6.3.Improving the robustness of laying hens and piglets against parasitic and bacterial infections

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USAMV Cluj Napoca, ROMANIA

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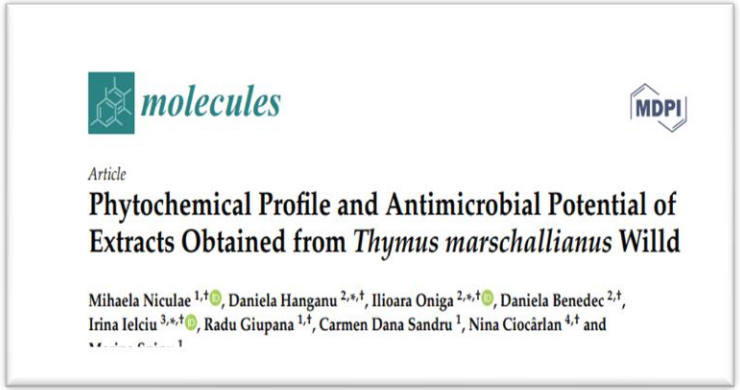


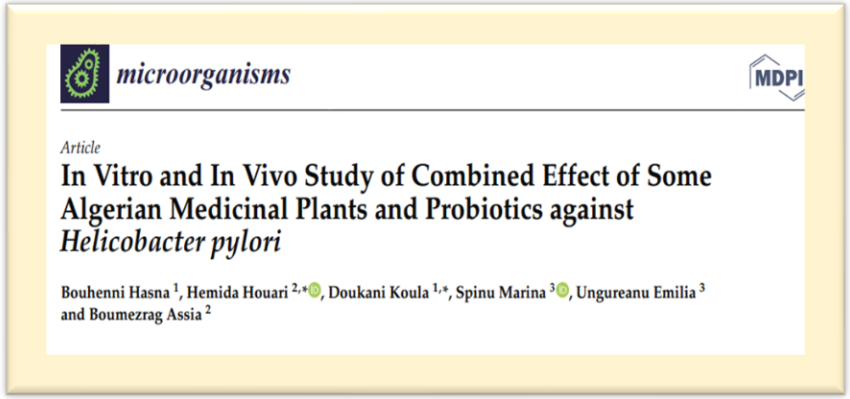
molecules

MDPI

Article

Phytochemical Profile and Antimicrobial Potential of Extracts Obtained from *Thymus marschallianus* Willd

Mihaela Niculae [1,†], Daniela Hanganu [2,*,†], Ilioara Oniga [2,*,†], Daniela Benedec [2,†], Irina Ielciu [3,*,†], Radu Giupana [1,†], Carmen Dana Sandru [1], Nina Ciocârlan [4,†] and



microorganisms

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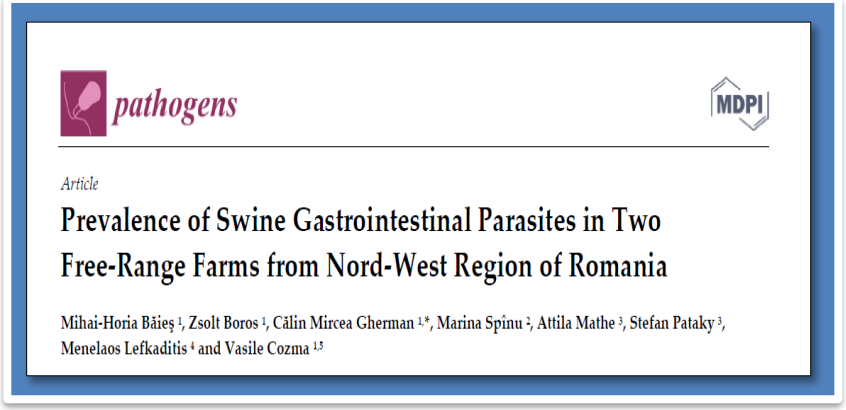
Article

In Vitro and In Vivo Study of Combined Effect of Some Algerian Medicinal Plants and Probiotics against *Helicobacter pylori*

Bouhenni Hasna [1], Hemida Houari [2,*], Doukani Koula [1,*], Spinu Marina [3], Ungureanu Emilia [3] and Boumezrag Assia [2]



pathogens

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Article

Prevalence of Swine Gastrointestinal Parasites in Two Free-Range Farms from Nord-West Region of Romania

Mihai-Horia Băieș [1], Zsolt Boros [1], Călin Mircea Gherman [1,*], Marina Spînu [2], Attila Mathe [3], Stefan Pataky [3], Menelaos Lefkaditis [4] and Vasile Cozma [1,5]

pathogens

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Article

The In Vitro Anticoccidial Activity of Some Herbal Extracts against *Eimeria* spp. Oocysts Isolated from Piglets

Mihai-Horia Băieș [1,†], Adriana Györke [1,†], Vlad-Dan Cotuțiu [1], Zsolt Boros [1], Anamaria Cozma-Petruț [2,*], Lorena Filip [2], Laurian Vlase [3], Ana-Maria Vlase [4], Gianina Crișan [4], Marina Spînu [5] and Vasile Cozma [1,6]

pathogens

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Article

The Effects of *Allium sativum* L., *Artemisia absinthium* L., *Cucurbita pepo* L., *Coriandrum sativum* L., *Satureja hortensis* L. and *Calendula officinalis* L. on the Embryogenesis of *Ascaris suum* Eggs during an in Vitro Experimental Study

Mihai-Horia Băieș [1], Călin Gherman [1], Zsolt Boros [1], Diana Olah [2], Ana-Maria Vlase [3], Anamaria Cozma-Petruț [4,*], Adriana Györke [1], Doina Miere [4], Laurian Vlase [5], Gianina Crișan [3], Marina Spînu [2] and Vasile Cozma [1,6]

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Increasing importance of anthelmintic resistance in European livestock: creation an meta-analysis of an open database

Hannah Rose Vineer,[1,a,*] Eric R. Morgan,[2,a,*] Hubertus Hertzberg,[3] David J. Bartley,[4] Antonio Bosco,[5] Johannes Charlier,[6] Christophe Chartier,[7] Edwin Claerebout,[8] Theo de Waal,[9] Guy Hendrickx,[10] Barbara Hinney,[11] Johan Höglund,[12] Jožica Ježek,[13] Martin Kašný,[14] Orla M. Keane,[15] María Martínez-Valladares,[16] Teresa Letra Mateus,[17,18] Jennifer McIntyre,[19] Marcin Mickiewicz,[20] Ana Maria Munoz,[21] Clare Joan Phythian,[22] Harm W. Ploeger,[23] Aleksandra Vergles Rataj,[24] Philip J. Skuce,[4] Stanislav Simin,[25] Smaragda Sotiraki,[26] Marina Spinu,[27] Snorre Stuen,[22] Stig Milan Thamsborg,[28] Jaroslav Vadlejch,[29] Marian Varady,[30]

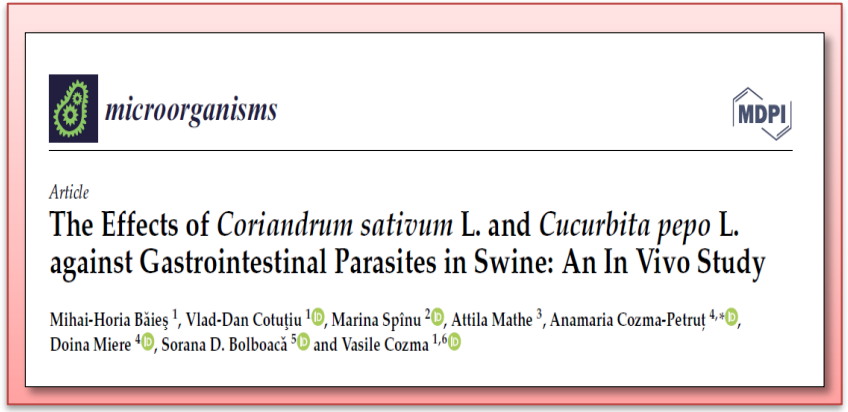
Journal of Infection and Public Health

Volume 7, Issue 4, July–August 2014, Pages 323-332

ELSEVIER

Detection of livestock-associated methicillin-resistant *Staphylococcus aureus* among swine workers in Romania

Aileen Huang [a], Anca E. Gurzau [b], Blake M. Hanson [c], Ashley E. Kates [c], Tara C. Smith [c], Melinda M. Pettigrew [c], Marina Spinu [d], Peter M. Rabinowitz [e]



microorganisms

MDPI

Article

The Effects of *Coriandrum sativum* L. and *Cucurbita pepo* L. against Gastrointestinal Parasites in Swine: An In Vivo Study

Mihai-Horia Băieș [1], Vlad-Dan Cotuțiu [1], Marina Spînu [2], Attila Mathe [3], Anamaria Cozma-Petruț [4,*], Doina Miere [4], Sorana D. Bolboacă [5] and Vasile Cozma [1,6]

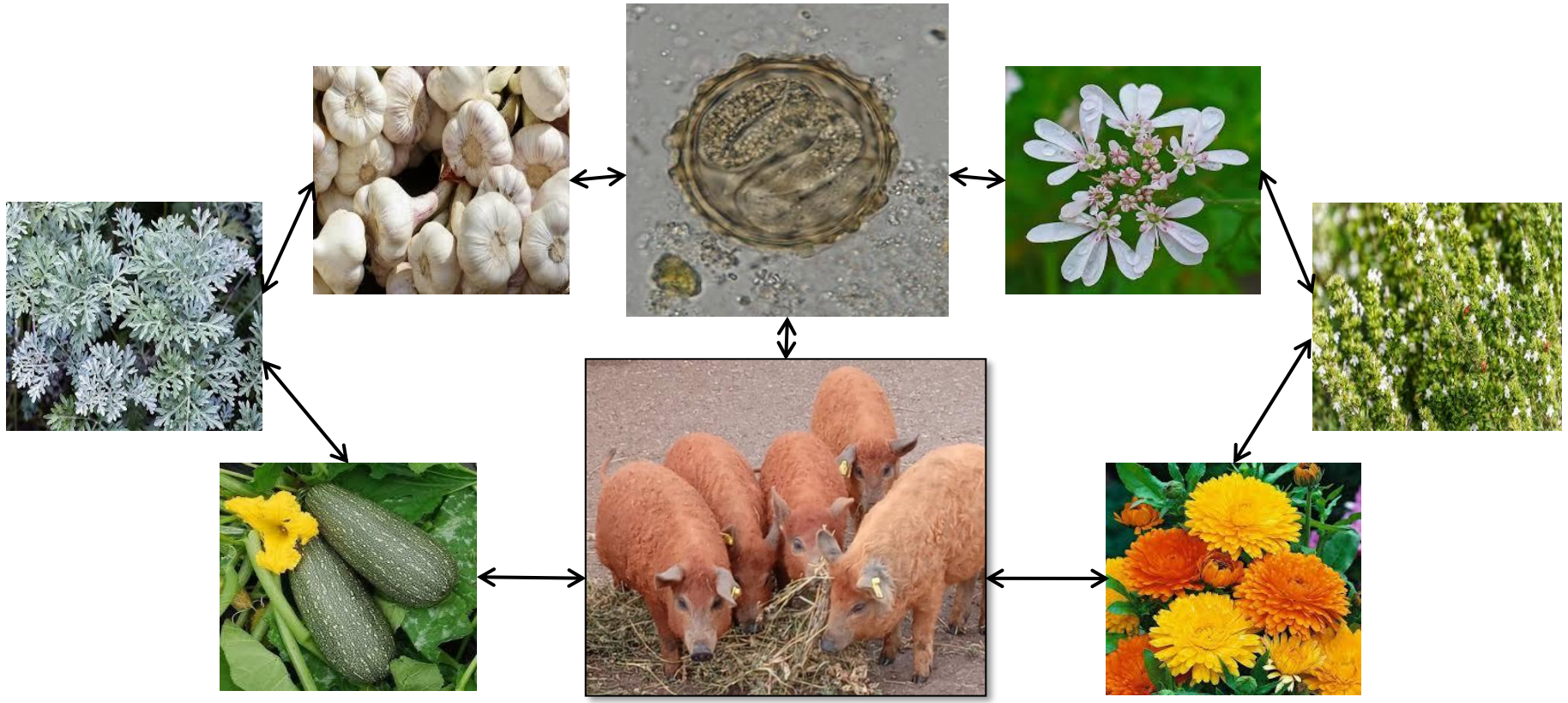
Introduction

- Parasitic diseases have a considerable effect on pig production, causing economic losses due to low immunity, high morbidity and mortality.
- Due to continuously increasing drug resistence in parasites and bacteria and prohibited use of antiparasitic and antibacterial medications in organic pig farming practices, phytotherapy could represent a valid, biologically available and cost effective alternative for parasite control.
- The use of phytotherapeutic remedies has notably increased over the past decade due to their biodegradability, decreased toxicity, environmentally friendliness, and to some extent their antiparasitic effect.

A

Aims

- **The primary objective of this research was to identify a plant-based formula that exhibits effectiveness in combating pig parasitoses without interfering with animal welfare and health.**
- **The present studies were designed to assess, *in vitro* and *in vivo,* the antiparasitic potential of *Allium sativum, Artemisia absinthium, Cucurbita pepo, Coriandrum sativum, Calendula officinalis, and Satureja hortensis* on naturally occurring gastrointestinal parasites of swine in two free-range (low-input) farms from Transylvania, and also to evaluate the parasitic prevalence in the same farms.**



Initial steps

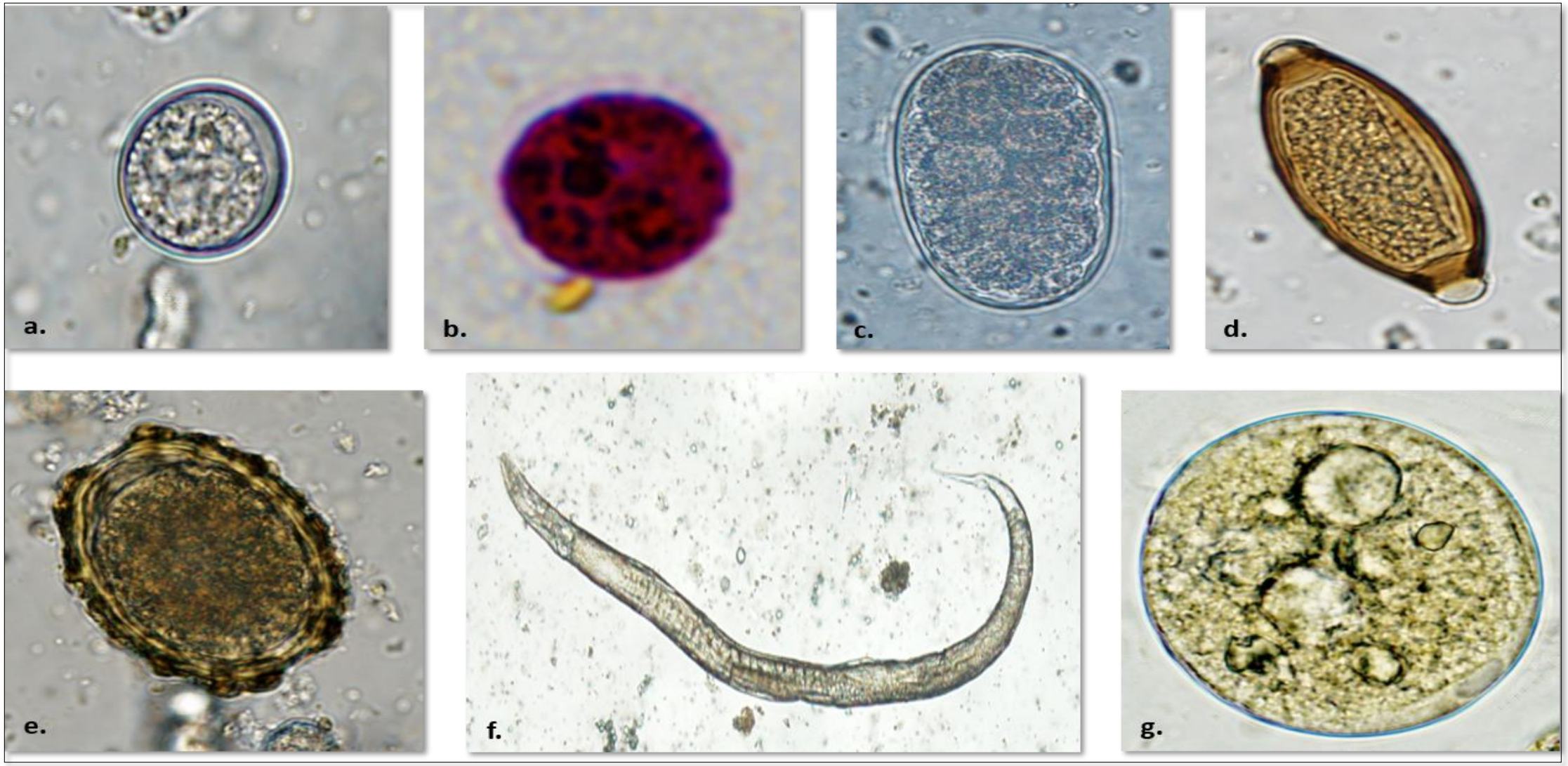
- Since parasitic diseases cause significant economic losses in swine industry and
- the number of free-range swine farms in Romania has increased in the last decades.
- **the current study segment aimed at identifying the parasitic profile of swine raised in two free-range (low-input) farms from Transylvania**.

Materials and methods

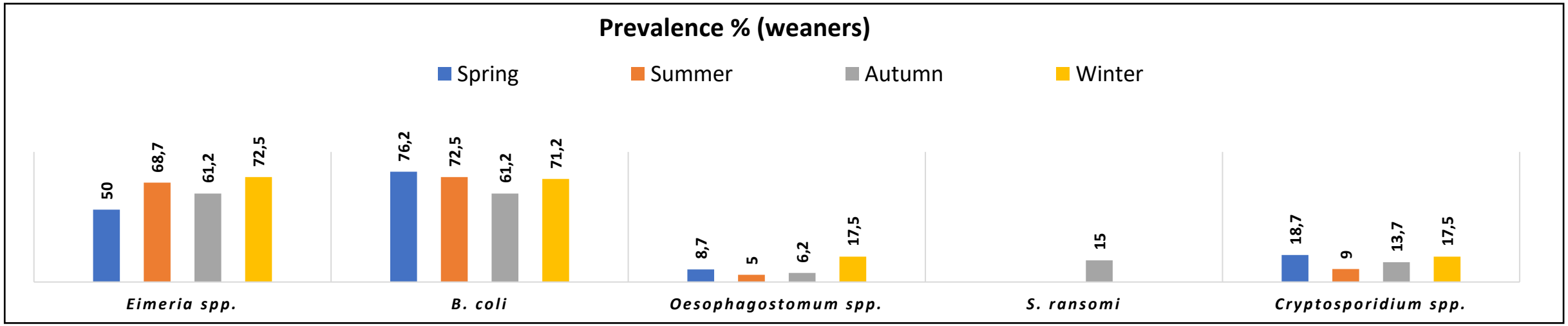
- 960 faecal samples were collected from weaners, fatteners, and sows, during the four seasons
- Coproparasitological examination methods: flotation (Willis, McMaster), active sedimentation, modified Ziehl-Neelsen stained fecal smear, modified Blagg technique and oocysts/eggs cultures.

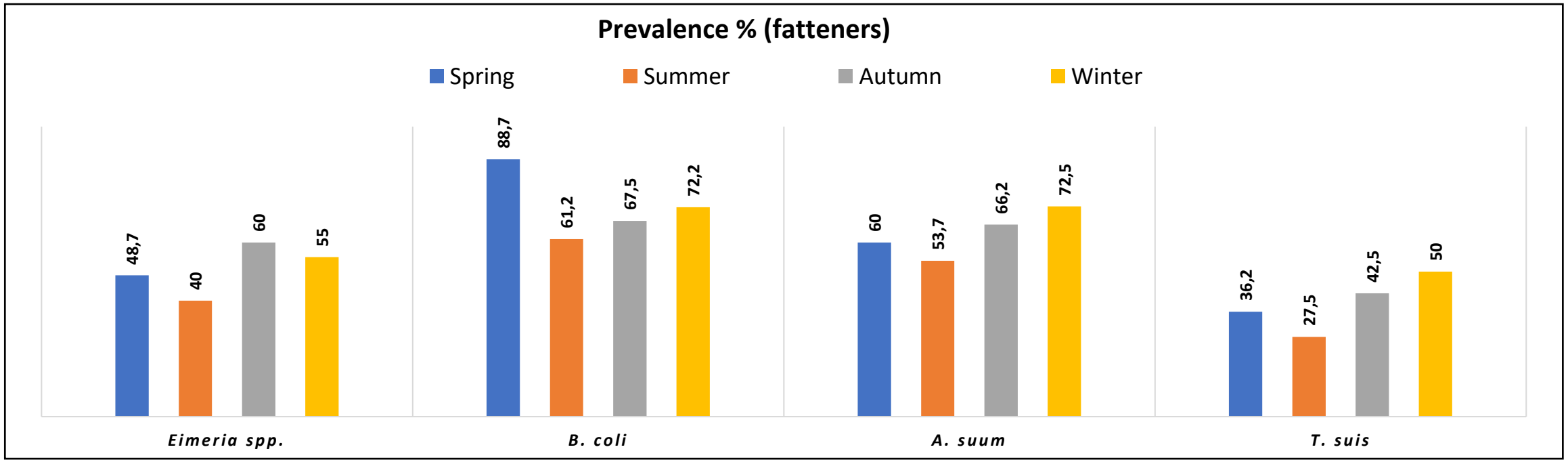
Results

Coproparasitological examination results: a- *Eimeria spp.* oocyst*, b- Cryptosporidium* spp. cyst, *c- Oesophagostomum spp.* egg, d- *T. suis egg, e- A. suum* egg*, f- S. ransomi* female and *g- B. coli.*

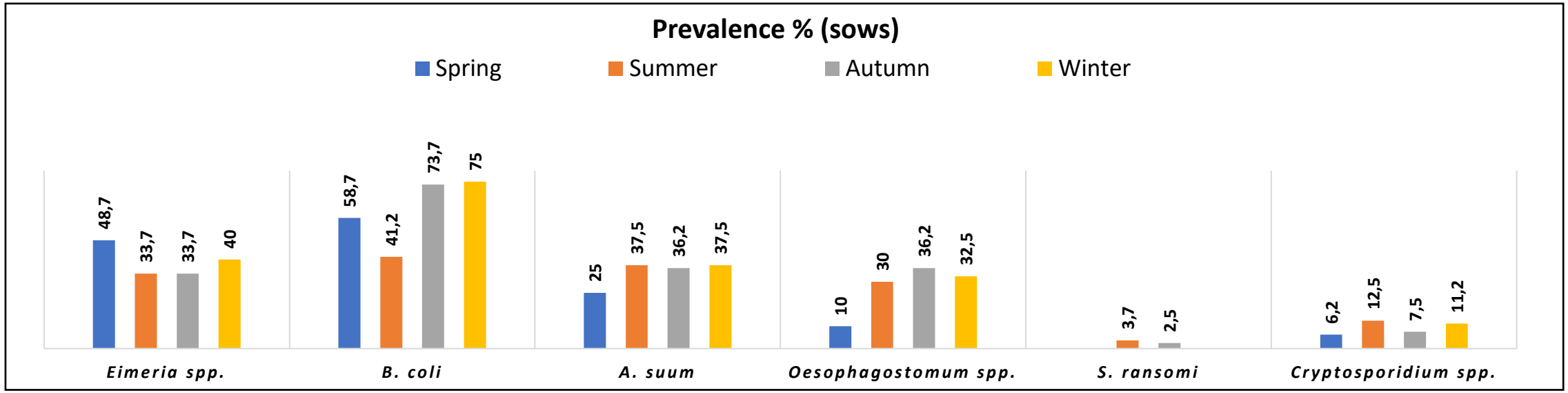


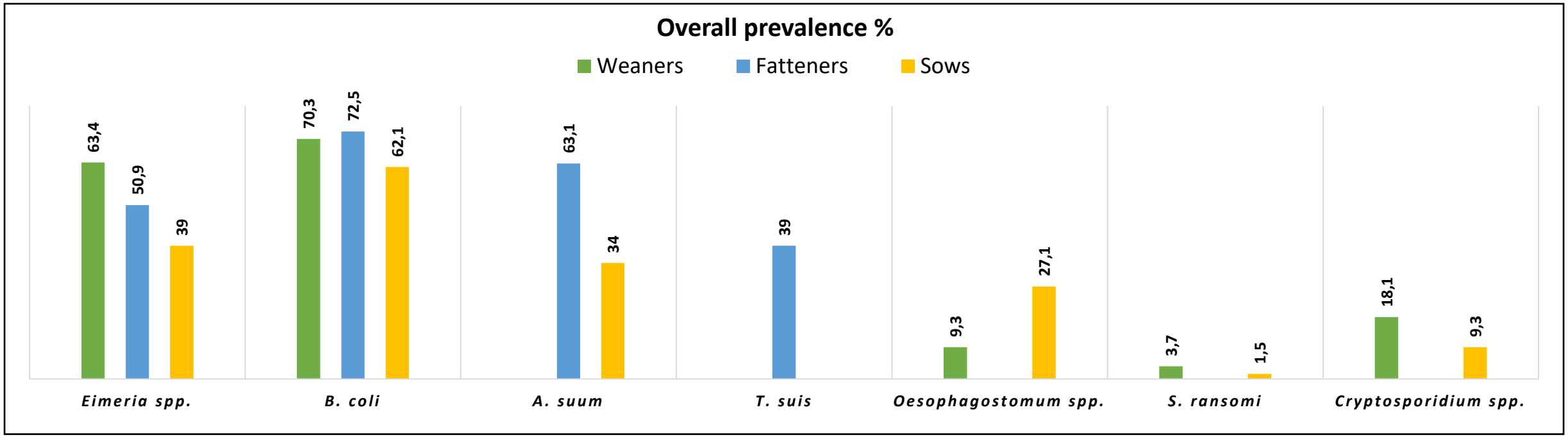
Results


Prevalence % (weaners)
Spring
Summer
Autumn
Winter
50
68,7
61,2
72,5
76,2
72,5
61,2
71,2
8,7
5
6,2
17,5
15
18,7
9
13,7
17,5
Eimeria spp.
B. coli
Oesophagostomum spp.
S. ransomi
Cryptosporidium spp.


Prevalence % (fatteners)
Spring
Summer
Autumn
Winter
48,7
40
60
55
88,7
61,2
67,5
72,2
60
53,7
66,2
72,5
36,2
27,5
42,5
50
Eimeria spp.
B. coli
A. suum
T. suis

Results


Prevalence % (sows)
Spring
Summer
Autumn
Winter
48,7
33,7
33,7
40
58,7
41,2
73,7
75
25
37,5
36,2
37,5
10
30
36,2
32,5
3,7
2,5
6,2
12,5
7,5
11,2
Eimeria spp.
B. coli
A. suum
Oesophagostomum spp.
S. ransomi
Cryptosporidium spp.


Overall prevalence %
Weaners
Fatteners
Sows
63,4
50,9
39
70,3
72,5
62,1
63,1
34
39
9,3
27,1
3,7
1,5
18,1
9,3
Eimeria spp.
B. coli
A. suum
T. suis
Oesophagostomum spp.
S. ransomi
Cryptosporidium spp.

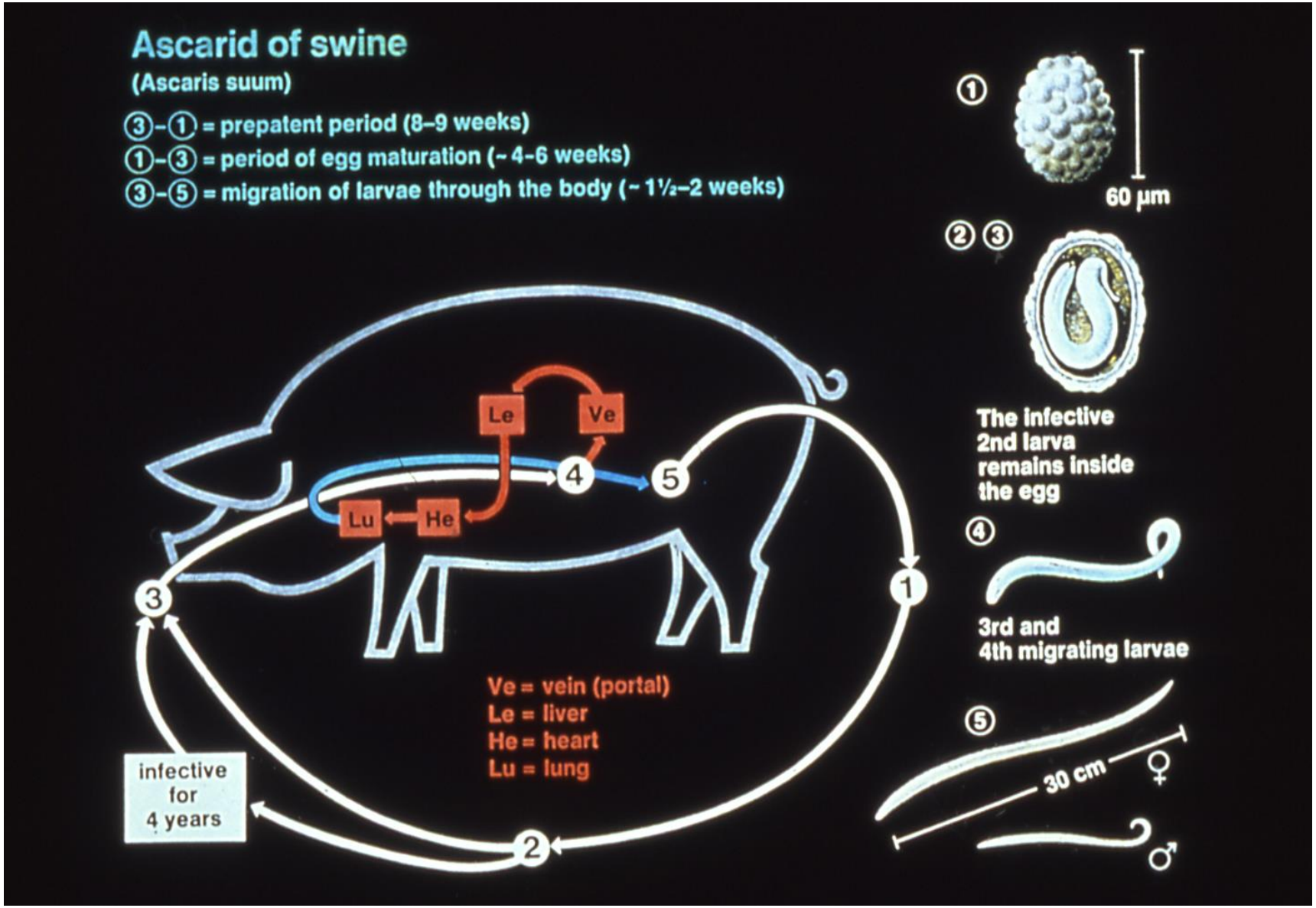
Conclusions

- This study provided essential information on Transylvania's distribution of gastrointestinal parasites in pigs.
- Different species of gastrointestinal parasites were present in most pigs reared in free-range farms in the study area
- Information of great value to farmers, policymakers, and researchers alike, leading to safer and healthier pork production for public consumption.
- Control strategies are needed to raise awareness among pig farmers about the impact of these parasites on the productivity and health of pigs as well as on human health.

The effects of *Allium sativum* L., *Artemisia absinthium* L., *Cucurbita pepo* L., *Coriandrum sativum* L., *Satureja hortensis* L. and *Calendula officinalis* L. on the embryogenesis of *Ascaris suum* eggs during an in vitro experimental study.

Background & Aim

- *Ascaris suum* is present in traditionally managed herds and on industrialized farms, especially in old fatteners and sows.
- Increasing resistance against antihelmintics redirected the research towards alternative, traditional therapies, medicinal plants included.
- **This study comparatively evaluated the *in vitro* effects of *Allium sativum*, *Artemisia absinthium L.*, *Cucurbita pepo*, *Coriandrum sativum*, *Satureja hortensis L.* and *Calendula officinalis* on inhibition of *A. suum* egg hatching and larval development.**

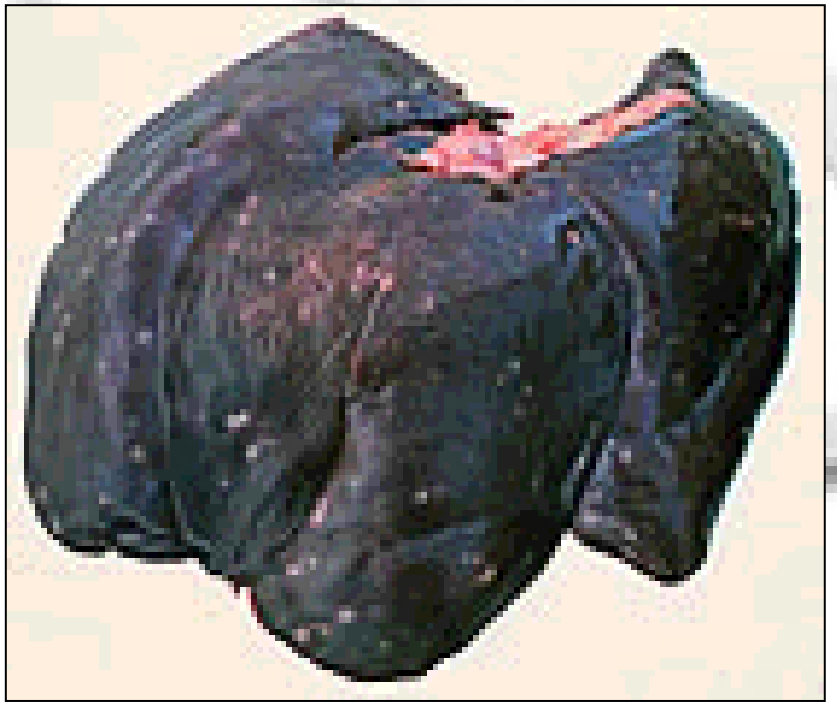

Ascarid of swine
(Ascaris suum)
③–① = prepatent period (8–9 weeks)
①–③ = period of egg maturation (~ 4–6 weeks)
③–⑤ = migration of larvae through the body (~ 1½–2 weeks)
Le
Ve
Lu
He
4
5
3
1
2
infective for 4 years
Ve = vein (portal)
Le = liver
He = heart
Lu = lung
①
60 µm
② ③
The infective 2nd larva remains inside the egg
④
3rd and 4th migrating larvae
⑤
30 cm
♀
♂

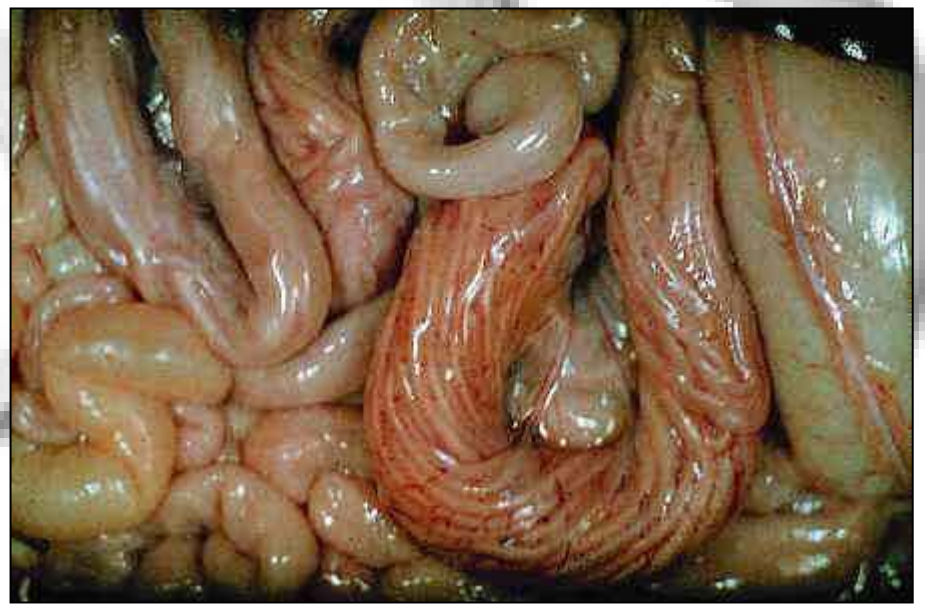
LESIONS

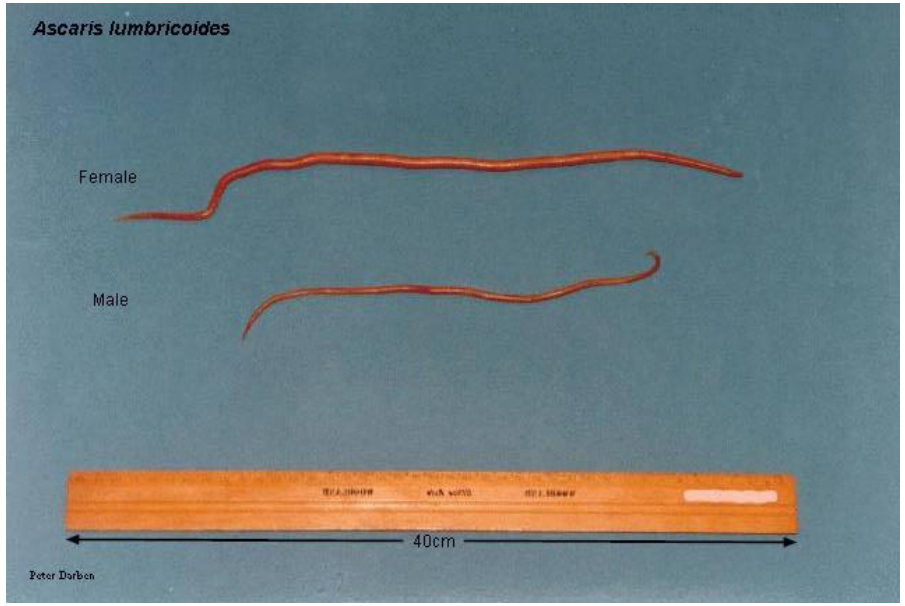
- Piglets: - catarrhal enteritis, obstruction of the small intestine, volvulus (a bowel obstruction in which a loop of bowel has abnormally twisted on itself) with gangrene of intestinal wall;

- the corpses are weak, with anemia, rickets, hyperkeratosis, jaundice, liver degeneration and cirrhosis;

- larval forms induce - pulmonary oedema, bronchial pneumonia, hemorrhage, liver necrosis.





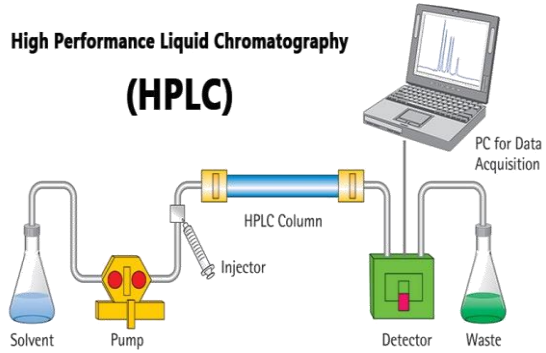


Ascaris lumbricoides

Materials and methods

1. Chemical analyses of medicinal plants

- High performance liquid chromatography coupled with mass spectrometry (HPLC/MS) was used for the analysis of biologically active compounds present in the plant extracts. All the procedures were performed at the Iuliu Haţieganu University of Medicine and Pharmacy, in Cluj-Napoca.

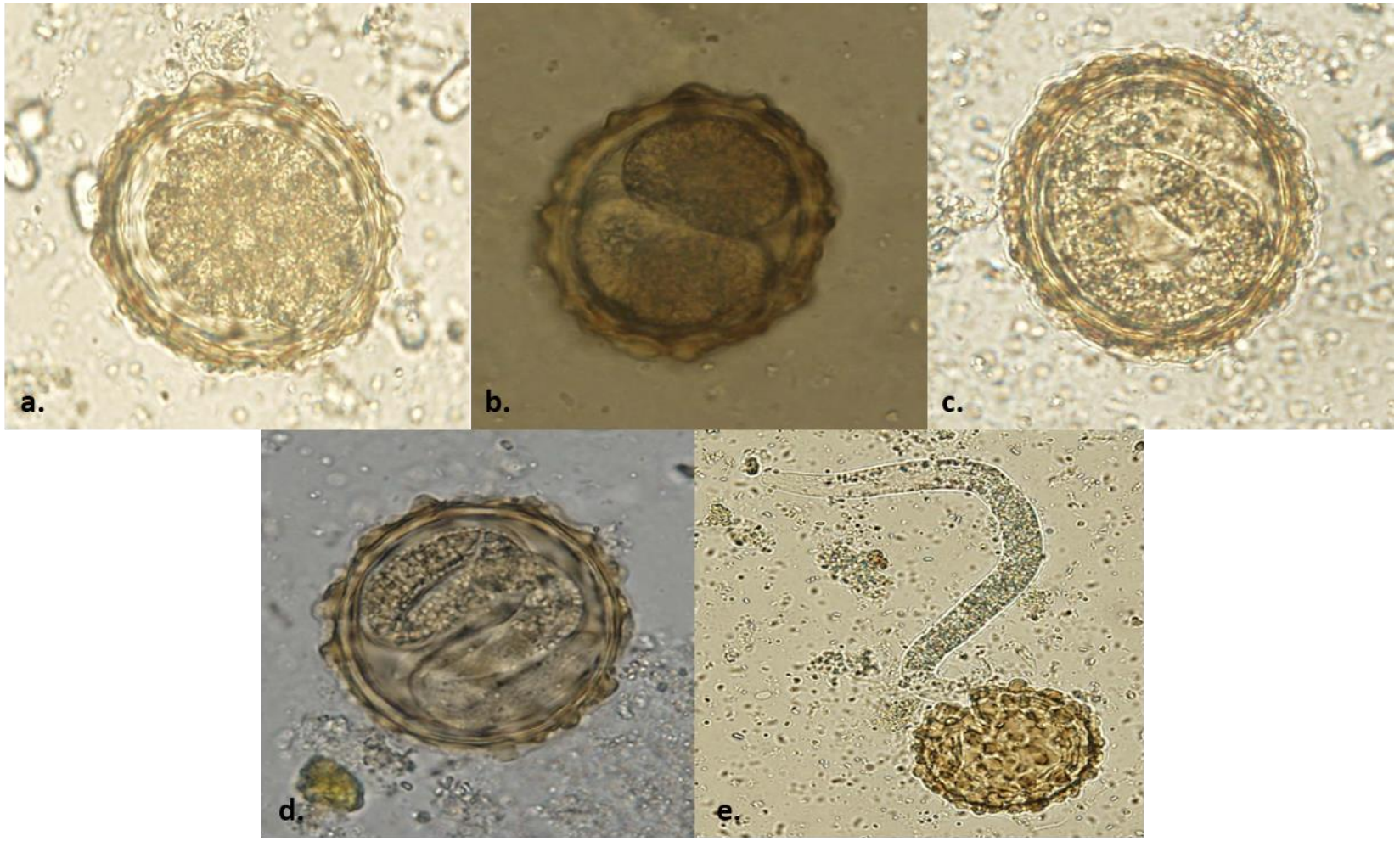


2. Experimental design

- *A. suum* eggs were collected from randomly sampled of traditionally maintained swine faeces. In 3 ml cell culture plates, the egg suspension (ES, $8x10^3$/ml) was divided in two control (C) (1C - 1ml ES + 1 ml distilled water, 2C- five plates of 1ml ES + 1ml ethanol of 70%, 35%, 17.5%, 8.75%, and 4.375%, respectively) and six experimental groups.
- The experimental (E, 1-6) groups included ES + each alcoholic plant extract (10%, 5%, 2.5%, 1.25%, 0.625%). Both C and E were performed in quintuplicate.
- All groups were incubated at 27 ℃ for a total of 21 days, *A. suum* eggs being examined after 2, 14 (L1) and 21 (L2/L3) days.

Materials and methods

3. Eggs Hatch Test/Larval Development Assay



(a) *Ascaris suum* unembryonated egg; (**b**) Egg of *A. suum* in two stage cell (early morula); (**c**) *A. suum* egg with L1 (larva); (**d**) *A. suum* egg with L 2/3 (larva); **(e)** Hatched L 2/3 (larva).

Results

1. Analysis of plant extracts

Bioactive Compounds		Vegetal Species and Plant Part Used for Extraction and HPLC-MS Analysis					
		A. absinthium	*S. hortensis*	*C. officinalis*	*A. sativum*	*C. sativum*	*C. pepo*
		herba	herba	herba	bulbus	fructus	semen
Polyphenols (µg/mL)	Chlorogenic acid	107.15	<LOQ	220.767	-	4.177	-
	Caffeic acid	-	<LOQ	-	1.221	-	-
	p-coumaric acid	0.621	1.464	-	-	0.501	-
	Ferulic acid	0.759	0.557	-	0.456	0.759	-
	Sinapic acid	-	-	-	0.228	-	-
	Vitexin	1.631	-	-	-	-	-
	Isoquercitrin	56.754	6.515	38.877	-	-	-
	Rutoside	3.826	<LOQ	18.819	-	<LOQ	-
	Quercitrin	1.113	0.365	<LOQ	-	-	-
	Quercetol	6.285	0.394	-	-	-	-
	Luteolin	1.159	6.621	-	-	-	-
	Kaempferol	3.666	-	-	-	-	-
	Apigenin	0.481	2.442	-	-	-	-
	Syringic acid	1.85	2.28	1.51	-	0.09	-
	Protocatechuic acid	1.32	0.95	0.67	-	-	-
	Vanillic acid	1.98	0.65	0.44	-	0.94	-

HPLC/MS—high performance liquid chromatography coupled with mass spectrometry; "-"—Not found; <LOQ—identified based on MS spectra but not determined quantitatively, below limit of quantification.

Results

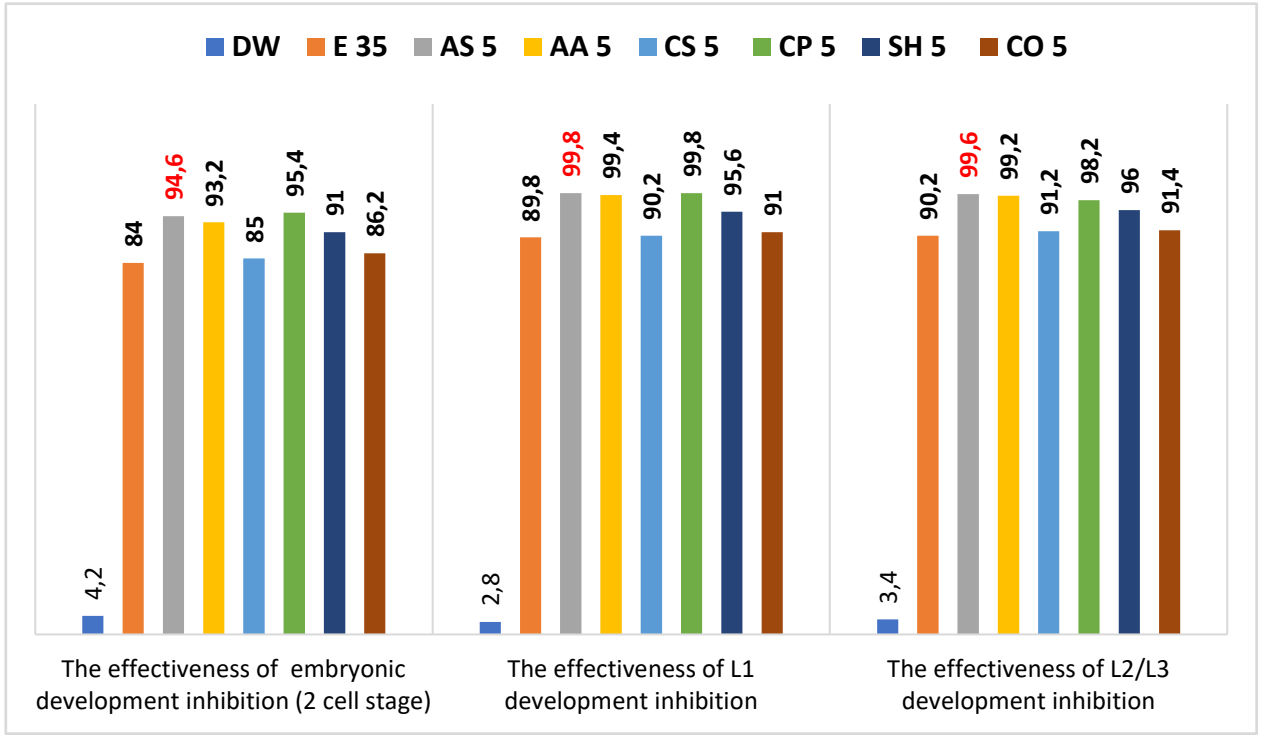
Bioactive Compounds		Vegetal Species and Plant Part Used for Extraction and HPLC-MS Analysis					
		A. absinthium	***S. hortensis***	***C. officinalis***	***A. sativum***	***C. sativum***	***C. pepo***
		herba	herba	herba	bulbus	fructus	semen
Tocopherols (ng/mL)	α-tocopherol	50.0	86.8	61.6	36.1	-	-
	γ-tocopherol	23.8	89.0	248.9	-	-	446.0
	Δ-tocopherol	5.0	13.2	9.3	-	-	23.2
Sterols (µg/mL)	Ergosterol	0.344	1.420	0.500	-	0.584	-
	Stigmasterol	34.831	14.215	72.888	-	9.675	22.024
	B-sitosterol	140.985	313.315	241.997	-	31.548	5.355
	Campesterol	3.329	6.140	1.635	-	1.780	0.358
Methoxylated flavones (ng/mL)	Jaceosidin	-	8820.76	-	-	-	-
	Hispidulin	3047.92	2483.00	-	-	-	-
	Eupatorin	976.53	-	-	-	-	-
	Casticin	15,384.14	-	-	-	-	-
	Acacetin		12691.97	-	-	-	-
Sesquiterpene lactones (ng/mL)	α-santonin	450.52	-	-	-	-	-
	Vulgarin	6499.39	-		-	-	-
Sulfoxide (µg/mL)	Aliin	-	-	-	14.726	-	-

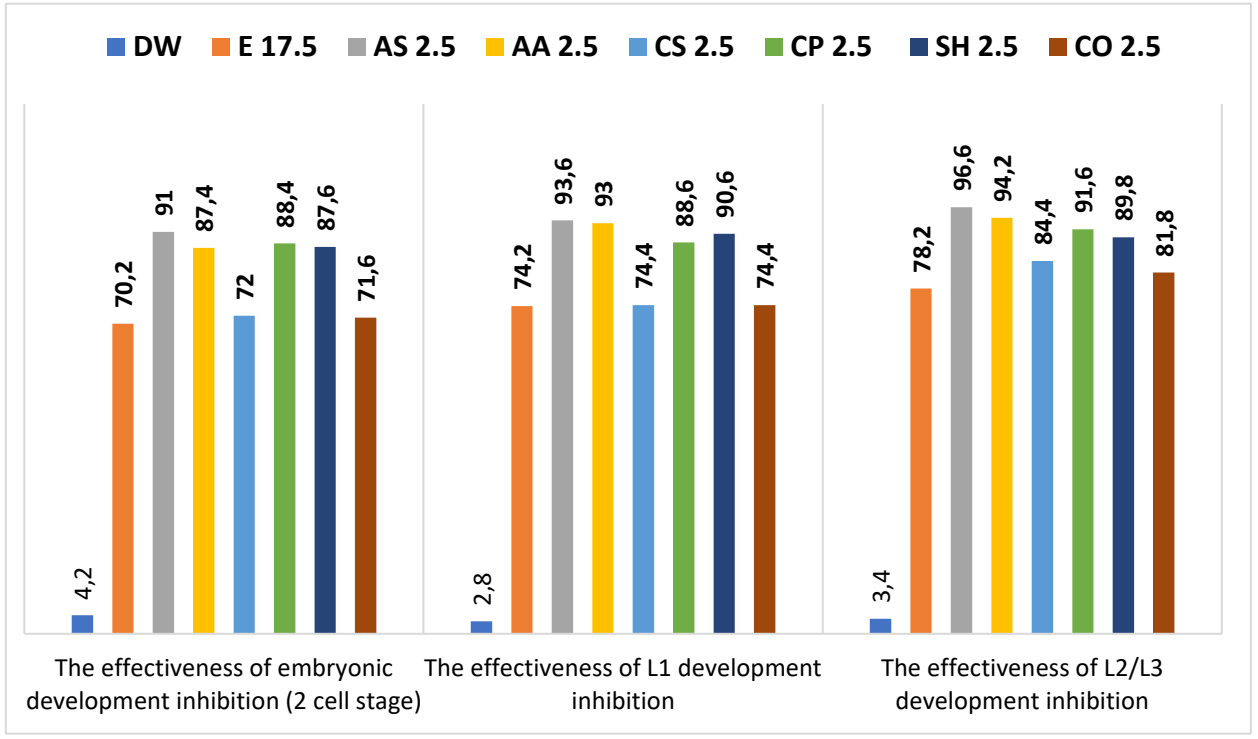
HPLC/MS—high performance liquid chromatography coupled with mass spectrometry; "-"—Not found; <LOQ—identified based on MS spectra but not determined quantitatively, below limit of quantification.

Results

2. Analysis of Plant Extracts Activity

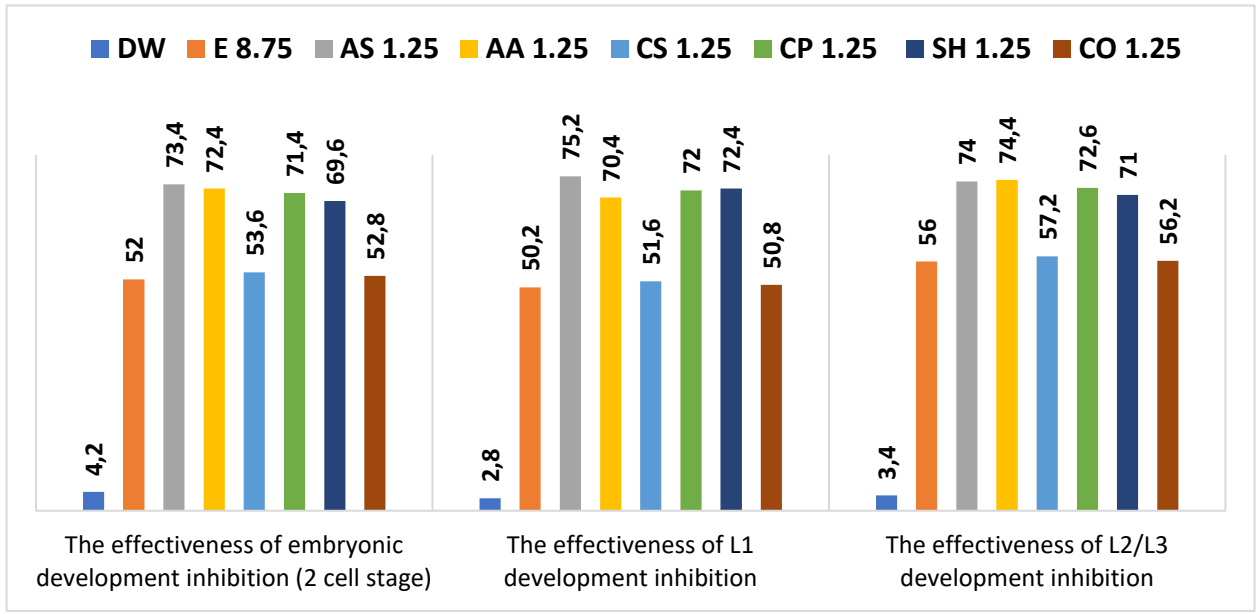
The efficacy of all tested plants, when compared to the control groups increased with concentration. Anti-embryogenic effects on the *A. suum* eggs were expressed by all plants, with more pronounced influence of the *A. sativum, A. absinthium, C. pepo* and *S. hortensis* extracts at all tested concentrations.

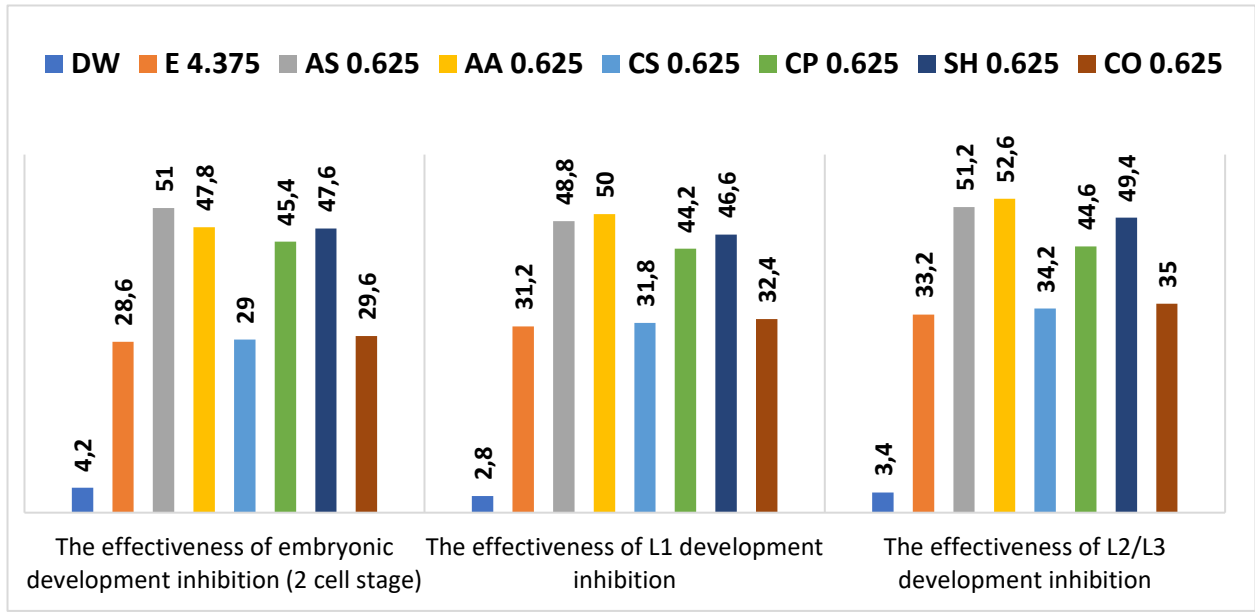


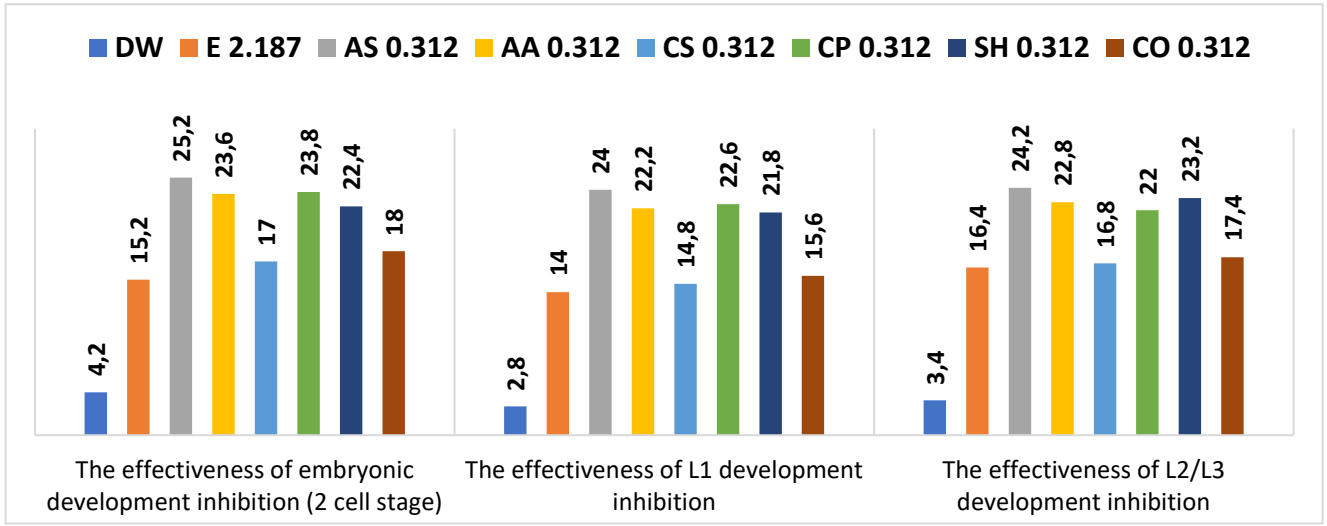


Percentage of embryogenesis inhibition at 5 % and 2.5 % concentration: Distilled water (DW), Ethanol (E), *A. sativum* L. (AS), *A. absinthium* L. (AA), *C. sativum* L. (CS), *C. pepo* L. (CP), *S. hortensis* L. (SH), *C. officinalis* L. (CO).

Results







Percentage of embryogenesis inhibition at 1.25 %, 0.625 % and 0.312 % concentration: Distilled water (DW), Ethanol (E), *A. sativum* L. (AS), *A. absinthium* L. (AA), *C. sativum* L. (CS), *C. pepo* L. (CP), *S. hortensis* L. (SH), *C. officinalis* L. (CO).

Conclusions

- In this study, all plants extracts showed various degrees of inhibitory effects on egg development. *A. sativum* L., *A. absinthium* L., *C. pepo* L. and *S. hortensis* L. extracts have shown the strongest anthelmintic activity.

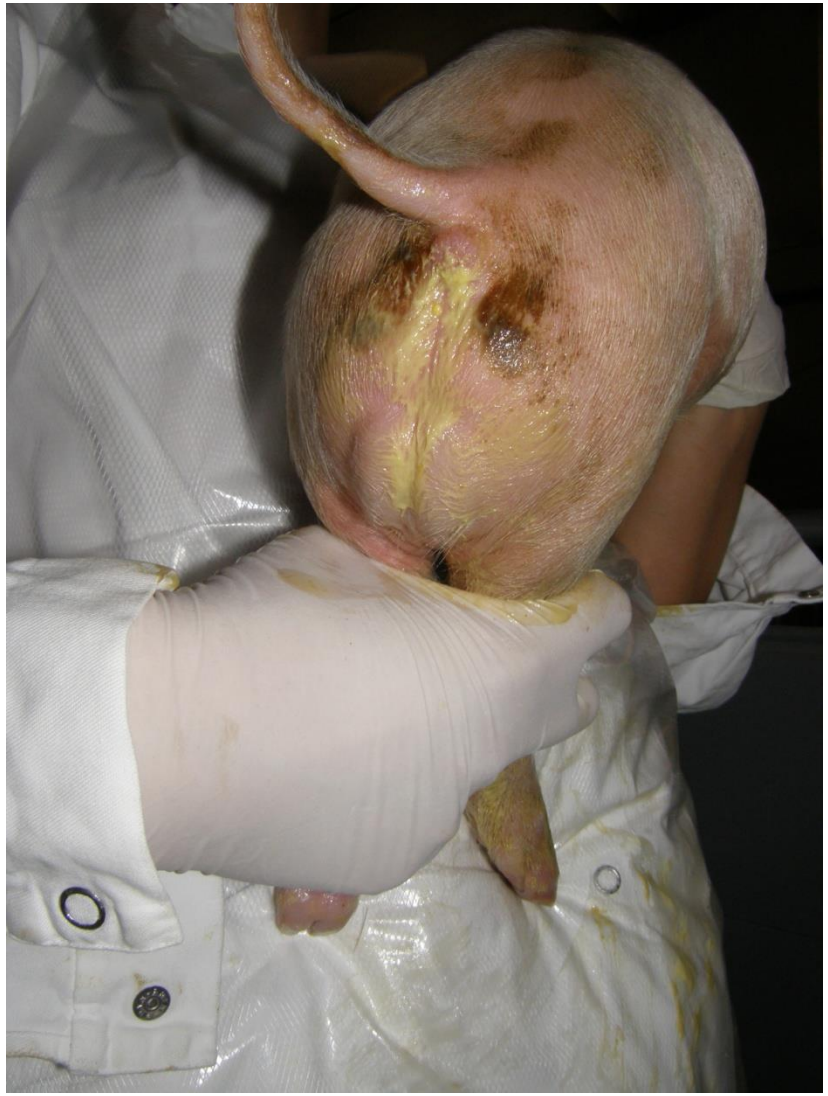
- In summary, based on the results of this study we suggested that some of these plant materials could be prospective sources for development of new antiparasitic herbal remedies.

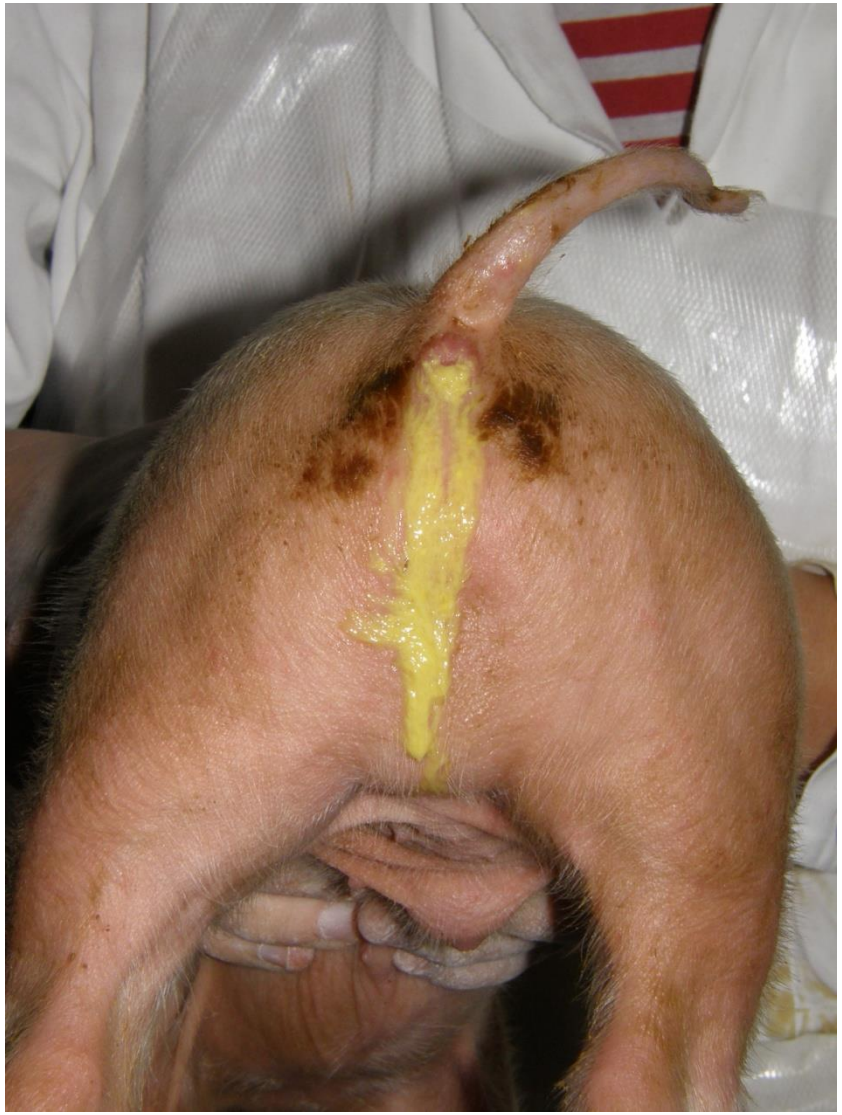
- *To our knowledge, this is the first ethno-pharmacological report based on the anthelmintic activity of medicinal plants traditionally used to treat A. suum infection in Romania.*

The *in vitro* anticoccidial activity of some herbal extracts against *Eimeria* spp. oocysts isolated from piglets.

Background & Aim

- Coccidiosis in certain livestock (birds and ruminants), caused by *Eimeria* spp., has a severe economic impact. In pigs, on the other hand, it is considered less significant, as natural infections are only sporadically related to clinical disease.
- Infections with *Eimeria* spp. are common in pigs worldwide, clinically affecting weaners and fatteners, manifested through diarrhea and weight loss.
- Lastly, an increased interest in safe and effective alternatives aimed at controlling coccidiosis has led to the use of plant extracts, essential oils, and traditional medicinal products, in organic swine farms in particular.
- **The aim of this study was to evaluate the effects of the alcoholic extracts from *Allium sativum* L. (garlic), *Artemisia absinthium* L. (wormwood), *Coriandrum sativum* L. (coriander), *Cucurbita pepo* L. (pumpkin), *Satureja hortensis* L. (summer savory), and *Calendula officinalis* L. (marigold) on the sporulation of *Eimeria suis* and *Eimeria debliecki* oocysts, isolated from piglets.**



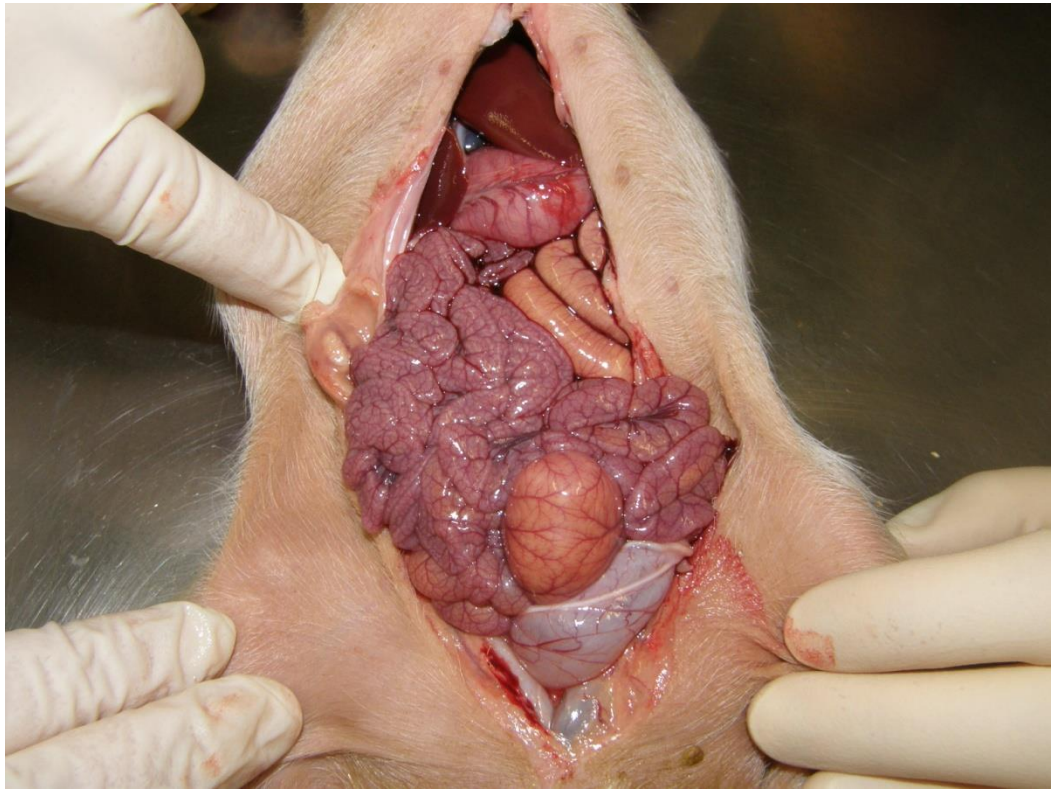


Photos: Prof. A. Joachim ,Vienne

Diagnostic

Postmortem examination reveals congestion of the jejunum and ileum.

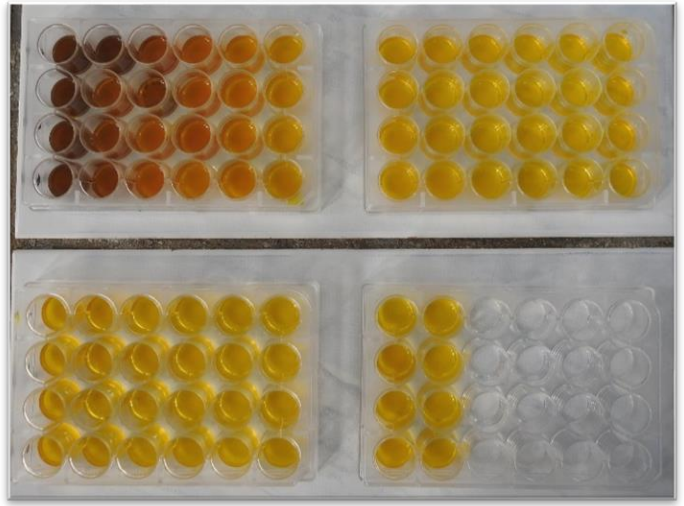
Coprology on a mixture of feces is useful

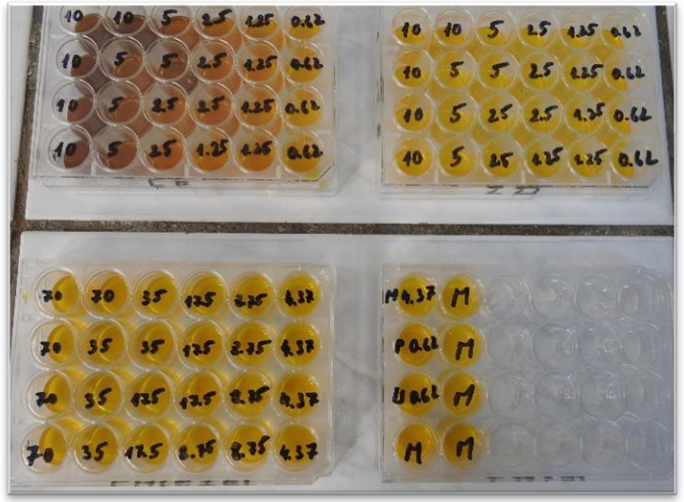


Materials and methods

Experimental design

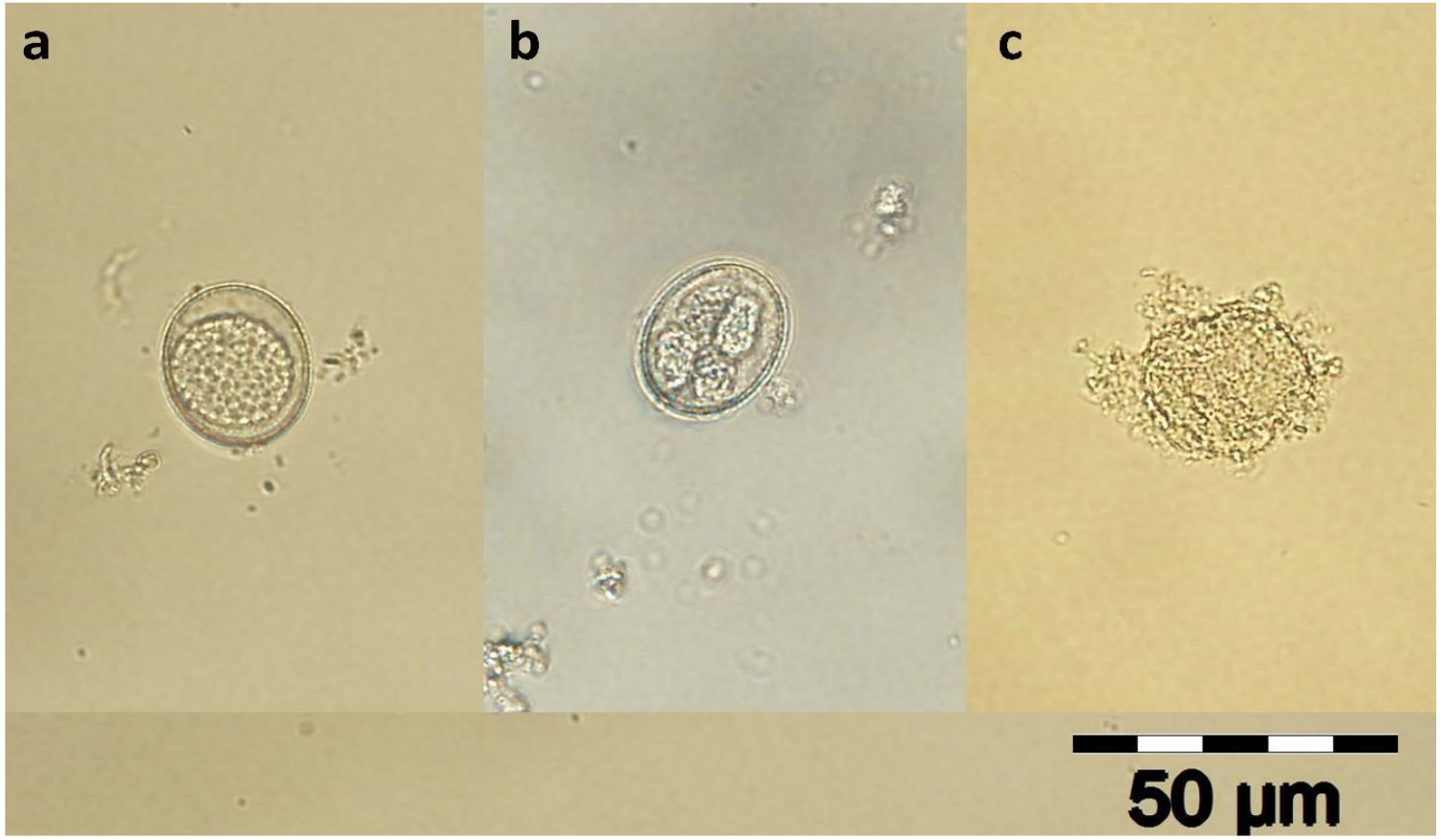
Groups	Concentration (%)	Abbreviations	Content/Well
Potassium dichromate	0.625	PD	1 mL SOS (0.5 mL OS + 0.5 mL 2.5% PD) + 1 mL DW
Ethanol	35	E 35	1 mL SOS + 1 mL 70% E
	17.5	E 17.5	1 mL SOS + 1 mL 35% E
	8.75	E 8.75	1 mL SOS + 1 mL 17.5% E
	4.375	E 4.375	1 mL SOS + 1 mL 8.75% E
	2.187	E 2.187	1 mL SOS + 1 mL 4.37% E
Alcoholic plant extracts	5	AS 5, AA 5, CS 5, CP 5, SH 5, CO 5	1 mL SOS + 1 mL 10% APE
	2.5	AS 2.5, AA 2.5, CS 2.5, CP 2.5, SH 2.5, CO 2.5	1 mL SOS + 1 mL 5% APE
	1.25	AS 1.25, AA 1.25, CS 1.25, CP 1.25, SH 1.25, CO 1.25	1 mL SOS + 1 mL 2.5% APE
	0.625	AS 0.625, AA 0.625, CS 0.625, CP 0.625, SH 0.625 CO 0.625	1 mL SOS + 1 mL 1.25% APE
	0.312	AS 0.312, AA 0.312, CS 0.312, CP 0.312, SH 0.312, CO 0.312	1 mL SOS + 1 mL 0.625% APE





OS—oocysts suspension, SOS—stock oocysts suspension (oocysts suspension mixed with potassium dichromate in equal volumes), PD—potassium dichromate, DW—distilled water, E—ethanol, APEs—alcoholic plant extract, AS—*A. sativum*, AA—*A. absinthium*, CP—*C. pepo*, CS—*C. sativum*, SH—*S. hortensis*, CO—*C. officinalis*.

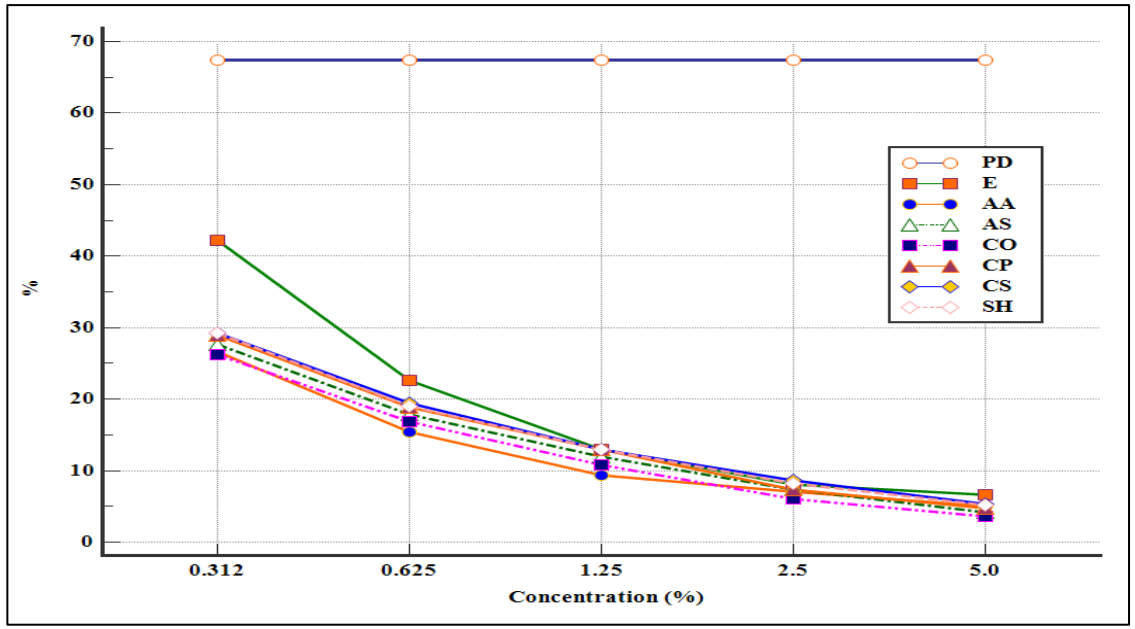
Materials and methods



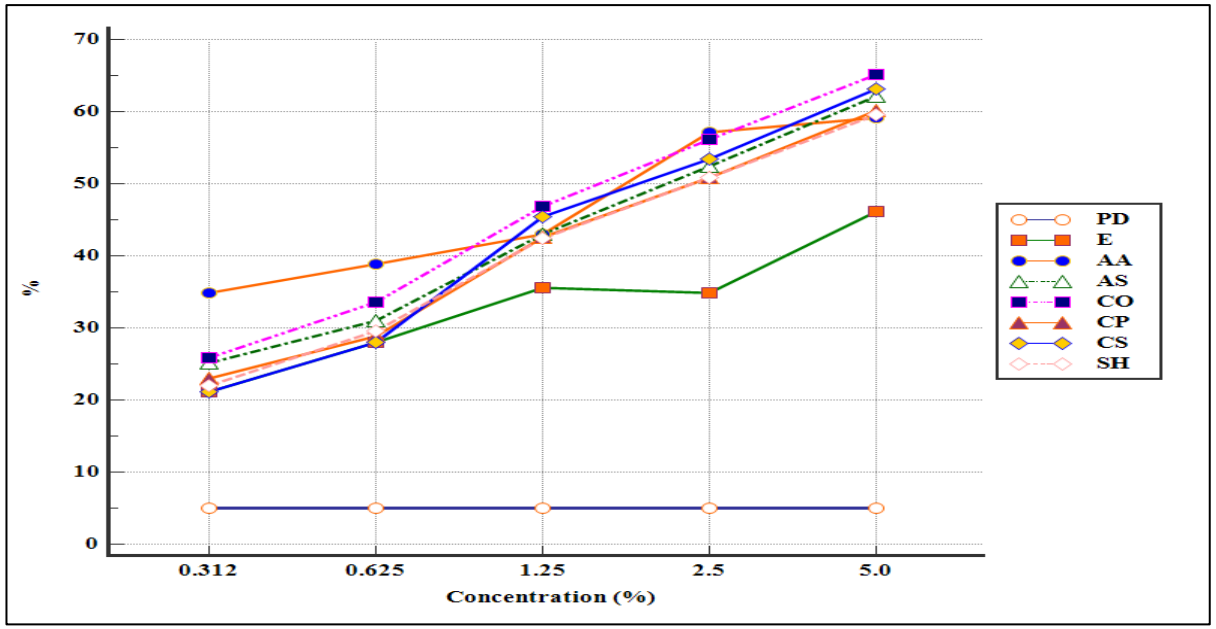
Unsporulated (**a**), sporulated (**b**), and destroyed oocyst (**c**) of *Eimeria suis* (400×). The concentration of 50 mg/mL of each APE produces complete oocyst wall destruction (**c**).

Results

In vitro antiparasitic activity of APE against *Eimeria* spp. oocysts



Sporulated oocysts after 96 h of incubation (PD—potassium dichromate, E—ethanol, AA— *A. absinthium*, AS— *A. sativum*, CO— C*. officinalis*, CP— *C. pepo*, CS— *C. sativum*, SH— *S. hortensis*).



Destroyed oocysts after 96 h of incubation (PD—potassium dichromate, E—ethanol, AA— *A. absinthium*, AS— *A. sativum*, CO— C*. officinalis*, CP— *C. pepo*, CS— *C. sativum*, SH— *S. hortensis*).

The LC_{50} (lethal concentration) of each APE after 72 and 96 h of incubation.

Time (hours)	AS (mg/mL)	AA (mg/mL)	CS (mg/mL)	CP (mg/mL)	SH (mg/mL)	CO (mg/mL)
72	28.84	31.62	28.18	33.11	35.48	24.55
96	21.88	18.62	20.42	23.44	23.99	16.98

AA— *A. absinthium*, AS— *A. sativum*, CO— C*. officinalis*, CP— *C. pepo*, CS— *C. sativum*, SH— *S. hortensis*

Results

The percentage of destroyed oocysts (mean ± SDM) from the experimental groups using Abbot formula.

Time (hours)	AS 5	AA 5	CS 5	CP 5	SH 5	CO 5
24	16.44 ± 5.22 ^{a}	9.23 ± 3.05 ^{a}	18.5 ± 3.59 ^{a}	15.6 ± 2.88 ^{a}	15.03 ± 2.72 ^{a}	18.94 ± 1.93 ^{a}
48	16.12 ± 6.38 ^{a}	6.98 ± 2.04 ^{a}	17.74 ± 3.34 ^{a}	13.05 ± 2.29 ^{a}	14.87 ± 2.54 ^{a}	18.06 ± 2.77 ^{a}
72	23.78 ± 5.47 ^{a}	13.2 ± 3.09 ^{a}	23.97 ± 3.74 ^{a}	21.97 ± 2.98 ^{a}	20.36 ± 2.88 ^{a}	27.5 ± 2.18 ^{a}
96	29.38 ± 3.16 ^{ab}	23.96 ± 3.71 ^{ab}	31.27 ± 4.42 ^{ab}	25.36 ± 2.38 ^{ab}	24.21 ± 2.45 ^{b}	**35.01 ± 1.93 ^{a}**
	AS 2.5	**AA 2.5**	**CS 2.5**	**CP 2.5**	**SH 2.5**	**CO 2.5**
24	15.94 ± 4.66 ^{a}	16.03 ± 4.82 ^{a}	18.96 ± 3.68 ^{a}	15.2 ± 2.92 ^{a}	14.1 ± 2.54 ^{a}	18.68 ± 2.07 ^{a}
48	14.72 ± 4.47 ^{a}	23.46 ± 4.79 ^{a}	18.78 ± 3.49 ^{a}	12.47 ± 2.94 ^{a}	13.03 ± 2.17 ^{a}	17.8 ± 2.55 ^{a}
72	25.79 ± 6.68 ^{a}	32.12 ± 3.81 ^{a}	25.6 ± 3.24 ^{a}	22.07 ± 2.82 ^{a}	22.64 ± 3.27 ^{a}	29.72 ± 3.14 ^{a}
96	26.45 ± 6.66 ^{a}	**33.82 ± 4.87 ^{a}**	28.08 ± 3.29 ^{a}	24.02 ± 3.28 ^{a}	24.15 ± 2.79 ^{a}	32.51 ± 1.72 ^{a}
	AS 1.25	**AA 1.25**	**CS 1.25**	**CP 1.25**	**SH 1.25**	**CO 1.25**
24	6.22 ± 3.28 ^{a}	9.44 ± 3.39 ^{a}	9.6 ± 2.29 ^{a}	4.99 ± 1.34 ^{a}	4.49 ± 1.02 ^{a}	8.5 ± 1.51 ^{a}
48	5.21 ± 3.21 ^{a}	6.85 ± 2.54 ^{a}	8.39 ± 2.7 ^{a}	3.97 ± 1.12 ^{a}	3.72 ± 0.99 ^{a}	8.39 ± 1.81 ^{a}
72	11.05 ± 3.17 ^{ab}	11.14 ± 2.93 ^{ab}	11.65 ± 2.23 ^{ab}	7.42 ± 1.83 ^{ab}	5.97 ± 1.19 ^{b}	14.18 ± 1.94 ^{a}
96	10.46 ± 4.28 ^{a}	10.41 ± 2.3 ^{a}	14.31 ± 2.52 ^{a}	10.23 ± 1.73 ^{a}	9.93 ± 1.88 ^{a}	**16.31 ± 1.76 ^{a}**
	AS 0.625	**AA 0.625**	**CS 0.625**	**CP 0.625**	**SH 0.625**	**CO 0.625**
24	4.77 ± 2.32 ^{a}	8.3 ± 3.8 ^{a}	8.84 ± 2.32 ^{a}	3.73 ± 1 ^{a}	3.21 ± 0.8 ^{a}	7.6 ± 1.41 ^{a}
48	3.33 ± 1.96 ^{a}	12.97 ± 3.24 ^{a}	10.43 ± 2.78 ^{a}	4.31 ± 1.29 ^{a}	3.81 ± 1.05 ^{a}	9.74 ± 1.87 ^{a}
72	4.14 ± 2.22 ^{ac}	14.17 ± 3.05 ^{a}	5.57 ± 1.88 ^{ac}	1.34 ± 0.46 ^{c}	3.9 ± 0.94 ^{bc}	8.71 ± 1.58 ^{ab}
96	3.82 ± 2.17 ^{bc}	**14.81 ± 2.99 ^{a}**	5.94 ± 1.36 ^{bc}	0.8 ± 0.12 ^{c}	1.89 ± 0.3 ^{c}	7.49 ± 1.34 ^{b}
	AS 0.312	**AA 0.312**	**CS 0.312**	**CP 0.312**	**SH 0.312**	**CO 0.312**
24	2.4 ± 1.48 ^{ab}	11.01 ± 4.16 ^{a}	5.22 ± 1.89 ^{ab}	1.24 ± 0.42 ^{b}	1.47 ± 0.38 ^{b}	4.75 ± 1.16 ^{ab}
48	3.58 ± 2.07 ^{b}	14.89 ± 3.44 ^{a}	6.57 ± 1.54 ^{b}	1.85 ± 0.51 ^{b}	2.1 ± 0.62 ^{b}	6.55 ± 1.32 ^{b}
72	3.31 ± 2.04 ^{bc}	14.92 ± 3.69 ^{a}	1.89 ± 0.65 ^{bc}	0.54 ± 0.12 ^{c}	0.5 ± 0.09 ^{c}	6.57 ± 1.31 ^{b}
96	5.06 ± 3.22 ^{bc}	**17.26 ± 3.09 ^{a}**	1.9 ± 0.65 ^{bc}	2.06 ± 0.58 ^{bc}	0.95 ± 0.2 ^{c}	5.74 ± 1 ^{b}

SDM—standard deviation of mean, AS (*A. sativum*), AA (*A. absinthium*), CP (*C. pepo*), CS (*C. sativum*), SH (*S. hortensis*), CO (*C. officinalis*).
Values with no common superscript in a column within an experiment were significantly different ($p \leq 0.05$).

Conclusions

- This study is one of the few performed on *Eimeria* spp. oocysts isolated from piglets. Statistical analysis showed that all plant extracts were effective in inhibiting the sporulation of both *E. suis* and *E. debliecki* oocysts as well as destroying them, while a minor, statistically non-significant percentage of oocysts remained sporulated.

- The alcoholic extracts of *C. officinalis*, *A. absinthium*, and *C. sativum* were the most potent and obtained the lowest LC50 values.

- As our *in vitro* results demonstrated that the APEs at higher concentrations had a dual effect, both inhibitory and destructive, their use as disinfectants in livestock shelters seems encouraging.

- In order to obtain the strongest anticoccidial effect, the implementation of herbal formulas which contain the most effective alcoholic plant extracts is needed. Further investigation on the isolation, purification, toxicity, and mechanism of action of the aforementioned major compounds (of tested plants) is required.

In vivo studies

I. *In vivo* assessment of the antiparasitic effects of *Allium sativum* and *Artemisia absinthium* against gastrointestinal parasites in swine, from low-input farms, in NW of Romania

II. The effects of *Coriandrum sativum* L. and *Cucurbita pepo* L. against gastrointestinal parasites in swine: An *in vivo* study

III. *Satureja hortensis* L. and *Calendula officinalis* L., two Romanian plants with *in vivo* antiparasitic potential on digestive parasites of pigs

Background & Aim

- ✓ Internal parasitic diseases of swine constitute one of the most important health issues in low-input livestock farming, affecting the welfare, reproduction performance and productivy of the infected animals.
- ✓ Phytotherapeutic remedies can be used for prophylaxis and therapy of digestive parasitosis and are a viable and sustainable alternative to chemical antiparasitics, but few of them have been subjected to scientific validation.
- ✓ Low-input swine farming in Romania adopted the traditionally use of the phytotherapy for controlling the pathogens in livestock.
- ✓ **The current studies aimed at evaluating, the *in vivo* antiparasitic activity of *Allium sativum*, *Artemisia absinthium L.*, *Cucurbita pepo*, *Coriandrum sativum*, *Satureja hortensis L.* and *Calendula officinalis* powders against digestive parasites in swine, in two low-input farms from Transylvania area.**

Materials and methods

- ✓ 2160 faecal samples were collected from weaners, fatteners, and sows.
- ✓ Different coproparasitological methods, including flotation (Willis, McMaster), centrifugal sedimentation, Ziehl-Neelsen staining as modified by Henricksen, a modified Blagg technique, and faecal cultures (nematode larvae/protozoan oocyst cultures) were involved during testing.

Materials and methods

1. Biochemical analyses of medicinal plants

- High performance liquid chromatography coupled with mass spectrometry (HPLC/MS) was used for the analysis of biologically active compounds present in the plant extracts. All the procedures were performed at the Iuliu Haţieganu University of Medicine and Pharmacy, in Cluj-Napoca.

2. Experimental design and swine husbandry

- For each farm and plant:
  - 3 control groups
    - 10 weaners, 10 fatteners and 10 sows
  - 3 experimental groups
    - 10 weaners, 10 fatteners and 10 sows
    - received *A. sativum* in a dosage of 180 mg/kg BW/day and *A. absinthium* in a dosage of 90 mg/kg BW/day for 10 consecutive days
    - received *C. sativum* in a dosage of 170 mg/kg BW/day and *C. pepo* in a dosage of 500 mg/kg BW/day for 10 consecutive days
    - received *C. officinalis* in a dosage of 140 mg/kg bw/day and *S. hortensis* in a dosage of 100 mg/kg BW/day for 10 consecutive days

3. Assessment of antiparasitic efficacy

Faecal egg count reduction test: **FECR (%) = 100 x (1–[T2/T1] x [C1/C2])**

- **T1** and **T2** are the mean pre- and post-treatment faecal egg counts (FEC) of a treated group
- **C1** and **C2** are the mean pre- and post-treatment FEC of control group

In vivo assessment of the antiparasitic effects of *Allium sativum* and *Artemisia absinthium* against gastrointestinal parasites in swine, from low-input farms, in NW of Romania

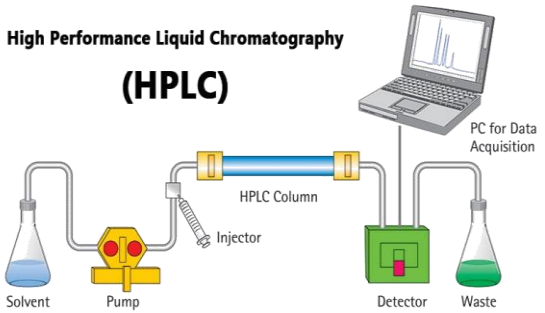
Results

Bioactive compounds		Vegetal species and plant part used for extraction and HPLC-MS analysis	
		Artemisia absinthium L.	*Allium sativum* L.
		herba	bulbus
Polyphenols (µg/mL)	Chlorogenic acid	107.15	-
	Caffeic acid	-	1.221
	p-coumaric acid	0.621	-
	Ferulic acid	0.759	0.456
	Sinapic acid	-	0.228
	Vitexin	1.631	-
	Isoquercitrin	56.754	-
	Rutoside	3.826	-
	Quercitrin	1.113	-
	Quercetol	6.285	-
	Luteolin	1.159	-
	Kaempferol	3.666	-
	Apigenin	0.481	-
	Syringic acid	1.85	-
	Protocatechuic acid	1.32	-
	Vanillic acid	1.98	-

Results

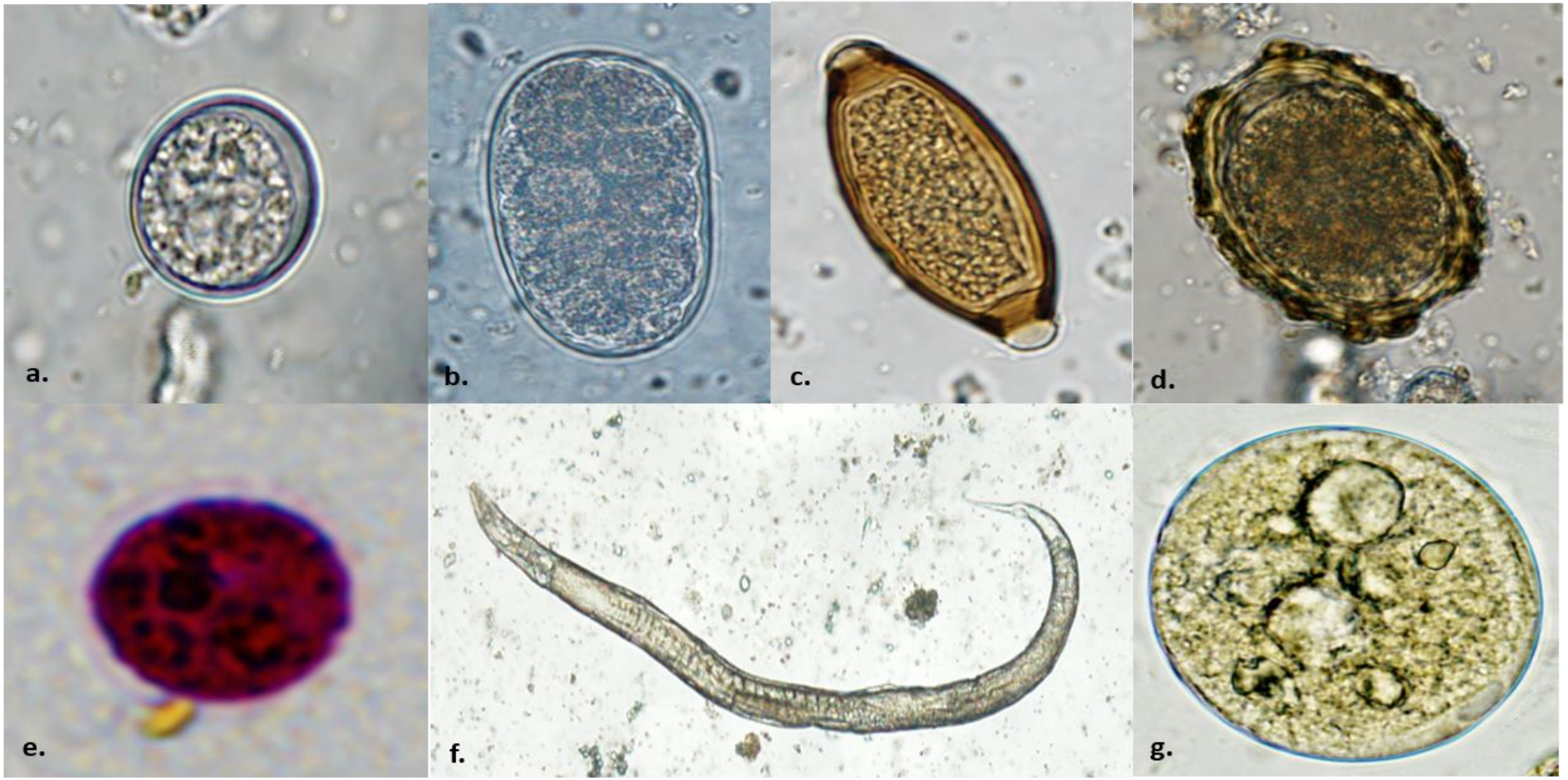
Bioactive compounds		Vegetal species and plant part used for extraction and HPLC-MS analysis	
		Artemisia absinthium L.	*Allium sativum* L.
		herba	bulbus
Tocopherols (ng/mL)	α-tocopherol	50.0	36.1
	γ-tocopherol	23.8	-
	Δ-tocopherol	5.0	-
Sterols (µg/mL)	Ergosterol	0.344	-
	Stigmasterol	34.831	-
	B-sitosterol	140.985	-
	Campesterol	3.329	-
Methyoxylated flavones (ng/mL)	Jaceosidin	-	-
	Hispidulin	3047.92	-
	Eupatorin	976.53	-
	Casticin	15384.14	-
	Acacetin	-	-
Sesquiterpene lactones (ng/ml)	α-santonin	450.52	-
	Vulgarin	6499.39	-
Sulfoxide (µg/mL)	Aliin	-	14.726

HPLC/MS—high performance liquid chromatography coupled with mass spectrometry; "-"—Not found;



Results

The coproparasitological examination revealed co-infections of up to five species of gastrointestinal parasites, namely *Eimeria* spp. (a), *Oesophagostomum* spp (b), *Trichuris suis* (c), *Ascaris suum* (d), *Cryptosporidium* spp. (e)*, S. ransomi (*f), and *Balantioides coli* (g).



Results

Percentage of faecal egg/oocyst/cyst count reduction (%) recorded on days 14, and 28 post-treatment in F1 and F2 farms (using FECR formula)

Parasite	*A. sativum* (14)						*A. sativum* (28)					
	Weaners		Fatteners		Sows		Weaners		Fatteners		Sows	
	F1	F2	F1	F2	F1	F2	F1	F2	F1	F2	F1	F2
Eimeria spp.	76.7	82.1	62.1	79.6	**100**	**100**	88.1	84.6	**20.0**	84.1	78.9	83.5
B. coli	59.8	74.2	**76.1**	75.1	82.3	66.3	**47.9**	72.3	66.7	69.8	55.8	67.8
A. suum	-	-	82.3	79.8	**87.6**	72.1	-	-	84.7	86.3	68.2	**62.8**
T. suis	-	-	66.7	**76.6**	-	-	-	-	63.9	**54.1**	-	-
Oesophagostomum spp.	**100**	-	-	-	**100**	87.5	88.7	-	-	-	67.3	**45.8**
S. ransomi	64.4	-	**100**	-	**100**	-	**57.3**	-	**100**	-	**100**	-
Parasite	***A. absinthium* (14)**						***A. absinthium* (28)**					
	Weaners		Fatteners		Sows		Weaners		Fatteners		Sows	
	F1	F2	F1	F2	F1	F2	F1	F2	F1	F2	F1	F2
Eimeria spp.	74.2	84.0	71.8	**33.1**	65.8	92.4	71.5	84.9	85.1	**100**	56.3	89.8
B. coli	72.1	**88.4**	60.3	37.7	58.7	88.0	63.3	80.6	46.9	71.9	**31.6**	85.1
A. suum	-	-	71.3	64.9	44.7	**80.5**	-	-	70.4	64.3	**30.2**	78.6
T. suis	-	-	50.4	**39.5**	-	-	-	-	49.9	**79.2**	-	-
Oesophagostomum spp.	33.2	-	-	-	49.5	63.1	**25.1**	-	-	-	43.8	**66.7**
S. ransomi	36.2	-	-	-	44.4	-	**31.3**	-	-	-	**69.1**	-

"-"= was not diagnosed

Conclusions

- This experiment was conducted between April and July 2021, on two free-range (low-input) Transylvanian farms, involving pigs of the Bazna and Mangalitza breeds.

- Both plant powders at the previously mentioned doses for 10 consecutive days had a strong antiprotozoal and anthelmintic activity, with *A. sativum* being more effective.

- *A. sativum* and *A. absinthium* have the potential of treating gastrointestinal parasitosis in swine.

- The antiparasitic efficacy can be attributed to the presence of polyphenols, tocopherols, flavonoids, sesquiterpene lactones and sulfoxide.

The effects of *Coriandrum sativum* L. and *Cucurbita pepo* L. against gastrointestinal parasites in swine: An *in vivo* study

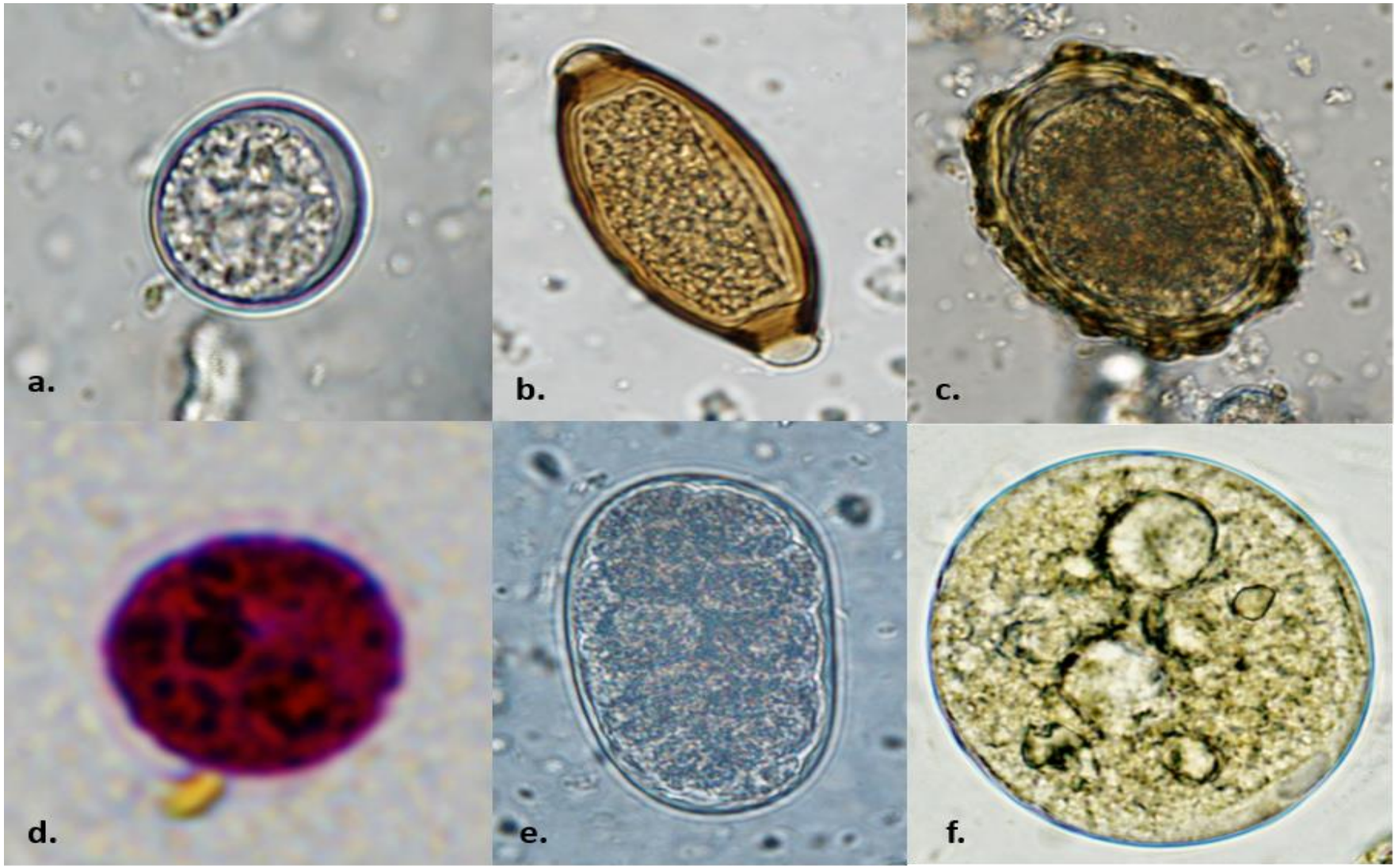
Results

Chemical class	Chemical compound	Plant species and plant part used for extract preparation and the results of HPLC-MS analysis	
		Coriandrum sativum L.	*Cucurbita pepo* L.
		fruit	seed
Polyphenols (µg/mL)	Chlorogenic acid	4.177	-
	p-coumaric acid	0.501	-
	Ferulic acid	0.759	-
	Rutoside	<LOQ	-
	Syringic acid	0.09	-
	Vanillic acid	0.94	-
Tocopherols (ng/mL)	γ-tocopherol	-	446.0
	Δ-tocopherol	-	23.2
Sterols (µg/mL)	Ergosterol	0.584	-
	Stigmasterol	9.675	22.024
	B-sitosterol	31.548	5.355
	Campesterol	1.780	0.358

HPLC/MS—high performance liquid chromatography coupled with mass spectrometry; "-"—Not found; <LOQ—identified based on MS spectra but not determined quantitatively, below limit of quantification.

Results

The examination revealed parasitic infections with **a**- *Eimeria spp.* oocyst*,* **b**- *T. suis egg, c- A. suum* egg, **d**- *Cryptosporidium* spp. cyst, **e**- *Oesophagostomum spp.* egg, and **f**- *B. coli*



Results

Percentage of faecal egg/oocyst/cyst count reduction (%) recorded on days 14, and 28 post-treatment in F1 and F2 farms (using FECR formula)

Parasite	*C. sativum* (14)						*C. sativum* (28)					
	Weaners		Fatteners		Sows		Weaners		Fatteners		Sows	
	F1	F2	F1	F2	F1	F2	F1	F2	F1	F2	F1	F2
Eimeria spp.	71.4	72.1	80	30.6	60	41.5	**100**	**25.4**	**100**	**100**	50	75.7
B. coli	29.6	68.9	44.4	62.4	**23.2**	74.2	**84.4**	79.5	50.4	20.1	67.4	31.2
A. suum	-	18.1	8.1	13.9	-	**0**	-	**30.3**	0	7.2	-	**0**
T. suis	-	0	0	0	-	-	-	3.3	0	0	-	-
Parasite	***C. pepo* (14)**						***C. pepo* (28)**					
	Weaners		Fatteners		Sows		Weaners		Fatteners		Sows	
	F1	F2	F1	F2	F1	F2	F1	F2	F1	F2	F1	F2
Eimeria spp.	11.6	**96.6**	13.9	33.3	45.4	**0**	24.9	94.7	35.9	**0**	61.1	0
B. coli	2.3	**59.5**	22.9	54.9	3.0	30.1	**0**	34.1	45.1	24.8	33.6	22.2
A. suum	77.4	80.9	83.5	79.7	87.1	**70.3**	79.7	**100**	84.5	95.9	85.9	88.9
T. suis	91.6	80.7	**50.1**	75.0	-	-	91.0	**100**	57.7	**100**	-	-

"-"= was not diagnosed; "0"= was identified, but had no efficacy

Conclusions

- This experiment was carried out between September and December 2021.
- Both plant powders at the previously mentioned doses for 10 consecutive days, were efficient against gastrointestinal parasites in swine. Coriander was more effective against protozoa while pumpkin showed better efficacy against helminths.
- Considering all the constraints of Romanian livestock farming, these results are a beacon of hope for better management and welfare practices in the swine farming.
- In addition, to the best of our knowledge, this is the first ethnopharmacological report on the antiparasitic effects of *C. pepo* and *C. sativum* traditionally used in Romania for treating protozoa and nematode infections in swine.

Satureja hortensis L. and *Calendula officinalis* L., two Romanian plants with *in vivo* antiparasitic potential on digestive parasites of pigs

Results

Chemical class	Chemical compound	Plant species and plant part used for extract preparation and the results of HPLC-MS analysis	
		Calendula officinalis L.	*Satureja hortensis* L.
		aerial part	aerial part
Polyphenols (µg/mL)	Chlorogenic acid	220.767	<LOQ
	Caffeic acid	-	<LOQ
	p-coumaric acid	-	1.464
	Ferulic acid	-	0.557
	Isoquercitrin	38.877	6.515
	Rutoside	18.819	<LOQ
	Quercitrin	<LOQ	0.365
	Quercetol	-	0.394
	Luteolin	-	6.621
	Apigenin	-	2.442
	Syringic acid	1.51	2.28
	Protocatechuic acid	0.67	0.95
	Vanillic acid	0.44	0.65

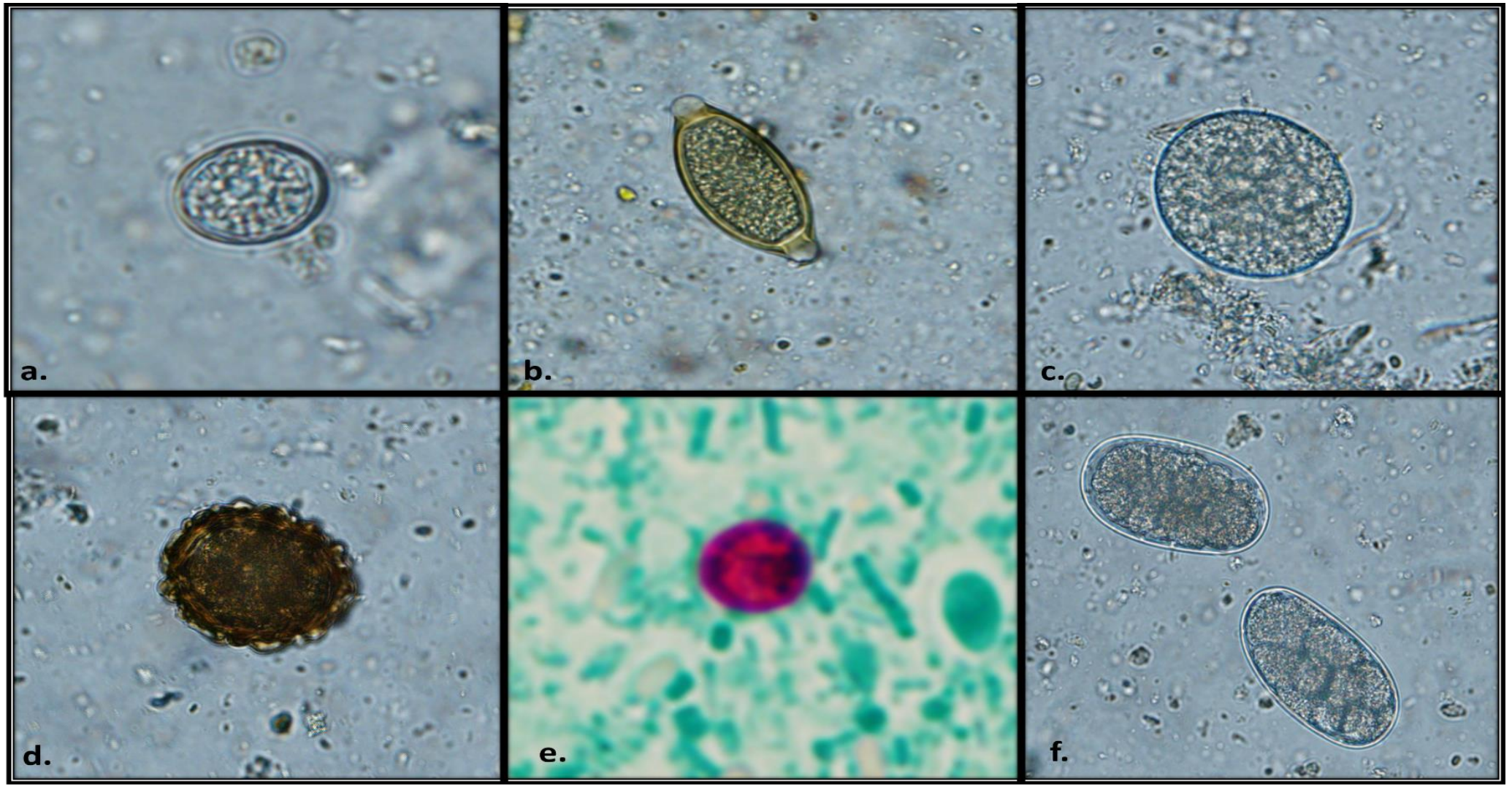
Results

Chemical class	Chemical compound	Plant species and plant part used for extract preparation and the results of HPLC-MS analysis	
		Calendula officinalis L.	*Satureja hortensis* L.
		aerial part	aerial part
Tocopherols (ng/mL)	α-tocopherol	61.6	86.8
	γ-tocopherol	248.9	89.0
	Δ-tocopherol	9.3	13.2
Sterols (µg/mL)	Ergosterol	0.500	1.420
	Stigmasterol	72.888	14.215
	B-sitosterol	241.997	313.315
	Campesterol	1.635	6.140
Methoxylated flavones (ng/mL)	Jaceosidin	-	8820.76
	Hispidulin	-	2483.00
	Acacetin	-	12691.97

HPLC/MS—high performance liquid chromatography coupled with mass spectrometry; "-"—Not found; <LOQ—identified based on MS spectra but not determined quantitatively, below limit of quantification.

Results

The examination revealed parasitic infections with **a**- *Eimeria* spp., **b**- *T. suis,* **c**- *B. coli*, **d**- *A. suum*, **e**- *Cryptosporidium* spp., and **f**- *Oesophagostomum spp.*



Results

Percentage of faecal egg/oocyst/cyst count reduction (%) recorded on days 14, and 28 post-treatment in F1 and F2 farms (using FECR formula)

Parasite	*C. officinalis* (14)						*C. officinalis* (28)					
	Weaners		Fatteners		Sows		Weaners		Fatteners		Sows	
	F1	F2	F1	F2	F1	F2	F1	F2	F1	F2	F1	F2
A. suum	-	-	**15.2**	10.3	-	49.9	-	-	54.2	34.9	-	**79.9**
T. suis	-	-	-	**8.2**	-	-	-	-	-	**20.3**	-	-
Oesophagostomum spp.	-	**60.5**	-	-	-	**28.6**	-	32.9	-	-	-	45.8
Eimeria spp.	91.8	42.5	**95.5**	75.9	-	74.9	72.5	57.1	88.9	**30.0**	-	76.5
B. coli	72.0	**90.9**	73.1	**53.6**	84.9	69.8	74.7	69.2	58.3	61.1	76.1	58.2
Parasite	***S. hortensis* (14)**						***S. hortensis* (28)**					
	Weaners		Fatteners		Sows		Weaners		Fatteners		Sows	
	F1	F2	F1	F2	F1	F2	F1	F2	F1	F2	F1	F2
A. suum	-	-	70.8	77.1	**91.1**	88.7	-	-	77.1	81.2	72.1	**59.7**
T. suis	-	-	**80.5**	84.0	-	-	-	-	**90.3**	87.1	-	-
Oesophagostomum spp.	-	-	-	-	80.2	**69.2**	-	-	-	-	**100**	83.7
Eimeria spp.	78.2	68.7	76.3	89.7	**25.1**	70.3	66.8	80.3	46.8	83.8	80.9	**94.1**
B. coli	80.1	88.4	**63.5**	74.7	70.2	70.5	83.6	**86.5**	72.2	71.2	70.7	74.6

"-"= was not diagnosed;

Conclusions

- The present experiment was conducted between April and June 2022.
- Both plant powders at the previously mentioned doses for 10 consecutive days, showed promising *in vivo* antiparasitic activity.
- *C. officinalis* had a strong antiprotozoal activity and mildly antihelmintic effects while *S. hortensis* was very effective against both helminths and protozoa infections.
- The antiparasitic efficacy can be attributed to the presence of polyphenols, sterols, tocopherols and flavonoids.
- The current study is the first report about the antiparasitic effects of *C. officinalis* and *S. hortensis* against digestive parasites of pigs, from Romania.

B

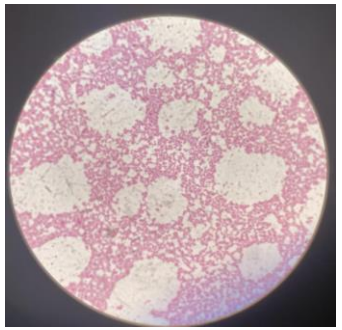
THE POTENTIAL OF ALCOHOLIC PLANT EXTRACTS TO CONTROL PATHOBIONTS OF SWINE ON A LOW-INPUT FARM

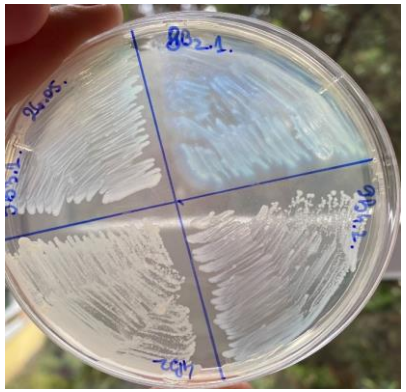
Aim of study

This research aimed to investigate the natural potential of locally available traditional medicinal plants in controling the antibiotic resistant bacterial load in swine raised on low-input outdoor farms from North Western and Central Romania.

TASK - Improving the robustness of laying hens and piglets against parasitic and bacterial infections by innovative feeding strategies and optimal use of outdoor area rich in vegetation

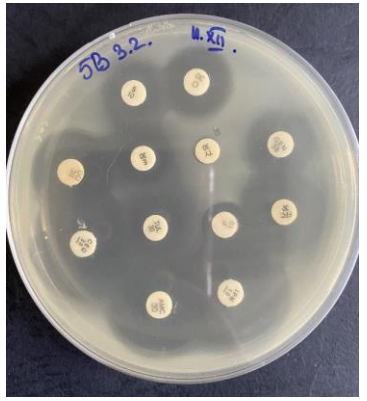
Materials and methods

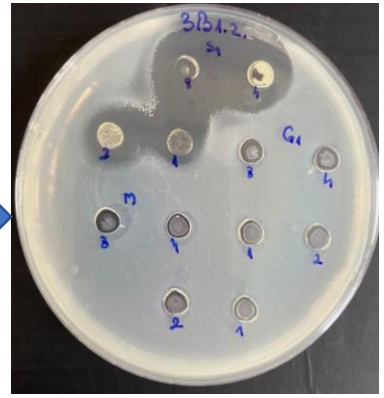




Aerobic bacterial strains (n=14) from the nasal cavities of extensively raised swine:

- ✓ biochemically identified (Vitek®2 Compact System)
- ✓ tested for susceptibility to antibiotics (n=12, antibiotic classes=6, Kirby-Bauer method)
- ✓ tested for susceptibility to plant extracts (aromatogram).

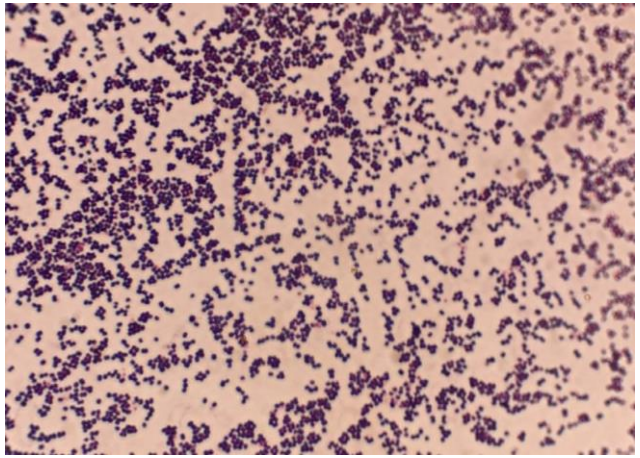




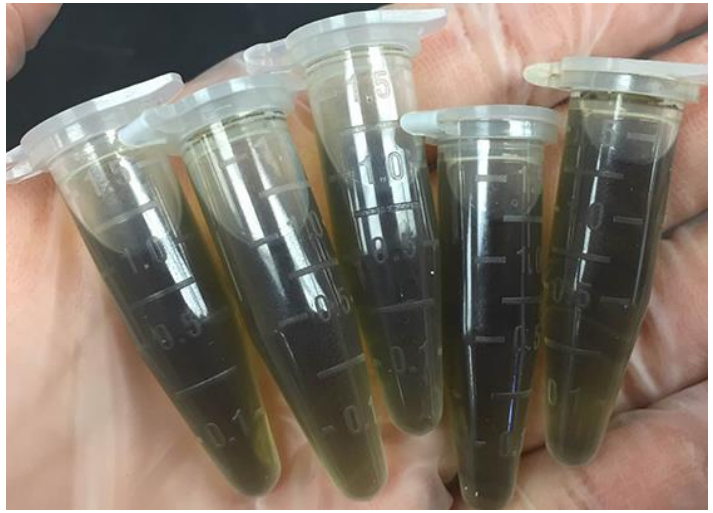
Materials and methods

AROMATOGRAM TECHNIQUE

- pure bacterial strains, 18-24h old diluted in broth to an optical turbidity of 0.5 McFarland
- Petry plates covered with the prepared solution
- 12 wells cut with a diameter of 6 mm
- 37.5 µl of alcoholic plant extract inoculated into each well
- 24h incubation at 37℃ - reading of inhibition diameters

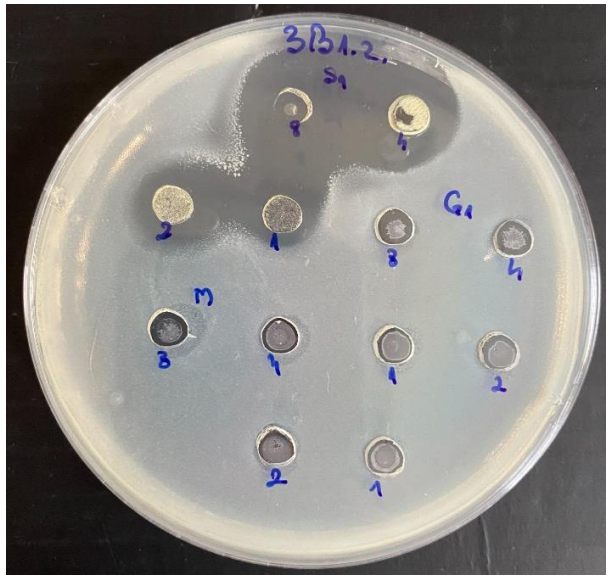


Stahylococcus vitulinus – Gram stain



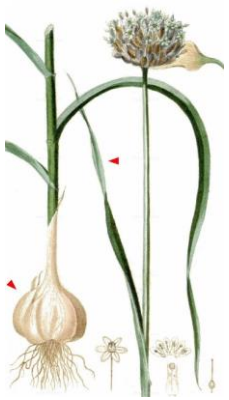
Alcoholic plant extracts

Aromatogram



Materials and methods

The active principles - identified by gas chromatography coupled with mass spectrometry in all tested plants.



Allium sativum - bulbs

Calendula officinalis - flowers

Coriandrum sativum -seeds

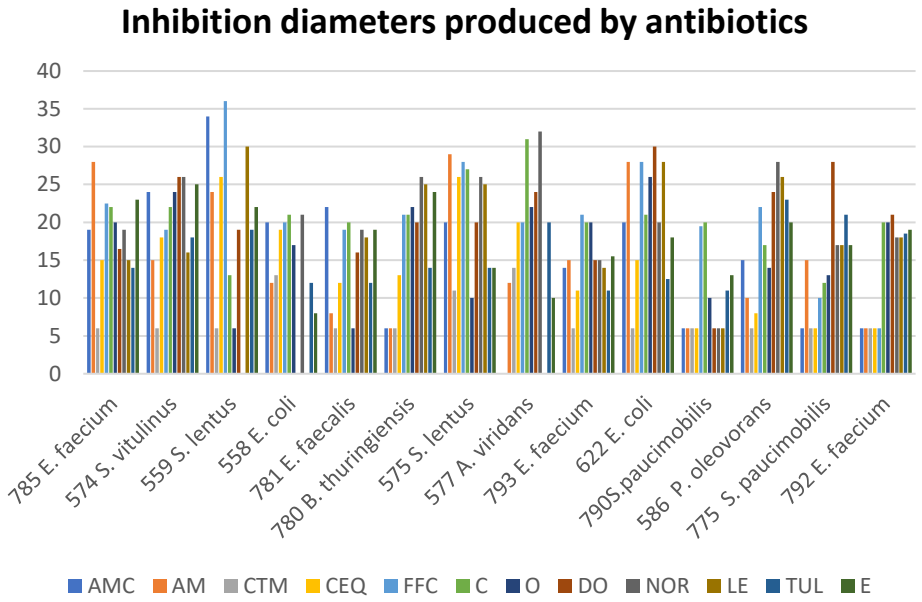
Artemisia absinthium – whole plant

Cucurbita pepo - seeds

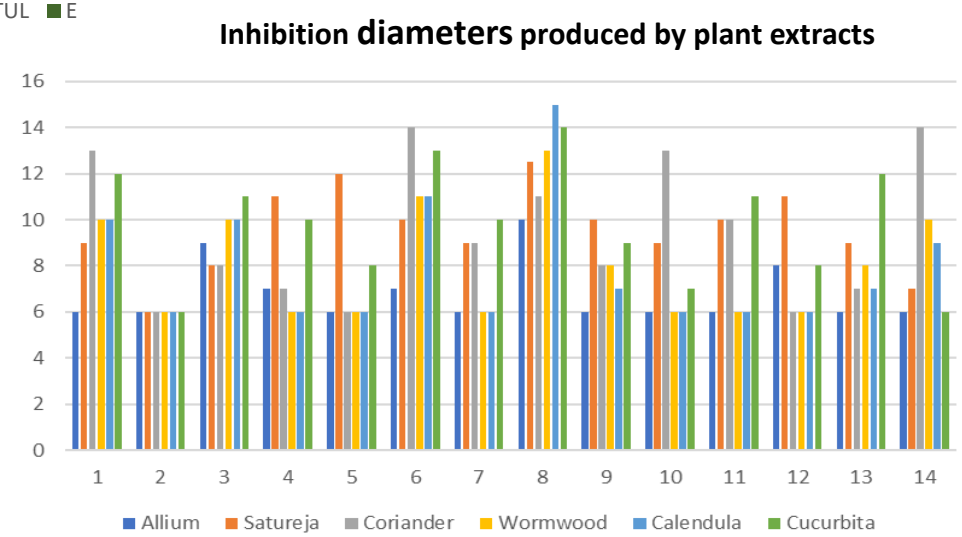
Satureja hortensis – whole plant

Results

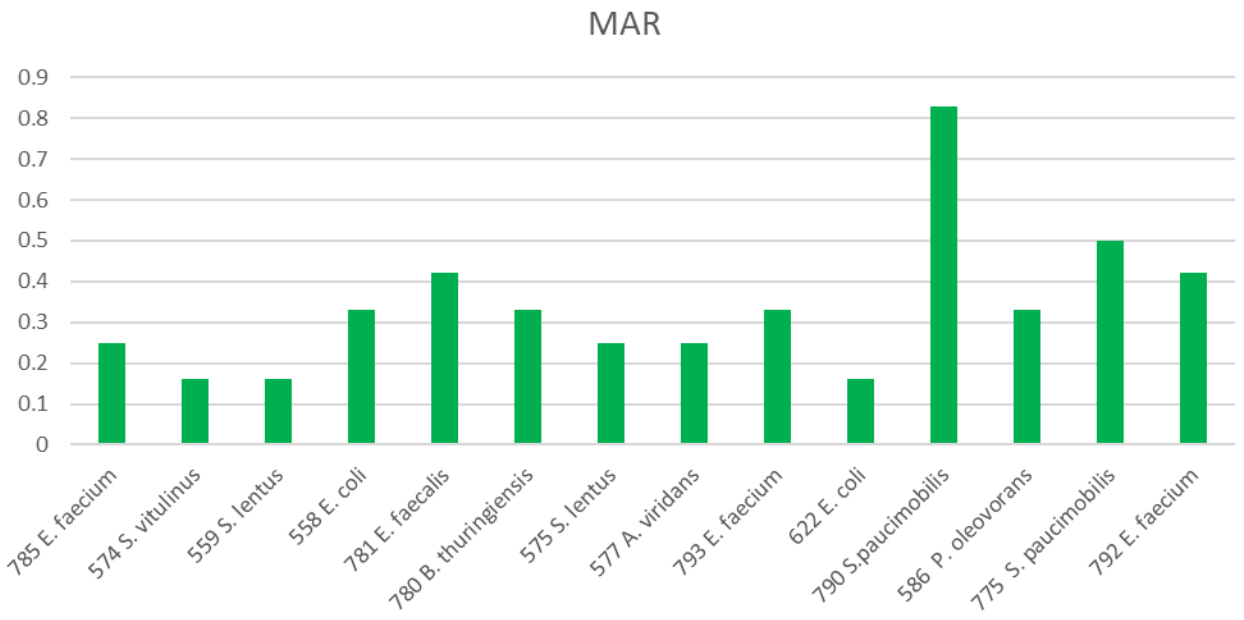
- **highest** average of inhibition diameters - chloramphenicol (20.75±0.92 mm) and norfloxacin (20.68±1.55 mm)
- **lowest** - cefotaxime (7.5±0.79 mm)



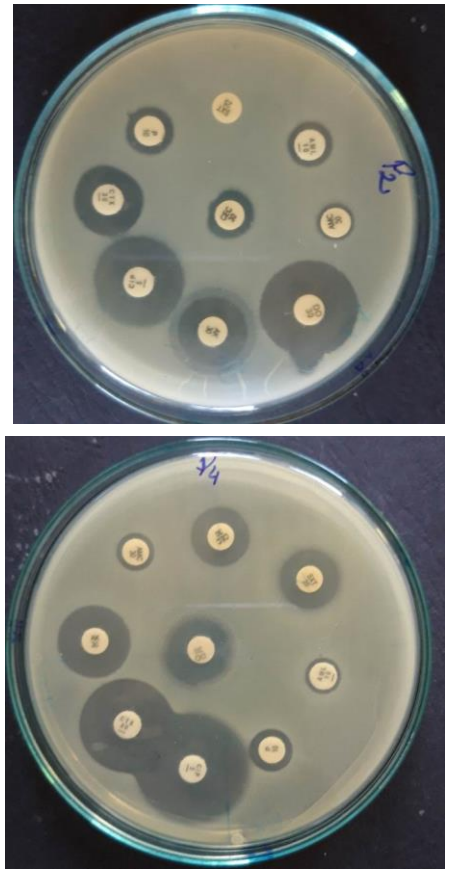
- **highest** average of inhibition diameters was of 11.11±0.68 mm for C. sativum extract and of 9.78±0.68 mm for C. pepo
- **lowest** average was found in Allium sativum - 6.86±0.35 mm.



Results



Multiple antibiotic resistance (MAR) index in studied bacterial strains



- The antibiogram indicated a multiple antibiotic resistance (MAR) index > 0.2 in 86% of the bacteria (overall MAR=0.34).

Conclusions

- Some of the tested plant extracts could display a considerable antimicrobial activity on pathobionts of swine.

- These plants could enhance the welfare of the animals by reducing the potentially pathogenic, antibiotic resistant bacterial load, as an alternative to classical antibiotic therapy.

C

EFFECTS OF *A. ABSITHIUM* SUPPLEMENTED FEED ON THE SPECIFIC CELL-MEDIATED RESPONSE IN PIGS FROM A LOW-INPUT FARM

Raising pigs in extensive system enhances their susceptibility to changes in micro- and macro-climate (uncontrollable stressfull factor)

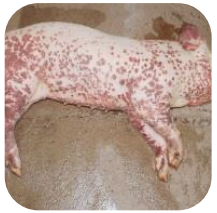
The infectious pressure is increased due to direct, unrestricted contact with the environment

Parasitic, bacterial and viral diseases cause major losses in swine, thus inducing a high health, welfare and also economic impact.

More and more wide-spreading free-range farming depends on the factors targeting environment protection, plant health, animal health, food safety, and consumer health.

Under immune suppressive circumstances it is important to define and use

imunestimulating/imunomodulating products of vegetal origin



Potentiate the host ability to control infection

Diminishes the allopatic/synthetic drug consumption

Prevents antibiotic resistance

Objectives

1. • Testing tolerance to **oral administration of *Artemisia absithium***
2. • Testing the ***in vitro*** **spontaneous and mitogen induced cell-mediated immune responsiveness**
3. • Testing the ***in vitro*** **effects of other plant extracts**

Materials and methods

Alcoholic plant extracts were prepared according to the provisions of German pharmacopoeia by the University of Pharmacy, Cluj-Napoca, Romania

Method 1. A new LC-MS method was used to identify 6 polyphenols in WS extracts: epicatechin, catechin, syringic acid, gallic acid, protocatechuic acid and vanilic acid.

Method 2. The MS signal was used only for qualitative analysis based on specific mass spectra of each polyphenol. The MS spectra obtained from a stanYesrd solution of polyphenols were integrated in a mass spectra library.

Dosages of *Artemisia absinthium* were established based on the literature

Materials and methods

The research was carried out on extensively raised Mangalitza suckling, weaned piglets and sows (n=10 for each group).

The feed supplemented with 5‰ *Artemisia absinthium* L and granulated was admninistered as daily ratio for 7-10 days/group.

Materials and methods

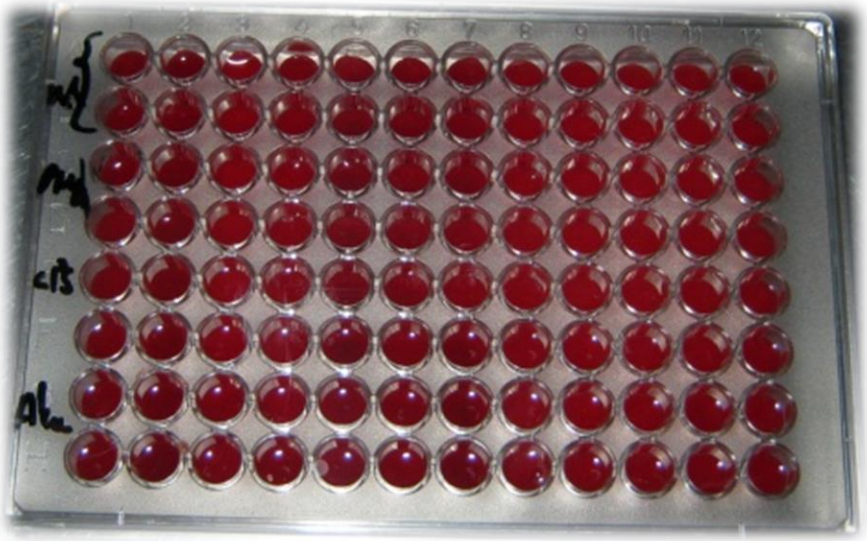
Blood was sampled before and after the end of oral treatment period; then it was mixed with RPMI1640 (1:4, Sigma Aldrich, USA), divided in 200µl aliquots in 96 well-plates and supplemented with alcoholic plant extracts (*Calendula officinalis, Satureja hortensis, Allium sativum, Coriandrum sativum, Cucurbita maxima,* 1.5 µl/well).

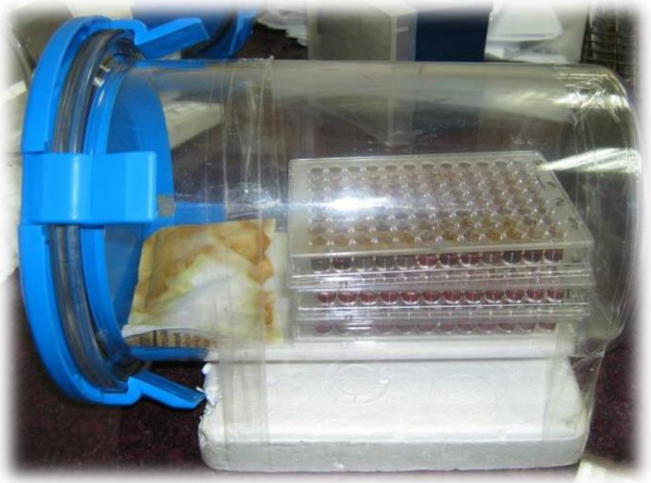





Materials and methods

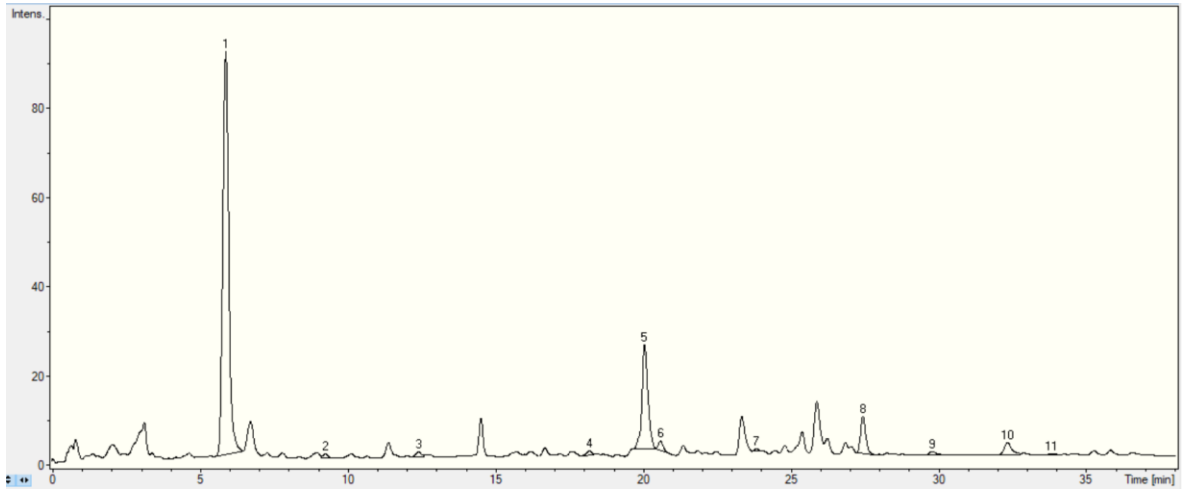
The plates were incubated at 37^{o}C for 48 h, residual glucose was quantified spectrophotometrically (SUMAL PE2, Karl Zeiss, Jena) and stimulation indices (SI %) were calculated.





The groups were compared by Student's t test for statistical significance of the results.

Results and discussions



The MS spectra obtained for poliphenols in *Artemisia absinthium*

Chlorogenic acid

antidiabetic effect, DNA protective effect, and neuroprotective effect. inhibitory activity against hepatitis B virus (HBV) in vivo and in vitro antioxidant

Polyphenols (method 1)					
Nr. pe cromatogramă	Compus	Nr.	Identified UV	Identified by qualitatuive MS	Concentration in the extract (µg/ml)
1	Clorogenic acid	4	Yes	Yes	107.150
2	p-cumaric acid	5	Yes	Yes	0.621
3	Ferulic acid	6	Yes	Yes	0.759
4	Vitexine	8	Yes	Yes	1.631
5	Isoquercitrine	11	Yes	Yes	56.754
6	Rutozid	12	Yes	Yes	3.826
7	Quercitrine	15	Yes	Yes	1.113
8	Quercetol	17	Yes	Yes	6.285
9	Luteoline	20	Yes	Yes	1.159
10	Kaempferol	21	Yes	Yes	3.666
11	Apigenine	22	Yes	Yes	0.481

Results and discussions

Polyphenols (method 2)	
Siringic acid	1.85 (µg/mL)
Protocatechuic acid	1.32 (µg/mL)
Vanilic acid	1.98 (µg/mL)

Metoxilate flavones	
Eupatorine	976.53 (ng/mL)
Casticine	15385.14 (ng/mL)
Hispidulin e	3047.92 (ng/mL)

Tocopherols	
Alpha-tocopherol	50.0 (ng/mL)
Gamma-tocopherol	23.8 (ng/mL)
Delta-tocopherol	5.0 (ng/mL)

The extract of *Artemisia annua L.* has been provides anti-inflammatory, antibacterial and antimicrobial properties which can be considered as a promising medicinal component in therapeutic applications.

Results and discussions

Sterols	
Ergosterol	344 (ng/mL)
Stigmasterol	34831 (ng/mL)
Beta-sitosterol	140985 (ng/mL)
Campesterol	3329 (ng/mL)

Sesquiterpen lactones	
Alfa-santonin	450.52 (ng/mL)
Vulgarin	6499.39 (ng/mL)

Vulgarin possesses strong and stable binding efficiency with multidrug resistant (MDR) *Acinetobacter baumannii eflux protein (*Ab-EP), a known pathogen for one health

Suvaithenamudhan, S.; Ananth, S.; Mariappan, V.; Dhayabaran, V.V.; Parthasarathy, S.; Ganesh, P.S.; Shankar, E.M. *In Silico* Evaluation of Bioactive Compounds of *Artemisia pallens* Targeting the Efflux Protein of Multidrug-Resistant *Acinetobacter baumannii* (LAC-4 Strain). *Molecules* **2022**, *27*, 5188

Beta-sitosterol exhibited the potential to inhibit the biosynthesis of peptidoglycan and prevent bacteria cell wall formation

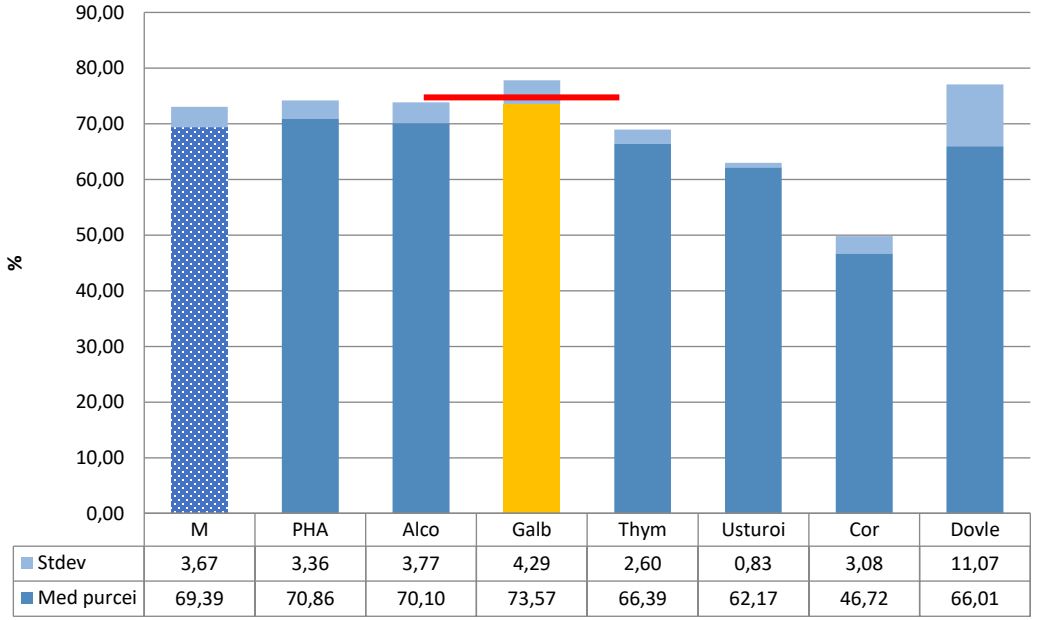
Evangelina IA, Herdiyati Y, Laviana A, Rikmasari R, Zubaedah C, Anisah, Kurnia D. Bio-Mechanism Inhibitory Prediction of β-Sitosterol from Kemangi (*Ocimum basilicum* L.) as an Inhibitor of MurA Enzyme of Oral Bacteria: In vitro and in silico Study. Adv Appl Bioinform Chem. 2021 Jun 23;14:103-115.

Results and discussions

The oral treatment with the *A. absithium* supplemented feed significantly (p<0.05-0.001) decreased all SI%, the least in suckling piglets.
Only the extract of *C.maxima* acted stimulating in suckling piglets (53.37±7.13%).

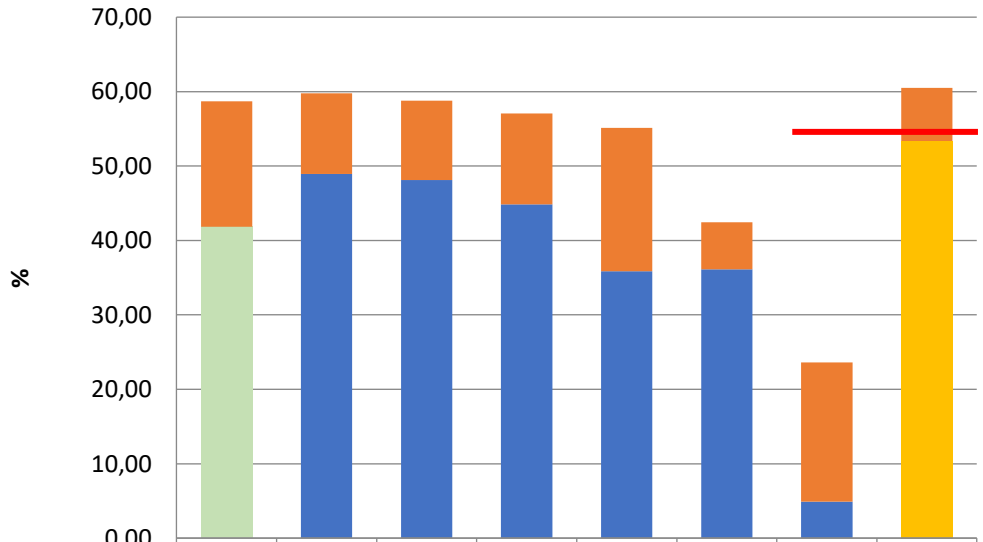
Before

Suckling piglets



	M	PHA	Alco	Galb	Thym	Usturoi	Cor	Dovle
Stdev	3,67	3,36	3,77	4,29	2,60	0,83	3,08	11,07
Med purcei	69,39	70,86	70,10	73,57	66,39	62,17	46,72	66,01

After



	M	PHA	Alco	Galb	Thym	Usturoi	Cor	Dovl
Stdev	16,89	10,82	10,64	12,23	19,27	6,29	18,68	7,13
Med purcei	41,83	48,94	48,13	44,84	35,85	36,14	4,92	53,37

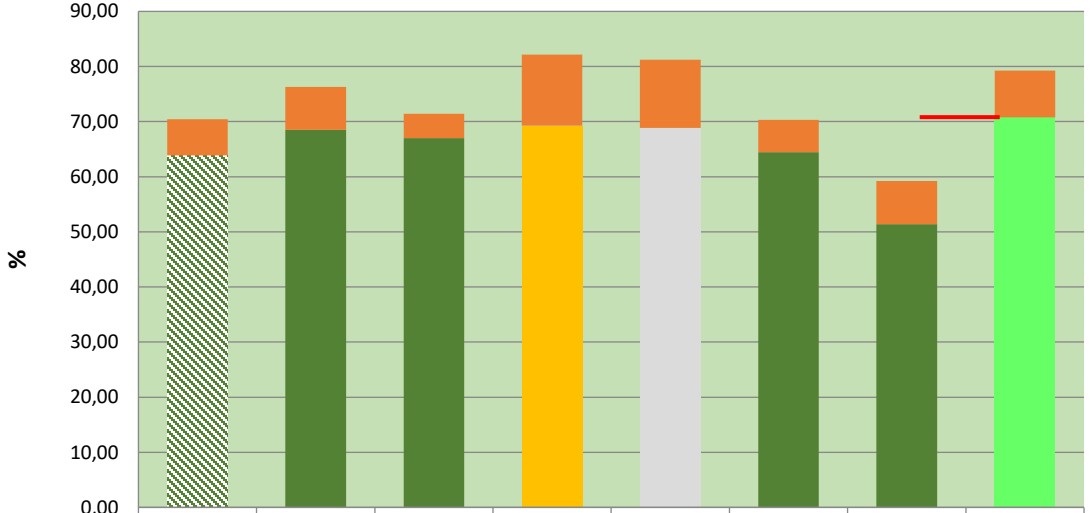
Results and discussions

None of the extracts acted stimulating, the lowest indices being recorded for *C. sativum*, within the negative range in weaners and sows.

Only the extract of *C.maxima* acted stimulating in suckling piglets (53.37±7.13%).

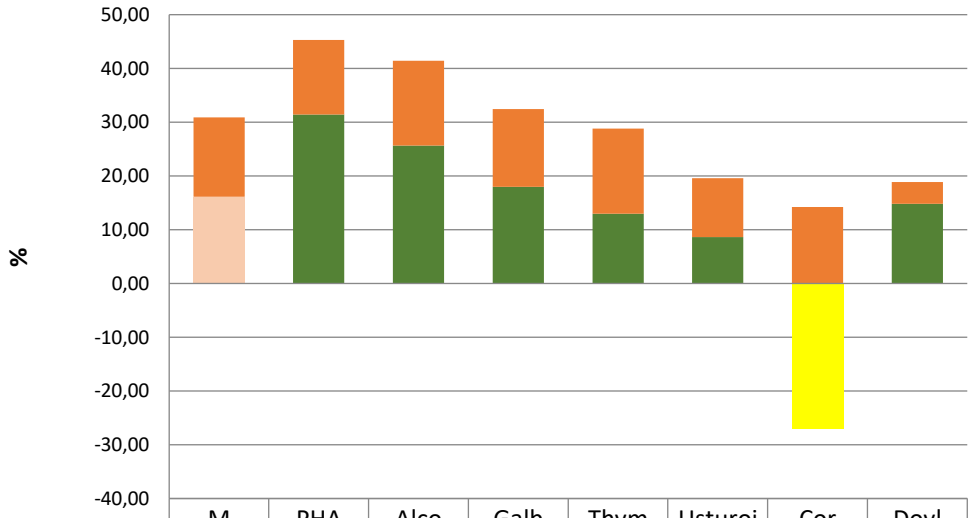
Before

Weaners



	M	PHA	Alco	Galb	Thym	Usturoi	Cor	Dovle
Stdev	6,53	7,78	4,37	12,86	12,38	5,82	7,88	8,47
Med intarc	63,88	68,50	67,01	69,29	68,83	64,47	51,36	70,77

After



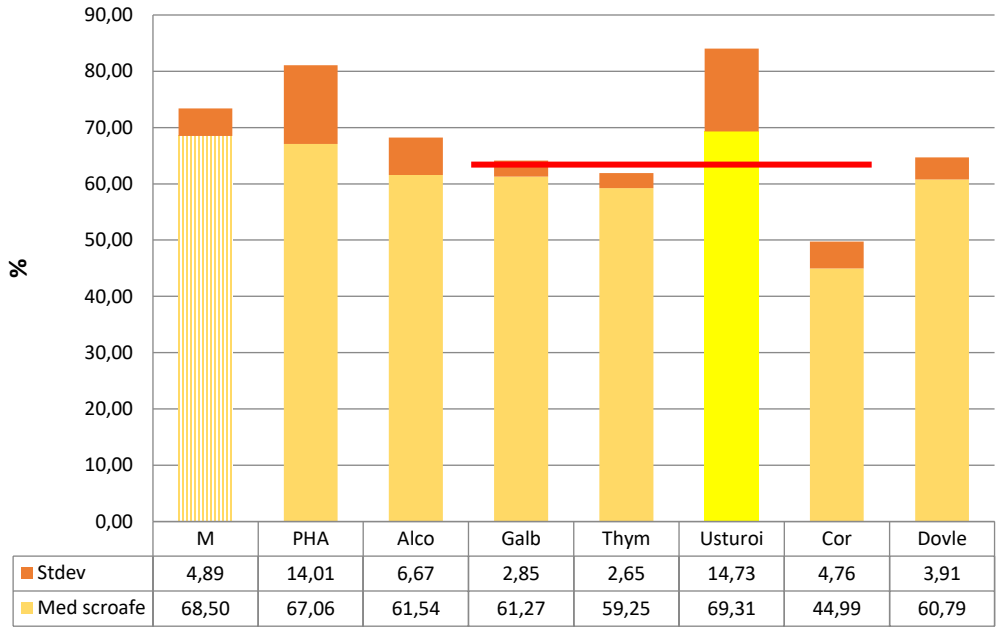
	M	PHA	Alco	Galb	Thym	Usturoi	Cor	Dovl
Stdev	14,71	13,88	15,76	14,46	15,86	10,95	14,24	4,07
Med intarc	16,18	31,47	25,69	18,00	12,98	8,67	-27,02	14,84

Results and discussions

None of the extracts acted stimulating, the lowest indices being recorded for *C. sativum*, within the negative range in weaners and sows.

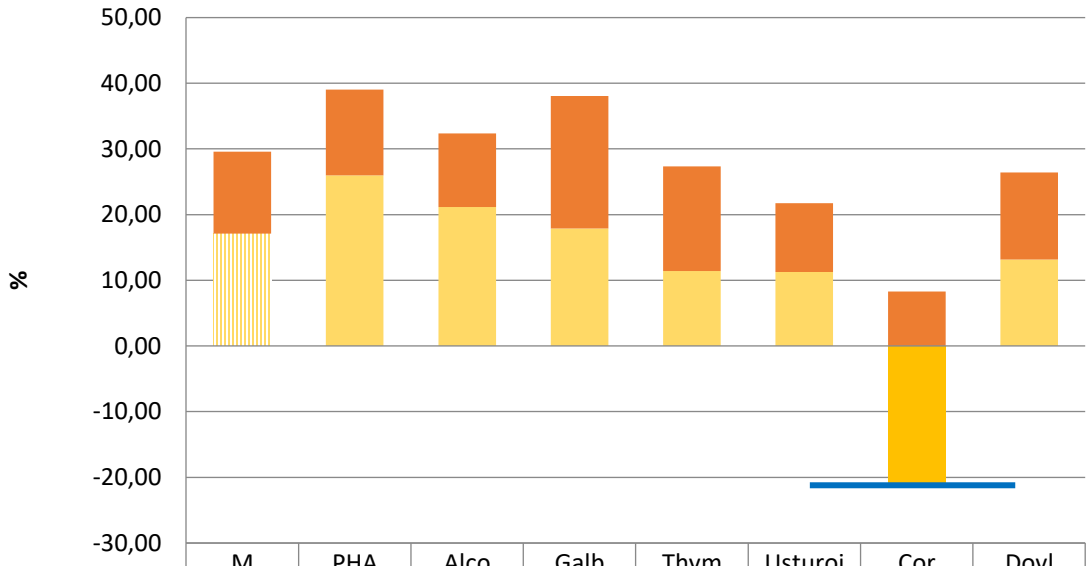
Before

Sows



	M	PHA	Alco	Galb	Thym	Usturoi	Cor	Dovle
Stdev	4,89	14,01	6,67	2,85	2,65	14,73	4,76	3,91
Med scroafe	68,50	67,06	61,54	61,27	59,25	69,31	44,99	60,79

After



	M	PHA	Alco	Galb	Thym	Usturoi	Cor	Dovl
Stdev	12,46	13,08	11,19	20,19	15,90	10,49	8,30	13,25
Med scroafe	17,10	25,96	21,15	17,87	11,42	11,26	-20,98	13,17

Conclusion

The results indicated negative effects of *A. absinthium* on the specific immune response when administered orally in pigs, suggesting the eventual reconsideration of its administration dosage/protocole.

Thank you for your attention!

PPILOW Romania team

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Pall Emoke
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