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PCR Detection of *Balantioides coli* in Domestic Pigs: A Preliminary Study

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INTRODUCTION

- *Balantioides coli* is a ciliated protozoan parasite with zoonotic potential.
- Domestic pigs are considered its main reservoir host.
- Aim of the study: to detect the presence of *B. coli* in fecal samples collected from domestic pigs using polymerase chain reaction (PCR) as a molecular diagnostic method.
- Individual fecal samples were processed for parasitic DNA extraction, followed by amplification of a specific genetic region of the protozoan.

2 MATERIAL AND METHOD

A. Animals studied

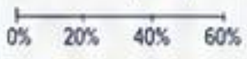
- 82 pigs.
- Crossbreeds of Landrace, Duroc, Pietrain, and Large White.
- 37 males and 45 females.
- Raised in an extensive system, in individual households, under variable conditions, some adequate and others less favorable.
- Age range: 4 to 40 months.

B. Age categories

Group 1
34 pigs, no more than 6 months **(41%)**

Group 2
23 pigs, 6 to 12 months **(28%)**

Group 3
25 pigs, older than 12 months **(31%)**



C. Sampling and processing

- 82 fecal samples collected in plastic containers.
- Labeled and transported under refrigerated conditions.
- Examined in the Clinic of Parasitology and Parasitic Diseases of the Faculty of Veterinary Medicine in Timişoara.
- Initially processed using the Willis method.
- Subsequently stored at −20 °C.
- Before DNA extraction, each sample was thawed at 4 °C.
- Fecal samples were subjected to parasitological examination using the Willis method and to the molecular PCR method.

D. Organization of samples into pools

- 82 fecal samples were grouped into 21 pools according to a group testing strategy.

- 20 pools contained four individual samples each.
- 1 pool contained two samples.

PCR protocol

- Genomic DNA isolation and purification were performed using the MagMAX™ CORE Nucleic Acid Purification Kit, according to the manufacturer's instructions.
- Extracted DNA was stored at −20 °C until PCR amplification.
- Identification targeted an approximately 410-base-pair fragment of the SSU rRNA gene and the ITS1–5.8S rRNA–ITS2 region.
- Primers used:
  - BSD (Forward Primer): 5'-GCTCCTACCGATACCGGGT-3'
  - BSRC (Reverse Primer): 5'-GCCGGTCATCTTACTTGATTTC-3'

(Zhang et al. (2024), Li et al. (2020), and Ponce-Gordo et al. (2008))

- PCR reaction final volume: 25 µL, containing:
  - 14.75 µL distilled water
  - 2 µL dNTP mixture
  - 2 µL $MgCl_2$
  - 2.5 µL 10× PCR buffer without Mg^{2+}
  - 0.5 µL of each primer
  - 0.25 µL Ex-Taq polymerase (5 U/µL)
  - 2.5 µL extracted DNA
- Amplification conditions:
  - Initial denaturation: 95 °C for 4 minutes
  - 35 cycles of: 95 °C for 1 minute, 56 °C for 1 minute, 72 °C for 1 minute
  - Final extension: 72 °C for 5 minutes
- PCR products analyzed by electrophoresis in 1.5% agarose gel stained with ethidium bromide [Li et al., 2020].

3 RESULTS AND DISCUSSIONS

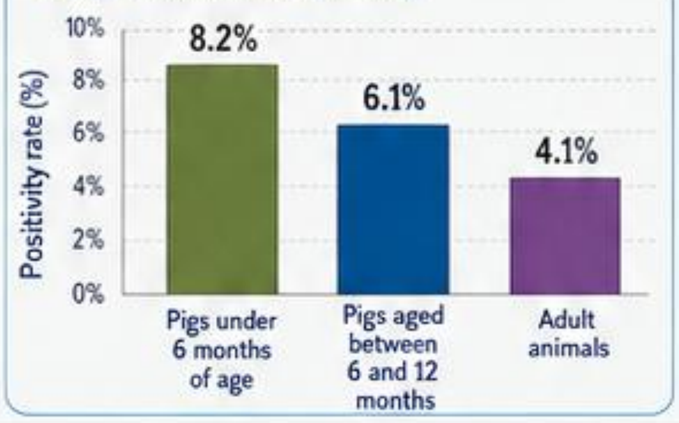
A. Main findings

Flotation (coproparasitological)
By flotation, *Balantioides coli* was identified in nine pigs.
Positivity rate by flotation: **18.4% (9/49)** relative to the total number of positive samples.
Positivity rate by flotation: **11% (9/82)** relative to the total number of examined samples.

VS.

PCR (molecular)
B. coli DNA was detected in **26.8% (22/82)** of the analyzed samples.

B. Age distribution of positivity



Interpretation summary

- The results indicate the presence of *Balantioides coli* infection in pigs raised under extensive/backyard farming conditions.
- The parasite remains present in swine populations and may be associated with husbandry conditions, hygiene standards, and sanitary management.
- Younger animals showed a higher frequency of infection, with a gradual decrease as age increases.

D. Comparisons with other studies

Study (Year)	Prevalence / Findings	Notes: Possible reasons for differences
Cioacă et al. (2025)	35.5% of pigs raised in backyard systems in Mehedinți County.	Local variations in husbandry practices and hygiene conditions.
Băieș et al. (2022)	70.31% in weaned piglets, 72.5% in fattening pigs, and 62.19% in sows; 960 fecal samples from pigs raised in an extensive system.	Differences in animal density, management practices, and larger sample size.
Thanasuwan et al. (2024)	276/324 pigs positive for gastrointestinal parasites (85.19% overall prevalence); *Balantioides coli* in 67 cases (20.68%); mixed infections 38.27%.	Diagnostic methods, geographical/climatic factors, and management-related factors.
Giaratana et al. (2021)	83 pigs positive, prevalence 46.89%, in Calabria, Italy.	Geographical/climatic and management-related factors.
Barbosa et al. (2016)	*B. coli cysts identified by direct examination in 22.4% of samples and by the Lutz technique in 21%; Faust and modified Sheather flotation techniques failed to detect the agent.*	Diagnostic methods can influence detection rate; flotation methods may miss infections.

Methodological takeaway

- The diagnostic method can significantly influence the detection rate.
- Flotation may underestimate the real prevalence.
- Molecular diagnosis provides higher specificity and allows confirmation of the etiological agent.
- Pooling may influence detection sensitivity, especially in samples with low parasitic load.
- Combining coproparasitological and molecular methods may provide a more complete assessment.

CONCLUSIONS

The study confirms the presence of *Balantioides coli* in pigs raised under extensive/backyard farming conditions, although at a lower level than that reported in most of the reviewed studies.

This indicates circulation of the protozoan in the investigated population and suggests either lower infection pressure or possible underestimation related to the diagnostic method used.

Considering the zoonotic potential of this parasite, detection in pigs is epidemiologically and public-health relevant, particularly in households where contact between animals, humans, and contaminated environments may occur frequently.

Periodic monitoring of pigs, combined coproparasitological and molecular methods, and improved hygiene conditions may help reduce transmission risk and improve understanding of the epidemiology of *B. coli* infection.

Molecular detection confirmed the circulation of *B. coli* in domestic pig populations and supports the role of swine as an important reservoir.

PCR provides a sensitive and specific approach for identifying *B. coli*, contributing to improved epidemiological diagnosis and assessment of zoonotic risk associated with direct or indirect contact with infected pigs.

These findings highlight the need for parasitological monitoring of pig herds and the implementation of appropriate hygiene and biosecurity measures on farms.