### **REPUBLIC OF AZERBAIJAN**

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### ABSTRACT

of the dissertation for the degree of Doctor of Philosophy

### BIOECOLOGICAL CHARACTERISTICS AND CHEMICAL PREVENTIVE MEASURES AGAINST PRIMITIVE INTESTINAL PARASITES (*EIMERIA*, *CRYPTOSPORIDIUM*) IN SHEEP IN THE LANKARAN-ASTARA ECONOMIC REGION

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### **INTRODUCTION**

**Relevance and degree of the completion of the topic.** Reforms in agriculture have been reflected in the development of animal husbandry, including sheep farming. The agricultural sector is one of the main sectors of the economy of our republic. It is necessary to study the spread areal of eymeriosis and cryptosporidiosis agents, which cause economic damage to animal husbandry, including sheep farming, which is the basis of this sector.

Therefore, it is important to study the spread of eymeriosis and cryptosporidiosis agents in the territory of the Lankaran-Astara economic region depending on species composition, age, season, and vertical zones, to study species belonging to the genera Eimeria and Cryptosporidium parasitizing together (associatively), and develop desinvasion measures against Eimeria agents.

Since the beginning of the 20th century, the species composition of coccidia parasitizing wild animals, domestic animals, and poultry, their distribution in different economic regions of Azerbaijan, as well as the ability of different species belonging to the genera Eimeria to live in the environment has been studied.<sup>1,2</sup>

Many researchers believe that cryptosporidium has a wide specificity within the sex limit of the host animal.<sup>3</sup>

The degree of infection of sheep with species belonging to the genera eimeria in the mountain, foothills, and plain zones was found

<sup>&</sup>lt;sup>1</sup>Musayev M.M., Yolçiyev Y.Y., Manafova Ş.H. Qərbi Azərbaycanda qaramalda parazitlik edən eymeriyaların növ tərkibi və bəzi ekoloji xüsusiyyətləri//Azərbaycan EA Xəbərləri, biologiya elmləri seriyası, – 1993, № 4-6, – s. 52-56

<sup>&</sup>lt;sup>2</sup>Məmmədov, İ.B. Naxçıvan Muxtar Respublikasında keçilərdə eymeriyaların yayılması və növ tərkibi // Kənd təsərrüfatının inkişafı: reallıqlar və perspektivlər, Beynəlxalq elmi–praktik konfrans, –Naxçıvan: Qeyrət, – 15 – 16 may, – 2015, – s. 24 – 28

<sup>&</sup>lt;sup>3</sup>Musayev, M.Ə., Qayibova, H.D. İnsanda superinfeksiya törədən koksidilər (Sporozoa, Apicomplexa) // Azərbaycan Zooloqlar Cəmiyyətinin I Qurultayının Materialları,-Bakı:Adiloğlu, -sentyabr, - 2003, - s. 22 - 26

to be different depending on the vertical zones in the eastern regions of Azerbaijan.<sup>4</sup>

The parasite-host relationship and the pathogenic effect of cryptosporidiosis agents on the lamb organism were also studied during experimental cryptosporidiosis of lambs.<sup>5,6</sup>

Taking into account the above, the goal was set to identify of species belonging to the genera eimeria and cryptosporidium parasitizing sheep in the Lankaran-Astara economic region of Azerbaijan (Masalli, Lankaran, Astara, Lerik districts), to study the distribution of causative agents of eymeriosis and cryptosporidiosis depending on age, season, vertical zones, to study associative invasions, to perform laboratory testing of desinvasion agents against eymeriosis agents (due to the high extensity and intensity) and their application in farms.

**Object and subject of the study.** The objects of the research were parasitizing species belonging to the genera Eimeria and Cryptosporidium sheep in private and farmer livestock (sheep) farms in the Lankaran-Astara economic region (Masalli, Lankaran, Astara, and Lerik districts). The subject of the research was the identification of Eimeria and Cryptosporidium in sheep in those regions, the study of the infection by age groups, seasons, vertical zones the

<sup>&</sup>lt;sup>4</sup>Musayev, M.Ə., Yolçiyev, Y.Y., Məmmədova, M.Ə. Azərbaycanın şərq rayonlarında zonalardan asılı olaraq qoyunların eymeriyalarla yoluxması // XX əsrin sonunda heyvanlar aləminin öyrənilməsi və qorunması. Akademik M.Ə. Musayevin anadan olmasının 80 illiyinə həsr olunmuş elmi konfransın materialları, - Bakı: Elm, - 2001, - səh. 53 - 56.

<sup>&</sup>lt;sup>5</sup>Ятусевич, А. И., Старовойтова, М.В. Паразито – хозяинные отношения при экспериментальном криптоспоридиозе ягнят // – Витебск: Ученые записки учреждения образования «Витебская ордена «Знак Почета» государственная академия ветеринарной медицины», – 2019. т. 55, вып. 2, – с. 88 – 92.

<sup>&</sup>lt;sup>6</sup>Ятусевич, А.И., Старовойтова, М.В. О патогенном влиянии криптоспоридий на организм ягнят // Международная научно – практическая конференция «Тенденции развития ветеринарной паразитологии на пространстве СНГ и других стран в начале XXI века», – Самарканд, [и.н.] – 28 – 30 апреля, – 2021, – с. 42 – 43.

clarification of the dynamics of associative infection, and carrying out desinvasion measures against the causative agents of eymeriosis.

**Purpose and tasks of the research.** In the Lankaran-Astara economic region, the identification of the species composition of primitive intestinal parasites (*Eimeria* and *Cryptosporidium*) of sheep, the study of the dependence of the extensity and intensity of the spread of parasites on age, season, and vertical zones, the associative parasitism of Eimeria and Cryptosporidium species, effective control (desinvasion) against the causative agents of eimeria were set as a goal of the research.

To achieve this goal, the following tasks were set:

- Identification of the species belonging to the genera Eimeria and Cryptosporidium parasitizing sheep;

- To determine the age and season dependence of the distribution dynamics of primitive intestinal parasites (*Eimeria* and *Cryptosporidium*) of sheep in the Lankaran-Astara economic region;

- To conduct a comparative analysis of the distribution dynamics of species belonging to the genera Eimeria and Cryptosporidium species in vertical zones of the region;

- To study associative parasitism of the species belonging to the genera Eimeria and Cryptosporidium

To test preventive and combat measures against the causative agents of Eymeriosis - desinvasion substances under laboratory and farm conditions, to estimate the economic efficiency, and to develop a recommendation based on the obtained results.

**Research methods.** In the course of the research, to determine infection with eimeria by the Darling-Fülleborn method and to determine infection with cryptosporidium oocysts, thin smears were prepared, stained with carbol fuchsin according to the Tsil-Nilson method. The amount of oocysts in 1 gram of feces was determined by Stoll's method.<sup>7,8,9,10</sup>

<sup>&</sup>lt;sup>7</sup>Крылов, М.В. Определитель паразитических простейших / М.В.Крылов. – Санкт – Петербург: Наука, – 1996. – 602 с.

Species belonging to the genera Eimeria and Cryptosporidium were identified, the dependence of their spread on vertical zones was determined and desinvasion agents against the causative agents of Eimeria were tested under laboratory and farm conditions.

### Main points presented to the defense of the dissertation:

- 1. The species composition of Eimeria and Cryptosporidium parasitizing sheep was identified.
- 2. The age and seasonal dynamics of eymeriosis and cryptosporidiosis of sheep were studied for different age groups.
- 3. The dependence of the distribution of species belonging to the genera Eimeria and Cryptosporidium on vertical zones (in plain, foothills, and mountainous zones) was studied.
- 4. Eymeriosis and Cryptosporidiosis were found to appear in a mixed form.
- 5. The desinvasion substances against the causative agents of Eymeriosis were tested, the economic efficiency was estimated and a recommendation was made based on the results.

**The scientific novelty of the research.** For the first time in Azerbaijan, primitive intestinal parasites of sheep (*Eimeria* and *Cryptosporidium*) were studied in the Lankaran-Astara economic region and the following scientific innovations were obtained as a result of the research:

Species belonging to the genera Eimeria and Cryptosporidium of sheep were identified for the first time;

For the first time, the age and seasonal dependence of the spread of the causative agents of sheep eymeriosis and

<sup>&</sup>lt;sup>8</sup>Никитин, В.Ф. Копроскопическая диагностика криптоспоридиоза и эймериоза телят // – Москва: Ветеринария, – 2002. № 9, – с. 27 – 31.

<sup>&</sup>lt;sup>9</sup>Сафиуллин, Р.Т. Удобрения органические. Методы паразитологического анализа. Методы определения ооцист и цист простейших/Р.Т.Сафиуллин. – Москва: Стандартинформ, – 2020. – 14 с.

<sup>&</sup>lt;sup>10</sup> Якубовский, М.В. Диагностика, терапия и профилактика паразитарных болезней животных / М.В. Якубовский, Н.Ф.Карасев; – Минск: Хата, – 2001. – 375 с.

cryptosporidiosis in the territory of the Lankaran-Astara economic region was studied on a scientific basis;

For the first time, the dependence of the spread of species belonging to the genera Eimeria and Cryptosporidiumon vertical zones was determined in parasitizing sheep in the Lankaran-Astara economic region;

Associative parasitizing of species belonging to the genera Eimeria and Cryptosporidium in sheep was studied for the first time;

For the first time, desinvasion substances against the causative agents of sheep eymeriosis were tested on a scientific basis, and when 8.0% sodium hydroxide was applied as a desinvasion substance against the causative agents of sheep eymeriosis, 37.23 AZN economic efficiency was obtained per sheep. Following the results, a recommendation called "On the prevention of eymeriosis of small horned animals" was made;

Theoretical and practical significance of the research. Complex protozoological studies were conducted for the first time in the Lankaran-Astara economic region of Azerbaijan. From the point of view of food safety, research carried out on increasing productivity and improving its quality in individual and private farms such as the identification of the species belonging to the genera Eimeria and Cryptosporidium, study of the infection dynamics of primitive intestinal parasites of sheep (*Eimeria* and *Cryptosporidium*) depending on the age of the host, seasons of the year, the dependence of invasions on bio-ecological characteristics, associative invasions, identification of Eimeria and Cryptosporidium species are of great importance. Preventive fighting (desinvasion) measures were developed on a scientific basis against the eymeriosis of small horned animals, including sheep. On the basis of certain provisions of the results of the research, a recommendation called "On the prevention of eymeriosis of small horned animals" was made. In the recommendation, measures to fight (desinvasion) against the causative agents of eymeriosis, which cause economic damage to sheep farming and are widespread in farms, are mentioned. Thus, based on the results obtained during the resolution of the issues

raised in conducting the research, the 8.0% solution of sodium hydroxide will be used in the farms for the purpose of desinvasion against the causative agents of sheep eymeriosis. The results of the research can be used as a tutorial for relevant specialties of higher and secondary schools, as literature in scientific works related to protozoological research of sheep.

**Approbation and application.** The obtained results and publications form the basis of the research conducted in 2018-2022. The results of the research were presented and discussed in the annual reports of the Parasitology Department of the Scientific Research Veterinary Institute and the doctoral students, as well as at the national and international (local and foreign) scientific-practical conferences.

The topic "Dynamics of infection of sheep with eimeria in Azerbaijan" was presented at the scientific-practical conference "Actual problems and innovative solutions in AIC" held in Makhachkala, Dagestan, in 2018, and the material was published. The topic "Age-dependent dynamics of sheep infection with eimeria in different villages of the Lerik region" was presented at the Republican scientific conference "Modern Problems of Biology" held in Sumgayit, in 2018, and the material was published.

The topic "About the Cryptosporidium parasite" was presented at the "XI International Conference of European Academy of Sciences and Research" held in Bonn, Germany, in 2019 and the material was published in the Proceedings of the conference. The topic "Age-dependent dynamics of Eimeria infection in sheep" was presented at the Republican Scientific Conference "Development Directions of the Agricultural Sector" held in Nakhchivan, in 2019, and the material was published. The topic "Dynamics of infection of sheep with Eimeria in the Lankaran region" was presented at the international scientific-practical conference "Concepts of sustainable development of science under modern conditions" held in Penza, Russia, in 2019, and the material was published.

The topic "Species composition and distribution dynamics of Eimeria parasitizing sheep in the southern region of Azerbaijan" was presented at the international scientific-practical conference entitled "Veterinary science in the 21st century - innovations towards future" dedicated to the 120th anniversary of the establishment of the Scientific Research Veterinary Institute held in Baku in 2021 and the material was published.

The topic " Identification of Eimeria and Cryptosporidium oocysts of sheep in the Lankaran-Astara economic region of Azerbaijan" was presented at the international scientific conference entitled "Theoretical Hypotheses and Empirical results" held in Oslo, Norway in 2023, and the material was published. The topic "Mono and associative infection of lambs with eimeriosis and cryptosporidiosis" was presented at the international scientific conference "Scientific results" held in Rome, Italy in 2023, and the material was published.

Based on the content of the dissertation work, 7 scientific articles (in the base of the third international index) and 8 conference materials were published. One recommendation was made by the decision of the Scientific Council of the Veterinary Scientific-Research Institute based on the results of the conducted research.

The organization where the dissertation work was performed. The research work was during the years 2018 – 2022 carried out in the laboratory of the Parasitology Department of the Scientific-Research Veterinary Institute of the Ministry of Agriculture of the Republic of Azerbaijan.

The total volume of the dissertation and separate structural sections in characters. The dissertation work is presented in 168 computer typing pages including tables, figures, and graphs, the volume consists of 211.593 characters. The dissertation consists of an introduction (11,114 characters), 6 chapters (176.892 characters), discussion of the study (19,763 characters), results (3272 characters), practical recommendations (of 552 characters) and a list of used literature. 172 literature resources were used, including 33 in Azerbaijani, 4 in Turkish, 100 in Russian, and 35 in English. The dissertation includes 10 figures, 53 tables, and 3 graphs.

### CHAPTER I LITERATURE REVIEW

In this chapter, the study of intestinal parasites of vertebrates in Azerbaijan and foreign countries, the structure, systematic classification, development cycle of the species belonging to the genus Eimeria and Cryptosporidium, physical and geographical characteristics of the regions where the research was carried out, information about the sheep genera kept on farms in the Lankaran-Astara economic region (Masalli, Lankaran, Astara, Lerik districts) are presented.

### CHAPTER II MATERIALS AND METHODS

Studies were conducted at the Parasitology Department of the Scientific-Research Veterinary Institute, on the materials - fecal samples collected from individual sheep farms of Masalli, Lankaran, Astara, and Lerik districts of the Lankaran-Astara economic region in 2018-2022. In total, 7301 animals (lambs, goats and sheep) were involved in the research. 5225 coprological examinations were conducted for the study of the causative agents of eimeriosis, and 4150 for the study of the causative agent of cryptosporidiosis.

The Darling-Fülleborn method was used to determine Eimeria infection in the samples collected during the research. To identify Cryptosporidium oocysts, thin smears were prepared from fecal samples and stained with carbol fuchsin according to the Tsil-Nilson method. The amount of oocysts in 1 gram of feces was determined by Stoll's method. BELL Solaris microscope, HD-CAM camera and Image Scope software to measure and photograph oocysts for identification of Eimeria and Cryptosporidium species, Motic No. 61105283 Binocular microscope to determine infection with eimeria and cryptosporidiosis agents, eimeria TRS-3756 thermostat was used for sporulation of oocysts. Species are assigned based on the morphological structures of the parasites. 50 specimens of each of the species belonging to the Eimeria genus, 30 and 50 specimens of

each of the species belonging to the Cryptosporidium genus were measured and the obtained results were processed statistically in the MS Exel 2016 program. Determining literatures were used in determining the species.<sup>7,11,12</sup>

In order to study the effect of desinvasion agents on hydroxide and formalin (according to formaldehyde, the main active substance in the composition) 2.0%, 4.0%, 6.0%, 8.0%, 10.0%, and 5.0% solutions of phenol (carbolic acid) were used for the comparison, and ordinary water was used as a control. Scales RADWAG Elektronic, Model: AS220.R2 were used to prepare different percentage solutions of sodium hydroxide. In order to continue the experiments, wood, and concrete with an area of  $10 \text{ cm}^2$ were taken as test objects under laboratory conditions. After determining the effect of desinvasion solutions of different concentrations on sporulated and unsporulated Eimeria oocysts under laboratory conditions, the desinvasion effect of those solutions was studied in an area of  $1m^2$ . An effective desinvasion substance (8.0%) sodium hydroxide) was tested under farm conditions, and based on the positive result obtained, the economic efficiency of the preparations used against the causative agents of sheep eimeria was estimated.

The statistical reliability of the difference between the tests was checked using the Student's t-test. The occurred changes were found to be statistically significant.<sup>13</sup>

<sup>&</sup>lt;sup>11</sup>Гаибова, Г.Д. Криптоспоридии (*Cryptosporidium, Coccidia, Apicomplexa*) животных в Азербайджане и обзор современных подходов к идентификации их видов // – Баку: Изв. НАН Азербайджана. Сер. биол. наук, – 2004. № 3–4, – с. 92 – 108.

<sup>&</sup>lt;sup>12</sup>Гаибова, Г.Д., Искендерова, Н.Г. Криптоспоридии (Cryptosporidiidae, Coccidea, Apicomplexa) домашних жвачных животных и человека в Азербайджане // – Тбилиси: Актуальные проблемы паразитологии в Грузии, – 2014. – с. 110 – 122.

<sup>&</sup>lt;sup>13</sup>Hesablama texnikasının və eksperimentin riyazi nəzəriyyəsinin elmi tədqiqatlarda tətbiqi / T.Ə.Babayev, A.R.Bünyatov, Q.C.Əfəndiyev [və b.]. – Bakı: Elm, – 1999. – 102 s. (s. 40-42)

### CHAPTER III IDENTIFICATION OF EIMERIA AND CRYPTOSPORIDIUM SPECIES PARASITIZING SHEEP IN THE LANKARAN-ASTARA ECONOMIC REGION

The species belonging to the genus Eimeria and Cryptosporidium are widespread in sheep. Infection occurs most often among lambs, and as a result, the basis for the

formation of pathological processes in organs and tissues is created. Identification of *Eymeriosis* and *Cryptosporidiosis* agents parasitizing sheep in individual and private sheep farms in the Lankaran-Astara economic region (Masalli, Lankaran, Astara, Lerik) was one of the issues raised during research. Therefore, it was relevant to determine the sizes and morphological structures of these pathogens.

Eymeriosis and Cryptosporidiosis are common protozoal infection diseases in vertebrates, which occur in small horned animals, including sheep, mainly in lambs, as well as in young animals, and it is accompanied by intestinal disorders (diarrhea). Decreased weight gain in infected animals, prolonged recovery time, and poor development of animals after recovery cause economic damage to sheep farms.

The research conducted in the sheep farms located in the Lankaran-Astara economic region (Masalli, Lankaran, Astara, Lerik districts), revealed 6 species of Eimeria: *Eimeria ahsata, E.faurei, E.arloingi, E.parva, E.inricata, E.ninaekohlyakimovae*.

*E. ahsata* oocysts found in sheep are oval in shape. The sizes (length and width) of the oocysts we found as a result of the research varied between 27.84-32.48x20.88-25.52  $\mu$ m (FI=1.27-1.44). The average size of oocysts was 30.81±1.63x23.39±2.03 (FI=1.32).

The sizes of sporocysts (length and width) varied between 16.24-20.88x9.28-11.60  $\mu$ m (FI=1.40-2.00). The average size of sporocysts was found to be 18.74±2.03x10.57±1.16 (FI=1.78).

*E.faurei* oocysts are egg-shaped and oval. The sizes (length and width) of the oocysts we found varied between 23.20-

27.84x16.24–20.88 μm (FI=1.33–1.43). The average size of oocysts was 25.30±1.95x18.34±1.95 (FI=1.38).

The sizes of sporocysts (length and width) varied between 13.92-16.24x6.96-11.60  $\mu$ m (FI=1.40-2.00). The average size of sporocysts was found to be 14.63±1.07x9.15±1.51 (FI=1.63).

Oocysts of *E.arloingi* are elliptical and oval. The sizes (length and width) of the oocysts we found varied between 25.52–30.16x16.24–20.88  $\mu$ m (FI=1.22–1.71). The average size of oocysts was 28.51±1.89x 18.92±1.90 (FI=1.52).

The sizes of sporocysts (length and width) varied between 11.60-16.24x6.96-9.28  $\mu$ m (FI=1.25-2.00). The average size of sporocysts was 13.74±1.61x8.68±1.03 (FI=1.61).

*E. parva* oocysts are round and elliptical. The sizes (length and width) of the oocysts we found varied between 11.60–16.24x11.60–13.93  $\mu$ m (FI=1.00–1.20). The average size of oocysts was 14.24±1.62x13.09±1.13 (FI=1.09).

The sizes of sporocysts (length and width) varied between 4.64-9.28x4.64-6.96  $\mu$ m (FI=1.00-1.50). The average size of sporocysts (length and width) was found to be 7.27±1.62x6.12±1.12 (FI=1.20).

Oocysts of *E. intricata* are oval and elliptical. The sizes (length and width) of the oocysts we found varied between  $48.72-53.36x30.16-34.80 \ \mu m$  (FI=1.47–1.62). The average size of oocysts was found to be  $51.18\pm1.72x \ 33.30\pm1.98$  (FI=1.54).

The sizes of sporocysts (length and width) varied between  $16.24-18.56x9.28-13.92 \mu m$  (FI=1.33-1.75). The average size of sporocysts was found to be  $17.63\pm1.15x11.74\pm1.72$  (FI=1.52).

Oocysts of *E.ninaekohlyakimovae* are oval and elliptical. The sizes (length and width) of the oocysts we found varied between  $16.24-20.88 \times 13.92-18.56 \ \mu m$  (FI=1.13-1.29). The average size of oocysts was found to be  $19.02\pm 1.69 \times 16.24\pm 1.17$  (FI=1.17).

The sizes of sporocysts (length and width) varied between 6.96-11.60x6.96-9.28  $\mu$ m (FI=1.00-1.33). The average size of sporocysts was found to be 9.28±1.41x7.89±1.15 (FI=1.19).

A comparison of the *Eimeria* species showed differences in

their morphological structures. The shapes of the oocysts of the species are different. The most important characteristic of the species is its pathogenicity. Pathogenicity depends on the biology of each species, the number of generations of the parasite, its reproduction, the toxicity of the substances secreted by the parasite, the host animal's immune status, associative invasions, the presence of various infections, etc. As a result of the influence of complex factors, the virulence of the parasite is sometimes weakened, and under favorable conditions, less pathogenic species can gain high virulence.

During the coprological examination of fecal samples of lambs and sheep of different age groups kept in private and farmer sheep farms in the Lankaran-Astara economic region (Masalli, Lankaran, Astara, Lerik districts), *Cryptosporidium parvum-like*, *Cryptosporidium andersoni-like*, *Cryptosporidium xiaoi-like* in different sizes, it was determined to be both oval and round in shape, and during the conducted research, the length and width were measured and recorded in µm.

*Cryptosporidium parvum-like*: The sizes (length and width) of the oocysts we found varied between  $4,0-5,5x3,5-5,0\mu m$  (FI=1.00–1.25). The average size of oocysts was  $4,8\pm0,53x4,2\pm0,5$  (FI=1.13).

*Cryptosporidium andersoni-like*: The sizes (length and width) of the oocysts we found varied between  $6,0-7,84x5,0-6,5\mu$ m (FI=1.08–1.26). The average size of oocysts was  $6,99\pm0,59x$   $5,91\pm0,47$  (FI=1.18).

*Cryptosporidium xiaoi-like*: The sizes (length and width) of the oocysts we found varied between  $2,94-4,42x2,94-4,42\mu$ m (FI=1.00–1.32). The average size of oocysts was  $3,96\pm10,48x$   $3,52\pm0,45$  (FI=1.13).

The study of the spread of eymeriosis and cryptosporidiosis within the farm is of great practical and theoretical importance. The practical importance of such work is primarily the timely determination of the origin of the disease and treatment by veterinarians with certain preparations. Its theoretical importance is to determine the patterns of formation of parasitofauna within the farm (Eimeria and Cryptosporidium found in sheep).

### **CHAPTER IV**

### DEPENDENCE OF *EIMERIA* AND *CRYPTOSPORIDIUM* INFECTION OF SHEEP ON AGE AND SEASON, MONO AND ASSOCIATIVE INFECTIONS IN THE LANKARAN -ASTARA ECONOMIC REGION

## 4.1. Characteristics of age and season dependence of infection with *Eimeria* and *Cryptosporidium* in sheep

The age dependence of the dynamics of invasion with *Eimeria* one of the primitive intestinal parasites was determined in sheep in the Lankaran-Astara economic region (Table 1).

Table 1

### Dynamics of invasion with *Eimeria ahsata, E.faurei, E.arloingi, E.parva, E.inricata, E.ninaekohlyakimovae* by age of sheep in the Lankaran-Astara economic region

Age of						
animais Indicators		Masalli	Lankaran	Astara	Lerik	Total
	Number of animals examined	96	88	96	92	372
1-30- day-old	Number of infected animals	36	29	28	24	117
	Infection percentage (IE, %)	37.5	33.0	29.2	26.1	31.5
2-4- month-	Number of animals examined	88	92	88	100	368
old	Number of infected animals	26	23	20	20	89

### **Continuation of Table 1**

	Infection percentage (IE, %)	29.5	25.0	22.7	20.0	24.2
5-7-	Number of animals examined	104	96	104	96	400
month- old	Number of infected animals	26	19	19	15	79
	Infection percentage (IE, %)	25.0	19.8	18.3	15.6	19.8
8-12- month- old	Number of animals examined	108	96	108	88	400
	Number of infected animals	21	15	15	10	61
	Infection percentage (IE, %)	19.4	15.6	13.9	11.4	15.3
Total num examined	ber of animals	396	372	396	376	1540
Total number of infected animals		109	86	82	69	346
Invasion extensity (IE, %)		27.5	23.1	20.7	18.4	22.5
Invasion intensity (Oocysts)		2-8	1-6	1-4	1-3	1-8

Thus, the extensity of eimeria invasion in lambs aged 1-30 days was 37.5%, in 2-4 month-old animals 29.5%, in 5-7-month-old sheep 25.0%, and in 8-12-month-old lambs 19.4% in the Masalli district. In 1-30-day-old, 2-4-month-old, 5-7-month-old, and 8-12-month-old lambs, the extensity was 33.0%, 25,0%, 19.8%, and 15.8%, respectively, in the Lankaran district. In the Astara district, a decrease was observed in the extensity in 1-30-day-old, 2-4-month-old, 5-7-month-old, 5-7-month-old, 3-12-month-old, 5-7-month-old, 2-4-month-old, 5-7-month-old, 3-12-month-old, 5-7-month-old, 2-4-month-old, 5-7-month-old, 3-12-month-old lambs that amounted to

29.2%, 22.7%, 18.3%, and 13.9%, respectively. In the Lerik district, the invasion extensity with Eimeria in 1-30-day-old, 2-4- month-old, 5-7- month-old, and 8-12-month-old lambs was equal to 26.1%, 20.0%, 15.6%, and 11.4%, respectively. The intensity of invasion in the studied animals was on average 2-8 oocysts in the Masalli district, 1-6 oocysts in the Lankaran district, 1-4 oocysts in the Astara district, and 1-3 oocysts in the Lerik district. The invasion was detected in 31.5% of 1-30-day-old lambs, 24.2% of 2-4-month-old lambs, 19.8% of 5-7-month-old lambs, and 15.3% of 8-12-month-old lambs in the Lankaran-Astara economic region. Among the examined animals, the extensity of invasion was 22.5%, and the intensity of invasion was 1-8 oocysts per field of view .

The intensity of invasion in the studied animals averaged 1-8 oocysts in spring, 1-3 oocysts in summer, 1-6 oocysts in autumn, and 1-5 oocysts in winter.

The age dependence of the dynamics of invasion with cryptosporidiosis caused by primitive intestinal parasites was determined in sheep in the Lankaran-Astara economic region (Table 2).

Table 2

# The dynamics of infection with *Cryptosporidium sp.* by the age of sheep in the Lankaran-Astara economic region

Age of	Indicators		<b>T</b> 1			
animals		Masalli	Lankaran	Astara	Lerik	Total
	Number of animals examined	96	88	96	92	372
1-30- day-old	Number of infected animals	30	24	24	20	98
	Infection percentage (IE, %)	31.3	27.3	25.0	21.7	26.3
	Number of animals examined	88	92	88	100	368

### **Continuation of Table 2**

2-4- month-	Number of infected animals	21	20	16	17	74
old	Infection percentage (IE, %)	23.9	21.7	18.2	17.0	20.1
5-7-	Number of animals examined	104	96	104	96	400
month- old	Number of infected animals	21	16	13	10	60
	Infection percentage(IE, %)	20.2	16.7	12.5	10.4	15.0
8-12- month- old	Number of animals examined	108	96	108	88	400
	Number of infected animals	14	9	10	6	39
	Infection percentage (IE, %)	13.0	9.4	9.3	6.8	9.8
Total number of examined animals		396	372	396	376	1540
Total number of infected animals		86	70	63	53	272
Invasion extensity (IE, %)		21.7	18.8	15.9	14.1	17.7
Invasion intensity (Oocysts)		1-5	1-4	1-3	1-2	1-5

Thus, the extensity of the infection of lambs aged 1-30 days was 31.3%, in 2-4 month-old lambs-23.9%, in 5-7-month-old lambs-20.2%, and in 8-12-month-old lambs 13.0% in the Masalli district. The extensity of the invasion in 1-30-day-old, 2-4-month-old, 5-7-month-old, and 8-12-month-old lambs was 27.3%, 21.7%, 16.7%, and 9.4%, respectively, in the Lankaran district. The extensity of the invasion in 1-30-day-old, 2-4-month-old, 5-7-month-old lambs was 25.0%, 18.2%, 12.5%, and 9.3%, respectively, in the Astara district. The extensity of the invasion in 1-30-day-old, 2-4-month-old, and 8-12-month-old lambs was 25.0%, 18.2%, 12.5%, and 9.3%, respectively, in the Astara district. The extensity of the invasion in 1-30-day-old, 2-4-month-old lambs was

21.7%, 17.0%, 10.4%, and 6.8%, respectively, in the Lerik district. The intensity of invasion in the studied animals is on average 1-5 oocysts in the Masalli district, 1-4 oocysts in the Lankaran district, 1-3 oocysts in the Astara district, and 1-3 oocysts in the Lerik district. The invasion was detected in 26.3% of 1-30-day-old lambs, 20.1% of 2-4-month-old lambs, 15.0% of 5-7-month-old lambs, and 9.8% of 8-12-month-old lambs in the Lankaran-Astara economic region. Among the examined animals, the extensity of invasion was 17.7% and the intensity of invasion was 1-5 oocysts per field of view.

Research shows that as lambs age, their infection rate decreases. This is due to their early infection. Intensive infection occurs due to oocysts that have entered the external environment during growth. Each ingested oocyst gives rise to a large number of oocysts in the intestines of lambs. Each time such re-infection and release of oocysts into the environment leads to infection of all lambs. In this period, not only the extensity of the invasion (percentage of infection by number) but at the same time, its intensity (infection of each individual with numerous oocysts) reaches the highest level.

In the Lankaran-Astara economic region, the dynamics of infection of sheep with Eimeria ranged around 33.8% in spring, 8.8% in summer, 28.6% in autumn, and 18.7% in winter (Graph 1).





The intensity of invasion in the studied animals averaged 1-8 oocysts in spring, 1-3 oocysts in summer, 1-6 oocysts in autumn, and 1-5 oocysts in winter.

In spring and autumn, humidity and temperature favorable for the development of oocysts have a positive effect on their development. In winter, the ambient temperature drops, the development of oocysts weakens and the probability of infection decreases, in summer, on the contrary.

The seasonal dynamics of the Cryptosporidiosis infection of sheep in the Lankaran-Astara economic region ranged around 27.5% in spring, 6.2% in summer, 22.8% in autumn, and 14.0% in winter.

The intensity of cryptosporidiosis invasion in the studied animals was on average 1-5 oocysts in the spring season,1-2 oocysts in summer, 1-4 oocysts in autumn, and 1-3 oocysts in winter (Graph 2).



# Graph 2. Dynamics of seasonal infection with *Cryptosporidium sp.* in the Lankaran-Astara economic region

Favorable conditions (environmental factors) for infection with eymeriosis and cryptosporidiosis in the studied farms of the Lankaran-Astara economic region were found. However, during the examinations, it was confirmed that the extensity of the infection reached a higher level in spring. The weakest infection with both invasions was established in summer, which is due to external environmental factors - high air temperature and low humidity. This is explained by the fact that most of the oocysts are destroyed in summer because of hot and dry weather.

## 4.2 Mono and associative invasions of lambs with Eimeria and Cryptosporidium

The dependence of associative infection with eimeria and cryptosporidium, which are primitive intestinal parasites, on the ages of lambs in the Lankaran-Astara economic region (Masalli, Lankaran, Astara, Lerik districts) was studied(Graph 3).



#### Graph 3. Associative invasions of lambs (%)

In the Masalli district, invasion with eimeria was 24.4%, 13.3% with cryptosporidiosis, and the associative invasion with both parasites was 8.9%; In the Lankaran district, 20.7%, 11.5%, and 5.7% invasion with eimeria, cryptosporidiosis, and associative invasion were found, respectively; In the lambs of the Astara district, the invasion with eimeria was 15.1%, with cryptosporidiosis was

8.6%, and mixed invasion associative both parasites was 4.3%, it was determined that the prevalence of eimeria infection in lambs in individual sheep farms in Lerik region was 11.8%, cryptosporidial infection was 5.9%, and mixed infection with both infections was 2.4%.

The intensity of eimeria and cryptosporidium invasions in lambs was on average 1-6 oocysts and 1-3 oocysts, respectively, in the sheep farms of the Masalli district; In the Lankaran district the intensity of the invasions with eimeria and cryptosporidium was 1-4 and 1-2 oocysts, respectively; In the Astara district, the intensity of eimeria and cryptosporidium invasions was 1-3 oocysts and 1-2 oocysts, In the Lerik district, the intensity of eimeria and cryptosporidium invasions was 1-2 oocysts and 1-1 oocysts, respectively. Thus, according to the results of the research, the incidence of invasions in lambs of sheep farms of the Masalli district is higher than in the Lankaran, Astara and Lerik districts. This depends on the climate of the Masalli district - air temperature and humidity, which is favorable for the development of eimeria and cryptosporidiosis agents.

### CHAPTER V INVASION OF SHEEP WITH EIMERIA AND CRYPTOSPORIDIUM SPECIES ON LOWLAND, FOOTHILL, AND MOUNTAINOUS ZONES

In order to study the dependence of the spread of Eimeria and cryptosporidium species on vertical zones, fecal samples taken from lambs and sheep of different age groups kept in individual sheep farms were examined coprologically in the lowland, foothill, and mountainous zones of the Lankaran, Masalli, Astara and Lerik districts.

As a result of the coprological examinations, it was found that the infection with primitive intestinal parasites was high in the farms located in the lowland zone, relatively low in the foothill zone, and weaker in the farms located in the mountainous zone. This is explained by the fact that after the causative agents fall into the external environment, the low temperature of the air leads to the weakening of their development or destruction. Compared to other vertical zones, the infection is relatively high in farms located in lowland zones.

The invasion extensity was examined depending on vertical zones. In the Masalli district, the extensity of invasion with evmeriosis in the lowland zone was 40.0% and with cryptosporidiosis - 31.6%; In the foothill zone-34.6% with eymeriosis and 28.2% with cryptosporidiosis; In the mountainous zone 23.5% with evmeriosis, 17.0% with cryptosporidiosis. In the Lankaran district, the extensity of invasion with eymeriosis was 36.3%, with cryptosporidiosis - 25.6% in the lowland zone; In the foothill zone, 27.1% with eymeriosis, 19.9% with cryptosporidiosis; In the mountainous zone, 13.0% with eymeriosis, 8.7% with cryptosporidiosis. In the Astara district, the extensity of invasion with eymeriosis in the lowland zone was 20.5%. with cryptosporidiosis - 14.1%; In the foothill zone, 9.0% with eymeriosis, 5.8% with cryptosporidiosis; In the mountainous zone, 5.8% with eymeriosis and 3.9% with cryptosporidiosis. In the Lerik district, the extensity of invasion with eymeriosis in the foothill zone was 7.5%, with cryptosporidiosis -2.7%; in the mountainous zone, 3.4% with eymeriosis and 1.4% with cryptosporidiosis. (Table 3).

Table 3

Climate zones	Exami- ned	Eimeria infe	etion	Cryptosporidium infection			
		Infected	IE (%)	Infected	IE (%)		
Masalli district							
Lowland zone	155	62	40.0	49	31.6		
Foothill zone	156	54	34.6	44	28.2		
Mountainous zone	153	36	23.5	26	17.0		

### The extensity of infection of sheep with eymeriosis and cryptosporidiosis in the lowland, foothill and mountainous zones

### **Continuation of Table 3**

Lankaran district								
Lowland zone	168	61	36.3	43	25.6			
Foothill zone	166	45	27.1	33	19.9			
Mountainous zone	161	21	13.0	14	8.7			
Astara district								
Lowland zone	156	32	20.5	22	14.1			
Foothill zone	155	14	9.0	9	5.8			
Mountainous zone	155	9	5.8	6	3.9			
	Lerik district							
Lowland zone	-	-	-	-	-			
Foothill zone	146	11	7,5	5	3,4			
Mountainous zone	148	4	2,7	2	1,4			

According to the coprological examinations, the intensity of invasion with evmeriosis in the lowland zone of the Masalli district was 3-9, and with cryptosporidiosis -1-4 oocysts. In the foothill zone, the intensity of invasion with eymeriosis- 2-7, with cryptosporidiosis was 1-3 oocysts. In the mountainous zone, the intensity of invasion with eymeriosis was 1-2, and with cryptosporidiosis 1-2 oocysts. In the Lankaran district, the intensity of invasion with evmeriosis in the lowland zone was 2-5 and with cryptosporidiosis - 1-3 oocysts. In the foothill zone, the intensity of invasion with eymeriosis was 1-4, with cryptosporidiosis - 1-2 oocysts. In the mountainous zone, the intensity of invasion with eymeriosis was 1-2 and with cryptosporidiosis -1-1 oocytes. In the Astara district, the intensity of invasion with eymeriosis was 1-2, with cryptosporidiosis - 1-1 oocytes in the lowland zone. In the foothill zone, the intensity of invasion with eymeriosis was 1-2 and with cryptosporidiosis - 1-1 oocytes. In the mountainous zone, the intensity of invasion with eymeriosis was 1-1 oocysts, and with cryptosporidiosis - 1-1 oocysts.

In the Lerik district, the intensity of invasion with eymeriosis was 1-1, with cryptosporidiosis - 1-1 oocytes in the foothill zone. In the mountainous zone, the intensity of invasion with eymeriosis was 1-1 oocysts, and with cryptosporidiosis - 1-1 oocysts.

In general, the results of our examinations confirm that among primitive intestinal parasites, Eymeriosis causative agents are more common than Cryptosporidiosis causative agents.

#### **CHAPTER VI**

### STUDY OF THE EFFECT OF DESINVASION SUBSTANCES ON EIMERIA OOCYSTS IN VITRO, TESTING THEM UNDER FARM CONDITIONS AND GAINED ECONOMIC EFFICIENCY

Despite substantial measures against invasive diseases, intestinal parasites including eymeria causes economic damage to sheep farming. In order to prevent eymeriosis, it is necessary to carry out desinvasion measures. In this regard, the research conducted is relevant.

### 6.1. Study of the effect of desinvasion agents on eimeria oocysts *in vitro*

Carrying out desinvasion measures is the most important link in the preven tion system, and it means the destruction of invasive disease agents in environmental objects, the neutralization of pathogens of parasitic diseases due to anti-epizootic measures that ensure the welfare of animals.

After eliminating any invasive disease occurring in farms, sanitization measures - desinvasion should be carried out there. These measures should be directed to the destruction of disease agents in the external environment or the weakening of the virulence of these agents. Detection of any parasitic disease in the early stages of its occurrence contributes to the prevention of its widespread or taking necessary measures to eliminate it.

During the research, the destructive effect of sodium hydroxide and formalin, and for comparison, phenol solutions with different percentages on sporulated and unsporulated Eimeria oocysts were studied under laboratory conditions (*in vitro*). For this purpose, different percentages (2.0%, 4.0%, 6.0%, 8.0%, 10.0%) solutions of the mentioned substances were used. Thus, the following changes

occurred in sporulated eimeria oocysts when viewed through a microscope (Figure 1).



Figure 1. The effect of sodium hydroxide on sporulated eimeria oocysts

When exposed to various percentage solutions of sodium hydroxide, their color became whitish-transparent, the oocysts were deformed, and in some cases, their outer covers and envelopes appeared to have a rough shape. Finally, the envelope of the oocysts gradually dissolved, disintegrating the internal structure. Due to the effect of sodium hydroxide, sporozoites protruded from a part of the envelope of some oocysts. Careful examination revealed that the sporozoites that came out of the sporulated eimeria oocysts were also deformed, and some of them were destroyed.

In order to clarify the effects of desinvasion agents on eimeria oocysts under laboratory conditions, wood and concrete were taken as test objects, and the experiment continued. Thus, 100% of eimeria oocysts (1100 oocysts) were destroyed in a 3-hour exposure to 8.0% sodium hydroxide in a concrete test object, 72.7% were destroyed by 8.0% formalin solutions (800 oocysts), and during the same period, 63.6% were destroyed by 5.0% phenol (700 oocysts). 100% of eimeria oocysts (1100 oocysts) were destroyed in 3-hour exposure to 8.0% sodium hydroxide in the wooden test object, and 63.6% (700

oocysts) were destroyed by 8.0% formalin and 5.0% phenol solutions during the same period of time. As a control, ordinary water was added to the Eimeria oocysts and no decrease in the number of oocysts was observed.

Unsporulated eimeria oocysts are more quickly affected by desinvasion substances than sporulated eimeria oocysts. This is explained by the relative sensitivity of their envelope to external environmental factors. The color of unsporulated eimeria oocysts became white and transparent, they were deformed, the envelope was mostly melted in the micropyle, and a furrow appeared from the micropyle into the oocyst, as a result, the structure was destroyed which was clearly visible through a microscope (Figure 2).



Figure 2. The effect of sodium hydroxide on unsporulated eimeria oocysts

After determining the effect of different percentage solutions of desinvasion substances on unsporulated and sporulated eimeria oocysts under laboratory conditions, 3 separate areas of  $1m^2$  were used for the experiments. 8.0% sodium hydroxide was applied to one of the experimental areas, 5.0% phenol solutions were applied to the 2nd area for comparison, and normal water was sprayed to the 3rd area as a control. 12 samples were taken from each isolated area and coprologically examined. 91.7% of eimeria oocysts were destroyed in the separated 1st area, 66.7% in the 2nd area, while eimeria oocysts were not destroyed in the control area.

## 6.2. Production test of desinvasion substances against causative agents of sheep Eimeria

The effects of 8.0% sodium hydroxide and 5.0% phenol as desinvasion agents on the causative agents of Eimeria in sheep were compared during the production test. The application of sodium hydroxide resulted in higher efficiency. The efficiency of the 8.0% sodium hydroxide preparation was 92.0%, and the efficiency of 5.0% phenol was 64.0%.

# 6.3. Estimation of the economic efficiency of desinvasion preparations applied against the causative agents of sheep Eimeria

Issues such as researching invasive diseases and developing complex control measures against these diseases are always relevant.

Based on the results obtained from the research carried out under farm conditions and desinvasion measures against eimeria pathogens of sheep, the economic damage and economic efficiency were estimated.

During the conducted experiments, it was found that the use of 8.0% sodium hydroxide as a desinvasion substance against the causative agents of Eimeria in sheep resulted in 37.23 manats profit per sheep, and the profit was 25.74 manats when a 5.0% phenol preparation was applied. Thus, the application of sodium hydroxide is more economically efficient as a desinvasion substance.

### RESULTS

 The spread of species belonging to the genera Eimeria and Cryptosporidium in sheep in the Lankaran-Astara economic region was studied for the first time. Six species of *Eimeria*: *Eimeria ahsata* (Honess.1942); *Eimeria faurei* (Moussu et Marotel. 1902; Martin. 1909); *Eimeria arloingi* (Marotel. 1905; Martin. 1909); *Eimeria parva* (Kotlan. Mocsu et Vajda. 1929); *Eimeria inricata* (Spiegi. 1925); *Eimeria ninaekohlyakimovae* (Jakimoff et Rastegaieff. 1930) and causative agent of cryptosporidiosis-*C.parvum* (Tyzzer, 1912)-like, *C.andersoni* (Lindsay, et al., 2000)-like, *C.xiaoi* (Fayer et Santín, 2009)-like were registered for the first time[7, 14].

- 2. In the Lankaran-Astara economic region, 31.5% of lambs infected with eimeria in 1-30-day-old lambs, 24.2% in 2-4-month-old lambs, 19.8% in 5-7-month-old lambs, and 15.3% in 8-12-month-old lambs were studied. The intensity of infestation in the examined animals was 2-8 oocysts in Masalli region, 1-6 oocysts in Lankaran region, 1-4 oocysts in Astara region, and 1-3 oocysts in Lerik region. In general, the intensity of invasion was 1-8 oocysts [1,2,3,4].
- 3. In the Lankaran-Astara economic region, 26.3% of 1-30-day-old lambs, 20.1% of 2-4-month-old lambs, 15.0% of 5-7-month-old lambs, and 9.8% of 8-12-month-old lambs were infected with cryptosporids. The intensity of infestation in the studied animals was on average 1-5 oocysts in Masalli region, 1-4 oocysts in Lankaran region, 1-3 oocysts in Astara region, and 1-3 oocysts in Lerik region [5, 6].
- 4. In the Lankaran-Astara economic region, the dynamics of infection of sheep with eimeria by seasons is 33.8% in spring, 8.8% in summer, 28.6% in autumn, 18.7% in winter, the dynamics of infection with cryptosporidis is 27.5% in spring, 6 in summer. .2%, 22.8% in autumn, and 14.0% in winter [8, 12].
- 5. In the Lankaran-Astara economic region, the total infection rate in sheep was 22.6% with eimeria and 16.8% with cryptosporidiosis. It has been studied as a result of the examinations that the associative invasion fluctuates between 8.9% and 2.4% [15].
- 6. Depending on the vertical zones, 40.0% with eimeria in the plain zone of Masalli region, 31.6% with cryptosporidis, 34.6% with eimeria in the foothills zone, 28.2% with cryptosporidis, 23.5% with eimeria in the mountainous zone, 17 with cryptosporidis, 0% infection was detected. 36.3% infection with eimeria, 25.6% with cryptosporidium, 27.1% with eimeria, 19.9% with

cryptosporidium, 13.0% with eimeria, 8.7% with cryptosporidium in the Mountainous zone of Lankaran region. 20.5% with eimeria in the Plain zone of Astara region, 14.1% with cryptosporidia, 9.0% with eimeria in the foothill zone, 5.8% with cryptosporidia, 5.8% with eimeria in the mountainous zone, 3.9% with cryptosporidia, Lerik district foothills 7.5% with eimeriosis in the zone, 3.4% with cryptosporidiosis, 2.7% with eimeriosis in the mountainous zone, and 1.4% with cryptosporidiosis were determined during the examinations. High intensity Masalli district 3-9 oocysts with eimeria and 1-4 oocysts with cryptosporidis in the plain zone of Masalli region, and the weakest intensity in Astara and Lerik regions in the mountainous zone, the intensity of infection was 1-1 oocysts with both eimeria and cryptosporidis were studied as a result of coprological examinations [9].

7. The effect of solutions of different concentrations of disinfestation agents on eimeria oocysts was studied *in vitro*. It was determined that the 8.0% solution of sodium alkali destroys eimeria oocysts by 100% in laboratory conditions, and the 8.0% solution of sodium alkali destroys eimeria oocysts by 91.7% in 1 m2 area. As a result of extensive production testing, the 8.0% solution of sodium alkali against the causative agents of eimeria in sheep was 92.0% effective, and 37.23 manats were saved for 1 sheep [10, 11, 13, 16].

### PRACTICAL RECOMMENDATIONS

- 1. The results of the research can be used as educational materials in the relevant specialties of higher and secondary schools, in scientific literature, and in the preparation of textbooks.
- 2. The 8.0% solution of sodium hydroxide had a high desinvasion effect against the causative agents of sheep eimeria. Taking into account that 37.23 manats of economic profit were obtained per sheep, it is more appropriate to apply it in large farms.
- 3. Based on the results of the research, the recommendation "On the

prevention of eymeriosis of small horned animals" was prepared and proposed to be applied to farms.

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