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Application of the PCR for diagnosis and monitoring of rotavirus in calves during treatment with the antiviral agent Triazavirin

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Abstract

Background: Rotavirus is a significant cause of morbidity and mortality in neonatal calves, leading to severe gastrointestinal and systemic illnesses. Current treatments primarily focus on symptomatic relief and prevention through vaccines, with limited use of antiviral agents. This article considers the issue of experiments using the antiviral agent Triazavirin in the treatment of rotavirus infection in calves, with tracking the amount of virus during the polymerase chain reaction examination in fecal samples.

Methods: The experiment was conducted at a Mordovian Republican Veterinary Laboratory in the National Research Mordovian State University named after N.P. Ogarev (Republic of Mordovia, Russia), between February and March 2023. Two groups of calves, experimental and control, were assessed. The experimental group received Triazavirin orally at a dose of 500 mg per day for 7 days. Before and after the experiment, fecal samples were taken from experimental calves for the PCR study of the ribonucleic acid of group A rotaviruses.

Results: Treatment with Triazavirin resulted in a reduction of the rotavirus load by an average of 35.2%. The PCR analyses before and after treatment indicated a substantial decrease in viral RNA in the majority of treated calves, with variable individual responses.

Conclusions: Triazavirin demonstrates potential as an effective antiviral treatment for rotavirus in calves, reducing viral load and possibly improving clinical outcomes. The use of PCR provided a reliable method for monitoring viral levels, thereby facilitating effective management of this infectious disease.

Introduction

Rotavirus infections in neonatal calves are a major veterinary concern worldwide due to their high prevalence and severe impact on the livestock industry. This viral pathogen is known for its rapid transmission and devastating effects on young animals, often leading to severe dehydration, diarrhea, and high mortality rates.

Rotavirus belongs to RNA viruses and affects calves at an early age, starting from 3-5 days of life [1, 2]. The animals at risk are the ones that have not received high-quality colostrum, with abnormalities in the time and process of feeding [3-5]. The disease is characterized by damage to the digestive system (intestines) [6, 7], the development of diarrhea [8], dehydration, impaired colostral immunity, depression, later tachycardia, and coma [9-11]. There is evidence of virus damage to the respiratory, central nervous, and cardiovascular systems, kidneys, and liver [12, 13]. Mortality among calves can reach 50% in this disease. Upon autopsy, pathological changes are noted in the organs of the digestive system, liver, kidneys, spleen, heart, bronchi, and lungs. The diagnosis is complex and includes laboratory research methods like polymerase chain reaction (PCR) and clinical data. This disease occurs in the form of mixed infections with the involvement of several pathogens, such as coronavirus, parvovirus, herpesvirus, reovirus, as well as enterobacterial agents [14, 15]. Prevention is reduced to vaccination, and the treatment is combined and symptomatic, with the use of serums. Rotavirus infection occurs in almost all agricultural enterprises and has a significant percentage of animal damage. Unfortunately, not all treatment regimens include antiviral agents [16, 17]. The main emphasis is on the use of antibacterial and vitamin preparations. Antiviral agents are practically not used [18-20]. However, these measures alone are often insufficient to control outbreaks, especially in densely populated farming environments. The development and implementation of effective antiviral treatments represent a critical need in veterinary medicine to enhance survival rates and improve animal welfare. Antiviral agents like Triazavirin could potentially offer a novel therapeutic avenue, reducing the viral load and mitigating the severity of the disease, thereby supporting the health and productivity of affected herds.

Thus, in our study, we conducted an experiment using the antiviral agent Triazavirin in the treatment of rotavirus infection in calves, tracking the amount of virus during the PCR test of fecal samples.

Methods

The study and experiments were conducted from February to March 2023 at a farm of the Streletsk LLC, Ruzaevsky district, Republic of Mordovia, Russia.

During the experiment, experimental groups of calves were formed, according to the principle of analogs. Pregnant cows (mothers) of experimental calves were vaccinated with the Kombovak-R vaccine, once, in December 2022. On the first day of life, the calves were injected with E-selenium. The animals were kept in the same hygienic conditions and had the same diet according to age and technology of keeping. Upon admission, two groups were formed considering the condition and age of the animals. Calves with symptoms of damage to the digestive and respiratory systems, with the presence of obvious clinical signs, such as dehydration, liquid gray-and-white feces, shortness of breath, lethargy, apathy, and nasal discharge, were selected for the experiment (Table 1).

No. of the experiment	Medication	Administration method	Administration schedule
1st experimental group	Triazavirin	orally	2 capsules (500 mg) per day (1 capsule twice a day) per head, for 7 days
2nd experimental group	Control	-	Received no treatment

Table 1: Experiment design.

Groups 1 and 2 included five and four heads of calves, aged 3-14 days. For treatment in the 1st experimental group, Triazavirin was used, administered orally (after feeding), at a dose of 2 capsules (500 mg) per day per head, for 7 days, while the 2nd control group received no treatment.

Before and after the experiment, fecal samples were taken from experimental calves for PCR examination of the RNA of group A rotaviruses (Rotavirus A). The study was conducted at the Mordovian Republican Veterinary Laboratory and N.P. Ogarev Mordovian State University, Agrarian Institute, Department of Morphology, Physiology, and Veterinary Pathology.

Results

During the experiment, qualitative and quantitative PCR studies were conducted before and after the use of Triazavirin (Table 2). Of the samples of five calves, rotavirus was detected in four animals during the study. After treatment with the antiviral agent, the following findings were noted: in sample No. 4, the virus was completely absent, in samples No. 5 and 2, the amount of the virus had decreased by 8.3 and 57.1%, respectively, in sample No. 1, we observed an increase in the amount of the virus by 24.6% (Table 2). The control group consisted of four animals, and we noted the presence of the virus in the calves.

No.	Result before the experiment		Result after the experiment		2nd control group	
	+/-	Average Ct	+/-	Average Ct	+/-	Average Ct
1	+	29.71	+	37.02	+	32.07
2	+	36.00	+	15.45	+	16.99
3	-	-	-	-	+	22.07
4	+	33.40	-	-	+	23.67
5	+	34.81	+	31.92		

Table 2: RNA of group A rotaviruses (Rotavirus A).

Table 2 Indicates a general reduction in viral load in the experimental group treated with Triazavirin, with one sample showing complete viral clearance and another showing a substantial reduction. One sample did show an increase in viral load, which might be an outlier or due to individual variability in response to treatment.

During the analysis of PCR studies, we examined the graphs attached to the study (Figures 1-3). The graphs contain a baseline, exponential curves reflecting the data, and a plateau.

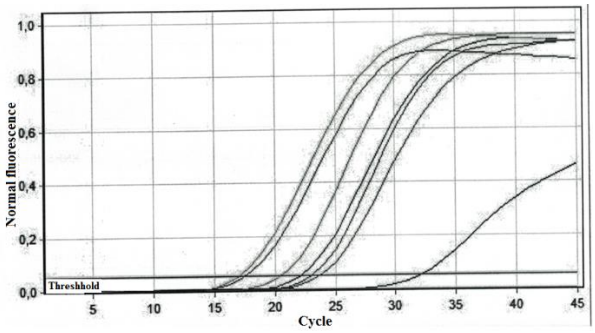

Figure 1: Graph of the PCR test, control group 2.

This graph represents the PCR results of the control group, showing baseline viral load levels without any intervention. The exponential curves reflect the amount of viral RNA detected, which is higher compared to the treated group, indicating no reduction in viral load.

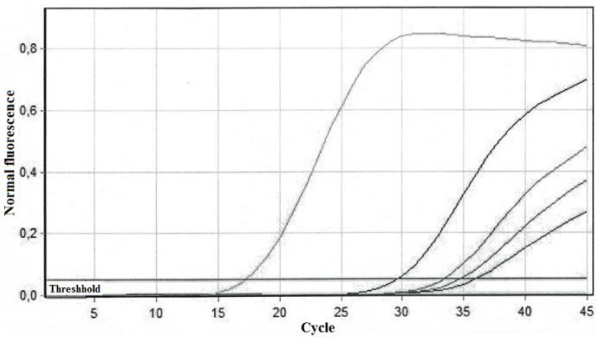

Figure 2: Graph of the PCR test, group 1, data before administering the medication.

This figure shows the initial viral load levels in the experimental group before Triazavirin administration. The exponential curves are similar to those in the control group, indicating comparable viral loads at the start of the experiment.

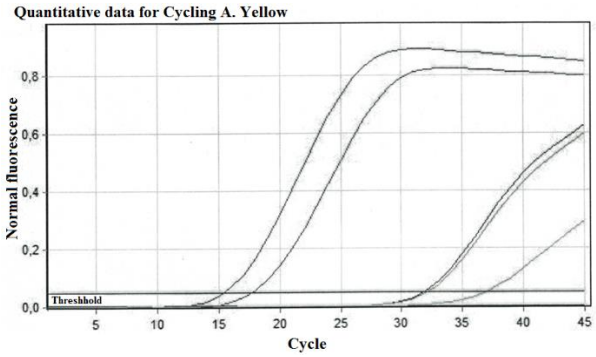

Figure 3: Graph of the PCR test, group 1, data after administering the medication.

Figures 2 and 3 demonstrate a significant change in the normal fluorescence levels among the test subjects of group 1, compared to the control group. The exponential curves are lower compared to the pre-treatment and control group, indicating a reduction in viral RNA levels after the administration of Triazavirin.

Discussion

Modern biotechnology offers a diagnostic method of genetic engineering, PCR. This method is the gold standard, as it allows for the detection of single pathogens in a sample for animal diseases. With this method, genetic material (DNA and RNA) is detected. Several studies reinforce the utility of PCR in veterinary medicine. For instance, research [21, 22] demonstrated that PCR could distinguish between closely related viral strains affecting cattle, thereby enabling more targeted treatment approaches.

The essence of the method is reduced to the cyclic multiple doubling of the DNA or RNA site of the pathogen and the accumulation of more genetic material for its detection. For RNA-containing viruses, reverse transcription PCR (RT-PCR) is used, where a DNA matrix with which PCR is already taking place is synthesized on the RNA matrix. The thermocycler performs 30-40 cycles of doubling the DNA section, as a result of which 10-12 copies of DNA are formed from 1 sample. The cycle includes several stages: 1: denaturation, 2: annealing, and 3: elongation. Next, the number of DNA copies is determined using fluorescence hybridization detection in real time. This allows not only to detect DNA or RNA in a sample (qualitative analysis) but also to measure the number of its copies and calculate the amount of the original matrix (quantitative analysis).

Viral, fungal pathogens, mycoplasma, chlamydia, and other bacteria present in biological material are studied. For example, research by Pansri et al., [23] employed qPCR to differentiate between bacterial and

viral causes of pneumonia in cattle, facilitating appropriate and timely treatment interventions.

The method has proven to be 100% specific, fast, and highly sensitive (detecting single pathogens). A comparative study [24] highlighted PCR's superiority over traditional culture techniques, which are slower and less sensitive, particularly for pathogens that are difficult to culture. A fragment of the DNA of the pathogen of the infection under study is sufficient for analysis, and the amount of the studied material can be several tens of microliters. This method makes it possible to identify the qualitative and quantitative presence of a pathogenic agent in the body, which allows not only for diagnosing the disease but also for monitoring the treatment of animals with various infectious diseases. In veterinary medicine, test systems are actively used to diagnose various diseases in pigs, cattle, cats, dogs, and birds. According to the results of our study, the use of antiviral agents, in particular Triazavirin, gives a positive result and leads to a decrease in the amount of the virus by 35.2%.

Triazavirin shows high efficacy (35.2%) when administered for 7 days in the indicated doses. The PCR test is the most effective means for detecting the virus while monitoring the treatment of a viral infection.

Author Contributions

Tatiana Reshetnikova conceptualized the study and supervised methodology, was responsible for writing the original draft. Vladimir Kuzmin was responsible for data curation, investigation and reviewing and editing of the paper. Tatyana Krylova aided with the formal analysis for this paper and visualization, also supervised the overall research.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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