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Prevalence of Intestinal Protozoa in Pigs of Northern Black Sea Region, Ukraine

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ABSTRACT

Intestinal protozoan parasites threaten the health and welfare of pigs and impair the sustainability of pig farms, resulting in monetary losses. The present study aimed to determine the distribution of protozoa in large white pigs in the farms of Odesa, Mykolaiv, and Kherson regions in Ukraine. The parasitological surveys were conducted from March 2020 to March 2022 on three types of farms, including four large farms (> 100 sows), six medium farms (25-100 sows), and eight small farms (< 25 sows). A total of 3938 fecal samples from pigs of various age groups, namely 0-2-month piglets, 2-4-month piglets, pigs on fattening, and sows, were examined. *Eimeria* and isospores were determined using McMaster's method in Raynaud's modification, cryptosporidia by staining smears, blastocysts by the method of ethyl acetate-formalin concentration, and direct microscopy. The recorded protozoa were species *Eimeria* spp., *Cystoisospora suis* (syn. *Isospora suis*), *Balantidium coli*, *Cryptosporidium* spp., *Blastocystis* spp. These species of protozoa were observed in 31.1%, 49.0%, and 58.8% of pigs in large, medium-sized, and small farms, respectively. The findings indicated that *Isospora suis* and *Eimeria* spp. were most commonly present in piglets aged 0-2 months in large (29.7% and 23.0%, respectively), medium (32.3%, 29.4%), and small farms (30.0%, 13.5%). *Balantidium coli* was not registered in pigs from large farms, and in piglets 2-4 months old from small farms, the prevalence of infestation was 17.2%. *Cryptosporidium* spp. and *Blastocystis* spp. were mostly recorded in piglets 2-4 months old from small farms (16.2%, 7.1%). In large and medium-sized farms of the Northern Black Sea region, mono infestations were recorded the most (73.6%, 72.0%), while mixed two-component infestation dominated (52.5%) small farms. Intestinal protozoa should be considered in the differential diagnosis of intestinal disorders as major factors or concomitant intestinal pathogens.

Keywords: Age, Animals, Black Sea, Intestinal protozoa, Prevalence

INTRODUCTION

Intestinal parasites have a significant negative impact on the efficiency of pig farming. Young animals are particularly vulnerable to parasitic infections as they can cause diarrhea and dehydration, and consequently the death of the animals. Parasites can also hinder weight gain in pigs, causing economic losses (Li et al., 2020). The economic effectiveness of combating parasitosis relies on the ability to minimize or eliminate losses and manage the costs associated with disease prevention and treatment. A crucial requirement for accurate profit and loss assessment is determining the ultimate loss resulting from parasitic infections in farm animals. This information is essential for precisely evaluating the financial impact and making informed decisions regarding disease control strategies (Michalski, 2007).

Internal parasites are highly prevalent in pigs, making it essential for every producer to be aware of their presence and the associated losses they can cause. The amount of loss is affected by several factors, the most important of which are the species of endoparasites, housing, feeding, geographical location, and breed of animals (Roepstorff et al., 2011; Zakir Abadura et al., 2022). At the beginning of the infestation, the animal loses its productive potential due to the inability to adapt to endoparasites. However, as the animals grow, they undergo physiological adjustments and their immune system develops, enabling them to better modulate any damage caused by parasites and compensate for the increase in weight (Weng et al., 2005; Lotfalizadeh et al., 2022). Protozoa are major biological barriers to efficient pig production but are often overlooked because clinical symptoms are rarely detected. Infected pigs experience a 5% reduction in daily feed intake and a 31% average daily growth, as well as an average 17% higher feed conversion ratio, compared to pigs on a parasite-free diet (Kipper et al., 2011; Ózsvári, 2018).

Gastrointestinal parasites are a major cause of reduced production efficiency in pigs. They impact productivity by directly competing for nutrients needed for optimal growth and reproduction. Additionally, these parasites can cause tissue damage (lesions) leading to organ culling in meat inspection, poor feed conversion, diarrhea, dehydration, or even

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death of animals (Kochanowski et al., 2017). The prevalence of gastrointestinal parasite infections in pigs varies based on different factors, including housing systems (intensive and semi-intensive), deworming practices, and pig management (Eijck and Borgsteede, 2005; Weng et al., 2005; Nwafor et al., 2019; Symeonidou et al., 2020; Adhikari et al., 2021). Free-range pig farming is common in rural areas of many developing countries despite some disadvantages, such as poor feed conversion, high mortality, and poor production (Kagira et al., 2010).

In most cases, gastrointestinal parasitic infections in pigs are subclinical, meaning they do not show noticeable symptoms. However, symptomatic infections can occur, particularly in young pigs. The most common mistakes of pig owners in the fight against parasitic infections are the lack of periodical examination of animal feces to identify specific parasitic problems on the farm, incorrect administration of antiparasitic drugs, and ineffective disinfection of premises (Balicka-Ramisz et al., 2020; Li et al., 2022; Sadr et al., 2022).

Pig endoparasitism indicates heterogeneity in terms of the involved parasite species and their pathogenicity (Roepstorff et al., 1998; Schubnella et al., 2016). Additionally, parasitized pigs tend to be more susceptible to infectious and non-infectious diseases, which undermine their health and welfare status (Greve, 2012). Acquisition of parasite-free pigs combined with good hygiene practices can minimize the initial infection pressure and further infection of the herd to a minimum (Joachim et al., 2001). The impact of endoparasites depends on the parasite load and the individual resistance of the animal, which can be influenced by environmental and nutritional factors. Endoparasitism can occur with or without clinical symptoms. A disease with a clinical manifestation can lead to death, especially in the initial phase of growth. The absence of clinical symptoms is important for production as if it remains unnoticed, it can lead to economic losses due to reduced pig productivity (Delsart et al., 2022).

The aim of the current research was to determine the prevalence of intestinal protozoa in pigs of different age groups depending managing system in the Northern Black Sea Region, Ukraine.

MATERIALS AND METHODS

Ethical approval

The current study was approved by the Scientific Council of the National Scientific Center "Institute of Experimental and Clinical Veterinary Medicine" of the National Academy of Agrarian Sciences of Ukraine. The experiments performed on animals do not contradict the current legislation of Ukraine (Article 26 of the Law of Ukraine 5456-VI of 16.10.2012 "On protection of animals from cruel treatment") and "General ethical principles of animal experiments", adopted by the First National Congress of Bioethics and international bioethical standards (materials of the IV European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Purposes, Simmonds, 2018; Kabene & Baadel, 2019). The research program was reviewed and approved by the Bioethics Commission of the National Research Center "Institute of Experimental and Clinical Veterinary Medicine" in the current order.

Sampling time and study design

From March 2020 to March 2022, a total of 3938 samples of feces from pigs of different age groups were examined. The samples were collected from Odesa, Mykolaiv, and Kherson regions. The main criteria for the selection of fecal samples were lack of appetite, presence of diarrhea, and inactivity. Animals in these farms were not subjected to any treatment or administration of antiprotozoal drugs. In the parasitology laboratory, 1267 fecal samples from pigs with different age groups from large farms were examined (> 100 sows), namely Bolgradskyi district (n = 320), Podilskyi (n = 295), Berezneguvatskyi (n = 402), Skadovsky (n = 250). Of the total fecal samples, 1774 were from medium farms (25-100 sows), namely Kodymskyi (n = 300), Baltskyi (n = 210), Tatarbunarskyi (n = 255), Veselinivskyi (n = 372), Arbuzynskyi (n = 306), and Ivanivskyi (n = 331) and 897 samples were from small farms (<25 sows) in 8 districts of the Northern Black Sea Region of Ukraine (Figure 1).

Figure 1. The studied farms of the Northern Black Sea region of Ukraine from 2020 to 2022.
Large (> 100 sows); Medium (25–100 sows); Small (<25 sows)

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Fecal sampling and parasitological analysis

Fecal samples from piglets aged 0-2 months old (n = 1245) were collected directly from the rectum. In each litter, samples were obtained from 3 to 5 piglets and combined into a single pooled sample. In addition, fattening pigs and sows were sampled directly from the rectum or from the floor immediately after defecation. Feces were analyzed using McMaster's method in Raynaud's modification, and the number of oocysts per gram (OPG) of fecal value was estimated according to the method of Raynaud (1970). Samples were examined at 100x magnification and in doubtful cases at 400x magnification. In order to determine cryptosporidia for coprological studies, 2 fecal samples from each animal were prepared on clean and degreased glass slides. Each sample was examined by creating a native smear according to the generally accepted method. The smears were stained according to the Kester and Romanovsky-Giems method, followed by microscopy at a magnification of x50. To isolate oocysts, a similar technique to the one described by Maddox-Hyttel et al. (2006). Blastocysts detection was carried out using the method of ethyl acetate-formalin concentration and direct microscopy (Vielma, 2019).

Statistical analysis

The chi-square test with Yates correction was used to compare the prevalence of intestinal protozoa in piglets and sows of different types of farms. The OPG values obtained in piglets and sows in different types of farms were compared using the Kruskal-Wallis test (in exceptional cases, the Kolmogorov-Smirnov test was used for the analysis of *I. suis* OPG in sows). Differences were considered statistically significant at $p < 0.05$. All data were analyzed using STATISTICA 7.1 (StatSoft).

RESULTS

In large farms (>100 sows), protozoa were detected in 31.1% of pigs from different age groups. In piglets aged 0-2 months old, the total infestation was 41.9%. Of all detected protozoa, *Isospora suis* was recorded at the highest rate (29.7%), while *Balantidium suis* (*B. suis*) was not detected (Table 1). The total infestation in piglets aged 2-4 months old was 38.9% and the most common was *Eimeria* spp. (21.3%), while the infestation of *Isospora suis* decreased by 17.4%. In fattening pigs (6-10 months) and sows, the total rates of infestation were 12.5% and 22.9%, respectively, with the dominance of *Eimeria* spp. 8.0% and 8.1%. In medium-sized farms (25–100 sows), out of 1774 examined animals of different age groups, the incidence of protozoa was 49.0%, which was 17.9% more than the incidence of pigs in large farms (Table 2).

Piglets aged 0-2 months old were most affected by oocysts of *Isospora suis* (32.3%) and *Cryptosporidium* spp. (13.1%), the total percentage of infestation was 59.4. The total infestation of piglets aged 2-4 months old amounted to 65.4% with the dominance of *Eimeria* spp. (29.4%) and *Cryptosporidium* spp. (10.3%). Infestation of fattening pigs with protozoa was 26.0%, and the prevalence rate of *Eimeria* spp. was 8.6%. The total infestation in sows was 23.7% and was dominated by *Blastocystis* sp (5.0%). Out of 897 examined pigs of different age groups in small farms (<25 sows), 58.8% were affected by protozoa (Table 3).

Piglets within the age range of 2-4 months (74.9%) and 0-2 months (67.1%) were the most infected groups of age, respectively. In fattening pigs and sows, the infestation percentage by protozoa was almost at the same level and amounted to 31.6% and 35.7%, respectively. Piglets aged 2-4 months old were most affected by *B.* suis (17.2%) and *Cryptosporidium* spp. (16.2%), compared to animals with large and medium farms. Pigs of all ages in large farms were dominated by mono infestation with protozoa, which ranged 57.4-73.6% (Graph 1). Double infestation was recorded in 22.7-32.1% of pigs, while the maximum rates of triple infestation (14.9%) were recorded in piglets of 2-4 months.

Single-component infestation in pigs from medium-sized farms was recorded at almost the same level as in pigs from large farms (Graph 2). However, the two-component infestation was recorded in a larger number of pigs with an incidence rate of 24.8-30.4%. The prevalence of protozoa caused by three pathogens was high only in piglets aged 2-4 months old (9.6%). In contrast to large and medium-sized farms, two-component infestation, as well as one-component infestation, was dominant in small farms, which was the highest in piglets aged 2–4 months old (52.5%, Graph 3). In sows, the extent of damage by triple infestation was recorded in 20% of animals.

Table 1. The prevalence of protozoa in pigs of large farms (>100 sows) located in the Northern Black Sea Region of Ukraine from 2020 to 2022.

Age groups	Animals (number)	The extent of the infestation (%)				
		Isospora suis	*Eimeria* spp.	*Balantidium suis*	*Cryptosporidium* spp.	*Blastocystis* spp.
0–2 months	415	29.7	23.2	–	6.9	1.9
2–4 months	380	12.3	21.3	–	3.1	2.1
on fattening	350	1.7	8.0	–	1.1	1.7
Sows	122	5.7	8.1	–	4.9	4.1

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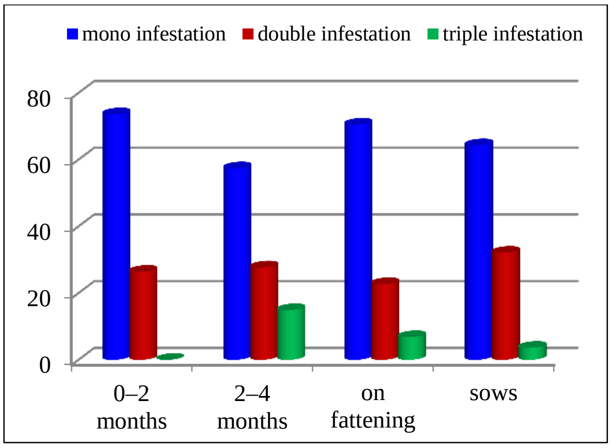
Table 2. The prevalence of protozoa in pigs of medium farms (25-100 sows) located in the Northern Black Sea Region of Ukraine from 2020 to 2022

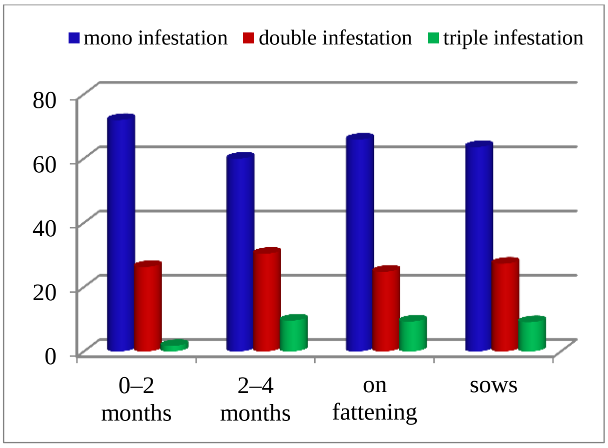
Age groups	Examined animals (Number)	The extent of the infestation (%)				
		Isospora suis	*Eimeria* spp.	*Balantidium suis*	*Cryptosporidium* spp.	*Blastocystis* spp.
0–2 months	510	32.3	4.3	5.7	13.1	3.3
2–4 months	630	14.4	29.4	6.5	10.3	4.1
on fattening	495	4.2	12.3	5.2	0.4	3.8
Sows	139	4.3	8.6	2.1	3.6	5.0

Table 3. The prevalence of protozoa in pigs of small farms (< 25 sows) located in the Northern Black Sea Region of Ukraine from 2020 to 2022

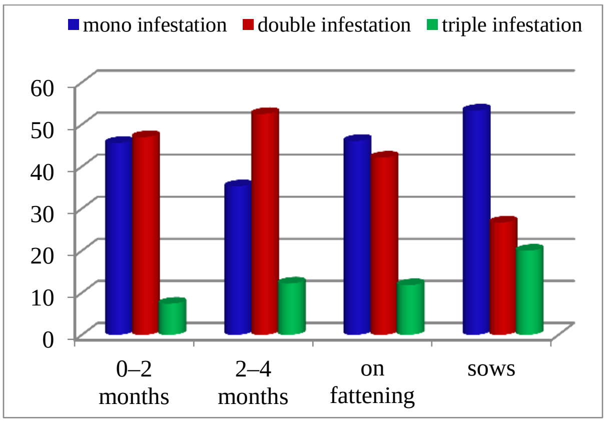
Age groups	Examined animals (Number)	The extent of the infestation (%)				
		Isospora suis	*Eimeria* spp.	*Balantidium suis*	*Cryptosporidium* spp.	*Blastocystis* spp.
0–2 months	320	30.0	7.5	11.2	12.8	5.6
2–4 months	295	20.6	13.5	17.2	16.2	7.1
on fattening	240	4.5	7.9	12.9	2.5	3.8
sows	42	2.3	11.9	9.5	4.7	7.1



Graph 1. Distribution of mono- and mixed protozoa in pigs of large farms (>100 sows) located in the Northern Black Sea Region of Ukraine from 2020 to 2022



Graph 2. Distribution of mono- and mixed protozoa in pigs of medium farms (25–100 sows) located in the Northern Black Sea Region of Ukraine from 2020 to 2022



Graph 3. Distribution of mono- and mixed protozoa in pigs of small farms (<25 sows) located in the Northern Black Sea Region of Ukraine from 2020 to 2022

DISCUSSION

Parasitism is an example of an antagonistic trophic relationship, where an organism exploits another species (known as the host) as a habitat and source of nutrients, either temporarily or permanently. It negatively affects the host’s condition, and can cause the host’s death (Vannier-Santos and Lenzi, 2011).

The effects of parasites on the body of the host depend on various factors, including the intensity of the infection, the age of the host, the host's immune response, its living conditions, feeding practices, and other environmental factors.

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All over the world, losses caused by parasitic diseases in animal husbandry are enormous (Symeonidou et al., 2020).

According to a study by Schubnell et al. (2016), parasite infections in pigs during the first months of life are highly prevalent. While helminths were uncommon, protozoan parasites were frequently detected and *I. suis* appears to be the most important parasite species associated with diarrhea or emaciation in the studied pig population.

On the territory of the Poltava region (Ukraine), eimeriosis and isosporosis were widespread protozoans with average infestation rates of 49.8% and 8.3%, respectively. Coccidiosis occurs more often as part of mixed invasions of the alimentary tract of pigs (73.2%) together with nematodes of the *Oesophagostomum genus*, *Ascaris suum*, *Trichuris suis* and the simplest of the *B. suis*. Eimeriosis and isosporosis mono invasions were recorded less. *Balantidium suis* (EI - 19.5%) are the main comembers of *Eimeria* and *isospores* in the intestines of pigs (Yevstafieva and Kovalenko, 2019).

The incidence of isosporosis and eimeriosis were significantly higher in young piglets, compared to adults. This implied that juveniles were highly susceptible to coccidia because of their weaker immunological response to severe infections. Several species of *Eimeria* and *Cystoisospora* could infect pigs, with *Cystoisospora suis* (syn. *Isospora suis*) being the predominant coccidia (Mundt et al., 2005; Worliczek et al., 2007). This apicomplexan protozoan mainly affects suckling piglets, which are unable to mount an adequate primary immune response (Koudela and Kucerová, 1999).

In a study performed by Symeonidou et al. (2020), approximately 19.1% of suckler piglets and 9.1% of weaned piglets were infested, while only four cases of *C. suis* infection were detected in sows. The prevalence of *Cryptosporidium suis* (*C. suis*) infections in pig populations varies depending on age and geographic location. In studies conducted in Europe, it has been observed that an increase in immunity against *C. suis* depended on age. The *C. suis* infections were more commonly found in suckling piglets, with *Isospora suis* being the predominant parasite in piglets aged 2-3 weeks, accounting for 26.9% of cases. *Cryptosporidium parvum*, another species of Cryptosporidium, was detected in 1.4% of the investigated piglets (Wieler et al., 2001).

Cryptosporidium suis in suckling piglets ranged from < 1% to > 40% (10.0% in Germany (Damriyasa and Bauer, 2006), 12.8% in Switzerland (Schubnell et al., 2016), 20.9% in Southern Germany (Wieler et al., 2001), and 42.9% in Poland (Kochanowski et al., 2017). For Scandinavian countries, *C. suis* infestation rate was 19.5% in Denmark, 4.5% in Finland, 31.8% in Iceland, 0.3% in Norway, and 20.1% in Sweden (Roepstorff et al., 1998).

The prevalence of intestinal parasites was investigated in intensive pig farms in Guangdong Province, China. The findings indicated that 24.9% of pigs were affected by coccidiosis (*Eimeria* spp. and *Isospora suis*) and 47.2% by *Balantidium coli* (*B. coli*). These infected pigs were mainly from farms without a strategic antiparasitic treatment regime (Weng et al., 2005). In some cases, *B. coli* was thought to be a major factor in porcine colitis although this role has not been fully elucidated (Szczotka-Bochniarz et al., 2021).

Giarratana et al. (2021) reported *Balantidium coli* in 46.89% of pigs, with a significantly higher prevalence in commercial hybrid pigs (64.84%) than in local breeding pigs (27.91%). The infection was more common in pigs raised in an intensive breeding system than in an extensive one. The prevalence of infection was lower in older animals than in young ones. The breeding system is likely to be a major factor in the distribution of parasites, as well as the sanitary and hygienic status of farms. Regarding the risk factors for *B. coli* parasitism, they increase with age. Prevalence increased significantly from 57% in suckler piglets to 100% in pigs older than one month (Hindsbo et al., 2000).

Cryptosporidum was confirmed in 27.7% of pig fecal samples. Most of the infected animals (42.1%) were 2 to 3 months old. The following types of parasites were identified, *Cryptosporidum scrofarum*, *C. suis,* and *C. parvum*. Asymptomatic infestations caused by *C. scrofarum* were observed in most herds. Mixed diseases caused by *C. suis* and *C. scrofarum* were rare, however, they were observed in 86% of positive animals (Rzeżutka et al., 2014).

In a study of intestinal parasites on intensive and extensive pig farms in Chongqing, China, *Eimeria spp.* was recorded in 16.53% of pigs, *Isopora suis* in 5.02%, *B. coli* in 22.79%, and *Cryptosporidium* spp. in 6.60%. *Balantidium coli* was the most common protozoan in all age groups of pigs. Season of the year, age of the animals, and treatment methods can affect infection rates (Lai et al., 2011).

Of 402 pigs from 55 farms, *B. coli* cysts, *Cryptosporidium* oocysts, and mixed infection were detected in 51.5%, 13.9%, and 7.2% of pigs, respectively. Among the pigs, the highest infection rates were observed in young pigs. Specifically, 58.9% of the young pigs were found to be infected with *Cryptosporidium* oocysts, 54.6% with *B. coli* cysts, and 58.6% with mixed infections (Yatswako et al., 2007). Parasite prevalence was 93.1% on family farms and 59.1% on industrial farms. Single infections were more frequent (32.5%) than multiple infections (12.1%). In both family and industrial farms, the most common parasites were *B. coli*, with prevalence rates of 71.6% and 46.4%, respectively. The *coccidia* parasites were also prevalent with a prevalence of 71.6% in family farms and 19.2% in industrial farms (Barbosa et al., 2015).

According to Kochanowski et al. (2017), single infestations are common in large and medium-sized farms (57.4-73.6%), while double infestation (26.7-47.0%) and triple infestation (7.4–20.0%) in small farms. A higher prevalence of parasites was found in small farms than in medium and large farms, except for the prevalence of *coccidia*, which was the highest in medium farms. Infection with several pathogens was recorded more often than with one parasite.

In domestic pigs, the diet and rearing conditions change dramatically during the life of the animal, which can cause a difference in the prevalence of mixed infections among different age groups. Masuda et al (2022) found that the

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prevalence of single infections caused by *Blastocystis suis* was 37.8%, while the prevalence of mixed infections was 57.3%. A high percentage of single infections (86.7%) was observed in sows, piglets, and weanlings, while mixed infections (83.3%) were observed in 3-5 month-old piglets and producer pigs.

Dashti et al. (2022) reported that *Blastocystis* spp. was significantly less common in intensively reared Iberian pigs (22.9%) than in their extensively reared counterparts (51.0%) or in intensively reared large white pigs (64.1%). Large white pigs indicated a significantly higher prevalence of *Giardia duodenalis*, *Cryptosporidium* spp., and *Eimeria bieneusi*. *Balantidium coli* was equally common (40.0-51.1%) in all three investigated pig populations.

The results of this study confirmed the difficulty in eliminating intestinal parasitism in pigs, even with regular and systematic antiparasitic prophylaxis. Several factors contribute to this challenge. Firstly, breeding animals, such as lactating sows, have a longer lifespan and can act as reservoirs for parasites, leading to re-infection of other age groups. Secondly, there may be shortcomings in generally accepted treatment schemes in intensive pig farming these schemes often involve the use of preventive substances in feed, regardless of the difference in body weight of sows between age groups, which may cause insufficient dosing. Finally, preventive schemes can be ineffective due to long time intervals (for example, more than 6 months) between the moments of introducing feed to sows, which can create an unprotected time window in the life of breeding stock (Weng et al., 2005; Barbosa et al., 2015).

Many factors can influence the prevalence and intensity of parasitic infection. Factors, such as herd size, floor type, use of an all-in/all-out system, or pen use related to the breeding system, are particularly important (Mejer and Roepstorff, 2006). Therefore, information about the factors affecting various production systems is important for farm development.

CONCLUSION

All over the world, on farms with different capacities, the prevalence of protozoa in pigs was a very serious problem that reduces the productivity of animals. The extent and intensity of parasitic diseases in pigs should be reduced by educating farmers and private animal owners on the value of intensive animal care, environmental sanitation, strategic deworming of pigs using effective broad-spectrum anthelmintics, biological control of parasites, and breaking their life cycle. Further research will be needed on the influence of climatic conditions in different zones of Ukraine on the spread of pig protozoa.

DECLARATIONS

Availability of data and materials

The authors confirm that the data supporting the findings of this study are available.

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None.

Authors' contributions

Mykola Bogach and Ihor Panikar participated in the data collection, analysis, preparation, and revision of the manuscript. Anatoliy Antipov та Volodymyr Goncharenko was involved in the collection of data and laboratory analysis**,** while Olena Bogach formatted and edited the manuscript. All authors read and approved the final manuscripts.

Competing interests

The authors have not declared any conflict of interest.

Ethical consideration

Ethical issues (including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy) have been checked by all the authors.

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