REPUBLIC OF AZERBAIJAN

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ABSTRACT

of the dissertation for the degree of Doctor of Philosophy

STUDY OF DISEASES OF ROTAVIRUS AND CORONAVIRUS ORIGIN IN CALVES IN THE NORTH-WESTERN REGION OF AZERBAIJAN AND DEVELOPMENT OF MEASURES FOR ERADICATION THEM

Specialty: 3109.01 – "Veterinary microbiology, virology, epizootology, mycotoxicology and mycology and immunology"

Field of Science: Biology

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INTRODUCTION

Relevance of the topic. The Republic of Azerbaijan is one of the most developed countries in the South Caucasus region, with significant economic potential, located on the Silk Road, and serving as a key trade and transport corridor between Europe and Asia. Its important geopolitical position enhances its significance.

The continuous expansion of the geography and volume of trade in live animals, birds, livestock products, and raw materials with foreign countries; increasing human migration; global warming; and the migration of millions of wild birds to Azerbaijan for wintering create favorable conditions for the rise of previously sporadic or exotic "imported" infectious and transboundary diseases in the country.

Some particularly dangerous diseases can cause significant economic damage, leading to a decrease in domestic livestock production, price imbalance, loss of domestic and foreign trade markets, and negatively affecting the social and economic status of regions or entire countries.

Reforms in the agrarian sector, especially in livestock and poultry farming, implemented in Azerbaijan in the early 1990s, caused fundamental changes in the epizootic situation of several infectious diseases. *This necessitated a thorough revision of the strategies and approaches to the treatment, prevention, and control of various infectious diseases in agricultural animals by state veterinary institutions*[1].

Alongside other areas of the agricultural production complex, one of the most important aspects of modern animal husbandry is the improvement of the breed composition of cattle. Calves obtained from pedigree animals, compared to

[1] 4 mart 2005-ci il tarixli 846-IIQD nömrəli Azərbaycan Respublikasının Qanunu (Azərbaycan Respublikasının Qanunvericilik Toplusu, 2005-ci il, № 4, maddə 271)

local breeds, grow faster, have higher daily weight gain, and produce more milk. Thus, the future development of this field largely depends on obtaining and preserving a healthy breeding generation and implementing robust epizootic measures against infectious diseases in farms.

Recently, certain achievements have been made in studying infectious diseases of bacterial and viral origin in calves. However, developing effective control systems and methods for several diseases is becoming increasingly important. Calf enteritis caused by rotavirus and coronavirus is among such diseases, frequently recorded in the country's livestock farms and leading to decreased breeding quality and mortality in newborn calves. *Combating such diseases presents challenges even in large farms with advanced management practices, as their etiology, clinical signs, epizootic characteristics, pathological anatomical changes, pathogenesis, diagnostic methods, and control measures differ significantly from those described in classical literature*[2].

In addition to pathogenic microorganisms, external factors affecting *the natural resistance and immunological reactivity of the organism play a significant role in the etiology of mass diarrhea in calves.*[3]

In most cases, it is difficult to identify a single etiological agent in diseases with diarrhea symptoms in newborn calves. Viruses, bacteria, and protozoa may contribute to the onset of the disease.

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[2] Mee J. F. Newborn dairy calf management. Veterinary Clinics of North America: Food Animal Practice. 2008; 24(1):1–17

[3] Pratelli, A., Cirone, F., Capozza, P., Trotta, A., Corrente, M., Balestrieri, A., Buonavoglia C. Bovine respiratory disease in beef calves supported long transport stress: An epidemiological study and strategies for control and prevention / Res. Vet. Sci. 2021;135:450–455

Therefore, the study, treatment, and prevention of rotavirus- and coronavirus-induced calf enteritis remains highly relevant.

Research goal and objectives. The research aimed to study the epizootic situation of rotavirus and coronavirus-induced calf enteritis in the northwestern region of the Republic of Azerbaijan, differentiate the disease from other diarrheal infections, identify the impact of seasonal factors on disease spread, and improve treatment and prevention methods.

To achieve this goal, the following objectives were defined:

- Study and analyze the current status of rotavirus and coronavirus-induced calf enteritis in livestock farms of the North-West region over recent years;
- Investigate the seasonal dynamics of these enteritis cases in livestock farms of the region;
- Determine the influence of age factor on the infection rate of calves with rotavirus and coronavirus-induced enteritis;
- Evaluate the effectiveness of immunomodulators in the treatment and prevention of these diseases;

Research methods. The main part of the research was conducted at "Gilan Dairy Farms LLC" in the Gabala district, located in the northwestern region of Azerbaijan. Additional studies were carried out at the Scientific Research Institute of Veterinary Medicine in the laboratories of "Virology" and "Veterinary Clinic and Infectious Diseases of Animals" departments. Samples were also collected from small farms in Qakh, Zagatala, and Ismayilli districts.

During the research period, fecal samples were collected from calves in various seasons and tested using express test methods to determine the seasonality of infection.

For testing, the express test kits "BoviD4/5, D4 Diarrhea AG" were used. Hemagglutination and delayed hemagglutination tests were conducted on fecal samples.

Bacterial infections were cultured in nutrient media and E. coli pathogens were identified. Microscopy was used for diagnosing cryptosporidiosis.

Main findings presented for defense.

- Studies on rotavirus and coronavirus infections in calves showed that seasonal factors, including temperature fluctuations and stress, are key contributors to the increasing spread of these diseases.
- Studying the effectiveness of colostrum and identifying that colostrum with BRIX value below 20 is insufficient to prevent diarrheal infections in calves was significant.
- The use of the immunostimulant "Vitalife" proved essential in the prevention and treatment of common calf infections caused by rotavirus and coronavirus.

Scientific novelty of the research. For the first time in Azerbaijan, widespread rotavirus and coronavirus infections in calves were studied in detail, and their correlation with seasonal factors and spread dynamics was established. These viruses were found to spread more commonly in spring, autumn, and winter. It was determined that environmental temperature changes act as a stress factor, increasing the disease's spread among animals.

It was also clarified that using colostrum with a BRIX value below 20, as measured by refractometer, leads to infections in calves. Therefore, colostrum with low BRIX value is considered poor quality for feeding newborn calves.

Various combinations of therapeutic and preventive measures were tested in disease control. The immunostimulant "Vitalife" was used in the treatment of rotavirus and coronavirus infections and was found to be effective.

Theoretical and practical significance of the study: The conducted research, based on experiments and observations, has proven useful in implementing preventive measures through the study of the epizootic situation of rotavirus- and

coronavirus-induced calf enteritis in livestock farms of the North-Western region.

In order to prevent rotavirus- and coronavirus-induced calf enteritis, the sensitivity of the causative agents to pharmaceutical preparations was determined, and the causes of disease outbreaks were identified, contributing to the prevention of economic losses in farms.

A treatment and prevention scheme against the diseases has been developed, which in turn has been beneficial for veterinarians.

The economic efficiency of the drugs used against rotavirus- and coronavirus-induced calf enteritis in livestock farms in the North-Western region has been comparatively assessed, and a guideline for the application of the most effective drugs in farms has been prepared.

Approval and Application: A total of 9 publications related to the topic of the dissertation have been published, 6 of which are scientific articles. The materials of the dissertation have been presented at the following international scientific-practical conferences - "8th International Virology Congress" (Gdansk, Poland, 2023), "One Health: Problems and Solutions" 3rd International Conference (Baku, 2023), "Scientific Research and Experimental Development" Congress (United Kingdom, 2023).

Volume and Structure of the Dissertation: The dissertation consists of an introduction, 6 chapters, conclusions, practical recommendations, a bibliography, and appendices. Its total volume is comprised of computer-typed pages. The dissertation includes 18 tables and 9 figures. Character count: 190356.

CHAPTER I
LITERATURE REVIEW

The first section of the dissertation provides a general overview of studies dedicated to the main causes of mortality in newborn calves, characteristics of diarrhea, and the evaluation of viruses.

The second section analyzes the results of studies focused on the causes of the spread, clinical symptoms, and epidemiology of coronavirus in newborn calves.

The third section presents a general overview of rotaviruses, analyzes research findings, and defines the level of study of the targeted problem and the necessary objectives to address it.

CHAPTER II
MATERIALS AND METHODS OF THE RESEARCH

2.1.**Research Area and Climate Characteristics.** For this study, statistical data on cases of diarrhea among calves born from imported "Holstein Friesian" breed cows at "Gilan Dairy Farms LLC" in the Gabala district of the North-Western region of the Republic, for the months of 2017–2020, were used. The farm houses about 2000 head of cattle, with around 900 dairy cows.

The research was also conducted at the Scientific Research Institute of Veterinary Medicine, in the “Virology” and “Veterinary Clinic and Infectious Diseases of Animals” laboratories, as well as in local rural farms in the Ismayilli, Gabala, and Zagatala districts.

2.2. Collected Samples. During the study, fecal samples were collected from calves during different seasons and tested using express test methods, with infection seasonality being recorded.

The fecal samples were taken only within 24 hours of the appearance of the first clinical symptoms, from 4 to 30-day-old calves, by inducing a reflex via rectal stimulation. The

number of samples collected per year was in 2020, 328 samples, in 2019, 342 samples, in 2018, 358 samples, in 2017, 357 samples.

To confirm diagnoses, pathological dissections were performed on deceased animals, using clinical and pathological examination methods.

2.3. Test procedure. For testing, "BoviD4/5, D4" Diarrhea AG express test kits were used according to the manufacturer's instructions. Hemagglutination and hemagglutination inhibition tests were conducted on fecal samples. Bacterial infections were cultured in nutrient media, and E. coli pathogens were identified. *Microscopy was used for the diagnosis of cryptosporidiosis*[4].

CHAPTER III
CLINICAL SIGNS OF ROTAVIRUS AND CORONAVIRUS DISEASES IN CALVES BASED ON SEASONAL DYNAMICS

3.1. Seasonal dynamics of rotavirus and coronavirus-related diseases in calves. The improvement of livestock farming, a key part of the national agricultural development strategy, makes the study of animal diseases a priority.

Understanding rotavirus- and coronavirus-induced diseases accompanied by diarrhea in young calves plays an important role in controlling these infections.

This study aimed to investigate the seasonal dynamics and clinical symptoms of rotavirus and coronavirus diseases in

[4] Michael B, Franz-Ferdinand R, Beate C. 2021.Prevalence of Worldwide Neonatal Calf Diarrhoea Caused by Bovine Rotavirus in Combination with Bovine Koronavirus, Escherichia coli K99 and Cryptosporidium spp.: A Meta-Analysis, MDPI Animals, 11(4), p.1014

calves. The experiments were carried out on farms located in the north-western region of the country.

Research findings indicated that the clinical signs of rotavirus- and coronavirus-induced diarrhea in calves in farms located in the North-Western region of the country are relatively similar. However, rotavirus infections were more common, though the mortality rate was lower compared to coronavirus infections. Secondary pneumonia was identified as a major clinical sign in coronavirus-infected calves.

The study showed that the incidence and mortality trends for both diseases vary across different seasons of the year. As a result, outbreaks become widespread during spring, autumn, and winter, causing significant damage to livestock farms.

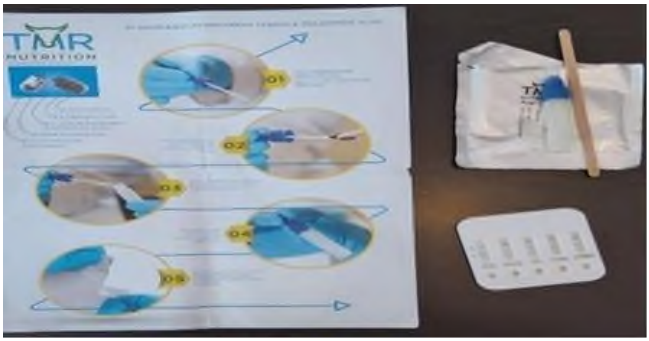

Figure 3.1. Determining the result of the express test for coronavirus and rotaviruses.

The Qabala district stands out for having a higher number of cold days throughout the year. As seen in Table 1, during the winter months, the highest temperatures range between 5–7°C, and in the summer months, the maximum reaches up to 29°C. Such climatic conditions are considered more favorable for enteroviruses.

Table 3.1. Temperature indicators recorded throughout the year in Qabala district.

Month	Yan	Fev	March	Apr	May	June	july	August	Sept	Oct	Nov	Dec
Max Tem	4.4	5.0	9.4	17.3	21.1	26.0	29.5	28.6	24.8	17.4	11.4	7.0
Min. Tem	-3.9	-2.8	1.0	6.7	11.2	15.4	18.2	17.4	13.9	8.5	3.0	-1.4
Sunny days	10.0	11.0	12.0	13.0	14.0	15.0	15.0	14.0	12.0	11.0	10.0	9.0

In Table 3.2, the monthly and seasonal indicators for calves that tested positive for rotavirus and coronavirus in 2020 are presented. The table also includes monthly mortality rates from diseases and a comparative analysis of deaths caused by rotavirus and coronavirus.

Table 3.2.

Indicators of calves positive for rotavirus and coronavirus in 2020 by month and season.

2020	1	2	3	3	5	6	7	8	9	10	11	12
Overall Positiv Reaction	35	33	31	45	18	5	3	2	23	38	46	49
Including coronavirus positive	20	22	18	25	11	4	2	1	14	22	25	27
Including rotavirus positive	15	11	13	20	7	1	1	1	9	16	21	22
Total Death	2	3	2	3	3	0	0	0	1	3	5	5
Death from rotavirus infections	0	1	1	1	1	0	0	0	0	1	2	1
Death from coronavirus infections	2	2	1	2	2	0	0	0	1	2	3	4

3.2. Clinical signs of rotavirus and coronavirus diseases in calves. For study the clinical symptoms and seasonal dynamics of rotavirus and coronavirus infections, 1,385 samples were tested between 2018 and 2021. These samples covered calves aged 0–90 days. *According to the studies by Qinghe Zhu et al., calf diarrhea is considered a multifactorial disease resulting from the combination of infectious and non-infectious complex risk factors. Influencing elements include enteropathogenic bacteria and viruses, environmental factors, the animal's immune status, genetic factors, nutrition, the course of labor, the structure of the calf's pen, maternal vaccination, and the calf's health condition.*[5] In addition to these factors, our research showed that diarrhea caused by rotavirus and coronavirus is among the most common types of calf diarrhea, and the frequency of these cases varies by season and year. According to our observations, diarrhea caused by rotavirus was most commonly recorded in calves aged 5 to 15 days. Literature describes watery, yellow diarrhea as a clinical sign of rotavirus infection in calves. Depression, shock, and dehydration are more commonly seen in calves younger than 5 days. Results from our analyses showed that depression, a decrease in suckling reflex, diarrhea, and dehydration were among the main clinical symptoms in infected calves.

Coronavirus-induced calf diarrhea was recorded in calves aged 2 to 21 days. It is generally noted that the epithelial cells of the small intestine and colon are susceptible to the virus. Additionally, coronavirus infection leads to watery diarrhea and may cause blood clots to appear in the feces. In our research, reduced appetite, fluid-electrolyte loss, dehydration, metabolic acidosis, and hypoglycemia were the most frequently observed clinical symptoms. Calves infected with coronavirus often developed pneumonia as a complication. Therefore, rotaviruses—especially during spring and

[5]Decaro N., Mari V., Desario C. Severe outbreak of bovine koronavirus infection in dairy cattle during the warmer season. Vet Microbiol. 2008;126 (1–3): 30–39.

winter months—were identified as the dominant viral pathogens causing diarrhea in newborn calves. The clinical symptoms of both rota- and coronaviruses were recorded from the first 10 days of the calves' lives, emphasizing the importance of controlling both viruses. During the research, samples were collected in various seasons and analyzed using express test kits. Based on the results, it was found that rotaviruses and coronaviruses cause diseases among calves belonging to “Gilan Dairy” Farms LLC, located in the northwest region of the country. While rotaviruses were associated with more infections compared to coronaviruses, the mortality rate was lower.
Rotaviruses and coronaviruses tend to cause mass infections among calves primarily in spring, autumn, and winter. Temperature changes in the environment can act as a stress factor, increasing the dynamics of disease spread among animals

CHAPTER IV

The Role of Colostrum Indicators in the Prevention of Coronavirus and Rotavirus-Associated Diarrhea in Calves

4.1. Determination of colostrum parameters in cows. The main objective of the study was to determine the correlation between colostrum quality indicators and the incidence of rotavirus and coronavirus infection, illness, and mortality in calves born to Holstein Friesian cows and heifers vaccinated with a combined (Bio Bos RRC) vaccine against rotavirus and coronavirus at "Gilan Dairy Farm" (GDF) LLC located in the Northwestern region of the Republic, considering the factors mentioned. It is hypothesized that feeding calves with colostrum with low BRIX values will result in IgG levels in the calf’s blood falling below 10 g/L, which would in turn be associated with increased incidence of disease and mortality in the calves.
In the second half of 2021, a trial group was created at GDF LLC consisting of a total of 50 expected calvings: 25 second or higher lactation Holstein Friesian cows and 25 first lactation heifers of the same breed. The quality of colostrum fed to the newborn calves was

analyzed, and cases of rotavirus and coronavirus infection, illness, and death were recorded.

Sample Collection. Immediately after the first milking, colostrum samples were collected from the cows within 30 minutes of calving into 50 ml polypropylene tubes and frozen at -20°C. Fecal samples were collected from all animals and stored at -20°C.

A portable optical refractometer (KERBL, Germany) was used to measure the dry matter content (BRIX). Additionally, fat, protein, lactose, and mineral (ash) values were analyzed using a Lactoscan milk analyzer. Newborn calves had their umbilical cords disinfected with betadine immediately after birth and were placed in individual pens after drying for 30 minutes in a clean straw area. Each calf was fed colostrum warmed to 38°C equivalent to 5% of its body weight within the first 2 hours and up to 10% within the next 6 hours. For quality analysis, 50 ml of the first colostrum milked within the first 2 hours was collected. Calves were monitored for 21 days post-birth, and those showing signs of diarrhea were tested using express test kits. For this purpose, the "Calf Test-5" express test kit from TMR Nutrition was used according to the instructions.

Among the vaccinated animals, 33 (66%) had high-quality colostrum, 9 (18%) had medium-quality colostrum, and 8 (16%) had low-quality colostrum. Of the high-quality colostrum, 25 were from cows with second or higher lactations, and 8 were from first-lactation heifers.

Out of the 50 calvings, 49 calves were born healthy, while one heifer delivered a stillborn calf. One cow gave birth to twins, resulting in a total of 50 calves from 50 cows. Among the 8 calves that received low-quality colostrum, diarrhea was observed in 5 (62.5%). Tables 4.1 and 4.2 show that all the affected calves were born to first-lactation heifers with low-quality colostrum. Among them, 2 (40%) were diagnosed with rotavirus and 1 with coronavirus. Both calves had severe disease. The calf diagnosed with coronavirus died despite treatment.

Table 4.1. Colostrum indicators of cows used in the experiment.

S/S	Identification number	Lactation number	Amount of colostrum obtained at first milking, liters	Colostrum QM% (BRIX)	Fatty %	Protein%	Minerals	Lactose percentage (%)	Ig conversion	Std. deviation
1	2	3	4	5	6	7	8	9	10	11
1	19101	1	6	24.4	6.5	14.1	1.2	2.6	50-80	0.08
2	1990	1	7	24	6.8	13.7	1.0	2.5	50-80	0.005
3	19103	1	9	22	5.7	13.8	1.1	1.4	50-80	0.004
4	1992	1	6	19	5.4	10.3	0.6	2.7	28-50	0.007
5	19111	1	8	24	6.3	14.5	1.5	2.5	50-80	0.005
6	19118	1	7	32	9	20.1	2	1.9	80<	0.006
7	1999	1	8	31.4	7	19.3	2.1	2	80<	0.005
8	1997	1	6	26	5.9	16.3	1.2	2.6	50-80	0.003
9	19119	1	5	19.6	5.1	11.1	0.6	2.7	28-50	0.006

Table 4.1-continue

1	2	3	4	5	6	7	8	9	10	11
10	19120	1	8	19	5	10.6	0.8	2.6	28-50	0.007
11	1980	1	7	31.9	6.6	21.8	1.1	2.4	80<	0.005
12	19121	1	8	32.8	6.7	22.7	1.2	2.2	80<	0.004
13	19122	1	6	31.8	6.5	21.9	1.1	2.3	80<	0.005
14	19104	1	8	31	6.7	20.8	1.2	2.4	80<	0.006
15	19105	1	7	19.2	5.2	10.5	0.8	2.7	28-50	0.004
16	19103	1	8	30	6.2	21.4	1.1	2.5	80<	0.004
17	1916	1	6	30.7	6.2	20.4	1.3	2.5	50-80	0.005
18	1912	1	7	31.1	6.7	20.8	1.2	2.4	80<	0.004
19	19127	1	6	19.3	5.5	10.1	1	2.7	20-50	0.006
20	1974	1	6	31.6	6.7	21.32	1.2	2.4	80<	0.005
21	1902	1	7	31.5	6.7	21.3	1	2.5	80<	0.006
22	1956	1	6	19.8	5.5	11.1	0.7	2.7	20-50	0.005
23	1908	1	8	32.3	6.8	22.9	1.3	2.3	80<	0.007
24	19125	1	6	31.5	6.6	21.3	1.2	2.4	80<	0.006

Table 4.1continue

1	2	3	4	5	6	7	8	9	10	11
25	1901	1	7	30.8	6.6	20.5	1.2	2.5	80<	0.005
26	18129	2	7	31.6	6.6	21.5	1	2.5	80<	0.005
27	17130	3	6	31.2	6.8	21.8	1.1	2.3	80<	0.006
28	18100	2	7	32.4	6.7	22.2	1.2	2.3	80<	0.007
29	18101	2	5	19.7	6.0	10.1	1.0	2.6	20-50	0.005
30	1887	2	7	30.7	6.6	20.7	1.2	2.5	80<	0.006
31	18115	2	6	32.1	6.8	22	1.1	2.2	80<	0.004
32	18124	2	8	31.2	6.1	21.6	1.0	2.4	80<	0.005
33	18120	2	6	27.8	6	17.6	0.9	2.6	50-80	0.004
34	18109	2	7	31.8	6.7	21.1	1.3	2.5	80<	0.006
35	18141	2	6	26.8	6.2	16.3	1	2.7	20-50	0.006
36	1809	2	6	30.7	6.6	21.2	1	2.5	80<	0.005
37	18112	2	7	30.9	6.6	20.7	1.2	2.4	80<	0.004
38	1854	2	5	27	5.6	17.4	0.9	2.7	50-80	0.005
39	18002	2	6	30.2	6.7	20	1.1	2.4	80<	0.006

Table 4.1continue

1	2	3	4	5	6	7	8	9	10	11
40	18120	2	7	32	6.8	21.8	1.1	2.3	80<	0.005
41	1865	2	6	23.9	6.3	14.1	0.9	2.6	50-80	0.004
42	18113	2	7	32.7	6.7	22.4	1.3	2.3	80<	0.007
43	18121	2	8	31	6.6	21	1.1	2.4	80<	0.005
44	1839	2	7	30.5	6.7	20.2	1.1	2.5	80<	0.008
45	17100	3	6	31.5	6.7	21.1	1.1	2.4	80<	0.007
46	17109	3	7	32.2	6.6	22.1	1.2	2.3	80<	0.005
47	17114	3	5	19.3	6.2	9.7	0.7	2.7	20-50	0.004
48	17001	3	7	31.6	6.7	21.3	1.2	2.4	80<	0.004
49	1788	3	6	30.7	6.6	19.9	1	2.5	80<	0.005
50	17127	3	8	31.7	6.7	21.5	1.2	2.3	80<	0.006

According to other sources, a Brix refractometer can be applied to detect the positive outcome of quality colostrum in calves. Therefore, the discussion of results refers to studies focused on the use of refractometry in calves.
The IgG concentration in colostrum has traditionally been considered a reliable indicator for evaluating colostrum quality before feeding calves. The IgG concentration in maternal colostrum significantly influences the acquisition of passive immunity; therefore, accurate measurement is crucial for proper colostrum management on the farm. In the present study, the average concentration of IgG in colostrum samples exceeded <80, which is considered a very good value.

Thus, the study determined that despite vaccination of Holstein Friesian cattle against rotavirus and coronavirus, low-quality colostrum was mainly observed in first-lactation cows, and calves fed with such colostrum were found to be susceptible to both diseases. The use of refractometers to analyze and evaluate colostrum quality proved to be practically significant. Based on the obtained measurements, it is possible to develop effective colostrum feeding protocols, which are crucial for livestock farming.

The experiment showed that when colostrum with a BRIX value below 20 (measured by refractometer) was used in feeding calves, diarrhea was observed in 62.4% of them. Of these, 40% were infected with rotavirus, 20% with coronavirus, and the remaining 40% with pathogens of various origins. For this reason, it is not recommended to use low-quality colostrum in feeding newborn calves.

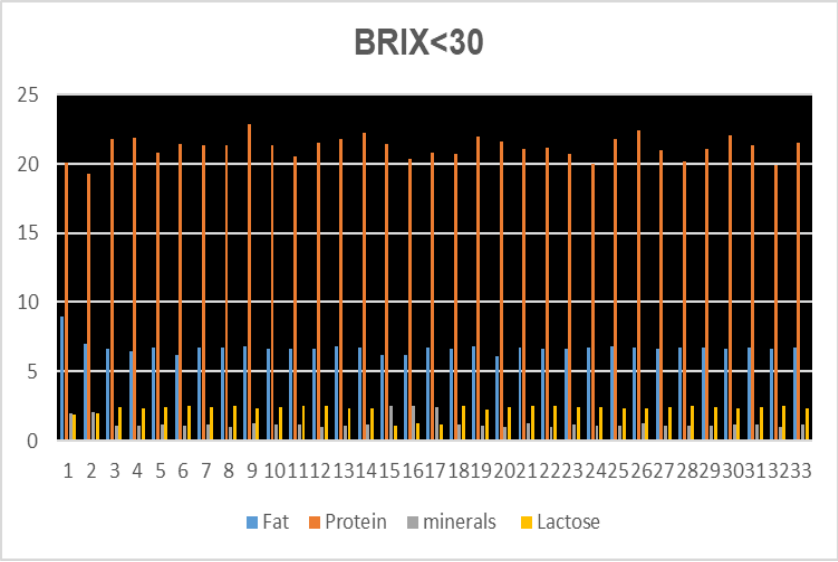

Figure 4.1. BRIX <30. Quality Indicator

CHAPTER V

PREVALENCE BY AGE GROUP AND DIFFERENTIAL DIAGNOSIS OF CALF DISEASES OF ROTAVIRUS AND CORONAVIRUS ORIGIN AMONG CALVES OF SMALL AND MEDIUM FARMERS IN THE NORTHWESTERN REGION (ISMAYILLI, GABALA, ZAGATALA DISTRICTS)

5.1. Prevalence and differential diagnosis of rotavirus and coronavirus-related calf diseases by age group. The aim of this study was to identify the prevalence of rotavirus and coronavirus-induced diarrhea in neonatal calves located in the northwestern region of the Republic using a rapid test method. The study was conducted on 45 local and crossbreed calves in 20 farms from the Ismayilli, Gabala, and Zagatala districts.

During the study, fecal samples were taken from calves showing clinical signs of diarrhea in 5 farms from Talystan village of Ismayilli

district, 5 farms from Nohur Qishlaq and Vandan villages of Gabala district, and 10 farms from Yeni Suvagil, Kurdemir, Aliabad, and Muxakh villages of Zagatala district, with 1–2 calves sampled per farm. The study was conducted between July and October 2023 and involved 45 calves from 20 farms. Of these calves, 28 were local breed, 11 were Simmental + local crossbreeds, 3 were Angus + local crossbreeds, and 3 were Swiss + local crossbreeds. Farms were selected to have 10–50 milking cows.

As clinical indicators, factors such as the feces' consistency, color, frequency of defecation, and organoleptic characteristics were recorded.

Rotaviruses were observed in 4 calves aged 3–10 days, 12 calves aged 11–20 days, 7 calves aged 21–28 days. Coronaviruses were observed in 0 calves aged 3–10 days, 3 calves aged 11–20 days, 0 calves aged 21–28 days.

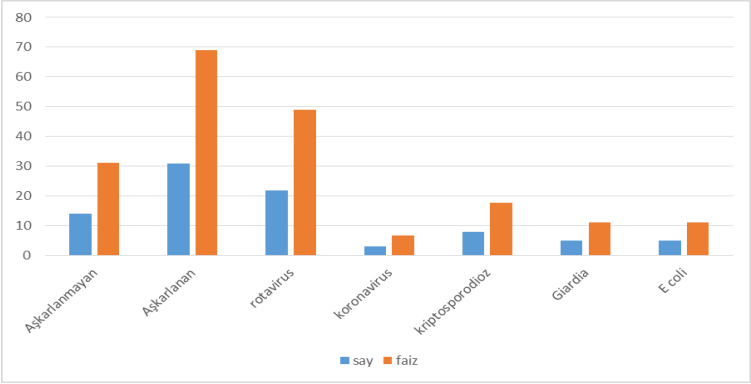

Figure 5.1. Diarrhea cases caused by different types of pathogens or non-pathogenic causes.

As seen in Figure 5.1, in 68.9% of diarrhea cases, a specific pathogen was identified. Among them, rotaviruses accounted for 48.9% and coronaviruses for 6.7%. The remaining 31.1% of diarrhea cases were considered non-pathogenic or caused by agents not included in the rapid test.

Table 5.1. Detection rates of pathogens in fecal samples and their percentage representation

Creator	Nümunənin sayı	%
Undetectable	14	31.1
Detected single and mixed	31	68.9
Including		
Rotavirus	22	48.9
coronavirus	3	6.7
Giardia	8	17.8
E coli	5	11.1

As shown in Table 5.1, no pathogens were detected in 14 (35.5%) of the 45 calves tested via rapid method, while a single or multiple pathogens were identified in 31 calves. Among them, Rotavirus was detected in 22 calves (48.9%), Coronavirus in 3 calves (6.7%), Cryptosporidium parvum in 8 calves (17.8%), Giardia in 5 calves (11.1%), E. coli K99 in 5 calves (11.1%). As presented in Table 2, rotavirus was detected in 15 calves as a single pathogen, in 1 calf together with E. coli, in 1 calf together with coronavirus, in 1 calf with Cryptosporidium, in 2 calves with Giardia, in 1 calf with both Cryptosporidium and coronavirus, in 1 calf with Cryptosporidium and Giardia. Coronaviruses were detected in 1 calf together with E. coli, in 1 calf with rotavirus, in 1 calf with Cryptosporidium and rotavirus. Rotaviruses were observed in 4 calves aged 3–10 days, 12 calves aged 11–20 days, 7 calves aged 21–28 days. Coronaviruses were observed in 0 calves aged 3–10 days, 3 calves aged 11–20 days, 0 calves aged 21–28 days. According to Table 5.2. in 125

blood samples HI titer was 1:64 in 125 samples, 1:32 in 95 samples, and below 1:16 in 125 samples.
olaraq.

Table 5.2. Distribution of titers in 125 blood samples.

Törədici	Say 45	%	3-10 günlük buzovlar	11-20 günlük buzovlar	21-28 günlük buzovlar
Negativ	14	31.11	5	6	3
pozitiv	31	68.9	6	15	10
Rotavirus	15	33.3	4	6	5
E coli	2	4.4	2		
E coli+rotavirus	1	2.2	-	1	-
E coli + Coronavirus	1	2.2	-	1	-
Kriptosporodioz	4	8.9	-	2	2
Giardia	1	2.2	-	-	1
Rotavirus+ coronavirus	1	2.2	-	1	-
Rotavirus+ kriptosporodioz	1	2.2	-	1	-
Rotovirus +Giardia	2	4.4	-	1	1
Kriptosporodioz+ rotovirus+ coronavirus	1	2.2	-	1	-
Kriptosporodioz, rotovirus, Giardia	1	2.2	-	-	1
Kriptosporodioz, E. coli+ Giardia	1	2.2	-	1	-

Tests showed that 64% of samples were positive and 36% negative. Based on these indicators, differential diagnosis has been determined to be a critical factor in the correct treatment and prevention of diarrhea caused by rotavirus, coronavirus, and *E. coli*.

By examining fecal samples using rapid test kits and diagnosing the disease in a timely manner, support is provided for future treatment and preventive measures, which not only positively affects the development of calves in farms but also prevents calf mortality and increases the economic profitability of farmers. The research shows that BRV and BCV are among the main causes of diarrhea in calves in the North-West region of the Republic (Ismayilli, Gabala, Zagatala), and both diseases, whether occurring individually or as mixed infections, result in economic losses and fatalities for farmers. During the study, the prevalence of rotaviruses in the North-West region of the Republic was found to be 48.9% (ranging from 0–53%). The prevalence of coronaviruses ranges from 6.7% (compared to 13–18% in Turkey). Infectious diseases accompanying rotavirus infections further aggravate the disease and increase losses in calves, and should be separately studied and closely monitored. In 14 out of 45 calves (35.5%), no pathogens were detected, whereas in 31 calves, either single or mixed pathogens were identified. Rotavirus was found among 22 calves (48.9%), Coronavirus was found among 3 calves (6.7%), Cryptosporidium parvum was found among 8 calves (17.8%), Giardia was found among 5 calves (11.1%). E. coli K99 was found among 5 calves (11.1%).

CHAPTER VI
MEASURES AGAINST ROTAVIRUS AND CORONAVIRUS DISEASES: TREATMENT AND PREVENTION

6.1. Treatment and preventive measures used to combat coronavirus and rotavirus. In previous years, the gastrointestinal diseases of newborn calves and mother cows were clearly linked to

the sanitary-hygienic conditions of housing and feeding. This led to the classification of the entire digestive pathology as non-infectious diseases. However, the characteristics of disease progression and its manifestations in well-fed farms suggest that infectious factors play a significant role in the etiology of these diseases.

In Azerbaijan, the role of viral and bacterial pathogens in the etiology of enteritis in newborn calves has not been fully studied. Therefore, differential diagnosis was necessary in the study.

To compensate for fluid loss during diarrhea, fluid-electrolyte therapy using isotonic solutions and serums is employed.

Glucose (i.v.) is appropriate in cases of hypothermia.

The volume of isotonic solution to be given is 1000 ml per 10 kg of body weight.

In cases of bacteremia and septicemia, antibiotics must be administered parenterally.

Immunoglobulin Therapy : In recent years, in addition to parenteral immunoglobulin therapy, the use of vitamin + IG + trace element preparations before colostrum feeding has become widespread.

Vitamins: To regulate vitamin balance in the body, vitamin complexes of groups A, D, E, and B are used.

Prevention: The basis of prevention consists of two essential aspects:

1) Ensuring that newborn calves receive sufficient high-quality colostrum immediately after birth (to provide passive immunity).

2) Maintaining cleanliness of the area where calves are kept during the neonatal period:

Hyperimmune Serum: To strengthen the immune system of calves, hyperimmune serum should be administered within 2 hours after birth. Indications for this include:

- Calves born from heifers;
- Calves born to cows purchased pregnant (6 months or more);
- Calves born from difficult births;
- Calves not receiving colostrum or accepting it poorly;
- Poor-quality colostrum;

- Widespread diarrhea and septicemia in the farm.

In farms with widespread disease, probiotics and immunomodulators are extensively used for preventive purposes.

Substance Name and Function

Lactoferrin – Antibacterial (Bacteriostatic) As shown in Table 6.1, oligosaccharide - prevents bacteria from adhering to the intestines. Interferon antiviral protection Trypsin inhibitor Prevents the breakdown of immunoglobulin in the intestine. IGF-1 promotes rapid development of the entire intestine.

Table 6.1 – Important Substances in Colostrum and Their Functions

Substance	Function
Lactoferrin	Antibacterial (Bacteriostatic)
Oligosaccharide	Prevents bacteria from attaching to intestinal walls
Interferon	Antiviral protection
Trypsin inhibitor	Prevents breakdown of immunoglobulin in the intestine
IGF-1	Promotes rapid development of the entire intestine

In colostrum, maternal antibodies are large protein molecules that are absorbed intact through the intestinal wall via pinocytosis within the first 24 hours after birth. After this 24-hour window, absorption ceases entirely. The amount of IgG in colostrum should be 50 g/L. Maternal antibodies remain in the calf's body for 8 weeks, providing protection against infections, although they are not detectable in the blood. By 4–5 weeks, immunoglobulin levels start to decrease, making calves more susceptible to infections. If colostrum intake is insufficient, the number of detectable antigens in the blood is lower, and calves get infected faster and more severely. However, with good colostrum intake, calves can be resistant to infections up to 6 months of age.

6.2. In the prevention of calf coronovirus disease administration of used immunostimulants.

Since antibiotics are not effective in the fight against viral diseases, the use of various immunostimulants is an important approach. Immunostimulants are substances (drugs and nutrients) that stimulate the immune system either by triggering its activation or by enhancing the activity of specific components of the immune system. A typical example of this is the granulocyte-macrophage colony-stimulating factor.

For this purpose, at "Gilan Dairy Farms" LLC, a substance called "Vitalife," which combines electrolytes and immunostimulants, was used in a group of newborn "Holstein-Friesian" calves that tested positive for coronavirus using an express test method (10 calves in each group). The study was conducted among calves born during the first six months of 2019.

"Vitalife" – Contains easily absorbable energy sources, electrolytes, and vitamins and minerals, along with the following complex immunostimulants.

From Table 6.2, it can be seen that in the group treated with "Vitalife," no deaths (0%) occurred among the 10 calves. 5 calves (50%) experienced a mild form of the disease, 3 (30%) moderate, and 2 (20%) severe. In the group treated with simple electrolytes, there was 1 death (10%), 4 calves (40%) had a severe form, 3 (30%) moderate, and 3 (30%) mild. The results showed that in cases of diarrhea caused by coronavirus and rotavirus, the use of preparations that include both electrolytes and biostimulants during the initial phase of the disease leads to a higher recovery rate and fewer complications in affected calves (Table 6.2). Therefore, using preparations containing biostimulants is recommended for treating calves infected with coronavirus. Acute gastrointestinal disorders in newborn calves may result from metabolic disturbances in the mother during pregnancy, poor nutrition, mastitis, improper calf-rearing practices, and most importantly, failure to feed colostrum in a timely manner.

Table 6.2: Clinical condition of calves during illness and disease outcomes.

N	Calf tag number	Dehydration rate %	Acidosis level 1-4	Skin elasticity test 0-15 in seconds	Clinical signs	Disease patogenesis	result
1	2	3	4	5	6	7	8
1	1921	5	2	7	There is a sucking reflex, the animal reacts to the environment the body temperature is 40C^{o}	light	recovered
2	1938	6	2	6	Has a weak sucking reflex, reacts poorly to the environment tem 39.8 C^{o}	middle	recovered
3	1996	5	1	5	The sucking reflex is moderate, the reaction to the environment is weak.Tem 39.9C^{o}	light	recovered
4	19115	7	2	6	Low sucking response, poor response to surroundings tem-40C^{o}	hard	recovered

Table 6.2-continue

1	2	3	4	5	6	7	8
5	19126	6	2	6	Good sucking response, responds to surroundings, tem 39.2	light	recovered
6	19670	4	1	5	Good sucking response, responds to surroundings,tem 39.8 C^o	light	recovered
7	19580	6	2	6	Weak sucking response, little response to surroundings,tem 39.9 C^o	middle	recovered
8	19130	8	2	8	Weak sucking response, little response to surroundings,tem 40 C^o	ağır	recovered
9	19210	6	1	7	Good sucking response, responds to surroundings,tem 39.5 C^o	middle	recovered
10	19520	5	1	5	Good sucking response, responds to surroundings,tem 39.1 C^o	light	recovered

Therefore, using preparations containing biostimulants is recommended for treating calves infected with coronavirus. Acute gastrointestinal disorders in newborn calves may result from metabolic disturbances in the mother during pregnancy, poor nutrition, mastitis, improper calf-rearing practices, and most importantly, failure to feed colostrum in a timely manner.

Preventing gastrointestinal diseases in newborn calves mainly relies on improving the feeding and housing technologies of pregnant cows, using therapeutic and preventive premixes, vaccinating pregnant cows with appropriate vaccines. Thus, a comprehensive set of measures is needed, including general preventive measures aimed at improving living conditions and preventing the accumulation and spread of pathogens, special preventive measures, and immunoprophylaxis of cows to prevent gastroenteritis in newborn calves. These gastrointestinal diseases in newborn calves are mostly observed on farms where there is a lack of attention to the care and feeding of cows, and where veterinary-sanitary rules are not followed during calving and newborn care. The normal physiological state of the mammary glands, especially the absence of mastitis, is crucial in preventing these diseases. Cows should be kept in production for at least 45 days; clinical examination and treatment of sick cows in the late pregnancy period should be conducted, along with pre-birth sanitation of animals and calving in pre-disinfected barns. Keeping newborn calves with cows for at least 3–4 days completely prevents gastrointestinal diseases. The first feeding of calves should take place within 2 hours of birth, followed by feeding at least 4 times a day. Calves older than 5 days should be moved to dispensaries, with each room containing 6–7 calves of no more than 2–3 days age difference.

Gastrointestinal bacterial infections cause significant economic losses to livestock farms and highlight the need for ongoing improvement and discovery of new, effective treatments and preventive methods. While the oral or parenteral use of antibiotics, sulfonamide and nitrofuran drugs, astringents, rehydration solutions, blood substitutes, natural gastric juice, salt mixture solutions, blood products, serums, colostrum immune-specific globulins, protein hydrolysates, plant-based decoctions and infusions, and probiotics may be helpful, they are not

always effective—especially in farms where diagnostic investigations are insufficient. At the same time, the specific cause plays a key role in multifactorial infectious pathology. In practice, the main condition for preventing any case, including epizootics, is to determine its specific cause. Furthermore, the uncontrolled and long-term use of antibiotics leads to the development of resistant bacterial strains, which worsen calf diseases. Therefore, studies have been conducted to develop a highly effective preparation for the treatment and prevention of acute gastrointestinal diseases in newborn calves—to improve efficiency and reduce labor and medication costs.

RESULTS

1. Coronaviruses and rotaviruses cause disease among calves belonging to "Gilan Dairy" Farms LLC located in the north-west of the republic, and tend to become widespread in the farms especially during spring, autumn, and winter seasons. Changes in environmental temperature act as stress factors, increasing the dynamics of disease spread among animals [1,7].
2. In the North-Western region of the republic (Ismayilli, Gabala, Zagatala), the primary factors causing diarrhea in calves are both diseases, occurring either as singular or mixed infections, leading to economic losses and fatalities for farmers. The prevalence of rotaviruses is 48.9% (ranging from 0–53%), while the prevalence of coronaviruses varies around 6.7% [2, 3].
3. Among 45 calves, no pathogens were detected in 14 (35.5%) calves, whereas in 31 calves, pathogens were detected either individually or as mixed infections. Specifically, rotavirus was detected in 22 calves (48.9%), coronavirus in 3 calves (6.7%), Cryptosporidium parvum in 8 calves (17.8%), Giardia in 5 calves (11.1%), and E. coli K99 in 5 calves (11.1%) [4,6].
4. When measured with a refractometer, colostrum with a BRIX value below 20 used in calf feeding resulted in 62.4% incidence of diarrhea in neonates. Among these, 40% were infected with rotavirus, 20% with coronavirus, and the remaining 40% with various other pathogens [5,8].
5. In the calf group treated with "Vitafife," there were no deaths (0%) out of 10 calves. 5 calves (50%) experienced mild illness, 3 calves (30%) moderate illness, and 2 calves (20%) severe

illness. In the group treated with a basic electrolyte, 1 calf (10%) died, while 4 calves (40%) had severe illness, 3 calves (30%) moderate illness, and 3 calves (30%) mild illness [9].

6. In cases of calf diarrhea caused by coronavirus, the early use of medications containing both electrolytes and biostimulants leads to a higher recovery rate, with a reduction in complications in sick calves [2].
7. As a result of the measures taken at “Gilan Dairy” LLC, 98.5% of newborn calves survived [8].

PRACTICAL RECOMMENDATIONS

1. Alongside rotavirus, secondary infectious diseases that exacerbate the illness and increase losses in calves should be studied separately and closely monitored.
2. It is not recommended to use low-quality colostrum in feeding newborn calves.
3. In the 7th month of pregnancy, cows should be transferred to the dry (non-lactating) section, supplied with vitamins and minerals, with a particular emphasis on vitamin E and selenium injections.
4. In barns with disease issues, newborn calves should be immediately separated from the mother, dried manually, nasal mucus should be cleared, and umbilical cord should be disinfected.
5. It is not advisable to transport newborn calves outside the barn.

LIST OF SCIENTIFIC WORKS PUBLISHED RELATED TO THE DISSERTATION TOPIC

1. Abbasov V, Zeynalova Sh. Clinical Signs of Seasonal Diseases Dynamics in Calves Caused by Rotavirus and Coronavirus Infection//Annual Research & Review in Biology, 2023, Volume 62, Issue 2, p.1-11.

Infection//Annual Research & Review in Biology, 2023, Volume 62, Issue 2, p.1-11.

2. Abbasov V., Zeynalova Sh. Infectious rotavirus and calf coronavirus// Bulletin of science and practice, 2023, No. 4, p.167-171.

3. Zeynalova Sh., Abbasov V. Clinical signs of seasonal diseases dynamics in calves caused by coronavirus and rotaviral infection// 8th European Congress of Virology. -Gdańsk (Poland), 2023, p.489.

4. Abbasov V., Zeynalova Sh. Spread of diseases rotavirus and coronavirus among calves in the north region of Azerbaijan/ 3rd International Azerbaijan Unified Health Conference, Khazar University. Baku, 2023, c.

5. Abbasov V., Zeynalova Sh. The role of colostural indicators in prevention of coronavirus and rotaviral diarrehea in calves. // One Health Journal /Veterinary medicine, 2023, v.1., №3, .p.13-18.

6. Abbasov V., Zeynalova Sh. The main cause of gastrointestinal infectous calves and their treatment//Scientific Research and Experimental Development-conference, 2023, p.7-8.

7. Abbasov V. Bioecological characteristics of Rotavirus and Coronavirus diseases in calves/ Nakhchivan State University. "Scientific works". Series of natural and medical sciences, 2023, No. 3(124), pp.144-147.

8. Abbasov V. Coronavirus of large cattle: epizootic status, genome structure, clinical symptoms and prevention-mini review/ Journal of young researcher, 2023, v. IX, № 3, -p.77-82

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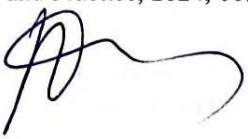

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