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EPIDEMIOLOGICAL MONITORING THE PESTE DES PETITS RUMINANTS IN THE REPUBLIC OF KAZAKHSTAN

Sarsenbay K. Abdrakhmanov, Yersyn Y. Mukhanbetkaliyev, Aizada A. Mukhanbetkaliyeva, Kanatzhan K. Beisembayev, Aslan A. Bayniyazov, Serik Narbayev, Kairat K. Yessenbayev

Faculty of Veterinary Medicine and Animal Husbandry Technology,
S. Seifullin Kazakh Agrotechnical Research University,
Astana, Republic of Kazakhstan

Corresponding author: Sarsenbay K. Abdrakhmanov, e-mail: s_abdrakhmanov@mail.ru
Co-authors: Yersyn Y. Mukhanbetkaliyev, e-mail: ersyn_1974@mail.ru
Aizada A. Mukhanbetkaliyeva, e-mail: aizada.1970@mail.ru
Kanatzhan K. Beisembayev, e-mail: kanatzhan.b@mail.ru
Aslan A. Bayniyazov, e-mail: aslan_b1973@mail.ru
Serik Narbayev, e-mail: narbaev_serik@mail.ru
Kairat K. Yessenbayev, e-mail: ykk0882@gmail.com

Abstract

Peste des petits ruminants (PPR) is a highly contagious viral disease of sheep and goats, as well as wild small ruminants, occurring mainly in acute or subacute clinical forms. PPR causes great economic damage to the small-scale sheep and goat farming and as the whole states. The article presents epidemiological monitoring and analysis of preventive measures carried out in Kazakhstan against PPR. The country's territory is conditionally divided into two safe zones (territories with and without vaccination). Specific PPR prevention is carried out in a safe zone with vaccination (buffer zone), which includes five regions and a city of republican significance (Almaty, Zhambyl, Zhetysu, Kyzylorda, Turkestan regions and Shymkent). In 2018-2022 from 3225570 to 6733974 sheep and goats were vaccinated in buffer zones annually. At the same time, vaccination coverage from the total number of susceptible animals was 15.2-36.8%.

Planned monitoring diagnostic studies have confirmed the epidemiological well-being of the country in terms of PPR. Over the past 5 years, 86,830 serologi-cal and 482 molecular genetic studies have been conducted, with negative results in all cases. Serological monitoring of «risk zones of possible infection» for the presence of antibodies to the PPR virus in susceptible pets also confirmed the absence of infection in the studied territories.

Key words: Epidemics situation; epidemiological monitoring; Kazakhstan; peste des petits ruminants; prevention; small ruminants.

Introduction

Peste des petits ruminants (PPR) – belongs to the group of cross-border infections, characterized by rapid spread, high contagiousness and mortality. The economic damage caused by this infection to goat and sheep farming is also enormous. In the most unfavorable cases, the incidence rate of PPR is 100%, and the mortality rate reaches 90%. The most susceptible to PPR is goats, among which mortality can reach 95%. In endemic areas, the mortality rate of the epidemic may be low, but there, too, the disease causes significant damage to herd productivity [1, 2].

Direct costs arise due to the death of animals, reduced productivity (dairy products, meat quality

and weight gain, the inability to remove wool and fluff), as well as the cost of quarantine measures. According to FAO estimates, the annual economic damage from this disease is more than $2 billion [3].

The Food and Agriculture Organization of the United Nations (FAO) and the World Organization for Animal Health (OIE) are mobilizing the international community as part of a new global initiative to eliminate PPR by 2030. The purpose of this FAO is to continuously improve the farming systems of small cattle by contributing to the eradication of this infection, strengthening food security and increasing the resilience of the population to external shocks of livelihoods in rural areas [4, 5].

In the official OIE data, 2018-2022, 54 countries of the world recognized as unfavorable and endemic by the PPR. During the specified period of time, 36 states were recognized as dysfunctional on the African continent, in Asia, PPR were registered in 16 countries and two more states (Bulgaria, Turkey) are located on the European continent. Out of 54 countries, China, Bhutan, Maldives, Kenya, Tunisia, Comoros and Uganda are considered endemic [6].

Currently, special attention is paid to China among the countries that are disadvantaged by the PPR, since this country has extensive common borders with Kazakhstan and bilateral trade and economic cooperation is very developed. The trade turnover between the countries, including the turnover of livestock products, is growing every year. Transport logistics is actively developing. All these factors increase the risk of infection entering the country from a neighboring state [7, 8]. In addition, a high risk of infection remains on the territory of the country, from countries such as Mongolia, Iran, India, Turkey, Afghanistan and Kyrgyzstan and Tajikistan, where the outbreak of the epidemic was previously recorded, which are unfavorable according to the PPR. The reason for this is the close geographical location and close trade and economic relations of Kazakhstan with these countries [9, 10].

In addition, the FAO reported that in recent years, the number of outbreaks of PPR detected on a global scale has decreased by two-thirds. This, reflecting the determination of the international community to defeat this highly contagious animal disease, gives hope that the goal of its elimination worldwide will be achieved by 2030.

The decrease in the foci of PPR is explained by the effectiveness of large-scale vaccination campaigns conducted in more than 50 countries. These measures were implemented with the support of FAO and its partners, funded by state secretaries, and in 2015-2018 alone, more than 300 million sheep and goats were vaccinated in 12 states [11, 12].

In many states bordering Kazakhstan (Kyrgyzstan, Turkmenistan, Iran, China) and countries with close trade and economic relations with us (Mongolia, Georgia, Turkey), mandatory vaccination of small cattle against the PPR is carried out. Such a measure is also carried out in Kazakhstan, in areas at risk of infection. The border zones with a high number and density of wild animals exposed to the plague of small cattle and small ruminants are the most dangerous for the penetration of the PPR [13].

The high degree of disadvantage of the countries bordering with Kazakhstan due to this epidemic, the PPR force to organize and carry out preventive measures to prevent penetration and spread on the territory of the country. Therefore, taking into account the peculiarities of animal husbandry in Kazakhstan and the need to combat this dangerous epidemic, it is very important to study and monitor the spread of the pathogen of the PPR, as well as to improve control measures [14, 15].

In this regard, the purpose of these studies was epidemiological monitoring and evaluation of the effectiveness of anti-plague measures of small ruminants carried out on the territory of the Republic of Kazakhstan.

Materials and Methods

The initial materials for the study were formed at the expense of their own data collected during visits to economic entities, as well as district and regional territorial inspections. In addition, reporting and review data of the Committee for Veterinary Control and Supervision of the Ministry of Agriculture of the Republic of Kazakhstan and statistical data of the Committee on Statistics of the Ministry of National Economy of the Republic of Kazakhstan were used as materials. When assessing the epidemic situation of small ruminant plague in the world and countries adjacent to the territory of Kazakhstan, official data of the World Animal Health Organization posted on the Rosselkhoznadzor website were used [16].

To conduct epidemiological studies on the

PPR and to analyze the epidemic situation, a comprehensive method of epidemiological studies was used. Monitoring studies aimed at identifying vectors of the plague virus in sick animals and small ruminants were conducted in a favorable vaccination zone. To do this, blood serum samples were taken from small cattle of different ages and sexes (from 2 to 6 months). In total, 1000 samples were taken from various farms of Almaty, Zhambyl regions and the city of Shymkent, including from one epidemiological unit to 30-50-100 samples. Studies, Id screen ® PRO Competition (ID.VET, France) was conducted by competitive enzyme immunoassay (ELISA) using a test system.

Results

Analysis of the epidemic situation of small ruminant plague in the world and trends in the spread of diseases in recent years in countries bordering the Republic of Kazakhstan indicates the presence of a high risk of small ruminant plague entering the territory of our country. Among them, the epidemic situation in Mongolia and China, as well as in Georgia, Turkey and Iran is of particular concern.

Based on the epidemic situation in these states regarding the PPR and the determination of the identified risk factors and possible routes of infection, given that our country is officially healthy for this infection, in accordance with the requirements of the World Organization for Animal Health, the territory of the Republic of Kazakhstan conditionally refers to 2 favorable zones, that is, vaccinated and unvaccinated territories (Fig. 1).

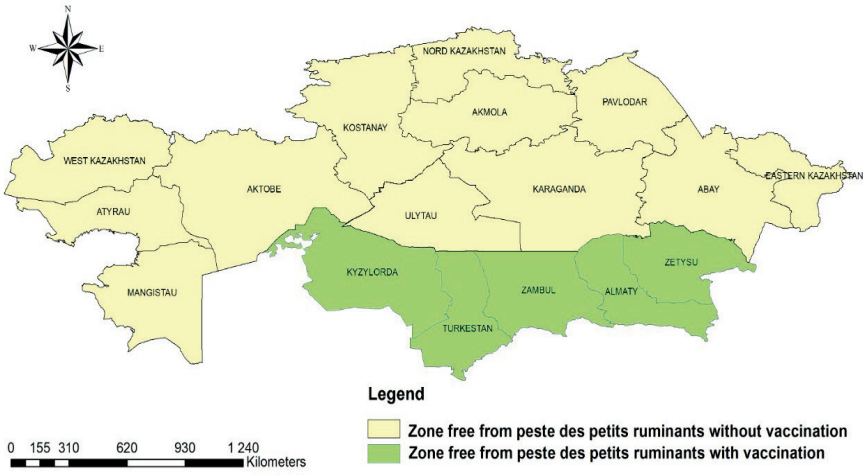

Figure 1 - Division of the territory of the Republic of Kazakhstan into vaccinated and non-vaccinated zones in relation to the PPR

The territory of the country, favorable without vaccination for the PPR, is the first zone and includes 12 regions of the republic: Abai, Akmola, Aktobe, Atyrau, East Kazakhstan, West Kazakhstan, Karaganda, Kostanay, Mangystau, Pavlodar, North Kazakhstan and Ulytau regions. The second favorable zone for the PPR (buffer zone), in which mandatory vaccination is carried out, includes the remaining 5 regions of the country and 1 city of republican significance (Almaty, Zhambyl, Zhetysu, Kyzylorda, Turkestan regions and the city of Shymkent). In accordance with the recommendations of the World Animal Health Organization, in the regions included in the favorable vaccination zone, susceptible animals are isolated from the rest of the country and neighboring countries with a different veterinary and sanitary status, in order to prevent the penetration of the PPR, taking into account geographical and physical barriers. Vaccination of susceptible animals in the buffer zone against the PPR is included in the list of mandatory voice measures and is funded by the state. Vaccination coverage of susceptible livestock, proper planning, organization and timely implementation of this event are the key to maintaining epidemiological well-being. In this regard, we evaluated the effectiveness of therapeutic measures in the country over the past 5 years (Table 1).

Table 1 - Vaccination of sheep and goats against PPR on the territory of the Republic of Kazakhstan (2018-2022)

Name of the region	2018			2019			2020			2021			2022		
	Number of sheep and goats, thousand heads	Number of vaccinated animals, thousand heads	Vaccination coverage, %	Number of sheep and goats, thousand heads	Number of vaccinated animals, thousand heads	Vaccination coverage, %	Number of sheep and goats, thousand heads	Number of vaccinated animals, thousand heads	Vaccination coverage, %	Number of sheep and goats, thousand heads	Number of vaccinated animals, thousand heads	Vaccination coverage, %	Number of sheep and goats, thousand heads	Number of vaccinated animals, thousand heads	Vaccination coverage, %
Abai	-	-	-	-	-	-	-	-	-	-	-	-	1 131,5	0	0
Akmola	511,2	0	0	522,2	0	0	530,2	0	0	539,4	0	0	573,4	0	0
Aktobe	1 074,5	0	0	1 109,4	0	0	1 127,1	0	0	1 153,4	0	0	1 312,0	0	0
Almaty	3 411,1	1450900	42,5	3 419,4	1 000,0	29,2	3 510,0	822,0	23,4	3 659,7	259,9	7,1	2 354,6	0	0
Atyrau	542,6	0	0	559,9	0	0	567,2	0	0	579,7	0	0	597,0	0	0
West Kazakh. region	1 155,6	0	0	1 147,9	0	0	1 130,6	0	0	1 188,8	0	0	1 306,6	0	0
Dzhambul	2 610,5	1163000	44,5	2 788,4	1 532,1	54,9	2 861,8	1 974,4	69,0	3 055,5	921,4	30,15	3 446,6	1 116,3	32,4
Zhetysu	-	-	-	-	-	-	-	-	-	-	-	-	1 701,5	0	0
Karaganda	933,1	0	0	930,8	0	0	924,5	0	0	950,9	0	0	738,5	0	0
Kostanay	436,6	0	0	454,4	0	0	463,6	0	0	471,5	0	0	465,7	0	0
Kyzylorda	586,7	240000	40,9	612839	151,5	24,7	620,9	151,7	24,4	698,6	151,2	21,6	731,6	224,3	30,7
Mangystau	373,2	0	0	387,3	0	0	384,4	0	0	419,9	0	0	311,0	0	0
Pavlodar	526,8	0	0	536,9	0	0	551,6	0	0	565,5	0	0	657,0	0	0
North Kazakh. region	386,6	0	0	404,2	0	0	419,3	0	0	433,4	0	0	463,2	0	0
Turkestan	4 112,0	3860074	93,9	4 088,2	2 371,0	58,0	4 290,6	2 438,7	56,8	4 602,5	2 045,8	44,4	4 530,1	1 870,0	41,3
Ulytau	-	-	-	-	-	-	-	-	-	-	-	-	266,7	0	0
East Kazakh. Region	1 663,5	20000	1,2	1 598,7	0	0	1 611,7	0	0	1 619,3	0	0	603,8	0	0
Astana	2,5	0	0	1,7	0	0	1,5	0	0	1,1	0	0	1,8	0	0
Almaty	2,4	0	0	2,3	0	0	1,5	0	0	4,5	0	0	1,7	0	0
Shymkent	-	-	-	113,2	63,1	55,8	95,4	65,6	55,8	109,3	80,0	73,2	82,6	15,0	18,2
Total:	18 329,0	6 734,0	36,8	18 677,9	5 117,8	27,4	19 092,0	5 452,4	28,6	20 042,0	3 443,2	17,2	21 276,8	3 225,6	15,2

As can be seen from the table, vaccination of susceptible animals against the PPR was carried out only in places included in the buffer zone. It should be noted here that the regions where vaccination was carried out changed in different periods.For example, due to the division of the Almaty region into two parts, the Zhetysu region is also included in the vaccination zone as a separate administrative unit, but neither the Almaty region nor the Zhetysu region are included in the vaccination plan for sheep and goats against the PPR for 2022. In addition, in 2018 the city of Shymkent was awarded the status of a city of republican significance and in connection with its separation from the Turkestan region, since 2019 the city of Shymkent as a separate administrative unit has been included in the action plan to combat the PPR.

In the period from 2018 to 2022, from 3,225,570 to 6,733,974 sheep and goats were vaccinated in buffer zones for 1 year. At the same time, up to 15.2-36.8% of the total number of susceptible animals were vaccinated.

The highest rate of vaccination coverage of animals prone to PPR was recorded in 2018 – 54.38%. This year, predisposed animals of Almaty, Zhambyl and Turkestan regions were mainly vaccinated and vaccination coverage in Almaty and Zhambyl regions amounted to 42.5-44.5%, in Turkestan region this figure reached 93.9%.

In 2019-2022, we are witnessing a general decrease in the number of vaccinations of susceptible livestock against the PPR. For example, in 2022, only 15.2% of the total number of susceptible animals were vaccinated. Separately, by region, vaccination coverage in Almaty region decreased from 42.5% (2017) to 0%, in Zhambyl region - from 69.0% (2020) to 32.4%, in Turkestan - from 93.9% (2018) to 41.3%.

In order to constantly confirm the epidemic well-being of the country, planned monitoring studies are conducted annually by the state for each notified infection. Based on this, at the next stage, we analyzed monitoring wax studies on the PPR conducted on the territory of the Republic of Kazakhstan in 2018-2022. These studies are conducted by the Republican State Enterprise on the right of economic management «National Reference Center for Veterinary Medicine» Federal State Budgetary Educational Institution of Higher Professional Education of the Ministry of Agriculture of the Republic of Kazakhstan and the results of their analysis are presented in Table 2.

Table 2 - Number of diagnostic studies of PPR in 2018-2022

Years	Number of serological research	Among them, the positive result	Number of molecular genetic research	Among them, the positive result
2018	33	0	115	0
2019	6063	0	140	0
2020	211	0	139	0
2021	41013	0	75	0
2022	41510	0	61	0
Total	86 830	0	482	0

As can be seen from the table, the number of studies varies greatly by year. As for serological reactions, if in 2018-2020 from 33 to 6063 samples were studied annually in the republic, then in 2021-2022 41013 and 41510 studies were conducted respectively. In total, 87027 blood serum samples of small cattle have been examined over the past 5 years and negative results have been obtained in all cases.

Also, in accordance with the monitoring research plan, it is planned to conduct molecular genetic studies. These studies were carried out by polymerase chain reaction (PCR) using special diagnostic kits. Data analysis showed that 482 molecular genetic studies have been conducted over the past 5 years. At the same time, from 61 to 140 samples are studied annually, and it should be noted here that all studies have shown negative results.

As already noted, over the past 2 years, the number of monitoring serological studies has significantly increased in order to strengthen control over the current epidemic situation of small ruminant plague. Data on the analysis of diagnostic studies for 2022 in the context of the regions of the state are presented in Table 3.

Table 3 - Analysis of monitoring studies of PPR conducted at the level of regions of Kazakhstan (2022)

Name of the region	Number of serological research	Among them, the positive result	Number of molecular genetic research	Among them, the positive result
Akmola	616	0	22	0
Aktobe	2 723	0	3	0
Almaty	6 074	0	3	0
Atyrau	444	0	0	0
West Kazakh. region	2 228	0	5	0
Dzhambul	5 165	0	4	0
Karaganda	1 922	0	5	0
Kostanay	1 731	0	6	0
Kyzylorda	3 896	0	0	0
Mangystau	1 871	0	0	0
Pavlodar	2 530	0	2	0
North Kazakh. region	1 884	0	7	0
Turkestan	5 363	0	1	0
East Kazakh. Region	5 063	0	3	0
Total	41 510	0	61	0

The table shows that sampling for serological studies from the regions was carried out taking into account the number of susceptible animals in each region and the presence of an area in the buffer zone where vaccination is carried out. So, in 2022, 50.0% of the studies (20,498 samples) were conducted by samples from Almaty, Zhambyl, Kyzylorda and Turkestan regions. The largest number of studies were conducted in Almaty (6074), the smallest - in Atyrau (444) regions. It should be noted that of the 61 planned studies on molecular genetic research, 22 (36.1%) were conducted with samples from the Akmola region. In addition, in the West Kazakhstan region, Karaganda, Kostanay and North Kazakhstan regions, 5-7 PCR samples were studied. As already mentioned, all serological and molecular genetic studies have shown negative results.

The World Organization for Animal Health, in accordance with the recommendations of Article 1.4.6 of the Continental Code of Animal Health (2018), should conduct surveillance (passive or active) on the PPR in order to confirm the historical suitability of the territory, state, proof of the absence of disease or source of infection. In this regard, in accordance with the tasks set, we conducted serological monitoring of the presence of antibodies to the PPR (unvaccinated sheep and goats) in susceptible domestic animals kept in farms of various forms of ownership (personal subsidiary farm, peasant farm) «in dangerous areas of possible manifestation of infection».

Blood serum samples of small cattle of different ages and sexes (from 2 to 6 months) the study, ID Screen® PRO Competition (ID.VET, France) was conducted by competitive enzyme immunoassay (ELISA) using a test system.

To conduct monitoring studies for the detection of antibodies to the PPR from neighboring countries, 1000 samples of blood sera from 2 regions (Almaty, Zhambyl) and Shymkent, belonging to the zones of increased risk of disease penetration, were selected (Table 4).

So, a total of 300 samples were taken in the Almaty region. Of these: Talgar district, Alatau rural district, Bereke village-23, Almalyk village – 42, Orman village – 14, Ryskulova village - 71 samples (total 150 samples for the district); Azat rural district of Enbekshikazakh district – 26, Rahat rural district-58, Uryktinsky rural district – 34. Kaynazarsky rural district district – 32 samples (150 samples in total for the district).

Zhambyl region-300 samples. Of these: Baizak district, Koktal village – 47, Sarykemer village – 53, Kostobe village – 31, Kyzylzhuldyk village - 33, Buryl village – 31 samples (total for the district 195 samples). Zhambylsky district rural district Birlesu – 35, Zhasorken rural district – 40, Enbek

rural district – 30 samples (total of 105 samples in the district).

There are 400 samples in the city of Shymkent, of which: Abai, Al–Farabi, Karatau and Enbek districts, 100 samples were taken from each administrative district.

According to the results of the work, All serological studies for the determination of antibodies against the PPR in the blood serum samples taken for the study showed a negative result.

That is, serological monitoring of the territory of the republic belonging to the buffer zone for the PPR confirms that at present these regions are favorable for the above infection, but at the same time, given the presence of risk factors contributing to the outbreak of the epidemic, there is a need to continue surveillance of the situation and conduct systematic screening studies.

Table 4 - Results of serological monitoring of buffer zones of the Republic of Kazakhstan by enzyme immunoassay, the presence of antibodies to the PPR in the body of small cattle

№	Region	District	Rural district	Locality	Type of ownership	Number of studies, heads		
						Total	Of these	
							negative	positive
1	Zhambyl	Baizak	Koctal	Koctal	PF (Kozhagulov M.)	47	47	0
2			Sarykemer	Sarykemer	PF (Tumashev)	53	53	0
3			Kostobe	Kostobe	PF (Umirbekov)	31	31	0
4			Krasnaya Zvezda	Krasnaya Zvezda	PF (Kostai A.)	33	33	0
5			Buryl	Buryl	PF (Tumaev E.)	31	31	0
6		Zhambyl	Birlesu	Birlesu	PF (Kukeev M.)	35	35	0
7			Jasorken	Jasorken	E. Beitkhanov	40	40	0
8				Yenbek	PF (Shangiev B.)	30	30	0
9	Almaty	Talgar	Alatau	Bereke	PF (Baibolov A.)	23	23	0
10			Alatau	Almalyk	PF (Zhakhanbekov Sh.)	42	42	0
11			Alatau	Orman	PF (Dzhaparov N.)	14	14	0
12			Alatau	Ryskulov	PF (Kotelnikov V.)	71	71	0
13		Enbekshikazak	Azat	Azat	PF (T. Nusipov)	8	8	0
14			Azat	Azat	PF (Abdullayev A.)	18	18	0
15			Raxat	Raxat	PF (Bukenov)	58	58	0
16			Orikti	Orikti	PF (Dauletov O.)	34	34	0
17				Kainazar	PF (Ashimov B.)	32	32	0
18	Shymkent	Abay	-	-	PF (Orhan zh.)	100	100	0
19		Al-Farabi		-	PSF («Lapiev»)	100	100	0
20		Karatau	-	-	PF (Oralbayev)	100	100	0
21		Enbekshi	-	-	PF (Mavlanov A.)	100	100	0
22						1000	1000	0

Note: [1] PSF is a personal subsidiary farm; 2 PF is a peasant farm.

Discussion

The results of the conducted studies show that the presence of factors contributing to the penetration of the PPR, such as the disadvantage of border states and institutions-economic partners, population density and density of susceptible farm animals, depending on the region of the state, can have a significant impact on the current epidemic situation and the dynamics of the epidemic process of PPR.

This is especially true in regions with a high risk of pathogen penetration from neighboring countries. In such regions of the country (Almaty, Zhambyl, Turkestan regions), along with a high density of susceptible animals, there is a high density of population and settlements. In this regard, the most important thing for the veterinary service of the country is systematic and purposeful work to prevent the importation of infection from outside and the formation of a buffer zone consisting of immune livestock in areas with the greatest risk of epidemic penetration.

To confirm the epidemiological well-being of the country, routine monitoring diagnostic studies for each notified infection are mandatory. The analysis of monitoring studies conducted in the territories of Almaty, Zhambyl regions and Shymkent showed that these regions are free from the PPR. Our research is confirmed by annual monitoring studies conducted by the Republican State Enterprise on the right of economic management «National Reference Center for Veterinary Medicine» in the state municipal enterprise on the right of economic management of the Ministry of Agriculture of the Republic of Kazakhstan.

Conclusion

The results of the study confirm the epidemiological suitability of the country's territory for the PPR. Currently, the PPR special control measures are the most effective way to prevent the penetration and spread of infection in controlled areas. The analysis of vaccination of susceptible animals in the territories included in the buffer zone showed that the level of vaccination coverage of susceptible animals varies annually, which is usually associated with a predictive assessment of the risk or reduction of the growing tension of the epidemic situation for the PPR in the region. In general, the downward trend in the proportion of vaccinated animals in the buffer zone over the past 2 years is based on a significant reduction in the intensity of the epidemic situation in the territories of disadvantaged countries bordering Kazakhstan (China) with Kazakhstan, and is economically justified. But such dynamics can also lead to unfavorable conditions, because as the number of unvaccinated animals increases, the risk of infection from the outside increases proportionally.

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SERINE PROTEASE OF TRICHINELLA SPIRALIS AND ITS POSSIBLE APPLICATION IN THE EARLY DIAGNOSIS OF TRICHINELLOSIS IN ANIMALS

Orken S. Akibekov[1], Aisarat M. Gajimuradova[2], Aibek Kh. Zhumalin[2], Sairan M. Duisenov[3], Nurtai N. Gubaidullin[1], Nasiphan A. Askarova[1]

[1]Faculty of Veterinary Medicine and Animal Husbandry Technology, NCJSC «Saken Seifullin Kazakh Agrotechnical Research University», Astana, Republic of Kazakhstan
[2]Research platform of agricultural biotechnology, NCJSC «Saken Seifullin Kazakh Agrotechnical Research University», Astana, Republic of Kazakhstan
[3]Branch «Karaganda NIVS», «KazNRVI» LLP, Karaganda, Republic of Kazakhstan

Corresponding author: Orken S. Akibekov, e-mail: orken.a.s@mail.ru
Co-authors: Aisarat M. Gajimuradova, e-mail: aisarat3878@mail.ru
Aibek Kh. Zhumalin, aibek_biotex@mail.ru
Sairan M. Duisenov, e-mail: kar_nivs@mail.ru
Nurtai N. Gubaidullin, e-mail: nur-tai.kz@mail.ru
Nasiphan A. Askarova, e-mail: nasiko.askarova@bk.ru

Abstract

Serine proteases of parasites play a significant role in the infection of a host organism. This is especially reflected in the process of immunosuppression, in particular, during IgG hydrolysis. The importance of the performed function ensures their presence at all stages of development, which makes them key targets for early diagnosis of trichinellosis. In this study, we demonstrated that serine protease transcripts are detected in 83% of cases when mice are infected with BALB/c on days 7 and 14 after infection and are identified by molecular methods. Two groups of mice were infected with different doses of larvae (100 and 250 larvae), it was shown that the dosage did not affect the effectiveness of serine protease detection in mice, which would provide a better diagnostic effect. The transcripts presence confirms the possibility of using serine protease as a protein for the diagnosis of trichinellosis in animals and humans.

Key words: recombinant protein; serine protease; parasites; *Trichinella;* trichinellosis.

Introduction

The genus *Trichinella* includes ten species and three different genotypes, which are capable of infecting more than 150 domestic and wild mammals [1, 2]. People become infected with trichinellosis when ingesting raw or undercooked meats infected with *Trichinella* larvae.

The main diagnostic methods today are serological tests, such as Western blotting and ELISA for the detection of antibodies to trichinella. These methods are valuable approaches to the diagnosis of human and animal trichinellosis [3].

The most commonly used antigens for the diagnosis of trichinellosis are excretory-secretory (ES) antigens of muscle larvae, however, cross-reactions with other parasites often occur. In addition, it should be taken into account that a variety of different antigens are expressed at different stages of development, which may explain why muscle larvae are not recognized by trichinella induced ES antibodies at the intestinal stage, and false negative results observed in the early stages of infection [4].

This is due to the fact that the biological function of the protein largely depends on the spatial structure of the protein. Different antigens can also be expressed at different stages of *T.spiralis* development. In total, 4691 proteins were identified at the adult larva, newborn larva and muscle stage, 1067 differentially expressed, including serine protease, DNase II, trypsin enzyme II of the protein family and paramiosin. These proteins are being actively studied by scientists as candidate molecules for early diagnosis, as well as for the creation of a vaccine against trichinellosis [5].

It is possible that the most relevant and

promising protein will be one that can be produced at all stages of the parasite's life cycle.

In Zhai C. and et.al. research the diagnostic effect of ES antigens at different stages of development was studied using ELISA. When mice were infected with 100 larvae after 10 dpi, antitrichinella IgG antibodies were detected using intestinal ES infectious antigens of larvae, but after 12 dpi, infection was already diagnosed with the help of antigens of encapsulated larvae [6].

The main proteins expressed at different stages of trichinella development are serine proteases [7, 8], which are a family of proteolytic enzymes that play many biological roles during parasite infestation: they are involved in worm invasion, migration, and proteolysis of various host tissues [9]. They can be important antigenic targets for the creation of serological and molecular tests for early diagnosis of the disease.

Recent studies have shown that several types of serine proteases are involved in invasion by *T.spiralis* larvae, but this factor is not fully understood. It is necessary to conduct additional studies on the stage choice of larval development, the excretion of *T.spiralis* serine proteases and to investigate their immunogenicity based on the primary, secondary and tertiary protein structures.

Thus, the objective of our research was to study the excretion of serine protease at different development stages and to predict the structure and spatial configuration of serine protease for the development of a test system based on a recombinant protein.

Materials and Methods

All activities involving animals were carried out in compliance with high standards of biosafety and animal welfare. All protocols are implemented in accordance with the *International Guiding Principles for Biomedical Research Involving Animals* [10].

The care and use of laboratory animals were approved by the Animal Ethics Committee of the Faculty of Veterinary Medicine and Animal Husbandry Technology of the NCJSC «S. Seifullin Kazakh Agrotechnical Research University» (KATRU), Astana, Kazakhstan (Protocol No. 2 of July 20, 2020).

The experiments were carried out on the basis of the Research Platform of Agricultural Biotechnology and the Joint Kazakh-Chinese Laboratory for Biological Safety NCJSC «Saken Seifullin Kazakh Agrotechnical Research University».

Infection of animals. T.spiralis was maintained by serial passaging of BALB/c mice in the Immunochemistry Laboratory of the Agricultural Biotechnology Research Platform. The larvae were collected by artificial digestion using a standard protocol. 12 mice aged three-four months were selected for the experiment. According to the principle of analogues, two groups of experimental animals were formed. The causative agents of *T. spiralis* trichinellosis were invaded to animals of first group of six mice at a dose of 100 larvae per head and second group of six mice at a dose of 250 larvae. Animals were infected by the introduction of a per os «digest» containing trichinella larvae using a disposable pipette (Figure 1).

Figure 1 – Infection procedure of BALB/c mice by oral administration of saline solution with larvae

Scheme of the experiment. On the 7th and 14th days, three mice from each group infected with T. spiralis were euthanized for pathoanatomic autopsy by sequential intramuscular injection of xylazine at a dose of 1.5 mg/kg and intravenous injection of anestofol at a dose of 7.5 mg/kg. After the muscle's dissection of animal carcasses, they were examined for the parasite's presence in accordance with WHO/OIE recommendations [11]. The small intestine was longitudinally dissected and washed three times with normal saline solution with ice, then cut with sharp scissors into 2 cm long fragments and cultured in normal saline solution at 37°C for 2.5 hours. Then the larvae released from the small intestine into a normal saline solution were collected by the Berman method [12].

Small intestine pieces

Mounting with a funnel

Figure 2 – Collection of larvae by the Baerman method from the small intestine with settling in a funnel

Diagnosis and isolation of the larvae of the causative agent of trichinellosis from animal muscle tissue samples was carried out by compressor trichinoscopy and digestion in artificial gastric juice (IHS), in accordance with methods of MUC 4.2.2747-10 «Methods of sanitary and parasitological examination of meat and meat products». The detected and isolated helminthological material was preserved in 70% ethanol solution.

Isolation of RNA. The total RNA was extracted using TRIzol reagent (Invitrogen, USA) [13] in accordance with the manufacturer's instructions. The RNA concentration was measured using NanoDrop 2000 (Thermo Scientific, USA). Total RNA was transcribed back into the first cDNA chain using ProtoScript II First Strand cDNA Synthesis Kit (New England BioLabs, England).

PCR conditions. The reaction was carried out on a VerityPro amplifier (Applied Biosystems, USA) using the following primers: Trich SP F: 5'-CAGTATTGTGGAAATCCTTATTTT-3'; R: 5'-TCAGTAAAAAGAGTCAAAA TT-3'. The composition of the reaction mixture included: Taq 5X MasterMix (New England BioLabs) – 5 µl. Primer (10 mM) F – 2 µl, primer R – 2 µl, mQ water – 15 µl, cDNA - 3 µl (100 ng), the total volume of the mixture is 25 µl. PCR mode: primary denaturation - 95℃ - 5 min. (1 cycle); denaturation 95℃ - 30 sec., annealing of primers 58℃ - 30 sec., elongation 72℃ - 60 sec. (30 cycles); final elongation - 72℃ - 5 min. (1 cycle).

Sequencing. The nucleotide sequence was determined using the BigDye Terminator v3.1 sequencing kit (ThermoFisher, USA) and the SeqStidio genetic analyzer (ThermoFisher, Applied Biosystems, USA). DNA sequences were collected and analyzed using a software package (Sequence Investigator, etc.), Finch TV v1.3.1. and using international nucleotide sequence databases (Blast, ENSEMBL, GeneBank, etc.).

Bioinformatic analysis. Bioinformatic methods were used to predict the structures and functions of the serine protease protein.

Statistical analysis. Statistical analysis was performed using GraphPad Prism 7.0. Statistical analysis was performed using Microsoft Excel 2010. P <0.05 was considered statistically significant.

Results

Larvae sampling. At the first stage of the study in mice of groups 1 and 2, 7 and 14 days after infection with freshly isolated larvae, the carcasses of mice were dissected and the intestines and

muscles of mice were examined for the presence of trichinella. Using the Berman method, live newborn larvae were sampled from the intestine. Repeatedly washed areas of the intestine and saline solution after flushing were examined for the presence of larvae. Consequently, egg laying and newborn larvae were found at 7 dpi in tissue and solution samples (Figure 3).

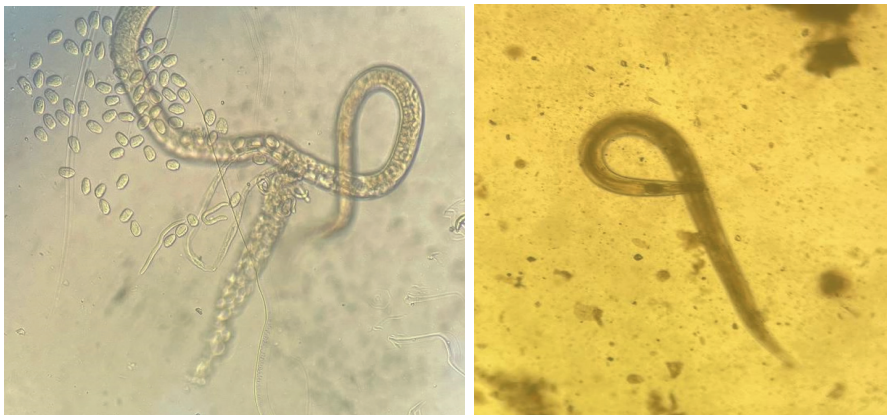

Figure 3 – Larva of *Trichinella spiralis* during life birth (A) and newborn larvae (B) at the stage of intestinal phase of infection

In mice of group 2 with a dosage of infection of 250 larvae, a larger number of newborn larvae were observed on the intestinal walls, whereas in group 1 with a lower dosage, larvae were found in flushes from the intestine at the egg–laying stage. This may indicate that with a higher dosage at the time of infection, the invasion process occurs faster and the larvae begin to enter the intestinal walls. It may also be due to the immune response of mice to infection, which is able to restrain larval infestations at a lower dose. It is important to note that both experimental groups did not have the presence of encapsulated larvae in the muscles at 7 dpi. However, quantitative differences in the two groups were revealed due to the difference in the dose of larvae (Table 1).

Table 1 – Intensity of invasion of mice in experimental groups at 7 dpi

Stage of infection	Group 1	Group 2
Intestinal 7dpi		
6.7±0.8 gr	5±1.5	13±2.1
Intensity of invasion	33.5±1.2	87.1±1.7

The intensity of the invasion was significantly different depending on a dose application, which confirms the dose-dependence during infection exposure, as we described in early studies [14].

Sampling at 14 dpi significantly differed in the larvae development stage and their localization in mice group 1 and 2. So after 14 days, no larvae were found in the intestines of mice from both groups, all larvae were localized in the muscles (Figure 4).

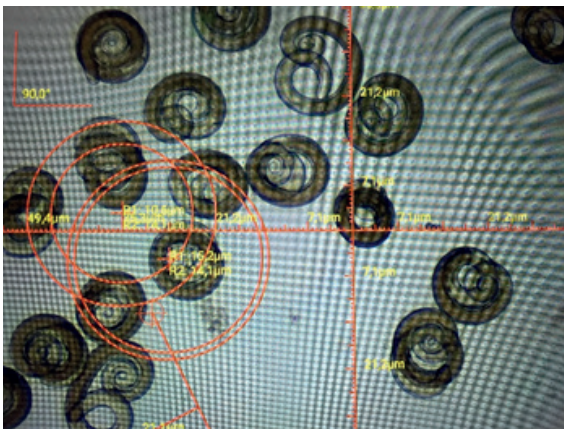

Figure 4 – Larvae of *Trichinella spiralis* from mouse muscle digestion at 14 dpi

In both group 1 and 2, the larvae reached the muscular stage, which did not show a dose-dependent effect. However, when microscoping muscles of mice from group 2, encapsulation of 4.2±1.3 larvae was already completed, when no encapsulation was detected in group 1. The intensity of invasion after 14 days averaged 20±5.7 larvae per 1 gram of muscle (Table 2).

Table 2 – invasion intensity of mice in experimental groups at 14 dpi

Stage of infection	Group 1	Group 2
Muscular 14 dpi	100 larvae	250 larvae
87.3±3.1 gr	17.2±4.1	23±7.3
Intensity of invasion	1501.6±12.7	2007.9±22.6

The invasion intensity differed slightly in the groups. However, studies have shown that the stage of larval development was different in both groups. The rate of larvae encapsulation in mice had been relatively fast in group 2, which explains the dose-dependency effect.

Molecular confirmation of serine protease gene transcripts presence. After studying the exact stage of larval development at different time periods after infection, the selected larvae were subjected to total RNA cleavage and isolation from all 12 larval samples.

The average RNA concentration was 873 ng/ml. After the reverse transcription reaction and cDNA synthesis, PCR was performed using a specific primer. The results of PCR were visualized by electrophoresis (Figure 5).

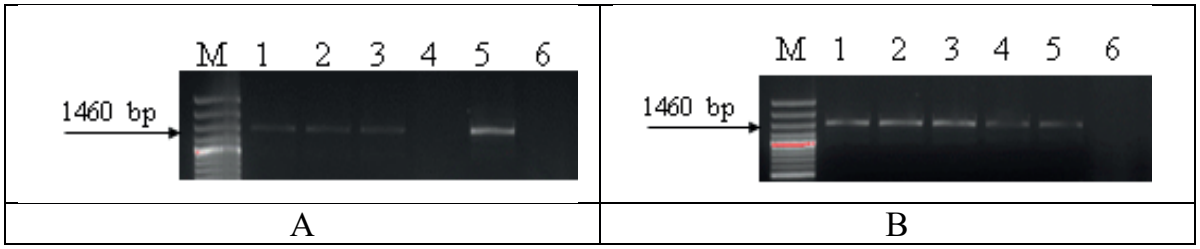

Figure 5 – Electrophoretic analysis of serine protease gene transcripts presence in larvae at 7 dpi (A) and 14 dpi (B)

According to molecular studies, the presence of serine protease transcripts was determined in all two groups of mice, regardless of the dose and the period of infection. Larvae selected at different stages of development and encapsulation in 83% showed the presence of transcripts of the serine protease gene. The data obtained are consistent with the data of recent studies of Song Y.Y. et.al. (2022), which showed that serine proteases play a key role in the invasion, growth and survival of T. spiralis in the host body and that they can be the main target molecules-candidates for the creation of vaccines and early diagnosis of trichinellosis [15].

After sequencing of the serine protease gene, the nucleotide sequence (1450 bp) was determined

ATTAGCATACGCATTAAGCATGAAAC
GCTGGCACCCCTTTGGCATACCTTTTCACA
ATGCATTTCTGTTGTTTTGTATTATTATTA
AGGAAACATTTTCACAGTATTGTGGAAAT
CCTTATTTTGAACCATATTTGACAAATCCA
CACTAATTCGAACCAAATTGTTGGTGAAT
GGGTTGCAAGGCCATATTCATTTCCATGG
ACTGTTCATGTATTAGCTCATATTTCTGGA
TTCTGGTATGAAATTCTTGTGGAGGCAGT
CTGATTTCTTTTGACTATACAAACGCCAGT
GATACTGTCCTCACTTCATCCCATTGTGTT
AGAGTAAACAATCGTCTTGTGGATGCAAA
TGCTATAACTGTGACAGCAGGTGCATTTA
ATATAAGGGAATTAAACGAACCCCACAG
AGTCACTTCAAAAGTCCTGGCATACATGT
CAGATAATTTTGGTGACGTCGGTAAACCA
AATGACGTCGCTATGTTGCGTTTAAAAGT
AAAGATTCCGCATTCTCACTACATCAGTT
CAGTCTGTTTGCCATATCCATTCCAAGAG
ATACCATATGGAGAAACGTGTTTTCTTTG
TTGGGGTTTCACTAGAGGAAGACCACTGT
CTGAATTGCGTCAGGTTGGAATCCCAATT
TTACGAAGCAGCAACTGCCGATTTACTGA
TGCGTATGATATTTTTTGCGCAGGTGATA
TGGGTGAAGGAAATTATTCTTTCCAAATT
GATTCGGGAGGACCTCTAGTTTGTAAATT
AAATGATTCCTATGTTCAAATTGGCATAG
TTAGTTTTGGTTACAACCATGCTGGAAAG

CACCACCCTGGTATTTAATCAAAAGTTCC
TTATTATTTGAATTGGATATACAATCAACT
GTCATCCGCTTCCTGATTCATTTAACTCTT
CAGATATCGGAGGCGAAGAATCTGATTGT
CCAGATGATTGTTACCACCCTTGGCGATC
CGTCTTCAAACATTTTAAACATCGCAAGG
CGTCATTCCGAAATCGTCCACCGTATTCA
CATTCACTCAGACTTACAATGAATGAGAA
TCGTCCACCACCACCTCCAGATTCTCAAA
ATTTTGATATGGAATCTTTGGAAAGTACT
GAAGGTGATCCTAGTGATTGGTCTCCATA
TTCAACTAACCAGCATTACCAATCAAATT
ATGATGGATCTCAGACAGGCAAAGGAAA
TCGTCCACCGTATTCACATTCACACAGAC
CTACAATGAATGAGAATCGTCCACCACCA
CCTCCAGATTCTCAAAATTTTGACTCAAA
TTACTGAAGCCATGCAGCTTTGCGGCATT
GTCATTCTTTAGTTTTGACGGAGCGCAAG
CATCACTCCAATACAATTATATATAAGGA
ATGGACTTTTAAAAGAATTAGCAATTAAT
ATAATTCTGTAAGTTTTTAAAATGCATTGT
ATGTTAATAAAATGAATTGCATCAC

Next, a bioinformatic analysis of the serine protease sequence was performed, including an alignment of the cDNA sequence of serine proteases in GeneBank with the determination of the amino acid sequence in the primary structure obtained in the Mega 11.0 program.

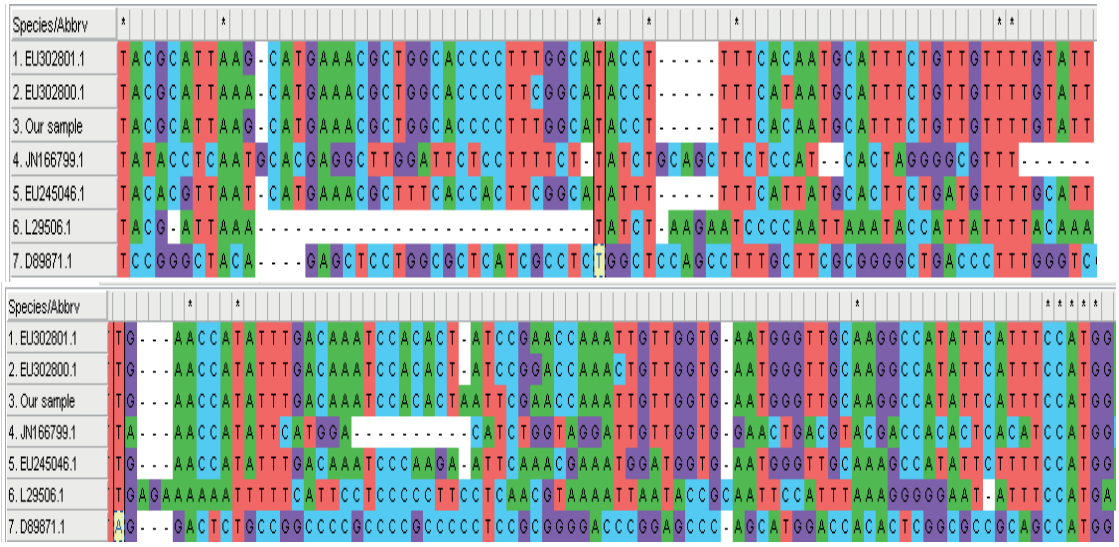

Figure 6 – Alignment of the nucleotide sequence of serine protease in the Mega 11.0 program

Based on the nucleotide sequence of the gene, the amino acid sequence of the protein was constructed:

#Sequence 1 Amino acid chain:

ISIRIKHETLAPLWHTFSQCISVVLYYY*GNIFTVLWKSLF*TIFDKSTLIRTKLLVNGLQGHI
HFHGLFMY*LIFLDSGMKFLWRQSDFF*LYKRQ*YCPHFIPLC*SKQSSCGCKCYNCDSRCI*Y
KGIKRTPQSHFKSPGIHVR*FW*RR*TK*RRYVAFKSKDSAFSLHQFSLFAISIPRDTIWRNVFSL
LGFH*RKTTV*IASGWNPNFTKQQLPIY*CV*YFLRR*YG*RKLFFPN*FGRTSSL*IK*FLCSNW
HS*FWLQPCWKAPPWYLIKSSLLFELDIQSTVIRFLIHLTLQISEAKNLIVQMIVTTLGDPSSNIL
NIARRHSEIVHRIHIHSDLQ*MRIVHHHLQILKILIWNLWKVLKVILVIGLHIQLTSITNQIMMDL
RQAKEIVHRIHIHTDLQ*MRIVHHHLQILKILTQITEAMQLCGIVIL*F*RSASITPIQLYIRNGLL
KELAINIIL*VFKMHCMLIK*IAS

A graphical representation of the protein subunits distribution in the structure of the larval cuticle membrane was obtained using the Phyre 2.0 Internet resource (Figure 7).

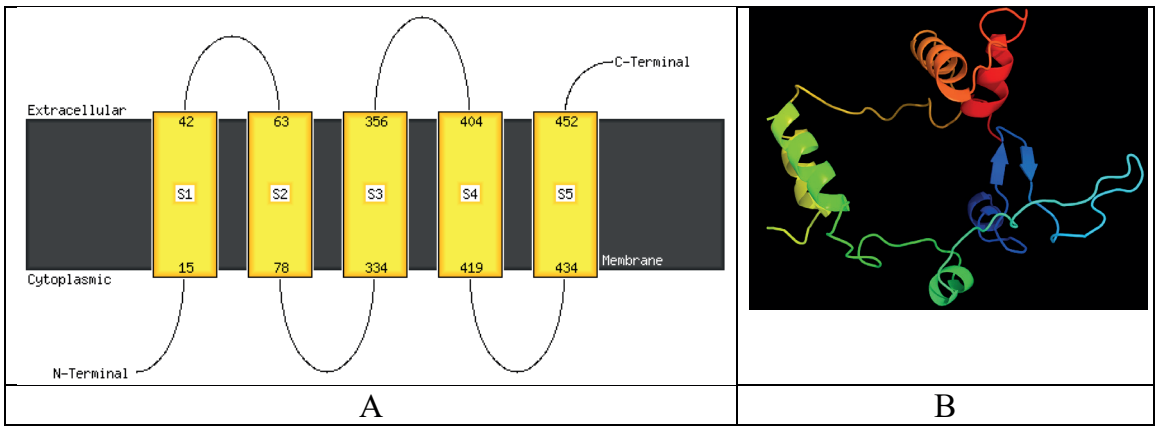

Figure 7 – Graphical representation of the distribution of protein structural units in the membrane thickness (A) and the spatial configuration of the protein (B)

According to the data, the serine protease consists of 1,450 bp, while the amino acid sequence includes 483 amino acids. The protein is represented by 5 subunits, alternately located in the thickness of the cuticle, while the sections of the molecule N-terminal end – 1-14, located in the cytoplasm, S1-S2 – 43-62 – associated with extracellular space, S2-S3 – 79-333 – located in the cytoplasm, S3-S4 – 357-403 – binds the protein to the extracellular space, S4-S5 – 420-433 – is concentrated in the cytoplasm and the C-terminal site – 31 amino acids - exits into the extracellular space. The location of the serine protease determines its functions as the main proteolytic enzyme involved in the process of larval invasion, in the processes of secretion, repair, morphogenesis and differentiation. Importantly, it is the exact protein that is able to cause an immune response to invasion in the host body. It has been proved that the immunosuppressive effect of parasites on the host organism largely depends on the activity of proteolytic enzymes capable of hydrolyzing the IgG hinge region and heavy chains of immunoglobulins of all classes, as well as cleaving interleukin 1β [16].

Discussion

In recent years, trichinellosis has become a new and emerging parasitic disease, and the severity of trichinellosis in humans ranges from subclinical to lethal [17]. Early diagnosis of infection is crucial for the timely and effective treatment of trichinellosis, since anthelmintic drugs are much more effective against adult helminths in the intestine than against encapsulated larvae in the muscles [18, 19]. Therefore, it is important to identify antigens recognized by the host's immune system at an early stage of infection. These immunodominant antigens can be developed as biomarkers for early diagnosis of trichinellosis or even as potential vaccines for better control of this zoonotic foodborne disease.

Serine proteinases are important members of the superfamily of proteolytic enzymes that are widely distributed in organisms. Serine proteases have two main structural folds: trypsin-like domains and subtilisin-like domains. Most trypsin-like domains play an important role in helping parasites to invade, digest, shed, and hydrolyze proteins [20].

Previous studies have shown that T**richuris muris** serine proteinase can disrupt the integrity of the cell membranes of the intestinal epithelium, which is associated with the hydrolysis of the mucous barrier of the intestinal surface of the host [21].

In our study, it was shown that serine protease is present in 83% of cases when infected with *Trichinella* larvae. This indicator is sufficient to identify the disease at different stages of development, including intestinal. The intestinal stage did not reveal a dependence on the dose of larvae during infection. However, the number of larvae accelerated the process of invasion and encapsulation in the muscles.

Sun G.G. and et.al. research it was shown that recombinant serine protease of trichinella has an immunogenic property, which proves the presence of specific antitrichinella IgG in 100% of infected pigs [22].

An earlier study of this group of scientists on mice showed that specific antitrichinella IgG in infected mice was detected by ELISA based on

recombinant serine protease protein after 7 dpi, and the level of positive antibodies reached 100% at 10 dpi, while ELISA on excretory-secretory antigens did not allow detecting 100% of infected mice up to 16 dpi [23].

Analysis of the protein sequence of serine protease showed that the protein is intracellular, has extracellular structures. The resulting complete amino acid sequence and spatial structure will help to better isolate this protein and understand its functions.

Recently, the conduct of proteomic analyses has been of great interest to scientists, since it allows us to determine not only the protein, but also its region as an immunogenic site using the example of the entire protein diversity represented in the ES serum. For example, scientists from China have identified 185 *T proteins.* and several enzymes (for example, adult-specific DNase II, serine protease and serine protease inhibitor) that can act as invasion-related proteins and early diagnostic antigens of trichinellosis [24].

Thus, parasitic serine proteases involved in reproduction, coagulation and associated with larval invasion of the intestinal mucosa may be potential targets for vaccines against trichinella, as well as antigens in the design of serological tests for early diagnosis of trichinellosis.

Conclusion

Studies have shown that serine protease is expressed in the larva of trichinella both at the stage of intestinal infection and at the stage of muscle larvae. Serine protease transcripts at 7 and 14 dpi were detected in 83% of infected mice. The invasion intensity showed a significant dose-dependence. The conducted bioinformatic analysis demonstrated that the location of serine protease in the membrane of the cuticle of larvae is a key factor in its use as a diagnostic component. Thus, serine protease is a promising protein for early serodiagnosis of trichinellosis.

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DEVELOPMENT OF THE TECHNOLOGY OF DEEP CULTIVATION OF A TRANSFORMED B. SUBTILIS STRAIN

Nurtas S. Tursynbayev[1], Nurlan N. Akhmetsadykov[1], Zhanat M. Batanova[1], Damir M. Khussainov[1], Yerkin A. Krykbayev[2]

[1]Faculty of Veterinary medicine, NJSC Kazakh National Agrarian Research University, Almaty, Republic of Kazakhstan
[2]LLP "Research and Production Enterprise "Antigen" Almaty, Republic of Kazakhstan

Corresponding author: Nurtas S. Tursynbayev, e-mail: tursynbayev_nurtas@mail.ru
Co-authors: Nurlan N. Akhmetsadykov e-mail: nurlan.akhmetsadykov@gmail.com
Zhanat M. Batanova batanova_77@mail.ru
Damir M. Khussainov e-mail: doctor-vet@mail.ru
Yerkin A. Krykbayev e-mail: krykbaev_e@mail.ru

Abstract

Deep cultivation of recombinant cultures in bioreactors makes it possible to accumulate a large number of specific antigens used in the production of immunobiological preparations and diagnostic test systems in a short period of time, taking into account stimulating and limiting growth factors. The main purpose of the research was to determine the influence of the factors of seed concentration, pH level and the level of dissolved oxygen on deep cultivation, with the control of accumulation kinetics by turbidimetry. During the research, it was found that the most optimal inoculation concentration is 1 million cells/cm^3 with a maximum final concentration of B. subtilis 4x106 cells/cm^3, the most optimal pH level is a dynamic mode from 8.0 to 6.0 with a maximum final concentration of 5x106 cells/cm^3, the most optimal level of dissolved oxygen is stationary mode with a minimum oxygen level of 25%, with a maximum final concentration of 4x106 cells/cm^3.

Bacteriological, technological and biochemical methods were used in the work to develop the modes of deep cultivation of the transformed B. subtilis strain with control of pH parameters and the level of dissolved oxygen.

The scientific significance of the research is based on the development of deep cultivation, with an assessment of external growth factors and their influence on the kinetics of accumulation of the transformed B. subtilis strain, which will provide effective conditions for the growth and production of microorganisms. The practical significance of the research is based on the possibility of optimizing the biotechnological stages of the cultivation of the transformed B. subtilis strain, which will positively affect the efficiency of submerged cultivation as the main method for the accumulation of specific antigens.

Key words: bioreactor; biotechnology; B. subtilis; cultivation; growth factors; microbiology; nutrient medium.

Introduction

Chlamydia abortus is a Gram-negative obligate intracellular bacterium responsible for abortion and reproductive problems. The disease has a high zoonotic potential and causes great economic damage to ruminant farmers [1]. The main method of combating this disease is the timely diagnosis and quarantine of animals. *Chlamydia psittaci (C. psittaci)* is distributed throughout the country and can be transmitted from animals to humans through close contact [2, 3]. Avian chlamydia (AC), mainly caused by *C. psittaci*, is an acute, severe or chronic asymptomatic disease of poultry, poultry and mammalian hosts. In the poultry industry, *C. psittaci* has been identified as the main causative agent of eye, respiratory, intestinal, and arthritis diseases, as well as abortions [4]. Moreover, a retrospective study has shown that *C. psittaci* may be a risk factor for atherosclerosis in poultry [5]. Thus, *C. psittac*i not only threatens the poultry industry and domestic animals, but also

causes serious economic damage to the poultry industry. However, *C. psittaci* infections are often underestimated due to the lack of rapid and reliable testing kits. In addition, there is a multi-infection of *Chlamydia pecorum (C.pecorum), Chlamydia gallinacean (C.gallinacean)* and *Chlamydia avium (C.avium)* in poultry [6, 7].

According to the OIE recommendation, an enzyme immunoassay based on recombinant proteins can be used as one of the main methods for diagnosing chlamydia in farm animals [8].

As the main carrier of recombinant genes, we chose the *Bacilus subtilis* strain, which is a widely used commercial strain with wide application in the field of bioengineering and biotechnology, since they are considered safe for use [9]. The main methods of cultivation of *B. subtilis* are stationary on solid nutrient media and submerged cultivation in bioreactors, each method has its advantages and disadvantages, primarily associated with high requirements for the availability of equipment and qualified personnel, only very recently, advances in the study of submerged cultivation penetrate into the field of hybrid modeling. [10]. The submerged culture method was chosen by us first of all with the possibility of combining modern recombinant technologies together with the technological stages of production in bioreactors, with low risks of fungal and bacterial contamination, and greater control over external parameters of cultivation. Over the past few decades, there has been significant progress in the production of pharmaceutical compounds using both microbial and mammalian cellular systems in large-scale bioreactors. For each new process, this includes scaling production steps from laboratory bioreactors (eg 250 ml to 2 L) to larger pilot and production bioreactors (eg 500 L to 15,000 L). However, scaling up is not a trivial task, since bioreactors are often considered one of the most difficult pieces of equipment to scale up [11]. Several factors exacerbate the complexity of scaling. These include the complexity of biological systems and significant costs, including media and reagents, associated with the transfer and testing of processes in large-scale bioreactors [12]. The environment in a large-scale bioreactor is also heterogeneous, which consequently leads to increased intercellular variability and greater process variability at scale. Similar process differences are observed between large bioreactors and downscale models and are one of the main limitations of downscale models, which may not be accurate indicators of production scale performance [13].

To obtain a transformed culture of *B. subtilis* for research, we chose the chlamydia outer membrane complex (COMC), which is a protein-rich insoluble shell of the outer membrane, which includes outer membrane proteins (OMPs), in particular, the main outer membrane protein (MOMP) and polymorphic membrane proteins (Pmps), which are used as the main candidates for vaccines against CD4 T cells and enzyme immunoassay diagnostic test systems [14].

Materials and Methods

2.1 Strain

B. subtilis strain transformed with specific fragments of Pmps (Polymorphic Membrane Proteins - polymorphic membrane proteins). The strain has the typical properties of bacilli, causing sporulation upon contact with oxygen.

2.2 Deep cultivation in bioreactor

Deep cultivation was carried out in a Bailun BlBio 30 bioreactor (Produced by Bailun Biotechnology Co Ltd (Bailun), China), with a volume of 30 liters, with automatic control systems for cultivation parameters, including temperature, pH, dissolved oxygen (DO), stirrer speed, etc. (Figures 1 and 2).

Figure 1 - Bioreactor for suspension cultivation

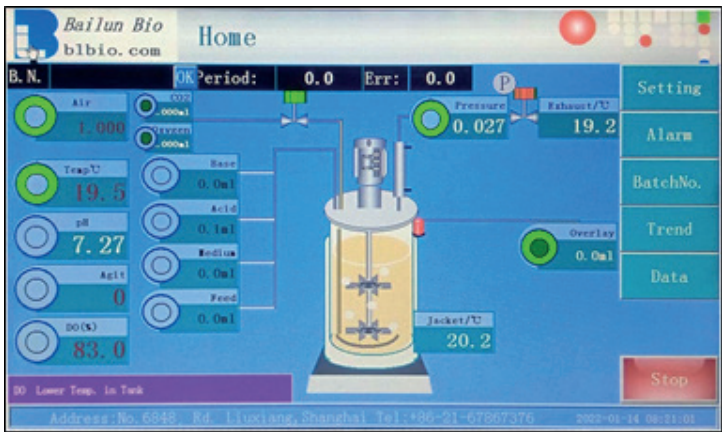

Figure 2 - Automatic control systems of the bioreactor

2.3 Growth phases of bacteria

Bacterial growth dynamics was examined based on the traditional 4 phases, (I) lag phase (II) logarithmic phase (III) stationary phase (IV) deceleration phase. The growth phases were determined by the concentration of viable bacteria incapable of spore formation per 1 cm^3. The growth phases were determined by microscopy, which will determine the degree of sporulation of strain B. subtilis, and the method of determining the number of cells using a densitometer, which will determine the total concentration in the samples.

2.4 Culture medium

In the studies, a modified Hottinger's digest was used, enriched with glucose, pH (7.4±0.1), containing NaCl - 0.5%, Na_2HPO_4 - 0.06%, peptone up to 1%. The prepared nutrient medium was tested for microbial and fungal contamination. This nutrient medium was developed and standardized at the Scientific and Production Enterprise "Antigen" and controlled by a system of standard operating procedures.

2.5 Concentration control

The concentration of streptococci was monitored using a DEN-1B MF-Units densitometer (Produced by GRANT USA INC.), measuring the turbidity of cell suspensions within the range of 0.0 - 6.0 McFarland units (McF) (0 - 180 x 107 cells/cm3), along with McFarland turbidity standards of 0.5; 1.0 and 2.0 ($BaSO_4$), which corresponds to a *B. subtilis* cell size of 0.25-1 micrometers in width and 2-4 micrometers in length, and varies

slightly during the sporulation stage. The turbidity of the samples taken from the bioreactor was determined, followed by taking into account the size of the cells.

Results

To develop the technology of deep cultivation of the transformed strain *B. subtilis,* 3 main areas of research were selected, (I) Culture inoculum regimes (II) Culture pH regimes (III) Dissolved oxygen culture regimes. The modes presented will make it possible to determine the main factors and aspects of deep cultivation, which will serve as the basis for the development of modern tools and methods of mathematical modeling. The timing of the growth phases was examined using a densitometer (Figure 3) and Gram staining (Figures 4 and 5).

Figure 3 - Densitometer McFarland DEN-1B

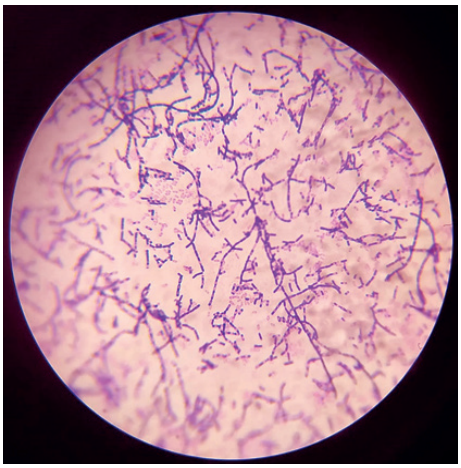

Figure 4 - Transient phase of sporulation

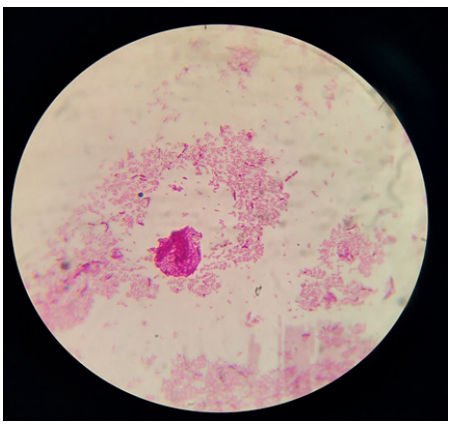

Figure 5 - the final phase of sporulation

Modes of seed concentration during cultivation

The development of modes of seed concentration during cultivation will allow the cost-effective use of seed material, taking into account 4 phases of bacterial growth. To work out the seed concentration, 5 modes were selected with an increasing concentration of bacterial cells per 1 cm3. These modes take into account the maximum and minimum seed concentration for the study. The results of the conducted studies are presented in table 1.

Table 1 - Results of testing modes of sowing concentration

№	Sowing concentration, cells/cm^3	Growth phase time, h				Maximum concentration cells/cm^3
		I	II	III	IV	
1.	100 thousand	20	72	96	108	2x10^6
2.	250 thousand	20	60	84	108	3x10^6
3.	500 thousand	20	56	72	108	4x10^6
4.	1 million	20	44	92	120	4x10^6
5.	2 million	20	44	92	120	4x10^6

According to the presented table, the most effective inoculation concentration is 1 million, where the maximum concentration reached 4x10^6 cells/cm^3, and the phase of logarithmic growth and stationary growth is the longest, which will allow efficient cultivation of strain *B. subtilis* in a bioreactor. Whereas low seed concentration cultivation regimes are characterized by slow reaching of the logarithmic and stationary growth phases, and fast reaching of the slow growth phase. Cultivation mode 5 with an inoculation concentration of 2 million cells/cm^3 does not differ from mode 4, so it is not advisable to spend extra money during cultivation.

pH levels during cultivation

The development of the pH level regimes will make it possible to take this factor into account during submerged cultivation in a bioreactor. The pH level during deep cultivation is one of the main factors that stimulate or limit the growth of bacteria. For the study, 5 modes were selected, where 3 modes are stationary, and 2 are dynamic with an increase / decrease in the pH level depending on the growth phases (Table 2).

Table 2 - results of testing the pH level modes

№	pH level	Growth phase time, h				Maximum concentration cells/cm^3
		I	II	III	IV	
1.	6,0	20	56	72	108	3x10^6
2.	7,0	20	56	72	108	3x10^6
3.	8,0	20	56	72	108	3x10^6
4.	6,0 → 8,0	20	72	96	108	2x10^6
5.	8,0 → 6,0	20	44	92	120	5x10^6

According to the results obtained, the most effective pH regimes for deep cultivation is dynamic regime 5, where the maximum concentration reached 5x106 cells/cm^3. In modes 1 to 3, the maximum concentration practically does not differ and is 3x106 cells/cm^3, while mode 4 turned out to be limiting and the maximum concentration was 2x106 cells/cm^3, this is due to the sensitivity of strain *B. subtilis* in the first phases to acidic environmental conditions, and the need for a pH level of 6.0 during growth phases 3 and 4.

Dissolved oxygen regimes during cultivation

The level of dissolved oxygen during deep cultivation is one of the most important factors, since the cultivated strain *B. subtilis* being a facultative aerobe is particularly susceptible to dissolved oxygen levels. For the study, there were 5 modes of the level of dissolved oxygen, 3 stationary and 2 dynamic. These modes take into account the effects of both low, high and varying dissolved oxygen conditions.

Table 3 - results of working out the modes of dissolved oxygen

№	Dissolved oxygen level	Growth phase time, h				Maximum concentration cells/cm^3
		I	II	III	IV	
1.	25%	20	44	92	120	4x10^6
2.	50%	20	44	78	92	2x10^6
3.	75%	20	44	60	78	2x10^6
4.	25% → 75%	20	44	60	78	2x10^6
5.	75% → 25%	20	44	60	78	2x10^6

According to the results obtained, the most effective regime is the lowest level of dissolved oxygen, where the maximum concentration was. It is worth noting that modes 1 and 4 reached growth phase 2 in the same amount of time, while phases 3 and 4 are strikingly different. And also, at high levels of dissolved oxygen, active spore formation is observed, limiting further growth o*f B. subtili*s.

Discussion

The use of recombinant technologies for the transformation of producer organisms makes it possible to obtain a high concentration of specific antigens, without the risk of fungal or extraneous bacterial contamination. The combined use of recombinant technologies, together with industrial methods of deep cultivation in a bioreactor, allows the most efficient accumulation of specific antigens. The use of modern recombinant technologies makes it possible to develop highly effective vaccines and diagnostic test systems. O'Neill and colleagues have developed a new vaccine based on recombinant major intrinsic protein and chlamydial protease activity factor proteins against *Chlamydia abortus* enzootic abortion in sheep. According to the results of their studies, these recombinant proteins are effective in inducing immune responses important for the treatment of chlamydial infections [15]. As a rule, diagnosing chlamydial infection by isolating the pathogen is difficult, since detection requires more than 2 weeks [16], which is why it is necessary to use modern diagnostic methods, such as ELISA. Specific detection of *Chlamydophila psittaci* can be analyzed using ELISA based on synthetic peptides. Recombinant major outer membrane protein [17] or outer membrane polymorphic protein [18, 19.]. A recently developed indirect ELISA based on recombinant proteins has shown its sensitivity and specificity for *Chlamydophila psittaci* [20]. The conducted studies will allow to determine the specific conditions of deep cultivation in bioreactors, as the main method for obtaining a large number of specific antigens, taking into account external and internal factors of growth, productivity, with the possibility of manual and automatic regulation of biotechnological stages. Deep cultivation in bioreactors is a modern high-performance solution that helps create unique conditions for each type of bacteria, including those in the presented studies, which will allow in the future to create libraries of cultivation models in accordance with the international Good Manufacturing Practice standard.

Conclusion

The most optimal parameters for submerged cultivation of the transformed *B. subtilis* strain were chosen: seed concentration of 1 million cells/cm³, at a dynamic pH level of 8.0→6.0 and at a level of dissolved oxygen of 25%, these cultivation parameters make it possible to obtain the concentration of the transformed strain *B. subtilis* within 4x106 and 5x106 cells/cm³.

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FATTY-ACID COMPOSITION OF IMPORTED NUTS SOLD IN THE MARKETS OF THE CITY OF ASTANA

Laura T. Auteleyeva, Balgabay S. Maikanov,
Ayana S. Smagulova

Faculty of Veterinary and Livestock Technology, S. Seifullin Kazakh Agrotechnical Research University, Astana, Republic of Kazakhstan

Corresponding author: Laura T. Auteleyeva, e-mail: laura_aut@list.ru
Co-authors: Balgabay S. Maikanov, e-mail: b.maikanov@kazatu.kz
Ayana S. Smagulova, e-mail: smagulovaayana28@gmail.com

Abstract

Nuts are high in protein, unsaturated fats, fiber, vitamins, and minerals. 70-80% of the fatty acids present in nuts and seeds are essential fatty acids, which are components of the plasma membrane and contain lecithin, a lipoprotein found in brain cells. The findings of investigations on the fatty acid makeup of several types of imported nuts in Astana's marketplaces are presented in this article. In this study, using the HPLC method, 17 samples of nuts imported from Uzbekistan, Iran, China have been analyzed. The experimental part was carried out in an accredited testing laboratory of Nutritest LLP, Almaty. As a result of the research, nut samples taken from the Shapagat, Alem, Sharyn, Astanalyk Bazaar, Eurasia-1 markets exceeded the norms of fatty acid composition from 1 to 20% in all samples. In addition, differences were found in the fatty acid profile of nuts of different types and origin. Peanuts from China, for example, have the highest quantities of palmitic acid, whereas cashews from Iran have the highest levels of stearic acid. Almonds also have the highest oleic acid values, while walnuts have the highest linoleic acid levels. These results may be useful in understanding the differences between nuts and choosing the most appropriate options for consumption based on individual needs and nutritional recommendations.

Key words: fatty acid; food safety; import; linoleic acid; oleic acid; polyunsaturated acids; nuts.

Introduction

Today, consumers are interested in a varied and balanced diet. As a result, the inclusion of nuts in the diet has increased dramatically due to their unique nutritional value, distinctive taste, aroma, nutritional properties and beneficial bioactive compounds. increasingly recognized [1].

Nuts are dry fruits with edible seeds and hard shells, with cashew nuts (Anacardium occidentale), walnuts (Juglans regia), almonds (Prunus dulcis), chestnuts (Castanea sativa), pistachios (Pistacia vera), and hazelnuts (Corylus avellana) being the most productive. They are high in protein, unsaturated fats, fiber, vitamins, and minerals. 70-80% of the fatty acids present in nuts and seeds are essential fatty acids, which are components of the plasma membrane and contain lecithin, a lipoprotein found in brain cells.

The ease of transport due to their estimate makes them indeed more prescribed for utilization in all circumstances. In expansion, nut utilization is regularly related with diminished hazard components for unremitting malady due to the composition of greasy acids, squalene, fiber, plant proteins, minerals, vitamins, carotenoids, and phytosterols with potential antioxidant impacts. Inquisitively, most of the cancer prevention agents in all nuts are within the shell, as appeared for almonds and peanuts, and these are misplaced when the skin is expelled. In expansion, in pistachios, most of the cancer prevention agents are crushed when the hard-shell splits [2].

The most commonly consumed are almonds, Brazil nuts, cashews, hazelnuts, pecans, peanuts, pine nuts, pistachios, walnuts and macadamia [3].

Due to their nutrient-rich composition, nut consumption has been associated with several health benefits such as improved lipid profile (lower cholesterol and triglyceride levels), improved

endothelial function and overall cardiovascular health, reduced glycemia and insulin resistance, diabetes prevention and delayed age-related cognitive decline [4].

The fatty acid composition of almonds is another noteworthy aspect, characterized by a substantial presence of monounsaturated (MUFA) fats at approximately 60%, along with polyunsaturated (PUFA) fats at around 30%. This composition predominantly encompasses oleic, linoleic, palmitic, or stearic acids [5, 6]. Summo et al.'s research further highlights the influence of genetic makeup on the fatty acid profile. While the essential fatty acids remain consistent across the examined varieties, variations can be identified in the individual quantities of each fatty acid, as well as in the cumulative content of unsaturated (mono- or polyunsaturated) and saturated (SFA) fractions. The polyunsaturated fatty acids in almonds not only give them nutritional value, but also make them more prone to self-oxidation, which speeds up spoilage and shortens shelf life. Thus, high levels of linoleic acid may indicate almond spoilage. For this reason, one of the most important quality indicators is the ratio of oleic and linoleic acids; high values of this ratio provide stability in oils and better nutritional value [7].

Cashews play a highly significant role as a nutritional source of fats, constituting approximately 47% [8] of their composition. Within cashews, there are 11 saturated fatty acids, amounting to 25.37% of the total content, featuring palmitic acid (12.20%), stearic acid (11.30%), arachidic acid (1.07%), and behenic acid (0.22%). Additionally, cashews consist of seven unsaturated fatty acids, which make up 71.98% of the overall content. These include oleic acid (51.47%), linoleic acid (19.66%), palmitoleic acid (0.36%), and eicosanoic acid (0.34%) [9]. Recent research underscores substantial variations in fat content and corresponding fatty acid profiles across different cultivation regions. Rico et al.'s study, examining 11 varieties of cashews, demonstrates that fat content ranges from 45.05 g/100 g in Vietnamese samples to 50.40 g/100 g in Kenyan samples. In terms of fatty acid profiles, oleic, linoleic, and palmitic acids emerge as the primary constituents [10].

Among the array of nut varieties, hazelnuts exhibit a notably elevated fat content, exceeding 60%. Certain scholarly sources even document fat levels surpassing 70%, contingent upon factors such as the specific cultivar or the position of the fruit. The lipid composition within hazelnuts predominantly comprises monounsaturated fatty acids (MUFAs), constituting approximately 80% of the cumulative fatty acid composition, with oleic acid prominently positioned as the predominant individual monounsaturated fatty acid. Additionally, the next significant fraction within hazelnut fat encompasses polyunsaturated fatty acids (PUFAs), primarily attributed to their linoleic acid composition. Nonetheless, certain research studies contend that saturated fatty acids (SFAs) might emerge as the second predominant cluster of fatty acids, influenced by the heightened presence of palmitic acid [11,12].

Like maximum different nuts, pistachios are excessive in fat, with available literature pointing out a value of around 50%, despite the fact that a few varieties may additionally have better fat content material, achieving values of up to 74.15%. Like different nuts, pistachio fat is rich in unsaturated fatty acids, namely MUFAs. This fraction specifically consists of oleic acid with the addition of palmitoleic acid, while the second maximum critical fraction, PUFA, in particular consists of linoleic acid [13]. As for SFAs, the fraction of minor fatty acids consists almost totally of palmitic acid [14].

The fats content of walnuts is very high, with average values that could only be exceeded with the aid of hazelnuts. Even though the fats content material is inside the 60% range, significant differences had been determined whilst comparing types. Values ranged from 49% to 82%. However, as referred to earlier, most reports show a fat cost of round 60%, with some version relying on the cultivar studied. Walnut fats is especially composed of unsaturated fatty acids, namely PUFAs, while MUFAs are the second one most vital type of fatty acids. Linoleic and linolenic acids are responsible for the excessive quantity of PUFAs, with oleic being the primary MUFA. In regards to the content of SFAs, palmitic and stearic acids are found in huge quantities [15,16,17].

Materials and Methods

The following samples were selected for the study: peanuts (China, Uzbekistan), cashews (Iran), almonds (Iran), walnuts (China, Greece, Kazakhstan). In total, 17 samples were examined, harvests of 2021 and 2022 in the markets of the city of Astana.

The experimental part was carried out in an accredited testing laboratory of Nutritest LLP, Almaty.

The comprehensive fatty acid composition analysis was conducted through high-performance liquid chromatography (HPLC) employing an HPLC Water liquid chromatograph (USA), following the guidelines outlined in MVI MN 1364-2000, titled "Methodology for the Gas Chromatographic Determination of Fatty Acids and Cholesterol in Food and Blood Serum." Experimental parameters within the laboratory encompassed a temperature range of 20.5-24.0 °C and a relative humidity range of 70-74%. Subsequent to the analysis, all gathered data underwent necessary adjustments and were subsequently presented as percentage values.

Results

The study analyzed the fatty acids contained in peanuts grown in Uzbekistan. As a result of determining the ratio of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) in peanut samples, it was found that the predominant acid among SFA (17.148%) was (C16: 0) palmitic acid, the content of which amounted to 11.216%. In the MUFA group (41.28%), oleic acid C18:1 prevailed, the content of which was 40.597%. Among PUFAs (41.387%), the proportion of linoleic acid was the largest and amounted to 39.877%.

According to Table 1, analysis of peanuts imported from China revealed that the saturated fatty acid (SFA) content in the sample was 18.931%, with palmitic acid (12.81%) being the predominant acid. At the same time, the proportion of monounsaturated fatty acids (MUFAs) was 40.23%, with similar proportions of oleic acid (39.4%) and linoleic acid (39.27%). Polyunsaturated fatty acids account for 40.835%

The available data indicate that analytical samples of peanuts imported from Uzbekistan and China contain (C16:0) saturated palmitic acid, (C18:0) stearic acid, (C18:1) oleic acid and (C18:2n6c) Linoleic fatty acids above standard values, twice the standard values in all samples.

Saturated fatty acids were mainly composed of 8 components, the total content of which does not exceed 18%. Among the saturated acids, palmitic acid (11–12%) and stearic acid (range 2–4%) were quantitatively distinguished. The remaining carboxylic acids are present in relatively small, sometimes trace, amounts in the oil. Of the unsaturated acids, oleic acid predominated in all cases. The relative content varied between 38 and 40% for many of the cultivars investigated. Among the polyunsaturated fatty acids, linoleic acid, which belongs to the ω-6 acid group, was conspicuous. Its relative content is 37-40%. For example, in peanuts grown in China [7] the level was 40.70%. This can be due not only to sorting, but also to weather, soil and agrotechnical conditions of plant cultivation.

Table 1 - Average concentration of fatty acids in peanuts imported from Uzbekistan and China

	Peanut Uzbekistan (n=3)	Peanut PRC (n=3)	Norm, %
Saturated fatty acids, %	17.148	18.931	
C14:0 Myristic	0.064	0.067	0.03
C15:0 pentadecanoic	0.032	0.028	
C16:0 palmitic	11.216	12.81	5.15
C17:0 margaric	0.063	0.050	
C18:0 stearic	2.67	3.896	1.10
C20:0 arachidic	0.060	0.064	
C21:0 geneucosan	0.023		
C23:0 tricosan	3.02	1.992	
Monounsaturated fatty acids, %	41.28	40.23	
C16:1 (cis-9) palmitoleic	0.047	0.037	0.01
C17:1 (cis-10) margaroleic	0.053	0.034	

C18:1 (cis-9) oleic	40.597	39.45	23.76
C20:1 (cis-11) eicosenoic	0.583	0.709	0.66
Polyunsaturated fatty acids, %	41.387	40.835	
C18:2 n6c linoleic	39.877	39.270	15.56
C18:3 n6 Y - linoleic	0.281	0.960	0
C20:3n3c (cis -11,14,17) eicosotriene	1.162	0.605	
C20:4 n6 arachidonic	0.067		

In the course of this study, the average concentration of SFA in walnuts from Uzbekistan was determined to be 11.23%, mainly with a high content (C16:0) of palmitic acid (6.68%). The fat present in the walnut from Uzbekistan is mainly composed of PUFAs, which is about 70% of the total fatty acids, and linoleic acid is the main polyunsaturated fatty acid. Monounsaturated fatty acids, which were about 20%, represent the second main fraction in walnut fat, almost exclusively due to the content of oleic acid.

The study found that the fat in Chinese walnuts contains mostly polyunsaturated fatty acids (67.418%), with the second most important type being monounsaturated fatty acids. Linoleic and Y-linolenic acids make up the majority of polyunsaturated fatty acids, with oleic acid (24%) being the most important monounsaturated fatty acid. The content of saturated fatty acids is an important part of the total fat composition, especially palmitic and stearic acids present in large amounts.

In each sample, the excess of the level of oleic acid was found to be two times higher, and the content of linoleic acid also exceeded the permissible norm by two times.

Table 2 - Average concentration of fatty acids in walnuts imported from Uzbekistan and China

	Walnut Uzbekistan (n=3)	Walnut PRC (n=3)	Norm
Saturated fatty acids, %	11.23	8.189	6.20
C14:0 Myristic	0.048	0.034	0.50
C15:0 pentadecanoic	0.019	0.027	
C16:0 palmitic	6,680	5.459	4.40
C17:0 margaric	0.041	0.046	
C18:0 stearic	4.277	2.508	1.30
C20:0 arachidic	0.166	0.115	
C21:0 geneucosan			
C23:0 tricosan			
Monounsaturated fatty acids, %	20.072	24.394	14.70
C16:1 (cis-9) palmitoleic	0.064	0.048	0.20
C17:1 (cis-10) margaroleic		0.046	
C18:1 (cis-9) oleic	19.980	24.142	11.0
C20:1 (cis-11) eicosenoic	0.128	0.155	1.10
Polyunsaturated fatty acids, %	68.698	67.418	40.40
C18:2 n6c linoleic	58.339	58.289	33.30
C18:3 n6 Y - linoleic	10.358	9.128	7.10
C20:3n3c (cis-11,14,17) eicosotriene			
C20:4 n6 arachidonic			

Studies of the fatty acid composition in cashew nuts gave the following results: where there is a high content of palmitic acid (9.195%), oleic (63.010%) and linoleic acid (16.976%). Cashews are considered a valuable product for their high content of monounsaturated fatty acids (63.401%).

At the same time, all indicators exceeded the established norms twice.

Almonds are rich in monounsaturated fatty acids, the share of which is 70.590% of the total content, among which 70% is oleic acid, which exceeds the allowable content twice. Almond kernels contained 6.487% palmitic acid, 0.312% palmitoleic acid, 1.857% stearic acid, 70.158% oleic acid, and 20.986% linoleic acid.

Table 3 - Average concentration of fatty acids in cashews and almonds imported from Iran

	Cashew Iran (n=2)	Norm	Almond Iran (n=3)	Norm
Saturated fatty acids, %	19.527	9.16	8.401	3.80
C14:0 Myristic	0.039	0.35	0.025	0
C15:0 pentadecanoic	0.025			
C16:0 palmitic	9.195	4.35	6.487	3.08
C17:0 margaric	0.123		0.038	
C18:0 stearic	10.109	2.97	1.857	0.70
C20:0 arachidic				0.01
C21:0 geneucosan	-		-	
C23:0 tricosan	0.037			
Monounsaturated fatty acids, %	63.401	27.32	70,590	31.55
C16:1 (cis-9) palmitoleic	0.274	0.32	0.312	0.24
C17:1 (cis-10) margaroleic	0.032		0.075	
C18:1 (cis-9) oleic	63,010	26.81	70.158	31.29
C20:1(cis-11) eicosenoic	0.085	0.14	0.046	0.01
Polyunsaturated fatty acids, %	17,073	7.84	21.009	12.33
C18:2n6c linoleic	16.976	7.66	20.986	12.32
C18:3n6 Y-linoleic	0.097	0.16	0.023	
C20:3n3c (cis-11,14,17) eicosotriene	-		-	
C20:4n6 arachidonic	-		-	

Discussion

Walnuts are prized for their high oil content. As you know, it contains polyunsaturated fatty acids such as oleic acid, linoleic acid and linolenic acid. Stearic acid and palmitic acid are types of saturated fatty acids. In fact, walnuts contain 40 to 500 times more omega-3 fatty acids than other nuts [18].

As a result of the study, the fatty acid composition of 17 walnut samples was analyzed and showed palmitic acid content (C16:0) in peanuts (China) was 12.8%, the highest among the samples evaluated in this study. Stearic acid content (C18:0) was highest in cashew nuts (Iran) 10.109%. In addition, the oleic acid content (C18:1) in almonds is 70.158%, the highest in walnuts, while walnuts have a low content of 20.1%. The highest linoleic acid content (C18:2) is 59.58% in nuts, the lowest is observed in cashew, where its density is only 16.976%.

Similar studies were carried out by foreign scientists, but depending on the variety and other factors, the results were as follows.

According to Cardassilaris, China's Shanxi province is experiencing a booming walnut culture, with particular emphasis on breeding varieties with a high oil content. Studies show that the oil content of different varieties from the same region varies from 59.4% to 71.5%, with an average of 65.9%. The variety "Xifu No. 1" stands out with the highest oil content - 71.5%. The average value of unsaturated fatty acids is about 92.4%, and the ratio of unsaturated to saturated acids is 7.6:1.

They also identified that among different varieties there were differences in the composition of fatty acids. The oleic acid-rich variety was "Jinglong No. 2" (40.5%), the linoleic acid-

rich variety was "Liaoning No. 1" (66.5%), and the linolenic acid-rich variety was "Sifu No. 1" (13.5%). The N-6: N-3 ratio was closest to 4:1 in Zhonglin No. 1 (4.6:1) [19].

According to the research conducted by Alshannaq A. and colleagues, pistachios, similar to the majority of other nut varieties, possess a significant fat content, with existing studies suggesting an approximate value of 50%. However, some variations might lead to certain strains having notably elevated fat levels, with measurements reaching as high as 74.15%. Comparable to other nuts, pistachios exhibit a substantial abundance of unsaturated fatty acids, specifically monounsaturated fatty acids (MUFAs). This particular category is primarily dominated by oleic acid, along with the inclusion of palmitoleic acid. The following consequential segment, polyunsaturated fatty acids (PUFAs), is predominantly constituted by linoleic acid [20]. Further findings from X. Fan et al. established that saturated fatty acids (SFAs), a fraction consisting of minor fatty acids, predominantly comprises palmitic acid [21].

Conclusion

In conclusion, this study examining the fatty acid composition of imported nuts within the city markets of Astana has furnished significant insights into the variety and distinctions within the composition of diverse types and origins of nuts. The findings underscored the substantial presence of essential fatty acids in nuts, which hold a crucial function in facilitating plasma membrane integrity and promoting brain cell functionality.

The study also found that nut samples taken from the markets exceeded the fatty acid composition, which can be important information for consumers and producers regarding product quality and safety. Differences in fatty acid composition between different types of nuts and their origins allow for conscious choices when selecting nuts.

Overall, the results of the study highlight the importance of nuts in the diet, due to their rich composition of vegetable protein, unsaturated fats, fiber, vitamins and minerals. Further research in this area may be useful to increase knowledge about the nutritional properties of nuts and their impact on human health.

This study highlights the importance of quality food analysis and composition control for food safety and consumer awareness. This data can be valuable to regulators and manufacturers in developing quality standards and controlling the production and import of nuts.

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VIABILITY AND ANTAGONISM OF CRYOPRESERVED LACTIC ACID BACTERIA

Kaiyrtay Kh. Almagambetov, Zinigul S. Sarmurzina, Anzhela A. Chshelkina, Daniyal Sh. Zhakenov, Bakhytgul J. Sadykova, Gulmira N. Bisenova, Serik M. Shaikhin

LLC "Republican collection of microorganisms", Astana, Republic of Kazakhstan

Corresponding author: Kaiyrtay Kh. Almagambetov, e-mail: rcmkz.list.ru
Co-authors: Zinigul S. Sarmurzina, e-mail: sarmurzina@list.ru
Anzhela A. Chshelkina, e-mail: ashhelkina@mail.ru
Daniyal Sh. Zhakenov, e-mail: zhakenov.daniyal@mail.ru
Bakhytgul J. Sadykova, e-mail: bakhytgul.sadykova130384@mail.ru
Gulmira N. Bisenova, e-mail: bissenova84@mail.ru
Serik M. Shaikhin, e-mail: rkm_shaikhin@mail.ru

Abstract

Preserving and advancing bioresources involving industrial microorganisms is of paramount importance for every nation. However, long-term storage of these strains often leads to diminished viability and biological activity. Thus, it is crucial to investigate the properties of cryopreserved strains stored at -80°C in a 10% glycerol solution within low-temperature refrigerators. This study aimed to comparatively analyze the viability of 129 lactic acid bacteria strains, including *Lactobacillus sp., Lactococcus sp.*, and *Pediococcus sp.*, cryopreserved from 2006 to 2020. Among them, 93 *Lactobacillus sp.* strains were categorized into three groups based on storage dates (2006-2007, 2013-2014, and 2017-2020). Viability titers were determined using the standard serial dilution method, counting microorganisms in CFU/ml. Regardless of the storage duration or species affiliation, the study identified lactic acid bacteria strains exhibiting both high (107 - 109 CFU/ml) and low (104-106 CFU/ml) viability titers. Additionally, the antagonistic activity of 33 *Lactobacillus sp.* strains was investigated using the delayed antagonism method, subdividing them into 17 strains with sufficiently high viability titers and 16 strains with low viability titers. The results revealed that 20% of strains with high viability titers and 27% with low viability titers exhibited relatively high antagonistic activity (with a zone of inhibition ranging from 10 to 18 mm). In both groups, strains with low antagonistic activity (with a zone of inhibition measuring 5-9 mm), particularly against Gram-positive and Gram-negative bacterial test-cultures, predominated. Significantly, 51% of lactobacilli strains demonstrated pronounced antagonism against *Candida albicans* ATCC- 885-653 test-culture. These findings underscore the practical importance of the study, emphasizing the necessity to analyze and select optimal concentrations of intracellular or extracellular cryopreservatives and to determine the initial viability titer when storing strains for cryopreservation. Tailoring cryopreservation solutions to each strain can enhance the preservation of their original properties, ultimately improving overall preservation quality.

Key words: Antagonism; cryopreservation; lactic acid bacteria; viability.

Introduction

The development and use of probiotics in young farm animals and poultry to increase resistance to intestinal infection, and immunomodulatory effect is actual in veterinary medicine [1]. Lactic acid bacteria, especially lactobacilli, are most often used as probiotic microorganisms.

Therefore, the preservation and replenishment of collections of lactic acid bacteria are necessary for the development of modern effective probiotic preparations based on them. More than 130 strains of lactobacilli of various species isolated from human organisms, fermented milk products, vegetation, etc. are deposited in the biobank of the Republican Collection of Microorganisms. The main task of the biobank of industrial microorganisms is to preserve the viability

and original biological properties of deposited strains of lactobacilli. Long-term storage of lactobacilli strains in the biobank is carried out by cryopreservation at minus 80°C in 10% glycerol solution.

Cryopreservation is one of the most widely used methods for long-term storage of microorganisms [2]. Eukaryotic and prokaryotic microorganisms quite stably retain viability and original biological properties both under conditions of cold stress and vacuum drying.

At the same time, it is known that during cryopreservation microbial cells are exposed not only to low temperatures but also to damaging physicochemical factors arising from water phase transitions, such as ice crystal formation, changes in the pH of the medium, and significantly changes intra- and extracellular osmotic and oncotic concentration gradients. Freezing and thawing with intracellular ice crystals and high concentrations of intra- and extracellular ingredients damage membrane structures. Free radicals and peroxide compounds formed as a result of oxidative reactions also disrupt the structure of polymers within cellular and intracellular membranes - lipids and proteins, and damage nucleic acids.

Analysis of literature data on the determination of viability of cryopreserved lactobacilli has shown the following. Some studies show stable preservation of viability and biological activity by lactobacilli under cryopreservation conditions [3, 4]. In other studies, it was revealed that some strains, regardless of the species affiliation of lactobacilli, lost their viability under conditions of low-temperature stress, their lgKOE/ml decreased by 1 - 2 orders of magnitude [5]. While some studies revealed better survival of bacterial cultures in case of slow cooling of cells [6], others showed that, on the contrary, at low cooling rates, most of the cells die at the freezing stage, possibly due to prolonged exposure to cold osmotic stress [7].

Preservation of the antagonistic activity of lactobacilli is a necessary condition for their use in the development of probiotic preparations [8]. It is conditioned by the production of organic acids (lactic, acetic), hydrogen peroxide, and lactocin production [9, 10].

It is known from the literature that the antagonistic activity of lactobacilli does not depend on their species affiliation and may differ between different strains within a species [11].

The antagonistic activity of lactobacilli to Gram-negative Enterobacteriaceae is well known and is the basis for the use of lactic acid bacteria in dysbiotic conditions as probiotics, both in medicine and veterinary medicine. At the same time, concerning yeasts of the genus Candida, several studies have revealed both high inhibitory activity and its absence in lactic acid bacteria. In vitro, co-cultivation showed a mutual inhibitory effect between *Candida albicans* and L*actobacillus plantarum*. The authors of the study explain this by competition for nutrients and colonization of the epithelial mucosa of the oral cavity and vagina [12, 13]. Also, the co-cultivation of the production strain of lactobacilli and Candida on a solid nutrient medium revealed a significant decrease in the number of colonies for all *Candida albicans* strains taken in the experiment compared to the control [14].

Preservation of viability of cryopreserved lactobacilli is not a guarantee of preservation of their antagonistic activity. Therefore, this study aimed to analyze both the viability and antagonistic activity of lactobacilli strains cryopreserved in a biobank. Commonly used methods for determining viability by serial dilutions [15, 16] and antagonistic activity of lactic acid bacteria against test cultures [17, 18, 19] were used.

Materials and Methods

The study was conducted at the Republican Collection of Microorganisms (RCM) biobank between April 2022 and June 2023. Microbiological procedures were performed within controlled environments using nutrient media, reagents, and light microscopy to examine Gram-stained micro-specimens. The research materials comprised cryopreserved lactic acid bacteria strains sourced from fermented dairy products, human and animal intestines, and plant surfaces, all stored in low-temperature refrigerators at -80°C.

Lactobacilli, lactococci, and paediococci were used for the determination of viability titre. The viability titre of 93 strains of lactobacilli represented by 13 species (*L. casei, L. brevis, L. fermentum, L. plantarum, etc.)*, 19 strains of lactococci (*L. lactis, L. diacetilactis*) and 17 strains of paediococci (*P. pentosaceus*) was studied.

Thirty-three cryopreserved strains of lactobacilli, including *L. case*i - 9, *L. acidophilus* - 6, *L. fermentum* - 6, *L. plantarum* - 5, *L. brevis* - 4, *L. cellobiosus* - 2 and *L. pentosus* - 1 strain were

used to determine antagonism.

The following test cultures were used to evaluate the antagonistic activity: E*scherichia coli* 157, *Staphylococcus aureus* 209 P, *Serratia marcescens* 221 F, *Salmonella typhimurium* TA 98 and *Candida albicans* ATCC- 885-653.

The following nutrient media were used for the cultivation of lactic acid bacteria and test cultures: MRS-1, MRS-4, Sabouraud, Czapek, Endo, MPA, MPB.

To determine the viability titre, the serial dilution method was used, which is a series of serial dilutions, each with the same dilution factor, with the diluted material from the previous step being used for the subsequent dilution. The number of bacteria present in the original sample was calculated by multiplying the number of colonies formed by the dilution factor, in CFU/ml.

The antagonistic activity of lactobacilli was studied by a modified delayed antagonism method - well diffusion method. The method is based on the diffusion of antibiotic substances formed by the tested strains of lactobacilli into the agar medium containing the test culture and inhibiting the growth of the latter. A 200 µl daily culture of lactobacilli grown on MRS-1 was placed in the volume of 200 µl into wells cut in the thickness of dense nutrient medium with the sowing of the test culture.

Results

Viability titres of cryopreserved lactic acid bacteria - lactobacilli, lactococci, and paediococci - were studied in a comparative aspect (Table 1). In 73.5% of the studied strains of lactobacilli, the viability titres were quite high ($4.1x10^7$ - $4.1x10^9$ CFU/ml), and low titres ($2.0x10^4$ - $4.8x10^6$ CFU/ml) were found in 26.5%. Among cryopreserved lactococci and paediococci, strains with viability titres of 10^7 - 10^9 CFU/ml were also predominant (in 79% and 94%, respectively). Low viability titres were detected in 21% of lactococci and 6% of paediococci.

Table 1 - Viability titre of lactic acid bacteria cryopreserved in 2006-2020

Number of lactic acid bacteria (n, %)	Viability titre (CFU/ml, %)	
Lactobacillus sp. n – 93	$2,0\times10^4$ - $4,8\times10^6$ 26,5%	$4,1\times10^7$ - $4,1\times10^9$ 73,5%
Lactococcus sp n- 19	$1,0\times10^4$ - $7,4\times10^6$ 21%	$3,5\times107$ - $1,2\times109$ 79%
Pediococcus sp. n-17	$1,0\times10^4$ - $6,2\times10^6$ 6%	$2,9\times10^7$ - $1,6\times10^9$ 94%

The viability titres of 93 strains of lactobacilli were studied separately in a comparative aspect by years of cryopreservation (Table 2). The viability titres of 3 groups of lactobacilli cryopreserved in 2006-2007 (37 strains), 2013-2014 (32 strains), and 2017-2020 (22 strains) were studied.

Table 2 - Viability titre of lactobacilli cryopreserved 2006-2020

Number of lactobacilli (n, %)	Viability titre (Mcr, CFU/ml, %)	
2006 - 2007 гг. n-39	$1,0\times10^4$ - $3,9\times10^6$ 25,6 %	$4,9\times10^7$ - $4,2\times10^9$ 74,4%
2013 - 2014 гг. n-32	$2,0\times10^4$ - $4,6\times10^6$ 37,5%	$3,0\times10^7$ - $2,7\times10^8$ 62,5%
2017 - 2020 гг. n-22	$1,0\times10^4$ - $8,0\times10^6$ 22,7%	$3,9\times10^7$ - $2,1\times10^9$ 77,3%

Comparative analysis of the viability titre of 93 strains of lactobacilli represented by 13 species (*L. casei, L. brevis, L. fermentum, L. plantarum, etc.*) cryopreserved in different years showed the following. Out of 39 strains of lactobacilli cryopreserved in 2006, 29 (74.4%) showed sufficient viability titre (10^7 -10^9 CFU/ml), and low titre (10^4 - 10^6) was detected in 10 strains (25.6%). The viability titer of lactobacilli cryopreserved in 2013 - 2014 in 20 cases (62.5%) was 107 - 108 CFU/ml, and in 12 (37.5%) - 104 - 106 CFU/ml. In the third group of lactobacilli cryopreserved in 2017 -2020, 16 (77.3%) of the 22 strains studied showed medium viability titre (10^7 - 10^9 CFU/ml). Low viability titre (104 - 105 CFU/ml) was detected in 5 strains (22.7%).

Figure 1 shows the result of determining the viability titre of L. plantarum 8RA-3pl+(15), it is equal to 6×10^8 CFU/ml. While in Streptococcus lactis AMS-23 (0048) and Streptococcus cremoris K-1 (0049) titres do not exceed 1×10^5 CFU/ml (Fig.2).

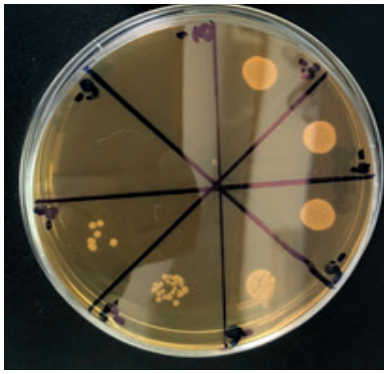


Figure 1 - Viability titre
L. plantarum 8RA-3pl+ (15) (0048)

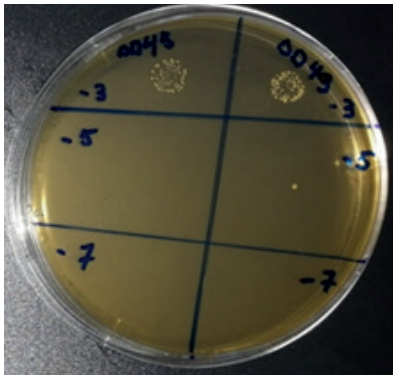


Figure 2 - Viability titres of Str. lactis
and *Str. cremoris (*0049)

Along with the preservation of viability (10^7 - 10^9 CFU/ml), it is important to preserve the antagonistic activity of cryopreserved lactobacilli strains. Therefore, the antagonistic activity of 33 lactobacilli strains was investigated, including *L. casei* - 9, *L. acidophilus* - 6, *L. fermentum* - 6, *L. plantarum* - 5, *L. brevis* - 4, *L. cellobiosus* - 2 and *L. pentosus* - 1 strain. The above strains cryopreserved in the RKM biobank in 2002 - 2020 were divided into 2 groups according to the value of viability titre: 16 strains with low (104 - 106 CFU/ml) and 17 with relatively high (10^7 - 10^9 CFU/ml) number of viable microbial cells. The results of the study of the antagonistic activity of cryopreserved strains of lactobacilli with high and low viability titre are shown in Table 3.

Table 3 - Antagonistic activity of cryopreserved lactobacilli

Groups studied, number of strains (n)	Growth suppression zone size (mm)		
	10-18 mm	5-9 mm	1-4 mm
High viability titre, n - 17	20,0%	42,3%	47,7%
Low viability titre, n - 16	27,5%	30,0%	42,5%

In our studies, the highest antagonistic activity towards test cultures (10 - 18 mm) was found in 20.0% of lactobacilli strains with relatively high viability titre and 27% with low titre. Low antagonistic activity (1 - 9 mm) was shown by 80% of strains with relatively high viability titre and 72.5% with low titre.

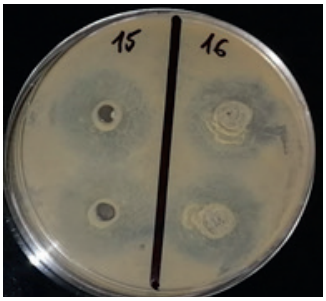
The antagonistic activity of the above 33 cryopreserved lactobacilli strains to each of the test cultures was also analyzed separately. The results of determining the antagonistic activity of the studied strains of lactobacilli to each of the test cultures are given below (Table 4).

Table 4 - Antagonistic activity of cryopreserved Lactobacillus strains to test cultures

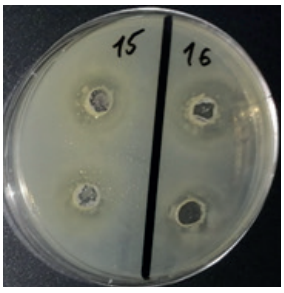
Test cultures	Number of lactobacilli strains (n) by zone of inhibition		
Zone of inhibition	10 - 18 mm	5 - 9 mm	1 - 4 mm
S. aureus 209P	4	15	14
S. marcescens 221 F	3	13	16
S. typhimurium TA98	1	15	17
E. coli 157	6	12	15
C. albicans ATCC- 885	17	4	12

There is a significant difference in the frequency of antagonistic activity of cryopreserved lactobacilli strains to gram-negative and gram-positive bacterial test cultures on the one hand and to fungi of the genus Candida on the other. Expressed antagonism (10 - 18 mm) to *Escherichia coli* 157 was detected in 6 strains, to *Staphylococcus aureus* 209 P in 4 strains, to *Salmonella typhimurium* TA 98 in 2 strains, to *Serratia marcescens* 221 F in 3 strains, and to *Candida albicans* ATCC- 885-653 in 17 strains. If to bacterial test-cultures the expressed antagonism (more than 10 mm) was revealed only in 6 - 18%, then to yeasts of Candida genus in 51% of cryopreserved strains of lactobacilli.

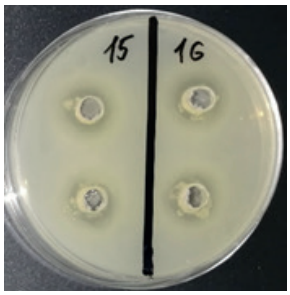
Figure 3 shows the results of the antagonistic activity of cryopreserved *L. plantarum* 8RA-3pl+ and *L. plantarum* 8RA-3pl- strains to test cultures. The antagonism to gram (+) and gram (-) bacterial test cultures is low but quite pronounced against fungi of the genus Candida.



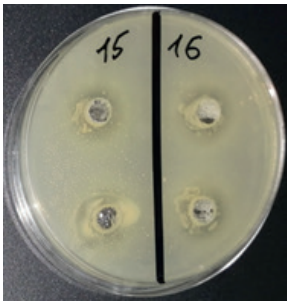
C. albicans ATCC- 885-653



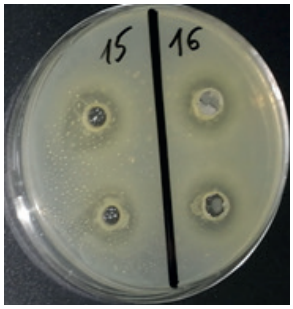
S. aureus 209 P



E. coli 157



S. marcescens 221 F



S. typhimurium TA 98

Figure 3 - Antagonistic activity of *L. plantarum* 8RA-3pl+ (15) and *L. plantarum* 8RA-3pl-(16) to test cultures

Discussion

A study of the viability of 129 strains of lactic acid bacteria (*Lactobacillus sp., Lactococcus sp.,* and *Pediococcus sp.*) cryopreserved between 2006 and 2020 revealed that microorganisms with high and low viability titres were equally found regardless of the storage dates. A separate study of the viability of 93 strains of *Lactobacillus sp.* divided into 3 groups according to storage dates (2006 - 2007, 2013 - 2014, and 2017 - 2020) also showed that the titre value did not depend on the duration of their storage in low-temperature conditions. Comparative analysis of the viability titre of cryopreserved lactobacilli of different species (*L. casei, L. fermentum, L. plantarum, etc.*) did not show any differences in titre depending on their species affiliation. This pattern was not only observed between different species of lactobacilli but also among strains within species. Thus, this study shows that there is no significant difference in the titres of viability and antagonistic activity between different taxonomic groups of lactic acid bacteria. At the same time, a difference in these indicators was found between strains within species. Such dynamics of viability and antagonism indicators may be due to different resistance to cold stress among strains of lactic acid bacteria.

The results obtained are consistent with other studies on the identification of cryo-resistant strains, on the determination of cryopreservation modes and concentrations of intra- and extracellular cryoprotectants necessary for optimal preservation of biological properties of lactic acid bacteria [11, 14, 20].

It is of practical interest that the study of antagonism of 33 strains of cryopreserved lactobacilli to test cultures revealed relatively high activity against fungi of the genus Candida, to a lesser extent against Gram (+) and Gram (-) bacteria. Thus, strains of lactobacilli with both low and relatively high titre of viability only in 20 -27.5% of cases showed pronounced antagonism to test cultures. However, in relation to *C. albicans*, more than half of the studied strains (51%) showed pronounced antagonism.

Conclusions

Viability titre values of lactic acid bacteria strains cryopreserved in different years are not related to storage duration.

No regular relationship between the antagonistic activity of the strains studied and the value of their viability titre was revealed. Cryopreserved strains of lactic acid bacteria can have rather high antagonistic activity at low viability titer and vice versa.

The antagonistic activity of the studied strains of lactobacilli is high in relation to *C. albicans* ATCC-885.

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WASTE AND BY-PRODUCTS FROM THE MEAT INDUSTRY AS A SOURCE OF BIOACTIVE COMPOUNDS

Anton Glimåker[1], Andriy Getya[2], Oksana Kravchenko[3], Galia Zamaratskaia[1]

[1]Swedish University of Agricultural Sciences, Sweden
[2]National University of Life and Environmental Sciences of Ukraine, Kyiv, Ukraine
3Poltava State Agrarian University, Poltava, Ukraine

Corresponding author: Galia Zamaratskaia, e-mail: galia.zamaratskaia@slu.se
Co-authors: Anton Glimåker, e-mail: angr0017@stud.slu.se
Andriy Getya, e-mail: getya@ukr.net
Oksana Kravchenko, e-mail: oksana_house@ukr.net

Abstract

The aim of this review was to summarize existing knowledge on bioactive peptides from the waste and by-products from meat industry and identify future directions of research. Reducing food waste and transforming it into food and feed is a step toward the achievement of economic development while reducing adverse effects on the environment. Meat production and consumption have increased in recent years. Above 20% of meat is wasted in Europe throughout the different steps in the production chain. Meat waste and by-products are rich sources of proteins, bioactive compounds, essential amino acids, vitamins and minerals, and have a potential to be further used for dietary and non-dietary purposes. Bioactive peptides often have health-promoting effects such as antioxidant, anti-hypertensive, anti-inflammatory, antimicrobial and antitumor activities when ingested and absorbed by human beings. Some bioactive peptides were shown to reduce the risk of development of cancer, diabetes and cardiovascular disease, which are among the most common diseases currently. Several methods to isolate bioactive peptides from meat waste and by-products were developed. Enzymatic hydrolysis can generate hydrolyzates and certain bioactive peptides from larger proteins, and the hydrolyzates can be used as dietary supplements or as an additive to increase the protein content of food. Bioactive peptides can be isolated from the hydrolyzates and used as functional food components, dietary supplements or medicines. We concluded that wastes and by-products from the meat industry present an opportunity for the generation of health-promoting bioactive compounds which can be successfully used in food and other relevant industries. However, existing research mainly is mainly focused on the influence of individual bioactive peptides on certain health parameters and is mainly performed using in vitro methods. Thus, future research should be directed to assess the long-term effects of bioactive compounds as a component of the whole diet.

Key words: animal protein; bioactive peptides; by-products; food security; food waste; meat.

Introduction

An extensive transformation of the existing food production chain and food consumption is vital for reaching the Sustainable Development Goals (SDGs) [1]. The livestock sector is responsible for approximately 14.5% of global greenhouse gas (GHG) emissions and GHG from animal-based foods are twice higher than GHG of plant-based foods [2, 3]. Thus, beef production is responsible for 35.3%, dairy cattle 30.1%, swine 9.5% and poultry 8.7% [4].

Food waste, unwanted and/or unused material of the primary production and/or consumption, is an important issue globally. In 2019, 931 million tons of edible foods were wasted [4]. Reducing food waste reduces the green gas emission, protects natural resources and increases global food security. The demand for food is constantly increasing because of increasing of human population and, consequently, food consumption. Along the production line of food,

from field to fork food waste occurs in all stages in all sectors. Reducing food waste in the meat industry is important for both economic and environmental reasons. It was estimated that 23% of the production in the meat sector in Europe 2018 through all stages, from primary production through post-harvest, manufacturing, distribution and consumption, is wasted [4]. From this, 64% of the waste occurs at the consumption stage.

A number of studies have emerged studying the quantities and types of meat waste generated during the production and consumption stages [5-7]. In those studies, different aspects were considered including reasons for food waste generation, strategies of prevention, and consumer's attitudes and behavior.

Most meat waste includes trimmings, cuttings, bones, collagen, carcasses, skins, fatty tissues, hoofs, internal organs, and blood. The definition of a by-product often depends on traditions, culture and religion; therefore, waste products and by-products definitions vary between geographical regions. Trimmings are meat portions which are left behind after the preparation of primal cuts from the carcass. Trimmings include fat, gristles, and meat, and are obtained by removal of muscle traces from the bones after the deboning process. Head meat, internal organs, major tendons and ligaments are not regarded as trimmings. Up to 30% of the live weigh of livestock animals can be considered as edible by-products.

Generally, waste from meat industry is rich in proteins and fat and contain a range of essential minerals and vitamins [8]. Protein-containing meat waste is an attractive material for the production of bioactive peptides with health-promoting properties [9]. Thus, meat waste generated can be potentially treated as a raw material for production of various biomaterials including food additives, medical preparations and feed material.

Therefore, the aim of the present study was to provide a summary of existing information on bioactive compounds from the waste and by-products from meat industry, identify future challenges and highlight areas where more research is needed.

Materials and methods

A literature study was conducted using the databases Scopus, Web of Science and PubMed. Peer-reviewed scientific publications have been selected using a combination of several keywords including: meat waste, meat by-products, animal bioactive peptides, meat bioactive compounds, by-product nutritional composition. Relevant networking websites, rapports, authority scientific opinions and conference proceedings have also been evaluated. Only publications written in English were included in the study.

Results and discussion

Categories of meat by-products

Food safety is a critical aspect as unsafe food poses global health threats. Even though by-products might be important sources of nutrients, their safety should be considered. To achieve safe products there are strict rules for the use of meat by-products.

Within the European Union, meat by-products are generally grouped into 3 categories based on their risk to human or animal health [10]. The categories are presented in Table 1. Category 1 includes by-products which have a very high risk, category 2 by-products have from medium to high risk and category 3 by-products have the lowest risk. Animal by-products posing a high risk should only be used for purposes outside the feed chain.

Waste content

Generally, waste from meat industry is rich in proteins and fat and contain a range of essential minerals and vitamins. Protein-containing meat waste is an attractive material to produce bioactive peptides with health-promoting properties [9]. Thus, meat waste and by-products can be potentially used to enhance the nutritional quality and functional value of foods. Edible fats separated during meat processing can be used in bakeries and confectionery, for cooking and frying, and for enhancing the flavor and texture of some foods [11]. Waste products can be used as a source of emulsifying and texturizing agents, colourants and the source of bioactive compounds [12].

Protein in waste and by-products from meat industry

Many meat by-products and waste are rich in proteins and amino acids. Blood is an example of a protein-rich waste product. The amount of blood generated during meat production is high and blood acts as a pollutant for the environment if disposed directly into water bodies [13].

However, the high protein content of blood makes it potentially useful in the food industry, as it can be used to enhance the nutritional value of other foods. The modern slaughterhouses in developed countries are equipped with waste management lines to separate blood from other parts and collect blood for animal feed or fertilizer purposes [14]. If the bovine blood has no infectivity risk like TSE, it may be used for pet foods and feeds for livestock [10]. If blood is hygienically collected and managed at approved slaughterhouses, it can be considered for human consumption [11].

Table 1- Meat by-products categories according to the European Union

Category	Risk	Examples of by-product	Examples of use
1	Very high	Material at a TSE risk, such as Specified Risk Material (e.g. bovine spinal cord). Pet animals, zoo and circus animals.	Destroyed by incineration, or by rendering followed by incineration (TSE suspects). Some material can be pressure-rendered and disposed of in an authorised landfill site. Some material to be used as a fuel for combustion, although there are no existing rules for this. Some material can also be used for the manufacture of medical devices.
2	Intermediate	Fallen stock, manure and digestive content, milk, colostrum	Some material can also be used for the manufacture of medical devices. Production of fuel, biodiesel, biogas. Some material can be pressure-rendeed and used for the production of organic fertilisers or in an approved composting or anaerobic digestion plant.
3	Low	Carcasses and parts of animals slaughtered which are not finally destined to human consumption but fit for human consumption. Hides, hair, feathers, bones	Production of pet food and organic fertilisers or soil improvers. Production of animal feedingstuffs, though TSE related restrictions on the feeding of processed animal protein restrict this.

Skin, bones and cartilages are also rich in proteins, such as collagen. Collagen is widely used in the food, pharmaceutical and cosmetic industries. In the food industry, collagen is used as a food additive and as a packaging material [15]. It is possible to extract collagen from the skin, ears and other waste material from meat industry, as it was demonstrated by many research groups. Collagen from rabbit by-products was also recently extracted and characterized [16]). This is important knowledge for Ukrainian industry as rabbit production will likely increase after the end of Russian military aggression [17].

Thus, efforts are needed to develop novel processes to efficiently use these waste and by-products and turn them into high-value material.

Bioactive peptides: definition and activity

Bioactive peptides are molecules of 3-20 amino acid residues with beneficial effects on health because of their potential biological activities. Those peptides are inactive when kept within the parent protein, and become active only after the cleavage of the proteins. The health-promoting activities include antimicrobial, antihypertensive, antioxidant, immunomodulatory and anti-inflammatory [18] (Figure 1). To perform their bioactivity, peptides must be absorbed, have low or no toxicity, and do not have an unpleasant taste. Bioactive peptides are easily excreted from the body and do not accumulate.

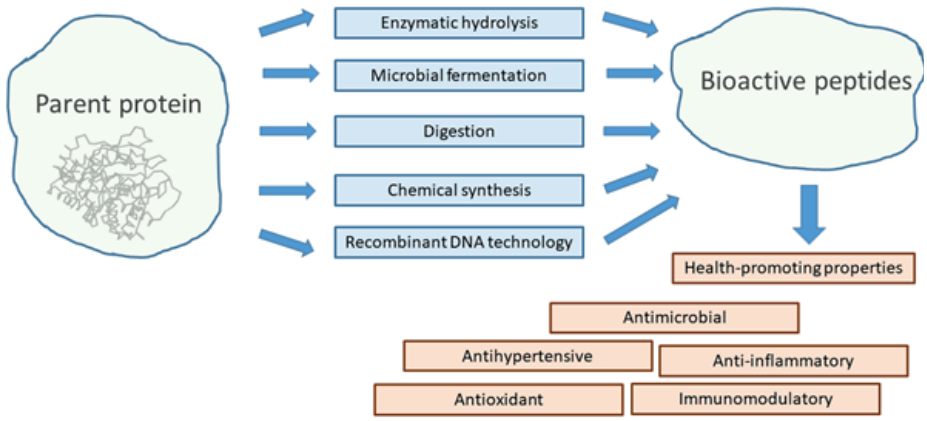

Figure 1 -Production methods and health-promoting properties of bioactive peptid

Generation of bioactive peptides from waste and by-products

Bioactive peptides can be isolated from their original protein through enzymatic hydrolysis and microbial fermentation (Figure 1). If the amino acid sequence of bioactive peptides is known, they can be produced by chemical synthesis or recombinant DNA technology [19] (Figure 1). Bioactive peptides can be isolated from various sources, including dairy and plant-based products, animal-based meat, and waste/by-products from the food industry. There are many types of bioactive peptides which can be produced using various methods. Some bioactive peptides are formed by endogenous enzymes in post-mortem meat, while other peptides - through microbial fermentation and chemical or enzymatic hydrolysis. The proteolytic enzymes originate from animals, microorganisms or plants and the type of proteolytic enzymes and substrate will determine the types of bioactive peptides generated. The most common way to produce bioactive peptides is through enzymatic hydrolysis, because of low amount of used toxic compounds, high specificity and mild conditions [20]. Another commonly used method to obtain bioactive peptides are microbial fermentation and *in vitro* digestion. In this study, the focus will be placed on enzymatic hydrolysis with proteases.

Enzymatic hydrolysis

During this process, the proteins are hydrolysed, and the bioactive compounds are released. Many proteolytic enzymes can be used, including commercially available pepsin, trypsin, chymotrypsin, corolase PP, papain, bromelain and pronase [11]. Those enzymes need different optimal conditions to perform their action [21]. Enzymatic hydrolysis of bioactive peptides using two types of enzymes, either enzymes extracted from microorganisms or plants, or digestive enzymes [22]. A combination of both types of enzymes can also be used, depending on the structure of the desired peptide.

The success of enzymatic hydrolysis is affected by pH, temperature, enzyme/substrate ratio, length of hydrolysis and enzyme deactivation (Table 2).

Table 2 - Some common commercial enzymes in generation of bioactive peptides, optimal conditions in the process and examples of use

Enzyme name	Optimum temperature, °C	Optimum pH	Origin	Meat by-products for protein hydrolysis	Reference
Papain	60-70	6-7	Papaya fruit	Animal muscle from bovine, porcine or deer Deer, sheep and pig blood Porcine liver	[18]
Bromelain	35-45	7	Pineapple fruit		
Ficain (ficin)	60	8	Fig tree	Camel meat, beef, and pork	[23]
Pronase	40-60	7.5	*Streptomyces griseus*	Bovine skin	[24]

A complete procedure includes peptide generation, isolation, characterization and bioactivity assays. The first step (generation of bioactive peptides) is basically a characterization of the meat by-products, which might be potentially used as a protein source. A *in silico* analysis is useful in characterization of the primary, secondary, tertiary and quaternary structure of the proteins. Then, the enzymatic digestion should be performde, where appropriate enzymes are used to generate the desired bioactive peptide [25]. The enzymes suitable for the digestion are determined by the *in-silico* analysis [26]. The hydrolyzates are generated and purified by different separation techniques, for example, ultrafiltration. Then, the hydrolysates are tested for their bioactivities. The hydrolyzates with desired properties are further purified by the use of chromatographic techniques such as ion chromatography (IEC), gel filtration chromatography (GFC) and high-performance liquid chromatography (HPLC). These methods separate the hydrolysate to peptidic fractions, which are further tested for their bioactivity using bioactivity assays. The bioactive peptide fractions with desired propertied are selected and characterized using mass spectrometry techniques such as MALDI-TOF and ESI-MS [27]. The sequenced bioactive peptides are then synthesised for additional bioavailability studies. The synthesis of the bioactive peptides is usually done by liquid-phase synthesis, Fmoc Solid-phase synthesis and Boc Solid-phase synthesis. Lastly, dose-response, safety and bioavailability studies *in vivo* are performed before the bioactive peptide are allowed to be used in the food industry [8].

Bioactive peptides prepared by this method with use of food-grade enzymes are recognized as safe. However, the disadvantages of this method is high cost, low number of commercially available enzymes and a limited peptide yield. Moreover, enzymatic hydrolysis is a time-consuming process and requires control over the temperature, pH, substrate and enzyme concentrations.

Fermentation

Fermentation is another method, which releases potential bioactive peptides by both natural and controlled fermentation. In the generation of bioactive peptides from dairy and plant foods, lactic acid bacteria (LAB) is often used. However, the activity of LAB is not efficient in meat products, and to the best of our knowledge, only limited data is available on bioactive peptides generated from meat [28]. Even lower number of studies were focused on the generation of bioactive peptides from meat by-products.

Health effects of bioactive peptides

The specific activity of the bioactive peptide is determined by its amino acid sequence, structure, chemical properties, and spatial structure of the peptide chain. Examples of health effects include but not limited to anti-hypertensive, antimicrobial, antioxidant, anti-inflammatory and antitumor activities [20].

Because of the increasing prevalence of hypertension and increased risk of the development of cardiovascular diseases, a lot of researcher are searching for approaches to prevent it by dietary meant. Reduction of blood pressure is important factor in prevention of hypertension. Renin-angiotensin system is responsible for regulation of the blood pressure. Angiotensin is converted to angiotensin I, then angiotensin I is converted to angiotensin II by angiotensin I converting enzyme (ACE). The generation of angiotensin II causes vasoconstriction. Inhibition of ACE leads to the limited formation of angiotensin II and thus lowers vasoconstriction [29]. Saiga et al. [30] demonstrated antihypertensive activity of chicken muscle extract after treatment with Aspergillus protease. The GFPTTKTYFPHF and VVYPWT peptide sequences have been shown to have anti-hypertensive activity. The GFPTTKTYFPHF peptide were found in the α-chain between fragment 34-46, VVYPWT peptide - between the fragments 34-39 on the β-chain of porcine hemoglobin. These peptides act as ACE inhibitors and exert their health effects by reduction of the blood pressure. Many of the anti-hypertensive bioactive peptides act as competitive inhibitors of the ACE enzyme. GFPTTKTYFPHF have an IC50 value of 4.9 μM and VVYPWT have an IC50 value of 6.0 μm from porcine blood. IC50 is a value indicating the concentration of inhibitor needed to inhibit 50% of a biological reaction, in this case, the ACE enzyme. ACE-inhibitory peptides corresponding to the sequences of porcine hemoglobin have also been identified [31]. ACE-inhibitory peptides (EACF and CDF) from rabbit meat proteins were also shown to have strong inhibitory effects [32]. In this in vitro study, EACF acted as a competitive ACE inhibitor with IC50 value of 41.1 μM, and CDF - as a non-competitive inhibitor with IC50 value of 192 μM [32]. The bovine fibrinogen-enriched protein fraction was also identified as a source of bioactive peptides with ACE-inhibitory activity [33].

Antimicrobial peptides reduce the growth of microorgansisms without side effects. The restricted use antibiotics have made antimicrobial peptides an attractive option against pathogens. Antimicrobial peptides from beef sarcoplasmic protein with activity against *Pseudomanas aeruginosa* were identified [34]. The peptide GLSDGEQ showed inhibitory effects against gram-negative and gram-positive bacteria, *Salmonella typhimurium, Bacillus cereus, Eschericha coli* and *Listeria monocytogenes* [20]. Hydrolysates from porcine blood proteins also demonstrated antimicrobial effect against *Bacillus cereus* [35].

Bioactive peptides with antioxidant activity might inhibit the effect of free radicals and reactive oxygen species. Antioxidants neutralize free radicals by donating electrons and stabilizes the free radicals making them less reactive, thus inhibiting the free radical ability to react with other substances in the human body [36]. The fact that some antibiotics cause side effects and oxidative damage, led to the search of new compounds with antioxidant properties. In this regard, bioactive peptides are an attractive option because of no side effects.

Bioactive peptides also continue to attract attention in clinical tumor therapy. Some bioactive peptides can act directly or indirectly on tumor cells and change the growth and apoptosis of the tumor cells. The antitumoral bioactive peptides generally act by inhibiting tumor angiogenesis and enhancing tumor cell apoptosis [37]. Currently, several peptides with antitumoral activities were isolated from bovine meat [34], and many studies indicated the potency of meat waste and by-products as a source of antitumor peptides [12].

Functional food with added bioactive peptides

Bioactive peptides from waste and by-products with health-promoting properties are promising ingredients for functional foods. Surprisingly, research on product development and effects of food with added bioactive peptides is limited. Wheat bread prepared with an enzymatic hydrolysate of bovine α- and β-globulins and fed to spontaneously hypertensive rats, led to a reduction of systolic blood pressure after 2 h of administration, although after 24 h, blood pressure increased [38]. When pork blood and liver hydrolysates were included as an ingredient in pork loaves, physico-chemical (water activity, lipid oxidations, color, texture and microbial qualities) and sensory properties were acceptable [39]. More studies are needed on improved processing, sensory evaluation and health effects of using bioactive peptides from meat industry as an ingredient in functional foods.

Conclusion

Some meat by-products are suitable for human consumption and can be consumed either directly or after processing. Some of meat by-products can be transformed into protein-rich feed for pets and livestock animals. Meat by-products and meat waste which are rich in proteins and produced in large amounts, can be used for the generation of bioactive peptides. Although there is a trend in the European Union to limit the consumption of animal-based proteins, meat by-products and waste have the potential to be used in the production of bioactive peptides and enhance food properties. Bioactive peptides can exert health-promoting properties and are considered as compounds for the development of functional foods. They might exert anti-hypertensive, antimicrobial, antioxidant, anti-inflammatory and antitumor activities. Bioactive peptides are released from parent proteins by several methods, among which enzymatic hydrolysis and fermentation are the most common. Enzymatic hydrolysis is performed using single or combination of enzyme to release the desired peptides. Some of these enzymes are derived from plants or microorganisms, some are animal digestive enzymes (trypsin and pepsin).

Because of increased demands for large-scale production of bioactive peptides from meat by-products and waste, more research is needed to identify their activity and to develop efficient, cheap and reliable methods for their generation. Moreover, future research should be directed to assess short- and long-term effects of bioactive compounds as a component of the whole diet and investigate possible effects of interactions with other food ingredient.

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ANTIBIOTIC RESISTANCE OF *STAPHYLOCOCCUS AUREUS* STRAINS ISOLATED FROM ANIMALS AND BIRDS IN THE TERRITORY OF KOSTANAY REGION

Gulnur K. Alieva[1], Raushan M. Rychshanova[1], Gulmira K. Murzakayeva[2], Anara M. Mendybayeva[1]

[1]Research Institute of Applied Biotechnology, A.Baitursunov Kostanay Regional University , Kostanay, Republic of Kazakhstan
[2]S. Seyfullin Kazakh Agrotechnical Research University, Astana, Republic of Kazakhstan

Corresponding author: Gulnur K. Alieva[1], e-mail: gukan.83@mail.ru
Co-authors: Raushan M. Rychshanova[1,] e-mail: raushan5888@mail.ru
Gulmira K. Murzakayeva[2], m.gumika@list.ru
Anara M. Mendybayeva[1], e-mail: jks1992@mail.ru

Abstract

This article reveals the features of the resistance of Staphylococcus aureus strains isolated from animals, birds and animal products in the territory of Kostanay region. Staphylococci can infect any tissue or organ of an animal, causing more than 100 different diseases. According to literary sources, more than a dozen species of non-pathogenic or conditionally pathogenic staphylococci are isolated from many agricultural and domestic animals and birds. For the first time in the Kostanay region, the analysis of antibiotic resistance of *staphylococcus* strains isolated in livestock farms and animal products was carried out.

The analysis of antibiotic resistance of *staphylococcus* strains isolated in livestock farms of Kostanay region and animal products was carried out. The study showed that the largest number of isolates of Staphylococcus aureus showed high resistance to beta-lactam antibiotics by an average of 55.3%, fluoroquinolones by 47.1% and tetracyclines by 45.6%.

Of the 69 studied isolates of Staphylococcus aureus, antibiotic resistance was shown by 43 (62.3%) isolates, 26 (37.7%) isolates were sensitive to all groups of AMD.

It was found that the largest number of isolates is 62.3% in the group of beta-lactams resistant to various antibiotics, the least resistant strains are 7.9% in the group of aminoglycosides and 20.3% in the group of sulfonamides.

Key words: antibiotics; bacteria; microbiology; sensitive; staphylococci; strains; resistance.

Introduction

Resistance is primarily referred as the ability of germs to tolerate therapeutic doses of antibiotics, sulfonamides, and nitrofurans, which would normally be fatal to other microbes.

The bacterial genome undergoes spontaneous changes, which promote the creation of resistant strains of microorganisms. The most recent research indicates that selective agents also have a role in the development of antibiotic-resistant bacteria, and that their connection to the DNA is not the only cause. Chemotherapeutic medications cause the death of susceptible bacteria during the selection process, whereas resistant germs survive, proliferate, and spread. Future bacterial generations encounter a barrier when acquired resistance becomes established. The kind and strain of the microbe determine the speed and stability of its development. development of the most Staphylococci, Escherichia coli, mycoplasma, proteus, and the blue pus bacillus are among the organisms that exhibit rapid and considerable antibiotic resistance [1, 3, 8].

Staphylococcus methicillin-resistant (MRS) is a marker for resistance to all lactam antibiotics, with vancomycin having the highest therapeutic importance. There are various subgroups of -lactam antibiotics, a significant class of antibiotics used in veterinary medicine. Based on

their methods of resistance, -lactam antibiotics can be divided into four major categories: penicillin, cephalosporins, monobactams, and carbapenems. The susceptibility of lactam antibiotics is closely related to their susceptibility, and their efficiency is negatively correlated with their propensity to produce resistance [2, 4, 5].

It is determined which antibiotic classes, such as aminoglycosides, tetracyclines, macrolides, and fluoroquinolones, isolates are susceptible to. Most isolates have been found to be susceptible to rifampicin (6.0%) and trimethoprim-sulfamethoxazole (20-40%).

Methicillin-resistant staphylococci were mostly investigated as pathogens in hospital acquired illnesses in recent years; however, this scenario has changed as these pathogens are spreading more and more in the population. They contribute to serious diseases arising from animals and items generated from animals by infecting both people and animals. This community is currently a major source of infections, acting as reservoirs in two instances. Since their initial discovery in the early 1980s, the incidence of MRSA infections has increased over time [7, 9].

Objectives: To isolate *Staphylococcus aureus* strains from animals, birds, and products derived from them in the Kostanay region and conduct phenotypic characterization, as well as assess susceptibility to antibiotics and the prevalence of resistant and multidrug-resistant staphylococci.

Materials and Methods

The Microbiology Laboratory of the Institute of Biotechnology at Kostanay A. Baitursynov State Universityconducted microbiological research from 2021 to 2023. Samples comprised milk and milk-derived products, animal and avian biological components, and animal-derived goods.

A 3% solution of erythritol salt, plasma from JSC "NPO Mikrogen," a combination of stains for Gram staining, and control strains (*S. aureus* ATCC 25923, *S. aureus subsp. aureus* ATSS 6538) were used to adapt the isolated strains. The following selective media were used in tests to identify staphylococci: salt agar, mannitol salt agar, milk-salt agar, mannitol-mannitol agar, Baird-Parker agar, CHROMagar Mastitis, CHROMagar, France, and blood agar, HiMedia, India. The study of antibiotic resistance by the Disco-diffusion method was conducted in the Muller-Hinton environment (Research Center of Pharmacotherapy, St. Petersburg).

Utilizing the "Staphy-test" test systems, biochemical validation of isolates was done (ERBA Lachema, Czech Republic). Traditional microbiological techniques were used to determine the biological properties of staphylococci.

- Disk diffusion was used to test the antibiotic susceptibility (Pasteur Epidemiology and Microbiology Research Institute, St. Petersburg). The following antibiotics were tested: ampicillin (10 μg), amoxicillin (25 μg), benzylpenicillin (10 IU), streptomycin (10 μg), cefoperazone (75 μg), cefoxitin (30 μg), kanamycin (30 μg), neomycin (30 μg), gentamicin (120 μg), tetracycline (30 μg), doxycycline (30 μg), ciprofloxacin (5 μg), norfloxacin (10 μg), erythromycin (15 μg), tylosin (15 μg), trimethoprim-sulfamethoxazole (1.25/23.75).

Equipment includes a drying cabinet (VK-75-01), an incubator, a thermostat (TC-1/80SPU), analytical balances (Precisa), adjustable volume pipettes (1 - 1000 l), and an OPTIKA B510BF binocular microscope.

Direct staining and gram staining were done on colonies made from cultured material. The smears, which were created as a thin layer, were used to identify gram-positive cocci. Onto salt agar were first streaked all of the samples. For 24-48 hours, the tubes were incubated at 37°C. The colonies were then harvested from the incubator and placed on specialized diagnostic media, including milk-salt agar, egg yolk mannitol salt agar, Baird-Parker agar, Chromagar Mastitis, and blood agar. The potential for plasma to coagulate allowed researchers to identify the coagulase activity of bacteria. It was confirmed that staphylococci grew on salt agar. The colonies were then transferred to blood agar and streaked with mannitol agar or milk-salt agar. Staphylococci colonies began to form after 24-48 hours of incubation at 37°C with samples in petri plates. Convex, 2.0–2.5 mm in diameter, and colored yellow, golden, lemon yellow, light green, white, or translucent, *Staphylococcus* colonies on mannitol agar. A method called disk diffusion methods (DDM) was employed to test the antibiotic susceptibility.

The cultured microorganism's suspension (also known as the inoculum) was made. Determining the suspension standard of the bacterial growth is one of the crucial steps in all test techniques. The bacterial suspension standard should have a concentration of 1.5–10 CFU/mL. The Biosan DEN-1 densitometer was used to calculate the

optical density. The basis for the instrument's operation is the measurement of optical density, and the output is then displayed in McFarland units.

Colony-forming units (CFU) of sterile isolates were modified using sterile isotonic saline to match the McFarland turbidity criterion of 0.5 for the creation of the inoculum. Direct suspension of colonies in sterile bacteriological saline was the technique employed. A number of the colonies that multiplied on the agar after 24 hours after being suspended in sterile bacteriological saline were chosen. Based on their morphological traits, similar colonies were grouped together. Within 15 to 20 minutes, the collected material was used after being suspended in sterile isotonic saline.

DDM, or disk diffusion method. Mueller-Hinton agar was made in accordance with the directions. The thickness of the agar layer in the Petri dish is one of the crucial details in figuring out the sensitivity of DDM. The agar layer should evenly cover the bottom of the Petri dish and be about 4 mm thick (plus or minus 0.5 mm). Before setting the plates down, the prepared agar needs time to firm. There shouldn't be any apparent condensation on the inside of the lid or the agar surface; it should be smooth and even.

The plates were slanted prior to incubation to prevent the disks from coming away from the agar surface. The plates were incubated at +37°C for 24 hours after the antibiotic disks were placed and left in place for 15 minutes. After incubation, the locations where the antibiotics had prevented microbial growth had been seen. This demonstrates the microorganism's susceptibility to the evaluated antibiotic (see Figure 1 in the source).

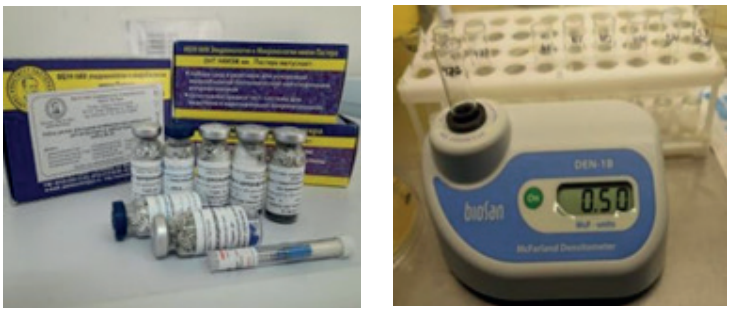

Figure 1 - Identification of susceptibility to antibacterial agents with disk diffusion method

This report outlines the outcomes obtained through the disk diffusion method, a means of identifying the responsiveness of bacteria to various antibiotics. Following the completion of the incubation period, Petri dishes were positioned upside down and examined against a dark backdrop to count bacterial colonies. A uniform and continuous layer of bacterial growth was observed on the agar surfaces. The area around the antibiotic disks displayed a clearly defined inhibition zone. The size of these zones, indicative of bacterial growth inhibition, was measured in millimeters using a caliper (see Figure 2).

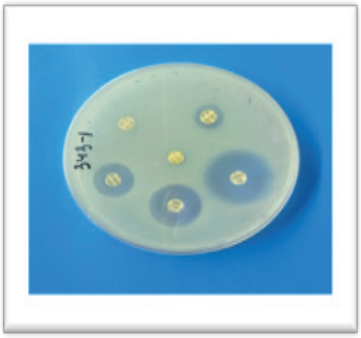

Figure 2 - Inhibition Zones of *Staphylococcus* Growth by Antibiotics

Results

In accordance with the criteria set by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) version 11.0 [10, 12], the Clinical and Laboratory Standards Institute (CLSI) [11, 13], and the "results of susceptibility testing to antimicrobial agents using a collection of discs," the following observations were made [14].

Research Results: A total of 69 isolated strains of *Staphylococcus aureus* underwent testing for their susceptibility to various categories of pharmaceutical agents, which included:

- β-lactam antibiotics (ampicillin, amoxicillin, benzylpenicillin, cefoperazone, cefoxitin).
- Aminoglycosides (streptomycin, kanamycin, neomycin, gentamicin).
- Tetracyclines (tetracycline, doxycycline).
- Macrolides (erythromycin, tilosin).
- Fluoroquinolones (cipro-loxacin, norfloxacin).
- Sulfonamides (trimethoprim/sulfamethoxazole).

Previous studies have consistently indicated that *S. aureus* is among the microorganisms with a notably high resistance to antibiotics [2, 3]. If S. aureus continues to prevail in medical settings, it signifies that the issue of drug resistance in *Staphylococcus* reservoirs is increasingly pressing, and current infection control measures are insufficient to curb the proliferation of these bacteria. Our conducted research provides further evidence of this reality.

Based on the results of testing the isolated strains of *Staphylococcus aureus,* the highest proportion of isolates exhibited resistance to amoxicillin - 41 (59.4%), ampicillin - 39 (56.2%), cefoxitin - 38 (55.1%), cefoperazone - 37 (53.6%), benzylpenicillin - 36 (52.2%), ciprofloxacin and tetracycline - 34 (49.2%), norfloxacin and tilosin - 31 (44.9%) (see Table 1).

Table 1 – Antibiotic Susceptibility of *S.aureus* Strain

Name	ABD drugs	Name of the ABD	Number of resistant strains	Number of resistant strains%	Group average %
S. aureus n=69	β-lactams	ampicillin	39	56,2±3,64	55,3±3,7
		amoxicillin	41	59,4±3,4	
		benzylpenicillin	36	52,2±4,0	
		cefoperazone	37	53,6±3,88	
		cefoxitin	38	55,1±3,76	
	amino-glycosides	streptomycin	6	8,7±7,64	7,9±5,5
		kanamycin	5	7,2±7,76	
		neomycin	0	0,0	
		gentamicin	11	15,9±7,03	
	tetracyclines	tetracycline	34	49,2±4,24	45,6±4,5
		doxycycline	29	42,0±4,85	
	macrolides	erythromycin	27	39,1±5,09	42,0±4,8
		tylosin	31	44,9±4,61	
	sulfonamides	sulfamethoxazole/	14	20,3±6,67	20,3±6,6
	fluoroquinols	trimethoprim	34	49,2±4,24	47,1±4,4
		ciprofloxacin	31	44,9±4,61	

Looking at the table, it's evident that most *Staphylococcus aureus* isolates displayed the highest resistance to β-lactam antibiotics, with 55.3% resistance, followed by fluoroquinolones at 47.1%, and tetracyclines at 45.6%.

Out of the 69 *Staphylococcus aureus* isolates subjected to testing, 43 (62.3%) exhibited resistance to antibiotics, while 26 (37.7%) isolates remained susceptible to all categories of antibiotics.

Among the different antibiotic classes, the lowest level of resistance was observed in the aminoglycosides group at 7.9%, and sulfonamides at 20.3%.

Among the 43 isolates showing resistance, 41 (95.3%) *Staphylococcus aureus* isolates displayed resistance to the β-lactam antibiotic class. Within this category, 5 (12.2%) isolates were resistant to a single antibiotic, 13 (36.1%) were resistant to two antibiotics, and 14 (34.1%) were resistant to three antibiotics. Additionally, 5 (12.2%) isolates were resistant to four antibiotics, and 4 (9.8%) isolates were resistant to all five antibiotics within the β-lactam class.

Furthermore, among the identified resistance patterns, 7 (16.3%) isolates out of the 43 *Staphylococcus aureus* isolates exhibited

resistance to a single β-lactam class, 12 (27.9%) isolates were resistant to two β-lactam classes, 9 (20.9%) isolates were resistant to three β-lactam classes, 7 (16.3%) isolates were resistant to four β-lactam classes, and 5 (11.6%) isolates showed resistance to all six groups (see Figure 3).

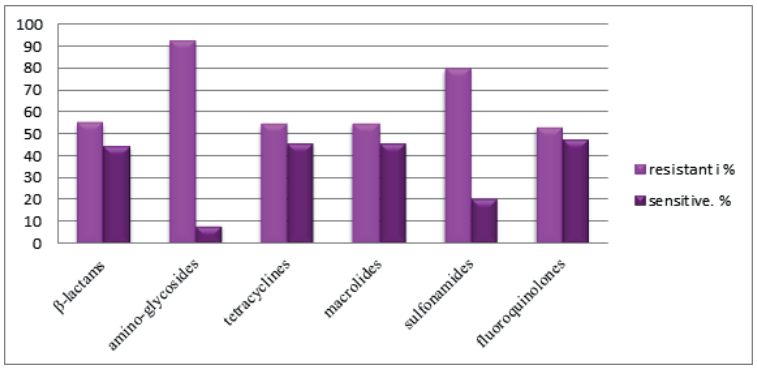

Figure 3- Correlation between the Susceptibility and Resistance of *S. aureus* Strains to Different Antibiotic Categories (%)

Discussion

Coagulase-negative staphylococci do not have the same pathogenicity as *Staphylococcus aureus*, but, according to researchers, they are highly resistant to anti-bacterial drugs. Coagulase-negative staphylococci act as an important reservoir of mobile genetic elements associated with resistance, which contribute to the rapid horizontal transfer of antimicrobial resistance genes and resistance genes between staphylococcus species.

Conclusion

A research investigation was carried out to evaluate how *Staphylococcus aureus* strains, collected from animals, birds, and agricultural products in the Kostanay region, respond to antibiotics. The study showed that the largest number of isolates of Staphylococcus aureus showed high resistance to beta-lactam antibiotics by an average of 55.3%, fluoroquinolones by 47.1% and tetracyclines by 45.6%.

Of the 69 studied isolates of Stapylococcus aureus, antibiotic resistance was shown by 43 (62.3%) isolates, 26 (37.7%) isolates were sensitive to all groups of AMD.

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THE EFFECTIVENESS OF UTERINE DISEASE DIAGNOSIS METHODS IN COWS DEPENDING ON POSTPARTUM DAYS

Isatay T. Jakupov[1], Aida B. Abultdinova[1], Gulnur K. Mamytbekova[1], Zhanargul Z. Zharkimbaeva[1], Gulzhan T. Yeszhanova[1], Albert G. Zabrodin[1]

[1]Faculty of Veterinary Medicine and Animal Husbundry Technology,
NJSC S. Seifullin Kazakh Agrotechnical University,
Astana, Kazakhstan

Corresponding author: Isatay T. Jakupov, e-mail: I.Jakupov@mail.ru
Co-authors: Aida B. Abultdinova[1], e-mail: Abultdinova-a@mail.ru,
Gulnur K. Mamytbekova[1], e-mail: gulnur4284@mail.ru
Zhanargul Z. Zharkimbaeva[1], e-mail: Zharkimbayeva89@mail.ru,
Gulzhan T. Yeszhanova[1], e-mail: Yeszhanova_astana@mail.ru
Albert G. Zabrodin[1], e-mail: albertzabrodinvetmed@gmail.com

Abstract

The article presents the results of the effectiveness of methods for diagnos-ing the uterus in cows on different days after calving. In the postpartum period in cows under conditions of weakened immunity and dystocia, inflammatory pro-cesses in the uterus can develop, most commonly manifesting in clinical and sub-clinical forms of endometritis. Our research shows that the rectal diagnostic method is impact in the first 30 day in milk (DIM), particularly when clinical signs are evident. In 60 DIM, the vaginal method demonstrated about 58.8% ef-fectiveness, by the using on "Metrastatum" device determine the degree of uterine involution and assess the obtained discharges. After 61 DIM the probability of detecting endometritis by laboratory methods increased from 6.3 to 25%. In this study, laboratory methods such as the Nagornyi-Kalinovsky test (NKT) and the Whiteside method (WM) were employed. Subclinical forms of inflammatory pro-cesses in the uterus in the absence of obvious visible signs imply the use of labor-atory methods based on identifying inflammatory markers. The definition of ef-fective and useful methods is an urgent task and requires additional research.

Key words: Cows; diagnostics; uterus; calving; inflammation; clinical methods, laboratory methods.

Introduction

In large livestock farms, the issue of impaired fertility in cows remains relevant, with the most common cause being inflammatory processes in the uterus after calving [1].

As practice shows, usually inflammatory processes of the reproductive organs are common among highly productive cows, according to research endometritis was 28% higher in cows with high milk productivity compared with low-productive females [2]. Inflammatory processes of the reproductive system have a negative impact on the effectiveness of insemination, lack of milk and offspring, which leads to significant economic losses in farms [3].

In this cause, timely diagnosis and treatment of postpartum disorders is important. Research is conducted annually with the aim of identifying new diag-nostic methods and enhancing those already in existence. Diseases of the uterus in cows are diagnosed using clinical, laboratory and biophysical research meth-ods [4,5,6].

Clinical methods include external: examination, palpation, internal - vagi-nal, rectal method, based on the study of the nature of discharge, consistency, to-pography of the genital organs of animals [7,8]. Laboratory methods are based on bacteriological, cytological, physico-chemical, biological, physical and hormo-nal studies of animal body fluids. The biophysical method is based on the use of ultrasonic scanners [9].

Carrying out a rectal examination in the first

10 days after birth has some difficulties, since the size of the uterus and its qualitative characteristics vary be-tween individual animals and depend on the days in milk [10].

For diagnose of the uterus diseases in the postpartum period, it is prefera-ble to conduct a vaginal examination using a vaginal speculum rather than rectal palpation of the uterus, but veterinarians in the farms rarely use it, since they overestimate the time and effort required for such diagnosis, and it is also neces-sary to follow the rules of asepsis and antiseptics.

To diagnose the condition of the genital organs in cows, a "Metrastatum" device has been developed by the degree of its immersion, the involution of the uterus is determined, and the form of inflammation is determined by the nature of the mucus taken to the bowl [11]. By using this device, 72.6% of acute postpar-tum endometritis in cows was diagnosed, while 65% were diagnosed by rectal ex-amination. However, the Metrastatum device is less specific for the diagnosis of subclinical pathologies of the uterus, when the uterus is located in the pelvic cavi-ty and a small amount of mucus is secreted [12,13].

Laboratory methods for diagnosing diseases of the uterus are based on the study of mucus, urine, vaginal smears, and blood. Using a cytological study of changes in the uterine mucosa, it is possible to diagnose inflammatory postpar-tum diseases from the very first day after calving, despite the fact that clinical signs and hematological changes appear only from 5-17 days after birth. Signs of the disease are: increased leukocyte infiltration of neutrophils and lymphocytes, dystrophic changes in the epithelial cells of the uterus and vagina, the presence of mucus and macrophages in the smear [14].

Proposed laboratory methods Golovan I.A. [15] detect latent endometritis using cyclic aromatic compounds (indole, skatole, phenol). Dudenko V.S. sug-gests examining 2 ml of mucus by exposure to a 20% solution of trichloroacetic acid, concentrated nitric acid and 33% sodium hydroxide solution [16]. Accord-ing to the method of Kalinovsky G.N. a 1% solution of acetic acid is used to de-termine mucopolysaccharides in mucus [17]; Phlegmatov N.A. recommends a bi-ological assay by studying the survival of diluted bovine semen in vaginal mucus [18]; method V.G. Gavrisha is based on determining the presence of histamine in urine - by mixing it with an aqueous solution of silver nitrate (lapis test) [19]; modified Foll test - for the detection of sulfur-containing amino acids in the con-tents of the uterus - using a 0.5% solution of lead acetate and a 20% solution of sodium hydroxide. These methods have both positive aspects and difficulties in use, it is not always possible to obtain a large amount of mucus, obtain urine, se-lection of reagents, recording of research results [20,21].

Ultrasound scanners are currently used to diagnose uterine pathology. They are used to determine pregnancy, diagnose pathologies of the reproductive system, and determine the condition of the internal structure of the uterus. This method is not always accessible to veterinarians; it is also difficult for them to di-agnose subclinical cases of diseases of the cows genital organs [22].

According to the International Dairy Federation, the European Cattle Breeders Association, the subclinical form of endometritis is detected in 20.0-25.0% of dairy cows [23]. The European Union calculated that losses from sub-clinical endometritis amount to 233 euros per head per year [24].

If the take into account that diseases of the uterus in cows are widespread, it becomes clear that without effective diagnostic and treatment methods it is not possible to carry out successful reproduction of the herd. The difficulty of diag-nosing chronic endometritis is due to the fact that the clinical signs of this disease are not expressed, and it is also difficult to differentiate from the physiological condition using clinical, rectal and vaginal methods. 30-60 days after calving, when the discharge stops, veterinarians differentiate only during the period of es-trus by individual (if visible) droplets of purulent [25]. Thus, the existing diag-nostic methods are laborious for wide application in veterinary practice, late de-tection of pathologies requires longer treatment, complications in the form of la-tent endometritis are observed, therefore, research on the development of early, simple, affordable methods for diagnosing calving and postpartum pathologies is relevant.

Material and Methods

The studies were conducted in the Department of Veterinary Medicine, Faculty of Veterinary Medicine and Animal Husbundry Technology at the S. Seifullin Kazakh Agrotechnical Research University, an also in several farms of the Akmola and North Kazakhstan regions of the

Republic of Kazakhstan.

Cows of 1-5 lactation Holstein-Friesian (n=1153), black-and-white breeds (n=142) were used in the experiments.

For diagnostic of the uterus condition, clinical, instrumental and laboratory methods were used. The clinical method included auxiliary instruments and devices, gynecological gloves, vaginal speculum; during instrumental examination, the "Metrostatum" device was used; for laboratory diagnostic methods: test tubes, stand, 1% acetic acid solution, 5% sodium hydroxide solution, measuring pipettes.

Diagnosis of acute endometritis was carried out clinically by visual examination of the vulva, tail root, by internal rectal examination, in which the topography, rigidity and consistency of the uterus were determined. During the vaginal examination, a Metrastatum device was used to determine the degree of involution of the genitals and determine the form of inflammation by the color of the discharge (from yellow-white, gray-brown to red-brown), consistency (from mucous, thick, viscous, the content of fibrin grains, necrotic mass or pieces of decayed tissues).

Chronic forms of endometritis were diagnosed by the state of the uterus, by the color of the discharge (cloudy, white); by the consistency of the discharge (creamy consistency); by the volume of discharge (0.5-1.2 ml); sedimentation, turbidity of the fluid (NMT), lemon-yellow staining of the contents of the test tube (WM).

According to the results of the analysis calving and after calving period, 5 experienced groups were formed: from 10-30 (n=31); 31-60 (n=17); 61-90 (n=16); 91-120 (n=16); 120 and more (n=11) days in milk (DIM).

Results

To study the prevalence of diseases of the reproductive organs in Holstein-Friesian (n=1153) and black-and-white (n=142) cows the monitoring, analysis of animals dispensary journal in agricultural formations of Akmola, North Kazakh-stan regions were performed. The results of the studies are shown in Table 1.

Table 1. Prevalence of reproductive organ diseases in Holstein-Friesian and Black-and-white cows

Breeds	Normal calving		Retained placenta		Uterus inflammation	
	n	%	n	%	n	%
Holstein Friesian (n=1153)	820	71,1	135	11,7	198	17,2
Black-and-white breed (n=142)	88	61,9	21	14,7	33	23,2

In 71.1% of calved Holstein-Frisian cows, the birth period proceeded with-out complications; the remaining 28.9% had such pathologies as retained placen-ta, uterine diseases, uterine subinvolution, endometritis. Of the 142 calved cows of the black-and-white breed, 61.9% had calving without complications, 14.7% had aftercalving detentions, and 23.2% had uterine diseases. To determine of the uterus diseases, the course and form of the pathology are important. To determine the course and form of endometritis in cows, the date and course of calving, the postpartum period, the nature of secretions, the state of the uterus and genital tract were learned.

The results of the diagnosis of uterine diseases depending on the days after calving are shown in Table 2.

Table 2. Effectiveness of diagnosing uterine diseases in cows using clinical and laboratory methods on different days after calving.

Days after calving	n	Transrectal examination		Vaginal examination		Laboratory tests			
		n	%	n	%	Nagornyi-Kalinovsky test		Whiteside Method	
						n	%	n	%
10-30	31	25	80,6	28	90,3	7	22,5	11	35,4
31-60	17	5	29,4	10	58,8	6	35,2	8	47,05
61-90	16	2	12,5	5	31,2	5	31,2	6	37,5
91-120	16	2	12,5	3	18,2	4	25	5	31,2
121 and more	11	1	9,09	2	18,1	2	18,1	4	36,3

The data in Table 2 show that in the diagnosis of endometritis, individual signs (enlargement of the uterus, the nature of secretions, their number) decrease or disappear with increasing days after calving. So in the interval of 10-30 DIM (n=31), endometritis was detected by rectal examination in 25 cows, in the interval of 31-60 DIM (n=17) in 5 cows, in the interval of 61-90 DIM (n=16) in 2 cows, in the interval of 91-120 DIM (n=16) in 2 cows, in the interval of 121 DIM and more (n=11) by one cow. Vaginal examination of cows in the interval of 10-30 DIM by the 31 examined cows, endometritis was detected in 90.3%, on 31-60 DIM in 58.8% of cows, on 61-90 DIM in 31.2%, on 91-120 DIM 18.2% and on 121 days or more about 18.1% of animals. When using laboratory methods in the same intervals after calving, by the Nagorny-Kalinovsky test about 18.1-35.2%, and by the Whiteside method about 31.2-47.05% cows with endometritis was revealed.

From 30 to 60 DIM, the effectiveness of the clinical rectal method decreas-es, a total of 29.4% of pathologies were detected, the method of vaginal diagnosis is more effective here, which determined 58.8% of pathologies. A vaginal exami-nation allows you to detect discharge from the cervix, with the help of the Metra-statum device, the degree of involution of the uterus and the properties of the dis-charge are determined. From 61 to 90 DIM in infertile cows, endometritis was de-tected by clinical methods and laboratory methods in 31.2-37.5% of cows. The probability of determining endometritis by laboratory methods increased from 6.3 to 25%.

Discussion

According to numerous studies [9, 10], the criterion for the diagnosis of endometritis by rectal examination is an increase in the size of the uterus, features of topography and consistency. Clinical diagnostic methods are most effective in acute endometritis and their effectiveness is 82-91%. Laboratory methods are most effective in the diagnosis of chronic endometritis [26].

Within the study, rectal and vaginal methods showed their effectiveness from 10 to 60 DIM, in the following from 61 to 90 DIM, vaginal examination was preferable to rectal, since it is possible to see mucus and evaluate its proper-ties. From 91 to 120 DIM or more, depending on the course of uterine diseases, 18.7% of patients were identified by vaginal examination, and 18.1-37.5% by la-boratory methods, which indicates the need for a comprehensive diagnosis of uterine diseases.

Conclusion

In conclusion, it can be noted that clinical methods show high diagnostic ef-ficiency for 10-60 DIM and from 61 to 90 DIM, vaginal examination using the "Metrastatum" device is more preferable, it determined 29.4% more cows with uterine disease than rectal. In addition, methods of clinical diagnosis of the geni-tals in cows are effective in acute and subacute course of endometritis. Laborato-ry methods make it possible to determine 18.1-37.5% of pathology; however, these methods require continued study and the search for new effective, fast methods that will be relevant in agricultural farms.

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Dear author!

The scientific journal "Bulletin of Science of S.Seifullin Kazakh Agrotechnical Research University: Veterinary Sciences" aims to be included in international databases such as Scopus, Web of Science and AGRIS (International information system for the Agricultural sciences and technology), etc. In this regard, the editorial board of the journal decided to consider and accept for publication from 2023 articles prepared in English.

Basis

In accordance with the order of the Minister of Education and Science of the Republic of Kazakhstan No. 170 dated April 30, 2020, the editorial office of the journal "Bulletin of Science of S. Seifullin Kazakh Agrotechnical Research University: Veterinary Sciences" has developed a website with an online system for submission and review of articles.

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Registration of the author is carried out via the following link: https://bulletinofscience.kazatu.edu.kz/index.php/veterinary-science

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Requirements for publication of scientific articles in the journal "Bulletin of Science of S. Seifullin Kazakh Agrotechnical Research University: Veterinary Sciences"

The editorial board of the journal asks the authors to familiarize themselves with the rules and adhere to them when preparing papers sent to the journal.

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2. Original articles up to 30,000 characters (10-15 printed pages);
3. Reports of foreign scientists.

The article should contain only original material, reflecting the results of research of the author/s, previously not published elsewhere.

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Articles are accepted with the originality of the text of at least 70% (checking is carried out using the Anti-plagiarism system).

Articles are accepted until the 20th day of each quarter (February 20, May 20, August 20, November 20).

The main requirements for the article:

- the relevance of the problem solved in the study;
- correctness of the experimental formulation and interpretation of the research result;
- the ability to reproduce experimental data;
- clarity and consistency of presentation;
- design of the article, in accordance with the requirements of the journal.

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Key words no more than 7 words or phrases separated by semicolons.

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Materials and methods. This section must meet the following criteria:

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- A brief description of the most significant findings that were identified in the Results section and their comparison with other studies on illustrative topics,
- Identification of problem areas, lack of some aspects;
- Future research directions.

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Articles in journals (print format)

Author, A. (Publication Year). Article title. Periodical title, Volume (Issue), pp.-pp.

Nevin, A. (1990). The changing teacher education special education. Teacher Education and Special Education: The Journal of the Teacher Education Division of the Council for Exceptional Children, 13(3-4), 147-148.

Articles in journals (electronic format)

Author, A. (Publication Year). Article title. Periodical Title, Volume (Issue), pp.-pp. DOI:XX.XXXXX or Retrieved from journal URL.

Jameson, J. (2013). E-Leadership in higher education: The fifth "age" of educational technology research. British Journal of Educational Technology, 44(6), 889-915. DOI:10.1111/bjet.12103

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IDENTIFICATION OF WHEAT GENES CONDITIONING RESISTANCE TO PATHOGENIC FUNGI

Aitbay K. Bulashev[1] (ID), Kairat N. Nabiyev[2] (ID)...

[1]Faculty of Veterinary Medicine and Livestock Technology NJSC S. Seifullin «Kazakh Agrotechnical Research University», Astana city, Republic of Kazakhstan;
[2]Faculty of Forestry, Wildlife and Environment NJSC «West Kazakhstan Innovation and Technology University», Uralsk, Republic of Kazakhstan

Corresponding author: Aitbay K. Bulashev, e-mail: tech@mail.ru
Co-authors: Kairat N. Nabiyeva, e-mail:naruk@mail.ru

Abstract

The author of the article, on the basis of his own research, proves that the presence of wheat resistance genes to pathogenic fungi is a key factor for use in breeding work. The article presents the results of identification of wheat genes Sr32, Bt9 and Bt10 responsible for drought resistance to pathogenic fungi that cause diseases of stem rust, as well as common smut ... [not less than 100 words and not more than 300 words].

Key words: resistance genes; stem rust; hard smut; pathogenic microscopic fungi; electrophoresis; PCR; wheat. (7 words or phrases).

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