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Scientific Report

Creation and Implementation of the Modern Treatment in Eimeria and Esophagostomiasis Invasion: Case Report in the Farms "Ulan", "Erlan", and "Balke"

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ABSTRACT

Introduction: Parasitic diseases in sheep reduce sheep-breeding productivity and decrease the economic efficiency of the industry. Our research aim was to study of the dynamics of clinical sings development in the manifestation of associated eimeriosis and esophagostomosis invasion, as well as concomitant immunodeficiency state in experimentally infested lambs, and to evaluate the efficiency of complex treatment approach for associated invasions.

Materials & Methods: A prospective study was conducted using helminthoscopic methods with the subsequent calculation of indicators of extensiveness and intensity of infestation, as well as haematological studies to determine of subpopulations of T- and B-lymphocytes.

Results: Due to the more active development of protozoa, the main symptoms of associated invasion were those typical for acute Eimeriosis. Progression of the disease was accompanied by an increase in the intensity of invasion both in relation to Eimeria and Esophagostomes. At the same time, in animals with the development of invasion, there was a decrease in the total number of leukocytes (up to 25%), including T- and B-lymphocytes, indicating a decrease in humoral defense of the lambs' organism. On the 24th day after infection, an increase in the level of T-lymphocytes (by 0.8%) was noted, due to an increase in T-helpers (by 2.0%). For treatment of associated infestation, Sulfamonomethoxine in combinationwith niacid was used. The use of these drugs in recommended doses and according to the developed scheme reduced the intensity of Eimeriosis invasion by 97-98% and esophagostomosis by 100%.

Conclusion: This study provides data on eimerioses and eimeriosis-esophagostomosis infestation in sheep. In describing eimerioses and helminthoses of sheep, the species composition, distribution, and seasonal age dynamics of infestation were determined. The developed method of treatment showed high therapeutic effect and can be recommended for use in all sheep farms of the Republic of Kazakhstan.

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1. Introduction

Small ruminant breeding is widespread all over the Kazakhstan due to the presence of large areas of inaccessible mountainous (7.2 million ha) and stony (18.2 million ha) pastures, which can be used only for small ruminants [1]. However, the main problem in pasture-based livestock production is the significant spread of parasitic diseases [2]. It leads to decreased animal productivity, high lamb losses, and development of immunodeficiency, which reduce the profitability of livestock production [1].

Abdrazakova et al. [3] reported that the most common parasitic diseases among goats and sheep in Kazakhstan are mixed invasions by helminthes and protozoa. It has to be noted that coccidiosis (eimeriosis) is most frequent in young sheep [2]. Hermosilla et al. [4] detected 5 *Eimeria's* species among 214 animals of different ages, with the biggest intensity of *E. ahsata* (64%), *E. infantrisata* (18.2%), *E. ovinoidalis* (56.5%), *E. crandallis* (39.7%), and *E. parva* (16.6%).

Akmambaeva et al. [2] found 18 species of parasites in sheep: 4 trematodes, 6 cestodes, and 8 nematodes. Amirov et al. [5] recorded associated variants of infestations in small ruminants. Win et al. [6] indicated the occurrence of parasitic agents in sheep in Myanmar: *Eimeria* spp. (96%), *Trichostrongyle* (77.1%), *Trichuris* spp. (35%), and *Moniezia expansa* (14%). Mixed invasion accounted for 84.8% (317 of 374 animals), while mono-infestations accounted for 15.2% (57 of 374 animals).

Helminthiasis can be supported by clinical symptoms, but in most cases they are asymptomatic [7, 8]. Chronic helminthiasis causes disturbances in defense responses and increases the development of secondary diseases development. Chauhan et al. [9] noted that only a limited number of parasites are unable to provoke effective immune reactions from in animals, while some species of parasites are able to use immune mechanisms for their accelerated reproduction. Most species of invasive agents secrete toxins with immunosuppressive action. Based on a fairly wide area of parasitic agents in the environment and the danger they pose to livestock in Kazakhstan, the research aim of our research to was study the dynamics of immunodeficiency state in lambs after experimental infestation; and to evaluate the effectiveness of complex treatment methods in associated eimeriosis and nematode infestation. Accordingly, following study objectives were formulated:

1) To analysis changes in the population of immunocompetent cells during the disease development and treatment flowing;

2) To study the efficiency of therapeutic drugs in reducing the invasion intensity.

2. Materials and Methods

2.1. Study design

The research was conducted several sheep farms of Beskaragai district, Abay region "Ulan", "Yerlan" and "Balke". The location of these farms is shown on the map (Figure 1).

The research was carried out in 2023 on 20 lambs (born in 2023), experimentally infected with *Eimeria* and esophagostomies.

2.2. Investigation experiment

Healthy lambs were selected for experimental infection: Helminthological tests confirmed the absence of nematode's eggs or *Eimeria* oocysts.

Infestation was carried out orally with invasive parasite's forms, esophagostome's larvae and *Eimeria*'s sporogonium, after their cultivating in a thermostat for 4-5 days at 20-22°C.

Fecal samples were sampled from control animals on days 7, 14, 24, and 30 after infection to detect parasites pathogenic forms and clinical signs of helmithosis'.

2.3. Helminthological study

Helminthological studies to detect eggs and oocysts were carried out by collecting feces in paper bags and preserving them with 2.5% potassium bicarbonate solution for further investigation in a core laboratory. Laboratory tests for detection of eggs and oocysts were carried out using the Darling flotation method [10]. Visualization and species identification were performed using an Olympus CX31 microscope equipped with phase contrast at a magnification of 400. Parasite species identification was performed by comparing the materials of the helminth identification and enumeration manual by Mifsut and Ballarin [11]. Traditional parasitological indices,including infestation intensity (II) and infestation extensiveness (IE) were also determined.

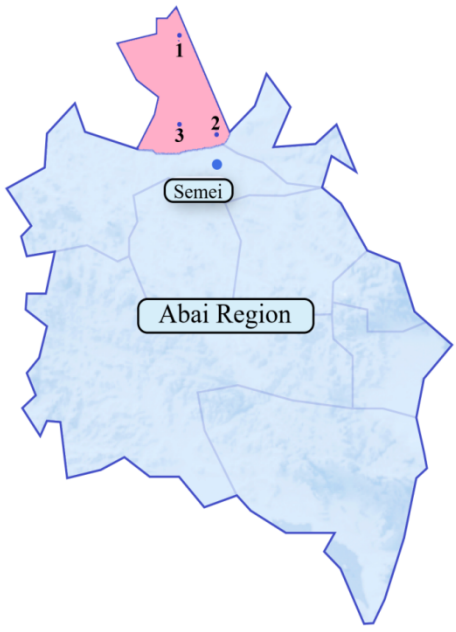

Figure 1. Territorial distribution of farms in which the research was conducted: 1. Farm "Ulan", Zhanasemey district. S. Ernazar; 2. Farm «Erlan», Zhanasemey district, Bokenchi village; 3. Farm "Balke", Beskaragai district, Birlik village. Birlik.

2.4. Hematology study

Blood for haematological (immunological) studies was collected from the jugular vein into vacuum tubes containing ethylenediaminetetetraacetic acid (EDTA). Quantitative characteristics of lymphocyte subpopulations (T- and B-lymphocytes, T-helpers, and T-suppressors) were carried out using monoclonal antibodies and flow fluorescent cytometer CytoFLEX LX (Beckman Coulte, USA). Blood sampling to determine the dynamics of the number of different lymphocyte subpopulations was performed at the following frequency.

Prior to experimental infection;

On days 7, 14, 24 and 30 after infection;

On days 10 and 24 after treatment.

In cases of animal mortality, pathological autopsy was performed according to Garcês and Pires [12] gaudiness.

2.5. Treatment approach

Treatment was conducted in separate cages with lattice floors, previously cleaned and disinfected to avoid re-infection of lambs. All drugs were chosen by the State Register of Veterinary Drugs and Feed Additives [13]: Sulfadimethoxine or sulfamonomethoxine was used for eimeriosis treatemt (once a day mixed with dry feed at a dose of 50 mg per a kg for two five-day courses with an interval of 3 days); and niacid (abamectin, avermectin B1) for esophagostomiasis treatment (subcutaneously once on the 5th day of the first course at a dose of 0.5 mL per a head). After infestation, all lambs received therapeutic treatment (Table 1). Two preparations in equal doses were used in the treatment against eimeriosis infestation.

Upon completion of treatment procedures, the effect of antiparasitic action was carried out by determining II and IE individually in each tested animal.

3. Results

3.1. Infestation experiment

The analysis of lambs' infestation was recorded by coprological analysis (Table 2).

In the first week after experimental infection, no clinical parasitological signs were detected in the experimental lambs. Only one lamb showed gastrointestinal disorders in the form of minor diarrhoea (which stopped in 24 hours). Therefore, this symptom was not associated with the manifestation of post-infection effects and was not considered. Also, no eggs and oocysts were carried out in the feces in this period. From 14th day, *Eimeria*'s oocysts were detected in the feces of most lambs in the experiment. The II was 80% (8 out of 10 lambs had sporogoniums in their feces). Clinical signs of eimeriosis of different intensities were detected in all animals: Depression, anemic mucous membranes, decreased appetite. In some lambs, diarrhea and increased temperature up to 40.5-41 ˚C were noted. No signs of esophagostomosis infestation were found in the control animals, and the lambs showed no characteristic clinical signs or presence of helminth eggs in feces.

From the 24th days after infection, the intensity of eimeriosis infestation was 100%, and esophagostome eggs were detected some animals (60%). Clinical signs observed in the lambs included general oppression, decreased appetite, immobility, anemia of visible mucous membranes; tachycardia (120-140 beats/min.); tachypnoea (40-52 movements/min.),and, in some animals, fixed high temperature (up to 41 ˚C). Intestinal peristalsis was increased, diarrhea was noted. As the sings were the same in all animals, we can assume that they were associated the development of eimeriosis. During this period (from 24th to 30th day), one lamb died.

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Table 1. Treatment schemes for experimentally infected lambs with associated eimeriosis and esophagostomosis infestation

Lamb's No.	Eimeriosis' Treatment Drug	Oesophagostomiasis Treatment Drug
1	Sulfadimethoxine	Niacid
2	Sulfadimethoxine	Niacid
3	Sulfadimethoxine	Niacid
4	Sulfadimethoxine	Control
5	Sulphamonomethoxine	Niacid
6	Sulphamonomethoxine	Niacid
7	Sulphamonomethoxine	Niacid
8	Sulphamonomethoxine	Niacid
9	Sulphamonomethoxine	Control

On the 30th day after invasion, the II of eimeriosis and esophagostomosis was 100%. Clinical signs in the animals during this period remained at the same level, with no noticeable changes.

3.2. Pathological changes study in lambs

Pathological autopsy revealed significant lesions in the small intestine. The mucosa of the duodenum and jejunum was thickened, with signs of inflammatory infiltration. Foci of hemorrhages and small grey-white ulcers were present on the mucosa. In the large intestine, there were practically no signs of esophagostomous invasion, though small thickenings in the mucosa were noted. No parasitic nematodes were found. So, the main pathological changes in the lambs during this period are associated with *Eimerias* active reproduction (Figure 2).

So, we observed the active disease flowing in young animals with mortality of 85-100% without treatment.

3.3. Immunological study

On seventh day after invasion, sheep showed a decrease in the level of T- and B-lymphocytes: Decrease in T-helper cells (by 2.4% or 74 cells/μL), the content of T-suppressors was reduced insignificantly (by 3.5% or 181 cells/μL). At 14th day, the lambs showed significant shifts in T- and B- immunity systems. At the same time, a decrease in T- and B-cells compared to the initial ones was observed. The number of T-lymphocytes was 59±4.2% or 2461±153 cells/μL ($P<0.001$),while B-lymphocytes were 12.7±2.1% or 332±41 cells/μL ($P<0.001$). The content of both T-helper cells and T-suppressors was reduced. Overall, immunosuppression was observed (Table 3).

The absolute number of lymphocytes was reduced compared with baseline and was 4595±253 ($P<0.001$). The II for both parasites was 100%, with extensiveness of infestation ranging from 1-10 to 80 *eimeria* and 10-15 esophagostome eggs in a microscope view-field.

Table 2. Dynamics of invasive agents' isolation after the experimental infection

Study Period	Intensity of Eimeria's Infestation	Intensity of Esophagostome's Invasion
Before invasion	0	0
7th day	0	0
14th days	346.2±124.3	0
24th day	894.7±221.6	5.4±2.3
30th days	1248.2 335.1	19.5±5.9

Figure 2. Pathological changes in study of the died lamb

Since attention during the studies was not paid to immune cells like monocytes and granulocytes, the focus remained on T- and B-lymphocytes fractions. After the 14th day, further decrease in the total number of leukocytes was observed. This indicates only a deep depression of the cellular immune defense system compared with the humoral defense.

From the 30th day onward, a stable increase in all cellular and humoral immune defense factors was observed, indicating an active response of the infected animals' organism against the infectious agents.

3.4. Cure approach in investigated animals

By the 10th day of sulfamonomethoxine application (Table 4), a significant reduction in the intensity of invasion was noticeable compared with the sulfadimethoxine preparation, by about 67%. Subsequently, 24 days after the start of treatment, animals administered sulfadimethoxine were unable to eliminate eimeriosis infestation. In contrast, animals treated with sulfamonomethoxine virtually ceased to release *Eimeria* oocysts into the environment. Since only one drug was used for the treatment of esophagostomiasis, animals numbered 4 and 9 served as untreated control.

During the treatment procedures, a positive tendency to decrease the intensity of esophagostomosis infestation was observed already on 10th day. By the 24th treatment-day, no esophagostomes eggs were found in the feces of experimental animals. In contrast, in control group, the IE had tendency to increase, even when treatment against eimeriosis was applied. The research protocols did not include the results for lamb No. 5, which died on the second day of treatment.

Table 3. Some immunobiological blood parameters in experimental infested lambs

Period	Total White Blood Cells Number	T-lymphocytes		T-helper Cells		T-suppressors		B-lymphocytes	
		%	Count	%	Count	%	Count	%	Count
Before infestation	2763±34	37.5±0.5	1145±21	20.5±0.2	536±15	15.7±0.5	511±13	16.5±0.4	513±8
7th day	2450±33	33.6±0.5	820±20	16.1±0.4	372±17	15.5±0.5	336±11	14.4±2.1	359±11
14th day	2225±103	27.3±0.5	547±33	13.1±0.4	315±25	14.2±0.6	152±8	6.7±0.5	184±16
24th day	2121±56	21±1.2	581±25	13.1±0.6	216±11	15.7±0.6	265±13	7.4±0.6	214±1
30th day	2570±15	21.4±0.3	727±2	14.0±0.2	305±9	15.2±0.3	321±9	14.6±0.6	326±22

Table 4. Results of treatment of experimentally infected lambs

Lamb's Nubber	Infestation Intensity at the 10th Day After Treatment Beginning		Infestation Intensity at the 24th Day After Treatment Beginning	
	Eimeria	Esophagostome	Eimeria	Esophagostome
1	376	4	57	0
2	527	8	44	0
3	504	6	49	0
4	621	16	51	41
5	-	-	-	-
6	341	5	2	0
7	359	4	1	0
8	284	7	1	0
9	376	19	0	54

As in the previous studies, blood samplings were carried out in parallel with II determination (Table 5).

So, positive dynamics of leucocyte count was observed, exceeding the levels recorded before the invasion. Although humoral immunity indices at the end of treatment were slightly higher than before the disease, the main increase in leucocytes was associated with such immune cells as neutrophils and monocytes. So, we can note the activating both cellular and humoral immune defense.

4. Discussion

The most frequent parasitic pathology widespread among small ruminants in the Republic of Kazakhstan are gastrointestinal tract diseases caused by nematodes (50.4-67%) and protozoa (82.1-87%). Adults are less susceptible to the disease because of developed resistance. However, new disease episodes can happen after transportation if other *Eimeriae* strains are present. When animals are infected with eimeriosis and esophagostomiasis, mass multiplication occurs in the intestine, destroying many intestinal epithelial cells [3, 14, 15].

We have conducted an experimental invasion in lambs. According to the obtained data, the first disease signs were detected between 7 and 14 days after invasion. And esophagostome invasion was delayed by 7-14 days compared with the faster development of eimeriasis. The main symptoms at experimental associative invasion were characterized as acute eimeriosis. These data are not entirely consistent with the development of immunological depression in invaded animals. There is no data about eimeriasis' negative effect on the immune system of animals in the literature. Just only Bouroutzika et al. [16] confirmed a significant immunosuppressive effect of eimeriosis on both humoral and cellular immune defense of the host organism. According to these data, both esophagostome and *Eimeria* are capable of suppressing host immune defense. However, a complete picture of the effect of associated invasions on the host immune system is not yet available due to the lack of data on the cellular component of the body's defense system [9]. Since the main defense mechanism against parasitic organisms is the production of specific antibodies [7], this study focused on lymphocytes as the main producers of humoral defense (antibody) components.

Table 5. Immunology cells checking in blood samples treated lambs

Treatment Day	Total Count of the White Blood Cells	T-lymphocytes		T-helper Cells		T-suppressors		B-lymphocytes	
		%	Count	%	Count	%	Count	%	Count
10th	3121±28	35.2±0.6	1120±17	20.5±0.2	565±6.5	17±0.6	453±19	17.2±0.2	458±11
24th	3242±59	39.5±1.2	1226±24	22.3±0.6	660±30	16.3±1	555±23	21.1±0.8	445±13

At the first post-invasion stage, we recorded a decrease in the total number of leukocytes, indirectly indicating suppression of the cellular component of the immune system. However, no consistent tendency was observed, as leucocytes' number varied in different animals. White et al. [17] and Perry et al. [18] confirmed that helminths are able to suppress hosts' immunity and can control the induction of regulatory T-cells. At the same time, the degree of suppression of the immune response correlates with the number of helminths in the host organism. This can be associated with the development of immunosuppression due to parasite's toxins action [9]. The maximum decrease in lymphocytes was observed up to 14th day after invasion.

After 14th day, growth of both T- and B-lymphocytes' fractions was observed. Considering the physiological peculiarities of immune system functioning, antibody production is typically observed on the 14th day in most diseases [7].

By the 20th day after invasion, there was a slight increase in the level of T-lymphocytes (by 0.8%) due to an increase in T-helpers (by 2.0%).

Between the 14th and 24th day, small changes in the humoral defense system were noted, reflecting the different life cycles of the parasites. The incubation period of esophagostomies is longer compared with *Eimeria* [19]. The main clinical signs and oocysts excretion in experimentally infected lambs occurred on the 14th day, while the first esophagostomes was recorded on the 24th day. It is this period, within one week, that causes a more smeared picture in the blood counts of different fractions of immunocompetent cells and the humoral immune response. Reduction of the associate invasion is complicated by the different nature of the invasive components [20]. In the conducted studies at experimental associated invasion, both esophagostome and *Eimeria* reached 100% invasion intensity. Clinical signs of esophagostomiasis in the lamb organism were smoothed, but when eimeriosis was treated, signs of esophagostomosis invasion developed. So, all prophylactic measures need to consider the physiology of both pathogens, but this is complicated by the fact that the pathogens belong to different classes of parasites [15]. However, it is possible that commensal connections can be formed between eimeriae and nematodes, favoring their simultaneous development in infected animals. Eimeriosis is caused by protozoa (an obligate intracellular parasites), while esophagostomiasis is a typical helminthiasis adult nematode with developing in the intestine. So, treating approaches in these diseases are somewhat different, as are the drugs used [1, 21]. In this way, anti-helminthic drugs are ineffective against eimeriosis, while anti-coccidial cure have practically no effect against helminths.

In the Register of Approved Veterinary Drugs in the Republic of Kazakhstan [13], no drug was found effective against both helminths (nematodes) and *Eimeria* (Protozoa). Almost all drugs, even those with complex action, target a particular class of parasitic organisms. Even drugs that are provided for the treatment of certain types of parasites, could not guarantee 100% effectiveness. Among sulfonamide drugs, sulfadimethoxin was significantly less effective against eimeriosis compared with sulfamonomethoxin, which practically allowed to stop the release of *Eimeria* oocysts from the organism of infected lambs completely. In addition, both drugs were able to stop the clinical manifestation of the disease after experimental infection.

The effectiveness of sulfonamide group drugs against *Eimeria* was confirmed by Filipenko and Soroka [21] and Bawm and Htun [22]. However, no effect was detected on the production of esophagostome eggs, confirmed by the increasing excretion dynamics in control lambs not treated with anti-helminthic drugs.

Niacid using in esophagostomiasis treatment had not been reported before our study (we haven't found data about this). However, the efficacy of niacid was found to be high. By the 24 days after treatment initiation, esophagostome egg excretion completely stopped in animals.

In experimental infestation, intensity invasion was 100 % for both pathogens, indicating high susceptibility of young sheep to eimeriosis and esophagostomosis. The dynamics of excretion of invasive agents from feces of infected lambs indicates a more rapid and active development of *Eimeria* than esophagostomosis.

Despite increased II, total leucopenia of almost 25% was observed in infected sheep. From the 24th day after infestation, gradual activation of humoral immune defense was observed, manifested by an increase in the B-lymphocytes fraction.

In treating associative invasion, no complex but drugs against fixed parasites (monoinvasions) have been used. For this purpose, it is necessary to use a complex treatment approach, taking into account pathogen's synergy and contraindications. So, we have used sulfonamide drug – sulfamonomethoxine – and a drug of the ivermectin group – niacid. This approach by the recommended scheme and doses allowed reducing the intensity of eimeriosis invasion by 97-98% and esophagostomosis by 100%.

Therapeutic procedures revealed positive dynamics in immune response in animals due to increasing T- and B-lymphocytes number.

4.1. Limitations

Due to the small size of control and experimental groups, the obtained results have to be vivificated in bigger groups. The research aim was not to study the manifestation of morbidity according to the parasites' number. Still, it was noticeable that in different lambs both the rate of increase of clinical signs and the degree of manifestation of the disease differed significantly. Since invasive agents obtained from one sick animal were used in the research, it could be assumed that the intensity of the disease manifestation depended only on the number of parasites ingested into the organism. Therefore, the next research stage will be the study of the parasite's number influence on the degree of clinical signs and the influence of the parasite's number on the level of the disease intensity according to the immunosuppressive effect of the parasite agents.

5. Conclusion

This study provides data on eimerioses and eimeriosis-esophagostomosis infestation in sheep. In describing eimerioses and helminthoses of sheep, the species composition, distribution, and seasonal age dynamics of infestation were determined. The developed method of treatment showed high therapeutic effect and can be recommended for use in all sheep farms of the Republic of Kazakhstan.

Ethical Considerations

Compliance with ethical guidelines

The research was considered by the Ethical Committee of the Shakarim University of Semey and accepted it as the research in the norms and principles of the Declaration of Helsinki Ethical Principles (Protocol No.: 3, 24 October, 2024).

Data availability

Supplemental or other research data can be obtained from the corresponding author after reasonable request.

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Authors' contributions

Conceptualization and methodology: Zhanat Nurzhumanova and Shyngys Suleimenov; Data collection, investigation and writing the original draft: Zhanat Nurzhumanova, Nurlygul Yessengulova, and Leila Kassymbekova; Review and editing: Assel Zhexenayeva, Altyn Zhubantaeva, and Shyngys Suleimenov; Data analysis: Zhanat Nurzhumanova, Assel Zhexenayeva, Altyn Zhubantaeva, and Shyngys Suleimenov; Supervision: Zhanat Nurzhumanova.

Conflict of interest

The authors declared no conflict of interest.

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