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Study of the Immunologic Value of Trichinella spiralis recombinant Protein Tsp-LE

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Abstract

Background and Aim. Trichinellosis is a serious zoonotic disease, with frequent outbreaks reported across various regions in Europe and Asia. However, current diagnostic tests based on excretory-secretory antigens (ES-Ag) and somatic antigens (S-Ag) lack adequate specificity and sensitivity. Moreover, the production of these antigens is both labor-intensive and costly.

Materials and Methods. The immunodiagnostic potential of a recombinant 11 kDa serine protease protein, previously characterized in earlier studies was assessed. This study detected infection in *Balb/c* mice as early as 14 days post-infection with *T. spiralis* larvae, yielding no false-positive results and an average infection level of 61±5.3 larvae per mouse.

Results. Comparative indirect ELISA analysis on mouse sera after the 3rd and 5th immunizations, alongside tests on S-Ag and S-Ag using sera from infected rabbits and pigs, demonstrated the high diagnostic accuracy of the recombinant protein. The protein revealed strong specificity for trichinellosis detection, showing no cross-reactivity with echinococcosis.

Conclusion. These findings support using rTsp-LE as a reliable component in developing ELISA test systems for trichinellosis detection.

Keywords: ELISA; Es-Ag; immunization; recombinant protein; S-Ag; *Trichinella spiralis.*

Introduction

Trichinellosis is a zoonotic disease caused by nematodes of the family *Trichinellidae,* affecting humans and a range of animals. The disease is registered in domestic animals such as pigs, dogs, and cats, as well as in numerous wild carnivores (including wolves, foxes, and bears), rodents (such as rats and mice), and humans [1]. Approximately 11 million people worldwide are estimated to be chronically infected with *Trichinella spiralis* [2].

In 2020, 181 cases of trichinellosis were reported across nine EU/EEA countries, with 117 cases confirmed and 64 classified as probable [3]. In China, from 2009 to 2020, cases of human trichinellosis have been primarily concentrated in southwestern regions, with eight outbreaks resulting in 479 cases and two fatalities [4]. Southeast Asia also shows variable seroprevalence rates of human trichinellosis, particularly in Laos and Vietnam, where rates range from 0% to 10.5% in certain villages. Additionally, in Cambodia, Laos, Malaysia, Thailand, and Vietnam, 13 outbreaks involving 1,604 cases and a mortality rate of less than 1% have been recorded over the past 21 years [5].

Given the epidemiological context, effective early diagnosis of trichinellosis in potentially high-risk domestic and wild animals is essential. However, routine testing of meat and animals for trichinellosis remains limited, primarily due to inflated costs, which can reach up to $3 per animal [6].

Early clinical diagnosis of trichinellosis is challenging due to the lack of pronounced clinical symptoms. Moreover, diagnosing the disease in its chronic stages proves even more difficult [7].

The detection of anti-Trichinella IgG via enzyme-linked immunosorbent assay (ELISA) using muscle excretory-secretory antigens (Es-Ag) from *Trichinella spiralis* larvae is the most used serologic method for diagnosing trichinellosis. However, a major limitation of current serologic tests is the potential for false negatives during early infection stages [8, 9, 10]. Immunoenzyme methods utilizing recombinant antibodies promotes the diagnostic process, eliminating the need for animal slaughter, reducing reliance on antigen availability, standardizing components, and enhancing both the quality and specificity of ELISA-based diagnostics [11, 12].

Sufficient literature supports the high sensitivity and specificity of ELISA with recombinant antigens. For instance, Wang et al. (2015) studied a recombinant 31 kDa protein and found a sensitivity of 96.67% and 96.87%, compared to 100% and 98.44% for ELISA using ES antigens. ELISA with recombinant antigens was able to detect antibodies in mice with heavy (500 larvae), moderate (300 larvae), and light (100 larvae) infections at 8, 12, and 14 days post-infection (dpi), respectively, whereas detection using ES antigens occurred at 10, 8, and 10 dpi, respectively [13]. In mice infected with 200 muscle larvae (ML) of *T. spiralis,* the rTsTryp-ELISA demonstrated a natural muscle protease sensitivity of 98.1%, compared to 94.2% for the ES antigen-ELISA. Additionally, the specificity of rTsTryp-ELISA was significantly higher than that of the ES antigen-ELISA, at 98.7% versus 95.4%, respectively [12]. Somatic antigens with molecular masses of 43 kDa (TsCSAg-43), 79 kDa (TsCSAg-79), and 101 kDa (TsCSAg-101), isolated by Supcharoengoon et al. (2022) from the sera of trichinellosis-infected pigs, showed IgG-ELISA sensitivities of 100%, with specificities of 97.77%, 95.54%, and 90.63%, respectively [14].

Y. Liu et al. (2021) developed a novel competitive enzyme-linked immunoassay (rCLP-cELISA) based on recombinant cystatin-like protein and monoclonal antibodies. This method demonstrated high sensitivity and specificity with human serum field samples and showed robust applicability with porcine and murine serum [15]. The expression of *Trichinella* antigens encoding proteins of 53 kDa [16, 17], 49 kDa [18, 13], 35.5 kDa [19], and 21 kDa [20] in heterologous systems has become a standard approach for generating antigens used in diagnostic test systems.

Recombinant antigens maintain great promise for several reasons: they exhibit high specificity, minimizing the risk of false negatives and cross-reactions. Also, the expression of recombinant antigens enables standardized production with high product purity and stability, while also reducing the risk of infection for personnel handling parasites.

The aim of our study was to evaluate the immunodiagnostic potential of recombinant *Trichinella spiralis* serine protease protein at various stages of infection in experimentally infected laboratory mice.

Materials and Methods

Ethical approval. All animal procedures were conducted following exacting standards of biosafety and animal welfare. Protocols adhered to the International Guidelines for Biomedical Research Involving Animals [21].

The care and use of laboratory animals were approved by the Animal Ethics Committee of the Faculty of Veterinary Medicine and Animal Husbandry Technology at S. Seifullin Kazakh Agrotechnical Research University (KATRU), Astana, Kazakhstan (Protocol No. 1, dated February 23, 2023).

Experiments were conducted at the Research Platform of Agricultural Biotechnology, NCJSC: "S. Seifullin Kazakh Agrotechnical Research University."

Infestation of animals T. spiralis larvae were maintained through serial passage in Soviet Chinchilla breed rabbits at the Joint Kazakh-Chinese Laboratory of Biological Safety.

Larvae were collected through artificial digestion following the standard protocol described previously [22]. For each species of trichinellosis, seven mice, aged three to four months, were selected for the experiment at 3, 5, 7-, 14-, 21-, and 30-days post-infection, with three mice forming the control group. Using the principle of analogs for *T. spiralis,* an experimental group of three mice was formed for each day of selection (18 in total), along with three control mice. The infection dose was 250 larvae per mouse, and the animals were orally infected using a disposable pipette containing *Trichinella larvae.*

Scheme of experiment. On the 3rd, 5th, 7th, 14th, 21st, and 30th days, three mice from each group were euthanized for pathological autopsy. Euthanasia was performed via sequential intramuscular injection of xylazine (Bioveta, Czech Republic) at a dose of 1.5 mg/kg, followed by intravenous injection of Anestofol ('VIK' LLC, Russia) at a dose of 7.5 mg/kg. Muscles were examined for the presence of parasites according to the guidelines of H.R. Gamble et al. (2019) [23]. The small intestine was dissected longitudinally, washed three times with ice-cold saline solution, then cut into 2-cm fragments and cultured in saline at 37 °C for 2.5 hours. Larvae that emerged from the small intestine into the physiological solution were collected using the Berman method [24].

Larvae isolation. Trichinella larvae were isolated from animal muscle tissue samples using compressor trichinelloscopy and digestion in artificial gastric juice (AGJ), following the methods outlined in Methodological Guidelines 4.2.2747-10 "Methods of Sanitary-Parasitological Examination of Meat and Meat Products" [25]. The detected and isolated helminthological material was preserved in a 70% ethanol solution.

Antiserum testing by indirect ELISA. ELISA was performed as previously described [13]. Briefly, 96-well ELISA plates (Corning, USA) were coated overnight at 4 °C with 100 µL of purified recombinant antigens (1 µg/mL) or ES antigens (1 µg/mL) in bicarbonate buffer (pH 9.6). After blocking with PBS containing 0.1% BSA for 1 hour at 37 °C, the following reagents were added sequentially and incubated for 1 hour at 37 °C: (1) mouse serum diluted 1:100 in PBST, and (2) HRP-conjugated anti-mouse IgG (Sigma, USA) diluted 1:5000. Reactions were detected by adding a one-component TMB substrate (tetramethylbenzidine, CJSC "HBO Immunotech", Russia), and the reaction was stopped by adding 50 µL/well of 2M H2SO4. Optical density (OD) values at 450 nm were measured using a microplate reader (BioSan, Lithuania). All samples were analyzed in duplicate. A ratio of test sample OD to negative control OD (S/N ratio) < 2.1 was considered negative, while S/N ≥ 2.1 was considered positive.

Statistical analysis. Mean and standard deviation were calculated using Microsoft Excel 2010. For each value, Student's r was calculated, and a p-value < 0.05 was considered statistically significant. Serological studies were carried out using the method described by T.S. Saiduldin (1981) [26].

Results

During experiments infecting laboratory Balb/c mice with *T. spiralis* larvae at a dose of 250 larvae, the phase of larval migration from the intestine to the muscle was observed. The results indicate that larval migration begins as early as day 14, which is consistent with multiple studies. Figure 1 shows the numerical values of larvae detected through postmortem examination of the mice.

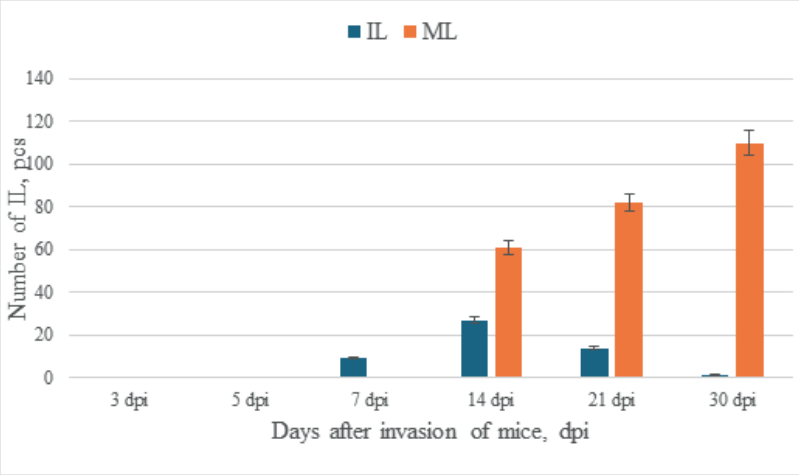
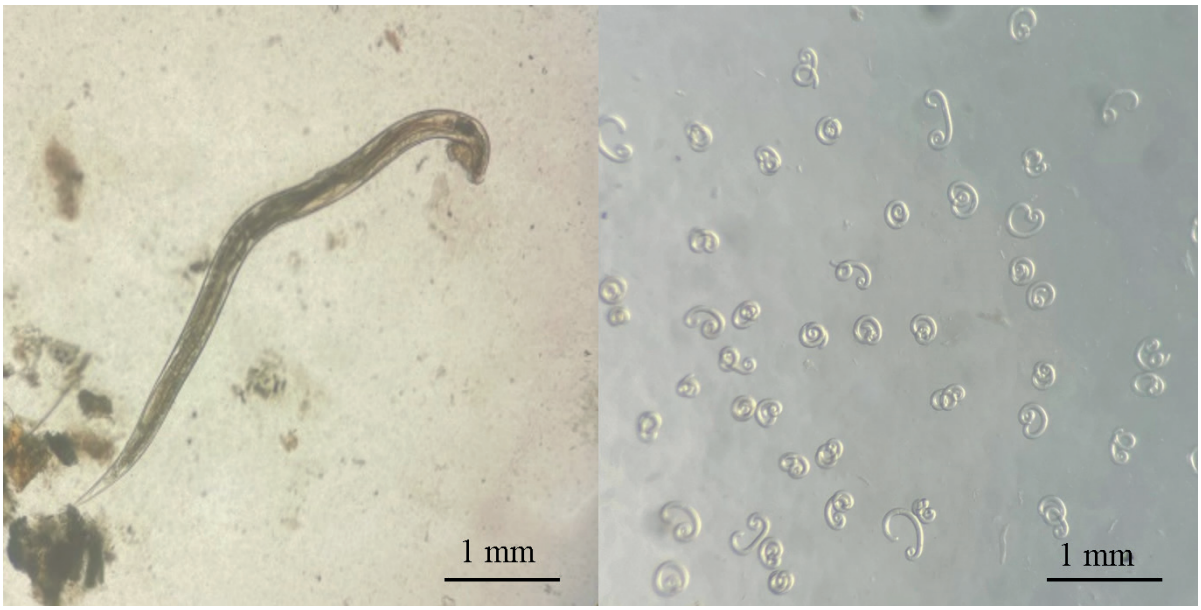


Figure 1 – Development of trichinella larvae in intestine and muscle

As shown in Figure 1, larvae were first detected in the intestines of infected mice at 7 dpi. However, by 14 dpi, active migration of T. spiralis larvae to the muscle was observed, with an average of 61±5.3 larvae. A significant decrease in the number of larvae in the intestine was noted by 21 dpi (14±2.08) ($p<0.01$), accompanied by a 34.4% increase in muscle larvae ($p<0.05$). By 30 dpi, the number of muscle larvae reached 110±4.3 ($p<0.01$), while the number of intestinal larvae decreased to 1.6±0.88 ($p<0.01$). Figure 2 shows muscle larvae isolated at 21 days post-infection.

On day 14, muscle larvae found in the muscles were both spirally twisted and hairpin-shaped, characteristics typical of the early muscle stage. This suggests ongoing larval migration from the intestine. Figure 2 shows muscle larvae isolated at 21 days post-infection.



T. spiralis larvae at 21 dpi found in mouse intestines

T. spiralis larvae at 21 dpi found in mouse muscles

Figure 2 – *Trichinella larvae* in the muscles of BALB/c mice (21 dpi)

Blood serum was collected from mice at each stage of infestation for antibody analysis using indirect ELISA. The results are shown in Table 1.

Table 1 – Antibody titer of mice infected with T.spiralis against ES-Ag measured by indirect ELIS AInventory /number of mice	Days after invasion of animals						Mean antibody titers
	3 days	5 days	7 days	14 days	21 days	30 days	
1	RA	RA	RA	1:800	1: 1600	1:3200	1:1600 (+18.9; -15.9)
2	RA	RA	1:100	1:800	1:3200	1:6400	1:3200 (+20.6; -17.1)
3	RA	RA	RA	1:400	1:1600	1:3200	1:980 (+12.5; -11.1)
Control group							
4	RA	RA	RA	RA	RA	RA	-
5	RA	RA	RA	RA	RA	RA	-
6	RA	RA	RA	RA	RA	RA	-

Note: Reaction absence

The tested method showed low efficiency in detecting trichinellosis during the intestinal phase of infection, despite the presence of larvae in the intestine. Antibodies were first detected at 14 days post-infection (dpi) with titers of 1:400 to 1:800 in some mice within the group. By day 30, titers had increased to 1:3200 and 1:6400, confirming a positive result. However, results obtained between days 7 and 14 could be interpreted as either positive or potentially false positive.

The recombinant serine protease protein Tsp-LE 11 kDa (PP099881.1), based on the cDNA sequence of the large exon of the *T. spiralis* serine protease gene (data not shown), was also evaluated for its diagnostic value. Studies were conducted on mouse, rabbit, and pig sera using Es-Ag, S-Ag, and the test antigen rTsp-LE.

An initial ELISA was performed using sera from mice immunized with the recombinant protein. The results are summarized in Table 2.

Table 2 – ELISA results using Es-Ag and rTsp-LE antigens in mice following two consecutive immunizations

Antigen	Average antibody titer	
	after the 3rd immunization	after the 5th immunization
ES-AG	1:300 (+41.4; -29.3)	1:570 (+23.1; -18.7)
Recombinant protein	1:570 (+23.1; -18.7)	1:2260 (+14.9; -13.0)
P	0.002	0.0001

During the first two immunizations, specific IgG was first detected in the infected mice serum after the third immunization with S-Ag, with OD 450 values of 0.324 and 0.234 at dilutions of 1:200 and 1:400, respectively. When using the recombinant antigen, a positive result was also observed after the third immunization at nearly the same titers of 1:400-1:800, with OD 450 values of 0.277 and 0.205, respectively. After the fifth immunization, detection occurred at titers of 1:400-1:800 and 1:1600-1:3200, with mean OD 450 values of 0.326 and 0.231 for ES-Ag and rTsp-LE, respectively. Despite the high specificity of ES-Ag, the ELISA values for detecting *trichinellosis* in mice using rTsp-LE are only slightly lower. However, it should be noted that the 11 kDa recombinant protein binds specifically

to the serine protease protein of *Trichinella* larvae, as evidenced by the absence of cross-reaction with *Echinococcus*, a commonly detected disease. Furthermore, this antigen was successfully used to diagnose animals infected with T. nativa, yielding results analogous to those obtained with *T. spiralis* (1:3200).

To study species specificity and the reaction of the obtained protein with antibodies from other animal species, indirect ELISA was performed using S-Ag, Es-Ag, and the recombinant protein. The results are presented in the table below.

Table 3 – Determination of antibody titer in sera of infected rabbits and pigs using Es-Ag, S-Ag and rTsp-LE

Antigens	Average antibody titer	
	Infested rabbits	Positive swine serum
S-Ag	1:20 480 (+14.1; -12.3)	1:6 400 (+32.0; -24.2)
ES-Ag	1:20 480 (+14.1; -12.3)	1:3 940 (+39.5; -28.2)
Recombinant protein	1:2 260 (+14.9; -13.0)	1:800 (+41.4; -29.3)
P	0.003	0.001

When sera from infected rabbits and pigs were tested using the three types of antigens, S-Ag showed a positive reaction with a mean titer of 1:20,480 in rabbits and 1:6,400 in pigs, with mean OD 450 values of 0.631 and 0.332, respectively. The results for ES-Ag were nearly identical to those for S-Ag, with titers of 1:20,480 in rabbits and 1:3,940 in pigs, and OD 450 values of 0.581 and 0.456, respectively. The recombinant Tsp-LE protein yielded positive results at mean titers of 1:2,600 in rabbits and 1:800 in pigs, with mean OD 450 values of 0.225 and 0.226, respectively.

Based on the results, the recombinant 11 kDa rTsp-LE protein demonstrates sufficient diagnostic value for detecting trichinellosis infection in different animal species. This protein can be utilized for the further development of a highly specific ELISA test.

Discussion and Conclusion

ES antigens derived from the muscle tissue of *T. spiralis* larvae are widely used as serodiagnostic agents for trichinellosis and are recommended by the International Commission on Trichinellosis (ICT) [27]. However, the process of obtaining ES-Ag requires harvesting muscle tissue larvae from experimentally infected laboratory animals, which can be resource-intensive and labor-intensive. Recombinant proteins offer a promising alternative to ES antigens, as they can be efficiently produced in large quantities using bacterial expression systems and used as antigens in sensitive, specific, and standardized ELISA tests for trichinellosis serodiagnosis. This approach could enhance the accuracy and convenience of diagnosing the disease. Previous studies [28, 29] have suggested that the serine protease of adult *T. spiralis* larvae plays a role in capsule formation and may help protect newborn larvae in the host bloodstream. These findings imply that serine proteases may be involved in intestinal mucosal invasion and could serve as potential targets for early detection of parasitic invasion, as well as for the development of vaccines against trichinellosis infection.

In our study, larvae were detected in the gut starting at 7 dpi, with their numbers increasing from 12 to 91 larvae by 30 dpi. Transformation into muscle tissue was observed between 21 and 30 dpi, and the number of larvae in the gut significantly decreased from 14 to 1.6 larvae. These findings suggest that signs of the disease can be detected between days 7 and 14 using the recombinant serine protease, enabling earlier diagnosis.

These findings are consistent with studies by O.N. Andreyanov, who demonstrated that at a dose of 100 T. spiralis larvae, survival in the intestine persists for 18-24 days [30]. Similarly, Pereverzeva et al. observed no intestinal, sexually mature *Trichinella* in mice after day 21 of infection. In their control group, the survival rates of intestinal *Trichinella* were: 54% after 4 days, 44% after 7 days, 21-30% after 15 days, 16.5% after 21 days, and 4-5% between 28-30 days [31], which aligns with our results.

No encapsulated larvae were observed by day 30 post-infection. These findings align with those of Pereverzeva, who reported the presence of semi-formed larvae in muscle tissue by day 21, suggesting ongoing transport from the intestine. Notably, even by day 35 post-infection, encapsulation in *Trichinella spiralis* was not observed in mice [32].

Serine protease can be expressed at high levels in encapsulated species (such as *T. spiralis, T. nativa, T. britovi,* and *T. nelsoni*) but at lower levels in non-encapsulated species (e.g., *T. pseudospiralis*) [33]. Previous studies have shown that both ES-Ag and S-Ag detect infection between days 8 and 14, with recombinant antigens exhibiting similar sensitivity [33, 34, 36]. However, recombinant antigens offer significantly higher specificity, and the more straightforward and standardized production process compared to ES-Ag and S-Ag makes them a preferable alternative for diagnostic applications.

In the present study, the recombinant rTsp-LE antigen demonstrated its diagnostic potential across three animal species (mice, rabbits, and pigs), with average antibody titers of 1:570, 1:2260, and 1:800, respectively. In comparison, when ES-Ag and S-Ag were used, the mean titers were much higher: 1:20480 for ES-Ag and S-Ag in rabbits, and 1:6400 and 1:3940 for pigs, respectively. These findings suggest that the recombinant 11 kDa *T. spiralis* antigen could serve as a viable alternative to ES-Ag and S-Ag for diagnosing trichinellosis. Additionally, no significant difference in antibody detection rates was observed when sera from mice infected with *T. nativa* were tested using the recombinant antigen, and there was no cross-reactivity with sera from *Echinococcosis*-infected animals.

According to the literature, antibodies are typically detectable 4-6 weeks after humans are infected from wild animals. A diagnostically significant result is a fourfold or greater increase in antibody titers in paired sera collected 14-20 days apart. Antibody levels peak between the 2nd and 4th months post-infection and then gradually decline, but can persist for up to 10 years or longer [34].

Garkavi et al. reported that in experimentally infected mice, total antibody concentrations peak at 6 weeks and again at 2-3 months, with IgM peaking on days 30 and 90, and IgA peaking on days 90 and 120 post-infection [35].

The recombinant serine protease protein has recently been actively studied as a component in vaccination. Mice vaccinated with the purified rTspSP-1.3 protein [36] showed an average 39% reduction in muscle larval load compared to the control group.

Recombinant rTsSP protein combined with cholera toxin subunit B (CTB) [37] induced a significant intestinal local sIgA response and a systemic *TsSP*-specific antibody response in vaccinated mice. Moreover, an increased number of goblet cells, acid mucins, and IgA-secreting cells were observed in the small intestine of vaccinated mice. Immune serum against rTsSP specifically recognized the cuticle of various stages of the worm, including muscle larvae, intestinal infective larvae, and adult worms. Vaccinated mice showed a 71.10% reduction in adult worm numbers at 9 dpi and a 62.10% reduction in muscle larvae at 42 dpi. Furthermore, rTsSP vaccination also inhibited the development of intestinal *T. spiralis* and reduced female fecundity.

These findings align with the study by Wang L. et al., who examined the 31 kDa antigen of T. spiralis and observed a slightly lower result compared to ES-Ag, with ELISA sensitivity and specificity of 96.67% and 96.87% for the recombinant protein, versus 100% and 98.44% for ES antigens [13]. In a study of four recombinant antigens for *Trichinella*, the r-P1 protein detected antibodies as early as 8 dpi, followed by the r-P2, r-P3, and r-P4 proteins at 10, 14, and 16 dpi, respectively, with antibody levels remaining elevated up to 45 dpi [38]. Similarly, the recombinant serine protease of *T. spiralis* (rTsSP-ZH68) was recognized by sera from infected mice at 8-10 dpi and by sera from early trichinellosis patients at 19 dpi [39].

The recombinant protein rTsp-LE, developed in this study, showed its effectiveness in detecting infection in experimentally infected animals. This antigen demonstrates promise not only for the development of ELISA-based diagnostic tests but also as a potential component for vaccines aimed at reducing the invasive load in animals.

Detection of the intestinal stage of *T. spiralis* on the 7th day post-infection (dpi) allows for the early identification of antibodies, enabling detection of the disease at an early stage. The studied recombinant protein demonstrates high diagnostic value, showing reliable results as early as after the third immunization. The absence of cross-reaction with echinococcosis, along with the ability to detect antibodies to *T. nativa,* supports the potential use of this protein in the development of an ELISA test. Future studies should focus on exploring the use of this protein not only in diagnostic applications but also as a component of a vaccine, or independently, to reduce the parasitic load during infection.

Authors' Contributions

OA: Designed and supervised the study and drafted the manuscript. AG: Statistical analysis and drafted the manuscript. AS, NA: Designed and conducted the study. DhZ: Bioinformatic analysis. FZ: Conducted the study and drafted the manuscript. NG, SYe: Conducted the study and bioinformatic analysis. All authors have read, reviewed, and approved the final manuscript.

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Conflicts of Interest

The authors declare that they have no competing interests.

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Meat Quality Control in Beef Production

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Abstract

The hazard analysis and critical control points (HACCP) system is primarily a risk assessment method focused on identifying and controlling the risk factors classified as the critical control points. Pathogenic microorganisms can enter the meat supply chain at various points along their path. Live animals can harbor various pathogens, while unhygienic conditions during slaughter and processing introduce external contamination risks, and cross-contamination can occur during handling and cooking. Traditionally, pathogen control is concentrated in the processing stage. Despite its effectiveness, approaches like HACCP do not fully satisfy the consumer demands for food safety. A more comprehensive approach targeting pathogen control at every stage of the supply chain that is, "from farm-to-table," offers broader coverage and greater integration.

Meat is one of the most perishable food products, providing an ideal environment for the growth of a wide range of pathogenic bacteria. Foodborne illnesses result from the consumption of bacteria, toxins, or cells produced by microorganisms present in food. Past outbreaks have been linked to various stages of the meat production process; hence, it is logical to develop a risk assessment model encompassing all stages, from raising food animals and processing carcasses to meat preparation and consumption.

The primary task in meat production is the evaluation of the microbiological quality of raw meat and the sanitary conditions at processing facilities based on the presence of indicator microorganisms in the meat, equipment, and processing plants. Accordingly, hygienic measures in meat production, processing, and retail aim to ensure meat safety, prevent rapid spoilage, and preserve its quality. HACCP, which heavily relies on prerequisite programs like good hygienic practice and sanitation standard operating procedures, provides enhanced hygiene standards essential for producing safe meat and meat products throughout the entire meat supply chain.

Keywords: critical control points; food products; HACCP; hygienic methods; microbiological quality; slaughterhouse.

Introduction

The hazard analysis and critical control points (HACCP) system is founded on three core principles: hazard identification, determination of critical control points, and implementation of risk mitigation procedures. Additionally, it involves the development of a monitoring system for evaluating the effectiveness of control measures. A critical control point is a stage in the production process where specific interventions can reduce the risk of adverse outcomes. Ineffective control methods should be revised accordingly [1].

For more than twenty years, food safety professionals have been so vigorously promoting HACCP food safety that they have actually overemphasized the usefulness of the HACCP concept [2]. The

global use and success of the HACCP system in the food industry has created false expectations that it can be used successfully at all stages of the food supply chain, from farm to table.

The long global evolution and use of HACCP in food processing facilities has provided a tremendous amount of documentation that the HACCP food safety system is very effective in combating identified foodborne hazards. Its ability to ensure food safety far exceeded that of the quality control system it replaced. The widespread success of HACCP has led regulators, policy makers and consumers to increasingly call for this remarkable tool to be utilized more effectively by applying it throughout the food chain from "farm to table." Simply put, the supply chain consists of seven stages: animal or crop production, slaughter or harvest, raw food production, processed food production, distribution, catering or retail operations, and consumption. The production of processed products is at the center of this supply chain. It is no coincidence that HACCP originated at the food processing stage - from farm to table.

Meat production is increasing worldwide and according to FAO/WHO estimates [3], it will reach 364 million tons by 2023. The growing demand for meat in developing countries is mainly the result of the rapid progress of societies that are becoming increasingly urbanized.

In recent years, there has been growing concern about the presence of pathogenic microorganisms in meat products despite increased hygiene measures for meat and processed meat products [4]. Contamination can occur during processing, through contact with equipment (e.g., grinders, belts, saws), through contact with food handlers (e.g., hands, knives), and through exposure to other environmental sources (e.g., air, water) [5].

Regulators require meat processing plants to implement hazard analysis and critical control point (HACCP) systems for meat production processes to reduce pathogens [6]. However, since it is difficult or impossible to completely eliminate pathogens from raw meat, the goal of HACCP for meat focuses on reducing and preventing microbial growth [7]. The HACCP system is now used in many regulated sectors of the food industry.

Monitoring microorganisms in meat products is an important step in HACCP programs [8] and proper storage of meat products is critical to control contamination of meat with spoilage microorganisms [9]. It has been proposed that HACCP systems in meat processing plants should be based on microbiological data, with the assessment of the number of indicator organisms in meat products at different stages of processing [10].

For raw meat products, safety and quality can be assessed using indicator microorganisms, including total aerobic platelet count (APC), coliform count (CC), and Escherichia coli (ECC) [11]. APC provides an estimate of the total bacterial population. Higher APC is generally associated with lower quality and reduced shelf life. The relationship between APC and the concentration of foodborne pathogens in raw meat is unclear. CC and ECC provide an estimate of faecal contamination and poor sanitation during processing. High CC and ECC values generally correlate with higher levels of foodborne pathogens originating from feces [12]. Meat can be further contaminated or cross-contaminated with various pathogenic bacteria after slaughter, such as during chilling, cutting, deboning, and slicing [13].

Thus, all processing conditions are important factors that can affect the microbiological quality. To improve the safety of finished meat products, more information is needed at the stage of contamination of carcasses or meat cuts in meat processing plants [14]. However, data on seasonal variations and meat type in microbiological testing of meat in different meat processing plants are limited. In particular, microbiological assessment of meat in meat processing plants that have implemented the HACCP system has not been carried out.

Quality Control Programs

The most effective strategy for reducing meat contamination and microbial growth is the implementation of quality control programs, such as good manufacturing practice (GMP) and HACCP. These programs involve identifying indicator microorganisms that can predict the presence of pathogenic microorganisms and spoilage-causing bacteria [15]. Notable bacterial foodborne pathogens, including *Salmonella, Campylobacter*, and *verocytotoxigenic Escherichia coli* (VTEC), can accumulate in and be excreted from the gastrointestinal tract of farm animals, including cattle. The typical sequence leading to beef-related foodborne illnesses involves pathogen transmission from cattle to humans through direct or indirect fecal contamination, subsequent cross-contamination, and/or proliferation during the production, processing, and consumption of beef and beef products.

The effective control of beef-borne pathogens necessitates a longitudinal, integrated approach based on the "meat chain" concept. This approach employs the GMP/GHP and HACCP principles, with shared responsibility among all participants in the meat supply chain. It also requires the consideration of resource availability, technical capabilities, consumer attitudes and behavior, and cost-effectiveness [16, 17, 18].

Meat as a Favorable Environment for Microorganism Growth

Meat and meat products provide an exceptionally favorable environment for the growth of pathogenic microorganisms [19]. Its perishable nature and nutrient-rich composition enable meat to support the proliferation of a wide range of pathogenic bacteria [20]. It is vulnerable to contamination at multiple stages, from primary production to the final consumption phase (from farm to fork). Contaminated meat is a major source of foodborne diseases and mortality caused by pathogens entering the body upon ingestion [21]. Foodborne illnesses result from the consumption of bacteria, toxins, or cells produced by microorganisms present in contaminated food [22]. Consequently, retail meat sales are frequently linked to foodborne illnesses if infectious doses are ingested during consumption [23, 24]. Epidemiological and microbiological studies identified cross-contamination during distribution and processing, along with subsequent bacterial growth, as significant contributors to foodborne diseases [25, 26, 27].

Meat and meat products are commonly implicated in food poisoning outbreaks. During production, processing, and storage, these products can become contaminated with pathogenic bacteria, such as *Listeria monocytogenes* [28, 29]. Hygiene and quality during meat handling and processing must be continuously monitored to ensure that meat products adhere to international quality and safety standards [30]. Given its rich nutrient profile, meat is a prime environment for the growth of pathogenic bacteria. The microbiological contamination of carcasses predominantly occurs during handling processes, including skinning, evisceration, processing, storage, and distribution in slaughterhouses and retail outlets [31].

In India, dietary habits have significantly shifted because of rapid urbanization and Westernization, leading to increased consumption of non-vegetarian foods. This shift has resulted in a rising meat demand. Concurrently, consumers have become more vigilant regarding meat quality, freshness, and health aspects [32]. The microbiological quality of meat and meat products is critical for public health. Numerous reports have documented foodborne disease outbreaks linked to meat consumption [33, 34, 35]. Fecal contamination, either through direct deposition or indirect contact via contaminated equipment, workers, facilities, and air, is a primary source of contamination [36]. Pathogens can contaminate meat at various stages of the slaughtering process [37, 38], necessitating appropriate control measures for eliminating or preventing microbial contamination. Among the pathogenic bacteria that can contaminate beef, *Salmonella spp., Listeria monocytogenes,* and *E. coli O157* are frequently associated with foodborne illnesses [39, 40]. It is now widely acknowledged that traditional meat inspection procedures cannot fully ensure that consumers are not exposed to infectious doses of meat-borne pathogens [41]. Therefore, meat inspection authorities worldwide are promoting or mandating the implementation of HACCP systems for meat production processes. While comprehensive HACCP systems should address physical, chemical, and microbiological hazards, the latter remains as a primary concern in meat production [42].

Critical Control Points

In the pre-harvest stage (on the farm), the global recycling of microbial pathogens can be mitigated through the strategic management of agricultural lands and livestock by-products. The fecal shedding of pathogens by farm animals is reduced by employing antimicrobial feed treatments, sourcing new animals from controlled environments, implementing biosecurity measures, optimizing animal welfare (including stress management), maintaining hygienic housing conditions, and utilizing prebiotics, probiotics, competitive exclusion strategies, and vaccination programs.

During the harvest stage (at the slaughterhouse), minimizing transportation time and ensuring optimal pre-slaughter conditions for animals can help reduce cross-contamination via transport vehicles or holding pens. The key measures include maintaining hygienic practices during slaughter and carcass processing, implementing effective sanitation procedures, and potentially applying treatments to hides and/or carcasses to prevent contamination.

In the post-harvest stage (processing, storage, distribution, and consumption), pathogen control in meat products often relies on bactericidal steps, such as cooking, or, where such steps are not employed, on the "multiple hurdles" concept of microbial control. Additional post-harvest risk reduction measures include effective cleaning and sanitation of all facilities involved in meat-processing, maintaining the cold chain, preventing cross-contamination during further processing or cooking, and educating consumers on food hygiene. The effectiveness of control measures varies depending on the pathogen and the stage of the meat supply chain. For instance, some pathogens in beef (e.g., *VTEC, Salmonella*) are most effectively controlled through primary production measures combined with enhanced slaughter hygiene. In contrast, other pathogens (e.g., *L. monocytogenes*) are best controlled during the processing and storage stages [43].

Quality monitoring systems are commonly implemented in the food industry to systematically assess indicators at specific points along the technological line and during meat handling [44]. In these systems, operations that significantly affect the microbiological quality of the product must be identified as critical control points (CCPs) and/or quality control points and managed to minimize contamination by pathogenic or spoilage bacteria [45]. Cleaning and disinfection are essential components of biosecurity on livestock farms, reducing animal exposure to foodborne pathogens [46, 47].

Management practices in feedlots can affect animal health, carcass quality, and, potentially, food safety. Feedlots, where cattle from various sources are mixed and housed at high densities, can be sources of contamination by *EHEC O157* (*enterohemorrhagic E. coli O157*) and *Salmonella* spp. Advanced enrichment and isolation techniques demonstrated that the EHEC O157 prevalence among cattle is significantly higher than that previously estimated, reaching 5.8% [48, 49]. Microbiological testing provides a means of assessing how effectively operators control slaughtering, processing, and production processes to minimize and control contamination [50]. The effectiveness of these systems has been demonstrated. Based on these indicators, corrective actions can also be taken to prevent or reduce potential contamination.

The safety and quality of meat products can be evaluated using various microbial indicators, such as the aerobic mesophilic count and coliforms [51]. The aerobic mesophilic count assesses the general microbial population in the environment and processing equipment, where high contamination levels indicate poor hygienic conditions [52]. Coliforms serve as indicators of inadequate sanitary conditions during food processing, production, and storage, with *E. coli* being a classic indicator of the potential presence of intestinal pathogens and a good measure of the sanitary quality of processed foods [53].

The Salmonella spp. transmission from animals to food is a significant concern for human health. It can only be controlled through rigorous cleanliness and adherence to hygienic procedures during slaughter and processing [54]. As natural reservoirs of pathogenic strains, cattle often contaminate ground beef with *EHEC O157* isolated from live animals, carcasses, and retail meat [55]. One study found that cattle housed in feedlots for less than 20 days were 3.4 times more likely to test positive for *EHEC O157* compared to those housed for longer periods [56]. Although *E. coli* is a common and usually harmless inhabitant of the gastrointestinal tract, some strains produce verotoxin that causes severe illness, including diarrhea, hemorrhagic colitis, and potentially fatal kidney failure [57, 58]. Stress responses in cattle, which are potentially linked to feed withdrawal and transportation, may exacerbate these issues. Additionally, *EHEC O157* has been detected in water bodies on many farms, suggesting that water sources may act as long-term reservoirs for this pathogen [59].

"Farm-to-Table" Concept

Meat is susceptible to contamination from both internal and external sources to the animal during slaughter and sale. Live animals can harbor a range of microorganisms on the surfaces that come into contact with their environment. The major contamination sources include animal hides and feces. Additional contamination sources can be the slaughter area, slaughterhouse environment, retail floors, air, and vehicles used for transporting meat from the slaughterhouse [60, 61]. Retail points also contribute to meat contamination [62]. In retail environments, tools like knives, wooden boards, and scales are common sources of bacterial contamination, particularly from *Staphylococcus aureus* and *Shigella species* [63]. Cross-contamination during transport not only affects cattle, but is also a significant source of pathogen transmission in poultry. Despite cleaning and sanitizing efforts, the crates and containers used for poultry transport often remain contaminated with *Salmonella* [64].

Considering that past disease outbreaks are linked to multiple production stages, it is advisable to create a thorough risk assessment model that addresses every stage, from animal rearing and carcass processing to meat preparation and consumption. In pork production, the supply chain encompasses the following stages: pre-harvest (on the farm, during transport, and storage), slaughter (skinning, evisceration, chilling, and production), processing (grinding, treatment, transport, storage, and distribution), consumption (cooking and eating), dose–response (exposure assessment, dose–response function, morbidity, and mortality), and human disease costs [65, 66].

The primary sources of the microbiological contamination of beef carcasses during slaughter include the leakage of intestinal contents and the cross-contamination from hides of slaughtered animals [67, 68]. Fecal contamination is a significant source of microorganisms [69]. The microbiological testing of carcasses is routinely used to verify the HACCP compliance in slaughterhouses. In the European Union (EU), this involves measuring aerobic colony counts and *Enterobacteriaceae* counts known as hygiene indicator organisms to ensure they are within established permissible limits [70].

Cutting and boning operations at meat-processing facilities involve extensive handling, which increases microbial risk due to the following reasons: (a) cross-contamination from hands and tools (e.g., knives, saws, and conveyors); and (b) the transfer of bacteria from the meat surface to its internal parts [71]. However, satisfactory results from slaughter line testing do not guarantee the meat product safety. Additionally, microbial contamination on surfaces in contact with food can sometimes exceed that on carcasses, leading to the accumulation of contamination during the cutting and processing stages [72, 73].

Slaughterhouses are a critical sector in the food industry in terms of potential foodborne illnesses and health hazards, particularly if the food hygiene principles are not strictly followed [74]. The hygiene practices of slaughterhouse workers play a crucial role in contamination. Dirty hands, clothing, and equipment used in carcass handling are contamination sources [75]. A study indicated that 48.4% of slaughterhouse workers did not cover their hair; 29% did not use aprons; and 64.5% wore jewelry (e.g., rings, bracelets, and watches) while working. These findings are consistent with data showing that 61.6% of workers did not cover their hair, and jewelry usage was not regulated [76].

The contamination in slaughterhouses and retail meat shops arises from the use of contaminated water, unhygienic practices (e.g., improper handling), and the use of contaminated surfaces and tools during meat-cutting operations [77].

Transportation can be a stressor for farm animals associated with the shedding of pathogenic bacteria like *EHEC O157* and *Salmonella* spp. in feces, leading to a contamination of trailer floors and bedding [78]. If trailers and bedding are not cleaned and disinfected between trips, they can spread contamination to other farms, the slaughterhouse environment, and other animals [79]. Hygiene rules apply not only during animal housing, but also during transportation. Cattle transported over long distances (more than 160.9 km) are twice as likely to test positive for *E. coli O157* at slaughter compared to those transported at shorter distances [80]. Ineffective vehicle cleaning and sanitizing contributed to contamination in 84% of cattle hides with *E. coli O157*, which had not been detected in any animal at the farm of origin [81].

Hygienic Methods and Quality Control of Meat and Meat Products

As widely recognized, the most significant threats to fresh meat products come from pathogenic bacteria capable of causing illness in humans, including *Salmonella, S. aureus, L. monocytogenes, Campylobacter,* and *E. coli O157*. Particularly, *E. coli O157* can cause food poisoning with only a small number of cells. The primary contamination sources are the slaughter animals themselves, workers, and the working environment. Air contamination through aerosols and water used in carcass processing plays a relatively minor role [82, 83]. Contaminants largely originate from an animal's skin and include microorganisms from the stomach and the intestines, which are released with feces [84]. Foodborne pathogens are a significant public health and economic concern in developed countries [85, 86].

A contamination level of up to 10^5 CFU/cm² indicates good hygienic conditions during slaughter, while higher levels suggest unsatisfactory conditions. Meat contaminated at 10^6 CFU/cm² indicates spoilage, characterized by unpleasant odor and reduced shelf life, while contamination reaching 10^7 CFU/cm² is associated with a noticeable slime formation [30]. Mold counts are used as the indicators of sanitary quality in food production facilities because molds can rapidly proliferate on food residues

adhering to surfaces, consequently posing a potential contamination risk. Some molds produce toxic metabolites known as mycotoxins, which can pose health risks to humans and animals [87].

Hygienic methods and quality control measures for meat and meat products are recommended in many countries, particularly in the food service sector [88]. Without proper hygiene control, slaughterhouse and meat-processing environments can become significant sources of bacterial contamination [89].

The primary objective in meat production is the assessment of the microbiological quality of raw meat and the sanitary conditions at processing facilities based on the presence of indicator microorganisms in the meat, equipment, and processing plants. Identifying the key contamination points and introducing microorganisms into meat allow specialists responsible for production hygiene to implement proper manufacturing practices at meat-processing facilities, thereby improving a product's microbiological quality [90]. Microbiological carcass testing is commonly used to verify the HACCP compliance in slaughterhouses. In the EU, this involves measuring the aerobic colony and *Enterobacteriaceae* counts, known as hygiene indicator organisms, to ensure they are within specified permissible limits [91].

Hygienic measures in meat production, processing, and retail aim to ensure meat safety, prevent rapid spoilage, and maintain quality. HACCP, which heavily relies on prerequisite programs, such as GHP and sanitation standard operating procedures, ensures improved production hygiene conditions necessary for safe meat and meat products throughout the meat supply chain. By the end of the 20th century, HACCP had been mandated, implemented, and continuously used by every meat company involved in international trade [92].

Under EU legislation that is, regulation EC nos. 852/2004, 853/2004, and 854/2004, food business operators at all stages of the production chain bear the primary legal responsibility for ensuring the safety of the food they produce. While HACCP-based procedures are not required at the primary production stage (on the farm), farmers are expected to produce food with hygiene considerations in mind using an approach similar to HACCP. Although the classic HACCP approach is not fully applicable to food safety control on meat farms, the HACCP principles can still be applied to identify hazards and potential corrective actions. Food safety systems for beef should include at least: (1) full animal traceability; (2) provision of clean, uncontaminated feed; (3) provision of clean, uncontaminated water; (4) hygienic animal housing/living conditions; (5) clean livestock; (6) animal health, welfare, and disease prevention; (7) proper waste disposal; (8) hygienic transport and handling; and (9) biosecurity [93, 94].

Foodborne diseases are more common in developing countries because of the widespread improper food handling and sanitation practices, inadequate food safety laws, weak regulatory systems, lack of financial resources for safer equipment, and insufficient education for food industry workers [95].

The bacterial composition of meat serves as an acceptable indicator of its hygienic quality. The poor infrastructure at slaughterhouses, unsanitary animal handling, and improper carcass handling contribute to high bacterial loads in meat. Therefore, evaluating bacterial counts can indicate potential threats to human health [96].

Conclusion

A higher meat contamination level by these microorganisms reflects the poor hygiene and sanitation practices applied at the slaughterhouse during transportation to meat-processing plants and during processing at meat plants [97].

However, the lack of specific critical control points that can eliminate or control the identified hazards hinders the effective use of HACCP throughout the supply chain. Food safety measures can be used at every stage of the supply chain, but most of these measures will be pre-programs rather than critical control points from the HACCP system [98]. The most pressing food safety issues in the food industry today are caused by the presence of Escherichia coli O157:H7 and Salmonella in raw meat and poultry products and in processed foods. Attempts to control these pathogens either at the "farm" or "table" level of the supply chain have failed due to the lack of effective control measures at these stages available at the food processing stage.

Cross-contamination and other poor food handling practices can also lead to foodborne illness. Thus, the idea that HACCP can be effectively applied from farm to fork has been reconsidered.

The microbiological quality of beef and meat products is highly dependent on the hygienic conditions prevailing during their production and processing. Without proper hygienic control, the environment in slaughterhouses and butcher shops can be an important source of microbiological contamination.

The impact of HACCP on process hygiene performance in slaughterhouses has been previously studied [99, 100]

Specific studies were conducted to determine whether mandatory implementation of HACCP systems in meat processing and retail establishments has any significant impact on their microbiological indicators of process hygiene [101]. The reduction in bacterial counts on food contact surfaces, butchers' hands and refrigeration units was found to provide strong evidence of improved process hygiene performance and justifies the adoption of GHP and SSOP in meat plants. This relatively large-scale study showed that the implementation of HACCP was associated with improved process hygiene in meat plants. However, this finding is necessarily limited in scope as it is not possible at this time to determine whether such improvements would have a similar positive effect on the incidence of meat borne diseases. Further research is clearly needed to establish this.

This information on the key microbiological contamination points in the beef processing chain will assist hygiene professionals in such establishments in developing appropriate hygiene procedures to prevent or reduce microbiological contamination of beef and meat products.

Authors' Contributions

GI: Conceptualized and designed the study, analyzed the data, and drafted the manuscript. SS: Conducted an extensive literature review and analyzed the data. Both authors reviewed and approved the final manuscript.

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UDC579.672 **Research article**

Analysis of microbial contamination in different types of meat in the Kostanay region

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Abstract

Background and Aim. Meat is a favourable environment for the growth and viability of pathogenic microorganisms. Bacteria in meat cause spoilage and increase the risk of foodborne toxic infections of various origins during consumption. The importance of monitoring and analysing microbial contamination is increasing due to increased requirements for food safety and public health protection. The aim of the study was to analyse the overall level of microbial contamination of different types of meat sampled from retail outlets in the Kostanay region.

Materials and Methods. A total of 30 meat samples, including pork, beef, and horse meat, were collected for analysis. To assess microbial contamination of the meat, the number of mesophilic aerobic and facultatively anaerobic microorganisms (QMAFAnM) and their species composition were determined.

Results. It was found that 96% of the meat sold at the retail level had elevated QMAFAnM, with coliforms isolated in 30% of the samples. *Salmonella spp.* and *Listeria monocytogenes* were not detected in the meat tested. Pork meat had the highest microbial contamination compared to beef and horse meat samples.

Conclusion. Exceeding the permitted levels of QMAFAnM and the presence of coliform bacteria indicate potential health risks for consumers and more effective measures are required to ensure food safety.

Keywords: coliforms; food safety; meat; microbial contamination; QMAFAnM.

Introduction

Food safety and consumer protection are fundamental priorities in government policies worldwide. Ensuring the safety of raw materials and food products is essential for maintaining public health. Food quality directly impacts population health, helping to reduce the risk of infectious and chronic diseases. This underscores the need for strict control over the production, storage, and distribution of food products.

Meat is an important component of a healthy diet, rich in high-quality protein essential for the normal functioning of all body systems [1]. The proteins in meat provide all the essential amino acids necessary for the growth and repair of tissues. Additionally, meat contains B-group vitamins, which play crucial roles in energy metabolism and various metabolic processes, helping maintain a healthy nervous system and cognitive abilities [2]. Meat is also abundant in micronutrients like magnesium, selenium, and zinc that support the immune system and are involved in producing hormones and enzymes [3].

Meat also supplies bioavailable forms of iron and phosphorus, which are essential for oxygen transport and bone mineralization, respectively [4]. The unique nutrient composition of meat makes it a key dietary component for sustaining cellular functions and biochemical processes critical to physiological health.

However, it should be noted that meat and meat products can potentially source several infectious diseases. In particular, meat derived from animals afflicted with illness has the capacity to result in human infection with zooanthroponotic diseases [5, 6].

The contamination of meat with microbial organisms represents a significant challenge to consumer health and the quality of food products in the livestock and food industries. Pathogenic bacteria, including *Escherichia coli, Listeria monocytogenes* and *Staphylococcus aureus*, may be present in poultry, pork, mutton and beef. Shiga-toxin-producing E. coli is frequently associated with beef and is responsible for a range of severe toxic infections, including haemorrhagic colitis and haemolytic uremic syndrome [7]. *L. monocytogenes* is a facultative anaerobic Gram-positive bacterium that is found in fresh meat and meat products. However, human listeriosis is mainly associated with ready-to-eat meat products with a long shelf life in the refrigerator [8]. A prevalence of 35% was observed for S. aureus in retail meat samples collected in 39 cities in China between 2011 and 2016 [7].

Furthermore, the contravention of storage conditions gives rise to the deterioration of foodstuffs and an augmented probability of contracting food poisoning upon consumption [7, 9]. Statistical evidence indicates that meat spoilage is responsible for approximately 400,000 deaths and over 600 million disease cases annually. The World Health Organization (WHO) has reported that a significant proportion of the global population has experienced food-related illness at some point in their lives, with over 30 diseases linked to poor food quality [10]. The relevance of this issue is also confirmed by data from Kazakhstan. In 2020, 37 cases of acute intestinal infections per 100,000 people were recorded, while in 2023, this figure increased to 55.37 cases per 100,000 people. The rise in morbidity highlights the need to tighten control over the quality of food products and improve sanitary and hygienic conditions at all stages of meat production and distribution [11].

The aim of our research is to analyse the overall level of microbial contamination of meat of different types of animals sampled from retail outlets in Kostanay region.

Materials and Methods

The study was conducted in the microbiological analysis laboratory of the Scientific Research Institute of Applied Biotechnology at A. Baitursynuly Kostanay Regional University. A total of 30 samples of chilled meat, including pork (n=10), beef (n=10), and horse meat (n=10), were collected from five different retail outlets in accordance with the GOST 31904-2012 standard “Food products. Methods of sampling for microbiological tests” [12].

Samples were delivered to the laboratory in sterile containers, with temperature control maintained to preserve sample integrity for microbiological analysis. Upon arrival, the samples were processed immediately under aseptic conditions to prevent any extraneous contamination.

The microbiological methods employed in the study were conducted in accordance with the standards outlined in GOST [13-18].

For the identification of coliform bacteria, samples were placed in meat-peptone broth (FBIS SRCAMB, Obolensk, Russia), followed by subculturing onto Endo agar and TBX agar (CHROMagar, Paris, France) and incubated at 37 °C for 24 hours. Colonies with characteristic morphology were subjected to further biochemical analysis.

For *S. aureus*, yolk-salt agar was used, with incubation at 37 °C for 24 hours, and then at room temperature for an additional 24 hours, with confirmatory tests based on coagulase activity.

Detection of *Salmonella* spp. included pre-enrichment in buffered peptone water (FBIS SRCAMB, Obolensk, Russia), selective enrichment in Rappaport-Vassiliadis broth (FBIS SRCAMB, Obolensk, Russia), and subsequent inoculation onto Salmonella agar (CHROMagar, Paris, France).

To identify *L. monocytogenes*, samples were placed in Fraser broth (FBIS SRCAMB, Obolensk, Russia), enriched, and incubated for two days, with the first day at 30 °C and the second at 37 °C, followed by subculturing onto Listeria chromogenic agar (CHROMagar, Paris, France) and Oxford agar (Condalab, Madrid, Spain).

To determine the total microbial number (QMAFAnM), which is the quantity of mesophilic aerobic and facultative-anaerobic microorganisms, we conducted studies using the classical deep culture method in accordance with the standards outlined in GOST 26670-91 [18].

The presence of total microbial contamination was assessed by inoculating serial dilutions of the samples onto QMAFAnM media (FBIS SRCAMB, Obolensk, Russia) and incubating at 37°Cfor 24 hours, followed by an additional 24 hours at room temperature.

The arithmetic mean of the number of colonies from all cultures was calculated using the counting process results. The number of microorganisms in 1.0 g ("X") of the product was calculated using the following formula:

$$X = n \times 10^m,$$

this formula defines the variables as follows: "n" represents the number of colonies counted on the dish, while "m" denotes the number of decimal dilutions.

The calculation result was expressed as a number between 1.0 and 9.9×10m. The obtained number of microorganisms was then subjected to a verification process in accordance with the relevant requirements outlined in "Customs Union Technical Regulations on the safety of food products 021/2011" [19].

Results

Among the microbiological indicators, we conducted studies to determine the presence of coliform bacteria. Characteristic growth of E. coli was observed on solid differential Endo medium, forming colonies with a metallic sheen, and on CHROMagar TBX chromogenic medium, where colonies appeared blue. *Proteus spp.* colonies on Endo agar were transparent with swarming growth and a distinctive odor. Growth of *E. coli* (7 strains) and *Proteus spp*. (2 strains) was detected (Figure 1).

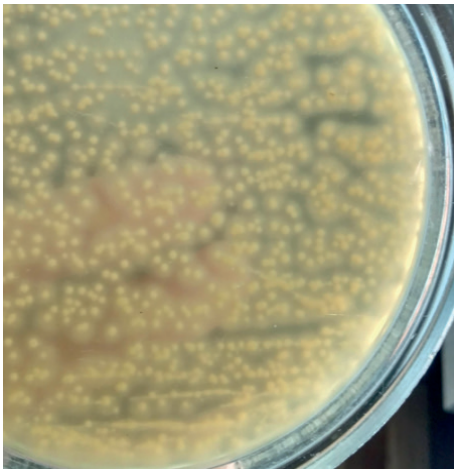
Growth of *E. coli* in sample No. 5 on Endo medium

Growth of *E. coli* in sample No. 7 on CHROMagar TBX medium

Growth of *Proteus* in sample No. 2on Endo medium

Figure 1 – Microbiological investigation of coliform bacteria

Research was also carried out to detect *S. aureus*. Staphylococci demonstrated characteristic growth on yolk-salt agar, forming colonies with "a rainbow halo" (indicating lecithinase production). A total of 4 strains coagulase-positive *S. aureus* were detected (Figure 2).



Growth of *S. aureus* in sample No. 26 on yolk-salt agar

Figure 2 – Microbiological investigation of *S. aureus*

The results of these studies demonstrated that *L. monocytogenesand Salmonella spp*. bacteria were not detected in any of the tested samples.

The number of colonies grown was enumerated in the cultures. The quantity of microbial colonies was determined by counting in cultures of dilutions at 10^2, 10^3,10^4, respectively (Figure 3).

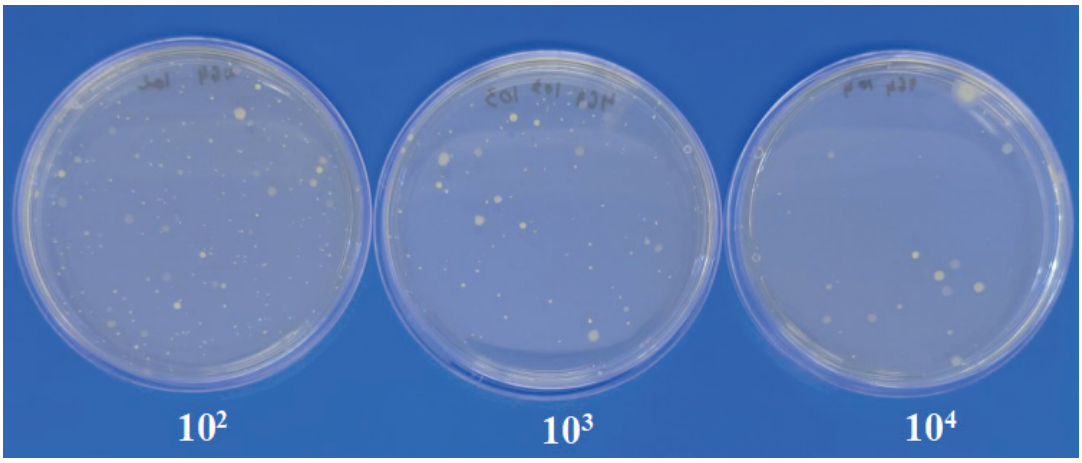


Figure 3 – Microbiological investigation QMAFAnM (dilutions: 10^2, 10^3, 10^4)

The findings of the study indicated that all 10 pork samples analysed exceeded the permissible level of QMAFAnM. Coliforms were identified in four samples (No. 2, No.8 - *Proteus spp.*, No.5, No.7 - *E. coli*). Two strains of S. aureus were isolated from samples No. 2 and No. 8. However, no evidence of *Salmonella spp.* or *Listeria spp.* bacteria was observed (Table 1).

Table 1 – Results of microbiological examination of pork

Sample №	QMAFAnM, CFU/g	Coliforms	*Salmonella spp.*	*L. monocytogenes*	*S. aureus*
1	11×10^4	–	–	–	–
2	15×10^4	+	–	–	+
3	5.4×10^4	–	–	–	–
4	7.9×10^4	–	–	–	–
5	8.2×10^4	+	–	–	–
6	8.5×10^4	–	–	–	–
7	35×10^4	+	–	–	–
8	27×10^4	+	–	–	+
9	10×10^4	–	–	–	–
10	9.7×10^4	–	–	–	–
Norma	1×10^4 CFU/g, max.	Not allowed in 0.001 g of product	Not allowed in 25 g of product	Not allowed in 25 g of product	Not allowed in 1 g of product

The analysis results of beef samples indicate that all tested meat samples exceed the permissible level of QMAFAnM. The isolation of coliform bacteria was observed in three samples (No.12, No.16, No.20 - *E. coli*). The tested samples did not show the presence of *S. aureus, Salmonella, or Listeria species* (Table 2).

Table 2 – Results of microbiological examination of beef

Sample №	QMAFAnM, CFU/g	Coliforms	*Salmonella spp.*	*L. monocytogenes*	*S. aureus*
11	5.9×10^4	–	–	–	–
12	15×10^4	+	–	–	–
13	24×10^4	–	–	–	–
14	11×10^4	–	–	–	–
15	22×10^4	–	–	–	–
16	3.4×10^4	+	–	–	–
17	1.2×10^4	–	–	–	–
18	9.7×10^4	–	–	–	–
19	6.8×10^4	–	–	–	–
20	5.6×10^4	+	–	–	–
Norma	1×10^4 CFU/g, max.	Not allowed in 0.001 g of product	Not allowed in 25 g of product	Not allowed in 25 g of product	Not allowed in 1 g of product

The data obtained indicated that nine samples of horse meat under investigation had exceeded the maximum permitted level of contaminants as defined by the QMAFAnM. In two samples, a positive reaction for the presence of coliform was identified (samples No.21 and No.29, which tested positive for E. coli). Two strains of *S. aureus* were isolated from samples No. 26 and No.28. No evidence of *Salmonella* or *Listeria bacteria* was found in any of the tested samples (Table 3).

Table 3 – Results of microbiological examination of horse meat

Sample №	QMAFAnM, CFU/g	Coliforms	*Salmonella spp.*	*L. monocytogenes*	*S. aureus*
21	12×10^5	+	–	–	–
22	5.7×10^5	–	–	–	–
23	5.2×10^5	–	–	–	–
24	5.0×10^5	–	–	–	–
25	7.3×10^5	–	–	–	–
26	11.1×10^5	+	–	–	+
27	6.3×10^5	–	–	–	–
28	10.1×10^5	–	–	–	+
29	6.6×10^5	+	–	–	–
30	7.9×10^5	–	–	–	–
Norma	1×10^4 CFU/g, max.	Not allowed in 0.001 g of product	Not allowed in 25 g of product	Not allowed in 25 g of product	Not allowed in 1 g of product

The results of the studies demonstrate notable discrepancies in QMAFAnM levels across diverse meat categories. The results indicated that the mean QMAFAnM value in pork was 13.77×10^4 CFU/g, the highest among all the meat types under investigation. This suggests that pork is more susceptible to microbial contamination than other meats. Beef demonstrated a mean QMAFAnM value of 10.46×10^4 CFU/g, which is considerably lower than pork but higher than horse meat. Horse meat exhibited the lowest level of QMAFAnM, at 7.72×10^4 CFU/g, and is the least susceptible to microbial contamination among the tested meats (Figure 3).

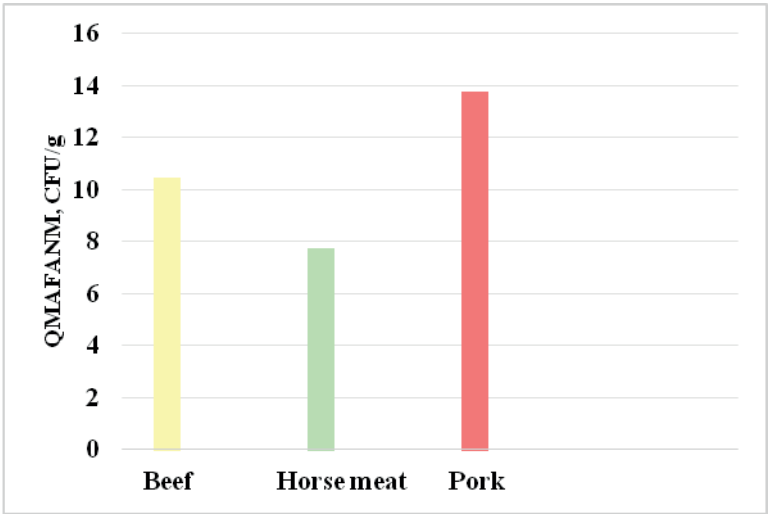


Figure 3 – Indicators of the level of microbial contamination in the studied meat samples

Discussion and Conclusion

The study's resultsrevealed notable differences in microbial contamination levels across different meat types. In all pork samples analyzed, the total microbial count (QMAFAnM) exceeded permissible limits, consistent with findings from other studies on microbial contamination in pork [20, 21]. Additionally, coliform bacteria and S.aureus were detected in pork samples, indicating potential health risks for consumers and possible lapses in hygiene protocols in the handling and processing of pork.

A similar contamination pattern was observed in the beef samples. All beef samples analyzed also had QMAFAnM levels above the acceptable standards, with coliforms present in three samples. This finding aligns with previous research [22] and may suggest deficiencies in the sanitary and hygienic processing of beef, potentially at various stages from production to storage. These issues highlight the need for more rigorous quality control measures for beef to prevent microbial contamination that could pose health risks.

The analysis of horsemeat samples showed that nine out of ten samples had QMAFAnM concentrations above permissible limits, though contamination levels were generally lower than those observed in pork and beef. Coliform bacteria and *S. aureus* were detected in two horsemeat samples, suggesting a degree of bacterial contamination but at a lower frequency than in the other meat types. This may reflect variations in the conditions under which horsemeat is handled and stored, yet underscores the importance of maintaining strict hygiene practices across all meat types.

Importantly, no samples from any of the meat types tested positive for the presence of pathogenic bacteria such as *Salmonella spp.* or *L. monocytogenes* indicating that while general bacterial contamination was an issue, there was no evidence of these specific pathogens in the samples.

To enhance the safety and quality of meat, adherence to microbiological control measures as set forth by the technical regulations of the Customs Union (021/2011,034/2013) [19, 23] is crucial. These regulations govern the production, storage, transportation, sale, and disposal of food products, ensuring safety standards are upheld at every stage in the supply chain.

The study results indicate the necessity for enhanced monitoring and regulation of the sanitary and hygienic conditions associated with the production, processing, and storage of meat of diverse types. The presence of elevated levels of QMAFAnM and the occurrence of coliform bacteria in meat products suggest potential risks to consumer health, underscoring the importance of implementing more rigorous measures to ensure food safety. Regular monitoring of microbial contamination and staff training in meat handling and storage practices are crucial strategies for reducing the risk of contamination.

Authors' Contribution

RR and AM: conceptualized and designed the study, conducted a comprehensive literature search, and analyzed the collected data. YuA, ME, and AG: carried out the research implementation and

analyzed the results. RR, ZA, and AG: performed the final editing and proofreading of the manuscript. All authors have read, reviewed, and approved the final version of the manuscript.

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Plants with radioprotective properties: current research and application prospects

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Abstract

Background and Aim. The objective of this study is to investigate plants capable of reducing radionuclide accumulation in animals and to develop scientifically substantiated dietary feed supplements suitable for the conditions of the Semipalatinsk Nuclear Test Site (SNTS). Plants with radioprotective properties play a critical role in the context of the former SNTS.

Materials and Methods. The selection criteria for the plants included their ability to bind and remove radionuclides as well as their antioxidant activity. Key species studied included the fruits of wild apple, rosehip, rowan, and hawthorn. The selected plants were harvested, dried, and processed into powdered and extract forms. A series of experiments were conducted on livestock to test various feed supplements enriched with radioprotective plants and dietary compounds such as pectin and potassium ferrocyanide. These radioprotective agents were incorporated into animal feed at concentrations of 5–7% to evaluate their effectiveness. Radiometric monitoring in experimental areas was carried out using instruments such as the RKS-01-SOLO radiometer-dosimeter, XFC-AT 6130 dosimeter-radiometer, and RAMON-02 radon monitor. Highly sensitive dosimeters were used to determine radionuclide levels in plants.

Results. The use of radioprotective plants as feed additives reduced radionuclide content in meat by 15% compared to the control group. The application of radioprotective agents facilitated the accelerated elimination of radionuclides from animals' bodies. In the experimental group, the radionuclide excretion period was reduced to 15 days compared to the control group.

Conclusion. The study results demonstrate the effectiveness of radioprotective plants in reducing radioactive substances in animals. These plants exhibit the ability to bind and remove radionuclides from animals' bodies. Feed additives based on these plants enhance productivity, quality, and safety of livestock products in areas affected by the former SNTS.

Keywords: antioxidants; biologically active substances; radioprotectors; radiation exposure; plants; radiation.

Introduction

The radioprotective properties of plants are a focal point of contemporary scientific research, particularly due to the increasing risk of radiation exposure to the environment and animal health. Substances with radioprotective properties are crucial for radiation protection. The aim of this study is to identify and consolidate effective radioprotective substances and plants capable of reducing radionuclide

accumulation in animals, as well as to develop scientifically substantiated feed additives and diets for animals raised in radioactively contaminated areas, such as the former Semipalatinsk Nuclear Test Site (SNTS). The implementation of such additives for livestock in the SNTS region is an essential measure. Ensuring the quality and safety of meat, dairy, and other livestock products in the context of the former SNTS remains a pressing issue in veterinary science. Therefore, the adoption and use of feeds with radioprotective properties to reduce radionuclide accumulation in animals is imperative [1].

Numerous medicinal herbs exhibit radioprotective properties or the ability to bind and remove radionuclides from the body. Herbs such as licorice, eleuthero, ginseng, nettle, rockrose, plantain, chamomile, and horse chestnut help stabilize cellular membranes, reduce swelling, and suppress autoimmune processes. These herbs protect the liver and kidneys from damage, enhance tissue regeneration, stimulate hematopoiesis, support intestinal epithelium health, and normalize the functions of the immune system, liver, spleen, and thymus.

Herbs like meadowsweet and eleuthero contribute to the normalization of the sympathetic and parasympathetic nervous systems' tone [2].

A complex of endogenous biologically active compounds, including amines, thiols, and other antioxidants, boosts overall resistance to radiation and suppresses the excessive accumulation of radiation-induced oxidative products that are harmful to living cells. Herbs such as kelp and flaxseed act as sorbents, promoting the elimination of radionuclides from the body.

Based on the above, the objective of this study is to analyze current scientific data on the radioprotective properties of plants and substances capable of reducing radionuclide accumulation in animals [3].

Explosions at the SNTS resulted in the release of numerous radioactive elements into the environment, the most radiologically significant of which include iodine-131, cesium-137, strontium-90, plutonium isotopes (239, 240, 241), and americium-241 [4].

Once inside the body, radionuclides act as harmful agents, damaging cells and requiring removal through any available means. Prolonged exposure to even minimal doses can lead to cellular changes, causing genetic mutations, malignant tumors, and various metabolic disorders, including those affecting digestion, hematopoiesis, and other bodily functions.

Our previous studies (2012–2014) revealed that soil, vegetation, water sources, and livestock products in the SNTS area remain significantly contaminated, representing a large-scale radioecological problem with adverse consequences stemming from the former test site.

Currently, researchers have estimated that animal diets in the SNTS region lack 30–50% of essential vitamins, macroelements, and microelements. Consumption of food contaminated with radioactive substances can result in human health issues. Thus, it is crucial in this region to use foods enriched with high-quality protein, vitamins, macroelements, microelements, and substances with radioprotective properties. A distinctive feature of these radioprotective foods is their ability to normalize metabolic processes, address the root causes of metabolic disturbances, and reduce radionuclide accumulation in the body [5].

Destructive, antioxidant, and anti-inflammatory actions: herbs such as licorice, eleuthero, ginseng, nettle, rockrose, plantain, chamomile, and horse chestnut stabilize cell membranes, reduce swelling, and suppress autoimmune processes. Antimutagenic actions: phenolic compounds, beta-carotene, vitamin E, and other antioxidants contribute to the prevention of radiation-induced mutations. Vasodilatory effects: phenolic compounds help normalize vascular permeability and expand blood vessels. Detoxification: all these plants facilitate detoxification processes, aiding in the elimination of harmful substances from the body. Hepato- and nephroprotective actions: these herbs protect the liver and kidneys from damage. Regeneration and hematopoiesis: they promote tissue regeneration, stimulate hematopoiesis, support epithelial recovery in the colon, and normalize the functions of the immune system, liver, spleen, and thymus. Nervous system regulation: herbs such as meadowsweet and eleuthero help normalize the tone of the sympathetic and parasympathetic nervous systems. Enhancing non-specific resistance: a complex of endogenous biologically active compounds, including amines, thiols, and other antioxidants, increases general radiation resistance and suppresses the excessive accumulation of radiation-induced oxidative products harmful to living cells. Sorption properties: herbs such as kelp and flaxseed act as sorbents, promoting the excretion of radionuclides from the body [6].

The radiation situation in the former test site area remains complex. More than 30 years have passed since the closure of the SNTS, but the effects of nuclear explosions may persist for several decades [7].

Pectin is a gelatinous substance commonly found in fruit jams or jellies. During digestion, pectin binds with radionuclides and toxic heavy metals, forming insoluble salts that are excreted through the intestines. Additionally, low-molecular-weight pectin fractions can enter the bloodstream, where they form complexes with radionuclides and are subsequently excreted via urine. The most popular sources of pectin include apples, blackberries, rosehips, pears, and nettle. Products made from these fruits and plants are rich in biologically active substances with radioprotective properties. Naturally, berries such as blackcurrants, cherries, and apricots are rich in organic acids and other biologically active compounds that enhance their radioprotective effects [8, 9].

The development of a new type of dietary supplement is based on the inclusion of fishmeal rich in essential macro- and microelements, biologically necessary vitamins, radioprotective plants such as sea buckthorn, Jerusalem artichoke, rosehips, earth pear, and pectin substances. The combination of these beneficial properties provides radioprotective and preventive effects, opening avenues for the creation of scientifically substantiated food products [10].

In veterinary practice, the use of substances with radioprotective properties is crucial for ensuring high-quality and safe products by minimizing radioactive substances in animals, particularly sheep raised in the SNTS area. Certain products, such as soy, carrots, beets, turnips, cabbage, and mushrooms, are effective in removing radionuclides from the body. Additionally, increasing the consumption of potassium-rich foods such as eggplants, green peas, potatoes, tomatoes, and watermelons can help reduce radiocesium accumulation in critical organs [11, 12].

Wormwood (Artemisia): In Karaganda, Kazakh specialists developed an anticancer drug from local wormwood. This medication, known as arglabin, is widely used in oncology clinics across the Commonwealth of Independent States (CIS) and is patented in 11 nations. The drug enables the treatment and prevention of liver, lung, and breast tumors. Currently, arglabin has received 11 million international orders for cancer treatment and is in demand in Russia, Ukraine, Belarus, Tajikistan, and Uzbekistan. Domestic demand reaches 2 million units [13].

A diet rich in vitamins is essential, as numerous studies indicate that the body's demand for vitamins increases even under low doses of ionizing radiation. Moreover, certain vitamins can help the body tolerate better elevated levels of radiation [14]. The primary sources of vitamin E are unrefined vegetable oils, such as soybean, corn, sunflower, and rosehip oils. Carotenoids, which are precursors to vitamin A, are found in carrots, red peppers, peaches, apricots, sea buckthorn, rowanberries, rosehips, pumpkins, and ripe tomatoes. Vitamin C is particularly abundant in rosehips, blackcurrants, citrus fruits, green peas, zucchini, carrots, beets, radishes, cauliflower, dill, and other similar foods.

Previous research has highlighted that a specially designed feed mixture rich in vitamins B1, B2, B6, B12, and C, as well as mineral substances, exhibits radioprotective properties. It reduces fattening times by threefold, improves meat quality, increases animal live weight gain by up to 20%, and decreases the overall dry feed consumption by 6–14%. This next-generation feed mixture, developed for agricultural animals, combines biologically valuable, therapeutic, preventive, and specialized products to produce high-nutritional-value meat. This is particularly significant for populations residing near the SNTS [15].

A horse feed with radioprotective and anthelmintic properties utilizes wild red apples rich in minerals and vitamins. This feed mixture includes potassium ferrocyanide and the anthelmintic gel Brovermectin. Potassium ferrocyanide aids in the excretion of the radioactive substance cesium-137 from the horse's body, while Brovermectin eliminates parasitic helminths. The primary nutritional components of the feed are proteins, carbohydrates, lipids, vitamins, and other biologically active compounds. The high pectin content in the feed enhances its digestibility and contributes to its radioprotective properties [16].

A mineral-salt block has been created using feed-grade salt supplemented with wild apple, potassium ferrocyanide, and anthelmintic agents. Under the influence of these developed mineral-salt additives, the infection rates of sheep with strongyloidiasis and eimeriosis decreased from 49.8–73.75% to 3–7%. When using the mineral-salt blocks, the infection rates fell from 49.8–73.75% to 2–4%, and the levels of radioactive substances in the animals decreased by 12% [17].

At present, there is a pressing need to systematize and consolidate accumulated experience in this field, as well as to identify promising directions for further research and practical application.

Materials and Methods

Radiometric monitoring employs devices and instruments subject to annual state calibration, including the RKS-01-SOLO radiometer-dosimeter, the MKS-AT6130 dosimeter-radiometer, and the RAMON-2 radon meter. Research requires the establishment of control and measurement stations. Samples of vegetation and feeds are collected from these control stations according to the standards of the Republic of Kazakhstan, specifically following the sanitary rules outlined in SanPiN 6.01.001-97 *Unified Rules for Sampling Environmental Objects,* which are designed for veterinary, agrochemical, control-toxicological laboratories under the Ministry of Agriculture and other organizations.

In order to ensure reliable laboratory data on the contamination of vegetation and feeds, strict adherence to sampling rules is essential. Vegetation samples must be collected after ground surveys at designated plots measuring 1 × 1 m. The sample mass should be at least 300 g. The aboveground parts of plants are cut at a height of 3 cm. Sampling of plant-based feeds is conducted in accordance with GOST 27262-87 *Plant Feeds: Sampling Methods*. For plant-based feeds, organoleptic properties are evaluated.

The products must be free from foreign odors, tastes, inclusions, or other defects. When assessing the safety of vegetation, general toxicological properties, bacterial contamination (including the presence of conditionally pathogenic and pathogenic microorganisms), and the content of heavy metals, pesticides, nitrites, nitrates, mycotoxins, and other harmful impurities are determined.

The radiological safety of vegetation concerning cesium-137 and strontium-90 is assessed according to permissible levels of specific radionuclide activity established by radiation safety standards and sanitary rules GN 2.6.1.054-96, approved by the decree of the State Sanitary and Epidemiological Surveillance on April 19, 1996, No. 7.

Sample preparation: Data for each sample must be recorded in a field passport. In the laboratory, the vegetation is ground and homogenized. The extract is prepared as follows: weigh 2 g of the sample and place it in a round-bottom flask, add 20 mL of a 3% nitric acid solution, and mix the solution on a reciprocating shaker (LIOP LS 120) for 60 min. Filter the resulting suspension through paper filters. The filtrate is then diluted 50-fold with distilled water and analyzed using an optical emission spectrometer.

Stage 1: The effect of radioprotective feed additives on animal growth and development

Group 1: standard feed. Group 2: feed supplemented with sea buckthorn and Jerusalem artichoke. Group 3: feed supplemented with wormwood and potassium ferrocyanide. Experiment duration: 4 months. Measured parameters: live weight gain, radionuclide content in meat, and total feed consumption. According to the results of the experiment using feed supplemented with wormwood and potassium ferrocyanide (Group 3), the animals in this group demonstrated the greatest live weight gain and the lowest radionuclide content in meat. These findings were utilized to develop recommendations for feed additives with radioprotective properties.

Stage 2: Development and testing of a new phytopreparation

A new radioprotective agent was developed based on local plants, including rosehip, sea buckthorn, and wormwood, and its efficacy was tested on animals. For three months, animals in the experimental groups were administered the new phytopreparation, while the control group received no such treatment.

Results

Reduction in radionuclide accumulation

In the experimental groups fed with the phytopreparation based on sea buckthorn, Jerusalem artichoke, wormwood, and potassium ferrocyanide, a significant reduction in radionuclide accumulation (cesium-137, strontium-90) in tissues was recorded. In the group receiving wormwood and potassium ferrocyanide, the radionuclide content in meat was within permissible limits, whereas the control group showed a 15% exceedance of the norm (Table 1).

Table 1 – Radionuclide content in animal tissues (Bq/kg)

Study parameters	Control	Experimental
Average radionuclide content	25% above norm	10% above norm
Cesium-137	212.5 ± 0.05	205 ± 0.004
Strontium-90	0.004 ± 0.001	0.002 ± 0.0001

In the experimental groups the average radionuclide content after application of radioprotectors and potassium ferrocyanide decreased by 90%, and in the control group by 75% i.e. cesium-137 in the control group was found 212,5 ± 0,05 Bq/kg, and in the experimental group 205 ± 0,004 Bq/kg, respectively, strontium-90 - 0,004 ± 0,001 Bq/kg and 0,002 ± 0,0001 Bq/kg.

Weight gain and product quality improvement

In groups of animals fed with radioprotective supplements (sea buckthorn, Jerusalem artichoke, wormwood), a significant increase in live weight was observed: 20% in the group receiving wormwood and potassium ferrocyanide and 15% in the group fed sea buckthorn and Jerusalem artichoke. Moreover, the quality of meat products improved due to a reduction in radionuclide content and an increase in nutritional value (Table 2).

Table 2 – Diet and radionuclide content in meat

Animal group	Weight gain	Radionuclide content in meat	Radionuclide increase in meat
Group 1 (standard feed), average weight: 320 kg			
Cesium-137 Strontium-90	336 ± 0.04 kg	213 ± 0.001 Bq/kg 0.003 ± 0.001 Bq/kg	15% above norm
Group 2 (sea buckthorn, Jerusalem artichoke), average weight: 331 kg			
Cesium-137 Strontium-90	381 ± 0.05 kg	210± 0.001 Bq/kg 0.01 ± 0.001 Bq/kg	5% above norm
Group 3 (wormwood, potassium ferrocyanide), average weight: 330 kg			
Cesium-137 Strontium-90	396 ± 0.02 kg	180± 0.001 Bq/kg -	Normal

In the group not receiving radioprotective preparations, the amount of cesium-137 was found to be 213 ± 0.001 Bq/kg, and strontium-90 was 0.003 ± 0.001 Bq/kg, which is 15% higher than the norm.

In the second group, which received radioprotectors (Jerusalem artichoke and sea buckthorn) along with feed, the levels of cesium-137 and strontium-90 decreased by 95%.

In the third group, which received wormwood and ferrocyanide along with feed, no radioactive substances were detected. Adding sea buckthorn, Jerusalem artichoke, and potassium ferrocyanide to the diet significantly reduced the levels of radionuclides in the animals' meat. The lowest radionuclide content was observed in group 3.

Acceleration of radionuclide elimination

The research results indicate that plants with radioprotective properties may play a crucial role in radiation protection, particularly in regions affected by nuclear testing. Phytopreparations accelerated radionuclide elimination in animals, reducing the excretion period from 30 days in the control group to 15 days in the experimental group. Studies focused on the radioprotective properties of local plants in Kazakhstan, the analysis of their active compounds, and their impact on radiation protection. Feed additives based on these plants showed potential as environmentally friendly alternatives to synthetic radioprotectors, requiring further laboratory and field trials to confirm their safety and efficacy. A deeper analysis of plant-based radioprotectors is essential to understand their mechanisms and enhance their utilization. Systematizing data on these agents will contribute to the development of new methods and technologies for effective radiation protection. Research on plant-based radioprotectors opens promising opportunities for safer and more sustainable radiation protection strategies.

Discussion and Conclusion

The results of the conducted studies have shown high efficiency of radioprotective preparations and radioprotective feeds in reducing radiation contamination of animal organism and improving the quality of livestock products. Prospectivity of the use of plant radioprotectors: plants with radioprotective properties represent a promising alternative to synthetic radioprotectors, providing an environmentally friendly and potentially safe solution for protection from radiation exposure.

Comprehensive studies are needed to fully understand the mechanisms of action of plant radioprotectors, and systematization and generalization of available data on radioprotectors will help in the development of new methods and technologies, as well as in the practical application of radioprotectors.

Research suggests that plants with radioprotective properties may be key elements in a defense strategy against radiation exposure. The importance of further study of these plants, including their mechanisms of action, efficacy and safety, is undeniable. Comprehensive studies and systematization of data are needed to better understand the potential of plant radioprotectors. Thus, the study of plant radioprotectors is an important step towards the development of safer and more effective methods of protection against radiation exposure, with significant implications for science and practice in this field.

1. Plants with radioprotective properties, such as sea buckthorn, wormwood, Jerusalem artichoke, and rosehip, demonstrate the ability to bind and excrete radionuclides from the body while protecting cells from radiation-induced damage.

2. Fodder mixtures enriched with radioprotective plants and substances (e.g., pectin, potassium ferrocyanide) not only reduce radiation levels in animals but also enhance their productivity, improve the quality and safety of livestock products.

3. Radioprotective preparations derived from local plants accelerate radionuclide excretion and promote tissue regeneration, making them a promising solution for use in radiation-contaminated areas, such as the former Semipalatinsk nuclear test site.

4. The use of radioprotective additives shortens the fattening period, increases weight gain, and improves the biochemical parameters of blood - an essential factor for ensuring food security in regions with heightened radiation risk.

Authors' Contributions

SD and AS: Conceptualized and designed the study, conducted a comprehensive literature search, analyzed the gathered data and drafted the manuscript. ShS, ZhS and ZH: Conducted the final revision and proofreading of the manuscript. All authors have read, reviewed, and approved the final manuscript.

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Antigenic Activity of Various *Rhodococcus equi* Strain Plasmids

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Abstract

Background and Aim. *Rhodococcus equi (R. equi)* infection is a fatal cause of equine *rhodococcosis.* Infections have also been reported in other species and humans. This study evaluated the immune response of R. equi in rabbits.

Materials and Methods. Different strains of R. equi (with and without plasmids) were administered to rabbits. Blood samples were collected from the ear veins of the rabbits at 7, 14, 21, and 28 days after the initial administration of the antigen, and the serum was examined for specific antibodies against *R. equi* plasmid antigens using the complement fixation reaction.

Results. The minimum antibody level was recorded on day 7. The average antibody level throughout the study period was slightly above the median, indicating a small asymmetry toward higher values. By day 28, the antibody level had reached 75% of values ≤4.25.

Conclusion. All strains of *R. equi* exhibited antigenic properties to varying degrees. The antibody level upon plasmid pVapN administration was higher than that of the other cases.

Keywords: *Rhodococcus equ*i; plasmid pVapB; plasmid pVapA; plasmid pVapN; antigenic activity.

Introduction

Rhodococcus equi (R. equi) is a pleomorphic Gram – positive *coccobacillus bacterium* commonly found in the gastrointestinal tract of horses. *R. equi* is recognized as an important pathogen based on reports of 3% mortality in foals, which is widespread globally [1, 2, 3]. *R. equi* is a primary lung pathogen in foals aged 6 months with high mortality rates [4, 5]. Pneumonia caused by *R. equi* can also occur in adult horses, especially in immunocompromised individuals, who can develop systemic infections [6].

In infected animals, chronic and purulent bronchopneumonia is typically observed and is associated with high mortality, especially in foals that have not been subjected to specific antibacterial therapy [3, 7]. Numerous studies using virulent strains of *R. equi* isolated from horses in different geographical regions worldwide have demonstrated the diversity of plasmid sizes and the predominance of different types of plasmids [8, 9, 10, 11, 12].

It has been established that only virulent strains of *R. equi*, which express the virulence-associated protein (VapA) of 15-17 kDa and possess a large virulence plasmid of 85-90 kDa containing the VapA gene, are pathogenic to horses. To date, 12 types of plasmids have been identified in VapA-positive strains from horses [11, 13, 14, 15]. VapA and VapB, which are closely related at the amino acid level, are located on the cell surface, and their expression is regulated by temperature and pH [16].

In studies on the source of intermediate virulence, VapB-positive strains have been isolated from the submandibular lymph nodes of pigs; these isolates were found to be of intermediate virulence in mice and contained one of five large plasmids sized 79±95 kb [17]. Virulent strains of *Rhodococcus equi* expressing the virulence-associated protein of 15–17 kDa (VapA) and possessing a large virulence plasmid (pVAPA) of 85–90 kb, containing the vapA gene are pathogenic to horses. Over the last two decades, after pVAPA, two types of host-associated virulence plasmids of *R. equi* have been discovered: the circular plasmid pVAPB, associated with isolates from pigs in 1995, and the recently identified linear plasmid pVAPN, linked to isolates from cattle and goats.

Molecular epidemiological studies of R. equi infections in foals on equine breeding farms in Japan and many countries around the world have been conducted over the past three decades, and epidemiological studies using the digestion schemes of plasmid DNA from virulent isolates with restriction enzymes have shown 14 different subtypes of pVAPA and their geographical preferences [18, 19, 20].

Virulent strains of *R. equi* isolated from sick foals and horse breeding environments exhibit a uniform plasmid pattern [21]. Certain types of plasmids are characteristic of specific geographical regions, which can be used in epidemiological studies [10, 11].

The aim of our research was to study the antigenic activity of various plasmids of the *R. equi* strain.

Materials and Methods

To study the antigenic activity of the plasmids of the *R. equi* strain, rabbits were immunized. We used the following strains, provided by Kitasato University (Japan):

1) The *Yokkaichi_P* strain contains no plasmids.
2) S4 cells containing the plasmid pVapB.
3) ATCC33701 cells carrying the plasmid pVapA.
4) Yokkaichi strain containing the plasmid pVapN.
5) The *R. equi* strain was isolated by us in 2023 [22].

Cultivation of *Rhodococcus equi* Suspensions: For inoculation, a 2-day culture of the strain in tubes was washed with saline solution to achieve a microbial cell concentration of 2–5 billion cells in 1.0 cm^3, according to the optical turbidity standard. This suspension was used to inoculate Tartakovsky flasks with NANAT medium [23]. The inoculated flasks were supplemented with 4.0–5.0 cm^3 of suspension and placed in a thermostatic incubator at 37 °C for 2 days. After 2 days, the cultured material was visually inspected for purity and typical growth characteristics.

Preparation of the antigens: The culture was washed with a sterile 0.5% phenolize saline solution, pH 7.0–7.2, using 25–30 cm^3 per flask. The resulting suspension, containing approximately 20–30 billion microbial cells in 1.0 cm^3 according to the turbidity standard, was filtered through a double-layered gauze filter into bottles. The purity of the gram-stained smears. The mixture was then heated in a water bath at +70 °C for 60 min.

After cooling, the heated suspension was stored in a refrigerator at +2 °C–4 °C, where it was also checked for purity and sterility.

Sterility determination: From the antigen stock, the samples were inoculated onto MPA and NANAT media. The cultures were incubated in a thermostat for 3 days at a temperature of 37 °C–38 °C. The cultures remained sterile throughout the observation period.

In each group, three rabbits weighing 2.0–2.5 kg were selected and subcutaneously injected with the antigen at a dose of 2 cm^3 twice, with an interval of 14 days. Blood samples were collected from the ear veins of the rabbits at 7, 14, 21, and 28 days following the first antigen administration, and the serum was tested for specific antibodies against the plasmid antigens of *Rhodococcus equi* using the prolonged complement fixation test (PCFT).

PCFT was performed under cold conditions in tubes with 0.2 cm^3 volumes of serum, antigen, and complement, with the hemolytic system in a titrated working dose. Each antigen was titrated with the corresponding rabbit serum using the checkerboard method in PCFT. Serum samples were collected from rabbits on days 0, 7, 14, 21, and 28 of the experiment.

Statistical Analysis: Data analysis was performed using R-Studio software, applying the non-parametric Kruskal–Wallis test. Statistical significance was assessed at a threshold level of $P < 0.05$ [24].

The experiments and methodology used to conduct research on laboratory animals comply with the requirements of biological safety and the ethical principles of animal experimentation in the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Conclusion of the Bioethics Commission of the Kazakh National Agrarian Research University dated November 2, 2022).

Results

The checkerboard titration of the antigen and rabbit serum before antigen administration yielded a negative result. The results of the studies, starting from the seventh day of the experiment, are presented in Table 1.

Table 1 – Antibody Levels in Rabbits Administered Different Strains (with no plasmids, plasmids) of *R. equi*

Days of the Experiment	Group number-Average antibody titer				
	1 - no plasmids	2 - pVapB	3 - pVapA	4 - pVapN	5 - *R.equi*
7	-	1:5	1:5	1:5	1:5
14	-	1:10	1:10	1:10	1:10
21	1:10	1:20	1:20	1:40	1:20
28	1:20	1:80	1:40	1:80	1:40

As can be seen from the table, when the antigen from the strain without plasmids was injected into the group of rabbits on days 7 and 14, no antibodies were detected in the PCFT, whereas in the groups of rabbits injected with pVapB, VapA, pVapN, and *R.equi*, the antibody titer was within 1:5 and 1:10, respectively.

From day 21 in the first group, antibody levels began to appear at 1:10 and peaked at 1:20 on day 28. On day 28 of the experiment, a significant increase in antibody titer was observed in rabbits in groups 2 and 4, i.e., when antigen from the strain carrying the pVapB and pVapN plasmids was administered.

Because of the studies in the first group, rabbit serum antigen had the highest titer at dilutions of 1:50, 1:100, and 1:150. In serum samples positive for the strain without plasmids, the indicator was 1:10. With a decrease or increase in antigen dilutions, a decrease or absence of antibody levels is observed. An increase or decrease in antibody titers is directly dependent on the dilution of the antigen.

The absence of anticomplementary properties was demonstrated in the reactions of antigen dilutions with physiological solutions.

The antigen dose with a high antibody response in the reactions was taken as the equivalent zone. In addition to the equivalent zone, a decrease in the reaction results was observed at other antigen dilutions.

Because of the reactions, the dilution of the antigen that gave the highest antibody titer in the serum was taken as the antigen unit (AU). This AU is the most effective antigen dilution.

It was established that 1 AU for an antigen without plasmids is 1:150, with a working dose of the antigen of 1:150 equivalent to 1 AU. Higher than 1 AU resulted in self-limiting reactions.

In the checkerboard titration of rabbit serum treated with the antigen from the plasmid pVapB, the following results were obtained: 1 AU was 1:250, and the working dose of the antigen was 1:200, which corresponds to 1.25 AU.

For the titration of rabbit sera and the antigen from the plasmid pVapA, 1 AU was 1:300, and the working dose was 1:250, resulting in 1.2 AU.

Regarding pVapN, 1 AU was 1:200, while the working dose was 1:250 (1.3 AU). The 1 AU of the antigen from the *R. equi s*train was 1:300, whereas the working dose was 1:250 (1.2 AU).

The minimum antibody level was recorded on day 7. 25% of the values were ≤2.0, which coincides with the level on day 14. The median value indicates the central tendency of the data. The average antibody level over the entire period was slightly above the median, indicating a small asymmetry toward higher values. By day 28, the antibody level had reached 75% of values ≤4.25.

A P value of >0.05 indicates the absence of significant differences between groups. Variations in antibody levels are more likely related to variability than to the action of the plasmids.

Plasmids did not have a significant effect on antibody levels. However, the antibody level upon the administration of the plasmid pVapN (3.0) was somewhat higher than that in the other cases.

A clear increase in antibody titers was observed in rabbits with increasing time after the administration of the strains. This indicates the formation of an immune response. The dynamics of antibody titers showed a significant increase in antibody levels as the observation time increased, indicating the activation of the immune response. The differences between days were statistically significant ($P < 0.01$).

Analysis of the impact of plasmids on antibody production indicated that the differences between groups with various plasmids and those without plasmids were not statistically significant ($P = 0.62$). Nevertheless, plasmid pVapN showed a slight trend toward increased antibody levels.

The values of the antibody titers exhibited a slight asymmetry toward an increase, suggesting a rise in the antibody levels in later days of the experiment.

Discussion and Conclusion

Pathogenic strains are classified as virulent or intermediate virulent based on the presence of plasmid genes encoding virulence-associated protein A (VapA) or B (VapB), respectively [25]. Strains lacking VapA or VapB are classified as avirulent. The highly conserved gene encoding the conjugative transfer protein, traA, is present in strains carrying the virulence plasmid.

The new linear virulence plasmid *R. equi* (pVapN) has been characterized in isolates from cattle [26] and identified in a single isolate from dogs [27], but it has not been described in other species. Epidemiological data indicate that strains carrying pVAPN are pathogenic to ruminants [19].

VapN-positive *R. equi* has been isolated from cattle in Japan. Although *R. equi* is generally considered to have low pathogenicity in cattle, the influence of certain predisposing factors can lead to widespread infection [28].

VapA is an important antigen involved in the humoral protective immune response to *R. equi* infections caused by virulent strains in foals [29].

In this study, specific *R. equi* strains that are known to be pathogenic in various animal species were used. In our experience with rabbits, they elicited an immune response characterized by a clear increase in antibody titers over time following the administration of the strains.

All strains of *R. equi* (with and without plasmids, as well as the strain itself) exhibited antigenic properties to varying degrees. The antigen from the R. equi strain without plasmids showed the lowest level of activity. The antibody level upon administration of the plasmid pVapN was somewhat higher than that in the other cases.

Statistical analysis showed that the differences between the means at each stage of the experiment were statistically significant ($P < 0.01$), confirming a progressive increase in the immune response and greater variability at later stages of follow-up.

“Antigenic unit” was used as a measure of antigenic activity in serological reactions. The highest antigen titer corresponding to the highest value of positive blood serum with antibodies of the corresponding type was used as the antigenic unit. The value of the antigenic unit is not affected by the activity of the specific blood serum used to determine it; thus, the antigenic unit can be determined with any positive blood serum by titration using the checkerboard method.

The results may help obtain hyperimmune (positive) serum for the development of diagnostic kits for *rhodococcosis*. This study should be considered as a pilot study for the further development of *R. equi* bioproducts in horses and goats.

Authors' Contributions

GI, EK, and BO: Conceptualized and designed the study, conducted a comprehensive literature search, analyzed the collected data, and prepared the manuscript. MZ, SK, and NM: Prepared materials for hyperimmunization’s and assessed antigen activity. UZ and BV: conducted the hyperimmunization of rabbits. All authors read, reviewed, and approved the final manuscript.

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experimental infection model in goats using virulent *Rhodococcus equi* containing pVAPN as a model for *Rhodococcus* infection in foals."

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Using indirect hemagglutination assay for the diagnosis of cattle brucellosis

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