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STUDY OF THE INTERFERON-INDUCING ACTIVITY OF A VACCINE STRAIN OF BOVINE RESPIRATORY SYNCYTIAL VIRUS

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ABSTRACT

Bovine respiratory syncytial virus (BRSV) is major viral component of bovine respiratory disease (BRD). BRD is a major cause of morbidity and mortality in all classes of cattle but particularly young beef and dairy calves.

The actuality of this infection is preserved regardless of the change in the technology of cattle breeding in our country at the present stage and therefore the study of immune factors is essential. Along with studying different immune factors, we proved that the vaccine strain "An-87"of the BRSV has a well-defined interferon-inducing activity. The highest values of interferon are found in the secretions of the respiratory tract in trache-ally applied antigen on the 3d day.

Key words: Bovine respiratory syncytial virus, bovine respiratory disease, interferon, vaccine strain.

Introduction

Bovine respiratory disease (BRD) remains one of the leading causes of beef and dairy calf morbidity and mortality in all over the world (USDA-APHIS-VS-CEAH, 2018). Therefore, the study of various components of the immune reactivity of animals has great practical application

Interferons are one of the important factors of antiviral immunity (Weber, 2020). Clinical trials have convincingly shown that interferons can be used successfully against viral infections and in cancer protection in humans. The species specificity makes them difficult to obtain as a ready-made preparation for treatment, regardless of the proposed methods of genetic engineering for their production. For these reasons, veterinary practice focuses on finding a suitable viral inducer of endogenous interferon. There are already a number of positive results in this direction.

For such a positive effect in calves point out Todd et al. (1971). Using a strain of BHB1, Ahl and Staub (1985) demonstrated interfering activity of a strain of BXB1 and PI-3. The result of these studies was the development of the commercial preparation Byferon (Straub, 1975).

Data on BRSV in this area are scarce. ElAzhary et al. (1981) in aerosol infection of calves with RSV proved interferon in the nasal secretion of animals with a peak on the third day. There is also and information on the use of interferon preparations to increase the immunobiological reactivity of animals (Sergienko and Nazaruk, 1991). Nazaruk et al. (1988) used interferons for the prevention and treatment of respiratory diseases in calves.

In the present study, we extended our studies of the immunity of BRSV by monitoring the interferon-inducing activity of a vaccine strain administered in different routes.

Materials and methods

Viruses. Bovine respiratory syncytial virus (BRSV) – vaccine strain "An-87" was attenuated in BHK21 tissue culture with a titer $10^{5,5}$ $TCID_{50}$/ml. Vesicular stomatitis virus (VSV) with a titer $10^{2,0}$ $TCID_{50}$/ml.

Biological experiment. Experimental work was carried out in 4-months-old calves, free of neutralizing antibodies to BRSV. The first group of three calves was inoculated intratracheally (IT) with $10^{5,5}$ TClD50 of "An-87"; the second group received $10^{5,5}$ $TClD_{50}$ of "An-87" subcutaneously (SC);

On the 3^{d}, 7th, 11th and 15th days after inoculation serum and nasal samples from each animal were collected.

Interferon (IFN) assay. Nasal secretions were collected by rinsing the calves' nostrils three times with 15–20 cm^3 of sterile saline with antibiotics and nystatin. The washing liquid was centrifuged at 3,500 rpm for 20 min. For experimental work supernatant liquid was used.

Serum samples were dialyzed against 0.1M HCl/KCl buffer with pH 2.0 at 4°C for 24 hours at a volume ratio of 1: 100. After that reverse dialysis against phosphate buffer with pH 7,2 for 24 h at 4°C was performed.

For the titration, a method for inhibition of $TCID_{50}$ in primary cell cultures of calf kidney and vesicular stomatitis virus (VSV) were used. The samples were twice diluted and 1 cm^3 of each dilution was inoculated in four tubes. The cell cultures with the samples were incubated for 18–20 h at 37°C. After that time tubes were infected with 0.1 cm^3 VSV (10 $TCID_{50}$) by absorption for 60 minutes at 30°C and 1 cm^3 growth medium (medium 199, 3% FCS, antibiotics) was added. Tubes with cell cultures were incubated again for 48–72 h at 37°C.

The interferon titer was calculated as the reciprocal of the last interferon dilution causing 50% inhibition of virus-induced cytopathic effect and was expressed as IFN units per volume

Several controls were administered – control of the viral dose (infected cultures, untreated with interferon), cell culture controls and eight controls for interferon identification.

Statistical evaluation. The Student t test was performed when comparison between groups was needed.

Results

The interferon production of the tested serum samples and nasal secretions of calves, vaccinated intratracheally and subcutaneously are presented in Fig. 1 and Fig. 2.

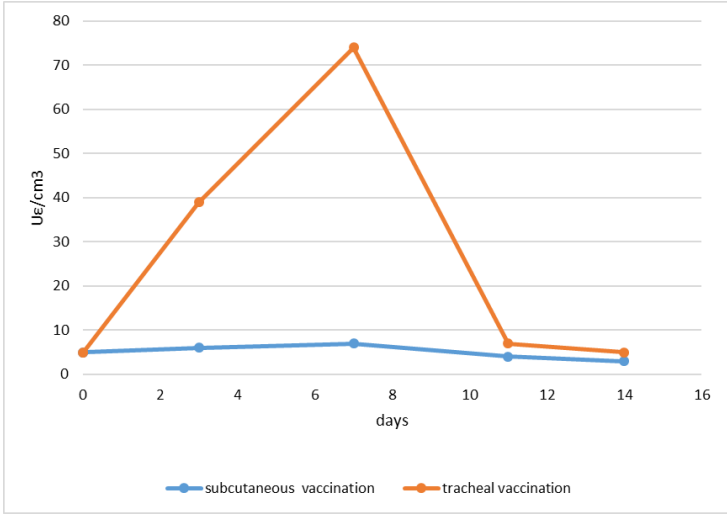

Figure 1: Serum interferon in calves vaccinated subcutaneously and tracheally with "An-87"

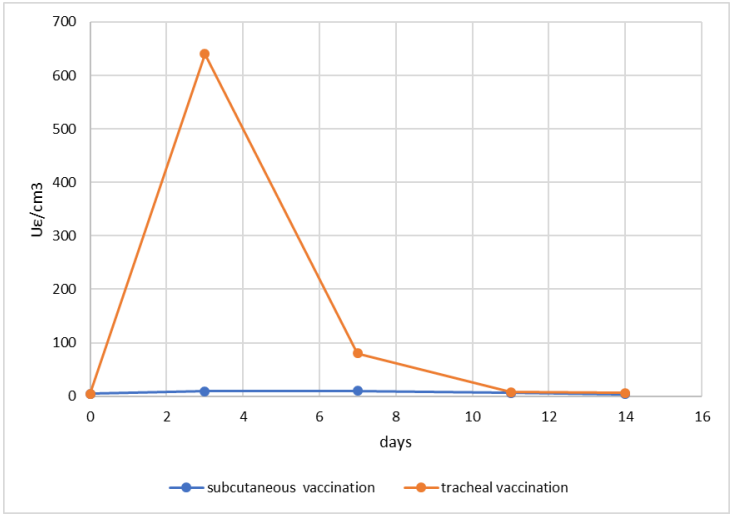

Figure 2: Dynamics of interferon formation in the nasal secretions of calves after subcutaneous and tracheal vaccination

The results show that serum interferon is detected from day 3 to day 7 in low titers in both groups of animals. Comparing the two groups of animals, higher levels of interferon were found in calves vaccinated tracheally ($p<0,01$)

The highest values of interferon in nasal secretions were observed on day 3 (640 IU/cm^3). After 11th day, interferon is no established. There is no interferon in the samples after subcutaneous administration of the vaccine strain

Positive samples with antiviral effect against VSV were additionally tested in the tests for identification of interferon – test for resistance to pH 2.0, test for heat resistance, test for species specificity, test after treatment with ether and trypsin and test for direction of inhibitory effect.

Discussion

Our studies of the interferon-inducing activity of the vaccine strain "An-87" confirm the better expressed immune response of calves after tracheal administration. The highest levels of interferon are found in the secretions of the respiratory tract during tracheal inoculation. We also proved serum interferon, but in lower titers. There is no evidence of interferon-inducing activity of the strain on subcutaneous administration. In regard to this Valarcher et al. (2003) demonstrated that, in contrast to wild-type BRSVs, recombinant BRSVs (rBRSVs) lacking the NS proteins are strong inducers of IFN-α/β in bovine nasal fibroblasts and broncho alveolar macrophages.

Interferons as immune factors have been extensively studied in recent years. But data about BRSV are scarce. The results of El Azhary et al. (1981) for the interferon activity of BRSV after aerosol infection reflect other dynamics of interferon in secretions and serum.

Nevertheless, these properties of our strain are positive for protection against infection in the tracheal route of inoculation of the virus.

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