Virus Research 310 (2022) 198671

Contents lists available at ScienceDirect

Virus Research

journal homepage: www.elsevier.com/locate/virusres

Inhibition of bovine and ovine capripoxviruses (*Lumpy skin disease virus* and *Sheeppox virus*) by ivermectin occurs at different stages of propagation in vitro

Eda Baldan Toker, Ozer Ates, Kadir Yeşilbağ *

Department of Virology, Bursa Uludag University, Faculty of Veterinary Medicine, 16059, Bursa, Turkey

ARTICLE INFO

Keywords:
Ivermectin
Antiviral efficiency
In vitro testing
Capripoxvirus
Lumpy skin disease
Sheeppox

ABSTRACT

Capripoxvirus diseases are listed as reportable diseases by World Organization for Animal Health (OIE). *Lumpy skin disease virus* (LSDV) and *sheeppox virus* (SPPV), which can only be distinguished by molecular analysis, cause moderately, severe, or sometimes fatal infections in cattle and sheep. Even though vaccines are the most effective way to control the infection, their effectiveness may decrease in some cases. Therefore, it is significant to explore antiviral drugs against these diseases along with the vaccine. This study aimed to investigate the antiviral efficiency of ivermectin (IVM) at different stages of in vitro replication of LSDV and SPPV. For this purpose, viral titers ($TCID_{50}$/mL) of the viruses not treated with IVM (0.0 μM) and treated with non-cytotoxic concentrations of IVM (1.0 and 2.5 μM) were compared during a nine-day (216 h) post-infection period by viral titration assay. At 2.5 μM concentrations of IVM, the mean viral titer was significantly ($P<0.05$) reduced by approximately three logs for the replication stage of LSDV and SPPV. To evaluate the antiviral activity of IVM against LSDV and SPPV by treatment at the virus attachment and penetration stages, the titers of the virus either untreated or treated with 2,5 μM IVM were compared by virus titration assay. The number of infectious virions for LSDV and SPPV were decreased by 99.82% and 99.87% at the viral replication stage, 68.38% and 25.01% at the attachment stage, and 57.83% and 0.0% at the penetration stage, respectively. It was determined that ivermectin is statistically more effective on LSDV than SPPV at the virus attachment and penetration stages ($P<0.05$). This study found that the drug IVM can inhibit capripoxviruses, including LSDV and SPPV at various stages of the propagation. Moreover, this research predicted the in vitro antiviral ability of IVM against capripoxvirus infections for the first time.

1. Introduction

Lumpy skin disease (LSD) and sheeppox (SPP) are common diseases of domestic ruminants. The World Organization for Animal Health (OIE) lists these diseases as notifiable because of their potential for considerable economic impact and fast spread. The morbidity rates of LSD in cattle can be 5–45%, while mortality is lower than 10%. The morbidity rate can reach 100% and can be much higher in unexpected outbreaks in Europe. While the morbidity rate of SPP is 70–90%, the mortality rate reaches 50% in adults and 100% in lambs which is more sensitive. Economic losses are due to decreased milk production, abortions and infertility, reduced growth rate, and deterioration of skin quality by deep pox lesions (Tuppurainen et al., 2017). Total production losses from LSD are calculated to be 45–65%, while SPP is estimated to cause reduced productivity of surviving animals and direct animal losses, with an average annual income loss of 30–43% (Kumar, 2011; Yeruham et al., 2007). It can take up to 6 years for flocks to recover from an outbreak of LSD or SPP (Garner et al., 2000). Vaccination campaigns and trade restrictions for the movement of live animals or their products also cause major economic losses. Importantly, capripoxvirus diseases are also a limiting factor for the genetic development of livestock, as high milk-producing cattle are more severely affected by infection than local breeds (Hailu, 2015; Tuppurainen et al., 2017).

Lumpy skin disease virus (LSDV) and Sheeppox virus (SPPV) are members of the *Capripoxvirus* genus in the family *Poxviridae* (ICTV, 2020). Although these two viruses are serologically indistinctive, they can be distinguished through molecular analysis. The genome of capripoxviruses is linear and has a dsDNA genome of about 154 kb. The

* Corresponding author.
E-mail address: kyesilbag@uludag.edu.tr (K. Yeşilbağ).

https://doi.org/10.1016/j.virusres.2021.198671
Received 7 November 2021; Received in revised form 26 December 2021; Accepted 28 December 2021
Available online 2 January 2022

capripoxvirus viral proteins bind to host glycosaminoglycans, and the virus enters the host cell via endocytosis. There are two different infectious virus particles, including intracellular mature and extracellular enveloped virions (ViralZone, 2021). Transmission of capripoxviruses can occur through aerosols, vectors, and contaminated feed and water. Capripoxvirus infections usually present with open clinical signs such as fever, ocular and nasal discharge, swelled lymph nodes, and rash mucosal membranes and skin. Clinical findings can be moderate, severe, or fatal. The severity of clinical signs varies depending on host susceptibility, immunity, and virulence of the virus strain (Babiuk et al., 2009; Skrypnyk et al., 2016). Control and eradication of capripoxvirus infections depend on the capacity of veterinary services, farmer's awareness, vector control, early detection of disease, animal movement restrictions, requirment for slaughtering the infected animals, and vaccination (Hamdi et al., 2021).

Capripoxviruses are endemic in the north of Equatorial Africa, the Middle East, Asia, and the Indian subcontinent. Also, LSD has spread into Southeast Europe, the Balkans, and the Caucasus as part of the Eurasian epidemic (OIE, 2013). Currently, there is no directly administered drug for the treatment of capripoxvirus infections. The most effective way is vaccination, despite some factors affecting vaccine effectiveness (EFSA, 2015, 2014; Tuppurainen et al., 2017). Ivermectin is a broad-spectrum anti-helminthic drug licenced for humans and animals. Also, ivermectin can stop or reduce the replication of various viruses with different inhibition mechanisms (Raza et al., 2020; Wagstaff et al., 2012; Yang et al., 2020). Recent studies have reported that ivermectin is proved to inhibit the replication of some RNA viruses (Human immunodeficiency virus type 1, West Nile virus, Dengue virus, Zika virus, Tick-borne encephalitis virus, Bovine viral diarrhea virus, Foot-and-mouth disease virus, Newcastle virus, Peste des petits ruminants virus, Bovine orthopneumovirus, Bovine respirovirus-3, Bovine coronavirus, and SARS-CoV-2) (Afzal et al., 2021; Azeem et al., 2015; Caly et al., 2020; Mastrangelo et al., 2012; Naeem et al., 2021; Yang et al., 2020; Yesilbag et al., 2021) and DNA viruses (Pseudorabies virus and Bovine herpesvirus type 1) (Lv et al., 2018; Raza et al., 2020; Yesilbag et al., 2021). This study evaluated the antiviral efficiency of ivermectin in the viral replication, virus attachment, and penetration stages of LSDV and SPPV.

2. Materials and methods

2.1. Compound of ivermectin

Injection solution containing ivermectin (IVM, 10 mg/mL) as an active component was used. This solution includes 22.23-dihydro-avermectin B 1a (~80%) and 22.23-dihydro-avermectin B 1b (~20%) (Avromec, Topkim, Turkey). For use in the tests, the stock solution of IVM (containing 1000 μM IVM) was prepared by vortexing for at least 10 min in Dulbecco's Modified Eagle's Medium (DMEM). Toxicity and efficiency results were also validated with a pure compound of IVM supplied by another commercial company (Pharmactive, Turkey).

2.2. Cell culture and viruses

Antiviral efficiency of IVM was applied on LSDV and SPPV-Bakirkoy strain. The 50% cell culture infectious dose (log_{10} $TCID_{50}$/mL) was 5.00 log10 $TCID_{50}$/mL for LSDV and 4.50 log_{10} $TCID_{50}$/mL for SPPV.

Propagation of stock viruses and determination of virus titers were carried out in the African green monkey kidney epithelial cell line (Vero). The cells were incubated in DMEM with 10% fetal calf serum (FCS) at 37 °C and 5% CO_2 atmosphere. DMEM has also contained 250 μL/mL Amphotericin B and 100 UI/mL Penicillin and 100 μg of Streptomycin.

2.3. Cell cytotoxicity assay for IVM

The cytotoxicity of IVM was assessed by calculating the number of viable cells in total cells. First, Vero cells (8×10^4cells/mL) were seeded on 24-well plates and cultured in DMEM containing 10% FCS for 24 h at 37 °C under 5% CO_2 atmospheric conditions. The medium was then removed, and the cells were treated with IVM at concentrations of 0.0, 1.0, 2.5, 5.0, 7.5, and 10.0 μM IVM diluted in DMEM for nine days under the same conditions. Every 24 h, cells treated with different concentrations of IVM were harvested individually by trypsinization. Detached cells in suspension were centrifuged, and the cell pellets were pipetted with 1 mL of DMEM. Finally, Vero cells stained with trypan blue were examined under the microscope and calculated by dividing the number of viable cells by the total cells.

2.4. Effect of IVM on replication of the viruses

The virus titers obtained from IVM-treated and untreated infected cells were compared to evaluate the effect of IVM on replication of LSDV and SPPV. Initially, 24-well plates were plated with Vero cells (8×10^4 cells/mL). The cell medium was removed when the cells covered 80% of the plate surface (after approximately 24 h). Then, 200 μl of a $100TCID_{50}$/mL suspension viruses were inoculated into the respective wells of the plates and incubated for two hours. For this purpose, the first three columns of the 24-well plate were inoculated with the virus to test non-cytotoxic IVM concentrations. The last column was not inoculated with the virus to perform cell control (see supplementary material-1). After incubation, the plate wells were washed three times with phosphate-buffered saline (PBS). Then 1 mL of a non-cytotoxic concentration (0.0, 1.0, and 2.5 μM) of IVM was added to each column on infected cells and incubated at 37 °C under a 5% CO_2 atmosphere. Infected cells were examined for cytopathogenic changes under an inverted microscope for nine days. Infected cells (together with cell supernatant) in wells to which 0.0, 1.0, and 2.5 μM IVM was added from day four post-infection were collected every 24 h in separate tubes at −80 °C. Before testing for viral titers, the tubes were thawed at 37 °C and centrifuged at 13000 rpm for 10 min at +4 °C. Thus, all the cells were lysed, and infectious viral particles were transferred to the supernatant. The viral titration assay was applied to IVM-treated viral suspensions collected at defined time points (96, 120, 144, 168, 192, and 216 h) to determine viral titers. Also, the efficacy of the commercial IVM solution was supported by performing the test procedure using a pure IVM compound at 2.5 μM concentration.

2.5. Effect of IVM on virus attachment

Viruses were treated with IVM to evaluate the antiviral efficacy of IVM in the cell-binding phase of LSDV and SPPV before inoculation into cell cultures. For this purpose, both of the viruses were diluted at $100TCID_{50}$ wherein the final dilution was prepared in DMEM containing 2.5 μM IVM for the IVM assay, while IVM-free DMEM was used for control wells. The virus dilutions were incubated at 37 °C for 1 hour. Then, 200 μL of these virus dilutions were inoculated onto Vero cells plated on the 24-well plates 24 h ago. Plates were incubated at 4 °C for 1 hour, and wells were washed three times with PBS to remove the unbound virus particles. DMEM without IVM and FCS was added on the cells, and the plates were incubated at 37 °C under 5% CO_2 conditions. Infected cells and supernatant were collected and stored at −80 °C until virus titration assay at the point where the level of cytopathogenic effects in control cells was 80% (at 10th days post-infection).

2.6. Effect of IVM on virus penetration

To determine the efficacy of IVM on the penetration phase of LSDV and SPPV into Vero cells entirely, the viruses were given time to enter the cell. For this purpose, 24-well plates were coated with Vero cells (8

$\times 10^4$ cells/mL). The viruses were diluted 100$TCID_{50}$, and 200 µl of a 100$TCID_{50}$/mL suspension were inoculated onto the cells in each of wells. Plates were incubated at 4 °C for 2 h, and infected cells were washed three times with PBS. Then, 500 µL of DMEM containing 2.5 µM IVM was added to the infected cells and incubated for 1 h at 37 °C. At the same time, 500 µL of DMEM without IVM and without FCS was added onto infected cells, which were prepared as virus control. Infected cell surfaces were washed with PBS (pH 3.00) to remove viruses still in the adsorption phase. One milliliter of DMEM without IVM and FCS was added to the cells and incubated at 37 °C under 5% CO_2 atmospheric conditions until 80% cytopathogenic effect (at 10th days post-infection) had developed in the virus control cells. Infected cells and supernatant in the same well were collected together and stored at −80 °C until virus titration assay.

2.7. Virus titration assay

The viral titers ($TCID_{50}$/mL) of LSDV and SPPV obtained in IVM-treated and untreated infected cells were compared simultaneously. Briefly, 0.1 mL of 10-fold diluted viral suspensions were added to a 96-well plate (four wells were used per dilution). Four infected and non-infected wells were used in each test plate as control wells. Vero cells (1.5×10^5 cells/mL, 50 µL suspension) were added, and plates were incubated at 37 °C under a 5% CO_2 atmosphere. Plates were examined under an inverted light microscope for the presence of cytopathogenic effects. $TCID_{50}$ titers were calculated using the Spearman-Kârber method.

2.8. Statistical analyses

All experiments were performed twice individually. Results were analyzed using IBM SPSS Statistics 23 software. Independent-Sample T-test was applied to compare the mean values of the test group's viral titers ($TCID_{50}$/mL) with the control. Pearson chi-square analysis was applied to evaluate whether ivermectin had more potent antiviral activity on LSDV or SPPV. Statistical significance was defined as $P<0.05$.

3. Results

3.1. The non-cytotoxic concentration of IVM on Vero cells

To determine the non-cytotoxic IVM concentrations in Vero cells, the percent viability values of cells treated with different concentrations of IVM were recorded for nine days (216 h). For Vero cells, 10 and 7.5 µM IVM were cytotoxic from day 2, while the 5 µM IVM concentration was found toxic from day 4. At the 216th hour of the experiment, the cell viability rates for 0.0 (untreated cells), 1.0, 2.5, 5.0, 7.5, and 10.0 µM IVM were 87.24%, 89.58%, 89.66%, 0.0%, 0.0%, and 0.0%, respectively (Fig. 1). Therefore, 1.0 µM and 2.5 µM IVM concentrations were chosen to evaluate the antiviral activity against LSDV and SPPV (Fig. 2). In addition, it was determined that the toxicity values of the pure IVM compound were similar to the commercial IVM solution (concentrations of 5 µM and above were cytotoxic).

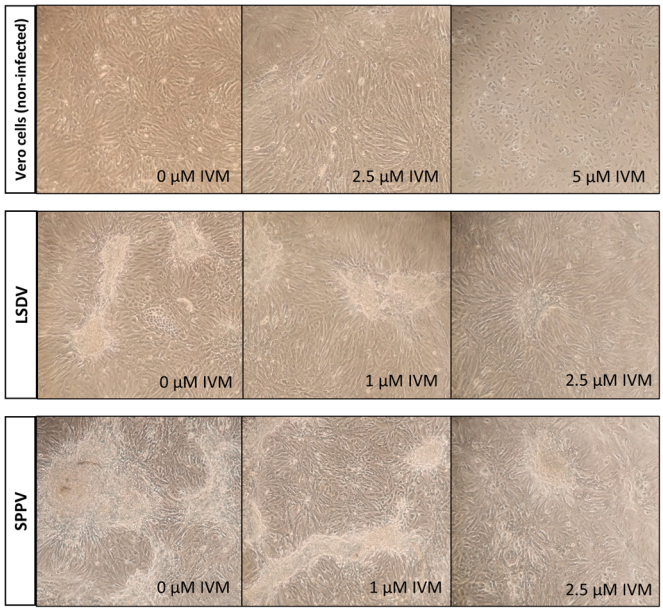

Fig. 2. Microscope images recorded in non-infected (non-IVM treated/ IVM treated) and virus-infected Vero cells (non-IVM treated/ IVM treated) on 144 h pi (x20 magnification). LSDV: Lumpy skin disease virus; SPPV: Sheeppox virus; IVM: Ivermectin.

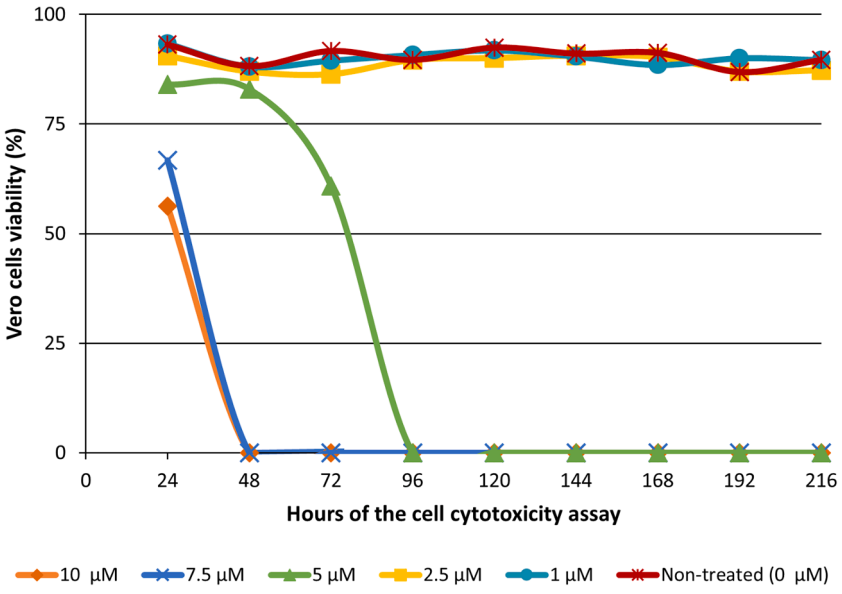

Fig. 1. Cell viability rates (%) of the Vero cells treated with various concentrations of ivermectin for nine days (216 h).

3.2. Inhibition of LSDV and SPPV replications by IVM

The antiviral efficacy of IVM on in vitro replication of LSDV and SPPV was evaluated by comparing viral titer levels in infected cells treated and not treated with IVM for nine days. Since the growth of LSDV and SPPV in cell culture was visually observed at microscope imaging starting the fourth day, viral titer assay was performed at defined time points (96 h, 120 h, 144 h, 168 h, 192 h, and 216 h p.i.) between the 4th and 9th days (Fig. 2). At the 216th hour, the mean viral titer of LSDV was 5.63 $\log_{10}$ $TCID_{50}$/mL at virus control (0.0 µM IVM) and was reduced to 5.00 $\log_{10}$ $TCID_{50}$/mL at 1.0 µM IVM and to 2.88 $\log_{10}$ $TCID_{50}$/mL at 2.5 µM IVM. For SPPV, the mean virus titer at the 216th hour was 5.50 $\log_{10}$ $TCID_{50}$/mL at 0.0 µM IVM and was reduced to 5.13 $\log_{10}$ $TCID_{50}$/mL at 1.0 µM IVM and to 2.63 $\log_{10}$ $TCID_{50}$/mL at 2.5 µM IVM (Fig. 3). In the presence of 1.0 µM IVM and 2.5 µM IVM, the number of infectious virions was reduced by 76.29% and 99.82% for LSDV, and 57.83% and 99.87% for SPPV, respectively. A significant statistical difference was observed for LSDV and SPPV, compared to the non-treated groups, when treated only with 2.5 µM IVM ($P<0.05$). Effect of commercial IVM solution on the replication of the viruses were further confirmed by the highest non-cytotoxic concentration (2.5 µM) of the pure IVM compound. At the 216th hours of treatment with pure IVM compound, the mean virus titers for LSDV and SPPV at 0.0 µM pure IVM were 5.38 $\log_{10}$ and 5.13 $\log_{10}$ $TCID_{50}$/mL, while it was reduced to 2.88 $\log_{10}$ and 3,00 $\log_{10}$ $TCID_{50}$/mL in the presence of 2.5 µM pure IVM, respectively. Because of this decrease in the titers, similar to commercial IVM solution, there were 99.68% and 99.25% decline in the number of infectious virions for LSDV and SPPV treated with 2.5 µM IVM, ($P<0.05$).

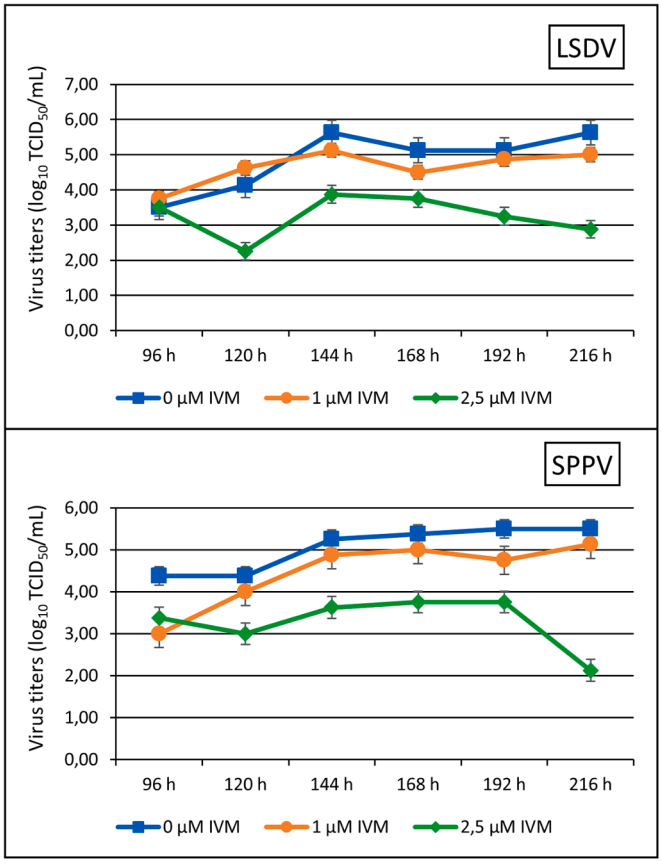

Fig. 3. Antiviral effect of ivermectin (IVM) against in vitro replication of Lumpy skin disease virus (LSDV) and Sheeppoxvirus (SPPV) in Vero cells. Virus titration assay was performed by homogenizing infected cells and their supernatant. The antiviral activity of IVM was determined using $TCID_{50}$.

3.3. Effect of IVM on virus attachment stage

The antiviral activity of IVM in the cell-binding phase of capripoxviruses was evaluated by treating the viruses with the highest dose of non-cytotoxic IVM. For this purpose, the viral titers of control viruses (0 µM IVM treatment) and infected cells treated with 2.5 µM IVM were compared in cell culture at the 10th day after virus inoculation. The mean titer value for LSDV in the virus control (0 µM IVM) was 5.63 $\log_{10}$ $TCID_{50}$/mL, while it was reduced to 5.13 $\log_{10}$ $TCID_{50}$/mL in the presence of 2.5 µM IVM. For SPPV, the mean virus titer was 5.75 $\log_{10}$ $TCID_{50}$/mL at virus control (0.0 µM IVM) and was 5.63 $\log_{10}$ $TCID_{50}$/mL at 2.5 µM IVM. There was a 68.38% and 25.01% reduction in the number of infectious virions for LSDV and SPPV treated with 2.5 µM IVM, respectively, compared to untreated infected cultures (Fig. 4). It was determined that ivermectin was statistically more effective on LSDV than SPPV at the virus attachment stage ($P<0.05$).

3.4. Effect of IVM on the virus penetration stage

On the 10th day post-infection (>80% cytopathogenic effect), mean viral titers were determined by the $TCID_{50}$ of untreated and IVM-treated cells to assess the impact of IVM on the penetration stage of capripoxviruses into Vero cells. For LSDV, the mean virus titer at 0.0 µM IVM concentration was 4.50 $\log_{10}$ $TCID_{50}$/mL, while it decreased to 4.13 $\log_{10}$ $TCID_{50}$/mL in the presence of 2.5 µM IVM. There was a 57.83% reduction in the number of infectious virions for LSDV due to a 0.37 $\log_{10}$ reduction in mean viral titer compared to untreated infected cultures (Fig. 4). IVM was found to have no effective antiviral activity (0.0% reduction) in the penetration phase of SPPV, as mean viral titers of both untreated infected cultures (0.0 µM) and SPPV treated with 2.5 µM IVM were 4.25 $\log_{10}$ $TCID_{50}$/mL.

4. Discussion

LSDV and SPPV, which belong to the capripoxvirus group, are listed as notifiable diseases by OIE. Vaccination is the most effective method to control and eradicate capripoxviruses in the desired area. Nevertheless, there is no antiviral drug that can be directly used to treat these infections. Ivermectin is a U.S. Food and Drug Administration (FDA)-approved drug for humans and animals and is commonly used as a broad-spectrum antiparasitic. In recent years, it has been reported to

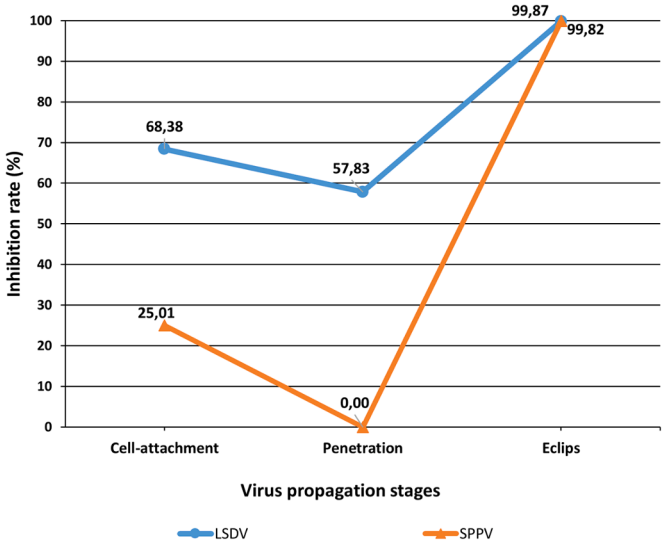

Fig. 4. Inhibition rates of ivermectin (IVM) in proliferation stage (virus attachment, penetration and replication) of Lumpy skin disease virus (LSDV) and Sheeppoxvirus (SPPV).

block the nuclear localization of viral proteins by inhibiting importin α/β (Wagstaff et al., 2012; Yang et al., 2020). This activity of ivermectin has given importance to research its antiviral activity. This led to the study of its efficacy as an antiviral drug against RNA viruses such as human immunodeficiency virus type 1 dengue virus (Wagstaff et al., 2012), West Nile virus, Zika virus (Mastrangelo et al., 2012), Newcastle disease virus (Azeem et al., 2015), Foot-and-mouth disease virus (Naeem et al., 2021), Peste des petits ruminants virus (Afzal et al., 2021), Bovine respiratory syncytial virus, Bovine parainfluenza virus type 3, Bovine viral diarrhea virus, Bovine coronavirus (Yesilbag et al., 2021), and SARS-CoV-2 (Caly et al., 2020). In addition, antiviral activity against the DNA viruses such as Pseudorabies (Lv et al., 2018) and Bovine herpesvirus type 1 (Raza et al., 2020; Yesilbag et al., 2021) was also reported.

This study evaluated the antiviral efficacy of non-cytotoxic IVM concentrations against LSDV and SPPV in Vero cells during attachment, penetration, and viral replication stages. Initially, the cytotoxicity of IVM on Vero cells was determined by calculating the percentages of viable cells/total cells, and the cells were found to have about 90% viability in 2.5 μM and below concentrations, similar to the pure IVM compound. In previous studies, IVM has been tested safely in different concentrations on various cell lines, such as at 5 μM in Vero/hSLAM (Caly et al., 2020), 5 μM (Yesilbag et al., 2021) and 25 μM (Raza et al., 2020) in MDBK, 0.6 μM (Varghese et al., 2016) and 3 μM (Lv et al., 2018) in BHK-21, 50 μM in chick primer fibroblastic cells (Azeem et al., 2015), 100 μg/mL in PK-15 cells (Wang et al., 2019). This difference in toxic concentrations may be due to differences in biological processing in different cell lines and experimental conditions.

To evaluate the antiviral efficacy of IVM in the early stages of Capripoxvirus replication, we treated the LSD and SPP viruses with IVM at the attachment and penetration stages. In the presence of 2.5 mM IVM, the number of infectious virions decreased by 68.38% at the virus attachment stage of LSDV, while 57.83% inhibition occurred at the penetration stage. For SPPV, there was a 25.01% reduction in the number of infectious virions at the virus attachment stage, while no antiviral effect (0.0% inhibition) was noted at the penetration stage. Compared to the viral titers obtained from viral infection stages in the host cell, it can be assumed that IVM shares limited impact on the early stages of host cell infection by capripoxviruses. No inhibitory effect of IVM on early stages of Bovine herpesvirus type 1 (Raza et al., 2020; Yesilbag et al., 2021), Pseudorabies virus (Lv et al., 2018), and some flaviviruses (Mastrangelo et al., 2012) were proposed in previous studies. Our data show that though limited, IVM's antiviral effect exists in the early stages of capripoxvirus replication, at least for LSDV and SPPV. Therefore, these data may lead to further studies to clarify the precise mechanism of action of IVM against capripoxvirus replication as well as other viruses having similar replication properties.

Two different concentrations of IVM were treated on Vero cells infected with LSDV and SPPV to determine whether the virus replication cycle is affected. At 2.5 μM concentrations of IVM, the number of infectious virions was significantly reduced by about three logs for LSDV (99.82% inhibition) and SPPV (99.87% inhibition). The effect on viral replication was also evaluated with the pure IVM compound, and the decrease in viral titers was confirmed. Our results showed that IVM affects the replication cycle of capripoxviruses more efficaciously in the post-entry stages than in the pre-entry stages, including virus attachment and penetration. There are only several reports on the antiviral effects of IVM on DNA viruses. Treatment with IVM has been reported to reduce the viral titer of bovine herpesvirus type 1 by four-fold (Raza et al., 2020; Yesilbag et al., 2021) and the titer of pseudorabies virus by three-fold (Lv et al., 2018). The mechanism of action for IVM on herpesviruses is proposed to depend on selective inhibition of UL42 protein, the accessory subunit of DNA polymerase (Lv et al., 2018; Raza et al., 2020). Considering the results of our study, IVM also inhibits replications of capripoxviruses, including LSDV and SPPV.

The maximum plasma concentration (C_{max}) after application of regular IVM doses (subcutaneous administration, 0.2 mg/kg) is about 0,096 μg/mL (Myers et al., 2021) and the tolerability for IVM application ranges to 20 times higher than the regular doses. Thus, clinical trials considering the short-term application of higher IVM concentration for the treatment of such infections should be a matter of future research.

In conclusion, this study shows that IVM may be considered for further studies to drive the possible treatment of capripoxvirus infections, i.e., LSD and SPP. For the first time, this research demonstrated the antiviral efficacy of IVM against capripoxviruses, ds-DNA viruses. Future studies could be beneficial for better understanding the molecular mechanism of action and opportunities for clinical treatment.

CRediT authorship contribution statement

Eda Baldan Toker: Validation, Investigation, Formal analysis, Writing – original draft. **Ozer Ates:** Resources, Investigation. **Kadir Yeşilbağ:** Conceptualization, Methodology, Supervision, Writing – review & editing.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

Acknowledgement

As a postdoctoral researcher, Dr. E.B. Toker was supported by the Scientific and Technological Research Council of Turkey (TUBİTAK, Project Number: 119 O 571). The authors thank to VETAL Animal Health Products Inc. (Adıyaman, Turkey) for cooperations, as well as Pharmactive Drug Co. (Istanbul, Turkey) and Dr. Figen Onuk Gören for supplying the pure compound of Ivermectin.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.virusres.2021.198671.

References

Afzal, S., Raza, S., Rabbani, M., Firyal, S., Altaf, I., Naeem, Z., 2021. Antiviral Potential of Ivermectin against Peste des Petits Ruminants Virus (PPRV). Pakistan J. Zool 1–4. https://doi.org/10.17582/journal.pjz/20200704060712.

Azeem, S., Ashraf, M., Rasheed, M.A., Anjum, A.A., Hameed, R., 2015. Evaluation of cytotoxicity and antiviral activity of ivermectin against Newcastle disease virus. Pak. J. Pharm. Sci. 28, 597–602.

Babiuk, S., Bowden, T.R., Parkyn, G., Dalman, B., Hoa, D.M., Long, N.T., Vu, P.P., Bieu, D.X., Copps, J., Boyle, D.B., 2009. Yemen and Vietnam capripoxviruses demonstrate a distinct host preference for goats compared with sheep. J. Gen. Virol. 90, 105–114. https://doi.org/10.1099/vir.0.004507-0.

Caly, L., Druce, J.D., Catton, M.G., Jans, D.A., Wagstaff, K.M., 2020. The FDA-approved drug ivermectin inhibits the replication of SARS-CoV-2 in vitro. Antivir. Res. J. 178, 104787.

EFSA, 2015. Scientific opinion on lumpy skin disease. EFSA J 13, 1–73. https://doi.org/10.2903/j.efsa.2015.3986.

EFSA, 2014. Scientific opinion on sheep and goat pox. EFSA J 12. https://doi.org/10.2903/j.efsa.2014.3885.

Garner, M.G., Sawarkar, S.D., Brett, E.K., Edwards, J.R., Kulkarni, V.B., Boyle, D.B., Singh, S.N., 2000. The extent and impact of sheep pox and goat pox in the state of Maharashtra. India. Trop. Anim. Health Prod. 32, 205–223. https://doi.org/10.1023/A:1005263601964.

Hailu, B., 2015. Economic importance and control techniques of lumpy skin diseases. Anim. Vet. Sci. 3 (58) https://doi.org/10.11648/j.avs.20150302.15.

Hamdi, J., Munyanduki, H., Tadlaoui, K.O., Harrak, M., El O.F., Fihri, 2021. Capripoxvirus infections in ruminants: a review. Microorganisms 9, 1–16. https://doi.org/10.3390/microorganisms9050902.

ICTV, 2020. Taxonomic information-international com1mittee on taxonomy of viruses [WWW Document]. https://talk.ictvonline.org/.

Kumar, S.M., 2011. An outbreak of lumpy skin disease in a Holstein Dairy Herd in Oman: a clinical report. Asian J. Anim. Vet. Adv. 6, 851–859.

Lv, C., Liu, W., Wang, B., Dang, R., Qiu, L., Ren, J., Yan, C., Yang, Z., Wang, X., 2018. Ivermectin inhibits DNA polymerase UL42 of pseudorabies virus entrance into the

nucleus and proliferation of the virus in vitro and vivo. Antiviral Res 159, 55–62. https://doi.org/10.1016/j.antiviral.2018.09.010.
Mastrangelo, E., Pezzullo, M., De burghgraeve, T., Kaptein, S., Pastorino, B., Dallmeier, K., De lamballerie, X., Neyts, J., Hanson, A.M., Frick, D.N., Bolognesi, M., Milani, M., 2012. Ivermectin is a potent inhibitor of flavivirus replication specifically targeting NS3 helicase activity: new prospects for an old drug. J. Antimicrob. Chemother. 67, 1884–1894. https://doi.org/10.1093/jac/dks147.
Myers, M.J., Howard, K.D., Kawalek, J.C., 2021. Pharmacokinetic comparison of six anthelmintics in sheep, goats, and cattle. J. Vet. Pharmacol. Ther. 44, 58–67. https://doi.org/10.1111/jvp.12897.
Naeem, Z., Raza, S., Afzal, S., Sheikh, A.A., Ali, M.M., Altaf, I., 2021. Antiviral potential of ivermectin against foot-and-mouth disease virus, serotype O, A and Asia-1. Microb. Pathog. 155, 104914 https://doi.org/10.1016/j.micpath.2021.104914.
OIE, 2013. Sheep Pox and Goat Pox Disease Card 1–5.
Raza, S., Shahin, F., Zhai, W., Li, H., Alvisi, G., Yang, K., Chen, X., Chen, Y., Chen, J., Hu, C., Chen, H., Guo, A., 2020. Ivermectin inhibits bovine herpesvirus 1 DNA polymerase nuclear import and interferes with viral replication. Microorganisms 8, 1–15. https://doi.org/10.3390/microorganisms8030409.
Skrypnyk, A., Kreindel, S., Masiulis, M., Zdravkova, A., Escher, M., Raizman, E., 2016. Emergence of lumpy skin disease in Asia and Europe. Empres. Heal. 360 (46), 24–26.
Tuppurainen, E.S.M., Venter, E.H., Shisler, J.L., Gari, G., Mekonnen, G.A., Juleff, N., Lyons, N.A., De Clercq, K., Upton, C., Bowden, T.R., Babiuk, S., Babiuk, L.A., 2017. Review: capripoxvirus diseases: current status and opportunities for control. Transbound. Emerg. Dis. 64, 729–745. https://doi.org/10.1111/tbed.12444.
Varghese, F.S., Kaukinen, P., Gläsker, S., Bespalov, M., Hanski, L., Wennerberg, K., Kümmerer, B.M., Ahola, T., 2016. Discovery of berberine, abamectin and ivermectin as antivirals against chikungunya and other alphaviruses. Antiviral Res. 126, 117–124. https://doi.org/10.1016/j.antiviral.2015.12.012.
ViralZone, 2021. SIB Swiss Institute of Bioinformatics [WWW Document]. https://viralzone.expasy.org/.
Wagstaff, K.M., Sivakumaran, H., Heaton, S.M., Harrich, D., Jans, D.A., 2012. Ivermectin is a specific inhibitor of importin α/β-mediated nuclear import able to inhibit replication of HIV-1 and dengue virus. Biochem. J. 443, 851–856. https://doi.org/10.1042/BJ20120150.
Wang, X., Lv, C., Ji, X., Wang, B., Qiu, L., Yang, Z., 2019. Ivermectin treatment inhibits the replication of Porcine circovirus 2 (PCV2) in vitro and mitigates the impact of viral infection in piglets. Virus Res. 263, 80–86. https://doi.org/10.1016/j.virusres.2019.01.010.
Yang, S.N.Y., Atkinson, S.C., Wang, C., Lee, A., Bogoyevitch, M.A., Borg, N.A., Jans, D.A., 2020. The broad spectrum antiviral ivermectin targets the host nuclear transport importin α/β1 heterodimer. Antiviral Res. 177, 104760 https://doi.org/10.1016/j.antiviral.2020.104760.
Yeruham, I., Yadin, H., Van Ham, M., Bumbarov, V., Soham, A., Perl, S., 2007. Economic and epidemiological aspects of an outbreak of sheeppox in a dairy sheep flock. Vet. Rec. 160, 236–237. https://doi.org/10.1136/vr.160.7.236.
Yesilbag, K., Toker, E.B., Ates, O., 2021. Ivermectin also inhibits the replication of bovine respiratory viruses (BRSV, BPIV-3, BoHV-1, BCoV and BVDV) in vitro. Virus Res. 297, 198384 https://doi.org/10.1016/j.virusres.2021.198384.