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Evidence of autophagic and Wnt/β-catenin signaling occurrence during Schmallenberg virus (SBV) infection on BHK-21 cells

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ABSTRACT

Schmallenberg virus (SBV) is a ruminant pathogen that is widely distributed around the world, but little is known about its interactions with permissive cells or about its pathogenetic mechanisms. This study highlighted, through a phenotypic approach, the changes in the expression of some autophagy and Wnt/β-catenin pathway markers that SBV causes on baby hamster kidney (BHK-21) cells. Western blot analysis revealed that SBV caused autophagy induction at 48 h post infection. Several markers, such as PI3K, Akt, and Wnt/β-catenin, were downregulated at the same time point. Furthermore, downregulation in the expression of PI3K, p-mTOR and Beclin-1 showed differences between multiplicity of infection (MOI) 0.05 and 0.5, but not between MOI 0.5 and 1.5. Exceptions for this trend were Akt and LC3-II, which progressively decreased depending on time, and β-catenin, whose expression almost disappeared regardless of MOI. The use of several chemical inducers and inhibitors has demonstrated the efficacy of late autophagy inhibitors (bafilomycin and chloroquine) in significantly lowering SBV infection and also preventing the changes caused by viral replication. Early autophagy inhibitors and inducers showed no effect on cellular viability or viral titers. Silencing the expression of Akt and β-catenin revealed a slight increase in the expression of viral glycoprotein Gc. These findings revealed the relationship that SBV has in important cellular regulatory pathways, expanding the knowledge about the cellular interactions of this virus and suggesting a central role for late stages of autophagy in the replication of this bunyavirus.

1. Introduction

Schmallenberg virus (SBV) is a small RNA virus belonging to the family *Peribunyaviridae*, genus *Orthobunyavirus*, whose host spectrum includes wild and domestic ruminants. The virus is transmitted to hosts through the bite of blood-sucking insects belonging to the *Culicoides* genus (especially *Culicoides imicola*) (Ferrara et al., 2024a; K Wernike et al., 2015a, 2015b). No direct virus transmission, even through breeding, has ever been established despite the virus has been detected transiently (in low levels) in semen following infection (Ponsart et al., 2014). The consequences of the infection in ruminants include fever, a reduction in milk production, and reproductive disorders, which may include abortion and neonatal malformations (such as hydrocephalus and arthrogryposis) (Lievaart-Peterson et al., 2015; Varela et al., 2013). In other species (such as pigs and wild boars), only seroconversion after exposure is reported (Ferrara et al., 2024b; Jiménez-Ruiz et al., 2022). The name "Schmallenberg" derives from the German town, where the first outbreak in ruminant population was reported in Europe in 2012 (Ferrara et al., 2023c; K Wernike et al., 2015a, 2015b). Since then, the virus has been reported throughout the European continent, becoming endemic, while nowadays it is considered a re-emerging and cosmopolitan virus (Ferrara et al., 2023c; Lievaart-Peterson et al., 2015).

The pathogenetic mechanisms by which the virus causes abortion and damage to the fetus are not yet known (Varela et al., 2013). Knowledge regarding the interaction between SBV and its host cells is also scarce. For example, SBV is known to induce apoptosis (via extrinsic and intrinsic pathways in a time and dose dependent manner) and interact with interferon signaling (up-regulating a total of 649

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IFN-stimulated genes) (Aksoy and Azkur, 2018; Barry et al., 2014). There is no evidence, however, of the relationship between this virus and other cellular signaling, like autophagy etc.

Autophagy is a conserved biological mechanism that involves sequestering and degrading intracellular components or foreign material in cytosolic vesicles (Choi et al., 2018). This pathway is quite conserved and includes early markers, such as the PI3K/Akt/mTOR axis with activation function, and late markers, such as LC3, linked to autophagosome activity (Blanco et al., 2020; Münz, 2017). Initially thought to be a process for maintaining cellular homeostasis, it is actually considered a defensive mechanism against pathogens (Zhang et al., 2018). Viruses have evolved strategies to induce or inhibit autophagy, with the purpose of promoting viral replication (Münz, 2017; Zhang et al., 2018). Even RNA viruses, over millennia of coevolution with host cells, have established complex interaction networks with cellular processes (Wang et al., 2018). The existing relationships between autophagic process and viral replication have been evidenced in the case of some bunyaviruses, such as severe fever with thrombocytopenia syndrome virus (SFTSV), that exploit autophagic flux for viral assembly and egress (Yan et al., 2021; Zhang et al., 2024). Other examples of known interactions between autophagy and RNA viruses include influenza virus and human cytomegalovirus (HCMV) (Bell et al., 2022; Zhao et al., 2018). However, there are no studies focused on SBV and the connections with the autophagic pathway, as well as there is no research that has investigated the role of the β-catenin pathway and SBV.

The Wnt/β-catenin pathway serves as a regulatory key during different cellular processes (Liu et al., 2022). Normally, β-catenin remains dormant in the cytoplasm because a specific degradation system sequesters it and transports it to the proteasomal system. The activation of the pathway, mediated by the Wnt ligand, inhibits the degradation system inducing β-catenin accumulation in the cytoplasm, and its translocation to the nucleus, that promotes the expression of genes related to cell maintenance, migration, proliferation, angiogenesis, and survival (Liu et al., 2022; Marineau et al., 2020). RNA viruses can stimulate or inhibit the Wnt/β-catenin pathway. For example, human cytomegalovirus (HCMV) suppresses the transcriptional activity of this pathway by triggering the sequestration and, eventually, the destruction of β-catenin (Angelova et al., 2012). Interplay between autophagy and Wnt/β-catenin signaling is described during several types of diseases, including some types of cancers and viral infections.

The aim of this work was to evaluate the expression changes of some autophagy and β-catenin pathway markers during SBV infection on its permissive cells. A further aim of this work was to evaluate the influence that the expression of these markers has on viral proliferation through the use of specific inhibitors and inducers.

2. Experimental procedures

2.1. Cell culture and infection

Baby hamster kidney fibroblasts (BHK-21) were cultured in Dulbecco's Modified Eagle's Medium (DMEM; Corning) supplemented with calf fetal serum and antibiotics (Corning). Cells were maintained in 25 cm flasks at 37∘C in an atmosphere containing 5 % CO_2 until the 90 % cell confluence. SBV (BH80/11–4 provided by Friedrich-Loeffler-Institut and Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale") were inoculated on confluent cells for different times (24, 48, and 72 h) using a multiplicity of infection (MOI) of 0.5. Moreover, the infection was performed using different MOI (0.05, 0.5, and 1.5) for 48 h. Infected and control cells from each experiment were washed with 1X PBS, scraped, and centrifuged.

2.2. Western blot assay

Pellets obtained from each time and MOI condition were solubilized in radioimmunoprecipitation assay buffer (RIPA) buffer containing protease and phosphatase inhibitors (Sigma). Protein quantification was performed using the Bradford assay (BioRad). Approximately 25 μg of protein were loaded with Laemmli buffer 5x in each well of pre-casted gels (BioRad). Once the sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was run, each gel was transferred to nitrocellulose or polyvinylidene fluoride (PVDF) membranes (BioRad). Following a blocking phase in 5 % bovine serum albumin (BSA), each membrane was incubated overnight with a panel of primary antibodies (including early and late stages of autophagy and β-catenin markers) eluted 1:1000 in BSA: mTOR, phospho-mTOR, PI3K, Akt, Beclin-1, LC3 I-II, β-catenin, GSK-3β, p-GSK-3β (Cell signaling). The expression of each marker was defined by comparing the expression of β-actin (Santa cruz) and β-tubulin (Cell signaling) used as a normalizer. Specific secondary HRP-conjugated anti-rabbit and anti-mouse antibodies, diluted 1:2000, were used for one hour before reading (Cell signaling). The visualization step was performed using Clarity Western ECL Substrate (BioRad) and a ChemiDoc Blot scanner (BioRad). Expression and densitometric analysis were assessed using Image Lab software (BioRad).

2.3. Immunofluorescence assay

Control and infected cells growth on slide glasses were fixed and permeabilized using paraformaldehyde and phosphate buffer saline (PBS) 1X with 0.1 % Triton, respectively. Each slide was incubated with two primary antibodies against LC3-II (Novus Biologicals, rabbit monoclonal antibody) and 1C11 mouse monoclonal antibody against SBV Gc protein (gently provided by the Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna-IZSLER, Brescia, Italy) (Kerstin Wernike et al., 2015a, 2015b). Appropriate secondary antibodies, labeled with Alexa Fluor 488 and Alexa Fluor 610, were used (Thermo Fisher Scientific). Nuclei were stained using 4′,6-diamidino-2-fenilindol (DAPI; Vector Laboratories). A fluorescent cell imager (ZOE Fluorescent Cell Imaging System, BioRad) was used to visualize the cells.

2.4. Inhibitors, inducers, siRNAs

Several reagents were used to investigate whether autophagy and the β-catenin pathway had a proviral or antiviral role. Briefly, infected cells were incubated with LY294002 (50 μM) and 3-Methyladenine (3-MA) (10 mM) as inhibitors of the early stage of autophagy (Ferrara et al. 2023a). Bafilomycin (100 nM) and chloroquine (50 μM) were used as inhibitors of late stages of autophagy. Rapamycin (50 nM) was used as an autophagy inducer (Ferrara et al., 2023b). The effects caused by the previously described reagents were evaluated through viral titer (following the Reed and Muench method) of the supernatant and the expression of the markers described in the previous subsection (Forte et al., 2021). Moreover, the experiments were repeated in 96-well plates with the same conditions to perform the 3-(4,5-Dimethylthiazol-2-yl)-2, 5 Diphenyltetrazolium Bromide (MTT) (Montagnaro et al., 2019). The MTT assay included the addition of MTT reagent (0.5 mg/mL; SERVA), the incubation at 37∘C, the solubilization with dimethylsulfoxide (DMSO), and the reading of the optical absorbance at 570 nm using a spectrophotometer (Multiskan™ GO Microplate Spectrophotometer, Thermo Fisher Scientific).

A specific monoclonal antibody directed against SBV Gc was used to further evaluate the expression of the viral protein following the use of the different chemicals (Wernike et al., 2015a, 2015b).

The same experiments were replicated in 6-well plates to assess the effects of Akt and β-catenin silencing on viral replication. Briefly, 10 pm of each siRNA were mixed with 250 μL of Optimem (Corning) and 7.5 μl of Lipofectamine (Thermo Fisher Scientific). Each mix was used to transfect BHK-21 cells before the infection with SBV (MOI 0.5, 48 h). The sequences of siRNAs targeting Akt (sense 5' GCGUGACCAUGAACGAGUUtt 3' and antisense 5'AACUCGUUCAUGGUCACGCgg 3') and β-catenin (sense 5' GGGUACGAGCUGCUAUGUUtt 3' and antisense

5'AACAUAGCAGCUCGUACCCtc) were provided by Thermo Fisher scientific. The siRNA efficacy was analyzed in Western blot using specific antibodies as previously described.

2.5. Statistical analysis

Each experiment consisted of three independent replicates. GraphPad Prism 6.0 (GraphPad Software, Inc., La Jolla, CA, USA) was used to perform one-way ANOVA, with results presented as mean standard deviation (SD). A p value < 0.05, 0.01, or 0.001 was considered statistically significant and shown graphically with one, two, or three asterisks, accordingly.

3. Results

3.1. SBV induced modifications to autophagy and Wnt/β-catenin pathways

Numerous autophagy markers showed decreasing expression levels with increasing infection time after infecting permissive cells with MOI 0.5. More specifically, Akt and PI3K were slightly reduced after 48 h of infection and then significantly reduced after 72 h (Fig. 1). The expressions over time of mTOR and phospho-mTOR were characterized by an increase at 48 and 24 h, respectively, followed by a strong reduction at 72 h for both markers (Fig. 1). Similarly, late markers of autophagy also underwent to a reduction in expression 72 h after infection (in the case of LC3-II also at 48 h) (Fig. 2). The expression of β-catenin was almost suppressed both 48 and 72 h after infection, meanwhile, the markers relating to the degradation system (GSK-3β and p-GSK-3β), underwent to a significant reduction only at 48 h (Fig. 3). The lower expression of LC3-II was evident by immunofluorescence as well, where a reduced number of cells caused by the cytopathic effect induced by the virus was observed (Fig. 4).

A partial correlation with MOI was found in the evaluation of the expression of early markers of autophagy. The latter, in fact, did not undergo significant changes when the MOI 0.05 was used (Fig. 5). The use of a higher viral dose (1.5) did not cause a further reduction in the expression of the autophagy markers (Fig. 5). The same trend was observed for all markers investigated, except for β-catenin and LC3 (Figs. 6 and 7). The expression of β-catenin, in fact, was almost undetectable regardless of the MOI, unlike what was observed for LC3-II, whose reduction correlated with the increase in MOI (Figs. 6 and 7).

3.2. Effects of selected inhibitors and inducers on SBV replication

The use of early autophagy inhibitors had no impact on viral titer or cell viability (Fig. 8). Similar results were also observed for rapamycin (inducer). These findings were confirmed during the densitometric analysis of gC expression. Moreover, no significant differences were found in the expression of the markers under study with the use of LY294002, 3-MA, and rapamycin (Fig. 8).

The use of late autophagy inhibitors, however, significantly reduced viral titers, significantly improving the viability of infected cells (Fig. 9). These outcomes were accentuated for bafilomycin. Western blot analysis confirmed these results by highlighting a reduced expression of gC for cells infected and treated with bafilomycin and chloroquine (Fig. 9). Furthermore, higher levels of β-catenin were observed in these cells (especially in the case of bafilomycin). Bafilomycin-treated cells clearly showed accumulation of LC3-I and LC3-II as a consequence of blocking autophagosome-lysosome fusion (Fig. 9).

The use of siRNA for Akt effectively reduced the expression of this protein, favoring viral replication (as demonstrated by the increased

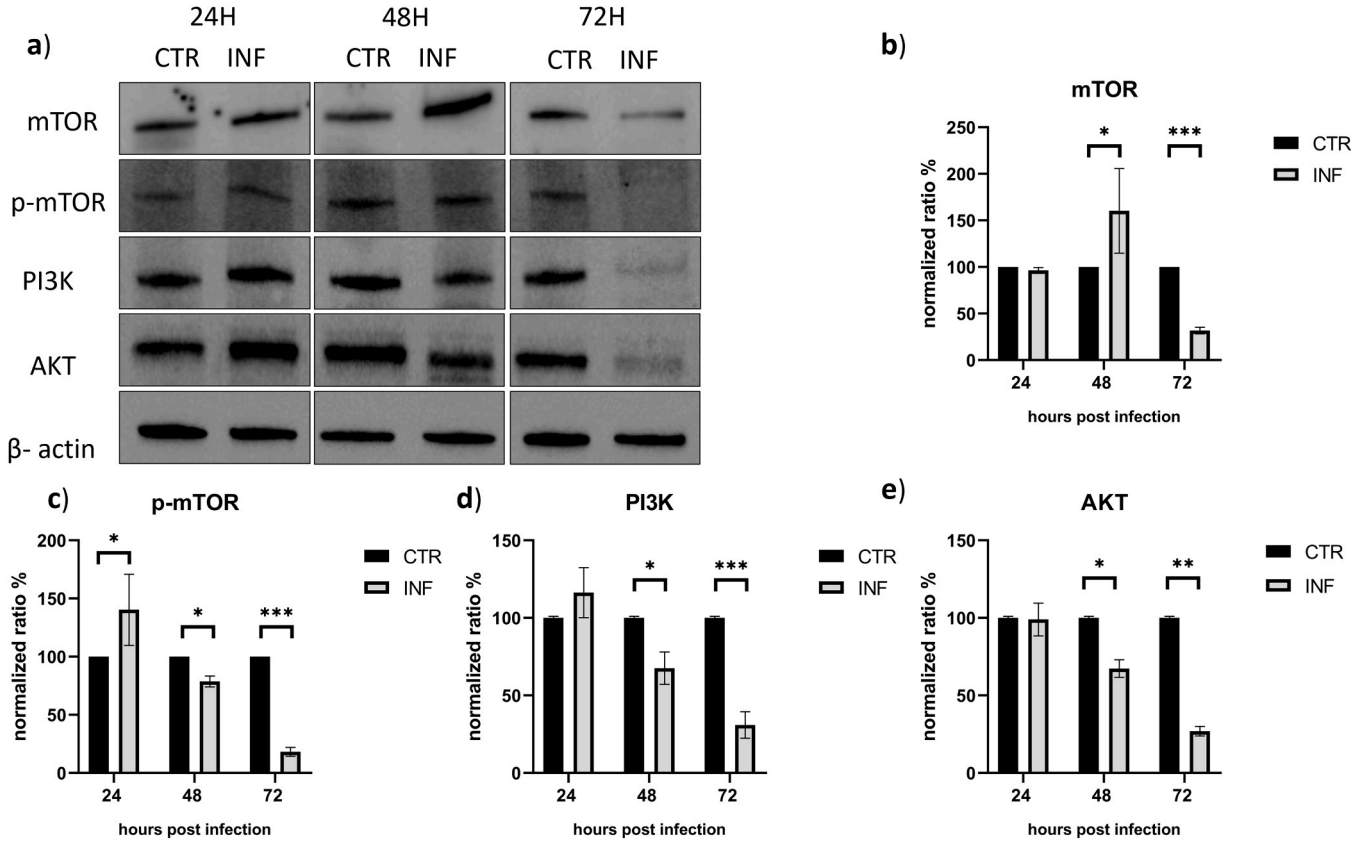

Fig. 1. Changes in the expression of several autophagy markers (PI3K, Akt, mTOR, p-mTOR) after SBV infection at different time points. BHK-21 were infected with SBV at different time-points (a). Protein expression of mTOR (b), p-mTOR (c), PI3K (d), Akt (e) was assessed by western blot analysis. All the independent experiments were performed three times, *P < 0.05, **P < 0.01, and ***P < 0.001.

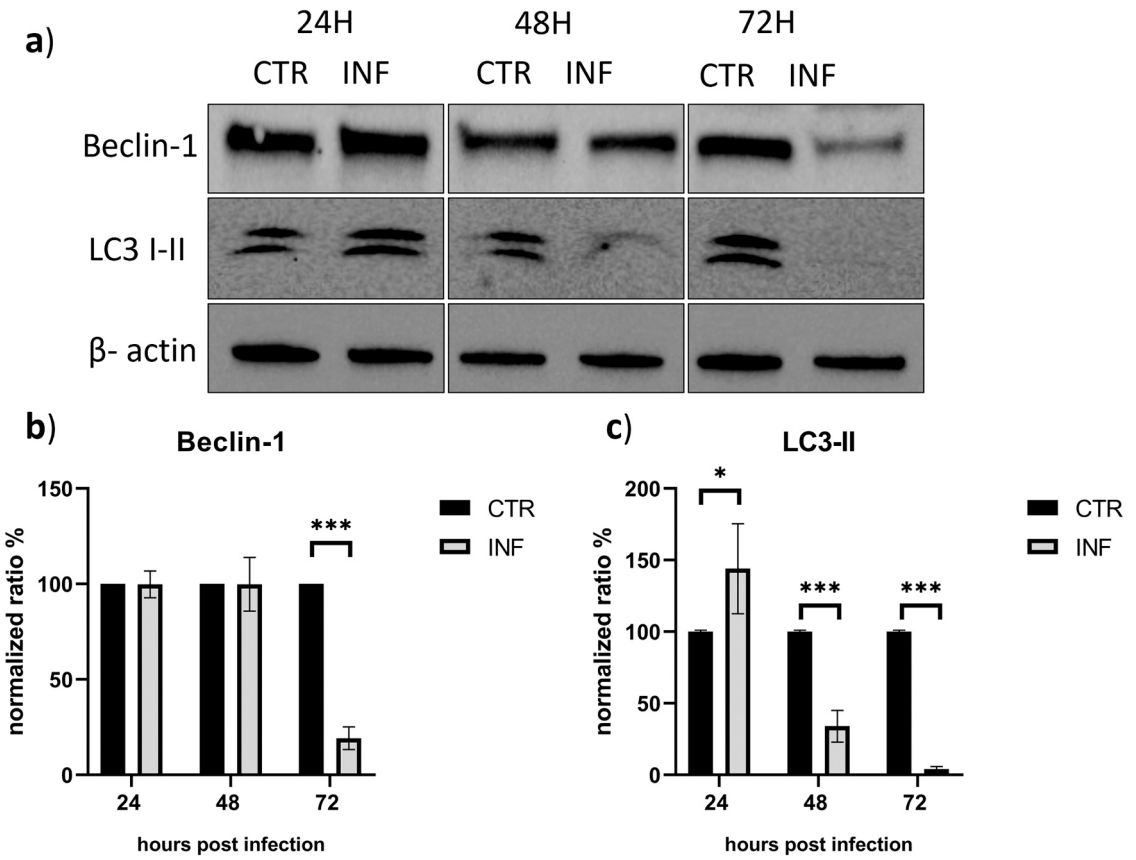

Fig. 2. Changes in the expression of several autophagy markers (LC3, Beclin-1) after SBV infection. BHK-21 were infected with SBV at different time-points (a). Protein expression of beclin-1 (b), and LC3-I-II (c) was assessed by western blot analysis. All the independent experiments were performed three times, *P < 0.05, **P < 0.01, and ***P < 0.001.

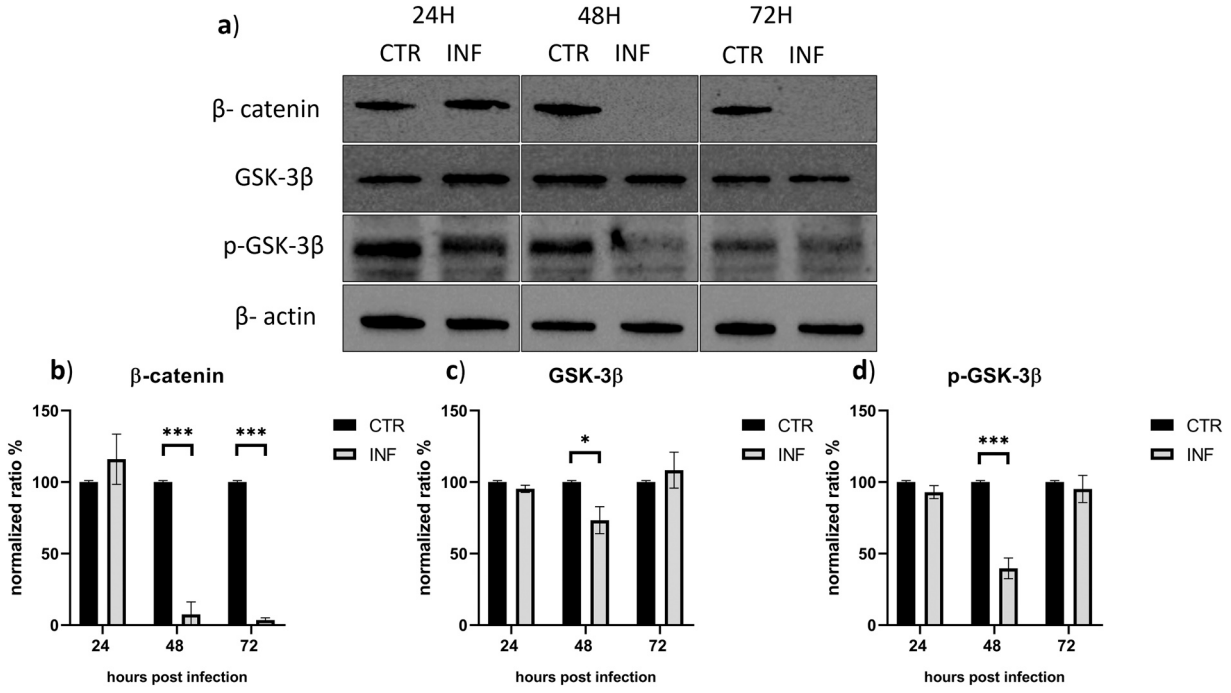

Fig. 3. Changes in the expression of several Wnt/β-catenin markers after SBV infection at different time points. BHK-21 were infected with SBV at different time-points (a). Protein expression of β-catenin (b), GKS-3β (c), and p-GSK-3β (d), was assessed by western blot analysis. All the independent experiments were performed three times, *P < 0.05, **P < 0.01, and ***P < 0.001.

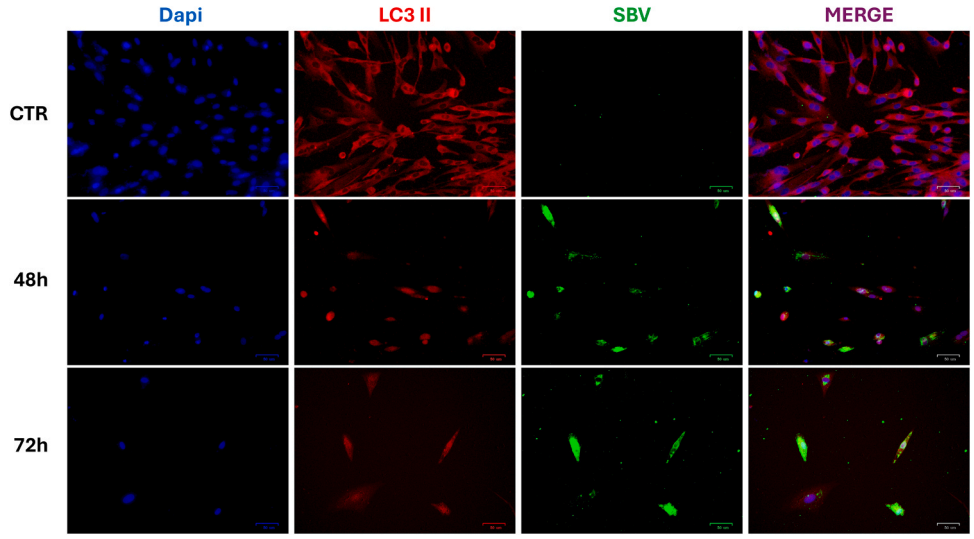

Fig. 4. Microscopy observation of BHK-21 cells infected with SBV. Cells were collected and marked with fluorescent probes using antibodies specific to LC3-II and glycoprotein C. A DNA stain (DAPI) was also applied. Autophagosomes were found prevalently in control cells. Green (viral glycoprotein) and red (autophagosomes) signals increase and decrease respectively, in a time-dependent manner.

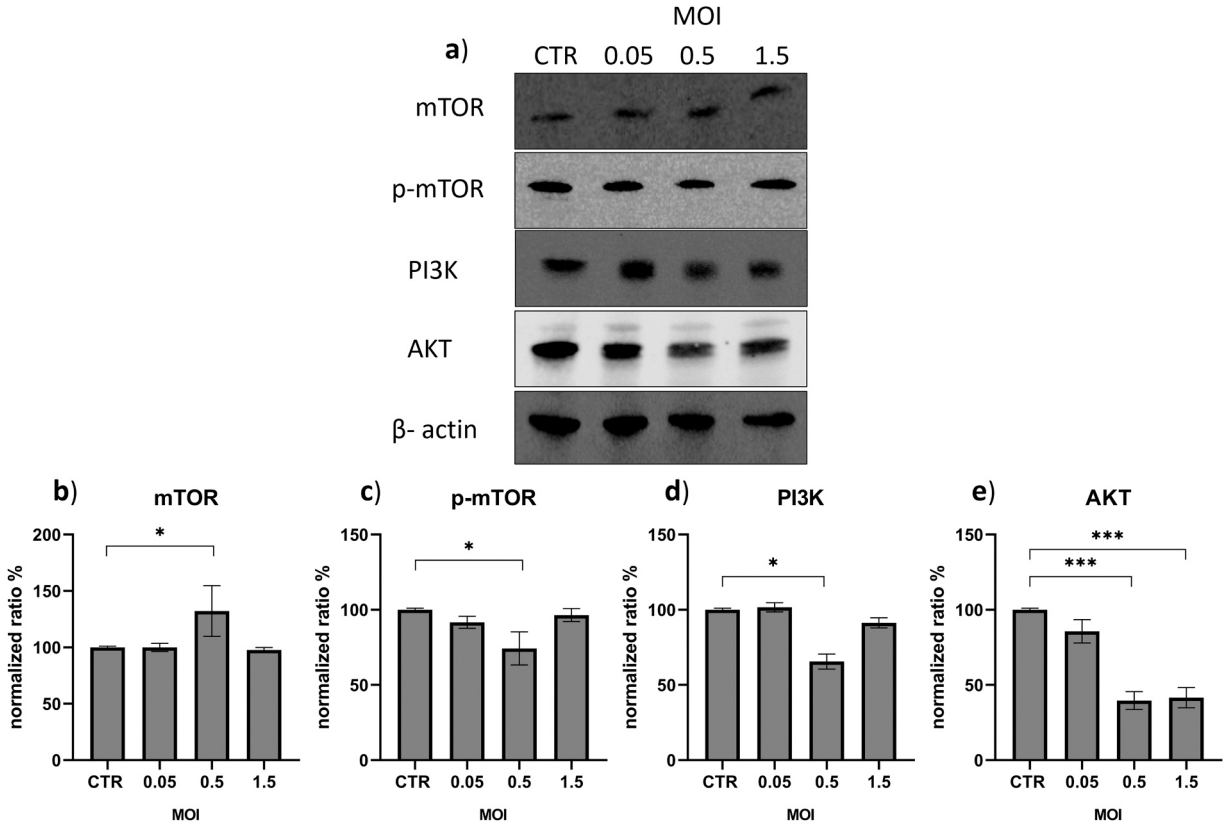

Fig. 5. Dose dependance in the change of expression of several autophagy markers (PI3K, Akt, mTOR, p-mTOR) during SBV infection. BHK-21 were infected with SBV at different MOI (a). Protein expression of mTOR (b), p-mTOR (c), PI3K (d), Akt (e) was assessed by western blot analysis. All the independent experiments were performed three times, *P < 0.05, **P < 0.01, and ***P < 0.001.

expression of Gc). Further evidence of the increase in viral replication was the reduction in β-catenin levels (Fig. 10). Similarly, the use of siRNA against β-catenin also obtained similar results in terms of gC expression. Inhibition of β-catenin expression also caused a reduction in LC3-II expression (Fig. 11). Whole blots and relative housekeeping have been included in the supplementary materials (Suppl. File 1).

4. Discussion

The results of the current study highlight how SBV impacts the PI3K/Akt/mTOR pathway, the Wnt/β-catenin signaling, and the autophagic process. Given its central role in the regulation of cell health and metabolism, PI3K/Akt/mTOR is a common player in the replication of several viruses (Blanco et al., 2020; Dunn and Connor, 2012). Several

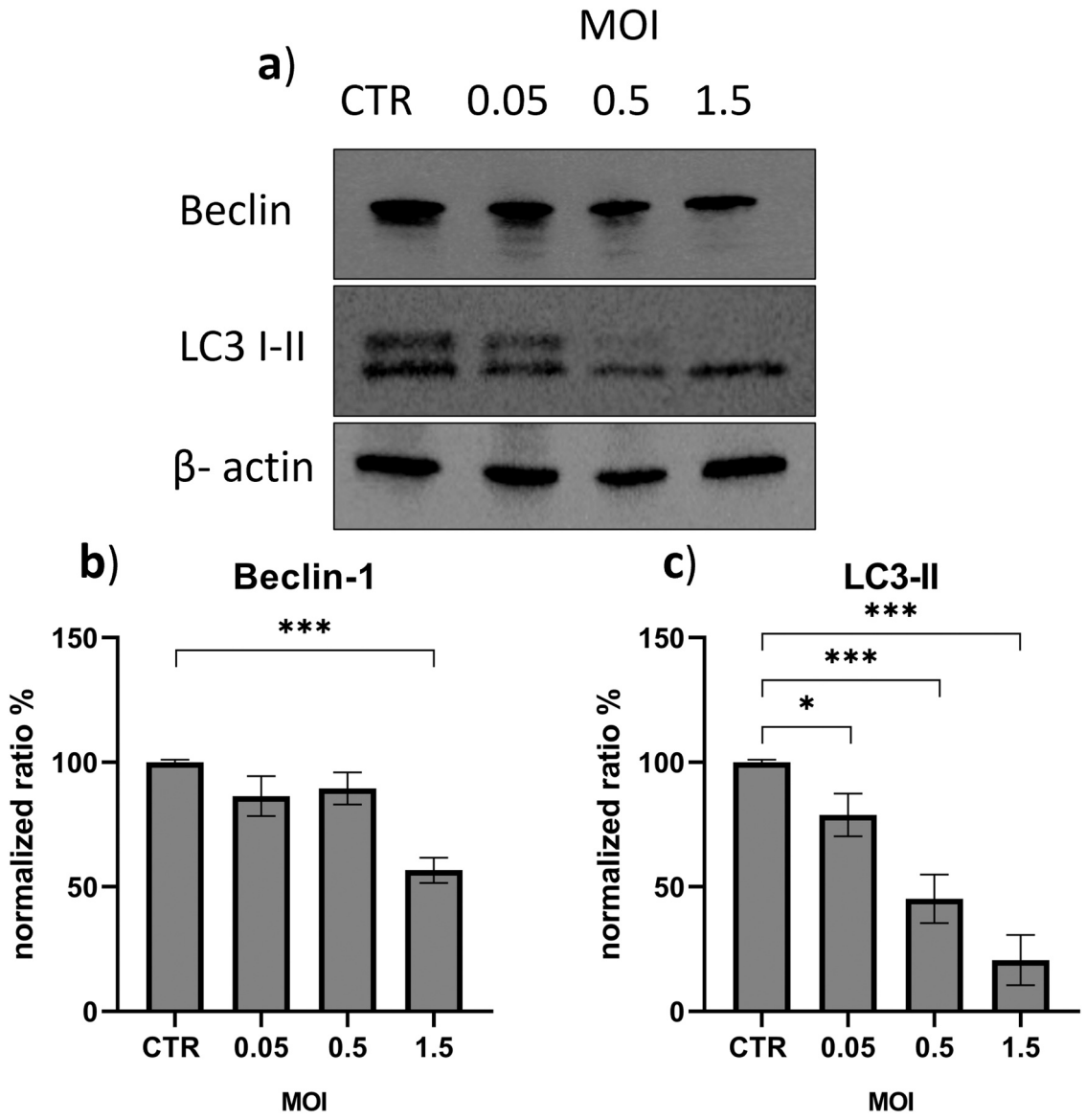

Fig. 6. Dose dependance in the change of expression of several autophagy markers (LC3, Beclin-1) during SBV infection. BHK-21 were infected with SBV at different MOI (a). Protein expression of beclin-1 (b), and LC3-I-II (c) was assessed by western blot analysis. All the independent experiments were performed three times, *P < 0.05, **P < 0.01, and ***P < 0.001.

RNA viruses inhibit PI3K/Akt/mTOR signaling during their proliferation. An example is the Rift Valley fever virus (RVFV) that causes a dephosphorylation of Akt and GSK3-β (Dunn and Connor, 2012). Dengue virus (DENV) can modulate the PI3K/Akt/mTOR pathway by a Bcl-2-linked mechanism, inducing apoptosis (Liu et al., 2014). The pathogenesis of Newcastle disease virus (NDV), a primary pathogen of chickens, is characterized by the phosphorylation of AKT both *in vitro* and *in vivo* (Fan et al., 2024; Wang et al., 2017). Several alphaviruses, including Semliki Forest virus (SFV), Ross River virus (RRV), and Chikungunya virus (CHIKV), trigger the PI3K/AKT/mTOR pathway in permissive cells (Van Huizen and McInerney, 2020). Hepatitis C virus (HCV) activates PI3K/Akt/mTOR signaling to improve its entry and replication (Pradhan et al., 2022; Shi et al., 2016). Scientific evidence highlights the contribution of this pathway to the arenavirus budding (Urata et al., 2012). In the case of SBV, changes to the pathway occurred 48 h after infection, coinciding with the onset of the cytopathic effect. As a consequence, it could be more closely linked to budding than viral entry. It remains to be clarified whether the downregulation of these markers was triggered by the cell (as a defense mechanism) or by the virus (mediated by some viral gene to promote its own replication). However, because inhibition/induction of this route with several drugs (LY294002, 3-MA, and rapamycin) had no effect on viral replication, it appeared that this pathway was not essential for the SBV life cycle. The same outcomes were observed also using Akt siRNA. Different results have been described in other studies. For example, the chemical inhibition of PI3K or AKT reduces replication of several alphaviruses (Ferrara et al. 2023c). The use of rapamycin enhances the HCMV proliferation *in vitro* (Altman et al., 2019). A recent study highlighted how porcine reproductive respiratory virus (PRRSV) is able to activate the PI3K/Akt/mTOR pathway during the early stages of infection and that the use of specific inhibitors (LY294002) drastically reduced viral replication (Zhu et al., 2013). The knockdown of Akt significantly reduced the HCV replication (Shi et al., 2016). The inhibition of PI3K activity by the supplementation of LY294002 and 3-MA improves the West Nile virus (WNV) infection in different cell lines (Tang et al., 2024). Even DNA viruses of veterinary importance, such as feline herpesvirus (FHV-1), stimulate Akt phosphorylation to facilitate their entrance and can compensate for a lack of the cellular Akt (due to natural processes or

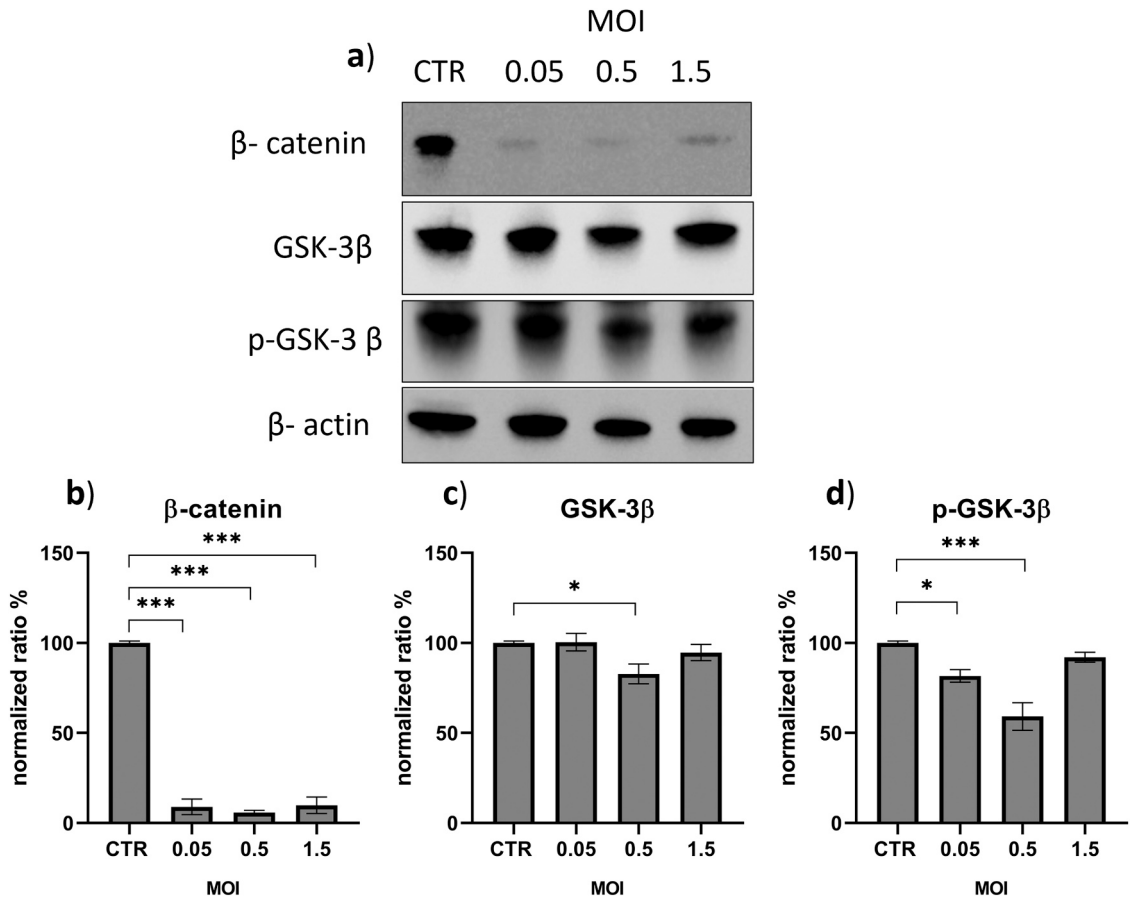

Fig. 7. Dose dependance in the change of expression of several Wnt/β-catenin markers during SBV infection. BHK-21 were infected with SBV at different MOI (a). Protein expression of β-catenin (b), GKS-3β (c), and p-GSK-3β (d), was assessed by western blot analysis. All the independent experiments were performed three times, *P < 0.05, **P < 0.01, and ***P < 0.001.

the use of specific inhibitors and siRNA) via a virus-encoded kinase (Ferrara et al., 2023a; Longobardi et al., 2024).

Our results indicated a time- and dose-dependent enhancement of the cellular autophagy. This process, fundamental for cell survival, is exploited by various RNA viruses for their assembly and budding. For example, picornaviruses leave infected cells in packages enveloped in autophagic membranes, nevertheless, herpesviruses incorporate autophagic membranes into their envelopes (Choi et al., 2018; Zhang et al., 2018). Paramyxoviruses and orthomyxoviruses guide autophagic membranes to the cell membrane, enhancing the integrity of the envelope they obtain (Wang et al., 2018; Zhang et al., 2018). Usutu virus, another arbovirus, induces autophagy and is targeted by the selective autophagy receptor p62/SQSTM1 for degradation (Nelemans et al., 2025). The influenza virus, through oxidative stress and mitochondrial damage, is also able to induce autophagy linked to apoptosis (Yeganeh et al., 2018; Zhang et al., 2021). Recent research found that SFTSV, another bunyavirus, triggers classical autophagy flux and utilizes autophagic vesicles for exocytosis (Yan et al., 2021; Zhang et al., 2024). Because autophagy is a critical mechanism for viral progeny development, inhibiting it with specific inhibitors significantly lowered viral titer and Gc expression. This result has also been reported in previous investigations on DNA (FHV-1, PRV, etc.) and RNA (RVFV, SFTSV, etc.) viruses (Ferrara et al., 2023b; Yan et al., 2021).

Another pathway that underwent changes during SBV infection in permissive cells was the Wnt/β-catenin. Regardless of the MOI used, 48 h after infection, an almost complete downregulation of β-catenin and a reduction in the expression of β-catenin degradation system were observed. Furthermore, when late autophagy inhibitors reduced SBV proliferation, β-catenin levels were normal, indicating a clear proportionality between SBV infection and β-catenin expression.

A study conducted on HCMV identified a degradation of β-catenin at 48 and 72 h after infection in dermal fibroblasts and human placental extravillous trophoblasts, while the transcription of the genes related to this protein was unchanged (Angelova et al., 2012). Porcine deltacoronavirus (PDCoV), an emerging coronavirus causing economic losses to swine industries worldwide, is able to trigger Wnt/β-catenin pathway activation, enhancing intestinal stem cell self-renewal (Zhang et al., 2025).

Similarly for this pathway, it is necessary to establish whether the phenomena we observed were either virus-mediated or a consequence of the antiviral response by the cell (β-catenin is also a component of IFN signaling). When β-catenin expression, a key component of cellular defense against viruses, was silenced by siRNA, the viral replication increased, supporting the hypothesis that these pathways are involved in the antiviral innate response. Similar features were observed for hepatitis B virus (HBV), CHIKV, influenza virus, and PRRSV (Chatterjee et al., 2023; Hillesheim et al., 2014; Tarnow and McLachlan, 2022; Wang et al., 2020). On the other hand, β-catenin has a proviral role during fowl adenovirus serotype 4 replication (FAdV-4) that induces the overexpression of the β-catenin (Wang et al., 2023). By increasing the accumulation and nuclear translocation of β-catenin and stimulating the expression of Wnt target genes, transmissible gastroenteritis virus (TGEV) infection triggered the Wnt/β-catenin pathway and the regeneration of intestinal epithelium (Yang et al., 2022).

Autophagy is also linked to the IFN signaling pathway, and deregulation can result in the occurrence of an antiviral state inside the cell. For example, tripartite motif 23 (TRIM23) is required for cGAS-STING-dependent antiviral autophagy of HSV-1 (Acharya et al., 2025).

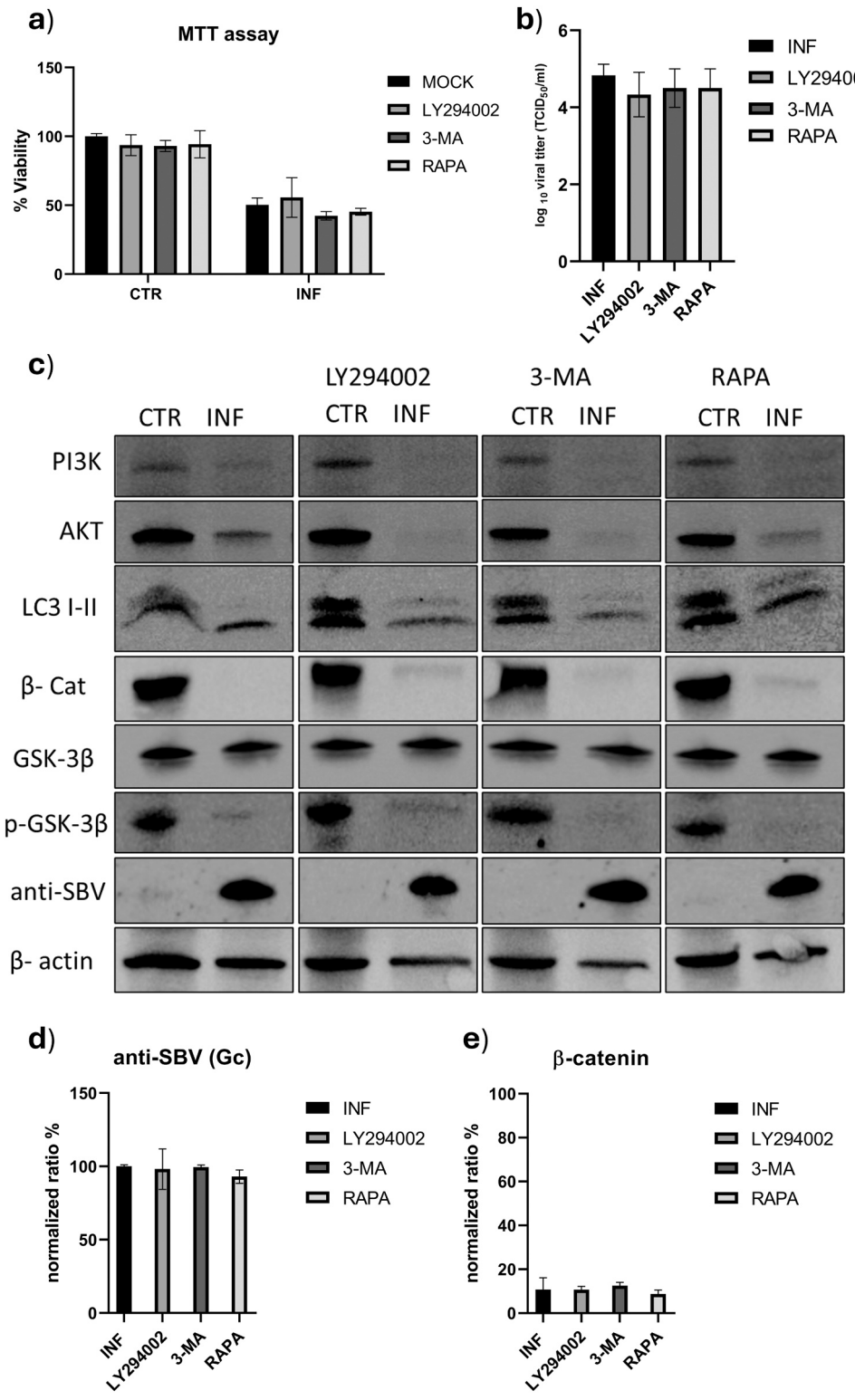

Fig. 8. Effects of the use of early autophagy inhibitors/inducers on SBV replication. The effects of early autophagy inhibitors/inducers on cell viability were assessed by MTT (a). The effects on viral replication were investigated by $TCID_{50}$ (b). Protein expression of several markers was assessed by western blot analysis (c). Glycoprotein C expression of infected cells was quantified using a specific monoclonal antibody (d). β-catenin levels were SBV replication-dependent (e). All the independent experiments were performed three times, *P < 0.05, **P < 0.01, and ***P < 0.001.

The study of viruses' interactions with these pathways is critical for determining how to inhibit viral replication by acting on them. Astragaloside IV is an example of how dysregulation of the Wnt/β-catenin pathway has been addressed in an antiviral activity by reducing the impact of the influenza virus on alveolar macrophages (Tang et al., 2025).

Recent studies have observed a decreased expression of β-catenin in human placentas of patients with spontaneous abortion (Chronopoulou et al., 2022). Alterations in the expression of the Wnt/β-catenin pathway are also associated with cartilage and bone development disorders in the

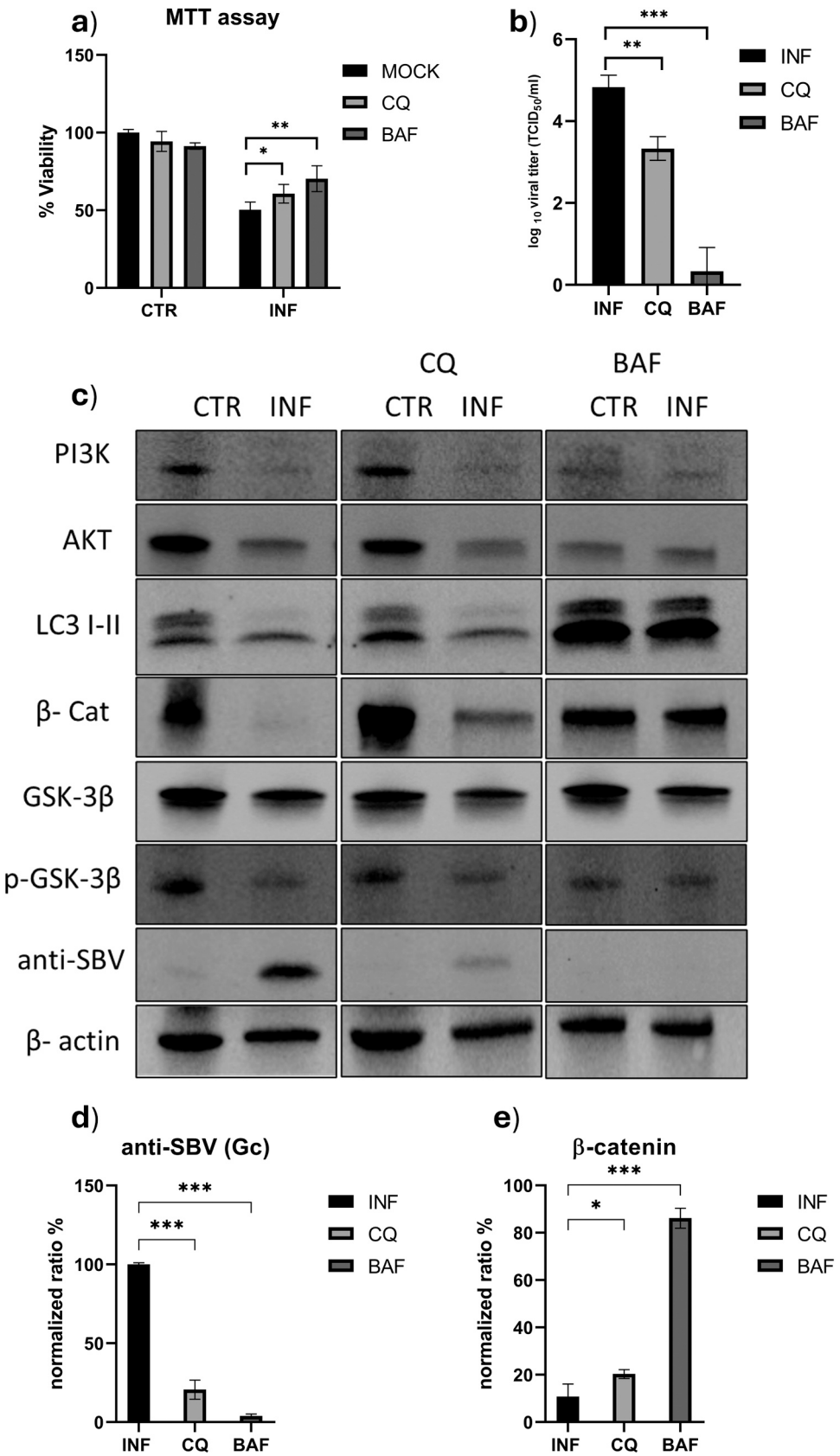

Fig. 9. Effects of the use of late autophagy inhibitors on SBV replication. The effects of late autophagy inhibitors on cell viability were assessed by MTT (a). The effects on viral replication were investigated by $TCID_{50}$ (b). Protein expression of several markers was assessed by western blot analysis (c). Glycoprotein C expression of infected cells was quantified using a specific monoclonal antibody (d). β-catenin levels were SBV replication-dependent (e). All the independent experiments were performed three times, *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$.

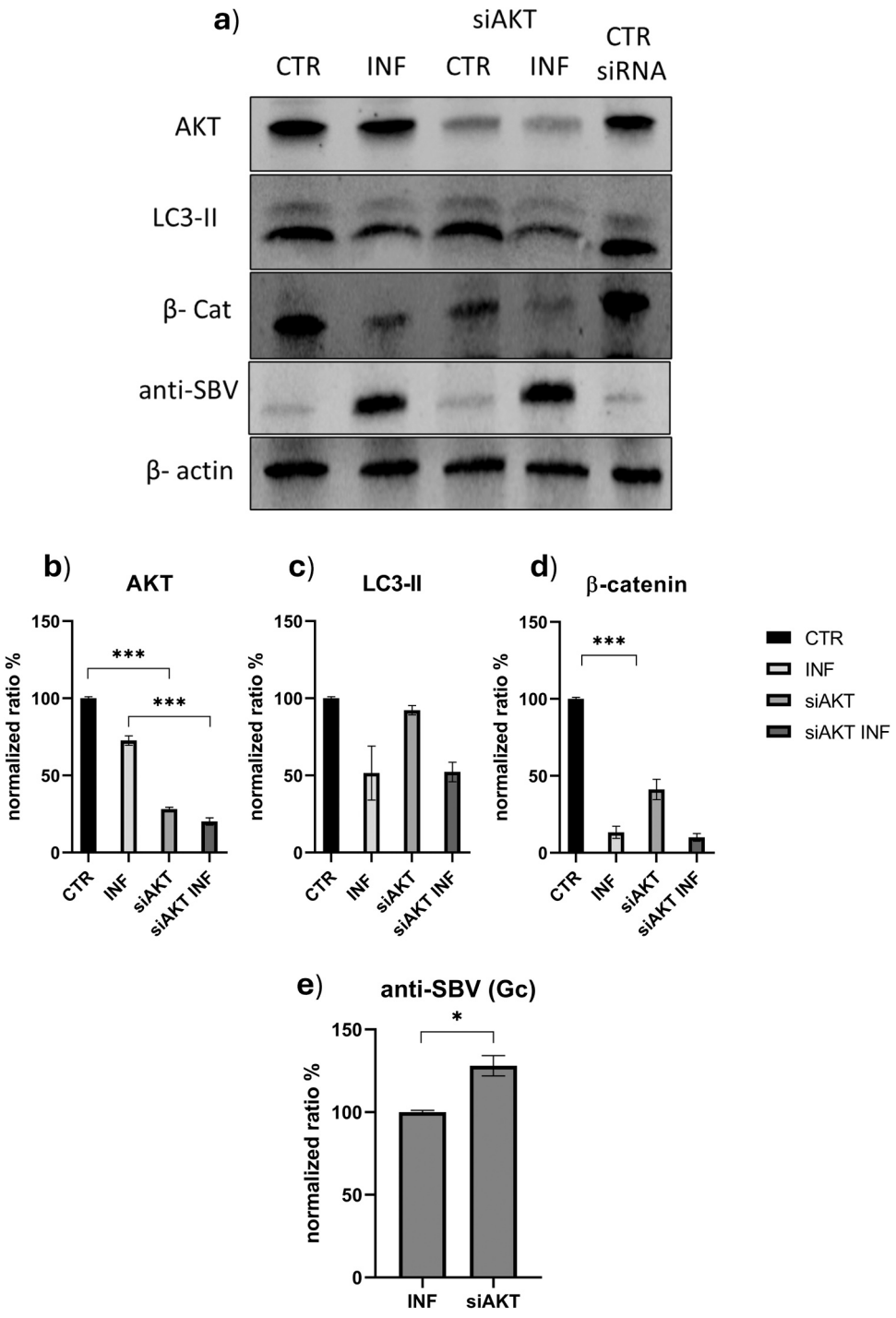

Fig. 10. Effects of the Akt silencing on SBV replication and the expression of several markers. Protein expression of several markers was assessed by western blot analysis after the silencing of Akt (a). Protein expression of Akt (b), LC3 I-II (c), and β-catenin (d), glycoprotein C (e) was assessed by western blot analysis. All the independent experiments were performed three times, *P < 0.05, **P < 0.01, and ***P < 0.001.

fetus (Chronopoulou et al., 2022; Ning et al., 2012). All these alterations are also common to the symptoms caused by SBV, which causes miscarriage and neonatal malformations characterized by hydrocephalus and artogryphosis (Li et al., 2015). The confirmation of the expression of Wnt/β-catenin pathway markers in vivo could suggest a role in pathogenic and abortive mechanism for SBV.

The scientific literature is characterized by a lack of studies on the pathogenesis of SBV and bunyaviruses in general. In vitro studies have shown that heparan sulfate proteoglycan was a major cellular attachment factor for the entry of SBV and that this virus causes apoptosis during its replication in permissive cells (Aksoy and Azkur, 2018; Murakami et al., 2017). Moreover, several transcriptomic studies have defined that the non-structural proteins (NSs) of SBV are capable of inhibiting the transcriptional machinery, blocking the antiviral response of the cell and the IFN synthesis (Blomström et al., 2015; Leventhal et al., 2021). The down-regulation of some markers observed in this study could be secondary to the same mechanism of inhibition of the cellular gene expression (Barry et al., 2014; Blomström et al., 2015). The present work has explored some aspects of the pathogenesis of SBV, providing previously undescribed features of the interactions that this

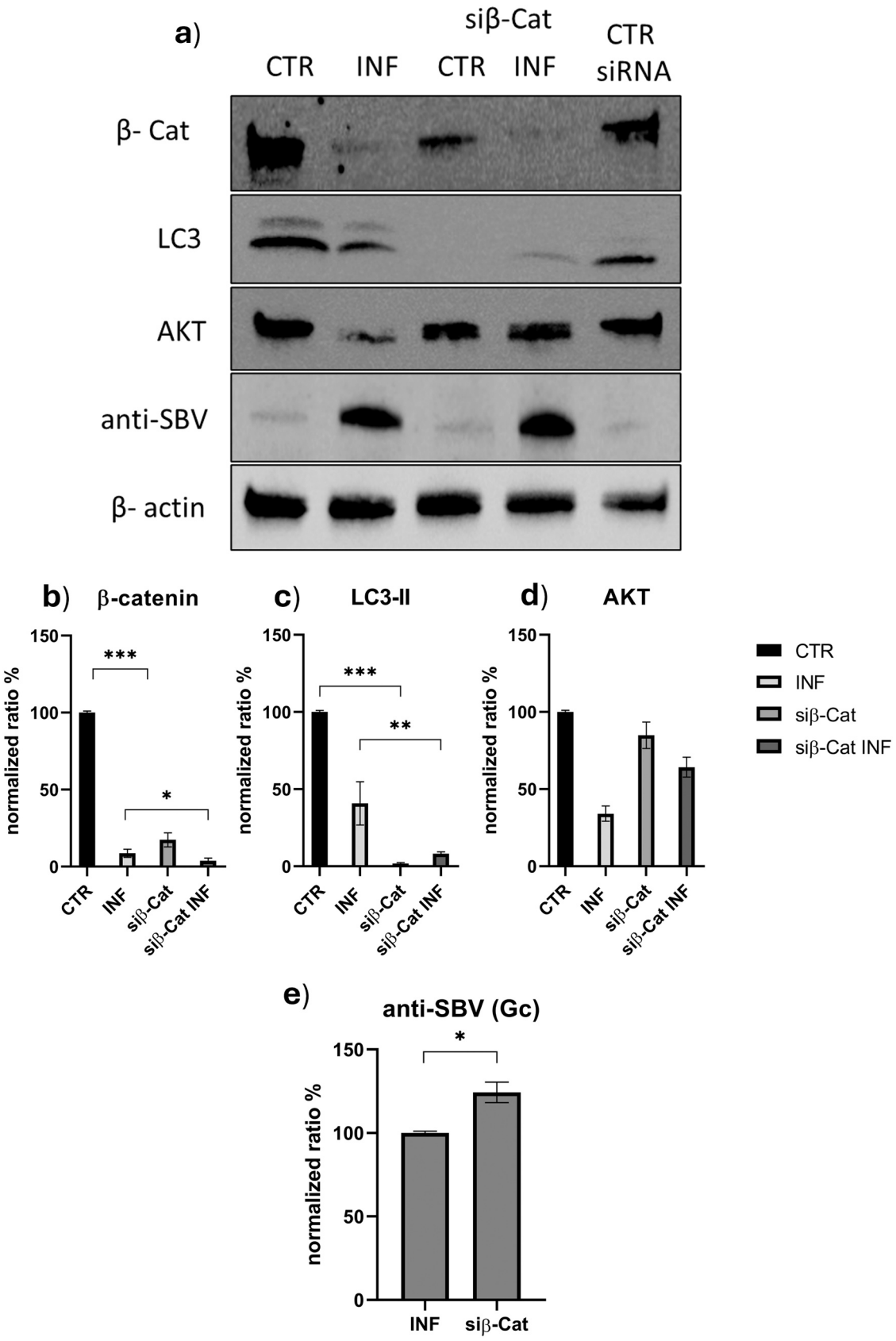

Fig. 11. Effects of the β-catenin silencing on SBV replication and the expression of several markers. Protein expression of several markers was assessed by western blot analysis after the silencing of β-catenin (a). Protein expression of β-catenin (a), LC3 I-II (b), Akt (c), glycoprotein C (d) was assessed by western blot analysis. All the independent experiments were performed three times, *P < 0.05, **P < 0.01, and ***P < 0.001.

virus carries out with autophagy and β-catenin.

CRediT authorship contribution statement

Ugo Pagnini: Writing – review & editing, Visualization, Resources, Formal analysis, Conceptualization. **Manuel Corsa:** Visualization, Resources, Methodology. **Hyun-Jin Shin:** Writing – original draft, Visualization, Formal analysis. **Francesco Origgi:** Writing – review & editing, Validation, Resources. **Davide Lelli:** Writing – original draft, Resources, Methodology. **Consiglia Longobardi:** Writing – original draft, Software, Resources, Methodology, Investigation. **Gianmarco Ferrara:** Writing – review & editing, Writing – original draft, Validation, Supervision, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Ethics approval statement

This research did not require ethical approval because it was only *in vitro* study.

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Supplementary file 1

Whole blots and relative housekeeping (actin or tubulin) used for Figs. 1, 2, 3, 5, 6, 7, 8, 9, 10, and 11.

Declaration of Competing Interest

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

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.vetmic.2025.110609.

Data availability

Data sharing is not applicable to this article as no datasets were generated or analysed during the current study.

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