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SEARCH FOR THE THERAPEUTIC POTENTIAL OF BIOLOGICALLY ACTIVE SUBSTANCES CONTAINED IN CONIFEROUS PLANTS

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Abstract

The paper presents scientific information about the problems in the search for therapeutic potential in coniferous plants widely used in folk medicine. Samples of coniferous plants growing in the mountain forests of the Western Altai in the eastern part of Kazakhstan were selected for the experiment: Baltic pine (*Pinus sylvestris* L.), European spruce *(Picea abies* L.), Siberian fir *(Abies sibírica*), Siberian pine (*Pinus sibirica* DuTour), and common juniper (*Juniperus communis* L.). During the study, the phytochemical composition of the components of P. sylvestris L. essential oils was determined by the indicators of sesquiterpenic fraction (69.76%), terpenoids (20.0%), and monoterpenes (5.51%). Using modern methods of studying biological activity (antimicrobial, antifungal, and anthelmintic), the authors established the presence of biological activity in the extracts of selected coniferous plants. In all coniferous plants, bactericidal activity was manifested in water decoctions, where biological preparations of Siberian fir and Baltic pine were more active. The oil extracts showed less bactericidal activity. Among them, extracts of Siberian pine and juniper were inactive, and the extract of European spruce was more active in its native form and 1:2 dilution. Among plants with a complete absence of bactericidal activity against the *Candida parapsilosis* opportunistic yeast, Siberian fir should be named. In other coniferous plants (European spruce, Baltic pine, juniper), bactericidal activity was detected only in oil solutions in a dilution of 1:2, and oil extract of Siberian pine in a dilution of 1:8 had a suppressive effect. Water/alcohol tincture of Siberian fir was found to have high fungicidal activity against *Aspergillusniger,* which continued to influence the growth of the micromycete at a dilution of 1:64 during the observation period. The presence of antiparasitic properties was observed in some plant extracts of coniferous forest plants. The best results were noted in alcohol tinctures of Baltic pine, juniper, and Siberian pine.

Key words: coniferous plants; biologically active substances; phytochemical composition; antimicrobial properties; antiparasitic effect; bactericidal activity; therapeutic potential.

Basic position and Introduction

Medicinal plants have long been used as a source of traditional medicinal remedies in almost all known civilizations [1]. Nature provides a significant supply of new phytochemicals, which are called natural products, and the development of medicines from them is a difficult task for attracting new potential customers [2].

Phytochemicals are substances produced mainly by plants that have different biological effects. In the pharmaceutical industry, plants are the main source of various active ingredients. They exhibit pharmacological effects that can be used for the treatment of bacterial and fungal infections, as well as chronic degenerative diseases such as

diabetes and cancer [3].

The traditional and pharmaceutical use of extracts of various coniferous trees against diabetes, neurological disorders, inflammation, and cancer has been described. Phytochemical components present in coniferous tree extracts are non-toxic at the therapeutic level, and polyphenolic compounds have significant biological activity. Stilbens, terpenes, alkaloids, lignins, and flavonoids, such as quercetin, rutin, resveratrol, pyrolytic carbon (PYC) compounds, and enzogenol, have sedative, antidiabetic, antitumor and anesthetic effects. In addition, phytochemicals present in coniferous tree extracts help regulate glucose and lipid metabolism, insulin secretion by stimulating β-cells, the NF-kB signaling pathway, inhibition of gluconeogenic enzymes, the protective effect of reactive oxygen species (ROS), as well as targeting and modulating cytokines that affect neuronal cells and reduce oxidative stress [4].

Aqueous extracts obtained from plant shoots collected in 2019 at the arboretum in Zelenka (Poland), including individual samples of *Picea abies* L., *Larix deciduas* Mill, *Pinus sylvestris* L., *Pseudotsuga menziesii*, and *Juniperus communis* L., are a rich source of phenols such as caffeic acid, ferulic acid, chlorogenic acid, 4-hydroxybenzoic acid, and many others. The obtained extracts showed antioxidant and antimicrobial properties *in vitro* [5].

Thus, the phytochemicals of coniferous trees with a biologically active effect can be used as an alternative to synthetic medicines. They can be reliably used in the future since they can be useful in the development of new therapeutic agents for the treatment of relevant pathologies [4].

In Kazakhstan, coniferous forests grow in the mountains of the Kazakh part of Altai, the Dzungarian Alatau, the eastern spurs of the Tien Shan, and the plains of the forest-steppe zone of Northern Kazakhstan [6, 7]. Residents of forested areas widely use the vegetative organs of woody plants (especially pine trees) for therapeutic and preventive purposes and therefore, the study of their biological effects is relevant.

The study aimed to evaluate the phytochemical composition of coniferous trees and study their antimicrobial properties and antiparasitic effects to establish their therapeutic potential.

Materials and methods

The object was the shoots of the following coniferous plants growing in the mountain forests of the Western Altai in the eastern part of Kazakhstan: Baltic pine (*P. sylvestris* L.), European spruce (*P. abies* L.), Siberian fir (*Abies sibírica*), Siberian pine (*Pinus sibirica* DuTour), juniper (*J. communis* L.). Branches of plants with healthy needles were selected.

The sampling of coniferous plants was carried out in the herbaceous pine forest (hP) located on the territory of the Ridder forestry, the Central Forestry (block 26, plot 17), the grass and fern fir forest (gfF) on the territory of the Fir part of the Butakovsky forestry (block 38 block, plot 40) and small clumps of Siberian pine were found locally in the areas of fir and aspen forests. The juniper samples were taken on the territory of the Ivanovo ridge, near the city of Ridder.

Pine needles were selected for analysis of the phytochemical composition of coniferous plants, given that in folk medicine, various pine organs are in great demand. Samples of all conifers for laboratory experiments were taken at a height of 1.7-1.8 m at the level of the respiratory organs of an adult tree from four sides (north, south, east, and west). The selected samples of plant needles were mixed to obtain an average sample and dried at room temperature for one week. To isolate the essential oil, the method of steam distillation (hydrodistillation method) was used. Chromato-mass spectrometric method was used to determine the component composition of essential oils. The analysis of pine needles essential oil was carried out on an Agilent 7890A gas chromatograph with an Agilent 5975C mass-selective detector in the laboratory of the Physico-chemical research methods engineering profile of the Chemical Faculty at the E.A.Buketov Karaganda State University [8].

Experimental studies on the analysis of the biological (antimicrobial, antifungal, antihelminthic) activity of extracts were carried out in the Agricultural Biotechnology Research Platform (NIP CKhB) of the S. Seifullin Kazakh Agrotechnical University (KATU) in 2020-2022.

The assessment of the biological activity of coniferous plant extracts was evaluated sequentially in several stages. Oil and water infusions, alcohol tinctures, and water decoctions were prepared from the biomass of coniferous

plants at a rate of 1:10. Infusions and tinctures were infused for 2 weeks in a dark place, and decoctions were prepared immediately before use. Sterilization filtration of the preparations was carried out using filters with a pore diameter of 0.45 nm. The extracts were stored at a temperature of 4-6°C for no more than 24 hours [9].

The analysis of the antimicrobial and antifungal activity of plant raw materials was carried out by the method of serial dilutions in agar and disk diffusion method. Determination of minimum suppressive concentrations (MSC) and minimum bactericidal concentrations (MBC) of aqueous plant extracts was carried out by sequential micro-dilutions in Mueller-Hinton broth [10-14].

To determine the bactericidal MSC, the extracts were tested against Escherichia coli, and for the antifungal MSC, the extracts were tested against opportunistic strains, opportunistic mycosis pathogens, *Candida parapsilosis* yeast strain 398.2 and *Aspergillus* niger mold.

Antihelminthic properties were tested on annelids (*Lumbricus terrestris* ringworms), which were used as a test object.

The MSC was determined visually by the absence of visible growth of microorganisms. Standard data were used to interpret the results of determining the sensitivity of microorganisms to antimicrobial agents [15].

To establish the presence of a helminthicidal effect, a proprietary method of accounting for the results was developed. Attention was paid to the naturalness of the behavior of worms, the desire to approach the wells or move away from them, the death of worms within a certain period, the presence and intensity of the smell of decomposition in case of death, and the presence and intensity of hemolysis. For the presence of each of the signs and its intensity, crosses were placed according to the principle: +++: a very pronounced sign, ++: a pronounced sign, +: a weakly pronounced sign. In the absence of results, it was marked with a minus. Then the total number of "+" was calculated and points were put down [7].

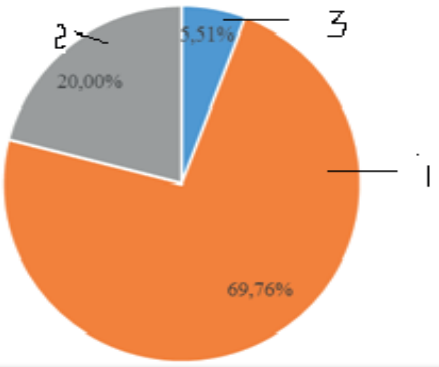
Results

Table 1 shows the indicators of the organoleptic properties of the essential oils obtained from Baltic pine needles. Figure 1 shows the composition of essential oils obtained from the same plant.

Table 1 – Indicators of organoleptic properties of Baltic pine needle essential oils

No.	Indicators	Characteristics
1	Color	light yellow color
2	Smell	pleasant pine odor
3	Physical condition	oily consistency
4	Weight of essential oil/100 g of needles	0.31± 0.04

The distribution of essential oil components in the examined samples is shown in the diagram. The average values of the established indicators of the essential oil components showed a high content of sesquiterpenic fraction (69.76%), an average content of terpenoids (20.0%), and a low content of monoterpenes (5.51% of the total content of the identified compounds). The total content (%) of the identified components of the essential oil of the Baltic pine was 95.27%.



1 – sesquiterpenic; 2 – terpenoids; 3 – monoterpenes

Figure 1- The composition of essential oils obtained from the sample *P. sylvestris* L.

To detect the bactericidal MSC, the extracts were tested against *E. coli*, and for the antifungal MSC, they were tested against opportunistic strains, pathogens of opportunistic mycoses, *C. parapsilosis* yeast strain 398.2 and *A. niger* mold fungi. MSC accounting was performed visually by the absence of visible growth of microorganisms (Tables 2-4).

Table 2 – Bactericidal MSC of coniferous plant extracts against E. coli (degree of dilution)

Type of raw materials	Oil extracts	Water-alcohol extracts	Water extracts	Water decoctions
Pinus sibirica DuTour	-	-	-	1:32
Picea abies L.	1:2	-	1:64	1:64
Pinus sylvestris L.	1:32	-	-	1:128
Ábies sibírica	1:512	-	-	1:256
Juniperus communis	-	1:128	1:32	1:2
Control	-	-	-	-

As can be seen from Table 2, several extracts of coniferous plants have no bactericidal or bacteriostatic activity at all, and most of them exhibit weakly expressed activity against *E. coli.* In all coniferous plants, bactericidal activity was manifested in water decoctions, with the biological preparations of Siberian fir and Baltic pine demonstrating more activity.

The oil extracts showed less bactericidal activity. Among them, extracts of Siberian pine and juniper were inactive, and the extract of European spruce was more active in its native form and 1:2 dilution.

Unexpectedly, water/alcohol tinctures of coniferous plants turned out to be inactive. Bactericidal activity was detected only in the juniper water/alcohol extract (Figure 2).

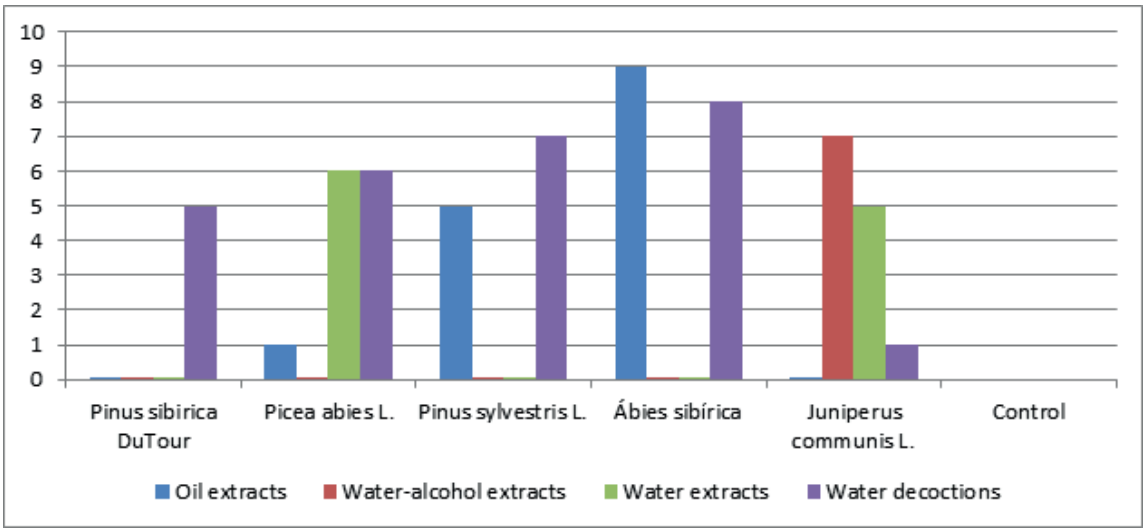


Figure 2 – MSC of coniferous plant extracts against E. coli bacteria (logarithm of dilution)

Table 3 – MSC of coniferous plant extracts against C.parapsilosis opportunistic yeast

Type of raw materials	Oil extracts	Water-alcohol extracts	Water extracts	Water decoctions
Pinus sibirica DuTour	1:8	-	-	-
Picea abies L.	1:2	-	-	-
Pinus sylvestris L.	1:2	-	-	-
Ábies sibírica	-	-	-	-
Juniperus communis	1:2	-	-	-
Control	-	-	-	-

As can be seen from Table 3, the absence of fungicidal or fungistatic activity against opportunistic yeast is recorded in a large number of plant extracts, except for oil extracts. Water decoctions and infusions, as well as tinctures of all analyzed coniferous plants, were inactive against *C.parapsilosis*.

Among plants with a complete absence of bactericidal activity against the *C. parapsilosis* opportunistic yeast, Siberian fir should be named. In other coniferous plants (European spruce, Baltic pine, and juniper), bactericidal activity was detected only in oil solutions in a 1:2 dilution. Only the oil extract of Siberian pine in a dilution of 1:8 had a suppressive effect (Figure 3).

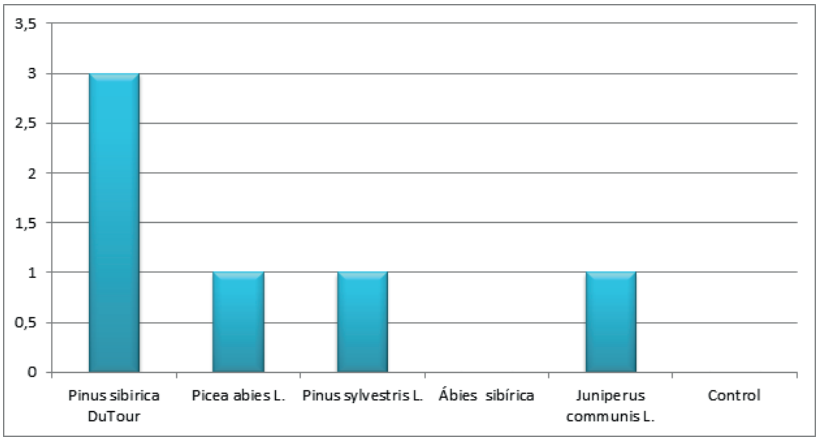


Figure 3 – MSC of oil extracts of coniferous plants against C. parapsilosis opportunistic yeast (logarithm of dilution)

Table 4 – MSC of coniferous plant extracts against the causative agent of opportunistic mold mycoses *A.niger*

Type of raw materials	Oil extracts	Water-alcohol extracts	Water extracts	Water decoctions
Pinus sibirica DuTour	1:4 $_{(f/s)}$	-	-	1:128 $_{(f/s)}$
Picea abies L.	-	-	-	-
Pinus sylvestris L.	1:4 $_{(f/s)}$	-	-	-
Ábies sibírica	-	1:64 $_{(f/c)}$	-	-
Juniperus communis L.	-	-	-	-
Control	-	-	-	-

Note: f/c is fungicidal, f/s is fungistatic

As can be seen from Table 4, a greater number of extracts of coniferous plants are characterized by the absence of fungicidal or fungistatic activity against the *A. niger* opportunistic mold fungi. Among the studied wild coniferous plants of the forest, the complete absence of fungicidal activity against opportunistic molds was recorded in two plants: European spruce and common juniper. For two oil extracts, the presence of fungistatic properties was observed only in native extracts (Siberian dwarf pine and Baltic pine). Aqueous extracts of all conifers did not inhibit the growth of micromycetes. Unexpectedly, the growth of *A. niger* was actively suppressed on the first day by an aqueous decoction of Siberian pine in dilution up to 1:128. This indicates a high level of suppression of mycelium growth by this decoction and the suppression of spore formation by the corresponding phytoncides.

The only preparation with high fungicidal activity against *A.niger* was a water/alcohol tincture of Siberian fir, which continued to influence the growth of the micromycete at a dilution of 1:64 during the observation period.

The disk diffusion method determined the MBC of biological preparations with the presence of antimicrobial or antifungal activity against three strains of microorganisms selected by us (Table 5).

Table 5 – MBC and minimal fungicidal concentrations of wild coniferous plant water decoctions

№	Name of vegetable raw materials	Diameter of the growth retardation zone, mm medium min/max		
		E. coli	*C. papapsilosis*	*Asp. niger*
8	*Pinus sibirica* DuTour	12,0 11,0-13,0	-	8,0 7,0-9,0
11	*Picea abies* L.	8,5 8,0-9,0	10,0 9,0-11,0	-
13	*Pinus sylvestris* L.	10,5 10,0-11,0	-	13,0 11-15
14	*Ábies sibírica*	10,0 9,0-11,0	-	30,0 28,0-32,0
28	*Juniperus communis*	7,0 6,0-8,0	-	-
	Control	-	-	-

As can be seen from Table 5, when MBC is exposed to the growth of *E. coli* and micromycetes, the diameter of the growth delay zone of microorganisms in most cases has limits from 7 to 15 mm, which indicates a weak sensitivity of microorganisms to antimicrobial and antifungal components of coniferous plant extracts. The obtained results allow us to state the presence of bacteriostatic properties in the aqueous extracts of wild coniferous plants included in the study. Such a general conclusion can be drawn from all the studied water decoctions, i.e. almost all of them have bacteriostatic properties against E. coli. Analysis of water decoctions of coniferous plants against opportunistic yeast *C. parapsilosis* showed the presence of a fungistatic effect only in the decoction of spruce. The remaining water decoctions not only did not have a fungicidal effect but also contributed to the active growth of yeast around the discs.

The detection of fungicidal properties of aquatic decoctions of coniferous forest plants against opportunistic mold fungi *A. niger* showed that the micromycete was quite sensitive to several decoctions (Baltic pine, Siberian pine) (the diameter of the lysis zone ranging from 8 to 15 mm), and in the presence of decoction of Siberian fir, the growth of *A. niger* almost completely stopped (Figure 4).

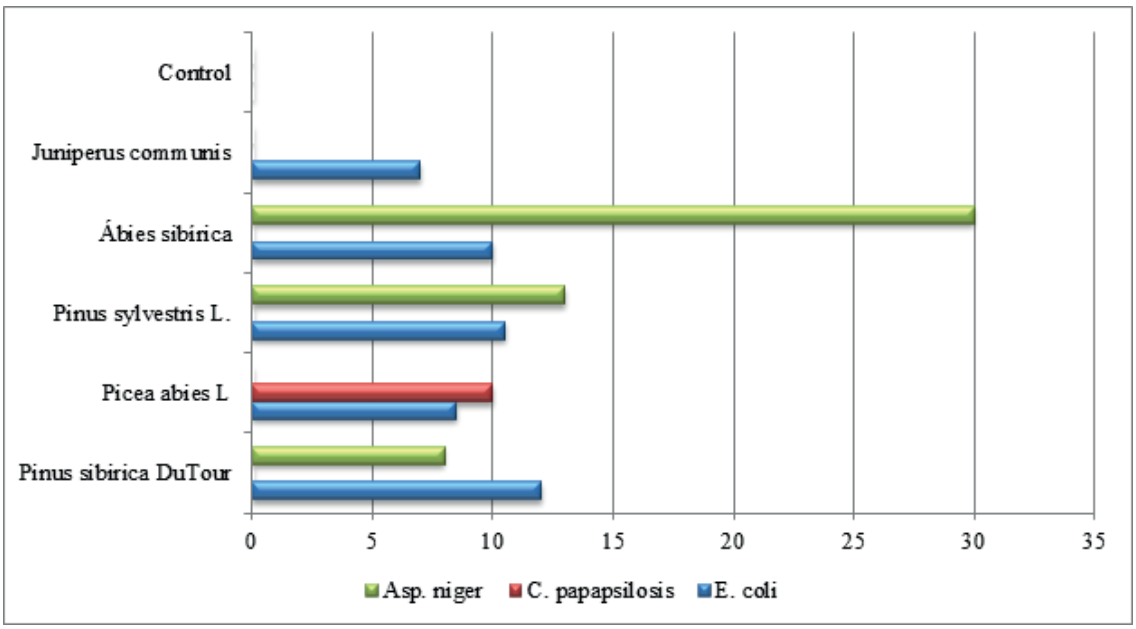


Figure 4 – The sensitivity of microflora to water decoctions of coniferous plants

As can be seen from Figure 3, only extracts from European spruce showed fungistatic activity against *the C. parapsilosis opportunistic yeast.* Water extracts of Siberian fir have pronounced fungicidal activity against the A. niger opportunistic mold fungi. Concerning the *E. coli* strain, all aqueous extracts showed a noticeable fungistatic effect.

The results obtained indicate the presence of various biological effects, in particular, antimicrobial and fungicidal activity, in coniferous plant extracts, which makes it possible to conclude that they can be used for the manufacture of preparations with a therapeutic effect against pathogens of infectious diseases. The study of antiparasitic activity by a modified technique involved the use of a test culture of earthworms. To determine the presence of a helminthicidal effect, helminths were transferred from Petri dishes to ready-made nutrient media with wells pre-filled with extracts. The behavior of worms on a solid nutrient medium was monitored every 3-6-12 hours for 3 days (Figure 5).

Figure 5 – The process of observing the behavior of worms in the study of antiparasitic properties of coniferous forest plant extracts

The results of the study of antiparasitic properties in plant extracts of coniferous plants in conditional scores are presented in Table 6.

Table 6 – Summary data on the presence of antihelminthic properties inwild forest plant extracts

№	Name of vegetable raw materials	Presence and intensity of antiparasitic properties (score)		
		oil	water-alcohol	water
1	*Pinus sibirica* DuTour	0,5	6,0	1,5
2	*Picea abies* L.	3,0	5,0	2,5
3	*Pinus sylvestris* L.	3,0	7,0	0
4	*Ábies sibírica*	4,0	4,0	0
5	*Juniperus communis* L.	0	7,0	5,0
	Control	0	1,0	0

As can be seen from the data obtained on the analysis of antiparasitic properties of coniferous forest plant extracts, the best results are obtained from alcohol tinctures of Baltic pine, juniper, and Siberian pine. Among the aqueous solutions, we noted some effects on the worms in juniper extracts and weaker effects from European spruce and Siberian pine. Among the oil extracts, extracts of Siberian fir can be distinguished. The following preparations did not affect the behavior of *L.terrestris:* aqueous extracts of Baltic pine and Siberian fir and oil extract of juniper.

Discussion

The resources of woody plants are divided into trunks (wood), branches, woody greens, and cones, where the needles are the most important part of medicinal purposes due to the accumulation of essential oils in them. Essential oils are a complex mixture of hydrocarbons and their derivatives that exhibit significant biological efficiency [16, 17]. For each region, information about the species diversity of resource plant species for the development of various industries is important. The resource potential of medicinal plants is the basis for the functioning of the pharmaceutical

industry. Essential oil species play an essential role in the production of many medicines because they have long been known as antispasmodic, diuretic, cholelitic, stimulating, and hepatoprotective medications [18, 19]. Thus, for example, the main phenolic components of *J. communis* extract are rutin, apigenin, isoscutellarein, hypolaetin, and protocatechuic acid. During an experiment on cancer cells of various origins, it was noted that *J. communis* extract consisting of a unique combination of phenolic compounds affected cancer cells using specific mechanisms of apoptosis [20].

According to experts, the collection of the Main Botanical Garden of the Institute of Botany and Phyto-introduction in Kazakhstan includes 1,115 taxa of medicinal plants of the world flora (1,071 species, 412 genera, 93 families). Among them, representatives of foreign flora equal 621 taxa (55.7%), Kazakh plants are represented by 452 taxa, and introduced flora (having a secondary area on the territory of Kazakhstan) is represented by 43 taxa. The vast majority of taxa in the collection are represented by annual or perennial grasses (annuals: 173 taxa, biennials: 55, herbaceous perennials: 712, herbaceous lianas: 20: total: 960 taxa). Hardy shrub forms account for less than 14% (155 taxa): semi-shrubs and small shrubsfor 57, woody lianas for6, shrubs for 75, and trees for 17 taxa [21].

The types of coniferous plants we selected are widespread in Kazakhstan and some of them are widely used among the population for therapeutic and preventive purposes in the form of water-based, alcohol-based, oil-based tinctures, decoctions, resins, balms, gels, etc. A potential candidate for these purposes is a coniferous tree extract (CTE) with an antibacterial effect [22]. According to our data, the essential oils of the Baltic pine contain the entire spectrum of important phytochemical components, including monoterpenes, terpenoids, and sesquiterpenes.

The analysis of the biological activity of phytochemical components of coniferous plants taken in the study showed that several preparations had an antimicrobial effect, others had antifungal properties, and some either did not show antagonistic properties at all or exhibited a stimulating effect and improved the growth of microorganisms.

Thus, we identified the bactericidal activity of water decoctions obtained from all coniferous plants. Moreover, the highest antimicrobial activity against *E. coli* was registered in water decoctions of Siberian fir and Baltic pine, and its absence was noted in oil extracts. Among the water/alcohol extracts, bactericidal activity was detected only in the juniper preparation. The MSC of juniper water/alcohol extract against the *E. coli* bacterium was manifested in a dilution of 1:128.

The absence of fungicidal or fungistatic activity against opportunistic yeast was recorded in water decoctions and infusions, as well as in water/alcohol extracts of all analyzed coniferous plants. Only oil extracts of plants that had a short-term fungistatic effect were active against *C.parapsilosis*. Our results are consistent with the data of other authors who claimed high resistance of C. parapsilosis yeast against fungicidal preparations [23, 24]. Among the plants with a complete absence of bactericidal activity against *C. parapsilosis* opportunistic yeast,Siberian fir should be named.

When studying the MSC of CPE against *A.niger* opportunistic mold fungi, it was found that none of the water and oil extracts showed fungistatic and fungicidal properties.

The analysis of antiparasitic properties in coniferous forest plant extracts showed the presence of the influence of the preparations on the behavior and vital activity of L.terrestris. The best results were obtained from the alcohol tinctures of Baltic pine, juniper, and Siberian pine; aqueous solutions of extracts of juniper, European spruce, and Siberian pine; and oil extracts of Siberian fir.

Conclusion

The results obtained indicate the presence of various biological effects in coniferous plant extracts, in particular, antimicrobial and fungicidal properties, which allows us to conclude that they can be used for the manufacture of preparations with a therapeutic effect against pathogens of infectious diseases.

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Түйін

Мақалада дәстүрлі медицинада кеңінен қолданылатын қылқан жапырақты өсімдіктердің емдік әлеуетін іздеудегі проблемалар туралы ғылыми ақпарат берілген. Эксперимент үшін Қазақстанның шығыс аумағындағы Батыс Алтайдың таулы ормандарында өсетін қылқан жапырақты өсімдіктердің үлгілері таңдалды: қарағай (*Pinus sylvestris* L.), кәдімгі шырша (P*icea abies* L.), сібір шыршасы (*Ábies sibírica*), сібір балқарағайы (*Pinus sibirica* DuTour), кәдімгі арша (*Juniperus communis* L.). Зерттеу барысында *Pinus sylvestris* L. эфир майларының компоненттерінің фитохимиялық құрамы сесквитерпендік фракция (69,76%), терпеноидтар (20,0%), монотерпендер (5,51%) бойынша анықталды. Биологиялық белсенділікті (микробқа қарсы, саңырауқұлаққа қарсы, антигельминтикалық) зерттеудің заманауи әдістерін қолдана отырып, біз таңдаған қылқан жапырақты өсімдіктердің сығындыларының биологиялық белсенділігінің болуын зерттедік. Барлық қылқан жапырақты өсімдіктерде бактерицидтік белсенділік сібір шыршасы мен қарағайдың биологиялық заттары белсендірек болған су қайнатпаларында көрінді. Май сығындылары бактерицидтік белсенділікті әлсіз деңгейінде көрсетті. Олардың ішінде сібір балқарағайы мен кәдімгі арша сығындылары белсенді емес, ал кәдімгі шырша сығындысы өзінің табиғи түрінде белсендірек болды және 1:2 сұйылтылған. Шартты-патогенді ашытқы Candida papapsilosiske қарсы бактерицидтік белсенділігі мүлдем жоқ өсімдіктердің арасында сібір шыршасын атап өткен жөн. Басқа қылқан жапырақты өсімдіктерде (шырша, қарағай, арша) бактерицидтік белсенділік тек 1:2 сұйылтудағы май ерітінділерінде анықталды, 1:8 сұйылтудағы сібір балқарағайының май сығындысы басым әсер етті. Сібір шыршасының су-спирт тұнбасы А.нигерге қарсы жоғары фунгицидтік белсенділікке ие болып шықты, ол бақылау кезеңінде 1:64 сұйылту кезінде микромицеттердің өсуіне әсер етуді жалғастырды. Қылқан жапырақты орман өсімдіктерінің кейбір өсімдік сығындыларында паразитке қарсы қасиеттердің болуы анықталды. Ең жақсы нәтиже кәдімгі қарағайдың, кәдімгі аршаның және сібір балқарағайының алкоголь тұнбаларында табылды.

Кілт сөздер: қылқан жапырақты өсімдіктер; биологиялық белсенді заттар; фитохимиялық құрамы; микробқа қарсы қасиеттері; антипаразиттік әсер; бактерицидтік белсенділік; емдік потенциал.

Аннотация

В статье представлена научная информация о проблемах при поиске терапевтического потенциала среди хвойных растений, широко используемых в народной медицине. Для эксперимента отобраны пробы хвойных растений, произрастающих в горных лесах Западного Алтая в восточной части Казахстана: сосны обыкновенной (*Pinus sylvestris* L.), ели обыкновенной *(Picea abies* L.), пихты сибирской (*Ábies sibírica*), кедра сибирского (*Pinus sibirica* DuTour), можжевельника сибирского. В ходе исследований был определен фитохимический состав компонентов эфирных масел *Pinus sylvestris* L. по показателям сесквитерпеновой фракции (69,76%), терпеноидов (20,0%), монотерпенов (5,51%). Используя современные методы изучения биологической активности (антимикробной, противогрибковой, антигельминтной), нами установлено наличие биологической активности экстрактов отобранных хвойных растений. У всех хвойных растений бактерицидная активность проявлялась у водных отваров, где более активными были биопрепараты пихты сибирской и сосны обыкновенной. Масляные экстракты проявили бактерицидную активность слабее. Среди них не активными были экстракты кедра сибирского и можжевельника обыкновенного, а экстракт ели обыкновенной был активнее в нативном виде и разведении 1:2. Среди растений с полным отсутствием бактерицидной активности против условно-патогенных дрожжей *Candida papapsilosis* следует назвать пихту сибирскую. У остальных хвойных растений (ель, сосна, можжевельник) бактерицидная активность выявлена только у масляных растворов в разведении 1:2, масляный экстракт кедра сибирского в разведении 1:8 оказывал подавляющий эффект. С высокой фунгицидной активностью в отношении A.niger оказалась водно-спиртовая настойка пихты сибирской, которая продолжала оказывать влияние на рост микромицета в разведении 1:64 в течение периода наблюдения. Наличие антипаразитарных свойств выявлено у некоторых растительных экстрактов хвойных растений леса. Лучшими результатами отличались спиртовые настойки сосны обыкновенной, можжевельника обыкновенного и кедра сибирского.

Ключевые слова: хвойные растения; биологически активные вещества; фитохимический состав; антимикробные свойства; антипаразитарный эффект; бактерицидная активность; терапевтический потенциал.

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OBTAINING OXYTETRACYCLINE CONJUGATES WITH PROTEIN CARRIERS

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Abstract

Oxytetracycline is widely used in veterinary medicine for the treatment of animals, as well as growth promoters. They can have adverse effects on human health through animal products if the rules for the use of antibiotics are not followed. The World Health Organization has established maximum residue limits of antibiotics in milk and meat, which require accurate, rapid and inexpensive methods to determine. Immunochromatographic assay (ICA) is ideal for this purpose due to its speed of analysis, high sensitivity and ease of use. This paper describes the results of study on the obtaining an oxytetracycline (OTC) conjugate with bovine serum albumin (BSA) and/or ovalbumin (OVA). OTC has been chemically purified from the antibiotic hydrochloride salt (OTC HC), which is widely used in animal husbandry and is more affordable than its chemically pure analogue. Spectrophotometric analysis of the prepared conjugates and immunization of laboratory animals showed the suitability of OTC, chemically purified from OTC HC, for crosslinking into BSA and/or OVA molecules and obtaining hapten-specific antibodies. The results obtained could be used for the manufacture of ICA components - labeled OTC-specific antibodies and test line antigen.

Key words: oxytetracycline; bovine serum albumin; ovalbumin; conjugate; antibiotic; high performance liquid chromatography; spectrophotometry.

Basic position and Introduction

Oxytetracycline (OTC) is one of the first tetracycline antibiotics described in the late 1940s. This drug is characterized by a wide spectrum of action against gram-negative and gram-positive microorganisms [1]. Tetracyclines, including chlortetracycline, oxytetracycline, and doxytetracycline are widely used in veterinary medicine due to their low cost compared to other antibiotics [2]. They are also used to stimulate the growth of fattening cattle and poultry [3]. For example, in Kazakhstan, such antibiotics as «Oxytetracycline hydrochloride for injections» (BioPharmGarant LLC, Russia), Oksirala 20% (Agio Pharmaceuticals Ltd., India), Ashoksi 10% (Ashish Life Science Pvt Ltd, India) are registered in the State Register of Veterinary Drugs and Feed Additives [4]. Residues of tetracyclines get into animal products in cases of non-compliance with the instructions for the use of antibiotics or the time of exposure of animals before slaughter or milk production [4]. The constant consumption of such products is fraught with serious health

consequences and could lead to allergic reactions and dysbacteriosis, and -may also cause nausea, vomiting, anaphylactic shock, and even death [5]. Violation of the use of antibiotics can lead to the emergence of antibiotic resistance genes in microorganisms, the transmission of such strains from animals to humans. [6]. In order to avoid harmful effects on human health, many countries have set maximum residue limits (MRL) of antibiotics in milk and meat. The World Health Organization (WHO) has established that the content of tetracyclines in food should be no more than 0.01 mg/kg [7]. Currently, there are several methods for determining antibiotic residues, each with its own advantages and disadvantages. For example, microbiological -tests are available for use in poorly equipped laboratories, but are characterized by low sensitivity and specificity [8,9]. Instrumental methods such as gas chromatography, high performance liquid chromatography (HPLC), chromatography–mass spectrometry is sensitive and highly specific, but require expensive equipment and trained personnel. In addition, they take a lot of time and are not suitable for routine analysis [10]. Recently, enzyme-linked immunosorbent assay (ELISA) has become increasingly popular for screening food products for contamination with antibacterial drugs. European Union (EU) Directive 2002/657 recommends this test for the determination of veterinary drug residues in livestock products in theEU. However, this test is not used in practice, since the equipment of domestic laboratories for veterinary and sanitary examination in food markets leaves much to be desired. Therefore, for practice, simple-to-perform, but sufficiently sensitive and specific tests are needed to determine the presence or absence of antibiotics in animal products in a few minutes. These tests include immunological methods based on the principle of thin layer chromatography, namely, immunochromatographic analysis (ICA) [11]. Antibiotics, including OTCs are haptens, and therefore competitive ICA is a suitable method for determining a contaminant in food products. The affordability of ICA tests for the detection of antibiotics is determined by the cost of its individual components, including the hapten conjugate with protein carriers. The use of chemically pure antibiotics in the preparation of the conjugate leads to a significant increase in the cost of analysis. In this regard, antibiotics for animals available on the veterinary medicine market are of particular interest.

The aim of our study was the use of veterinary oxytetracycline hydrochloride (OCT HC) for the preparation of the antibiotic conjugate with bovine serum albumin (BSA) and/or ovalbumin (OVA) as well as specific antibodies, which are the main ICA reagents.

Materials and methods

Laboratory animals. The Soviet chinchilla rabbits (3 heads, males, 6 months old, body weight 3300-3500 g) and outbred mice (3 heads, males, 2 months old, body weight 20-25g). Experiments with animals were approved by the Animal Ethics Committee, Faculty of Veterinary and Animal Husbandry Technology, S. Seifullin Kazakh Agrotechnical University, and were carried out in accordance with the Rules for the maintenance and care of laboratory rodents and rabbits (Interstate standard, GOST 33216-2014), as well as International Guiding Principles for Biomedical Research Involving Animals.

Reagents used to prepare antibiotic-protein conjugates. OTC HC for veterinary medicine (CJSC RPE Agrofarm, Russia), bovine serum albumin (BSA) (Jackson Immuno Research, USA) and/or ovalbumin (OVA) (Sigma-Aldrich, USA).

Chemical purification of the antibiotic. Three g of OTC HC was poured into a 0.5 L three-necked flask equipped with an addition funnel, a gas outlet tube, and a ground glass stopper. The antibiotic was dissolved in 50 ml of bidistilled water (pH=7.01) at a temperature of 30-35°C. Then, 100 ml of 7% sodium bicarbonate solution was added dropwise over 1 hour. Carbon dioxide released during the reaction of hydrochloric acid with sodium bicarbonate entered the calcium hydroxide solution. The mixture was stirred for 8 hours at room temperature at low speed of the magnetic rotor. After separation, the antibiotic was filtered on a Schott funnel. Subsequently, the filtrate was washed with distilled water and dried under vacuum.

Determination of purity and quantitative content of purified OTC using HPLC. Tetrahydrofuran, methanol, acetonitrile in ratios of 50:150:800 was used as the mobile phase - eluent, respectively. 20 mg of OTC substance was dissolved in 25 ml of 0.01 M hydrochloric acid. Endcapped octadecylsilyl silica gel for chromatography with a pore diameter of 5 μm

was used as a sorbent for the chromatographic column. The following parameters were used: chromatographic column temperature, 50°C; eluent solvent pumping rate, 1.3 ml/min; amount of sample used, 10 µl. The result of the analysis was recorded at an optical density of 254 nm.

Conjugation of OTC with protein carriers was carried out using the methods described by T. Wongtangprasert et al. (2014) [12], Nail L. et al. (2014) [13], and Birader K. et al. (2021) [14].

Optical spectrophotometry was used to identify the hapten-protein conjugate. Briefly, 100 µl of test substance solution was poured into a clean cuvette. Then, it was placed in a holder, and a reference spectrum was recorded with the radiation source (dark spectrum) turned on and off. Then, the spectrum of light that passed through the sample was recorded in 10 repetitions in accordance with the manufacturer's instructions attached to the device. The absorption of light by the studied samples at each wavelength was calculated in the Spectra Suite program using the standard formula and obtained in the form of a graph-spectrum.

Studying the immunogenicity of the OTC-protein conjugate. On day 0 of immunization, mice were intraperitoneally injected with OTC-BSA at a dose of 25 µg in 0.1 ml of complete Freund's adjuvant (CFA). On the 7th, 15th, 22nd and 32nd days of immunization, the same dose of antigen was administered in incomplete Freund's adjuvant (IFA). Antiserum was separated from blood taken from the tail vein on the 38th day of immunization and stored at -20°C until use.

Immunization of rabbits was carried out by 4-fold subcutaneous injection of OTC-BSA into the back area at a dose of 500 µg in 1.0 ml of adjuvant at several points with an interval of 10 days. On the 0th day of injection CFA was used, and on subsequent days IFA was used. Ear vein blood samples were taken 5 days after the last immunization. The separated immune sera were stored at -20°C until use.

Indirect ELISA (i-ELISA). The wells of a 96-well immunoassay plate (Suzhou CellPro Biotechnology Co., Suzhou, China) sensitized with OTC-OVA (1 µg/ml) in bicarbonate buffer, pH 9.6. The plate was incubated at +4°C overnight. The plate was washed 3 times with 300 µl of Tween-20 phosphate-buffered saline per well to remove unbound antigen. Then, two-fold dilutions of mouse and/or rabbit serum were prepared in 8 wells, starting from a dilution of 1:100 (0.1 ml), and incubated at +37ºC for 60±5 minutes. Blood serum samples of animals taken before immunization were used as a control. After incubation, the plate was washed as described above to remove non-specifically bound antibodies, and anti-mouse (Jackson ImmunoResearch Inc., Pennsylvania, USA) and/or anti-rabbit (Jackson ImmunoResearch Inc., Pennsylvania, USA) conjugates were added to the wells in a volume of 0.1 ml and incubated at +37ºC for 1 hour. The washing procedure was repeated to remove unbound reaction products. 0.1 ml of a stabilized solution of 3,3',5,5'-tetramethylbenzidine hydrochloride with hydrogen peroxide (CJSC "NVO Immunotech", Moscow, Russia) was added to the wells and the plate was incubated for 10-15 minutes at room temperature. The reaction was stopped by adding a solution of 0.5 M sulfuric acid to the plate wells. The results of the ELISA were taken into account using a spectrophotometer (Hangzhou Allsheng Instruments Co., Hangzhou, China) with a vertical light flux at a wavelength of 450 nm. The reaction was considered positive if the optical density (OD) of the immune serum exceeded the OD value of the control serum at a dilution of 1:200 by at least two times.

Results

The yield of chemically purified antibiotic from OTC HC for veterinary medicine (CJSC RPE Agrofarm, Russia) was more than 90%. The results of the study of the purity and quantitative content of the antibiotic using HPLC are shown in Fig.1.

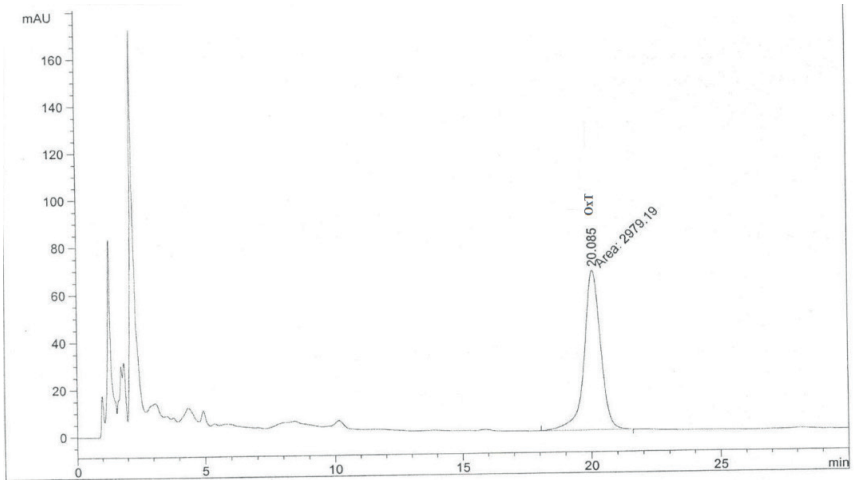


Figure 1 – HPLC chromatogram of purified OTC

As can be seen from Figure 1, the HPLC chromatogram of purified OTC contains several non-volume peaks identified as residual impurities and/or oxidation products in air during separation. At the same time, the content of the target component in the obtained substance was 96% in terms of dry matter using the ChemStation software.

The identification of the obtained compound was carried out using IR spectroscopy by determining the main signals of functional groups in pressed tablets of potassium bromide (Fig. 2).

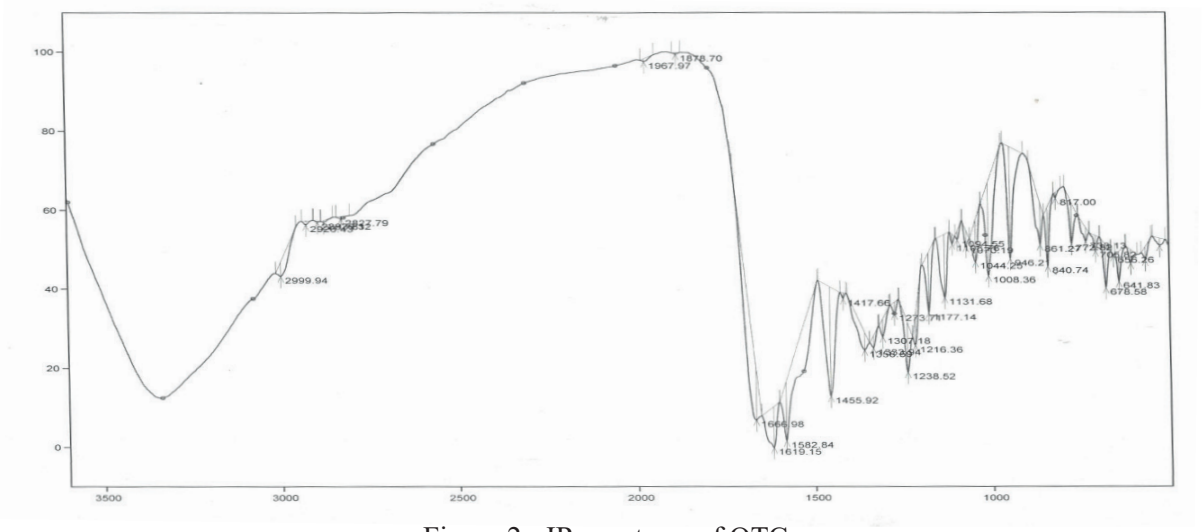
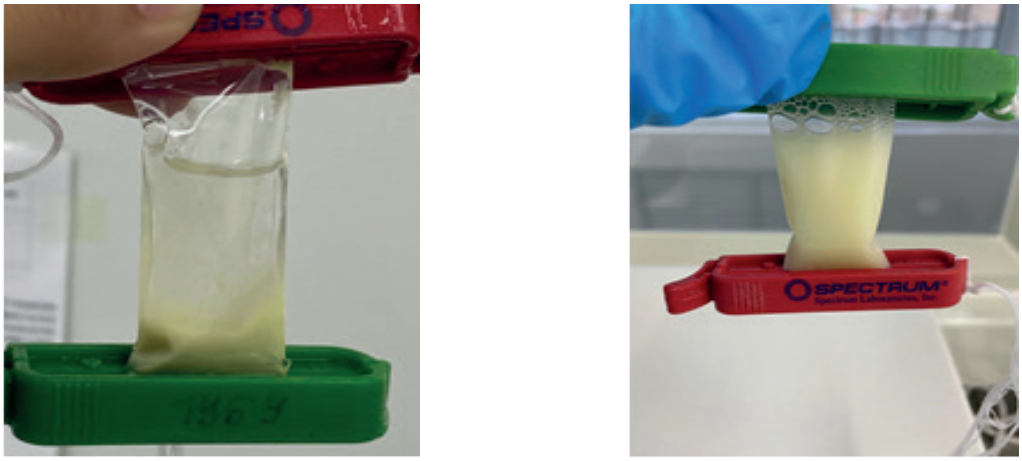


Figure 2 - IR spectrum of OTC

Analysis of Figure 2 shows that the spectrum of purified OTC contains signals at 3300-3600 cm-1, which are characteristic of hydroxyl groups (OH– groups) and are contained in the molecule of the test substance in the amount of two units. Stretching vibrations of 3200 cm-1 and 1238 cm-1 correspond to the phenolic fragment of the molecule, 1666 cm-1 - to the C=O carbonyl group associated with the cycle and 1619 cm-1, which indicates the presence of an amino group (NH2-group). Signals from 2999 to 2827 indicate the presence of aliphatic bonds in the molecule.

Chemically purified OTC as well as veterinary medicine OTC HC have been used to prepare antibiotic conjugates with BSA and/or OVA. Methods by L. Nail et al. (2014) and K. Birader et al. (2021) used for this purpose did not give the desired results due to the denaturation of proteins with the formation of a precipitate (Fig.3-a). An antibiotic-carrier conjugate was obtained by T. Wongtangprasert et al. (2014) only when purified OTC was used (Fig. 3-b).



a b

Figure 3 - Appearance of OTC-BSA conjugates:
a - OTC HC for veterinary medicine; b - chemically purified OTC

Spectrophotometric analysis was used to establish the crosslinking of the purified OTC into the carrier molecules (BSA, OVA). For this purpose, OTC, carrier proteins and the prepared conjugate were studied using UV optical photometry in the wavelength range of 200-1100 nm (Fig. 4, 5, 6).

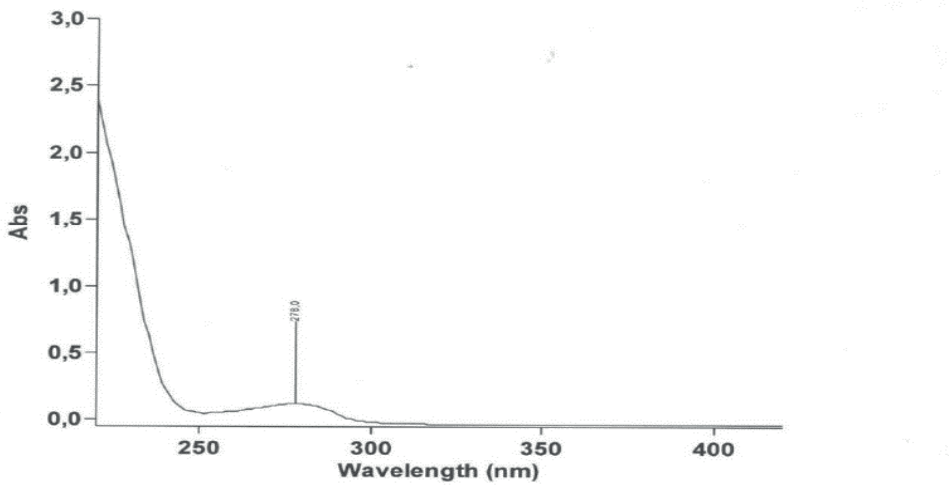
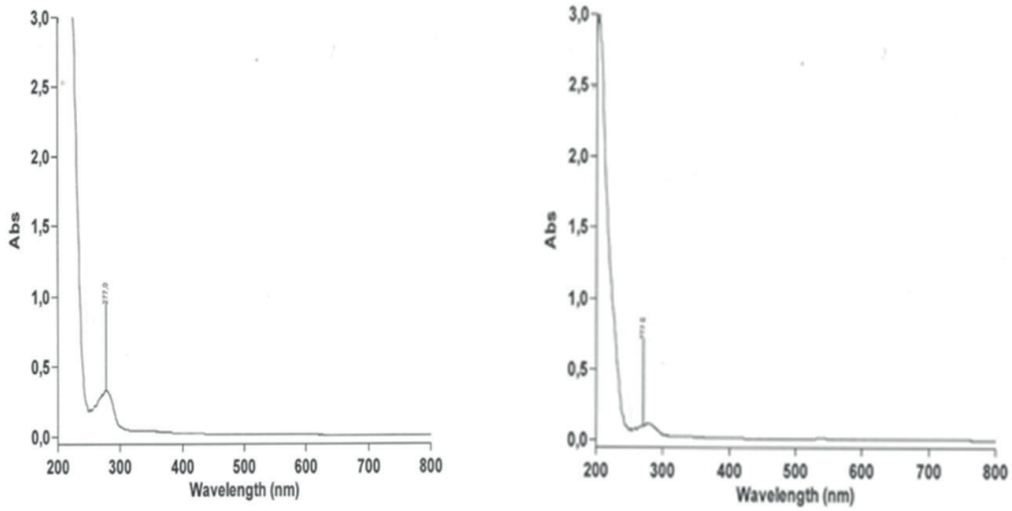


Figure 4 - UV spectrum of purified OTC

Figure 4 shows that the UV spectra of the chemically purified antibiotic contain one peak at a wavelength of 278 nm.

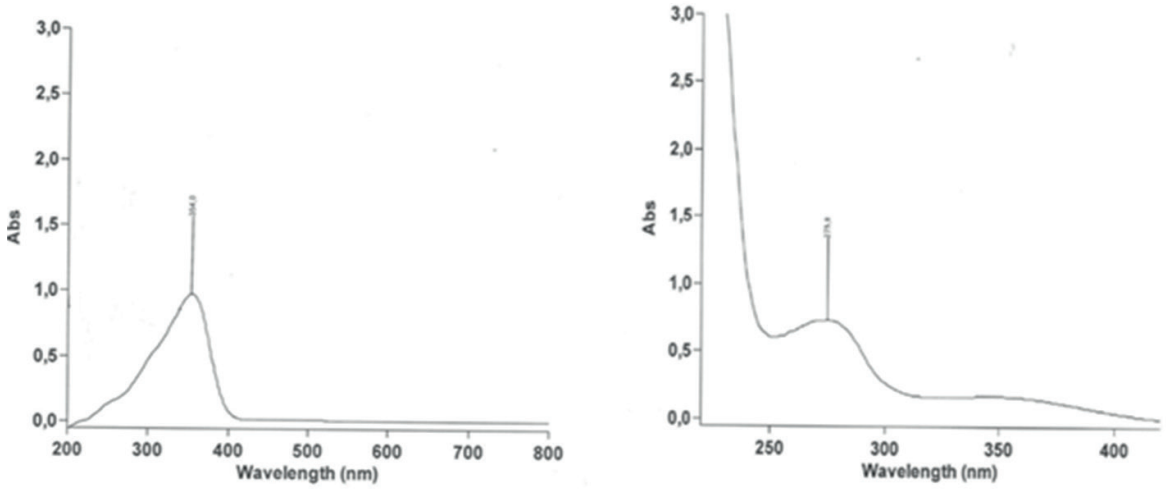
Spectrophotometric analysis of carrier proteins showed the presence of one peak in both the UV spectrum of BSA and OVA at 277 nm and 271 nm, respectively (Fig. 5).



a b

Figure 5 - UV spectra of proteins: a - BSA, b – OVA

Spectrograms of antibiotic conjugates with carrier proteins are shown in Fig.6.



a b

Figure 6 - UV spectra of conjugates: a - OTC-BSA, b - OTC-OVA

In the spectra of optical photometry, there are continuous signals with characteristic peaks of the initial substance, which testify the homogeneity of the final products obtained (Fig. 6). In the spectral regions, the presence of signals characteristic of the substrates of protein polymers is demonstrated, while the presence of the initial compounds of an antibiotic nature is not noticed, which is due to the complete absorption of low molecular weight compounds by macromolecules. The obtained UV spectra prove the suitability of OTC, purified from the hydrochloride salt of the antibiotic, for the preparation of a conjugate with BSA and/or OVA.

Figure 7 shows the results of studying the immunogenicity of OTC crosslinked into carrier protein molecules in mice and rabbits.

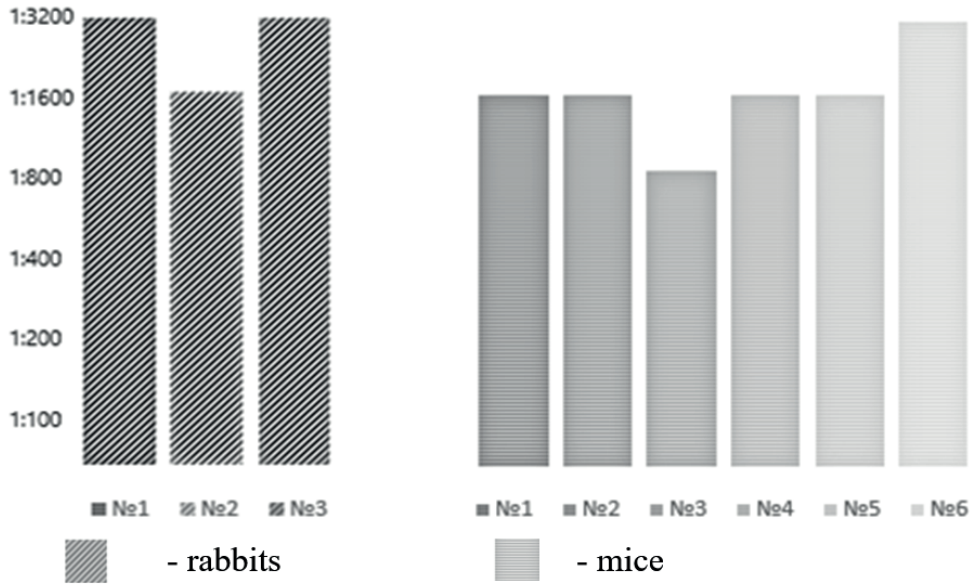


Figure 7 - Titers of anti-OTC antibodies by i-ELISA

Studies of antisera samples showed that BSA- coupled OTC was recognized by the immune system of laboratory animals as a separate epitope of the integral antigen and stimulated B-lymphocytes to synthesize antibiotic-specific antibodies. This is evidenced by the titers of antibodies against OTC by i-ELISA in samples of both mouse and rabbit antisera. It follows from the diagram that antibodies specific to the antibiotic were detected within the range of blood serum dilutions from 1:1600 to 1:3200, which is evidence of the immunogenicity of the antibiotic purified from its hydrochloride salt.

Discussion

The veterinary practice of the country needs simple, but sufficiently sensitive and specific tests to determine the presence of antibiotics in food products in a few minutes, which is very important for the reliable protection of public health. Such tests can be developed on the basis of competitive ICA, the main reagents of which are antibodies specific to various antibiotics and their conjugates with high molecular weight carriers. The difficulty in creating ICA tests for determining the MRL of antibiotics lies in the preparation of stable conjugates of hapten with carriers and the production of highly specific antibodies, since antibiotics, including OTC, are small substances with a molecular weight in the range of 460–434 Da [15]. In addition, imported ICA tests available on the market of veterinary drugs remain expensive for small producers of meat and milk in Kazakhstan, which produce more than 90% of milk and about 70% of meat in slaughter weight. One of the main components of ICA is an antibiotic-carrier conjugate, which is used in the diagnosticum design not only as a test line reagent, but also as an immunogen for obtaining specific antibodies. The use of chemically pure antibiotics for these purposes makes a significant contribution to the rise in the cost of the test system. In this study we propose a method for purifying OTC from its hydrochloride salt, which is widely used in veterinary practice and is affordable. Purified OTC conjugated with OVA served as an antigen in i-ELISA, while an antibiotic cross-linked into a BSA molecule was used as an immunogen. The results of spectrophotometric analysis showed the formation of a whole molecule consisting of a chemically purified antibiotic and OVA and/or BSA. The last conjugate showed its immunogenicity for the body of laboratory animals, which made it possible to obtain mouse and rabbit antisera with titers against the antibiotic hapten in the range of 1:1600 - 1:3200.

Thus, the developed method for obtaining chemically pure OTC from its commercial hydrochloride salt could reduce the cost of ICA tests designed to detect antibiotic residues in food.

Conclusion

- A method for obtaining chemically pure OTC from OTC HC for veterinary use has been developed, and OTC-BSA and/or OTC-OVA conjugates have been prepared;
- The homogeneity of the conjugates has been proven by HPLC and spectrophotometric analysis;
- OTC-BSA, as an immunogenic preparation, may be used to obtain oxytetracycline-specific antibodies;
- The obtained results will be used in our further study in order to design a domestic competitive ICA test for the detection of OTC in livestock products.

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Аннотация

Окситетрациклин широко используется в ветеринарии для лечения животных, а также в качестве стимуляторов роста. При несоблюдении правил использования антибиотиков, они могут оказывать неблагоприятные последствия на здоровье людей при употреблении продуктов животноводства. Всемирная организация здравоохранения установила предельно-допустимые концентрации антибиотиков в молоке и мясе для определения которых требуются точные, быстрые и недорогие методы. Для этой цели идеально подходит иммунохроматографический анализ (ИХА), благодаря скорости анализа, высокой чувствительности и простоте в применении. В настоящей работе описаны результаты исследований по получению конъюгата окситетрациклина (ОТЦ) с бычьим сывороточным альбумином (БСА) и/или овальбумином (ОВА). ОТЦ был химически очищен из гидрохлоридной соли антибиотика (ОТЦ ГХ), который широко используется в животноводстве и по цене более доступен, чем его химически чистый аналог. Спектрофотометрический анализ приготовленных конъюгатов и иммунизация ими лабораторных животных показали пригодность ОТЦ, химически очищенного из ОТЦ ГХ, для сшивки в молекулы БСА и/или ОВА и получения гаптен-специфичных антител. Полученные результаты могут быть использованы при изготовлении компонентов ИХА - меченых ОТЦ-специфичных антител и антигена тестовой линии.

Ключевые слова: окситетрациклин; бычий сывороточный альбумин; овальбумин; конъюгат; антибиотик; высокоэффективная жидкостная хроматография; спектрофотометрия.

Түйін

Окситетрациклин ветеринарияда малды емдеу үшін, сондай-ақ өсу стимуляторлары ретінде кеңінен қолданылады. Егер мал шаруашылығында антибиотиктерді қолдану ережелері сақталмаса, олар тағам өнімдері арқылы адамдардың денсаулығына кері әсерін тигізуі мүмкін. Дүниежүзілік денсаулық сақтау ұйымы сүт пен етте антибиотиктердің шекті рұқсат етілген-мөлшерлерін белгіледі, ал оларды анықтау үшін дәл, жылдам және арзан әдістер қажет. Осы мақсатта нәтиже жылдамдығына, жоғары сезімталдығына және қолданудың қарапайымдылығына байланысты иммунохроматографиялық талдау (ИХТ) өте қолайлы әдіс. Бұл жұмыста окситетрациклиннің (ОТЦ) бұқа сарысуы альбуминімен (БСА) және/немесе овальбуминмен (ОВА) конъюгатын дайындау бойынша зерттеу нәтижелері баяндалған. ОТЦ мал шаруашылығында кеңінен қолданылатын және оның химиялық таза аналогына қарағанда қол жетімді болып табылатын антибиотиктің гидрохлоридті тұзынан (ОТЦ ГХ) химиялық әдіспен тазартылған. Дайындалған конъюгаттарды спектрофотометриялық зерттеу және олармен зертханалық жануарларды иммунизациялау ОТЦ ГХ-нен химиялық тазартылған ОТЦ-нің БСА және/немесе ОВА молекулаларымен қосыла алуына және гаптенге тән антиденелерді түзе алуына жарамдылығын көрсетті. Алынған нәтижелер ИХТ компоненттерін, атап айтсақ тест жүйесінің ОТЦ-мен таңбаланған арнайы антиденелерін және сынық сызығының антигенін өндіруде пайдаланылуы мүмкін.

Кілт сөздер: окситетрациклин; бұқа сарысуы альбумині; овальбумин; конъюгат; антибиотик; өнімділігі жоғары сұйық хроматография; спектрофотометрия.

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STUDIES OF FISH AND FISH PRODUCTS FOR THE PRESENCE OF HEAVY METAL SALT AND RADIONUCLIDES IN WATER BODIES OF THE WEST KAZAKHSTAN REGION

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Abstract

With the increase in production and processing industries, as well as the extraction of minerals in the territory of the Republic of Kazakhstan, it leads to pollution of the environment, ecology, wildlife and water resources of our country. All production capacities in agriculture and animal husbandry require water resources.

As part of the research work, diagnostics were carried out on the content of salts of heavy metals and radioactive substances in the water bodies of the West Kazakhstan region, where they originate with transboundary rivers and an oil and gas field (KPO) is being produced. To carry out our research work, samples of fish products were taken from 15 reservoirs and 3 objects of domestic trade for laboratory testing for the content of salts of heavy metals (Pb, Cd) and radionuclides (Sr-90, Cs-134). Radioactive substances accumulate in the bones and muscles of fish, and subsequently enter the human body through ingestion. The risk of developing adverse effects on human health depends on the radiation dose. If the radiation dose is low, or if exposure occurs over a long period of time, the risk is much lower as the human body repairs damaged cells and molecules. Lead and cadmium harm the human nervous system and its mental development, it can cause kidney damage and lead to bone disease. It is necessary to constantly monitor the content of salts of heavy metals and radionuclides in fish and livestock products, since safe products are a guarantee of public health.

Key words: Heavy metal salts; radionuclides; fish products; reservoirs; WKR.

Basic position and Introduction

In the last century, as a result of technological progress, the balance of heavy metals in the environment was disturbed, and there was a global pollution of nature with heavy metals. The most common are mercury, lead, cadmium, arsenic, copper and others. As a result, they interact with the plants and fauna of water bodies with the effluents of industrial plants entering water bodies.

As a result of such interactions, heavy metals can accumulate in aquatic organisms, leading to death or disruption of life, and through trophic chains, including humans, can lead to various diseases [1].

The study of the elemental composition of aquatic organisms allows us to solve a number of ecological, physiological, veterinary and practical problems. This factor is responsible for the appearance of a significant number of works on the content of trace elements of heavy metals in commercial aquatic organisms in the last decade. Fish are capable of accumulating heavy metals in the aquatic ecosystem, even if their amount does not exceed the established limit. Pathological changes at the molecular, organism, and population levels can occur under the influence of toxic substances in fish. Since fish is a staple food, human health is at great risk [2].

Environmental pollution is a very pressing issue. The Republic of Kazakhstan is one of the states where many petrochemical complexes, ore deposits contribute to pollution of salts of heavy metals and radionuclides into the environment. Due to the increase in production capacity, urbanization of the population and the development of agriculture and fisheries, the study of water resources is an important issue. During the study of the dynamics of toxicological and radiological contamination of fish muscle tissue (predators and benthophages) from fish farms and commercial water bodies of West Kazakhstan region. Food fish products must be made from aquatic biological resources extracted (caught) from safe areas of extraction (catching) in accordance with the data of planned monitoring of safety of aquatic biological resources, carried out by the authorized bodies of the member states, and aquaculture objects, originating from farms (enterprises) that are safe in veterinary respect.

The aquatic environment contaminated with heavy metals has a negative impact on hydrobionts, leads to an increase in ecological consequences and poses a threat not only to living organisms living in water, but also to human health. In the summer season on the background of high functional activity of the digestive system, the sensitivity of fish carbohydrases to the action of heavy metals increases [11]. The relevance of this approach is confirmed by the works carried out by scientific teams in many countries of the world [3].

For an objective assessment of the industrial impact on the pollution of hydrobionts with toxicants, it is necessary to consider the ionic-salt composition of the water of the water body under study. With increasing salinity, hardness, alkalinity of water toxicity of metals decreases, which increases the tolerance and resistance of aquatic organisms to high concentrations of metals [4].

The presence of significant fluctuations in hydrochemical indicators (salinity, hardness, alkalinity) leads to a variety of correlation relationships between biotic and abiotic factors. A certain dependence in the accumulation capacity of fish on the type of food in the Zhaik-Caspian basin in terms of lead accumulation is dominated by predators, to a greater extent catfish - 33 %, to a lesser extent pikeperch - 27 %. Lead in the muscles of benthophages is contained in similar concentrations, amounting to 20% [5,6].

Materials and methods

On contamination of fish with salts of heavy metals and radionuclides in the reservoirs Derkul, Solyanka, Embulatovka, Chizha, Barbastau hollow. Muratsay, Berezovka, Chagan, Bagyrlay, Ashysay, Shyngyrlau, Shiderti, Kaldygayly on Aydyn Island and Livkino fish nursery.

Figure 1 - Sampling Process for Laboratory Research

Sampling was carried out at the facilities of the domestic trade markets Ayazhan, El-Yrysy and Mirlan in the city of Uralsk. Processing of macrozoobenthos samples were carried out according to the standard methodological manual [6].

Toxicological and radiological studies were conducted in accordance with GOST 17319-2019 in the branch of RGP on PCV Republican Veterinary Laboratory of West Kazakhstan region. A total of 84 fish samples were examined, such as crucian carp, perch, roach, bream, ide, rudd, silver bream, podust, pike, tench, carp, zander.

Micronutrients (Lead and Cadmium) in fish muscle were determined on an atomic absorption spectrometer according to the methodological guidelines [7].

Statistical processing of data to identify the main trends and biotic parameters was carried out using Excel and Statistica [8].

Maximum permissible levels (MPL) of toxicants in fish were evaluated according to current sanitary standards [9].

Results

Residual amounts of salts of heavy metals were studied on fish samples from 15 West Kazakhstan Region reservoirs. The results of the studies are given in Figures 2 and 3. Note: the number of fish is given in parentheses.

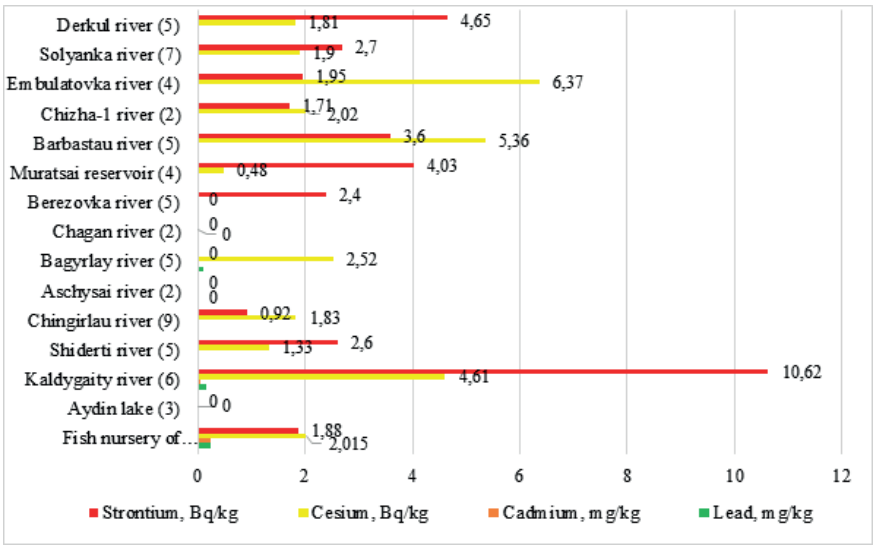

Figure 2 - Content of toxic elements and radionuclides in fish from WKR reservoirs

It follows from Figure 2 that heavy metals lead and cadmium in muscle samples of fish living in the fish nursery of «Livkino» LLP contained 0.25 mg/kg and 0.245 mg/kg Hg, Cd, Cu and Zn are considered the most dangerous in ecotoxicological respect elements [13] Kaldygaity river - 0.148 mg/kg and 0.04 mg/kg; Shyngyrlau river - 0.02 mg/kg and 0.002 mg/kg; Bagyrlay river - 0.1 mg/kg and 0.0375 mg/kg respectively. In invertebrate animals, the TM ions can also come by dermal absorption [12].

The content of caesium 137 and strontium 90 radionuclides in fish from the fish nursery of «Livkino» LLP was within 2.015 (±14.0) Bq/kg and 1.88 (±14.85) Bq/kg; Kaldygaiti river - 4.61 (±8.31) Bq/kg and 10.62 (±29.11) Bq/kg; Shiderti river -1.33 (±5.9) Bq/kg and 2.6 (±11.83) Bq/kg; Shyngyrlau river - 1.83 (±6.7) Bq/kg and 0.92 (±11.56) Bq/kg; Dv. Muratsay - 0.48 (±2.0) Bq/kg and 4.03 (±18.42) Bq/kg; Barbastau river - 5.36 (±8.86) Bq/kg and 3.6 (±15.2) Bq/kg; Chizha river - 2.02 (±15.2) Bq/kg; Shingirlau river - 1.83 (±6.7) Bq/kg and 0.92 (±11.56 Bq/kg). Chizha-1 - 2.02 (±9.57) Bq/kg and 1.71(±15.85) Bq/kg; Embulatovka river - 6.37 (±15.6) Bq/kg and 1.95 (±35.8); Solyanka river - 1.9 (±6.42) Bq/kg and 2.7(±19.15) Bq/kg; Derkul river 1.81 (±5.7) Bq/kg and 4.65 (±17.61) Bq/kg, respectively [19,20]. Cesium 137 and strontium 90 were detected - only in the inhabitants of Bagyrlay river (2,52 (±9,17) Bq/kg) and Berezovka river (2,4 (±7,8) Bq/kg). Thus, in WKO average degree of contamination of fish and fish products with residual amounts of radionuclides does not exceed MAC (caesium 137 - 2,48 Bq/kg; strontium 90 - 3,08 Bq/kg). In all sample's heavy metals, namely arsenic and

mercury, were not detected.

Lead belongs to hazard class I, its peculiarities of stay and migration in natural waters are explained by the fact that it reacts relatively easily with water impurities to form less soluble compounds. Its concentration in water is low and does not exceed 10 µg/L aquatic plants can also retain lead well. Lead accumulates insignificantly in organs and tissues, so it is relatively harmless for humans in this link of the trophic chain. According to many Russian scientists, methylated lead compounds in fish of unpolluted water bodies are relatively rare. In regions with industrial emissions, the accumulation of tetramethyl lead in fish tissues is efficient and rapid, so acute and chronic exposure to lead occurs at pollution levels of 0.1-0.5 µg/L. Hydrobionts are capable of accumulating TM to concentrations tens and even thousands of times greater than their content in the environment [14,15,16].

Cadmium is one of the toxic heavy metals; it is less toxic to plants than mercury. It is dangerous to living organisms because it can replace zinc in enzymes with metals in their active centers, which leads to drastic disruption of enzymatic processes. This metal accumulates in the tissues of the internal organs of aquatic plants and fish [17,18].

The acute cadmium toxicity limit for freshwater fish ranges from 0.09 to 105 µg/L. Note: The number of fish is given in parentheses.

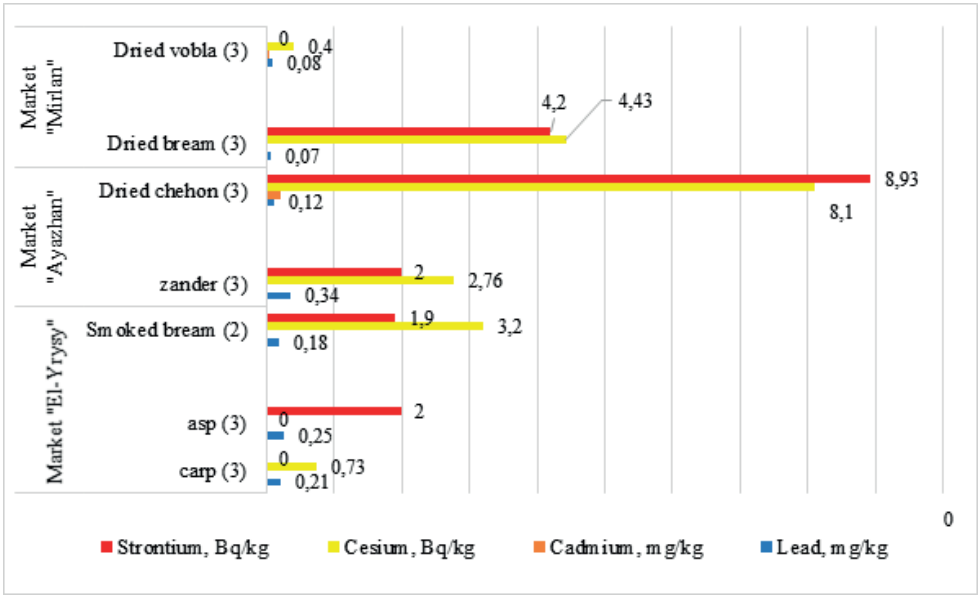

Figure 3 - Content of toxic elements and radionuclides in fish and fish products from retail outlets

The data of Fig. 3 indicate that the content of heavy metals and radionuclides in the examined samples of fish and fish products does not exceed the MPC. Thus, according to GOST 32161-2013 "Food products. Method for Determination of Cesium Cs-137" and GOST 32163-2013 "Food products. Method for Determination of Strontium Sr-90" cesium 137 and strontium 90 must not exceed 130.00 Bq/kg. and 100.00 Bq/kg, respectively.

Discussion

The monitoring of heavy metal contamination is one of the main tasks in ensuring the safety of food of animal origin. Heavy metals such as Pb, Cd and Hg, and the radionuclides Sr and Cs are toxic elements with the highest accumulation factor, but whose presence in food is strongly influenced by environmental conditions. Exposure to metallic pollutants is particularly important in the consumption of fish, whose integration into the habitat and the biomagnification process may pose a health risk to the consumer. In the present study, it was found that the limiting value set for Sr was in the studied river Kaldagaiti (>10.62bq/kg) Derkul (>4.65bq/kg) and Cs in water samples Barbastau and Embulatovka (>6.37bq/kg, >5.36bq/kg). Cd and Pb concentrations in all analysed samples were within the permissible limits (0.148-0.2mg/kg) in Bagirlay, Shyngirlau rivers, however, it should be noted that in case of Cd and its compounds, the quality standards depend on water hardness [19].

However, it should be noted that the maximum allowable concentrations (MAC) of Pb, Cd and Hg were not exceeded in any of the rivers studied. The Pb and Cd content in the water samples from these surveyed lakes was in line with the results of the survey, as shown by the review of available sources. Analysis of bottom sediments showed a significant quantitative differentiation of the investigated metals between the individual water bodies and a significantly higher content of metals in sediments compared to concentrations in water. The results of this study are in agreement with observations by other authors that heavy metal pollutants entering surface waters are predominantly deposited in bottom sediments. This mechanism is influenced by the structure of the sediment, which, unlike the water column, is stable and immobile, preventing rapid resuspension and biochemical dissolution of metal bonds back into the water column.

Assessment of the degree of contamination of bottom sediments with heavy metals on the basis of geochemical criteria according to Boyakovskaya classification allows to refer sediments to class I, which includes sediments considered to be uncontaminated. It should be noted that the content of all investigated metals in bottom sediments of this lake did not exceed the geochemical background determined for Pb, Cd and Hg. showed that the content of heavy metals in fish tissues largely depends on the balance between absorption rate and elimination rate. And it depends on environmental conditions and the duration of life of fish. In young fish, characterized by higher metabolic activity and food intake rate, metal impurities can accumulate in tissues and organs to a greater extent than in older fish. Fish as a food product must comply with the requirements established in the legislation On the Technical Regulation of the Eurasian Economic Union On the Safety of Fish and Fish Products for Foodstuffs, i.e. the permissible content of Pb, Cd and Hg. Comparison of Pb, Cd and Hg content in the muscles of the studied fish species with the content of these metals in the muscles of different commercial fish species showed that the content of the mentioned metals in the muscles of the three studied fish species was low. Health risk assessment associated with the content of Hg, Pb, and radionuclides Sr and Cs in the muscles of the three studied fish species sampled from the rivers was conducted in the laboratory "Republican Veterinary Laboratory" of the Committee for Veterinary Control and Supervision of the Ministry of Agriculture of the Republic of Kazakhstan and the test report results give us the results of the study. That in all investigated fish the investigated heavy metals, i.e. Pb, Cd and radionuclides Sr,Cs , did not pose a threat to the health of consumers consuming such fish species [20].

Conclusion

Summarizing the data obtained, we can state that the content of heavy metals and radionuclides in some specimens of fish and fish products WKR exceeds MPC (GOST 31903-2012), which is a consequence of industrial waste entering the water bodies.

Fish tissues can accumulate toxic substances, since they are the main species of hydrobionts and act as one of the last links in the food chain.

The contents of heavy metals (lead, cadmium, arsenic, and mercury) and radionuclides (cesium 137 and strontium 90) in fish and fish products consumed by WKR residents were within acceptable limits.

Information on finansing

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Түйін

Өндіріс пен өңдеу өнеркәсібінің ұлғаюымен, сондай-ақ Қазақстан Республикасының аумағында пайдалы қазбалардың өндірілуімен еліміздің қоршаған ортасының, экологиясының, жануарлар дүниесі мен су ресурстарының ластануына әкеліп соғады. Ауыл шаруашылығы мен мал шаруашылығындағы барлық өндірістік қуаттар су ресурстарын қажет етеді. Ғылыми-зерттеу жұмыстарының шеңберінде Батыс Қазақстан облысының трансшекаралық өзендерден бастау алатын және мұнай-газ кен орны (ҚПО) өндірілетін су айдындарындағы ауыр металдар мен радиоактивті заттардың тұздарының құрамы бойынша диагностика жүргізілді. Зерттеу жұмысымызды жүзеге асыру үшін ауыр металдар (Pb, Cd) тұздарының және радионуклидтердің (Sr-90, Cs-134) құрамын зертханалық тексеру үшін 15 су қоймасы мен 3 ішкі сауда объектілерінен балық өнімдерінің сынамалары алынды. Радиоактивті заттар балықтардың сүйектері мен бұлшықеттерінде жиналып, кейіннен адам ағзасына жұту арқылы түседі. Адам денсаулығына жағымсыз әсер ету қаупі сәулелену дозасына байланысты. Егер сәулелену дозасы төмен болса немесе әсер ету ұзақ уақыт бойы болса, адам ағзасы зақымдалған жасушалар мен молекулаларды қалпына келтіретіндіктен, қауіп әлдеқайда аз болады. Қорғасын мен кадмий адамның жүйке жүйесіне және оның психикалық дамуына зиянын тигізеді, ол бүйректі зақымдап, сүйек ауруына әкеледі. Қауіпсіз өнімдер халық денсаулығының кепілі болғандықтан, балық пен мал шаруашылығы өнімдерінде ауыр металдар мен радионуклидтердің тұздарының мөлшерін үнемі бақылау қажет.

Кілт сөздер: ауыр металл тұздары; радионуклидтер; балық өнімдері; су қоймалары; БҚО.

Аннотация

С увеличением производственных и перерабатывающих производств, а также добыча полезных ископаемых на территории Республики Казахстан приводит к загрязнению окружающей среды экологии, животного мира и водных ресурсов нашей страны. Во всех производственных мощностях в сельском хозяйстве и животноводстве необходимо водные ресурсы. В рамках научно-исследовательской работы проведена диагностика по содержанию солей тяжелых металлов и радиоактивных веществ в водоемах Западно-Казахстанской области, где они берут начало с трансграничными реками и ведется добыча нефтегазового месторождения (КРО). Для выполнения научно-исследовательской работы были отобраны пробы рыб и рыбной продукции из 15 водоемов и 3-х объектах внутренней торговли для лабораторного исследования на содержание солей тяжелых металлов (Pb, Cd) и радионуклидов (Sr-90, Cs-134). Радиоактивные вещества накапливаются в костях и мышцах рыб, и последовательно попадают в организм человека при употреблении в пищу. Риск развития неблагоприятных последствий для здоровья человека зависит от дозы облучения. Если доза облучения низкая или если облучение имеет место в течение длительного периода времени, риск значительно ниже, поскольку организм человека восстанавливает поврежденные клетки и молекулы. Свинец и кадмии наносит вред нервной системе человека и его умственному развитию может вызвать нарушение работы почек и привести к болезням костей. Необходим постоянный контроль над содержаниям солей тяжелых металлов и радионуклидов в продукции рыбоводства, так как безопасная продукция залог здоровья населения.

Ключевые слова: соли тяжелых металлов; радионуклиды; рыбная продукция; водоемы; ЗКО.

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THE PREVALENCE OF INVASIVE DISEASES AMONG CATS IN URALSK

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Abstract

The frequent spread of intestinal worms among carnivorous animals is found in a number of diseases that are dangerous to the human body. Intestinal worming infection rates are high among stray cats and dogs as well as domesticated animals.

In the research work, studies were conducted to differentiate intestinal worming in cats brought in from micro-districts of Uralsk, West Kazakhstan region. The results of the studies determined the extent of intestinal worming among cats, their species composition, seasonal dynamics of infections and the level of the age of the disease.

The city of Uralsk revealed a widespread of cat helminth infections. (IE-36.1%). By intestinal helminth infestation, the primary *toxocariasis Toxocara mystax*) is 19,7%, and by spread *toxocariasis* (*Toxascaris leonina*) is 12,4%.

The degree of the extensiveness of infestation, depending on the time of the year, was determined; it was found that in summer and autumn, the infestation reaches a high degree of extensiveness.

It was found that intestinal worm infestation in cats is associated with age. *Toxocariasis* is more common in 6-month-old cats. And with age, the incidence of this worming disease decreases significantly. In cats over five years old, this worming disease is sporadic.

Key words: the prevalence of invasive disease; cats; animal helminths; carnivores; helminthiasis; infestation; toxocariasis.

Basic position and introduction

It is known that endoparasites of domestic animals are widespread throughout the world. In our country, they are also ubiquitous. The only reason for the spread of infestation is considered to be the constant uncontrolled growth of dogs and cats.

These circumstances indicate that it is necessary to pay attention to causing significant damage to animals and preventing people from contracting infectious diseases.

Today, cats exist in every family and are more closely related to humans than dogs. Endoparasites found in cats, including intestinal helminth infections, are dangerous and widespread, and some of their species are not well studied [1].

Diseases caused by helminth infections become very serious and dangerous; they occur in marginal animals and adult animals.

Every 2nd, cat daily excretes several hundred to several thousand helminth eggs into the environment, many of which enter the premises together with clothes and shoes. Therefore, helminth infections are widespread even in animals that have not left the house [2-6].

Studying the species composition of feline helminths, the spread of helminths, the

extensiveness of infestations, and age and seasonal dynamics is necessary for checking the epizootiology of helminthiases of domestic animals (carnivores) and the epidemiology of parasitic diseases.

This will be the reason for correctly and effectively applying preventive and therapeutic measures against infestation. Therefore, studying the features of the spread of diseases spread by helminth infections common among cats and the organization of efforts to combat them is an urgent current problem [7-12].

Regarding roundworm infections, Toxocara canis is the most common ascariasis in dogs. It is highly pathogenic, and the consequences of the infection often have the character of complications. During larval migration, various tissues (liver, lung, heart, kidney) may show granulomatous lesions. The pulmonary phase can be fatal in puppies if the dog is heavily infected before birth, and death occurs within a week after birth. This is the most serious impact of Tochosaga infection. After the worms have progressed to the adult stage in the small intestine, the most important clinical symptoms are abdominal bloating characterized by mild diarrhoea. Adult worms often escape and become infected by faeces or vomit. Other roundworms do not migrate Toxascaris leonina in dogs and, less frequently, in cats, so the pathological significance is not particularly pronounced [13-16].

Two species, Taenia taeniaeformis and Dipylidium caninum are common in cats.

In many cases, clinical signs go undetected. The infection will be associated with diarrhoea and stunted development, but gastrointestinal parasitism of roundworms can often be significant. Humans can accidentally become infected with Ancylostoma caninum zoonosis, Ancylostoma braziliense and Uncinaria stenocephala, which can cause self-limiting dermatological lesions in the form of migrating skin larvae. In addition, Ancylostoma ceylanicum, which usually causes ankylostomas in cats, also affects humans [17].

An analysis of studies by scientists has shown that roundworms in dogs and cats rank first in the frequency of occurrence compared to other intestinal helminth infections [18-20].

Materials and methods

Following the purpose of the research work, it was found to study the characteristics of the spread of the main intestinal helminthiasis of cats in the West Kazakhstan region, the city of Urals, and to organize their prevention and treatment. Cats infected with random helminths, caught from various small areas in the city of Ural, West Kazakhstan region, were taken as the object of the study. The blood and excrement of cats in which helminths were detected were included as research materials.

The research work was carried out in the period of 2020-2022 at the "Zhardem-Vet" educational-scientific production centre of West Kazakhstan Agricultural and Technical University, named after Zhangir Khan. During the research, 310 faeces and 80 blood samples from cats of different breeds, sexes and ages were studied. Two hundred fifty-six cats of other species, sexes and ages, randomly infected with intestinal helminths, caught at different times from different districts of the city of Urals, were studied.

For the study, freshly separated stool samples were taken from domestic animals; during the collection of stool samples, anamnesis data were collected, as well as gender, age, type and time of the study were determined.

The Fülleborn flotation method was used in order to determine the presence of eggs of Toxocara mystax, Toxascaris leonina, Uncinaria stenocephala, and Dipilidium caninum in coprological research.

The eggs of the helminths mentioned above were counted using the Tracha method in the VIGIS counting chamber.

Results

The results of the distribution of the main intestinal worms of cats in the West Kazakhstan region, Ural city, gave the following data (Fig.1).

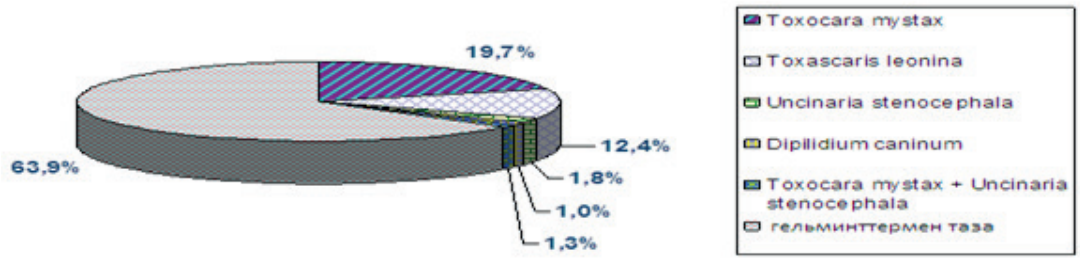

Figure 1 - Distribution of main intestinal worms of cats in the city of Ural, West Kazakhstan region

As a result of the analysis of the conducted studies, the average extensiveness of infestation in helminthiasis of cats was found to be 36.1%, while the high prevalence of intestinal helminths was 19.7% caused by Toxocara mystax. Toxasquirosis (the causative agent of *Toxascaris leonina*) took 2nd place in terms of the majority - 12.4%. After that, uncinariasis (the causative agent of *Ucinaria stenocephala*) was 1.8%, and dipilidios is (the causative agent of *Dipilidium caninum*) was 1%.

There were also cases of mixed infestation of *Toxocara mystax* and *Uncinaria stenocephala* - 1.3% (Fig 1).

The seasonal dynamics of infection of cats with the main types of helminths were as follows.

As a result of the study throughout the year, the degree of cat infestation was different (Fig 2).

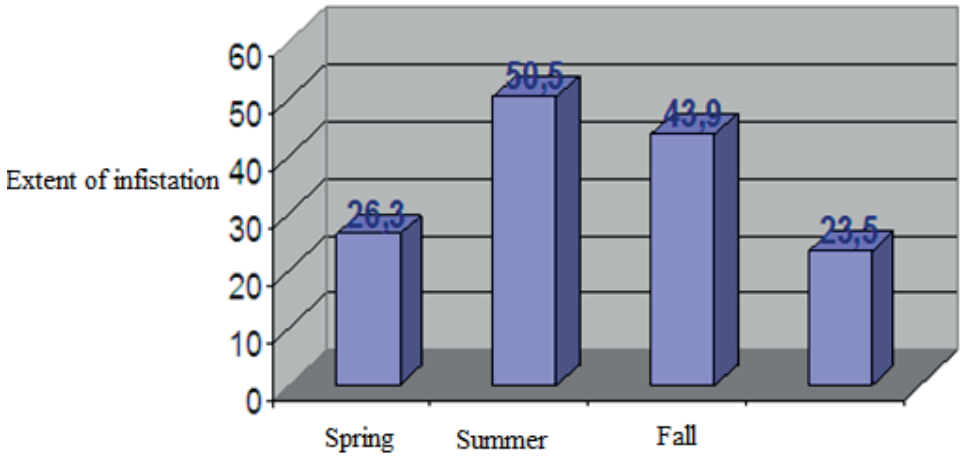

Figure 2 - Seasonal dynamics of infection of cats with the main types of helminths

The dependence of the extent of infestation on the season was determined, and the size of infestation was 50.5% and 43.9% in summer and autumn and reached a high level, while the extent of infestation decreased to 23.5% in winter.

In the spring months, it is observed that the degree of infection of cats with helminths increases to a small extent.

The upper limit of infection of cats with *toxocariasis* helminths is 23.8% in summer and 20.4% in autumn. And it was found that the extent of invasion is significantly lower. If it is 15.7% in winter, it rises to 18.9% in spring.

The lowest infection rate of *toxocariasis* in cats was observed in the winter months - 8.8%. And if the spread of infestation increased to 10.5% throughout the year, it reached the upper limit - 15.8% in the summer months and 14.3% in the autumn.

Infection with *uncinariasis* in cats was 3.9% in summer, while the extent of infestation decreased by 2% in autumn.

Uncinariosis was not registered in winter, and the rate of infection in spring was 1%.

In cats, the rate of infection with dipilidiosis was 1.9% in summer, 1% in autumn, and 0.9% in winter.

Mixed infestation with *toxocariasis* and *uncinariasis* was recorded at 2.9% in summer and 2% in autumn.

Seasonal dynamics of infection rate in cats are clearly natural.

It is believed that the condition of a great extent of invasion in summer and autumn is due

to the appearance of helminth eggs and favourable environmental conditions for the infection of cats.

The dynamics of infection in cats by age provided the following data (Table 1).

Age characteristics of animals have a significant effect on the degree of infection with intestinal worms.

According to these data, the animals in the study were divided into age groups: 1 - 6 months, 7 - 12 months, 1 - 3 years, 3 - 5 years and over five years.

Table 1 - Infectivity of animals by age

Age characteristic	Number of examined animals	Number of infected animals	*IE %
1-6 months	76	35	46,0
7-12 months	50	22	44,0
1-3 years	47	16	34,0
3-5 years	41	11	26,8
5 years and older	42	8	19,0
Everything	256	92	35,9

**extent of infestation*

35 out of 76 head mares were infected between 1 and 6 months of age (IE - 46.0%)

And 50 cats between the ages of 7-12 months were examined, and 22 (IE - 44.0%) of them were infected.

In the course of the study, 16 animals out of 47 were infected between 1 and 3 years of age, accounting for 34.0%.

Out of 41 examined animals aged 3-5 years, the number of infected animals was 11 (26.8% IE).

And 42 animals over five years of age were examined, and the number of infected ones was 8 (IE - 19.0%).

Based on the results of the research, it was determined that the infection of cats with intestinal worms depends on their age. Some types of intestinal worms affect the generation of animals. For example, toxocariasis is common in 6-month-old cats. And due to the increase in age, infection with this worm disease decreases significantly; this worm disease occurs very rarely in cats over five years old.

There is a definite relationship between the age of the cat and its susceptibility to *toxocariasis* - up to 6 months of age, it is rarely registered, and as the age of the cat increases, an increase in an infestation is observed.

Infection of uncinariasis and dipylidiasis was recorded in animals aged one year, and the mixed infestation was recorded only in young cats under 3 years of age.

There is no significant difference in the degree of contamination of cats with intestinal worms in different districts of the Western Urals city.

A slightly higher degree of infection was observed in the village of Zachagan; the extent of infestation was 42%. After that, the central market is in "Mirlan" district (IE - 37.8%), "Myasokombinat" (IE - 35.3%), Selektsionny (IE - 34.4%), 6th sub-district (IE - 32.8 %) and in the territory of Orda and Gagarin streets (IE - 31.5%).

Discussion

In the city of Uralsk, a wide spread of helminthiasis, the most common in cats, was revealed, with the calculation of their invasive extensiveness, the percentage structure of which is reflected in our scientific study of IE – 36.1% of helminths occurring in cats.

The degrees of helminth infection in cats imported to different micro districts in the West Kazakhstan region, Uralsk, were revealed. Including in the village of Zashagan, the Central market "Mirlan", and the Meat-processing areas, helminths had a high degree of IE.

Accordingly, regarding the prevalence of helminthiasis, *Toxocara mystax* ranks first, accounting for 19.7%, and the causative agent *Toxascaris leonina* ranks second, accounting for 12.4%. And *Uncinaria stenocephala* – 1.8% - and *Dipilidium caninum*-1%. It was also found that they occur in mixed invasions: *Toxocara mystax* and *Uncinaria stenocephala* – 1.3%.

Also, the seasonal dynamics of infection of cats with helminths have been relatively calculated for the city of Uralsk. While the percentage of summer and autumn seasonal invasion increases,

this indicator decreases in the winter and spring seasons.

Depending on the age, percentage degrees of invasion expansion was detected in cats aged 1-6 months to 3-5 years.

Conclusion

It has been established that helminthiasis of cats is widespread in the city of Uralsk. (IE - 36.1%). *Toxocarosis (the causative agent of Toxocara mystax)* is in first place in terms of infection with intestinal worms - 19.7%. *Toxascariosis (the causative agent of which is Toxascaris leonina)* is in the 2nd place in terms of distribution - 12.4%. Accordingly, uncinariasis (causing agent *Uncinaria stenocephala*) – 1.8% and dipilidiosis (causing agent *Dipilidium caninum* – 1%) are found in the following places. At the same time, a mixed infestation of *Toxocara mystax* and *Uncinaria stenocephala* was detected - 1.3%.

It was found that there is no significant difference in the degree of contamination of cats caught for intestinal worms in different areas of the city. According to the degree of contamination, the extent of infestation in the territory of Zachagan district is 42%. Central Market «Mirlan» (IE - 37.8%), «Myasokombinat» district (IE - 35.3%), «Selektsionny» district (IE - 34.4%), in the 6th sub-district (IE - 32.8%) and Orda and was registered in the territory of Gagarin street (IE - 31.5%).

It has been determined that the seasonal dynamics of infection in cats depending on the time of year. In summer and autumn, the amount of infestation increased by 43.9-50.5%, and in winter-spring, it significantly decreased by 23.5-26.3%.

It was found that the high degree of contamination is more common in 1-6-month-old cows, making up 46.0%. And in cats from 7 to 12 months, the extent of the infestation is 44.0%; from 1 to 3 years - 34.0%; from 3 to 5 years - 26.8%. In cats older than five years, the infection rate was 19.0%.

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Аннотация

Частое распространение кишечных гельминтов среди плотоядных животных обнаруживается в ряду заболеваний, представляющих опасность для организма человека. Среди бездомных собак и кошек, а также домашних животных, содержащихся в домашних условиях, показатели заражения кишечными гельминтами показывают высокие результаты.

В научной работе были проведены исследования с целью дифференциации кишечных гельминтов у кошек, завезенных из микрорайонов города Уральска Западно-Казахстанской области, в результатах исследований определена степень распространения кишечных гельминтов среди кошек и их видовой состав, сезонная динамика заражений и уровень возрастного заражения.

По городу Уральск выявлено широкое распространение гельминтозов кошек. (ИЭ-36,1%). По заражению кишечными гельминтами первичный токсокароз (возбудитель *Toxocara mystax*) составляет 19,7%, а по распространению токсаскариоз (возбудитель *Toxascaris leonina*) – 12,4%.

Определена степень экстенсивности инвазии в зависимости от времени года, установлено, что в летнее и осеннее время инвазия достигает высокой степени экстенсивности.

Было обнаружено, что заражение кошек кишечными глистами связано с возрастом. Токсокароз чаще встречается у 6-месячных кошек. А с возрастом заболеваемость данным гельминтным заболеванием значительно снижается, у кошек старше 5 лет это заболевание встречается очень редко.

Ключевые слова: распространенность инвазивных заболеваний; кошки; гельминты животных; плотоядные животные; гельминтоз; инвазия; токсокароз.

Түйін

Етқоректі жануарлар арасында ішек құрттарының жиі таралуы адам ағзасы үшін қауіп тудыратын аурулар қатарынан табылуда. Иесіз қалған ит-мысықтар, сонымен қатар үй жағдайында ұсталатын үй жануарлары арасында да ішек құрттарымен залалдану көрсеткіштері жоғары нәтижені көрсетуде.

Зерттеу жұмысында Батыс Қазақстан облысы, Орал қаласының шағын аудандарынан ауланып әкелінген мысықтарды ішек құрт ауруларын дифференциациялау мақсатында зерттеулер жүргізіліп, зерттеу нәтижелерінде мысықтар арасында ішек құрттарының таралу дәрежесі, олардың түрлік құрамы, зарарланудың маусымдық динамикасы және жас ерекшеліктеріне байланысты залалдану деңгейі айқындалды.

Орал қаласы бойынша мысықтардың гельминтоздары кеңінен таралғаны анықталып отыр. (ИЭ – 36,1%). Ішек құрттармен зарарлануы бойынша бастапқы кезекте токсокароз (қоздырғышы *Toxocara mystax*) – 19,7%-ды құраса, ал таралуы бойынша токсаскариоз (қоздырғышы T*oxascaris leonina*) – 12,4% көрсетіп отыр.

Инвазияның экстенсивтілігінің жыл мезгіліне байланыстылығы айқындалып, жазғы және күзгі уақыттарда инвазия экстенсивтілігі жоғары дәрежеге дейін жететіндігі анықталды.

Мысықтардың ішек құрттарымен зарарлануы жасына байланысты екені анықталды. Токсокароз 6 айлық мысықтарда жиі кездеседі. Ал жасының ұлғаюына байланысты аталған құрт ауруымен зарарлану айтарлықтай төмендейді, 5 жастан жоғары мысықтарда бұл құрт ауруы өте сирек жағдайда кездесіп отыр.

Кілт сөздер: инвазиялық аурулардың тарауы; мысықтар; жануарлардың гельминттері; ет қоректілер; гельминтоз; инвазия; токсокароз.

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VETERINARY SANITARY EXAMINATION AND HELMINTOSIS COMPARATIVE MONITORING OF FISH OF WATER SOURCES OF KORGALZYN DISTRICT, AKMOLA REGION

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Abstract

This article presents the results of studies on helminthological diseases of carp species living in the rivers Nura, Tengiz, Birtaban, Shalkar, Uyaly, Sholak, Alkasor of the Korgalzhyn region. 226 studied fish samples were subjected to comparative monitoring using helminthological methods.

When examining the fish of the reservoirs of the Korgalzhyn district of the Akmola region, 41.1% of the fish were found unfit for organoleptic indicators.

As a result of laboratory studies, fish samples taken from the Sholak, Alkasor and Uyaly rivers were recognized as unsuitable, while the indicators of the studied fish from the Nura river and lakes Shalkar, Tengiz, Birtaban were within the normal range.

According to the study, 6.4% of diplostomiasis in Lake Shalkar and 28.6% of opisthorchiasis in Lake Alkasor are affected by helminthiases. 16.6% postdiplostomiasis in the lakes Shalkar and Birtaban, 10.5% opisthorchiasis in the Nura River. In Lake Sholak, ligulase was 9.1%.

Key words: Fresh water fish; monitoring; opisthorchosis; helminths; epidemiological situation; zoonosis; Korgalzhyn district.

Basic position and Introduction

Fish meat can be a reservoir of many invasive diseases that threaten humans, such as diphyllobotryosis, opisthorchosis, clonorchosis, and metagonimia. Liverworms of the family Opisthorchiidae are considered causative agents of serious diseases all over the world. Opisthorchids Opisthorchis felineus, O. viverrini, Clonorchis sinensis, and Metorchis bilis infect the liver of mammals, including humans [1]. Therefore, great attention is paid to the quality of the fish, as well as compliance with regulatory legal documents. Trematodes carried by fish are an important cause of diseases in Kazakhstan. The number of cases of opisthorchiasis (infection with parasites of the opisthorchiidae family) in humans reached 2521 reported cases (17 cases per 100,000 population) in 2002 and gradually decreased to 1225 cases in 2011 (7.4 cases per 100,000 population) [2].

Opisthorchiasis is a dangerous parasitic disease of humans and fish-eating mammals, caused by the trematode Opisthorchis felineus (Plathelminthes, Trematoda), also known as the feline or liver worm. Infection with this parasite occurs when eating fish infected with parasites of the

Cyprinidae family (order Cyprinidae). Therefore, when eating slightly salted or undercooked (fried or boiled) fish, the risk of human infection with this pathogenic parasite is very high [3].

The fight against zooanthroponotic helminthiases, which are widespread in the Republic of Kazakhstan, is one of the state tasks in the field of health and veterinary medicine. On the territory of Kazakhstan, there are natural foci of many dangerous parasitic diseases, which requires the veterinary service to take effective measures to combat, and prevent the spread of these diseases to other regions of Kazakhstan [4].

Since parasites show great potential for adapting to environmental changes, it is important to understand that one approach to detecting parasites is not enough. With the development of aquaculture, the amount of fish consumed for human nutrition increases, and therefore the risk of human infection with invasive diseases, especially opisthorchiasis, ligulosis, diphyllobothriasis, etc., increases [5].

In addition, it is known that fish in the early stages of development are most sensitive to the effects of toxic environmental factors, and prone to mass death from infections and invasions when exposed to pollutants [6, 7].

Infection of fish with helminths is typical in spring and summer. Usually, the disease is registered among fry and juvenile fish in spawning ponds. Adult fish are less intensively infected [8].

Nematode parasites of the family Anisakidae infect late hosts, piscivorous birds and mammals, through secondary intermediate hosts such as first intermediate hosts, copepods and fish. However, in people who consume raw or undercooked fish, it can lead to a nematode infection called anisakidosis [9].

The most common are trematodes, cestodosis, and nematodes caused by various parasitic worms. These parasites are found in fish in natural water bodies, as well as in ponds and spawning grounds. The most common microorganisms in fish and fish products [10].

The most dangerous helminthiasis among trematodes is opisthorchiasis. Opisthorchiasis is a biogelmintic caused by Opisthorchis felineus, a trematode belonging to the Opisthorchidae family. Infection of humans, cats, dogs, foxes, Arctic foxes, pigs occurs when eating fish of the carp family infected with opisthorchiasis larvae [11].

Material and methods

Fish sampling was carried out on the rivers Nura, Teniz, Birtaban, Shalkar, Uyaly, Sholak, and Alkasor of the Korgalzhyn region. A total of 226 specimens of fish were examined for helminthological diseases (carp, perch, silver carp, pike perch, carp, pike, and tortas).

The research work was carried out in the in May-July NGO "Fishery", in the city of Nur-Sultan, the Kazakh Agricultural Technical University named after Saken Seifullin, the Faculty of Veterinary Medicine and Livestock Technology, the Department of Hunting and Fisheries and the laboratory of the Department "Veterinary Sanitation" and the "National Reference Center veterinary medicine" in Nur-Sultan.

The study of diseases such as ligulosis and opisthorchiasis was carried out by cutting and dissecting the obtained material, in which the presence of tongues and other helminths in the abdominal cavity of fish was studied, studied by placing them in compression glass [12].

The following laboratory research methods were used:

- to detect eggs of nematodes and cestodes - using the Fulleborn method [13], the Kotelnikov-Khrenov method [14], and other flotation methods in various modifications;

- detection of trematode eggs - by sedimentation with sequential washing.

To quantify the degree of damage to fish by parasites, we used such indicators as the intensity and extensiveness of infection, as well as the index of abundance of parasites.

Laboratory research: Bacterioscopy. Determination of the concentration of hydrogen ions (pH) . Qualitative reaction to hydrogen sulfide. Determination of the content of aminoammonium nitrogen. Reaction with copper sulfate in broth, reaction with peroxidase. Reaction to gaseous ammonia (according to Eber) . Determination of ammonia with Nessler's reagent[15].

Results

Table 1 - Organoleptic indicators of fish

Reservoir	Indicators					
	Mucus	Scales	Eye	Mouth	Gills	Consistency
River Nura n=34	Abundant, transparent, odorless	Smooth, shiny, hard to get	Convex, clean, transparent cornea	Closed	The color is bright red, the mucus is viscous and transparent	Tight; fish does not bend; meat is difficult to separate from bones
Lake Teniz n=21	Transparent, odorless	Shiny, hard to extract	Protrudin, clean	Closed	Bright red, clear mucus	The fish bends slightly, the meat barely separates from the bones
Lake Birtaban n=36	Fluffy, natural fishy smell	Shiny, snugly fitting to the body	Protrudin, transparent	Semi - open	Bright red, cream transparent	Meat is difficult to separate from bones
Lake Shalkar n=42	Abundant, transparent, odorless	Smooth, shiny, hard to get	Convex, clean, transparent cornea	Closed	The color is bright red, the mucus is viscous and transparent	Elastic, the fish bends slightly, the meat barely separates from the bones
River Uyaly n=32	Cloudy, gray color	Easily torn	Down, transparent	Half - open	Bright red, creamy, cloudy	The fish bends slightly, the meat barely separates from the bones
Lake Alkasor n=7	Transparent, odorless	Shiny, hard to extract	Convex, clean	Closed	The color is bright red, the mucus is transparent	Tight; fish does not bend; meat is difficult to separate from bones
Lake Sholak n=54	Slightly cloudy, natural fishy smell	Shiny, stretches easily	Clean, slightly lowered, transparent cornea	Half - open	The color is dark red, the mucus is viscous, indistinct	Elastic, the fish bends slightly, the meat barely separates from the bones

As can be seen from Table 1, the indicators of fish taken from the rivers Nura and Shalkar, Teniz and Birtaban lakes were fresh. Mechanically intact, gills are red, eyes are transparent, intact, and the smell is characteristic of live fish. The scales are shiny, close to the skin, the skin is spotless and close to the muscles. The fins are rigid, the abdominal cavity is not swollen, and the internal organs are easily separated.

The organoleptic characteristics of fish from the Sholak and Alkasor lakes and the Uyaly river were questionable. Fish from Lake Shortan had cloudy mucus, scales were easily removed. The gills were dark red in color, the mucus was viscous and cloudy, and the gill covers were not tightly closed.

Table 2 - Physical and chemical indicators of fish

Reservoir	Indicators				
	Bacterioscopy	pH	Eber ammonia reaction	Hydrogen sulfide reaction	Peroxidase reaction
River Nura n=34	The smear is poorly colored, absent in deep layers, on the surface of up to 10 microbial bodies	6,8±0,02	negative	negative	Filtered blue is colored green

Lake Teniz n=21	There are no microbial bodies in the deep layers, up to 15 in the surface	6,6±0,05	negative	negative	Filtered blue is colored green
Lake Birtaban n=36	There are no deep layers, and up to 12 microbial bodies in the surface	6,6±0,08	negative	negative	Filtered blue is colored green
Lake Shalkar n=42	The smear is poorly colored, absent in deep layers, on the surface of up to 10 microbial bodies	6,7±0,04	negative	negative	Filtered blue is colored green
River Uyaly n=32	Up to 20 m/d in deep layers, up to 35 microbial bodies in the surface layer	6,8±0,04	Weak positive	positive	The filter remained unchanged; the reaction is negative
Lake Alkasor n=7	Up to 25 m/d in deep layers, up to 40 microbial bodies in the surface layer	6,9±0,09	Weak positive	positive	The filter remained unchanged; the reaction is negative
Lake Sholak n=54	The smear is well colored, in deep layers up to 9 microbial bodies, on the surface up to 20 microbial bodies	7,1±0,05	Weak positive	positive	The filter remained unchanged; the reaction is negative

As can be seen from Table 2, biochemical parameters of fish examined from Nura river and Shalkar, Teniz and Birtaban lakes were within the normal range. Thus, smears were poorly stained, no microbial bodies were found in deep layers, up to 15 microbial bodies in surface layers, pH was normal, peroxidase reaction was positive, ammonia and hydrogen sulfide were negative.

At bacterioscopy of smears from marks in samples of fish from lakes Sholak, Alkasor and river Uyaly smears were well stained, up to 25 microbial bodies in deep layers, up to 40 microbial bodies in surface layers, pH index was doubtful, reaction on peroxidase was negative, filtered blue-green color was not stained. The reaction of ammonia was weakly positive-forming a quickly disappearing cloud, in hydrogen sulfide-positive, a small brown spot appeared on the paper (Table 2).

Table 3 - Infection of fish with helminthiases in reservoirs of the Korgalzhyn region

Reservoir	Type of fish	Number of fish		Type of parasite	Extensiveness, %	Intensity, экз.
		Researched	Invasive			
Nura River	Carp (*Carassius*)	7	Not detected	-	-	-
	Walleye (*Sander lucioperca*)	8	Not detected	-	-	-
	Taban (*Abramis brama*)	19	2	*Metacercariae of the family Opistorchidae*	10,5	1-2
Sea Lake	Torta (*Rutilus rutilus*)	10	Not detected	-	-	-
	Pike (*Esox lucius*)	11	Not detected	-	-	-

Birtaban Lake	Silver carp (*Carassius gibelio*)	6	Not detected	-	-	-
	Pike perch (*Sander lucioperca*)	24	4	*Posthodiplostomum cuticola*	16,6	1-2
	Perch (*Percafluviatilis*)	6	Not detected	-	-	-
Lake Shalkar	Torta (*Rutilus rutilus*)	5	Not detected	-	-	-
	Perch (*Percaflu viatilis*)	31	2	*Diplostomum spathaceum*	6,4	1-2
	Pike (*Esox lucius*)	6	Not detected	-		
Uyaly river	Carassius gibelio (*Carassius gibelio*)	32	4	*Posthodiplostomum cuticola*	12,5	1-2
Lake Alkasor	Torta (*Rutilus rutilus*)	7	2	*Metacercariae of the family Opistorchidae*	28,6	1-2
Lake Sholak	Perch (*Percafluviatilis*)	7	Not detected	-		
	Carassius gibelio (*Carassius gibelio*)	16	2	*Posthodiplostomum cuticola*	12,5	1-2
	Pike (*Esox lucius*)	9	Not detected	-		
	Cyprinus	22	2	*Ligutidae*	9,1	1-2

Fish in Shalkar Lake and Sholak Lake are infected with helminths, and the extensiveness of *Posthodiplostomum cuticola* is 12.5, and the intensity is 1-2. Single eggs of P*osthodiplostomum cuticola* with the extensiveness of 12.5, intensity 1-2 (figure–1). The mechanism of transmission and life cycle of the helminth *Posthodiplostomum cuticola* is not fully understood. Helminth eggs must mature in freshwater before they are ingested by fish, and the eggs have been shown to be larval-free, in which case the fish can become an accidental intermediate host without further infection of the definitive hosts (fish-eating birds).

Figure 1 - Fish infected with Posthodiplostomum cuticola

When studying perch in Lake Shalkar, cercariae diplostomum cercariae were found in the eye with an extensiveness of 8.3% and an intensity of 28-32 points. 10.5% are infected with metacercariae of trematodes of the family Opistorchidae of the flounder of the Nura River with an infection rate of 1-2 specimens. Diplostomes are common diseases of pond fish in Kazakhstan. Most natural reservoirs are main centers of diplostomic invasion. The main carriers of pathogens of diplostomiasis are fish-eating birds (gulls, terns, mergansers), bringing an invasive source into fishery ponds. In our studies, the percentage of infection was negligible, 8.3%, but the intensity of invasion was high.

According to the research results, metacercariae of trematodes of the diplostomum family were more common in the eyes of fish, Posthodiplostomum cuticola in the skin and subcutaneous tissue, and plerocercariae of the Ligutidae family – in the abdominal cavity.

In order to study compliance with safety requirements for human health in terms of parasitism, the selection and volume of fish are carried out in accordance with the following requirements:

► Guidelines 3.2.1756-03 “Prevention of parasitic diseases. Epidemiological control of parasitic diseases”.

► State standard 7631-2008 “Fish, fish products and products from them. Method for determining organoleptic and physical indicators.

► State standard 31339-2006 “Fish, fish products and products from them. Acceptance Rules and Sampling Methods"

Discussions

Organoleptic indicators of all fish samples studied were at a normal level. During the study of samples, invasive diseases were identified in fish of lakes of Korgalzhyn district, i.e. opisthorchiasis and diphyllobotriosis.

It is known that these types of invasive diseases are dangerous for the human body. Therefore, fish caught in these lakes will definitely cause diseases in humans if they enter the human body without passing a veterinary sanitary examination. Given the fact that in some cases the population catches fish directly from these lakes and uses carp as food, the fish of these lakes is unambiguously dangerous to the health of the population. It turned out that fish affected by invasive diseases have low sensory and biochemical indicators.

Taking into account these points, it is necessary to carry out a strict veterinary and sanitary examination of fish taken from lakes in these regions. It is necessary to conduct propaganda work on the fact that it is dangerous for the population to use food sequence without processing unexplored fish.

The scales are shiny, the eyes are soft, the mucus is clean, with a characteristic smell. The gill covers are located close to each other, and the color of the gills is from red to dark red, in some specimens there are remnants of silt and debris under the gill covers. The internal organs are not damaged, they are clearly visible, and the abdomen does not swell. Physical and chemical parameters were within normal limits.

Conclusion

Microscopic examination of surface smears revealed only cocci and bacilli; no microbes were found in the deeper layers of the smears. The pH of all samples was constant from 6.61 ± 0.001 to 6.78 ± 0.001. When peroxidase was detected, the gill filtrate turned blue-green and brown, which indicates the good quality of the studied samples. When determining ammonia with Nessler's reagent, the extracts obtained from the samples acquired a greenish-yellow color and remained transparent.

According to research data, 6.4% of diplostomiasis in Lake Shalkar and 28.6% of opisthorchiasis in Lake Alkasor are affected by helminthiases. In lakes Shalkar and Birtaban, postdiplostomiasis accounts for 16.6%, opisthorchiasis - 10.5% in the Nura River. In Lake Sholak, ligulase was 9.1%.

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Аннотация

В данной статье представлены результаты исследований по гельминтологическим заболеваниям карповых видов, обитающих в реках Нура, Тенгиз, Биртабан, Шалкар, Уялы, Шолак, Алкасор Коргалжынского района. Сравнительному мониторингу с использованием гельминтологических методов подвергли 226 исследованных проб рыб.

При осмотре рыб водоемов Коргалжынского района Акмолинской области по органолептическим показателям 41,1% рыб были признаны негодными.

Пробы рыбы, отобранные из озер Шолак, Алкасор и реки Уялы, в результате лабораторных исследований признаны негодными, в то время как показатели изученной рыбы из реки Нуры и озер Шалкар, Тенгиз, Биртабан находились в пределах нормы.

По данным исследования, гельминтозами поражено 6,4% диплостомозов в озере Шалкар и 28,6% описторхозов в озере Алкасор. 16,6% постдиплостомоз в озерах Шалкар и Биртабан, 10,5% описторхоз в реке Нура. В озере Шолак лигулаза составила 9,1%.

Ключевые слова: пресноводная рыба; мониторинг; описторхоз; гельминты; эпидемиологическая ситуация; зооноз; Коргалжынский район.

Түйін

Бұл мақалада Қорғалжын ауданына қарасты Нұра, Теңіз, Біртабан, Шалқар, Ұялы, Шолақ, Алқасор өзен-көлдерінде мекендейтін тұқы тектес балықтардың гельминтологиялық ауруларына жүргізілген зерттеу нәтижелері келтірілген. Жалпы зерттелген 226 балық сынамасына салыстырмалы мониторинг жұмыстары жасалып, гельминтологиялық әдістер қолданылды.

Ақмола облысы Қорғалжын ауданы су көздері балықтарын сезімдік көрсеткіштері бойынша зерттегенде балықтардың 41,1% жарамсыз деп танылды.

Шолақ, Алқасор көлдерінен және Ұялы өзенінен алынған балық сынамалары зертханалық зерттеу нәтижесінде жарамсыз деп танылып, ал Нұра өзені және Шалқар, Теңіз, Біртабан көлдерінен зерттелген балықтардың көрсеткіштері қалыпты шектерде болды.

Зерттеу бойынша балықтардың гельминтоздарға шалдығуы Шалқар көлінде диплостомоз ЭИ 6,4%, Алқасор көлінде описторхоз 28,6%. Шалқар және Біртабан көлінде постдиплостомоз 16,6%, Нұра өзенінде описторхоз 10,5%. Шолақ көлінде лигулез 9,1%-ды құрады.

Кілт сөздер: тұщы су балығы; мониторинг; описторхоз; гельминттер; эпидемиологиялық жағдай; зооноз; Қорғалжын ауданы.

Dear author!

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

Establishment

In accordance with the order of the Minister of Education and Science of the Republic of Kazakhstan No. 170 dated April 30, 2020, the editors of the journal "Bulletin of Science of S. Seifullin Kazakh Agrotechnical University -Veterinary Sciences" developed a website with an online system for submitting and reviewing articles.

In this regard, when submitting an article for publication in a journal, it is necessary to register as an author on the journal's website and upload the article proposed for consideration on the online platform. Author registration is carried out at the following link: (video instruction is attached) https://bulletinofscience.kazatu.edu.kz/index.php/veterinary-science

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

The editors of the journal ask the authors to familiarize themselves with the rules and adhere to them when preparing papers sent to the journal.

New. The author's material, reflecting the results of scientific research, should contribute to the development of the field of veterinary sciences, within which this material is prepared.

Logical presentation. The text of the manuscript should be structured in such a way that readers understand the purpose and objectives, the theory and methodology of the research, the results obtained by the author, and the author's contribution to the development of a particular area of scientific knowledge.

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Further below, the text of the annotation is placed in a line. The volume of the abstract is at least 100 words. In the article, the Abstract is compiled **in English, Kazakh, Russian** (the placement of the abstract depends on the original language).

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Discussion. Discussion and interpretation of the results, including in the context of previous studies.

• A brief description of the most significant findings that were identified in the Results section and their comparison with other studies on illustrative topics,

• Identification of problem areas, lack of some aspects;

• Future research directions.

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3 Krasnova, T.V. Ancient Russian toponymy of the Yelets land [Text]: monograph. - Yelets: Publishing House of Yelets State University, 2004. - 157)

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

Articles in journals (electronic format)

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

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

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Tables are placed according to the text. The tables are numbered in the order of references in the text. The numbering heading of the table is typed in a non-bold font with left alignment (for example, Table 1). The subject heading (if available) is placed on the same line in a non-bold font with a left alignment. The reference to the table in the main text is made in a non-bold font in brackets - for example, (Table 1). If the table has a large volume, it can be placed on a separate page, and in the case when it has a significant width on a page with landscape orientation.

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SAMPLE DESIGN OF THE ARTICLE

UDC (ӘОЖ), (УДК) 577.2:577.29

IDENTIFICATION OF WHEAT GENES THAT CAUSE RESISTANCE TO PATHOGENIC FUNGI

***Ivanov Ivan Ivanovich*[1]**
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Associate Professor,
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E-mail: tech@mail.ru

Abstract: The author of the article based on the actual research proves that the presence of wheat resistance genes to pathogenic fungi is a key factor for use in breeding work. The article presents the results of the identification of wheat genes Sr32, Bt9, and Bt10 responsible for drought resistance to pathogenic fungi that cause diseases of stem rust, as well as hard smut ... [at least 100 words].

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

The main text of the article should contain the following structural elements:
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- Materials and methods;
- Results;
- Discussion;
- Conclusion;
- Information about financing (if available);
- List of references according to AUSS 7.1-2003 SSILP;
- References, according to API standards.

***Then is followed by annotations in two languages**

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