Part 1. Veterinary medicine

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THE EFFECTIVENESS OF CURRENT TREATMENT METHODS FOR COCCIDIOSIS IN PIGS

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Summary. Intestinal dysbiosis and coccidiosis are common conditions in piglets, characterized by disturbances to the microbial community and reduced productivity in animals. Consequently, there is an urgent need to identify effective treatment regimens that eliminate pathogens, restore the intestinal microbiota, and enhance the body's resistance. This study aimed to develop and compare a comprehensive treatment regimen involving sulfadimethoxine and a *Bacillus* spore-forming probiotic for suckling piglets with coccidiosis. Piglets suffering from coccidiosis in the first experimental group were administered sulfadimethoxine orally at a dose of 25 mg/kg once daily for five days. Animals in the second experimental group were administered sulfadimethoxine at the same dose and a *Bacillus* spore-forming probiotic at a dose of two milliliters per animal for 21 days. The results demonstrate that including the *Bacillus* spore-forming probiotic in the treatment regimen normalizes the intestinal microbiota, as evidenced by a reduction in opportunistic microflora and a significant increase in obligate anaerobe populations. Complete clearance of coccidia and elimination of *Candida albicans* was achieved in the second experimental group (sulfadimethoxine + *Bacillus*), confirming a pronounced therapeutic effect compared to monotherapy (sulfadimethoxine)

Keywords: piglets, gut microbiota, sulfadimethoxine, *Bacillus* spore-forming probiotic

Introduction. Coccidiosis is a common parasitic disease in pigs that often goes unnoticed in its early stages. However, its impact on the health of piglets and the overall economics of pig farming can be quite significant. It is a protozoan disease primarily caused by *Cystoisospora suis*, which affects the intestinal mucosa of young piglets, leading to impaired absorption, stunted growth, and increased susceptibility to secondary infections (Hinney et al., 2020; Jankowska-Makosa et al., 2023; Bohach, Paliy and Bogach, 2024).

Despite the low mortality rate of pigs from coccidiosis, it can lead to significant economic losses due to reduced daily weight gain (up to a 20% loss in body weight), poor feed conversion, and deteriorated performance indicators in infected animals during subsequent stages of production (Bogach, Paliy and Bogach, 2022; De Alencar Rezende et al., 2026). Furthermore, coccidiosis directly compromises piglets' immunity. It increases the risk of infection with other enteropathogens, such as rotavirus, transmissible gastroenteritis virus, *Clostridium*, and *Escherichia coli*, leading to a more severe clinical course and increased mortality (Chaudhary, Parajuli and Dhakal, 2023; Bohach et al., 2023; Han et al., 2024).

One of the traditionally used strategies for controlling bacterial and protozoal infections in piglets is the use of sulfonamide antibiotics, such as sulfadimethoxine, a broad-spectrum antimicrobial agent that acts bacteriostatically by competitively inhibiting folic acid synthesis (Joachim and Mundt, 2011). Against the backdrop of antimicrobial use, the use of probiotics has become widespread (Gujvinska and Paliy, 2018; Zhao et al., 2025). Probiotic microorganisms of the genus *Bacillus* are of interest as a means of optimizing the intestinal microbiota, increasing resistance to pathogens, and improving growth parameters. *Bacillus* probiotics — spore-forming strains such as *B. subtilis, B. pumilus, B. amyloliquefaciens*, and others — are capable of surviving in the aggressive conditions of the digestive tract, potentially improving the structure of the intestinal mucosa and enhancing barrier and enzymatic functions (Vieira et al., 2021; Mazur-Kuśnirek et al., 2023; Tang et al., 2024).

The combined use of sulfadimethoxine and *Bacillus* probiotics for piglets is a comprehensive approach. This drug has antiparasitic and antimicrobial effects and is aimed at eliminating or suppressing pathogenic microflora and parasites, while probiotics help maintain and restore beneficial gut microbiota, especially after antibiotic therapy. This strategy may be accompanied by a reduction in the negative consequences of antibiotic-induced dysbiosis, a decrease in clinical manifestations of diarrhea, and a faster restoration of normal digestive tract function, which is critical during the weaning period and contributes to improved performance indicators in young animals.

The study **aimed** to develop and comparatively apply a comprehensive treatment regimen using the drug sulfadimethoxine and the *Bacillus* spore-forming probiotic for coccidiosis in piglets.

Materials and methods. A study was conducted at a pig farm in Poltava Region to determine the therapeutic efficacy of eimeriostats against coccidiosis in piglets. The efficacy of the drugs was evaluated in 60 infected Large White piglets, aged 14–21 days and weighing 3.5–6.5 kg. The piglets were divided into three groups: two experimental groups of 20 piglets each and one control group of 20 piglets. Ten experimental piglets were selected for coprological and microbiological studies, and 10 g of fecal samples were collected from their rectums. Coccidial oocysts were identified using the Fulleborn flotation method (DSSU, 2009). The intensity of *Cystoisospora suis* infection in the piglets ranged from 88.9 to 92.2 oocysts per gram of feces. Microbiological studies were conducted to determine the intestinal microbiota of the piglets before and after treatment for coccidiosis. Intestinal microbiocenosis in piglets with coccidiosis was determined before and after treatment, in accordance with DSTU 8703-2:2017 (SE 'UkrNDNC', 2018). Laboratory studies were conducted at the Laboratory of Swine Diseases of the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' (Kharkiv, Ukraine) using modern methods.

The piglets in group Experimental I were given the drug sulfadimethoxine orally at a dose of 25 mg/kg once daily for five days. The piglets in group Experimental II were given sulfadimethoxine at the same dose and a *Bacillus* spore-forming probiotic at a dose of two milliliters per animal for 21 days. The control group of piglets received no treatment.

The *Bacillus* spore-forming probiotic contains five strains: *B. amyloliquefaciens* ALB 65, *B. pumilus* UNCSM-026, *B. subtilis* UNCSM-020, *B. subtilis* var. *mesentericus* UNCSM-031, and *B. licheniformis* UNCSM-033.

Helminth and coccidial fecal examinations in animals were conducted before administration and on the 7th, 14th, and 21st days after administration, in accordance with DSTU 5079:2008 (DSSU, 2009). The anticoccidial activity of the drug was assessed based on prevalence reduction (PR) and intensity reduction (IR).

All manipulations with experimental animals were carried out in accordance with the 'European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes' (CE, 1986) and Council Directive 2010/63/EU (CEC, 2010), and under Art. 26 of the Law of Ukraine No. 3447-IV of 21.02.2006 'About protection of animals from cruel treatment' (VRU, 2006) and basic bioethical principles (Simmonds, 2017). Under the current procedure, the research program was reviewed and approved by the Bioethics Committee of the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' (Kharkiv, Ukraine).

The results were processed by methods of variation statistics. To compare mean values Student's *t*-test was used (Van Emden, 2019).

Results and discussion. The treatment of piglets in experimental groups I and II for cystoisosporiasis resulted in a significant reduction in the prevalence of the disease, with a decrease of 1.41 times and 1.38 times, respectively ($p \leq 0.001$), just 7 days after treatment when compared to baseline values. By the 14th day, there was also a notable reduction in the intensity of infection, observed at 6.68 times and 5.3 times, respectively. Three weeks after the initiation of treatment, coproscopy of fecal samples showed no oocysts of the pathogen *Cystoisospora suis* in suckling piglets (Table 1). In contrast, the control group continued to shed oocysts throughout the entire experimental period.

After two weeks, a significant increase in the efficacy of the administered drugs was observed in the two experimental groups, reaching 85.0% and 81.0%, respectively. By the 21st day, this figure had increased to 100% relative to baseline values. Since clinical recovery was not recorded in the experimental animals of the two groups, the overall efficacy of the drugs increased significantly to 80.0% and 85.0% by the 14th day, indicating the onset of widespread clinical recovery among the animals. After 21 days, the therapeutic efficacy of the two treatment regimens for piglets reached 100%.

Table 1 — Comparative efficacy of sulfadimethoxine in the treatment of coccidiosis in piglets aged 14–21 days (M ± m; n = 20)

Animal group	Before treatment	On the 7th day			On the 14th day			On the 21st day		
	MI, oocyst	P, %	PR, %	MI, oocyst	IR, %	PR, %	MI, oocyst	IR, %	PR, %	MI, oocyst
Experimental I	88.9 ± 0.48	28.9	10.0	63.2 ± 0.53***	85.0	80.0	13.3 ± 0.48***	100.0	100.0	0***
Experimental II	92.2 ± 0.31	27.9	10.0	66.5 ± 0.30***	81.0	85.0	17.5 ± 0.38***	100.0	100.0	0***
Control	90.5 ± 0.34	–	–	92.4 ± 0.36***	–	–	97.0 ± 0.55***	–	–	104.3 ± 0.54***

Notes: Experimental I — sulfadimethoxine; Experimental II — sulfadimethoxine + *Bacillus* spore-forming probiotic; *** — the difference in values is statistically significant at $p \leq 0.001$ compared to the corresponding values before treatment.

Coccidial infection significantly reduced bacterial diversity in the intestines of sick piglets. Analysis of the microbial community composition indicates the presence of marked dysbiosis in both experimental groups of infected suckling piglets before treatment. This is confirmed by a significant increase in the levels of opportunistic pathogens, particularly *Staphylococcus* spp. (up to 10.1–14.6×10^8 CFU/g), *Proteus* spp. (up to 15.5–19.2×10^7 CFU/g), *Shigella* spp. (up to 9.3–12.5×10^6 CFU/g), *Klebsiella* spp. (up to 17.3–21.4×10^6 CFU/g), as well as the yeast-like fungus *Candida albicans* (13.1–16.1×10^4 CFU/g), anaerobic representatives of the genus *Clostridium* spp. (4.3–6.2×10^5 CFU/g), which significantly exceed the physiological norms for healthy intestinal microflora (Table 2).

Table 2 — Species composition of the large intestine microbiome in suckling piglets before treatment (M ± m; n = 20)

Microorganisms	Animal group	
	Microbiota indicators, CFU/g	
	Experimental I	**Experimental II**
E. coli	28.1±0.52×10^7	34.2±0.47×10^7
Shigella spp.	9.3±0.44×10^6	12.5±0.46×10^6
Proteus spp.	15.5±0.50×10^7	19.2±0.45×10^7
Klebsiella spp.	21.4±0.39×10^6	17.3±0.47×10^6
Clostridium spp.	6.2±0.32×10^5	4.3±0.34×10^5
Staphylococcus spp.	14.6±0.47×10^8	10.1±0.49×10^8
Enterococcus spp.	28.3±0.49×10^7	34.3±0.52×10^7
Candida albicans	16.1±0.52×10^4	13.1±0.44×10^4
Lactobacillus spp.	5.4±0.37×10^4	8.2±0.42×10^4
Bifidobacterium spp.	31.3±0.45×10^5	29.3±0.54×10^5

Notes: Experimental I — sulfadimethoxine; Experimental II — sulfadimethoxine + *Bacillus* spore-forming probiotic.

At the same time, a reduced content of obligate microflora — *Lactobacillus* spp. (5.4–8.2×10^4 CFU/g) — and a relatively unstable level of *Bifidobacterium* spp. (29.3–31.3×10^5 CFU/g) were observed, which is a characteristic sign of an imbalance in the intestinal microbiota. Furthermore, the imbalance between different groups of microorganisms (an increase in opportunistic flora against a background of decreased protective microflora) confirms a disruption in the gut's colonization resistance.

The established state of the microbiota justifies the need for anticoccidial and corrective therapy aimed at eliminating opportunistic flora and restoring the normal microbiota.

A comparative analysis of the results indicates that the use of sulfadimethoxine in Experimental I is less effective in regulating the intestinal microbiota compared to its combination with the *Bacillus* spore-forming probiotic (Experimental II).

In Experimental I, significantly higher levels of opportunistic microflora persist. Specifically, the number of *Staphylococcus* spp. in the first experimental group is 6.3×10^7 CFU/g vs 19.2×10^3 CFU/g in the second group; *Proteus* spp. — 7.1×10^6 vs 26.3×10^5 CFU/g; *Klebsiella* spp.— 5.2×10^6 vs 14.4×10^5 CFU/g, and *Shigella* spp.—16.6×10^5 vs 10.2×10^5 CFU/g. Similarly, the level of *Enterococcus* in Experimental I (37.4×10^5 CFU/g) exceeds the corresponding value in Experimental II (31.5×10^4 CFU/g). *Candida albicans* fungi were detected only in the first experimental group (15.2×10^4 CFU/g), whereas they were absent in the second experimental group. In Experimental I, a high level of *E. coli* (35.2×10^6 CFU/g) persists compared to Experimental II (21.4×10^5 CFU/g), which may indicate incomplete normalization of the animals' intestinal microbiota (Table 3).

Table 3 — Species composition of the large intestine microbiome in suckling piglets after treatment (M ± m; n = 20)

Microorganisms	Animal group	
	Microbiota indicators, CFU/g	
	Experimental I	**Experimental II**
E. coli	35.2±0.61×10^6***	21.4±0.68×10^5***
Shigella spp.	16.6±0.52×10^5***	10.2±0.45×10^5***
Proteus spp.	7.1±0.36×10^6***	26.3±0.67×10^5***
Klebsiella spp.	5.2±0.40×10^6***	14.4±0.51×10^5***
Clostridium spp.	11.4±0.46×10^3***	8.1±0.45×10^3***
Staphylococcus spp.	6.3±0.39×10^7***	19.2±0.58×10^3***
Enterococcus spp.	37.4±0.58×10^5***	31.5±0.66×10^4**
Candida albicans	15.2±0.45×10^4	absent
Lactobacillus spp.	3.3±0.36×10^5***	28.7±0.65×10^8***
Bifidobacterium spp.	24.2±0.52×10^6***	37.5±0.68×10^{10}***

Notes: Experimental I — sulfadimethoxine; Experimental II — sulfadimethoxine + *Bacillus* spore-forming probiotic; **, *** — the difference in values is statistically significant at $p \leq 0.01$ and $p \leq 0.001$, respectively, compared to pre-treatment values.

Monotherapy with sulfadimethoxine (Experimental I) is insufficiently effective for normalizing the intestinal microbiota, as evidenced by the persistence of high levels of opportunistic microorganisms and low numbers of obligate flora. In contrast, combined use with the *Bacillus* spore-forming probiotic (Experimental II) ensures marked suppression of pathogenic microflora and active restoration of the normal microbiota, confirming the advantage of complex therapy in correcting dysbiosis.

Coccidial infection directly or indirectly affects the composition of the gut microbiota in animals (Gong et al., 2021; Hinney et al., 2021). Dysbiosis in animals with coccidiosis was characterized by reduced microbiota diversity, a deficiency of obligate anaerobes, and excessive growth of opportunistic microorganisms (Lu et al., 2021; Buffoni et al., 2026). The pathogen *Cystoisospora suis* enriches the bacterial population, including *Enterococcus* spp., *Escherichia* spp., *Shigella* spp., *Staphylococcus* spp., and others (Cui et al., 2017). This was precisely the pattern observed in the baseline state, which was partially maintained after treatment in Experimental I, where high levels of *Staphylococcus* spp., *Proteus* spp., *Klebsiella* spp., and other opportunistic bacteria were noted.

In Experimental II, conversely, a significant decrease in the abundance of pathogenic microflora and a simultaneous increase in obligate microorganisms (*Lactobacillus* spp., *Bifidobacterium* spp.) were noted. This is consistent with the literature, according to which *Bacillus* probiotics are capable of inhibiting the growth of pathogenic bacteria, restoring microbial balance, and increasing the colonization resistance of the intestine (Hu et al., 2014; Jiang et al., 2022). The therapy administered to the two experimental groups facilitated the effective treatment of piglets for coccidiosis. Complete clearance of coccidia from the animals' bodies was observed, indicating the high therapeutic efficacy of the treatment regimen used.

Bacteria of the genus *Bifidobacterium* play a key role in maintaining intestinal homeostasis, and a decrease in their numbers is one of the most characteristic markers of dysbiosis (Tojo et al., 2014; Kim et al., 2025). The sharp increase in their numbers observed in the second experimental group indicates effective restoration of the normal microbiota and stabilization of the microbial ecosystem. Similarly, members of the genus *Lactobacillus* are key probiotic microorganisms widely used to correct dysbiotic disorders (Sanders et al., 2019). It is known that anticoccidial therapy without the use of probiotics often leads to a disruption of the microbial balance, as it suppresses both pathogenic and normal microflora, which explains the insufficient effectiveness of Experimental I, where the restoration of the obligate microbiota was limited and dysbiotic changes persisted.

The inclusion of the *Bacillus* spore-forming probiotic in the treatment regimen (Experimental II) demonstrated high efficacy in correcting dysbiotic disturbances of the gut microbiota. It was established that the use of the probiotic contributed to a marked suppression of opportunistic and pathogenic microflora, in particular a significant reduction in the levels of *Staphylococcus* spp., *Proteus* spp., *Klebsiella* spp., *Shigella* spp., as well as the complete elimination of *Candida albicans* fungi. This effect may be associated with the antagonistic activity of bacteria of the genus *Bacillus*, which produce a wide range of biologically active substances (bacteriocins, enzymes, organic acids) capable of inhibiting the growth of pathogenic microorganisms (Khalid et al., 2021; Liu et al., 2023). At the same time, in Experimental II, a sharp increase in the abundance of obligate microflora — *Lactobacillus* spp. and *Bifidobacterium* spp. — was observed, indicating the restoration of the intestinal microbiota. Probiotic *Bacillus* strains contribute to the normalization of the microbiota not only through direct antagonistic action but also by creating favorable conditions for the growth of indigenous microflora, including lowering the pH of the environment and competing for adhesion receptors (Wang et al., 2021).

Recent studies (Elisashvili, Kachlishvili and Chikindas, 2019; Sudan, Zhan and Li, 2022) have shown that *Bacillus* spore-forming probiotics are highly stable in the gastrointestinal tract. They can undergo transitory colonization and have a pronounced immunomodulatory effect. These characteristics ensure the effectiveness of *Bacillus* spore-forming probiotics in restoring microbial balance, even against the backdrop of anticoccidial therapy, and contribute to increased intestinal colonization resistance. This is manifested by the displacement of pathogenic microflora and the stabilization of microbial homeostasis.

These findings suggest the advisability of incorporating *Bacillus* spore-forming probiotics into complex therapies for dysbiosis, with the goal of normalizing microbiota and enhancing intestinal colonization resistance.

Conclusions: 1. Research has shown that suckling piglets have pronounced dysbiotic disturbances in their gut microbiota, manifested by increased levels of opportunistic pathogens (e. g., *Staphylococcus, Proteus, Klebsiella, Shigella*, and *Candida albicans*) and decreased numbers of obligate microflora (e. g., *Lactobacillus* and *Bifidobacterium*).

2. Treating piglets with sulfadimethoxine led to a full recovery from coccidiosis, but it did not fully normalize the microbiocenosis. This is evidenced by the persistence of dysbiotic changes and the insufficient restoration of normal microflora. The combined use of sulfadimethoxine and the *Bacillus* spore-forming probiotic is significantly more effective. It promotes treatment of coccidiosis, suppresses pathogenic and opportunistic microflora, eliminates *Candida albicans* fungi, and restores symbiotic microorganisms.

Declaration of competing interest. The authors declare that they have no conflict of interest.

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