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Efficacy of a modified albendazole and ivermectin formulation on treatment and the seasonal dynamics of sheep helminthosis

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ABSTRACT

The aim of this study was to develop a complex antiparasitic compound containing albendazole (ABZ) and ivermectin (IVM) based on solid dispersion to increase the bioavailability of the active substances, to evaluate the efficacy of the formulation, and to monitor the effect of its use on the seasonal dynamics of sheep helminthosis for one year. To obtain the complex, a mechanochemical technology with joint grinding of ABZ, IVM, and polymer (polyvinylpyrrolidone, PVP) was used. The formulation was then used to study its efficacy in sheep helminthosis using coprological methods. Additionally, the sheep were treated both in spring and autumn to study the seasonal dynamics of infection with nematodes, cestodes, and trematodes. The suspension, administered at a rate of 0.2 mg IVM and 3 mg ABZ/kg body weight, showed 100% efficacy against the entire complex of helminths in both spring and autumn treatments. Furthermore, sheep admitted to winter stall maintenance presented fairly low rates of extensiveness (10.0–13.7%) and intensity (9.5–14.7 EPG) of infection with all types of helminths. The present study showed that administrations of the newly formulated anthelminthic (CAPS-AI) twice in the year, i.e., in the spring (before going out to pasture) and in the fall (before placing them in stalls), ensured a relatively low level of sheep helminthoses, making it possible to prevent the clinical manifestation of helminthosis and subsequently economic losses.

Introduction

One of the factors negatively affecting the development of livestock farming is infectious diseases, in particular helminthic infections (7, 8). It is known that depending on the natural and geographical conditions of the area and the organization of treatment and preventive measures in different years, certain types of zooparasites may dominate. Therefore, there is a need for constant monitoring of the epizootic situation regarding parasitic infections, and the efficacy of control measures (8, 18, 19, 20, 22).

Sheep helminthoses, in most cases, are present in the form of mixed infections; therefore, the use of complex drugs with a wide spectrum of antiparasitic activity is justified. These drugs mainly contain benzimidazoles (e.g., albendazole, ABZ) and macrolides (e.g., ivermectin, IVM) (5, 6), the main disadvantage of which is low solubility in water (32). Various physical and chemical methods have been developed to improve the solubility of these substances (33). The spread of mechanochemistry methods to combine several substances has led to the production of so-called complex drugs, the use of which allows affecting the entire spectrum of parasites, which implies a reduction in the volume of drug use and the frequency of manipulations with animals (21, 23, 24). Notably, we have introduced a technology for the mechanochemical modification of antiparasitic substances using lipophilic polymers, which enables the preparation of solid dispersions (SDs) with enhanced solubility, improved bioavailability, and high efficiency (12). Continuing research in this direction has made it possible to significantly reduce the dose of the active substance (AS) without reducing its effectiveness. For instance, we showed that SD based on a composition of ABZ and water-soluble polymers at a rate of 3 mg per kg of body weight (BW) provides 100% effectiveness against different helminths of sheep and horses (25, 26). This study aimed to develop an optimal complex antiparasitic compound containing ABZ and IVM with the highest water solubility of the active substances, to evaluate the efficacy of the formulation after oral treatment, and to monitor the effect of its use on the seasonal dynamics of sheep helminthosis during one year.

Materials and Methods

To obtain the SD of ABZ or IVM with polyvinylpyrrolidone, the following AS and materials were used:

- ABZ - methyl [5-(propylthio)-1H-benzimidazol-2-yl]-carbamate. Substance (99%) produced by Changzhou Jialing Medicine Industry Co., Ltd (Changzhou, China). Batch No.: 200611. Solubility in water: 0.76 mg/L.
- IVM – substance (sodium 97.5%) produced by Shandong Qilu King-Phar Pharmaceutical Co., Ltd. (Shandong, China). Batch No.: 0316040073. Solubility in water: 4.0 mg/L
- Polyvinylpyrrolidone (PVP) KoVidone*K30 produced by Boai NKY Pharmaceuticals Ltd. (Boai, China). Batch No.: GH240528182. Molecular weight Mw: ~ 30 kDa.
- Sodium carboxymethyl cellulose (Na-CMC; CEKOL® 700) produced by CP Kelco (Finland). Batch No.: AA6297891.CAS 9004-32-4.

Mechanochemical technology for the preparation of SDs: The process of obtaining an SD of the composition ABZ: PVP (1:9) was carried out in a drum mounted on LE-101 roller mill with an 800 mL volume with joint (weight ratio of ABZ to PVP as 10.0 g / 90.0 g) mechanical grinding of components using 32 steel balls (diameter 25 mm, ball weight 54 g). The pre-mixed components were immersed in a drum, which was mounted on mill rollers rotating at a speed of 70–75 rpm. Samples of the resulting SD were taken after 2, 4, and 5 hours to analyze changes in ABZ solubility. Upon completion of the mechanical treatment process, quantitatively free-flowing SD powder with the composition ABZ: PVP (1:9) was unloaded from the drum (9). Similarly, SD of the composition IVM: PVP (1:9) was obtained from 10.0 g of IVM and 90.0 g of PVP.

It should be mentioned that the optimal ratio of AS (i.e., ABZ, IVM) and polymer (i.e., PVP) was selected based on our extensive experience in previous studies (12, 13), Based on these studies, after testing AS: PVP ratios of 1:2 to 1:20 we found the ratio 1:9 optimal in terms of solubility parameters, economic, toxicological indicators and anthelmintic activity. However, in some studies to obtain more concentrated compositions, we also used compositions of 1:4 and 1:5, in particular in the case of substances that have a solubility of about or more than 10 mg/L, e.g., IVM and praziquantel.

Study of physical and chemical properties of SDs: The obtained SDs of ABZ and IVM were analyzed to confirm the content of ABZ and IVM in the obtained material and the corresponding suspensions, since we previously demonstrated the possibility of the destruction of fenbendazole during its mechanochemical modification in an organic solvent environment (13, 14). To assess changes in solubility, the SD sample of ABZ: PVP (1:9) was analyzed using reverse-phase High Performance Liquid Chromatography (HPLC). Briefly, a 0.5 g sample was dissolved in 10 mL of distilled water and stirred in a shaker-incubator at 25°C and 180 rpm for 3 hours. After incubation, the suspension was centrifuged, and the concentration of ABZ in the supernatant was measured. The analysis was performed on an Agilent 1100 Diode Array with Analytical Column Separon SGX C18 (150×3.3 mm, 5 µm) at 30°C. The mobile phase consisted of acetonitrile and acetate buffer (pH 3.4) in a 1:1 ratio, applied in isocratic mode with a flow rate of 1 mL/min. The injection volume was 1 µL, and detection was carried out at a wavelength of 230.8 nm. Results were compared against a standard ABZ solution prepared in DMSO (Chemically pure STP TU KOMP 2-451-11 supplied by Komponent-Reaktiv, Moscow, Russia).

The structure of the obtained SDs was also confirmed using the following physicochemical methods (12):

- Infrared spectroscopy (IR) study was carried out on an ultraviolet-visible spectrophotometer (Shimadzu 2600, Shimadzu Corporation, Kyoto, Japan) confirmed the chemical stability of the ABZ substance, and the shift of its characteristic bands confirmed the formation of inclusion complexes.

- X-ray diffraction phase analysis was carried out on a DRON-4 diffractometer (Bourevestnik, Saint Petersburg, Russia) using CuKα radiation confirmed the loss of crystallinity of the ABZ substance and micronization of the particles of the original components.

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- Scanning electron microscopy (SEM), carried out on a desktop electron microscope Hitachi TM 4000 Plus, showed that significant micronization of ABZ and PVP particles was detected during mechanical processing, namely, crushing of spherical PVP particles (2–300 µm) with a decrease in the average particle size with increasing duration of mechanical treatment (39 µm for 1 hour, 25 µm for 2 hours, 19 µm for 3 hours). In this case, ABZ particles (~5 µm) were homogeneously distributed on the PVP surface.

Preparation of the newly formulated antiparasitic complex (CAPS-IA) suspension: By physically mixing SDs of ABZ and IVM, taken in weight ratios of 15:1 (due to the parasiticidal activity of the medicinal substances), with the addition of 1% Na-CMC to the mixture, a dry suspension concentrate (CAPS-IA) was obtained, which was stored in a hermetically sealed container. Approximately 30–60 minutes before use, the concentrate was mixed with water in weight ratios of 1:9 to form a 10% stable viscous suspension, which was not transformed within 3 days. The suspension was fed to sheep individually based on the active substance per animal weight.

Study of the parasiticidal activity: The efficacy studies of the CAPS-IA suspension were conducted during 2023–2024 on a flock of sheep at the Altai Experimental Agriculture Organization in the Shebalinsky district of the Altai Republic, Russia. The model flock of sheep was formed from young animals born last year (10–12 months), the age group most susceptible to infestation with helminths. The conditions of sheep farming and the species composition of animal helminths in the Shebalinsky district are typical for the territories of the Central and Northern Altai; the results of studies on this model flock can be extrapolated to all farms in the region. Coprological sampling was performed every two months, collecting 20–25 fecal samples per session randomly, as a representative of the flock, for ovolarvoscopic examination to determine the level and structure of helminth infections.

To detect gastrointestinal helminth eggs, a flotation method using ammonium nitrate solution (specific gravity ~1.3) was applied. Pulmonary helminths were assessed using the Baermann technique, while *Dicrocoelium* eggs were detected using a sequential sedimentation method. Egg and larval counts were conducted using a VIGIS chamber, functionally equivalent to the McMaster counting chamber (16).

The suspension CAPS-IA was prescribed for helminthosis on 108 heads of sheep in the form of a 10% aqueous suspension per kg of BW, according to AS: 0.2 mg IVM and 3 mg ABZ. This dose was proved to have the highest parasiticidal activity in previous studies (23, 25).

The effectiveness assessment was carried out according to the “Critical Test” method, after preliminary helminth-ovoscopic examinations; the control in the experiment was the indicators of infection of animals before treatment. The parasiticidal effectiveness was assessed by examining feces using ovo- and larvoscopy 20 days after using the drug in accordance with established guidelines for follow-up efficacy evaluation (16). Based on the results of coprological examinations, infection indicators were derived: EI, % – extent of infection, proportion of infected animals; AI is the arithmetic mean of the number of eggs or larvae (in case of lungworm) in 1 gram of feces (EPG) per one examined animal. Based on the EI values of individual helminth taxa, the structural index of the helminth complex was calculated (19). To assess the parasiticidal activity of the drugs, the following indicators were calculated: EE, % – extensive effectiveness, the proportion of animals freed from parasites in relation to the control (untreated); IE% – intensity efficiency, reduction in the average number of eggs relative to the control. The number of *Moniezia* spp. eggs in samples are conventionally taken as an indirect variable indicator of infection.

Statistical Analysis: Since the helminth infection rates were over-dispersed, the nonparametric Mann–Whitney U test was used to compare the number of eggs or larvae in the fecal samples. Empirical and critical values of the U test of samples (Uemp/Ucrit) with the reliability of the difference in the infection intensity (II) rates at $P \leq 0.05$ were calculated. Calculations were performed using SAS/Stat software (SAS ver. 9, System for Windows).

Results

The resulting SDs of ABZ and IVM were obtained after mechanical treatment (m/t) as free-flowing beige powders with significantly enhanced water solubility as determined by HPLC, comparing the areas of the chromatograms of the original ABZ and IVM with their SD (Figure 1). Analysis of the data in Figure 1 showed an increase in the solubility of ABZ and IVM in their SDs, which is shown in Table 1. Such an increase in solubility suggested a corresponding increase in biological activity, as we have shown in our previous studies (12, 13). Therefore, these SDs were used to prepare the dosage form in the suspension CAPS-IA. These dispersions were then used to formulate the CAPS-IA complex antiparasitic suspension by combining them with an aqueous solution containing 1% Na-CMC. As expected, the mechanochemical modification of selected substances with PVP increased their solubility significantly, which is explained by the lipophilic properties of PVP (13). Table 1 summarizes the solubility profiles of the pure substances (ABZ and IVM) and their corresponding SDs with PVP.

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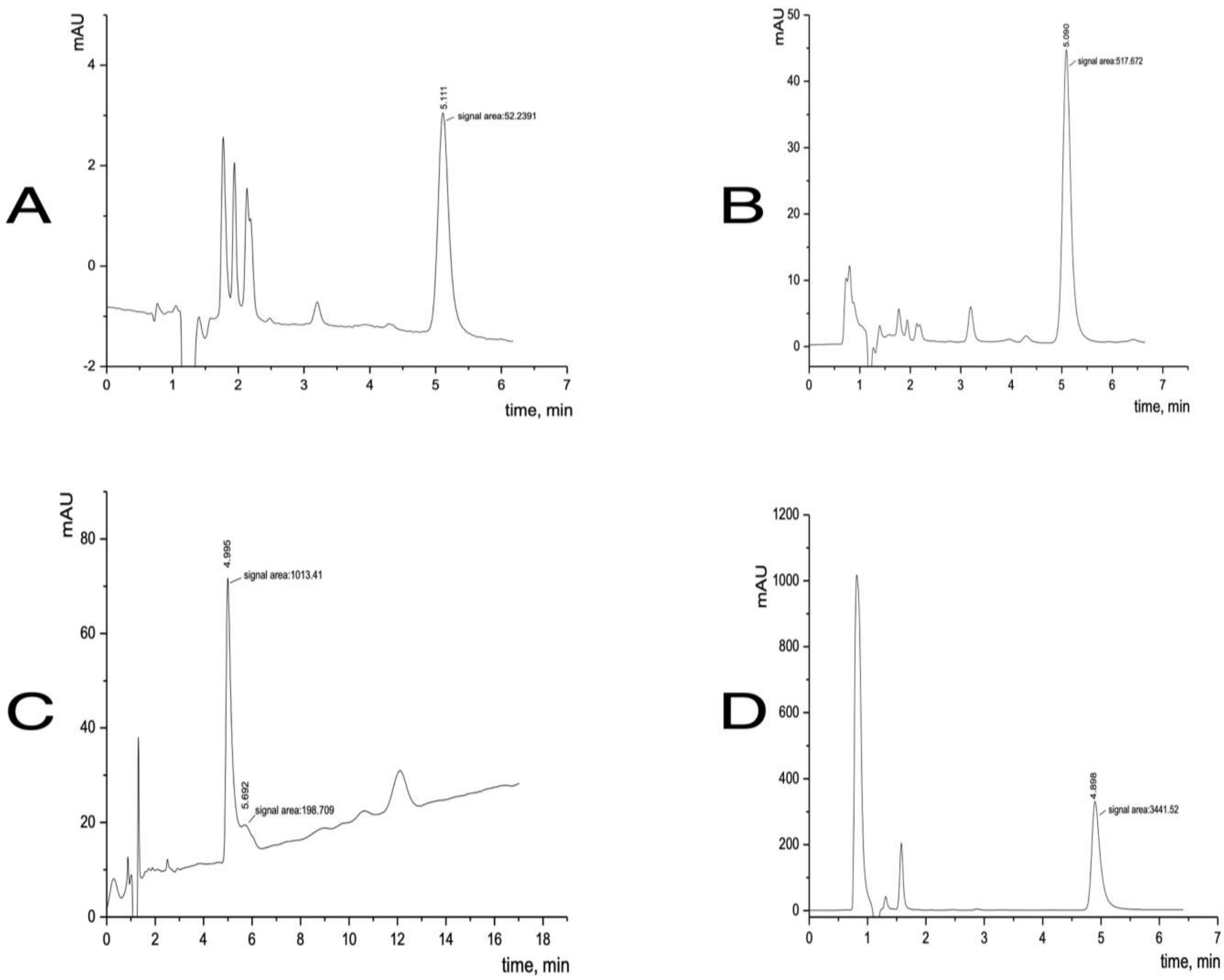

Figure 1. High Performance Liquid Chromatography (HPLC) chromatograms of the initial ivermectin (A), solid dispersion of ivermectin: polyvinylpyrrolidone (1:9) composition after 2 hours of m/t (B), the initial albendazole (C), solid dispersion of albendazole: polyvinylpyrrolidone (1:9) composition after 5 hours of m/t (D).

Table 1. The solubility of albendazole (ABZ), ivermectin (IVM) substances, and their solid dispersions (SD) with polyvinylpyrrolidone (PVP).

No	Samples and methods for obtaining them	Solubility	
		absolute mg/L	Increasing ratio
	SD of ABZ		
1	ABZ initial substance	0.76	–
2	Physical mixture composition ABZ:PVP (1:9), without m/t*	1.5	2.0
3	SD of composition ABZ:PVP (1:9), m/t 2h	5.9	7.8
4	SD of composition ABZ:PVP (1:9), m/t 4h	9.7	12.8
5	SD of composition ABZ:PVP (1:9), m/t 5h	9.8	12.9
	SD of IVM		
1	IVM initial substance	4.0	–
2	Physical mixture composition IVM:PVP (1:9), without m/t	8.8	2.2
3	SD of composition IVM:PVP (1:9), m/t 2h	36.4	9.1
4	SD of composition IVM:PVP (1:9), m/t 4h	55.8	14.0
5	SD of composition IVM:PVP (1:9), m/t 5h	59.3	14.8

*- mechanical treatment (m/t).

The data from the study of the IR spectra of ABZ, PVP, their physical mixture (without mechanical processing), and their SD composition ABZ: PVP (1:9) are shown in Figure 2. Analysis of the spectra shows that no noticeable shifts in the absorption bands are observed after mechanical processing, but only a decrease in the relative intensity of the ABZ bands compared to the intensity of the polymer bands, which may indicate the formation of inclusion complexes and is consistent with an increase in solubility. The data of X-ray phase analysis confirmed the loss of crystallinity of the ABZ substance and micronization of the particles of the original components. From these data, it follows that there is a loss of crystalline phase in the mechanically processed mixture (Figure 3). It is known from the literature (31, 33) that a decrease in crystallinity and micronization of drug substances leads to an increase in their solubility in water, bioavailability, and pharmacological activity.

The data of SEM showed that the substance of ABZ was a partly amorphized powder with an average particle size of 20–30 μm, and PVP consisted of spherical particles with an average size of 10–100 μm. Under mechanochemical processing, a destruction of ABZ and PVP particles occurred, followed by the formation of polydisperse powder, mainly consisting of particles of irregular shape and size of 5–20 μm and their aggregates (Figure 4).

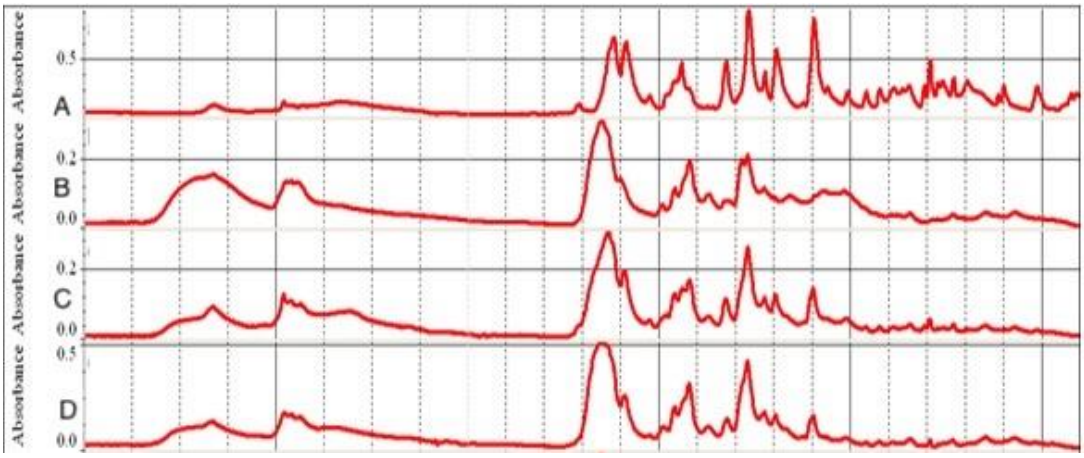

Figure 2. Infrared spectroscopy spectra of different substances examined in this study. A) albendazole, B) polyvinylpyrrolidone, C) physical mixture of albendazole and polyvinylpyrrolidone, D) solid dispersion of albendazole: polyvinylpyrrolidone (1:9).

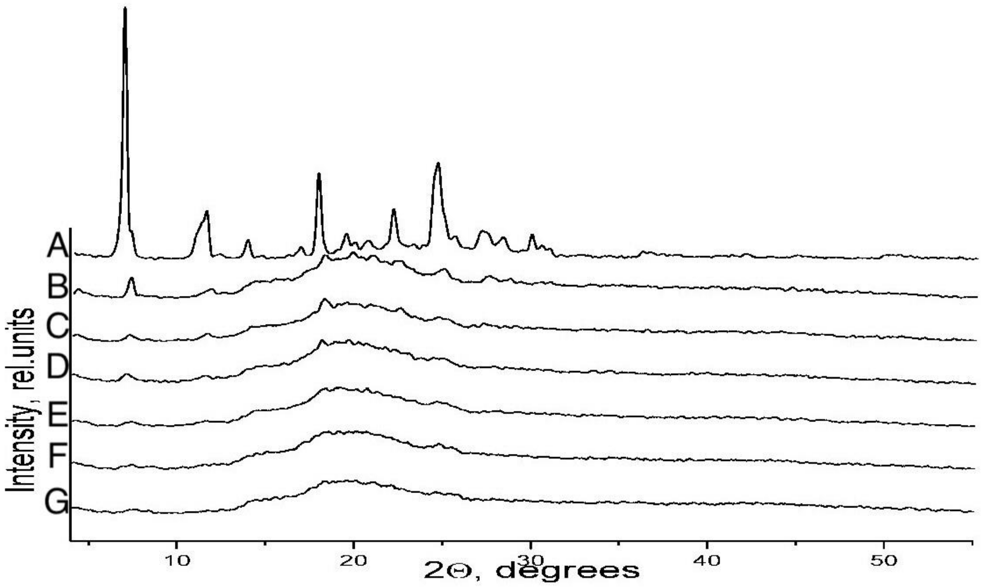

Figure 3. X-ray diffraction patterns of different substances examined in this study using DRON. A) albendazole. B) physical mixture of albendazole and polyvinylpyrrolidone, solid dispersion of albendazole: polyvinylpyrrolidone (1:9) after mechanical treatment in the mill for 1h (C), 2h (D), 3h (E), 4h (F), 5h (G).

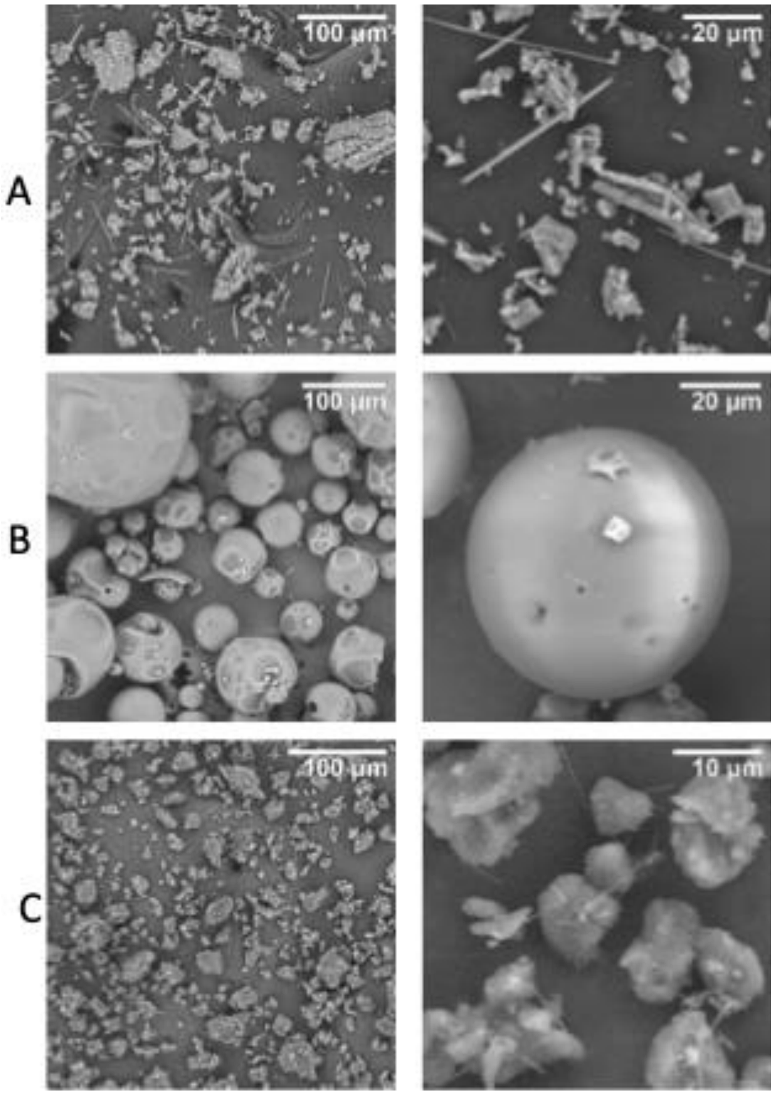

Figure 4. Scanning electron microscopy micrographs of different substances examined in this study. A) albendazole, B) polyvinylpyrrolidone, C) solid dispersion of albendazole: polyvinylpyrrolidone composition 1:9 after mechanical treatment in the mill for 5 hours.

Study of the parasiticidal activity: The parasiticidal effectiveness of the drug is presented in Table 2. Suspension CAPS-IA at a dose of 0.8 mL, based on AS: 0.2 mg IVM and 3 mg ABZ per kg BW in both spring and autumn treatments, showed 100% effectiveness against the entire complex of helminths.

The seasonal dynamics of helminth infections in sheep are shown in Table 3, which presents coprological monitoring data collected during a 35-day period after treatment in May and a 40-day period in October. The extent of infection of sheep with helminths of the suborder Strongylata during the use of parasiticide during the year ranged from 10.0 to 65.0%, with an AI from 9.5 to 253.6. A statistically significant increase in the number of gastrointestinal strongyles eggs in fecal samples was observed in March-May (Uemp 14.5/Ucrit 19.5; $P \leq 0.05$ and 29.5/30; $P \leq 0.01$) and the summer-autumn periods, i.e., August-October (0/6; $P \leq 0.01$ and 16.5/18; $P \leq 0.05$). Infection by intestinal nematodes of the genus *Trichuris* fluctuated at an insignificant level from 10.0 to 35.0%. Eggs of *Moniezia* spp. and *Dicrocoelium* spp. were detected in the summer; their infection reached 25%, and the maximum number of eggs was recorded in August (1/2; $P \leq 0.05$). Infection of sheep with pulmonary strongyles of the genus *Protostrongylus* throughout the year was at a relatively low level (1.9–25.0%) with no statistically meaningful difference. According to the results of ovolarvoscopic studies, in the annual structure of the helminth complex (Figure 5), the share of nematodes is 88.2, trematodes – 4.8, and cestodes – 7%.

Figure 6 shows the variation in helminth infection rates over time following treatment with CAPS-IA. In the winter–spring period, the overall extent of helminth infection remained high, reaching 75.0% in May. Following CAPS-IA therapy, a notable reduction was observed, i.e., by June, the infection rate had dropped to 45%. This decline demonstrates the efficacy of treatment, although some reinfection likely occurred due to pasture exposure. By the end of the grazing season (October), 65% of the animals were infected with helminths. Following

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treatment, however, the extent of infection of sheep with all helminths was 10.0% in November and 13.6% in December, with a composition of 10% gastrointestinal strongylids and 5.0% protostrongyles. As a result, animals entered the winter with a low rate of infection intensity, although in January, a slight increase in the rate (20.0%) was observed.

Figure 7 illustrates the monthly dynamics of helminth infection intensity (measured as eggs and larvae per gram of feces) in sheep treated with CAPS-IA. During the winter–spring period (January to May), the total infection intensity ranged between 35.9 and 104.4 EPG. Following the first treatment, the intensity dropped to 44.4 by June (6/13; $P \leq 0.01$). However, a mild increase was noted in July, reaching 71.5 EPG (3.5/6; $P \leq 0.05$). By the end of the grazing season, the infection intensity peaked at 296.3 EPG (4/21; $P \leq 0.01$ and 17.5/19.5; $P \leq 0.05$). Thirty-five days after the second CAPS-IA therapy in October, a marked decrease was observed in November, with intensity falling to 9.5 EPG (3.5/25; $P \leq 0.01$). Fifty days later, in December, a slight increase was recorded, with a total intensity of 14.7 EPG, comprising 12.8 from gastrointestinal strongylids and 1.9 from *Protostrongylus* spp. Although the animals entered winter with relatively low parasite loads, a modest resurgence was detected in January, with infection intensity rising to 31.3 EPG.

Table 2. Efficacy of the newly formulated suspension (CAPS-AI) against sheep parasitosis according to different variables.

	Animal group	Feeding period	Quantity treatments	*n* examined animals	Infected EI[a] %	Avg. EPG[b]	EE[c] %	IE[d] %
Gastrointestinal strongyles infections								
1	Before treatment	May	108	20	65.0	87.6±11.3	-	-
2	After treatment	– \\ –	-	20	0.0	0.0	100	100
3	Before treatment	October	105	20	45.0	253.6±33.2	-	-
4	After treatment	– \\ –	-	20	0.0	0.0	100	100
Trichurosis								
5	Before treatment	May	108	20	35.0	8.5±3.6	-	-
6	After treatment	– \\ –	-	20	0.0	0.0	100	100
7	Before treatment	October	105	20	10.0	2.3±1.6	-	-
8	After treatment	– \\ –	-	20	0.0	0.0	100	100
Monieziosis								
9	Before treatment	October	105	20	20.0	15.2±4.8	-	-
10	After treatment	– \\ –	-	25	0.0	0.0	100	-
Dicrocoeliosis								
11	Before treatment	October	105	20	15.0	8.1±3.9	-	-
12	After treatment	– \\ –	-	25	0.0	0	100	100
Pulmonary strongylosis								
13	Before treatment	October	105	20	25.0	10.0±2.5	-	-
14	After treatment	– \\ –	-	25	0.0	0.0	100	100

[a] EI: extent of infection, [b] EPG: egg per gram of feces,[c] EE: extensive effectiveness, [d] IE: intensity efficiency.

Table 3. Monthly dynamics of helminthoses in sheep treated with the newly formulated suspension (CAPS-AI) in May and October.

Month	*n*	Gastrointestinal strongyles*		*Nematodirus*		*Trichuris*		*Moniezia*		*Dicrocoelium*		*Protostrongylus*	
		EI[a],%	AI [b]	EI,%	AI	EI,%	AI	EI,%	AI	EI,%	AI	EI,%	AI
February	20	50.0	18.5	15.0	4.7	35.0	5.7	0	0	0	0	15.0	7.0
March	20	50.0	41.2**	20.0	4.9	30.0	7.2	0	0	0	0	20.0	6.0
May	20	65.0	87.6*	20.0	3.8	35.0	8.5	0	0	0	0	20.0	4.5
June	20	25.0	30.8	10.0	1.4	15.0	1.4	0	0	15.0	6.6	15.0	4.2
July	20	30.0	31.8	0	0	20.0	6.6	15.0	18.3	20.0	10.5	15.0	4.3
August	20	40.0	186.5**	25.0	6.5	35.0	8.1	25.0	20.5	25.0	21.5*	20.0	6.3
October	20	45.0	253.6*	15.0	7.1	10.0	2.3	20.0	15.2	15.0	8.1	25.0	10.0
November	20	10.0	9.5**	0	0	0	0	0	0	0	0	0	0
December	20	10.0	11.4	5.0	1.4	0	0	0	0	0	0	5.0	1.9
January	25	16.0	21.8	8.0	1.9	12.0	2.4	0	0	0	0	12.0	5.2
Total	205	34.1	69.3	11.8	3.2	19.2	4.2	6.0	5.4	7.5	4.7	19.4	4.5

[*]Gastrointestinal strongyles excluding *Nematodirus*; difference from the previous month, [a] EI: extent of infection, [b] AI: extent of infection, arithmetic mean of the number of eggs or lungworm larvae in 1 gram of feces. *: $P \leq 0.05$, **: $P \leq 0.01$.

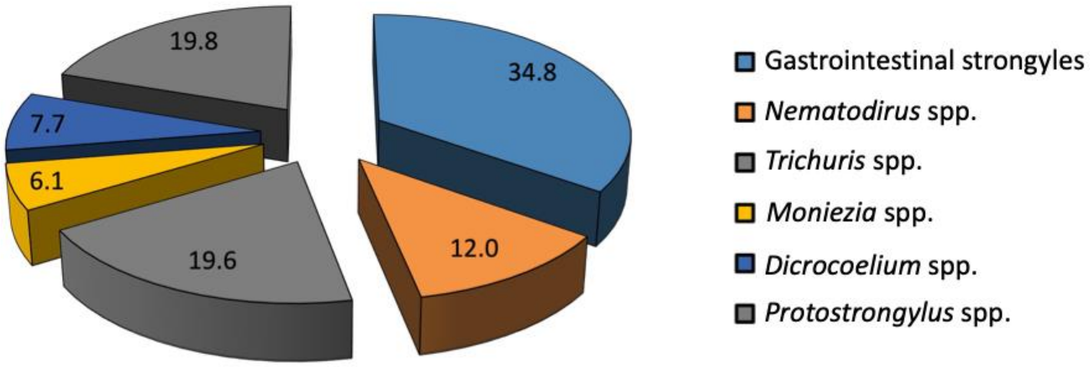

Figure 5. The average annual structure of the helminth complex of sheep in this study.

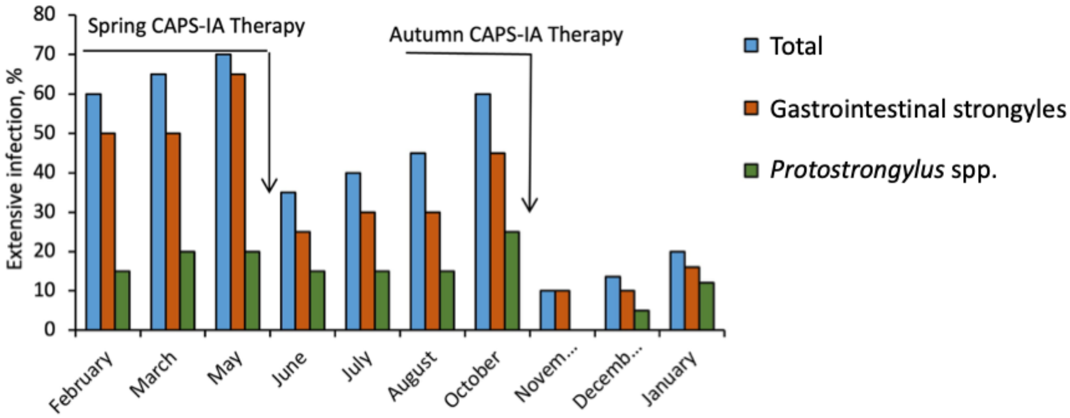

Figure 6. Dynamics of the abundance of sheep helminthoses during the study period.
CAPS-AI is the newly formulated suspension.

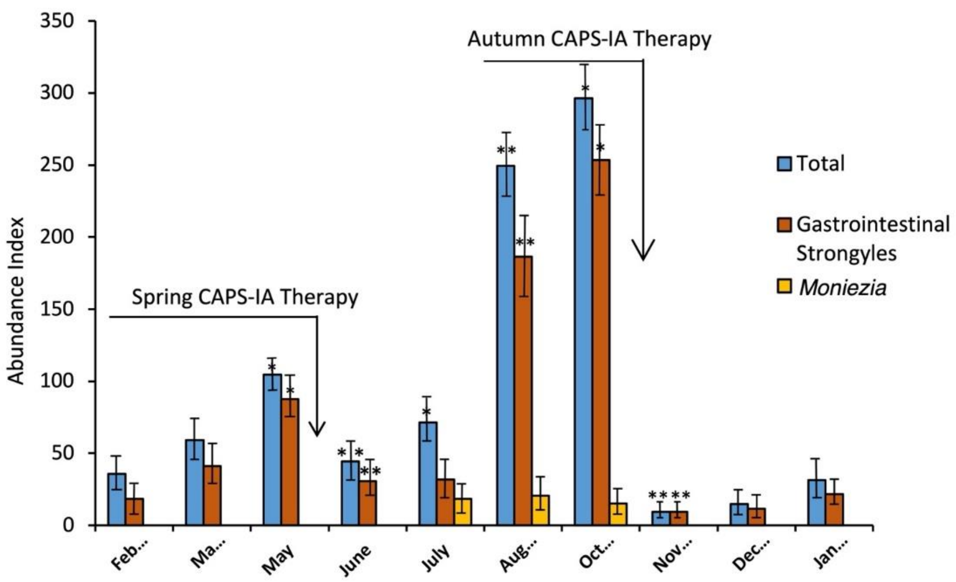

Figure 7. Dynamics of the intensity of infection of sheep according to different helminthoses.
Difference from the previous month * $P \leq 0.05$, ** $P \leq 0.01$. CAPS-AI is the newly formulated suspension.

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Discussion and Conclusion

The present study showed that administrations of our newly formulated anthelminthic CAPS-AI twice in the year, i.e., in the spring (before going out to pasture) and in the fall (before placing them in stalls), ensured a relatively low level of sheep helminthoses, making it possible to prevent the clinical manifestation of helminthosis and subsequently economic losses. In particular, we showed that SDs of ABZ and IVM with PVP increased solubility, almost 12–15 times higher compared to that of the active substances, and were highly effective for the control of various endoparasite taxa, including gastrointestinal nematodes, lungworm, lancet liver fluke, and tapeworm. This increase in the bioavailability of the obtained SD and CAPS-IA, and therefore their biological activity, was repeatedly observed in previous studies (23). Similarly, oral administration of drugs based on mechanically modified AS macrolides and benzimidazoles for helminthosis in farm animals invariably demonstrates high parasiticidal activity (1, 24, 28). In line with these expectations, the CAPS-IA aqueous suspension, containing 0.2 mg IVM and 3 mg ABZ per kg BW, achieved 100% effectiveness against the primary helminth species complex in sheep, according to ovolarvoscopy results from both spring and autumn treatments. It should be noted that the absence of eggs or larvae in feces does not mean 100% removal of the larval stages and arrested hypobiotic larvae, as surviving individuals will continue their function in the infection process.

A key prerequisite for the rational planning of antiparasitic control strategies is a clear understanding of both the taxonomic composition of the helminth community and the seasonal dynamics of infection. In our study, conducted in the Shebalinsky district, we found that nematodes comprised the majority of the helminth fauna in sheep, accounting for 86.2%. In contrast, trematodes represented 7.7% and cestodes 6.1% of the total helminth population (Figure 5). Gastrointestinal strongylids were the dominant group in terms of structural index values, accounting for 46.8% of the total. Pulmonary strongylids (19.8%) and *Trichuris* spp. (19.6%) were also present in notable proportions, while *Dicrocoelium* spp. and *Moniezia* spp. were found at lower levels, representing 7.7% and 6.1%, respectively. Similar structural patterns of helminth infection had previously been recorded in sheep raised in the Altai Mountains (7, 21).

During the year, the infection of sheep with gastrointestinal nematodes of the order Strongylida ranged from 25 to 65%, with a significant increase in AI indicators in the spring and autumn, with a maximum of 253.6 EPG in October. In addition, the analysis showed that sheep were infected with *Trichuris* spp. (10–35%), eggs of *Moniezia* spp. and *Dicrocoelium* spp. were found in the summer-autumn period, and their infection reached a maximum of 25% in August. Sheep were infected with *Protostrongylus* spp. from 5 to 25%. The average annual level of infection intensity in sheep by gastrointestinal strongyles was 34.1%, *Trichuris* spp. 19.2%, *Moniezia* spp. 6.0%, *Dicrocoelium* spp. 7.5% and *Protostrongylus* spp. 19.4%. All these indicate a relatively low level and pronounced seasonal dynamics of animal infection with helminths in farms in the South of Siberia, where the transhumance-pasture system of sheep farming is practiced (winter and summer pastures). Similarly, in sheep farms of the region, with similar natural and climatic conditions, but with different nature of pasture use and the degree of anthropogenic pressure, significant differences in animal infection with helminths are noted (21). However, in other regions of the planet, in areas with more favorable conditions for the development of helminths, a higher level of parasite numbers is formed with less pronounced seasonal fluctuations in infection (30, 32).

Conducted studies of the helminth complex of sheep indicate that when carrying out antiparasitic measures, it is necessary to pay special attention to intestinal and pulmonary strongylosis, monieziosis, and dicrocoeliosis, which need the use of drugs with a wide spectrum of parasiticidal action. At the same time, the use of a complex drug with different mechanisms of action of substances (benzimidazoles and macrolides) makes it possible to slow down the process of developing resistance of helminths to therapeutic agents (27, 29). The use of such drugs against the backdrop of a unified approach to their use can significantly optimize the system of antiparasitic measures. The implementation of this approach inevitably leads to a reduction in the volume of therapeutic agents, the frequency of manipulations with animals, and, in general, the costs of antiparasitic measures. At the same time, such an approach involves regular monitoring of the infected individuals, the results of which regulate the nature of intervention in the parasitic system, i.e., preparations, timing, frequency, etc. In sheep farms in the region, under favorable epizootic conditions for major helminthoses, a scheme of double (spring and autumn) use of complex therapeutic agents with a wide spectrum of action can be implemented on young animals of the current and previous year of birth, and adult ewes over 2 years old as a refugium source for preserving helminths susceptible to parasiticidal drugs. It is widely accepted that the creation of refugia when controlling helminthic infections of ruminants significantly slows down the development of resistance in helminths to parasiticidal drugs (10, 15).

Forty-five days after spring therapy, the infection of animals with helminths quickly recovered due to infection on the pasture and reached EI 35.0%, AI 44.4%. The rapid restoration of the level of helminth infection on a summer pasture is a natural process and is characteristic of other farm animals (17). At the end of the pasture season, infection rates reached EI 65%, AI 296.3, and after autumn therapy in October, there was a slow rise, but in January of the following year, they decreased to EI 20.0% and AI 31.3. Since at the start of winter, animals showed low extensity and intensity rates, the further slight increase could be due to the development of surviving larval forms of parasites capable of hypobiosis.

As a limitation, we did not study the pharmacokinetic properties of the CAPS-IA solution and modified IVM and ABZ individually. Furthermore, the long-term evaluation of eggs/larvae reappearance period as an early indicator of anthelmintic resistance development (3) will be advantageous in understanding whether the administration of mechanochemically modified formulations would also be helpful in the prevention or slowdown of anthelminthic resistance. The conducted complex of physicochemical studies of the obtained SDs of ABZ and IVM with PVP showed that their joint mechanical treatment allowed not only to micronize the components, reduce the degree of their crystallinity, but also to form inclusion complexes of the "drug-polymer" type with increased solubility in water, high bioavailability and parasitological activity. Such a scientific and practical approach made it possible to develop a suspension dosage form, CAPS-IA, which is convenient for use and has shown a high parasitological effect. The experiment conducted on a model flock to control animal infestation with helminths by double therapy with complex parasitocidal agents can be recommended and implemented by veterinary practice in the region.

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Ethical Statement

The study was conducted in accordance with the requirements of the European Convention for the Protection of Vertebrate Animals used for Experimental and Scientific Purposes (ETS No. 123, Strasbourg, 1986), and with the approval of the FAEC Commission on Biological Ethics (210, 12.09.2022).

Conflict of Interest

The authors declared that there is no conflict of interest.

Author Contributions

VM and SK conceived and planned the experiments. IB and DK carried out the parasitological experiments. MK, SK, and MI carried out the chemical experiments and analyses of solid dispersions. VM and AS contributed to the interpretation of the results. VM took the lead in writing the manuscript. SK and AS writing: original draft, review & editing. All authors provided critical feedback and helped shape the research, analysis, and manuscript.

Data Availability Statement

All data generated or analyzed during this study are included in this published article. Any additional data are available from the corresponding author (S.K.) on request.

Animal Welfare

The authors confirm that they have adhered to ARRIVE Guidelines to protect animals used for scientific purposes.

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