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Evaluation of the in vitro Effect of the Interferon Produced by Bovine Ephemeral Fever Virus on Foot and Mouth Disease Virus

Ehab El-Sayed Ibrahim[1*], Albehwar A. A.[2], Heba Attia[1], Mohamed A. Abdrabo[3] and Mohamed Hassan Khodeir[2]

[1]Department of Foot and Mouth disease Vaccine Research; Veterinary Serum and Vaccine Research Institute (VSVRI), Abasia, Cairo; Agricultural Research Center (ARC), Egypt

[2]Department of Pet Animal Vaccine Research; Veterinary Serum and Vaccine Research Institute (VSVRI), Abasia, Cairo; Agricultural Research Center (ARC), Egypt

[3]Central Laboratory for Evaluation of Veterinary Biologics, Abasia, Cairo; Agricultural Research Center (ARC), Egypt

***Corresponding Author:** Ehab El-Sayed Ibrahim, **E-Mail:** ehabelsayed@hotmail.com

ABSTRACT

This paper presents a potential strategy for the development of an antiviral agent against FMDV, specifically O Pan-Asia-2, A Iran 05 and SAT2/EGY/2012. Examining the potential use of interferon from the Bovine Ephemeral Fever Virus (BEFV) as a therapy is an intriguing approach. In order to test the cell toxicity and anti-FMDV in African green monkey kidney cell (Vero) and Baby Hamster Kidney Cell (BHK) cell lines up to serial dilutions of 10^{-7}, BEFV was used to produce interferon in Vero and MDBK cell lines 12, 24, and 48 hours after cell infection. The in vitro findings showed that the cell safety and the ability of the prepared interferon to exhibit antiviral effects against various three FMDV serotypes, especially when administered shortly before or concurrently with the virus, are encouraging. The highest anti-FMDV effective dilution of the obtained interferon, either by Vero or MDBK cells, ranged from 10^{-3} to 10^{-4}. This suggests that the interferon may be able to activate cellular antiviral mechanisms and disrupt FMDV infection. The next critical step will be conducting further *in vivo* studies to evaluate the efficacy, safety, and potential side effects of the interferon-based treatment. This will provide important insights into virus-host interactions and the broader therapeutic potential of this approach. Some key areas for future research could include: expanding the testing of the interferon against additional FMDV serotypes to assess its breadth of coverage; Optimizing the administration parameters, such as timing, dose, and route, to maximize the antiviral effect, Investigating the specific mechanisms by which the interferon inhibits FMDV replication and spread, Evaluating the interferon's impact on disease progression and transmission in animal models, Assessing any potential negative effects or toxicity in the treated animals. The development of effective antivirals against FMDV is critical for controlling outbreaks and protecting livestock. This research represents a promising line of investigation that warrants further study.

Keywords: Bovine Ephemeral Fever, Interferon, Foot and Mouth Disease, MDBK cells.

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INTRODUCTION

Foot and Mouth Disease (FMD), also known as hoof-and-mouth disease (HMD), is a highly contagious and sometimes fatal viral disease that affects cloven-hoofed animals, including domestic and wild bovids. The disease causes a high fever followed by the formation of vesicle inside the mouth and near the hooves, which can rupture and lead to lameness **(Arzt *et al.*, 2011).** FMD has severe implications for animal farming due to its high infectivity and the Easley spread through contact with contaminated equipment, vehicles, clothing, feed, and even predators. Containing FMD outbreaks require extensive efforts in vaccination, trade restrictions, quarantines, monitoring and the culling of both infected and uninfected animals. The highly contagious nature of FMD and its severe economic impact on animal farming makes it a significant concern for the agricultural industry, necessitating robust disease management strategies and ongoing research into effective control measures.

A class of signaling proteins called interferons (IFNs) is produced and secreted by host cells in reaction to the presence of several viruses. Generally, a virus-infected cell releases interferons, which trigger neighboring cells to strengthen their defenses against viruses **(De Andrea *et al.*, 2002)**. IFNs are members of a broad class of proteins called cytokines, which are molecules that allow cells to communicate with one another and activate the immune system's defense mechanisms, which aid in the elimination of infections. Because they shield cells from viral infections, interferons have the capacity to "interfere" with viral replication **(Parkin and Cohen, 2001)**.

With a focus on the FMDV, IFNs are utilized as a therapeutic option to control viral diseases. Because of their attractive therapeutic options, IFNs are regarded as the first line of defense against viral diseases. The highly infectious pathogen FMDV, which affects animals with cloven hooves, is extremely susceptible to the antiviral effects of interferons. Expressed using an adenovirus vector, type I, type II, and type III IFNs have been shown to efficiently prevent FMDV multiplication in vitro, protect challenged mice the day following IFN therapy, and in certain circumstances even induce sterile immunity. Currently, vaccination is the only way to stop the rapidly spreading FMD, but it takes 5-7 days for an adaptive immune response to develop. The best course of action would be to use persistent IFN activity to prevent virus reproduction and spread while the vaccine-stimulated adaptive immune response is growing. This strategy to successfully regulate FMD in animal hosts has been hampered by issues with delivery mechanisms and the comparatively short half-life of IFN proteins in vivo **(Diaz-San *et al.*, 2021**; **Lee *et al.*, 2023)**.

The effect of interferon-gamma (IFN-γ) on the replication of FMDV was studied in vitro using persistently infected epithelial cells isolated from FMDV-infected cattle. The researchers initially treated primary bovine thyroid (BTY) cells with varying doses of recombinant bovine IFN-γ. They then measured the cytokine's activity by detecting viral antigen in cell supernatants and analyzing viral RNA expression, comparing treated cells to untreated controls. The results showed that pretreatment with IFN-γ profoundly affected FMDV growth in BTY cells. Viral replication was inhibited at IFN-γ concentrations above 2.5 u/ml, with the effect being both dose-dependent and related to the duration of exposure. Analysis of the inhibition mechanism suggested that IFN-γ did not suppress FMDV replication through the induction of nitric oxide. It was found that continuous IFN-γ treatment severely restricted FMDV replication or even eliminated the virus from persistently infected bovine epithelial cells, indicating that a cytokine-mediated pathway may play a role in clearing persistent FMDV infections in vivo. These findings provide valuable insights into the potential of IFN-γ as an antiviral agent against FMDV and its possible involvement in the clearance of persistent infections, which could inform the development of novel treatment strategies (**Zhang *et al.*, 2002).**

According to **He *et al.*, (2016)**, bovine ephemeral fever virus (BEFV) is a member of the genus Ephemerovirus within the family Rhabdoviridae. According to **Nandi and Negi (1999)**, the disease's clinical signs include dyspnea, abrupt fever onset, depression, difficulty swallowing, serous nasal and ocular discharge, stiffness, and lameness. The massive synthesis of IFNS was thought to be the cause of the widespread inflammation and toxicity linked to BEF illness **(St. George, 1993)**.

A class of cytokines known as IFNs is essential to the immune system's defense against viral infections. Based on their cell surface receptors, they are divided into three primary types: type I, type II, and type III. IFNs are among the first lines of defense against invasive infections because of their potent antiviral capabilities **(Lin and Young, 2014)**. A "cytokine storm" is a complicated immunological response that viral infections can cause by releasing a variety of inflammatory mediators. **Dubovi *et al.*, (2011)** Toll-like receptors (TLRs) can be stimulated by extracellular macromolecules, which in turn cause IFNs to be secreted. Fascinatingly, when administered therapeutically, natural IFN components seem to be less hazardous than synthesized IFN molecules (**Cheon *et al.*, 2014)**.

Desai (2016) showed that type I IFNs are a specific subclass of IFNs that are induced during viral infections by viral replication products, such as double-stranded RNA. These IFNs exert their functions by binding to specific cell surface receptors and triggering a cascade of events: alerting the immune system to the presence of the virus or pathogen, helping the immune system recognize the virus/pathogen, activating immune cells to attack the infected cells, Inhibiting the growth and division of virus and cancer cells and enhancing the ability of healthy cells to fight the infection (**Stephanie, 2020).**

In summary, interferons represent a critical component of the innate and adaptive immune response against viral infections, and their multifaceted mechanisms of action make them an important area of study in immunology and infectious disease research.

The purpose of this work was to explore the possibility of BEFV-induced interferon as a natural, affordable antiviral treatment for FMDV. The inhibitory effects of BEFV-induced interferon on FMDV in MDBK (Madin-Darby Bovine Kidney) cell culture were investigated in vitro by the researchers. The goal of this study was to investigate natural antiviral substitutes that would lessen the large financial losses caused by FMDV epidemics. In order to inform the planning of a more in-depth study on the potential therapeutic uses of this all-natural antiviral strategy, the researchers assessed BEFV-induced interferon's capacity to impede FMDV replication in cell culture.

This in vitro investigation represents an essential step in assessing the viability of BEFV interferon as a potential management strategy for FMDV infections. The findings from this preliminary work could inform the development of future trials and research focused on leveraging BEFV-induced interferon as a cost-effective, natural alternative for mitigating the substantial economic impact of FMDV.

MATERIALS AND METHODS

1.Viruses

- Bovine Ephemeral Fever Virus **(Albehwar *et al.*, 2018),** with a titer of 10^6 $TCID_{50}$/ml, was propagated on MDBK cell culture and used to induce interferon through the present study.
- Utilizing the Foot and Mouth disease viruses (O Pan-Asia-2, A Iran 05, and SAT2/EGY/2012), to examine the *in vitro* antiviral efficacy of BEFV-induced interferon **Shabana and Abd El-Sadek (2021).**

The Veterinary Serum and Vaccine Research Institute (VSVRI) provided these viruses.

2. Cell Cultures and Media

BEFV interferon was produced using MDBK and Vero cells.

- The antiviral effect of the produced BEFV interferon on FMDV was assessed using Vero and Baby Hamster Kidney (BHK) cells **(Eweis *et al.*, 2022).**
- The Capricorn Company's Minimum Essential Media (MEM), which was enhanced with 10% Newborn Calf Serum (NCS) that was imported from Australia, was used to propagate and maintain all of the cell cultures that VSVRI provided.

3. BEFV-induced activation of interferon

Interferon-producing samples were taken out of confluent Vero and MDBK cell cultures that were propagated in 25-ml tissue culture flasks. According to **Tzipori's (1975)** technique, these flasks were infected with BEFV at a multiplicity of infection of 2:1, and the virus was left to adsorb for an hour at 37 °C. The infected media were collected 12, 24, and 48 hours after cell infection, and they were centrifuged at 800 rpm for five minutes in a cool centrifuge, according to **Tsunetsugu-Yokota (2008).** After that, they were exposed to UV light with a wave length of 254 nm for 20 minutes.

4. Cytotoxicity assay of the prepared BEFV interferon

Confluent Vero cell cultures prepared in microtiter plates were inoculated with tenfold dilutions (up to 10^7) of each preparation to investigate the cytotoxicity of BEFV interferon. Six wells were used for the inoculation of each dilution, with the non-inoculated wells serving as the normal cell control.

5. Examination of BEFV interferon's antiviral impact on FMDV

FMDV was introduced into sets of BHK and Vero cell culture plates using 25 µl/well of 100 $TCID_{50}$ of the virus. The cells were then allowed to adsorb for an hour before being washed with PBS. Following this, 150 µl of maintenance medium was added to each of the five tissue culture wells containing the prepared interferon concentrations (undiluted up to 10^7) in the first set of plates; in the second set of plates, the interferon was added first, and then FMDV one hour later; in the third set of plates, both BEFV and FMDV interferon were inoculated simultaneously; the test included normal cells and untreated virus controls, and each plate was inspected under a microscope each day.

6. Quantification of cell protection by interferon

BEFV interferon-induced protection of BHK and Vero cells against FMDV was measured. According to **Stewart (2012),** a number of viruses can produce apparent cell damage, and any cytopathic effect can be used to gauge how well interferon is shielding cells. This assay technique has been used to test almost all kinds of interferons against a wide range of viruses and has the benefits of simplicity, speed, and economy of samples and supplies.

RESULTS

Table 1: BEFV-induced interferon's antiviral impact against FMDV serotype (O Pan-Asia-2):

Interferon dilution	FMDV (O) on BHK cell						FMDV (O) on Vero cell					
	MDBK generated interferon after			Vero generated interferon after			MDBK generated interferon after			Vero generated interferon after		
	12HPCI*	24HPCI	48HPCI	12HPCI	24HPCI	48HPCI	12HPCI*	24HPCI	48HPCI	12HPCI*	24HPCI	48HPCI
FMDV inoculated in BHK and Vero cells one hour before BEFV interferon												
Undiluted	+	+	+	+	+	+	+	+	+	+	+	+
10^{-1}	+	+	+	+	+	+	+	+	+	+	+	+
10^{-2}	+	+	+	-	+	+	+	+	+	-	+	+
10^{-3}	+	+	+	-	+	+	-	-	+	-	-	-
10^{-4}	-	-	-	-	-	-	-	-	-	-	-	-
FMDV inoculated in BHK and Vero cells one hour after BEFV interferon												
Undiluted	+	+	+	+	+	+	+	+	+	+	+	+
10^{-1}	+	+	+	+	+	+	+	+	+	+	+	+
10^{-2}	+	+	+	+	+	+	+	+	+	+	+	+
10^{-3}	-	+	+	+	+	+	-	+	+	+	+	+
10^{-4}	-	+	+	+	+	+	-	+	+	+	+	+
10^{-5}	-	-	+	+	+	+	-	-	-	-	-	-
10^{-6}	-	-	-	-	-	-	-	-	-	-	-	-
FMDV inoculated in BHK and Vero cells simultaneously with BEFV interferon												
Undiluted	+	+	+	+	+	+	+	+	+	+	+	+
10^{-1}	+	+	+	+	+	+	+	+	+	+	+	+
10^{-2}	+	+	+	+	+	+	+	+	+	+	+	+
10^{-3}	+	+	+	+	+	+	+	+	+	+	+	+
10^{-4}	+	+	+	+	+	+	+	+	+	+	+	+
10^{-5}	+	+	+	+	+	+	-	-	+	-	-	+
10^{-6}	-	-	-	-	-	-	-	-	-	-	-	-

Table 2: BEFV-induced interferon's antiviral impact against FMDV serotype (A Iran 05):

Interferon dilution	FMDV (A) on BHK cell						FMDV (A) on Vero cell					
	MDBK generated interferon after			Vero generated interferon after			MDBK generated interferon after			Vero generated interferon after		
	12HPCI*	24HPCI	48HPCI	12HPCI	24HPCI	48HPCI	12HPCI*	24HPCI	48HPCI	12HPCI*	24HPCI	48HPCI
FMDV inoculated in BHK and Vero cells one hour before BEFV interferon												
Undiluted	+	+	+	+	+	+	+	+	+	+	+	+
10^{-1}	+	+	+	+	+	+	+	+	+	+	+	+
10^{-2}	+	+	+	-	+	+	+	+	+	-	+	+
10^{-3}	-	-	+	-	+	+	-	-	-	-	-	+
10^{-4}	-	-	-	-	-	-	-	-	-	-	-	-
FMDV inoculated in BHK and Vero cells one hour after BEFV interferon												
Undiluted	+	+	+	+	+	+	+	+	+	+	+	+
10^{-1}	+	+	+	+	+	+	+	+	+	+	+	+
10^{-2}	+	+	+	+	+	+	+	+	+	+	+	+
10^{-3}	-	+	+	+	+	+	-	+	+	+	+	+
10^{-4}	-	+	+	+	+	+	-	-	+	-	-	+
10^{-5}	-	-	-	-	-	-	-	-	-	-	-	-
FMDV inoculated in BHK and Vero cells simultaneously with BEFV interferon												
Undiluted	+	+	+	+	+	+	+	+	+	+	+	+
10^{-1}	+	+	+	+	+	+	+	+	+	+	+	+
10^{-2}	+	+	+	+	+	+	+	+	+	+	+	+
10^{-3}	+	+	+	+	+	+	+	+	+	+	+	+
10^{-4}	+	+	+	+	+	+	-	+	+	-	-	+
10^{-5}	-	-	+	-	-	+	-	-	-	-	-	+
10^{-6}	-	-	-	-	-	-	-	-	-	-	-	-

Table 3: BEFV-induced interferon's antiviral impact against FMDV serotype (SAT2/EGY/2012):

Interferon dilution	FMDV (SAT2) on BHK cell						FMDV (SAT2) on Vero cell					
	MDBK generated interferon after			Vero generated interferon after			MDBK generated interferon after			Vero generated interferon after		
	12HPCI*	24HPCI	48HPCI	12HPCI	24HPCI	48HPCI	12HPCI*	24HPCI	48HPCI	12HPCI*	24HPCI	48HPCI
FMDV inoculated in BHK and Vero cells one hour before BEFV interferon												
Undiluted	+	+	+	+	+	+	+	+	+	+	+	+
10^{-1}	+	+	+	+	+	+	+	+	+	+	+	+
10^{-2}	+	+	+	-	+	+	-	+	+	-	+	+
10^{-3}	-	-	-	-	-	-	-	-	-	-	-	-
FMDV inoculated in BHK and Vero cells one hour after BEFV interferon												
Undiluted	+	+	+	+	+	+	+	+	+	+	+	+
10^{-1}	+	+	+	+	+	+	+	+	+	+	+	+
10^{-2}	+	+	+	+	+	+	+	+	+	+	+	+
10^{-3}	-	-	+	+	+	+	-	-	+	-	+	+
10^{-4}	-	-	+	-	-	+	-	-	-	-	-	-
10^{-5}	-	-	-	-	-	-	-	-	-	-	-	-
FMDV inoculated in BHK and Vero cells simultaneously with BEFV interferon												
Undiluted	+	+	+	+	+	+	+	+	+	+	+	+
10^{-1}	+	+	+	+	+	+	+	+	+	+	+	+
10^{-2}	+	+	+	+	+	+	+	+	+	+	+	+
10^{-3}	+	+	+	+	+	+	+	+	+	+	+	+
10^{-4}	-	+	+	+	+	+	-	-	+	+	+	+
10^{-5}	-	-	-	-	-	-	-	-	-	-	-	-

*HPCI= hours post cell infection with BEFV. +: Antiviral Effect -: No antiviral Effect

DISCUSSION

The Picornaviridae family illness FMDV is the most infectious among animals with cloven hooves. It can result in severe financial losses and cause an acute illness that spreads quickly, causing fever, lameness, and vesicular sores on the feet, lips, snout, and teats **(Grubman and Baxt, 2004)**. Programs for controlling FMD should incorporate quick steps to restrict and manage the disease's spread. They currently encourage the creation of immunomodulatory and/or antiviral drugs in order to address these needs (**The Royal Society, 2002; Gamil and Soliman, 2021).**

This work was done as a preliminary in vitro investigation to find out the antiviral effect of interferon generated by BEFV against three serotypes of FMDV in MDBK and Vero cell cultures. It was discovered that type II pIFN (pIFN-γ) exhibits antiviral activity against FMDV in cell culture (**Moraes *et al.*, 2007).** Such work seems to be supported by **Saber *et al.*, (2022),** who found that the interferon generated by the bovine ephemeral fever virus was able to significantly reduce the replication of the RVFV in cell culture experiments. Specifically. The presence of the bovine ephemeral fever virus interferon led to a dose-dependent decrease in RVFV titers in infected cell cultures. At the highest interferon concentrations tested, viral titers were reduced by over 90% compared to control cultures without interferon. The antiviral effect was observed across multiple strains and serotypes of RVFV. The interferon appeared to inhibit an early stage of the RVFV replication cycle, likely by blocking viral entry or uncoating.

The obtained results of the current study are demonstrated in Tables 1, 2 and 3 and show the ability of interferon generated by BEFV to inhibit replication of FMDV in Vero cell cultures. Inoculation of the FMDV serotype O Pan Asia1 on BHK one hour before BEFV-induced interferon showed antiviral effects at dilution 10^{-3} with MDBK-generated interferon after 12, 24, and 48 hours post-cell infection (HPCI) and antiviral effects at dilutions 10^{-1}, 10^{-3},10^{-3} with Vero-generated interferon after 12, 24, and 48 hours of HPCI, respectively. But on Vero cells one hour before BEFV-induced interferon showed antiviral effects at dilution 10^{-2}, 10^{-2},10^{-3} with MDBK-generated interferon after 12, 24, 48 HPCI and antiviral effects at dilution 10^{-1}, 10^{-2},10^{-2} with Vero-generated interferon after 12, 24, 48 HPCI, respectively (Table 1).

Also, in the case of inoculation of the FMDV serotype A Iran 05 on BHK one hour before BEFV-induced interferon, antiviral effects at dilutions of 10^{-2}, 10^{-2}, 10^{-3} with MDBK-generated interferon after 12, 24, and 48 hours post-cell infection (HPCI) and antiviral effects at dilutions of 10^{-1}, 10^{-3},10^{-3} with Vero-generated interferon after 12, 24, and 48 hours of HPCI, respectively. But on Vero cells one hour before BEFV-induced interferon showed antiviral effects at dilution 10^{-2} with MDBK-generated interferon after 12, 24, 48

HPCI and antiviral effects at dilution 10^{-1}, 10^{-2},10^{-3} with Vero-generated interferon after 12, 24, 48 HPCI, respectively (Table 2).

On the other side, inoculation of the FMDV serotype SAT2 on BHK one hour before BEFV-induced interferon showed antiviral effects at dilution 10^{-2} with MDBK-generated interferon after 12, 24, and 48 HPCI and antiviral effects at dilutions 10^{-1}, $10^{-2,}$ and $10^{-2,}$ respectively, with Vero-generated interferon after 12, 24, and 48 HPCI, respectively. But on Vero cells, one hour before BEFV-induced interferon showed antiviral effects at dilutions of 10^{-1}, 10^{-2},10^{-2} with both MDBK and Vero cell-generated interferon after 12, 24, and 48 HPCI, respectively (Table 3).

Katze *et al.*, (2002) observed that interferon-stimulated gene expression and activity can trigger an antiviral state in virus-infected cells and surrounding tissues when interferons are applied, indicating that BEFV-induced interferon may act as a preventive measure to shield animals from RVFV outbreaks. The ability of BEFV-induced interferon to generate an antiviral milieu supports the possibility that it can protect untainted animals from infection during pandemics.

Regarding inoculation of the FMDV serotype O Pan Asia1 on BHK one hour after BEFV-induced interferon showed antiviral effects at dilution 10^{-2},10^{-4} and 10^{-5} with MDBK-generated interferon after 12, 24, and 48 HPCI, respectively and antiviral effects at dilution 10^{-5} with Vero-generated interferon after 12, 24, and 48 HPCI, respectively. But on Vero cells, one hour after BEFV-induced interferon showed antiviral effects at dilutions of 10^{-2}, $10^{-4,}$ and 10^{-4} with MDBK-generated interferon after 12, 24, and 48 HPCI and antiviral effects at dilutions of 10^{-4} with Vero-generated interferon after the same HPCI, respectively (Table 1).

Also, the inoculation of the FMDV serotype A Iran 05 on BHK one hour after BEFV-induced interferon showed antiviral effects at dilutions of 10^{-2}, 10^{-4}, 10^{-4} with MDBK-generated interferon after 12, 24, and 48 HPCI, respectively and antiviral effects at dilutions of 10^{-4} with Vero-generated interferon after 12, 24, and 48 HPCI. But on Vero cells, one hour after BEFV-induced interferon showed antiviral effects at dilutions of 10^{-2}, 10^{-3},10^{-4} with MDBK-generated interferon after 12, 24, and 48 HPCI, respectively and antiviral effects at dilutions of 10^{-3}, 10^{-3},10^{-4} with Vero-generated interferon after the same HPCI, respectively (Table 2).

But in the case of inoculation of the FMDV serotype SAT2 on BHK one hour after BEFV-induced interferon showed antiviral effects at dilutions of 10^{-2}, 10^{-2},10^{-4} with MDBK-generated interferon after 12, 24, and 48 hours post-cell infection (HPCI), respectively and antiviral effects at dilutions of 10^{-3}, 10^{-3},10^{-4} with Vero-generated interferon after 12, 24, and 48 hours of HPCI, respectively. But on Vero cells one hour after BEFV-induced interferon showed antiviral effects at dilution 10^{-2}, 10^{-2},10^{-3} with MDBK-generated interferon after 12, 24, 48 HPCI, respectively and antiviral effects at dilution 10^{-2}, 10^{-3},10^{-3} with Vero-generated interferon after 12, 24, 48 HPCI, respectively (Table 3).

In this respect, it was shown that pretreatment of cells with IFN-α/β can dramatically inhibit FMDV replication **(Ahl and Rump, 1976; Chinsangaram *et al.*, 1999 and 2001)**. These findings also corroborate earlier research from **Zhang *et al.*, (2002)**, who demonstrated that pretreatment of primary bovine thyroid cells with bovine IFN-γ significantly decreased FMDV RNA and protein synthesis. This process was found to involve at least two IFN-α/β-stimulated gene products (ISGs), namely double-stranded RNA-dependent protein kinase (PKR) and 2′,5′ oligoadenylate synthetase (OAS)/RNase L **(De los Santos *et al.*, 2006)**. It was noticed that the interferon generated by Vero cells infected with BEFV increases over time, from 24 to 48 hours post-infection, and MDBK cells have a higher ability to produce interferon compared to Vero cells. Interferon is usually thought to have non-virus-type-specific antiviral actions. According to **Charles (1991)**, administering a particular type or subspecies of interferon can frequently trigger an antiviral state that is efficacious against a broad spectrum of animal viruses, both DNA- and RNA-based. This suggests that the interferon induced by BEFV infection, regardless of the cell type, has the potential to provide broad-spectrum antiviral protection, including against unrelated viruses like FMDV. The increased interferon production over time and the higher interferon-generating capacity of MDBK cells further support the idea that BEFV-induced interferon could be leveraged as a prophylactic measure against FMDV outbreaks. The non-specific antiviral nature of interferon is a key point that strengthens the proposed cross-protective potential against RVFV, as observed in the initial in vitro findings. This emphasizes the need for further research to validate these mechanisms in animal models and explore practical applications (**Medina *et al.*, 2020)**.

Regarding simultaneous inoculation of the FMDV serotype O Pan Asia1 on BHK with BEFV-induced interferon, it showed antiviral effects at dilution 10^{-5} with both MDBK and Vero cell-generated interferon after 12, 24, and 48 HPCI. But Vero cells with BEFV-induced interferon showed antiviral effects at dilutions of 10^{-4}, 10^{-4},10^{-5} with both MDBK and Vero cell-generated interferon after 12, 24, and 48 HPCI,

respectively (Table 1). Also, in the case of inoculation of the FMDV serotype A Iran 05 on BHK simultaneously with BEFV-induced interferon, it showed antiviral effects at dilutions of 10^{-4}, 10^{-4},10^{-5} with both MDBK and Vero cell-generated interferon after 12, 24, and 48 HPCI, respectively. But on Vero cells, BEFV-induced interferon showed antiviral effects at dilutions of 10^{-3}, $10^{-4,}$ and 10^{-4} with MDBK-generated interferon after 12, 24, and 48 HPCI, respectively and antiviral effects at dilutions of 10^{-3}, 10^{-3},10^{-5} with Vero-generated interferon after 12, 24, and 48 HPCI, respectively (Table 2).

But the inoculation of the FMDV serotype SAT2 on BHK simultaneously with BEFV-induced interferon showed antiviral effects at dilutions of 10^{-3}, 10^{-4},10^{-4} with MDBK-generated interferon after 12, 24, and 48 HPCI, respectively and antiviral effects at dilutions of 10^{-4} with Vero-generated interferon after 12, 24, and 48 HPCI. But Vero cells with BEFV-induced interferon showed antiviral effects at dilutions of 10^{-3}, 10^{-3}, 10^{-4} with MDBK-generated interferon after 12, 24, and 48 HPCI, respectively and antiviral effects at dilutions of 10^{-4} with Vero-generated interferon after 12, 24, and 48 HPCI (Table 3).

These results seem to be supported by **Pestka *et al.*, (1987); Sarker and Sen (1998); Stark *et al.*, (1998) and Medina *et al.*, (2020)**, who discovered that simultaneous inoculation of both groups resulted in the best antiviral effect of BEFV-induced interferon against FMDV, followed by a subsequent interferon inoculation after virus inoculation. Interferons can inhibit the growth of viruses within cells and modify the nonspecific and specific immune responses to viral antigens in body fluids and on cell surfaces. The synthesis of viral polypeptides is the first stage of the virus multiplication cycle that is usually blocked for many viruses that are susceptible to the antiviral activity of interferon in cell culture.

It was concluded that BEFV-induced interferon provides broad-spectrum antiviral protection, including FMDV. The observed synergistic effect when inoculating the interferon simultaneously with the virus, followed by additional interferon one hour before virus inoculation, suggests interferon could potentially be used both as a prophylactic measure and as a treatment. The known mechanisms of interferon-mediated antiviral activity provide a strong biological basis for this cross-protection. Explaining the action of interferons, Interferons are powerful pleiotropic cytokines that, when activated in response to viral and other infections, broadly modify cellular processes. These modifications include adjustments to the nutritional microenvironment, protein synthesis, proliferation, and membrane composition. According to recent data, rewiring the cell's metabolism in response to IFN promotes antiviral responses **(Jorg, 2016)**.

CONCLUSIONS

The obtained results of the current study emphasize the need to provide a natural anti-FMD viral agent in addition to using BEFV-interferon to control the spread of the disease, as interferons are known to act via autocrine and paracrine pathways to induce an antiviral state in infected cells and in neighboring cells containing interferon receptors. As the initial line of defense against viral infection, interferons' primary function is to locally prevent viral spread. Therefore, more research is needed.

Conflict of interest

The authors declare that they have no conflict of interest.

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