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Co-infections with gastrointestinal parasites in a free-range swine farm from the Transylvania area

Mihai-Horia Băieş[1]✉, Zsolt Boros[1], Călin Gherman[1], Marina Spinu[1], Attila Mathe[2], Vasile Cozma[1,3]

1 – Department of Parasitology and Parasitic Diseases, Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, Mănăştur street, 3-5, 400372, Cluj-Napoca, Romania.

2 – Agricultural Research and Development Station of Turda, Agriculturii street, 27, 401100, Turda, Romania.

3 – Academy of Agricultural and Forestry Sciences Gheorghe Ionescu-Sisești (A.S.A.S), Mărăști Boulevard, 61, 011464, Bucharest, Romania.

Correspondence: Tel.: + 40758563438; Email: mihai-horia.baies@usamvcluj.ro

Abstract. Parasitic diseases cause significant economic losses in swine by diminishing productions, diseases and mortality. In the last decade, an increase in the number of free-range swine farms was noticed in Romania, further complicating the epidemiology of parasitic diseases.

The current study aimed to identify the parasitic profile of swine raised on a free-range farm from the Transylvania area.

Sixty faecal samples collected from piglets, fattening pigs, and sows were investigated by flotation (Willis and McMaster), active sedimentation, Ziehl-Neelsen stain modified by Henricksen, and modified Blagg methods. The coproparasitological examination revealed parasitic infections with *Balantidium coli, Eimeria* spp./*Isospora suis*, *Ascaris suum* and *Trichocephalus suis*. The number of oocysts (OPG), cysts (CPG) and eggs (EPG) were counted per gram of faeces. Prevalence (P) and the average intensity (I) of infection varied according to age and category of investigated swine. During the spring season, the broadest spectrum of parasites was identified in fattening pigs: *B. coli* (P = 40%, I = 300 CPG), *Eimeria* spp./*I. suis* (P = 90%, I = 700 OPG), *A. suum* (P = 70%, I = 200 EPG) and *T. suis* (P = 60%, I = 800 EPG). Similar infection rates were observed in piglets: *B. coli* (P = 40%, I = 400 CPG) and *Eimeria* spp./*I. suis* (P = 90%, I = 1.000 OPG), and sows: *B. coli* (P = 30%, I = 200 CPG) and *Eimeria* spp./*I. suis* (P = 90%, I = 9.100 OPG). In the autumn season, the highest number of parasite species was identified in fattening pigs: *B. coli* (P= 40%, I=50 CPG), *Eimeria* spp./*I. suis* (P=100%, I=1700 OPG), *A. suum* (P=100%, I= 5940 EPG), and *T. suis* (P=20%, I= 225 EPG), followed by sows: *B. coli* (P= 90%, I= 400), *Eimeria* spp./*I. suis* (P= 30%, I= 467 OPG), and *A. suum* (P=60%, I=533 EPG) and piglets: *B. coli* (P=20%, I=225 CPG) and *Eimeria* spp./*I. suis* (P=100%, I= 31.480 OPG). Furthermore, the study showed statistical significance between seasons and age groups for all diagnosed parasites.

However, further research is required to understand better the epidemiology of these infections in swine from Transylvania.

Keywords: Digestive parasites; Swine; Epidemiology; Free-range farm.

Co-infecții/infestații cu paraziți gastro-intestinali într-o fermă de porci free-range din zona Transilvaniei

Rezumat. Bolile parazitare cauzează pierderi economice semnificative la porcine prin diminuarea producțiilor, de asemenea prin creșterea susceptibilității pentru bolile infecțioase și mortalitate. În ultimul deceniu, în România s-a observat o creștere a numărului de ferme free-range, complicând și mai mult epidemiologia bolilor parazitare la suine.

Studiul actual a avut ca scop identificarea profilului parazitar al suinelor crescute într-o fermă free-range din Transilvania.

Un număr de 60 de probe de fecale au fost colectate de la purcei, grăsuni și scroafe. Metodele coproparazitologice utilizate au fost următoarele: flotația (Willis și McMaster), sedimentarea activă, coloratia Ziehl-Neelsen modificată de Henricksen și Blagg modificată.

Examenul coproparazitologic a evidențiat infecții/infestații parazitare cu *Balantidium coli, Eimeria spp./Isospora suis, Ascaris suum* și *Trichocephalus suis*. Numărul de oochisturi (OPG), chisturi (CPG) și ouă (OPG) au fost numărate per gram de fecale. Prevalența (P) și intensitatea medie (I) a infecției/infestații au variat în funcție de vârstă și categorie de porcine investigate. În timpul sezonului de primăvară, cel mai larg spectru de paraziți a fost identificat la grăsuni: *B. coli* (P = 40%, I = 300 CPG), *Eimeria spp./I. suis* (P = 90%, I = 700 OPG), *A. suum* (P = 70%, I = 200 EPG) și *T. suis* (P = 60%, I = 800 EPG). Rate similare de infecție/infestație au fost observate la purcei: *B. coli* (P = 40%, I = 400 CPG) și *Eimeria spp./I. suis* (P = 90%, I = 1.000 OPG) și scroafe: *B. coli* (P = 30%, I = 200 CPG) și *Eimeria spp./I. suis* (P = 90%, I = 9.100 OPG). În sezonul de toamnă, cel mai mare număr de specii de paraziți a fost identificat la grăsuni: *B. coli* (P = 40%, I = 50 CPG), *Eimeria spp./I. suis* (P = 100%, I = 1700 OPG), *A. suum* (P = 100%, I = 5940 EPG), și *T. suis* (P = 20%, I = 225 EPG), urmată de scroafe: *B. coli* (P = 90%, I = 400), *Eimeria spp./I. suis* (P = 30%, I = 467 OPG) și *A. suum* (P = 60%, I = 533 EPG) și purcei: *B. coli* (P=20%, I=225 CPG) și *Eimeria spp./I. suis* (P=100%, I= 31.480 OPG). În plus, studiul a arătat semnificația statistică între anotimpuri și grupe de vârstă pentru toți paraziții diagnosticați.

Cu toate acestea, sunt necesare cercetări suplimentare pentru o mai bună înțelegere a epidemiologiei acestor infecții/infestații parazitare la porcii din Transilvania.

Cuvinte cheie: Paraziți digestivi; Suine; Epidemiologie; Fermă free-range.

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Introduction

In Romania, the vast majority of swine are raised in low input systems. Recently an increase in the number of free-range farms has been registered in this country (Ichim, 2012). Organic farming depends on the ecological factors targeting environment protection, plant health, animal health, food safety, and consumer health (Suteu, 2011). Free-range is a type of farming where the animals, for at least part of the day, can roam freely outdoors, rather than being confined in an enclosure for 24 hours each day. On many farms, the outdoor areas have fences, thereby technically making this an enclosure. However, free-range systems usually offer the opportunity for extensive locomotion and sunlight that otherwise are prevented by indoor housing systems (Constantin, 2012). Swine infections with gastrointestinal parasites are widely reported worldwide and are influenced by the type of swine management practices (Nansen and Roepstorff, 1999). Free-range swine keeping is common in the rural areas of many developing countries despite its shortcomings like poor food conversion, high mortality rates, inferior products, and the risk of spreading zoonotic diseases (Kagira et al., 2010). Parasitic infections cause significant economic losses in swine raising farms by reducing production, morbidity, and mortality (Kochanowski et al., 2017). These conditions can cause intestinal malabsorption, impaired fertility, delayed or incomplete immunity after vaccination protocols, and negative effects on the meat quality (Lai et al., 2011).

Pigs may harbour numerous intestinal parasites, most commonly protozoa (*Balantidium coli*, *Entamoeba* spp., *Cryptosporidium* spp.) and nematodes (*Ascaris suum*, *Trichuris suis*) (Joachim et al., 2001). Most of the time, the course of such parasitic infections is subclinical, but symptomatic infections may occur, particularly in growing pigs (Weng et al., 2005). Swine with parasitic infections tend to be more susceptible to diseases, which undermine their health and welfare status (Greve et al., 2012). The most frequent mistakes in fighting parasite infections in swine include a lack of faecal sample testings, improper administration of antiparasitic drugs, a low dose of deworming medicines, ineffective disinfection, or the wrong choice of disinfectant agent (Balicka-Ramisz et al., 2020).

The current study aimed to identify the parasitic profile of swine from three age categories raised on a free-range farm from Transylvania.

Materials and methods

Romania has a temperate-continental climate of transitional type, with four clearly defined seasons (Trusca and Alecu, 2005). The samples were collected from a free-range farm located in Cluj County, an area with lots of pastures and forests. The animals had a fresh source of water from the local watering source. The shelters are periodically cleaned throughout each year. The animals have access to outdoor areas all the time.

The free-range farm breads two races of swine, meaning Mangalita and Basna. Both races were included in the current study. The 60 animals, representing 20 piglets (10 Basna, 10 Mangalita) of 6 to 8 weeks of age, 20 fattening pigs (10 Basna, 10 Mangalita) of 4 to 5 months, and 20 sows (10 Basna and 10 Mangalita) between 2 and 5 years, originated from a total of 140 animals. The animals were chosen randomly based on their age, and none of them had any clinical symptoms.

The animals were dewormed during June of 2020, sows with injectable Ivermectin (0.3 mg/kg body weight), fattening pigs with Levamisole, injection (5 mg/kg body weight), and piglets orally with Toltrazuril (20 mg/kg body weight). Sixty samples of faecal matter were collected from this free-range farm during the spring and autumn of 2020. The faecal samples were collected from six swine pens (10 animals/pen) in clean sample containers. The samples were examined macroscopically (colour, consistency, the presence of macroscopic parasites). Afterwards, the containers were numbered and stored at 2-8°C until testing.

All the collected faecal samples were subjected to individual coproparasitological examinations. The methods used are as follows: active sedimentation (5 g of faeces, 5-minute centrifugation, 2000 rotation/minute), flotation (Willis) (5 g of faecal matter in 25 ml of supersaturated sodium chloride solution, 20 minutes), Ziehl-Neelsen stain modified by Henricksen (used methyl alcohol 96%, carbol fuchsin, sulfuric acid, and malachite green), modified Blagg (2 g faecal matter, 10 ml glycerinated formaldehyde alcoholic solution, 2 ml ethylic ether) and McMaster (flotation fluid, counting chambers, faecal egg-count). All the used methods followed and respected the protocols described by Mircean et al. in 2011.

Statistical analyses: Prevalence and its 95% confidence interval of parasites infection in swine were calculated for each group and season. Student t-test was used to analyse the differences among groups and different time points using Excel.

Then, the mean value and standard deviation of the mean were calculated for the number of oocysts, cysts and eggs per gram of faeces. A value of $p \leq 0.05$ was considered statistically significant. Statistical analysis was performed with Epitools and Excel program.

Results and discussions

The coproparasitological examination revealed co-infection with several species of parasites meaning, *Ascaris suum* (figure 4), *Trichocephalus suis* (figure 3), *Balantidium coli* (figure 2), and *Eimeria* spp./*Isospora suis* (figure 1) during spring and autumn. All the tested animals came out negative in sedimentation, Ziehl-Neelsen stain modified by Henricksen and Blagg methods. The Flotation and McMaster methods revealed that the prevalence and the average intensity of infections varied according to age and swine category.

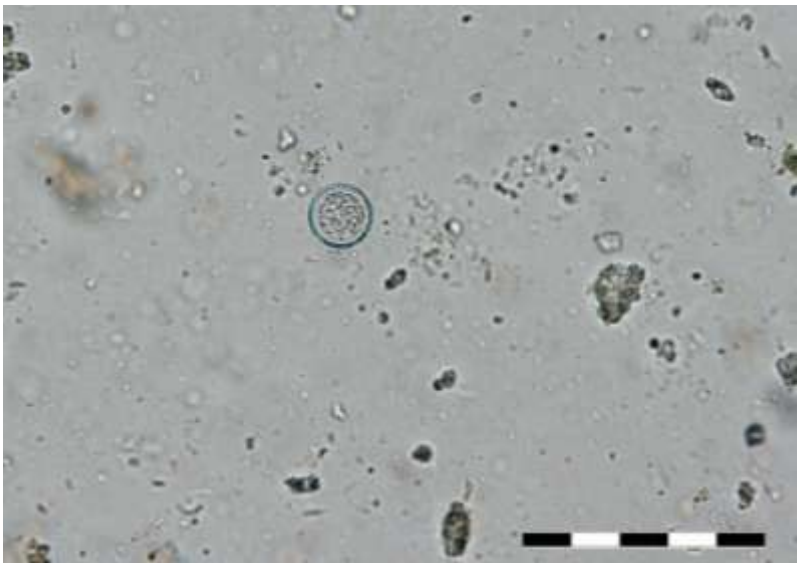

Figure 1. Oocyst of *Eimeria* spp. (23 x 18 µm) using the flotation method. 200X

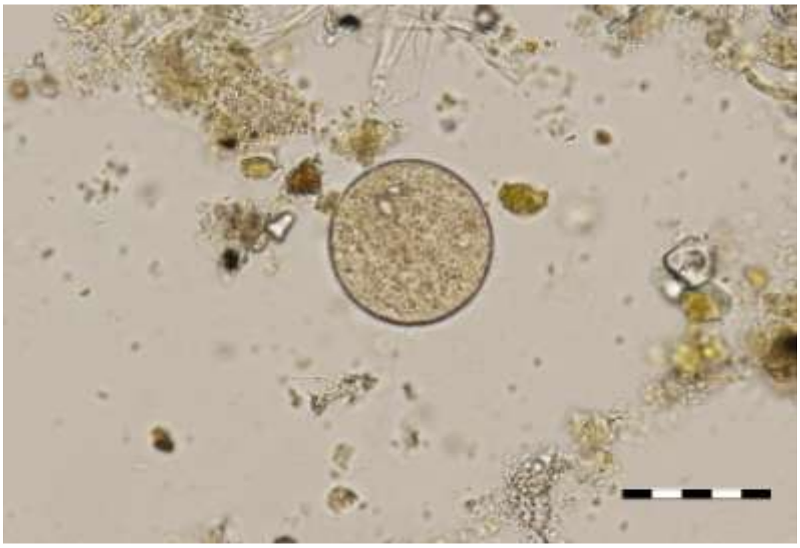

Figure 2. Cyst of *Balantidium coli* (40x 60 µm) using the flotation method. 400X

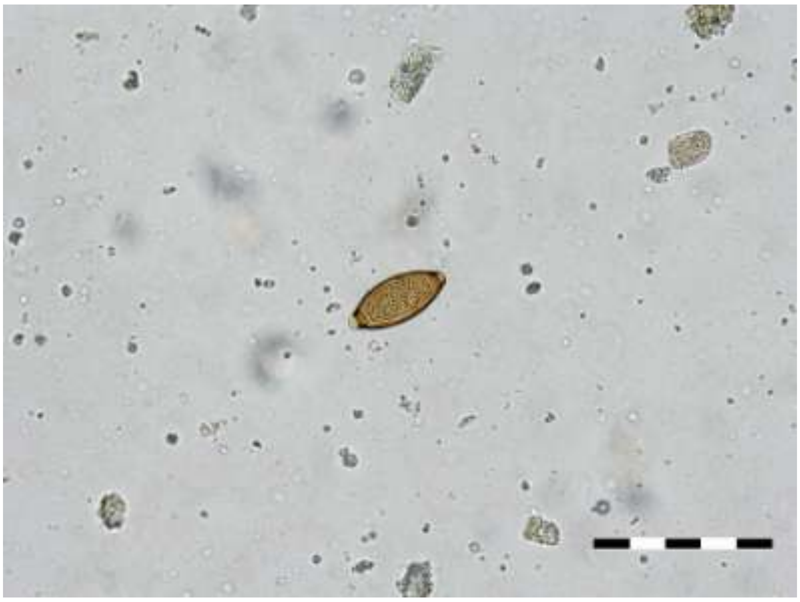

Figure 3. Egg of *Trichocephalus suis* (55 x 29 µm) using the flotation method. 200X

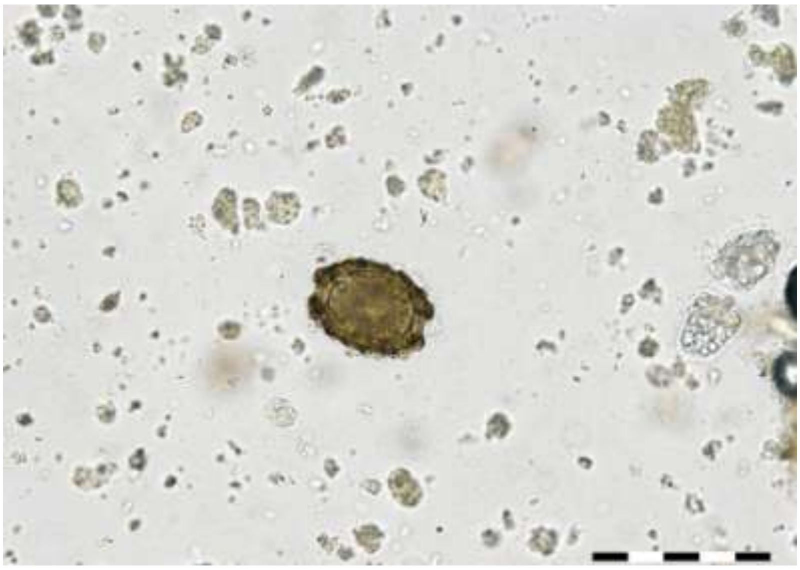

Figure 4. Egg of *Ascaris suum* (56 x 42 µm) using the flotation method 200X

In piglets, only *B. coli* (P = 40%, I = 400 CPG during spring, P=20%, I= 225 CPG during autumn) and *Eimeria* spp./*I. suis* (P = 90%, I = 1.000 OPG during spring, P=100%, I=31.480 OPG during autumn) were identified, as seen in table 1.

In fattening pigs, *B. coli* (P = 40%, I = 300 CPG during spring, P= 40%, I= 50 CPG during autumn), *Eimeria* spp./*I. suis* (P = 90%, I = 700 OPG in spring, P=100%, I=1700 OPG in autumn), *A. suum* (P = 70%, I = 200 EPG in spring, P=100%, I=5940 EPG during autumn) and *T. suis* (P = 60%, I = 800 EPG in spring, P=20%, I=225 EPG in autumn) were diagnosed, as seen in table 2.

In sows, *B. coli* (P = 30%, I = 200 CPG in spring, P=90%, I=400 CPG in autumn) and *Eimeria* spp./*I. suis* (P = 90%, I = 9.100 OPG in spring, P=30%, I=467 OPG in summer) and *A. suum* (P=60%, I=533 EPG in autumn) were identified, as seen in table 3.

Table 1. The prevalence, average intensity and standard deviation in piglets. CPG= Cyst per gram of faeces; OPG= Oocyst per gram of faeces. P-value is the probability value

	Piglets (spring season)		Piglets (autumn season)	
Parasite	*Eimeria spp./Isospora suis*	*Balantidium coli*	*Eimeria spp./Isospora suis*	*Balantidium coli*
Prevalence %	90 (56.6-98.2)	40 (16.8-68.7)	100	20 (5.67-50.98)
Average intensity (OPG/ CPG)	1000	400	31480	225
Standard deviation (OPG/CPG)	± 212.13	± 163.30	± 85023.51	± 247.49
p-value	0.004628	0.532	0.004628	0.532

Table 2. The prevalence, average intensity and standard deviation in fattening pigs. EPG= Egg per gram of faeces; CPG= Cyst per gram of faecal matter; OPG= Oocyst per gram of faeces. P-value is the probability value

Fattening pigs (spring season)				
Parasite	*Eimeria spp./Isospora suis*	*Balantidium coli*	*Ascaris suum*	*Trichocephalus suis*
Prevalence %	90 (56.6-98.2)	40 (16.8-68.7)	70 (39.7-89.2)	60 (31.3-83.2)
Average intensity (OPG/CPG/EPG)	700	300	200	800
Standard deviation (OPG/CPG/EPG)	± 193.65	± 81.65	± 81.65	± 178.89
Fattening pigs (autumn season)				
Parasite	*Eimeria spp./Isospora suis*	*Balantidium coli*	*Ascaris suum*	*Trichocephalus suis*
Prevalence %	100	40 (16.8-68.7)	100	20 (5.67-50.98)
Average intensity (OPG/CPG/EPG)	1700	50	5940	225
Standard deviation (OPG/CPG/EPG)	±1170.95	0	±2225.21	± 247.49
p-value	0.009749	0.009	3.95241E-12	0.000143

Table 3. The prevalence, average intensity and standard deviation in sows. EPG= Egg per gram of faeces; CPG= Cyst per gram of faecal matter; OPG= Oocyst per gram of faeces. P-value is the probability value

	Sows (spring season)		Sows (autumn season)		
Parasite	*Eimeria spp./Isospora suis*	*Balantidium coli*	*Eimeria spp./Isospora suis*	*Balantidium coli*	*Ascaris suum*
Prevalence %	90 (56.6-98.2)	30 (10.8-60.3)	30 (10.8-60.3)	90 (56.6-98.2)	60 (31.3-83.2)
Average intensity (OPG/CPG))	9100	200	467	400	533
Standard deviation (OPG/CPG)	± 1747.14	± 100	± 230.94	± 579.87	± 628.23
p-value	1.0622E-10	0.86	1.0622E-10	0.86	0.03472

Similar studies were conducted on piglets in several European and African countries. A good example, in this case, is the study conducted in Poland by Kochanowski et al. in 2017, where the flotation method revealed a prevalence of 31.4% for *Eimeria* spp./*Isospora suis*. Nwafor et al. (2019), during their study in South Africa, found a prevalence of 88% for Coccidia in young piglets by using the flotation method. *Balantidium coli* was found in piglets from Bangladesh and Grece, with a prevalence of 28.6%, respectively 2.6% in the flotation method (Dey et al., 2014; Symeonidou et al., 2020).

In fattening pigs, the prevalence rates differ from study to study, and it depends on the identified parasite species. In Poland, the presence of Coccidia was identified in fattening pigs by the flotation method, the revealed prevalence being 7.1% (Kochanowski et al., 2017). A second study from South Africa in 2019 indicated a higher prevalence (75%) for Coccidia (flotation) in the same category of animals (Nwafor et al., 2019). Regarding *B. coli* in this category of animals, a few studies show that the prevalence rates for this parasite species can vary between 13.5% and 52.4% (Dey et al., 2014; Symeonidou et al., 2020). In fattening pigs, infections with *A. suum* and *T. suis* are the most common. Regarding *A. suum*, the prevalence revealed by the flotation test can differ from one country to another. In South Africa, a study performed in 2019 showed a prevalence of 63.9% for A. suum in fattening pigs (Nwafor et al., 2019). A later study (2020) from Denmark indicated a much lower prevalence of only 28% (Pietrosemoli and Tang, 2020). The prevalence of *T. suis* can vary from 2.9%, as shown in Poland, up to 63.9%, found in South Africa (Kochanowski et al., 2017; Nwafor et al., 2019).

In sows, the current study found only co-infection with Coccidia and *B. coli*. These two parasitic infections were found in other similar studies as well. A study from Poland in 2017 showed a prevalence rate of 17.1 % in the flotation method (Kochanowski et al., 2017). Still, another study from South Africa in 2019 revealed a higher prevalence, respectively 43.8% (Nwafor et al., 2019). *Balantidium coli* infection was found in a study in 2014 in Bangladesh, and the flotation method indicated a prevalence rate of 38.5%. The latest study from 2020 in Greece showed a prevalence of 81.3% in sows for this parasite species.

The economic losses generated by parasitic infections in swine generate a low interest from the research community than required to successfully manage and reduce their impact in swine breeding systems (Nwafor et al., 2019).

Conclusions

All the three age categories from this free-range farm had parasitic co-infections. The broadest spectrum of parasites was identified in fattening pigs in both seasons. Only fattening pigs had associated co-infections with *B. coli* and *Eimeria* spp./*I. suis, A. suum* and *T. suis* during spring and autumn. Piglets did not have helminthic infections, and sows had a co-infection with *A. suum* only during autumn. The study showed statistical significance between seasons and age groups for all diagnosed parasites. Even if the prevalence and the average intensity values were high, clinically, all the swine were asymptomatic, meaning there was no correlation between parasitic infections and clinical symptoms.

Acknowledgements

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