvious inhibitory effect for TGEV and PDCoV infection (Figs. 2C and 3B). Furthermore, the cells infected with TGEV or PDCoV for 1 h, 12.5 μM of LJ001 were added to ST cells, the miscible liquids were detected after infected for 1, 6, 12 and 24 hpi. The results showed that LJ001 had no obvious effect at the early stages of TGEV and PDCoV replication, while its inhibitory effect was apparent at the viral replication cycle (Fig. 4). Previous research showed that LJ001 acts on VSV during the viral-entry stage, further studies indicated LJ001 targeted the virus-cell fusion binding to lipid membranes of VSV in an irreversible manner (Wolf et al., 2010; Vigant et al., 2013). These previous results differ with our observations of the LJ001 effects on the viral replication stage, which may be because of the difference of the lipid membrane of TGEV and PDCoV with other enveloped viruses in biophysiological and physiological properties. However, the specific mechanism needs further investigation. Future experiments will determine if LJ001 can be used not only as a treatment but also for preventing TGEV and PDCoV infection.

TGEV and PDCoV belong to different genera of coronavirus, encoding distinct nucleotide sequence and amino acid sequence (Zhu et al., 2018). The clinical symptoms of PDCoV are similar to that of TGEV. Histologic lesions are observed in all sections of small intestine, while the pathogenesis mechanism behind TGEV and PDCoV infections remain largely unknown (Koonpaew et al., 2019). No treatments or vaccines are available for PDCoV currently. In this study, LJ001 was described to have the antiviral activity against TGEV or PDCoV at a micromolar range. Recent years, the emerging and re-emerging coronaviruses cause severe threat to global public health, and new drugs were constantly discovered to anti-coronaviruses. Prior research showed saracatinib can inhibit middle east respiratory syndrome-coronavirus (MERS-CoV) at the early stages of the viral life cycle by suppressing the Src-family of tyrosine kinases (SFK) signaling pathways (Shin et al., 2018). Agostini reports showed that antiviral remdesivir (GS-5734) could inhibit several coronaviruses replication, including severe acute respiratory syndrome coronavirus (SARS-CoV), murine hepatitis virus (MHV) and MERS-CoV. They further demonstrated that this antiviral activity was mediated by the viral polymerase and the proofreading exonuclease (Agostini et al., 2018). However, the exact mechanism of the antiviral activity of LJ001 on TGEV or PDCoV replication need further study.

In summary, this study suggested that LJ001 showed antiviral activity at the replication stage of TGEV and PDCoV. LJ001 has the potential to serve as a new therapeutic agent for coronaviruses infections. Further study is still needed to understand the underlying antiviral mechanism of LJ001 on PDCoV and TGEV infection.

Author Statement

Hui Hu and Guoyu Yang designed and funded the study; Yunfei Zhang performed the experiments; Yunfei Zhang and Lu Xia analyzed the results and drafted the manuscript; Yixin Yuan, Qianqian Li and Li Han participated in correcting the manuscript. All the authors have reviewed the final version of the manuscript and approve it for publication. No conflict of interest exits in the submission of this manuscript, and manuscript is approved by all authors for publication. I would like to declare that the work described was original research that has not been published previously, and not under consideration for publication elsewhere, in whole or in part.

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Declaration of Competing Interest

The authors report no declarations of interest.

Acknowledgments

Not applicable.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.virusres.2020.198167.
References

Agostini, M.L., Andreu, E.L., Sima, A.C., Graham, R.L., Sheahan, T.P., Lu, X., Smith, E.C., Case, J.B., Feng, J.Y., Jordan, B., Ray, A.S., Ghibar, T., Siegel, D., Mackman, R.L., Clarke, M.O., Baric, R.S., Denison, M.R., 2018. Coronavirus susceptibility to the antiviral remdesivir (GS-5734) is mediated by the viral polymerase and the proofreading exoribonuclease. mBio 9 (2), e00221–18.

Balmer, B.F., Powers, R.L., Zhang, T.H., Lee, J., Vigant, F., Lee, B., Jung, M.E., Purcell, M.K., Seokvik, K., Aguilar, H.C., 2017. Inhibition of an aquatic rhabdovirus demonstrates promise of a broad-spectrum antiviral for use in aquaculture. J. Virol. 91 (4), 02181–16.

Hu, H., Jung, K., Vlasova, A.N., Chepngeno, J., Lu, Z., Wang, Q., Saif, L.J., 2015. Isolation and characterization of porcine deltacoronavirus from pigs with diarrhea in the United States. J. Clin. Microbiol. 53 (5), 1537–1548.

Hu, H., Jung, K., Vlasova, A.N., Saif, L.J., 2016. Experimental infection of gnotobiotic pigs with porcine deltacoronavirus strain OH-FD22. Arch. Virol. 161 (12), 3431–3434.

Huang, Y.W., Dickerman, A.W., Pineyro, P., Li, L., Fang, L., Kiehne, R., Opriessnig, T., Meng, X.J., 2013. Origin, evolution, and genotyping of emergent porcine epidemic diarrhea virus strains in the United States. Mbio 4 (5), e00737–00715.

Janetanakit, T., Lumyai, M., Bunpapong, N., Boonypatisitnop, S., Chaiyawong, S., Nonthabenjanvan, N., Kesaengsaksinwut, S., Amonin, A., 2016. Porcine deltacoronavirus, Thailand. 2015. Emerging Infect. Dis. 22 (4), 759–765.

Jin, X.H., Zheng, L.L., Song, M.R., Xu, W.S., Kou, Y.N., Zhou, Y., Zhang, L.W., Zhu, Y.N., Wang, Q., Vlasova, A.N., Saif, L.J., 2015. Isolation and characterization of porcine deltacoronavirus from pigs with diarrhea in Korea. Virol. Sin. 33 (2), 131–140.

Lee, J.H., Jung, H.C., Nguyen, V.G., Moon, H.J., Kim, H.K., Park, S.J., Lee, C.H., Lee, G.E., Park, B.K., 2016. Detection and phylogenetic analysis of porcine deltacoronavirus in Korean swine farms. Transbound. Emerg. Dis. 63 (3), 248–252.

Ma, Y., Zhang, Y., Liang, X., Lou, F., Oglesbee, M., Krakowka, S., Li, J., 2015. Origin, evolution, and virulence of porcine deltacoronaviruses in the United States. Mbio 6, e00664–15.

Niederwerder, M.C., Hesse, R.A., 2018. Swine enteric coronavirus disease: a review of 4 years with porcine epidemic diarrhea virus and porcine deltacoronavirus in the United States and Canada. Transbound. Emerg. Dis. 65 (5), 660–675.

Shin, J.S., Jung, E., Kim, M., Baric, R.S., Go, Y.Y., 2018. Saracatinib inhibits middle respiratory syndrome-coronavirus replication in vitro. Viruses. 10 (6), 283.

Vigant, F., Lee, J., Hollmann, A., Tanmer, L.B., Akyol Ataman, Z., Yun, T., Shai, G., Aguilar, H.C., Zhang, D., Meriwether, D., Roman-Souza, G., Robinson, L.R., Jueltich, T.L., Buckkowdski, H., Chou, S., Castanho, M.A.R.B., Wolf, M.C., Smith, J.K., Banyard, A., Kielian, M., Reddy, S., Wenk, M.R., Seike, M., Santos, N.C., Freiberg, A.N., Jung, M.E., Lee, B., 2013. A mechanistic paradigm for broad-spectrum antivirals that target virus-cell fusion. PLoS Pathog. 9, e1003297.

Wang, L., Byrum, B., Zhang, Y., 2014. Porcine coronavirus HKU15 detected in 9 US states, 2014. Emerging Infect. Dis. 20 (9), 1594–1595.

Wang, Q., Vlasova, A.N., Kennedy, S.P., Saif, L.J., 2019. Emerging and re-emerging coronaviruses in pigs. Curr. Opin. Virol. 34, 39–49.

Wolf, M.C., Freiberg, A.N., Zhang, T., Akyol-Ataman, Z., Grock, A., Hong, P.W., Li, J., Watson, N.F., Fang, A.Q., Aguilar, H.C., Honko, A.N., Darnoiseaux, R., Miller, J.P., Woodson, S.E., Chantasirivisal, S., Fontanes, V., Negrete, O.A., Krogstad, P., Dasqupta, A., Moscona, A., Hendley, L.E., Whelan, S.P., Faull, K.F., Holbrook, M.R., Jung, M.E., Lee, B., 2010. A broad-spectrum antiviral targeting entry of enveloped viruses. Proc. Natl. Acad. Sci. U.S.A. 107 (7), 3157–3162.

Woo, P.C.P., Lau, S.K.P., Lam, C.S.F., Lau, C.C.Y., Tsang, A.K.L., Lau, J.H.N., Bai, R., Teng, J.L.L., Tsang, C.C.C., Wang, M., Zheng, B.J., Chan, K.H., Yuen, K.Y., 2012. Discovery of seven novel mammalian and avian coronaviruses in the genus Deltacoronavirus supports bat coronaviruses as the gene source of Alphacoronavirus and Betacoronavirus and avian coronaviruses as the gene source of Gammacoronavirus and Deltacoronavirus. J. Virol. 86 (7), 3995–4008.

Xu, Z., Zhong, H., Zhou, Q., Da, Y., Chen, L., Zhang, Y., Xue, C., Cao, Y., 2018. A highly pathogenic strain of porcine deltacoronavirus caused watery diarrhea in newborn piglets. Virol. Sin. 33 (2), 131–141.

Yang, Y.L., Yu, J.Q., Jordan, R., Ray, A.S., Cihlar, T., Siegel, D., Mackman, R.L., King, A.M.Q., Adams, M.J., Carstens, E.B., Lefkowitz, E.J., 2012. Virus Taxonomy: Classification and Nomenclature of Viruses: Ninth Report of the International Committee on Taxonomy of Viruses. Academic Press.

Koonsap, S., Teeravechyan, S., Frantz, P.N., Chailangkarn, T., Jongkueawsattana, A., 2019. PEDV and PDCoV pathogenesis: the interplay between host innate immune responses and porcine enteric coronaviruses. Front. Vet. Sci. 6, 34.

Lee, J.H., Chung, H.C., Nguyen, V.G., Moon, H.J., Kim, H.K., Park, S.J., Lee, C.H., Lee, G.E., Park, B.K., 2016. Detection and phylogenetic analysis of porcine deltacoronavirus in Korean swine farms, 2015. Transbound. Emerg. Dis. 63 (3), 248–252.